ISSN 1948-5204 (online)

World Journal of

Gastrointestinal Oncology

World J Gastrointest Oncol 2022 January 15; 14(1): 1-368

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 14 Number 1 January 15, 2022

REVIEW

MINIREVIEWS

World Journal of Gastrointestinal Oncology

Monthly Volume 14 Number 1 January 15, 2022

Contents

World Journal of Gastrointestinal Oncology

Monthly Volume 14 Number 1 January 15, 2022

Contents

World Journal of Gastrointestinal Oncology

Monthly Volume 14 Number 1 January 15, 2022

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Florin Burada

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

PUBLICATION DATE
January 15, 2022

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 1-18

DOI: 10.4251/wjgo.v14.i1.1

ISSN 1948-5204 (online)

REVIEW

Molecular regulatory mechanism of ferroptosis and its role in gastrointestinal oncology: Progress and updates

Fan Yang, Si-Yu Sun, Sheng Wang, Jin-Tao Guo, Xiang Liu, Nan Ge, Guo-Xin Wang

ORCID number: Fan Yang 0000-0002-5032-6450; Si-Yu Sun 0000-0002-7308-0473; Sheng Wang 0000-0002-1531-7655; Jin-Tao Guo 0000-0001-5722-6359; Xiang Liu 0000-0001-7538-6786; Nan Ge 0000-0002-5764-7054; Guo-Xin Wang 0000-0002-5928-3370.

Author contributions: Yang F wrote the paper; Sun SY, Wang S, Guo JT, Liu X, Ge N, and Wang GX collected the data.

Conflict-of-interest statement: The authors have no conflict of interests to declare for this article.

Supported by China Postdoctoral Science Foundation, No. 2020M670101ZX; Doctoral Scientific Research Foundation of Liaoning Province, No. 2019-BS-276; Science and Technology Program of Shenyang, No. 19-112-4-103; Youth Support Foundation of China Medical University, No. QGZ2018058; Scientific Fund of Shengjing Hospital, No. 201801; and 345 Talent Project of Shengjing Hospital, No. 52-30C.

Country/Territory of origin: China

Specialty type: Gastroenterology and Hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Fan Yang, Si-Yu Sun, Sheng Wang, Jin-Tao Guo, Xiang Liu, Nan Ge, Guo-Xin Wang, Department of Gastroenterology, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China

Corresponding author: Si-Yu Sun, MD, PhD, Chief Doctor, Doctor, Professor, Department of Gastroenterology, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Shenyang 110004, Liaoning Province, China. sun-siyu@163.com

Abstract

Gastrointestinal (GI) tumors, including liver, pancreatic, gastric, and colorectal cancers, have a high incidence rate and low survival rate due to the lack of effective therapeutic methods and frequent relapses. Surgery and postoperative chemoradiotherapy have largely reduced the fatality rates for most GI tumors, but these therapeutic approaches result in poor prognoses due to severe adverse reactions and the development of drug resistance. Recent studies have shown that ferroptosis plays an important role in the onset and progression of GI tumors. Ferroptosis is a new non-apoptotic form of cell death, which is iron-dependent, non-apoptotic cell death characterized by the accumulation of lipid reactive oxygen species (ROS). The activation of ferroptosis can lead to tumor cell death. Thus, regulating ferroptosis in tumor cells may become a new therapeutic approach for tumors, making it become a research hotspot. Current studies suggest that ferroptosis is mainly triggered by the accumulation of lipid ROS. Furthermore, several studies have indicated that ferroptosis may be a new approach for the treatment of GI tumors. Here, we review current research progress on the mechanism of ferroptosis, current inducers and inhibitors of ferroptosis, and the role of ferroptosis in GI tumors to propose new methods for the treatment of such tumors.

Key Words: Ferroptosis; Gastrointestinal oncology; Hepatocellular carcinoma; Pancreatic cancer; Gastric cancer; Colorectal cancer

Core Tip: Ferroptosis refers to cell death triggered by iron-dependent lipid peroxidation. Recent studies have demonstrated that ferroptosis is involved in the onset and

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: April 20, 2021
Peer-review started: April 20, 2021
First decision: July 29, 2021
Revised: August 4, 2021
Accepted: December 7, 2021
Article in press: December 7, 2021
Published online: January 15, 2022

P-Reviewer: Jeong KY
S-Editor: Wang JL
L-Editor: Filipodia
P-Editor: Wang JL

progression of numerous gastrointestinal (GI) tumors. Hence, inducing ferroptosis in tumor cells may become a new therapeutic strategy against GI tumors. Here, we review the molecular mechanism of ferroptosis and its role in GI tumors, with the aim of providing new research directions and ideas for the treatment of GI tumors.

Citation: Yang F, Sun SY, Wang S, Guo JT, Liu X, Ge N, Wang GX. Molecular regulatory mechanism of ferroptosis and its role in gastrointestinal oncology: Progress and updates. *World J Gastrointest Oncol* 2022; 14(1): 1-18
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/1.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.1

INTRODUCTION

Cell death is a basic life process that is pivotal to the development and homeostasis of multicellular organisms. Functionally, cell death can be categorized into accidental cell death (ACD) and regulated cell death (RCD). ACD refers to instantaneous and catastrophic cell death due to severe physical (*e.g.*, high pressure, high temperature, and hypertonicity), chemical (*e.g.*, drastic pH fluctuations), or mechanical (*e.g.*, shear force) damage. In contrast, RCD is triggered *via* specific molecular mechanisms and can be modulated (delayed or accelerated) *via* pharmacologic or genetic interventions [1]. RCD can be further categorized by onset mechanism as apoptosis, autophagic cell death, paraptosis, mitotic catastrophe, oncosis, pyroptosis, autoschizis, necroptosis, entosis, or ferroptosis[1,2].

Ferroptosis is iron-dependent, non-apoptotic cell death characterized by the accumulation of free iron and lipid reactive oxygen species (ROS)[3]. Studies have shown that the free iron concentration in gastrointestinal (GI) tumor cells is higher than that of normal cells, and the survival of tumor cells is highly dependent on the abnormally activated antioxidant system[2,3]. Additionally, in recent years, a large number of studies have shown that the activation of ferroptosis can lead to GI tumor cell death[1-4]. Thus, regulating ferroptosis in tumor cells may become a new therapeutic approach for GI tumors. Therefore, ferroptosis has become a research hotspot.

Here, we summarize recent research progress on the mechanism of ferroptosis and its role in GI tumors to expand ideas on clinical tumor treatment.

DISCOVERY AND CHARACTERISTICS OF FERROPTOSIS

In 2003, Dolma *et al*[4] identified a new compound while screening for compounds with killing effects against tumor cells. The identified compound, erastin, which selectively kills tumor cells expressing RASV12 protein, a mutated form of RAS. However, the erastin-mediated killing mechanism is different from that of previously known compounds, *i.e.* it does not cause nuclear morphological changes, DNA fragmentation, or caspase-3 activation, and its cell-killing process cannot be reversed by caspase inhibitors[2]. Then Yang *et al*[5] and Yagoda *et al*[6] found that erastin-mediated cell death is inhibited by iron chelators and is accompanied by elevated intracellular ROS levels. Additionally, both studies identified RAS-selective lethal (RSL) compounds, RSL and RSL3, which trigger this type of cell death[3]. In 2012, Dixon *et al*[3] named this type of cell death ferroptosis, which is iron-dependent, non-apoptotic cell death characterized by intracellular ROS accumulation. In 2018, the Nomenclature Committee on Cell Death defined ferroptosis as a form of glutathione peroxidase 4 (GPX4)-regulated RCD that is triggered by oxidative stress in the intracellular microenvironment and can be inhibited by iron chelators and lipophilic antioxidants[1].

Ferroptosis is a novel type of iron-dependent cell death with genetic, biochemical, and morphological features different from other forms of cell death including apoptosis, unregulated necrosis, and necroptosis[3]. The ultra-micromorphological features of ferroptosis include cell membrane disruption and blebbing, mitochondrial shrinkage, increased mitochondrial bilayer density, reduced or absent mitochondrial cristae, outer mitochondrial membrane disruption, normal nuclear size, and the

absence of chromatin condensation[7]. The main biochemical characteristics of ferroptosis include iron and ROS accumulation, protein kinase activation, cystine/glutamate antiporter inhibition, reduced cystine uptake and glutathione (GSH) synthesis, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidation[8].

Ferroptosis, modulated by specific pathways, is involved in various biological processes and exhibits unique gene expression and molecular regulatory systems. Current studies suggest that ferroptosis is mainly caused by the imbalance between lipid ROS generation and detoxification in cells. The accumulation of lipid ROS when the cellular antioxidant capacity is reduced can result in oxidative stress-induced cell death, *i.e.* ferroptosis[9].

MECHANISM AND REGULATION OF FERROPTOSIS

Ferroptosis is mainly regulated by the following three mechanisms[7]: (1) regulation of iron metabolic pathways such as autophagy-related genes 5 and 7 (ATG5/ATG7)-nuclear receptor coactivator 4 (NCOA4) pathway[10] and p62-Kelch-like epichlorohydrin-associated protein-1 (Keap1)-nuclear factor erythroid 2-related factor 2 (NRF2) pathway[11]; (2) regulation of lipid metabolic pathways such as the p53-serine acetyltransferase 1-arachidonate-15-lipoxygenase pathway[7], acyl-CoA synthase long-chain family member 4 (ACSL4)[12], lysophosphatidylcholine acyltransferase 3 (LPCAT3)[13], and 15-lipoxygenase/phosphatidylethanolamine (PE)-binding protein-1 (15-LOX/PEBP1)[14]; and (3) regulation of the GSH/GPX4 pathway such as the cystine/glutamate antiporter system (System X_c^-)[15], transsulfuration pathway[16], and mevalonate pathway[17]. Dysregulation of these three regulatory pathways eventually significantly reduce GPX4 activity and increase intracellular lipid ROS levels, thereby leading to reduced cellular antioxidant capacity, additional lipid ROS accumulation, oxidative damage to the cell membrane, and ferroptosis. Ferroptosis suppressor protein 1 (FSP1) can inhibit lipid peroxidation and ferroptosis by directly eliminating lipid ROS independent of GPX4[18] (Figure 1).

Regulation of iron metabolism

Iron is an essential trace element in the human body. Iron deficiency can cause anemia and iron-dependent enzyme abnormalities. However, iron accumulation can lead to tissue damage and increase the risk of developing various diseases (*e.g.*, tumors). ROS that accumulate during cell metabolism mainly include superoxide radical anions (O^{2-}) and hydrogen peroxide (H_2O_2), which are converted by free Fe^{2+} ions to hydroxyl free radicals (HO^-) that subsequently generate lipid peroxides by oxidizing macromolecules, especially lipid molecules (*e.g.*, polyunsaturated fatty acids, PUFAs). These reactions, which involve iron and generate hydroxyl or alkoxy radicals (RO^-), are termed Fenton reactions[19]. Intracellular accumulation of lipid peroxides without timely elimination cause oxidative damage to DNA, proteins, and the cell membrane, eventually leading to ferroptosis[20].

Therefore, iron ions are indispensable for the accumulation of lipid peroxides and the initiation of ferroptotic pathways. The absorption, distribution, metabolism, transformation, and excretion of iron ions are closely associated with the onset of ferroptosis[21]. Dietary iron is mainly absorbed as ferric (Fe^{3+}) ions in the duodenum and upper jejunum, where it is transported to the blood by transferrin. Some Fe^{3+} ions are transported by binding to the membrane receptor transferrin receptor 1 (TFR1), which are packaged into endosomes. There, Fe^{3+} ions are reduced to Fe^{2+} ions by the metalloreductase, six-transmembrane epithelial antigen of the prostate 3. Finally, Fe^{2+} ions are delivered by solute carrier family 11a2/divalent metal transporter 1 from endosomes into the cytoplasmic labile iron pool. Intracellular iron storage mainly occurs in the form of iron-protein complexes comprising ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1), while the remaining excess Fe^{2+} ions are oxidized to Fe^{3+} ions and transported out of cells by ferroportin on the cell membrane[22].

Both increased iron uptake and decreased iron elimination can enhance the sensitivity of cells to oxidative damage and ferroptosis *via* the Fenton reaction. Supplementation with exogenous iron ions but not other divalent metal ions can accelerate erastin-induced ferroptosis[3]. Cells with mutated RAS show significantly increased iron uptake and significantly decreased iron storage capacity following the onset of ferroptosis[23]. The intracellular level of labile iron (Fe^{2+} ions) is also a key factor affecting lipid peroxidation and ferroptosis. Upon exposure to different ferroptosis inducers, the intracellular Fe^{2+} ion level increases and various transport proteins associated with iron metabolism (*e.g.*, ferritin and TFR1) are rearranged after

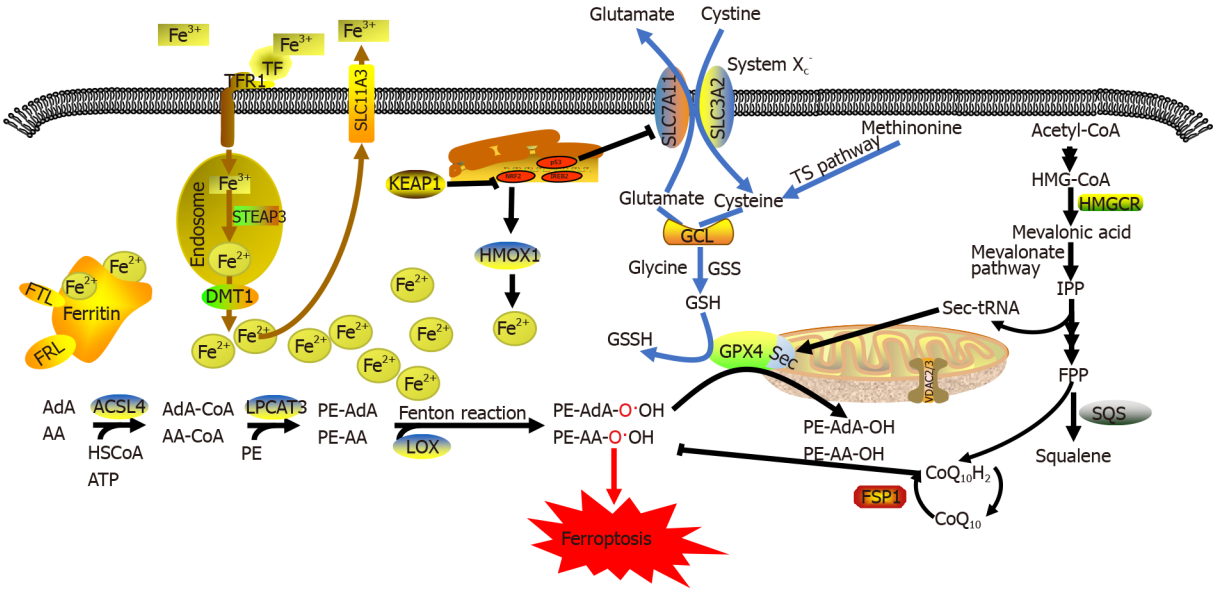


Figure 1 Molecular regulation mechanism of ferroptosis. ART: Artesunate; DHA: Dihydroartemisinin; GPX4: Glutathione peroxidase 4; GSH: Glutathione; HMOX1: Heme oxygenase 1; PUFAs: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SQS: Squalene synthase; VDAC: Voltage-dependent anion channel.

ferroptosis[21]. Iron overload and ferroptosis can be inhibited by knocking out genes encoding transferrin receptors or upregulating the expression of iron-storage proteins. Inhibiting the main transcription factor that regulates iron metabolism, iron-responsive element-binding protein 2 (also known as iron regulatory protein 2), can significantly upregulate the expression of genes associated with iron metabolism (*e.g.*, FTH1 and FTL) and inhibit erastin-induced ferroptosis[24]. Blocking iron transport by knocking out the ferroportin gene SLC11A3 exacerbates erastin-induced ferroptosis in neuroblastoma cells[25]. Furthermore, Yang *et al*[26] observed that phosphorylase kinase catalytic subunit γ2 (PHKG2) positively regulates ferroptosis by modulating the free Fe^{2+} ion level, while inhibiting PHKG2 expression exhibits an iron-chelating effect. Autophagy can also regulate the cellular sensitivity to ferroptosis by affecting iron metabolism[27]. Ferritin-selective autophagy (ferritinophagy) enhances cellular sensitivity to ferroptosis by controlling the level of available iron[28]. NCOA4 is a selective cargo receptor that delivers ferritin to autophagosomes, where ferritin is degraded and free iron is released into the cytoplasm. Downregulating NCOA4 expression reduces the sensitivity of human fibrosarcoma cells (HT-1080) and human pancreatic cancer cells (PANC1) to ferroptosis. This process is regulated by autophagy-related genes, ATG5 and ATG7[10]. Other proteins that affect iron metabolism, such as NRF2[11], heat shock protein beta-1[29], and CDGSH iron-sulfur domain-containing protein 1 (CISD1, also referred to as mitoNEET)[30] can also affect cellular sensitivity to ferroptosis.

Iron chelators can directly act on iron-containing enzymes, most likely lipoxygenases, because they can catalyze PUFA oxidation and be directly inactivated by lipophilic iron chelators. Dixon *et al*[31] suggested that iron is extremely prone to electron exchange under aerobic conditions. Thus, the inhibition of ferroptosis by iron chelators may be attributed to the fact that iron is a cofactor of numerous important metalloenzymes and that iron chelators prevent electron transfer from iron to oxides, thereby inhibiting oxygen free radical generation and preventing ferroptosis by inhibiting lipid peroxidation. Therefore, regulating iron metabolism and ferritinophagy may serve as a new target and approach for modulating ferroptosis.

Regulation of lipid metabolism

Lipids are important regulators of cell death. In mammals, both apoptotic and non-apoptotic pathways can be induced, regulated, or inhibited by different lipid signals [34]. For example, increasing the intracellular saturated fatty acid-to-monounsaturated fatty acid ratio can trigger apoptotic pathways. Increased long-chain fatty acid levels can trigger necrotic pathways[32], and exogenous monosaturated fatty acids can reduce cell death *via* acyl-CoA synthetase long chain family member 3 (ACSL3). All of these pathways exert a lipotoxic effect[33].

Unlike other forms of cell death, ferroptosis does not require an effector (*e.g.*, pore-forming proteins). Instead, lipid-mediated oxidative stress and subsequent membrane damage are key factors leading to the onset of ferroptosis. In particular, PUFAs, which contain bis-allylic protons that are vulnerable to hydrogen abstraction, are more likely to form lipid peroxides and induce ferroptosis[26]. PUFA abundance and localization determine the degree of lipid peroxidation in cells, and thus, the extent of ferroptosis [7].

The intracellular accumulation of lipid peroxides is the core process of ferroptosis. Lipid peroxidation in cells may be enzymatic or non-enzymatic. Non-enzymatic lipid peroxidation, also known as lipid autoxidation, is a free radical-mediated chain reaction, in which PUFAs are oxidized to lipid hydroperoxides by hydroxyl radicals generated *via* the Fenton reaction[19]. In contrast, enzymatic lipid peroxidation refers to lipoxygenase (LOX)-catalyzed generation of various lipid hydroperoxides from free PUFAs. Then lipid hydroperoxides are catalyzed by Fe^{2+} ions to generate free alkoxy radicals, which participate in the next lipid peroxidation reaction. Continuous PUFA oxidation and depletion alters the fluid mosaic structure of cell membrane and increases its permeability, eventually leading to cell death[17,20].

Fe^{2+} ions participate in the formation of free radicals and are an important catalyst in lipid peroxidation. Free PUFAs serve as substrates for the synthesis of lipid signaling mediators, but they must be esterified to membrane phospholipids and oxidized to become ferroptotic signals[17]. These toxic mediators are sparsely distributed within the cell membrane, mitochondrial membranes, lysosomal membranes, and endoplasmic reticulum membranes[26]. A lipidomic study uncovered that lipid metabolism disorders are closely associated with ferroptosis, where the key phospholipids—PEs—which contain arachidonic acid (C20:4) or its derivative adrenic acid (C22:4), are oxidized to ox-phosphatidylethanolamines (ox-PEs) that induce the onset of ferroptosis[34]. PUFAs are converted to coenzyme A derivatives, which are incorporated into phospholipids to become ferroptotic signals. Thus, the regulatory enzymes involved in PUFA biosynthesis from membrane phospholipids can trigger or prevent ferroptosis. Indeed, PUFA formation requires various lipid metabolism enzymes, such as ACSL4[14], LPCAT3[13], and 15-LOX/PEBP1[14]. In addition, lipid peroxidation promotes ferroptosis due to the generation of toxic aldehydes, such as 4-hydroxy-2-nonenal and malondialdehyde that can inactivate some proteins involved in normal physiological functions[35].

PUFAs are activated by ACSL4 and transported by LPCAT3 to the inner and outer leaflets of the cell membrane, where they undergo esterification and participate in the oxidation of negatively charged membrane phospholipids. Under normal circumstances, 15-LOX/PEBP1 and GPX4 co-regulate the oxidation of esterified fatty acids, but during oxidant/antioxidant imbalance, long-chain PUFAs in the cell membrane are often oxidized and trigger ferroptosis, especially when being induced by other factors such as RSL3[22].

ACSL4, which belongs to the long-chain acyl-CoA synthetase family, catalyzes the activation of fatty acids to form fatty acyl CoA in the body. It is also the key enzyme required in the first step of fatty acid catabolism. Previous studies have revealed that knocking out enzymes of the ACSL family other than ACSL4 in mouse embryonic fibroblasts does not cause ferroptosis[12]. Unlike other members of the ACSL family, ACSL4 can activate long-chain PUFAs and participate in the synthesis of membrane phospholipids. For example, ACSL4 catalyzes the conversion of arachidonic acid and adrenergic acid to arachidonoyl-CoA and adrenyl-CoA, respectively, which participate in the synthesis of negatively charged membrane phospholipids (*e.g.*, phosphatidylethanolamines and phosphatidylinositol) and their incorporation into the cell membrane. LPCAT3 knockout cells display only a slight alleviation of ferroptosis compared to ACSL4 knockout cells. Additionally, ACSL4 is required for lipid peroxides to inhibit GPX4[12,36]. These results suggest that ACSL4 may be a crucial determinant of ferroptosis. Another study revealed that thiazolidinediones exhibit a protective effect on ACSL4-knockout embryonic fibroblasts. The combination of thiazolidinediones and RSL3 alleviated membrane lipid oxidation and cell death and significantly improved the survival of ACSL4-knockout mice[12]. Hence, ACSL4 inhibition may be a new target for the treatment of diseases associated with ferroptosis.

LOXs are non-heme, iron-containing enzymatic effector proteins essential for mediating the formation of ferroptosis-related peroxides. Knocking out LOXs, which prefer free PUFAs as substrates, can alleviate erastin-induced ferroptosis and cellular damage[3]. Vitamin E can inhibit LOX activity, which provides a foundation for the protective effect of vitamin E against ferroptosis[37]. Current studies suggest that LOXs primarily form a complex with PEBP1, which allosterically regulate LOXs to accommodate the ferroptotic signal sn2-15-Hydroperoxy-eicasotetraenoyl-phosphati-

dylethanolamines (sn2-15-HpETE-PE) at the catalytic site. Two major LOX subtypes mediate lipid peroxidation: 15-LO1 and 15-LO2. These two LOX subtypes have tissue-specific distribution patterns. For example, 15-LO1 is highly expressed in human aortic endothelial cells, while 15-LO2 is highly expressed in renal tubular endothelial cells and neuronal cells[14]. A previous redox metabolomic analysis revealed the similarity between 15-LO1 and 15-LO2. Both enzymes are involved in ferroptosis-associated diseases, such as traumatic brain injury, asthma, and acute renal ischemic injury[7]. LOX-mediated free PUFA oxidation requires 15-LOX/PEBP1 complex formation. In this complex, PEBP1 allosterically regulates LOXs and initiates downstream phospholipase A_2-related oxidation pathways for specific PUFAs. PEBP1, also known as RAF1 kinase inhibitor protein, is a small scaffold protein that binds to RAF1 and inhibits activity under steady-state conditions. 15-LOs are newly identified partners of PEBP1. 15-LO/PEBP1 complexes allosterically activate LOXs, which convert 15-hydroxyperoxyeicosatetraenoic acid (15-HpETE) to the pro-ferroptotic signal, 15-HpETE-PE, thereby triggering ferroptosis[38]. The mechanism by which the LOX/PEBP1 complex selects specific PUFAs for oxidation among diverse unsaturated fatty acids remains unknown. Clearly, this issue urgently needs to be addressed in investigating the regulatory mechanism of ferroptosis.

Regulation of amino acid metabolism

Amino acid metabolism is an important component of metabolic networks, and amino acid metabolism disorders are closely associated with ferroptosis[7]. GSH is an important antioxidant and free radical scavenger in the body. Many free radicals produced *via* metabolism can damage cell membranes, attack biological macromolecules, promote aging, and induce the onset of tumors or atherosclerosis. Functionally, GSH can bind and convert harmful, toxic molecules (*e.g.*, free radicals and heavy metals) into harmless substances that can be excreted from the body[39]. GSH is a tripeptide consisting of three amino acid residues: glutamic acid, cysteine, and glycine. It exists in reduced (G-SH) and oxidized (G-S-S-G) forms and is the first line of defense for free-radical scavenging in the body due to the presence of an active sulfhydryl (-SH) group that is susceptible to oxidization and dehydrogenation. Together with non-enzymatic antioxidants (reduced nicotinamide adenine dinucleotide phosphate/nicotinamide adenine dinucleoside phosphate), GSH exerts a strong protective effect on the body[40]. The synthesis of GSH requires cysteine as the starting material. Therefore, cellular resistance to lipid oxidation relies on intracellular cysteine levels, which are mainly produced by the System X_c^- and transsulfuration pathways.

System X_c^- plays an important role in maintaining GSH homeostasis and distribution. This molecule is a disulfide-linked heterodimer that comprises the regulatory subunit solute carrier family 3 member 2 (SLC3A2) and the catalytic subunit solute carrier family 7 member 11 (SLC7A11). System X_c^- promotes a 1:1 cystine and glutamic acid exchange across the plasma membrane. Cystine is reduced to cysteine upon entering cells[41]. Thus, System X_c^- regulates GSH synthesis by affecting extracellular glutamic acid levels[42]. A previous study found that System X_c^--knockout mice have significantly lower glutamic acid levels around neurons and a milder drug-induced neurotoxic response than normal mice[43]. Previous pharmacological studies revealed that erastin, sulfasalazine, and high glutamic acid concentrations induce ferroptosis by inhibiting System X_c^-[3,44]. These findings indicate that System X_c^- may mediate ferroptosis initiation by affecting glutamic acid uptake and GSH synthesis.

Methionine can be converted to adenosylhomocysteine and cysteine in cells *via* the transsulfuration pathway[16]. During cysteine insufficiency, homocysteine is converted to cystathionine (a cysteine precursor), which eventually enters the cysteine pool *via* the transsulfuration pathway. Numerous studies have demonstrated that more than 40% of cysteine in mammals is obtained from food. Cysteine is mainly used to synthesize GSH, antioxidant peptides, and thioredoxin (Trx) in the body. Under oxidative stress, cystathionine-b-synthetase promotes the conversion of methionine to cysteine and subsequent GSH synthesis, thereby protecting cells from oxidative stress-induced damage[16,45]. Hence, cysteine can be synthesized in cells *via* the transsulfuration pathway even when intracellular System X_c^- is inhibited, indicating that ferroptosis inducers, which inhibit System X_c^-, cannot completely and effectively kill cells. Hayano *et al*[46] showed that inhibiting cysteinyl-tRNA synthetase (CARS) expression using RNA interference upregulates the transsulfuration pathway and enhances cellular resistance to erastin-induced ferroptosis but is unable to inhibit RSL3- or buthionine sulfoximine-induced ferroptosis, suggesting that the transsulfuration pathway negatively regulates ferroptosis.

Glutamic acid and glutamine are additional ferroptosis regulators. A high extracellular concentration of glutamic acid can inhibit System X_c^- and trigger ferroptosis. Ottestad-Hansen *et al*[43] found that knocking out System X_c^- protects mice against neurotoxic injuries caused by glutamic acid accumulation. Additionally, iron chelators and ferroptosis inhibitors can inhibit glutamic acid-mediated neurotoxicity. Glutamine naturally exists in human tissues and plasma at substantial concentrations. Its degradation fuels the tricarboxylic acid cycle and provides fundamental materials for biosynthetic processes. During glutamine deficiency or the inhibition of glutamine degradation, ROS accumulation, lipid peroxidation, and ferroptosis cannot be induced by depleting cysteine or blocking cystine uptake, probably because the product of glutamine degradation, α-ketoglutarate, is essential for the onset of ferroptosis[3]. However, not all glutamic acid metabolic pathways can induce ferroptosis. The first step of glutamic acid metabolism is the conversion of glutamine into glutamic acid by the glutaminases GLS1 and GLS2. These glutaminases have similar structures and enzymatic properties, but only GLS2 can induce ferroptosis, probably because GLS2 is a transcriptional target of p53. Indeed, GLS2 upregulation can induce p53-dependent ferroptosis[47,48]. Under certain circumstances, p53 can suppress ferroptosis by blocking dipeptidyl-peptidase-4 activity in a transcription-independent manner[49]. Inhibiting glutamine degradation has been demonstrated to alleviate cardiac, renal, and brain injury caused by ischemia-reperfusion in an experimental model[50]. Hence, the regulation of glutamine anabolism may provide new approaches for alleviating ferroptosis-induced organ injuries.

In addition, oxidant/antioxidant imbalance may also induce ferroptosis[7]. ROS levels in the body are regulated by an antioxidative defense system comprising antioxidants, such as Nrf2, GPX4, and catalase. However, inhibitors of the antioxidative system (*e.g.*, superoxide dismutase inhibitors and thioredoxin reductase inhibitors) can induce human epithelial/fibroblast cell death only when intracellular GSH is depleted [51], indicating that erastin may induce ferroptosis by interacting with a specific downstream target of GSH. GPX4, which belongs to the GPX antioxidative defense system, is a key enzyme in maintaining the balance between GSH and GS-SG. High SLC7A11 expression in various tumor types increases cystine uptake and GPX4 synthesis in cells, thereby promoting tumor growth by reducing cellular oxidative stress and inhibiting ferroptosis[41].

GPX4 is a GSH-dependent enzyme. Selenocysteine is an amino acid within the catalytic center of GPX4, but since it is encoded by a UGA codon (which is also a stop codon), selenocysteine needs to be inserted into GPX4 by a specific carrier. Selenocysteine-specific tRNA (sec-tRNA) contains isopentenyladenosine and can decode the selenocysteine UGA codon, thereby allowing the accurate insertion of selenocysteine into corresponding proteins. Importantly, sec-tRNA maturation can also be regulated by the mevalonate pathway acting on GPX4 because its maturation requires tRNA-isopentenyltransferase to catalyze the transfer of the isopentenyl group of isopentenyl pyrophosphate (IPP) to the specific adenine sites of sec-tRNA precursors. Since IPP is an important product of the mevalonate pathway, inhibitors of the mevalonate pathway (*e.g.*, statins) can inhibit sec-tRNA maturation and GPX4 synthesis[16,17], thereby affecting the progression of ferroptosis. IPP and mevalonate pathway inhibitors regulate the onset of ferroptosis by affecting GPX4. At present, GPX4 is a key target to induce ferroptosis and is activated by numerous ferroptosis inducers, such as erastin and RSL3. Erastin inhibits GPX4 activity by depleting GSH, while RSL3 directly inhibits GPX4 activity[7], resulting in lipid peroxide accumulation that triggers ferroptosis. Additionally, other ferroptosis inducers (*e.g.*, diphenylene iodonium (DPI), DPI7, DPI10, and DPI12) exert similar effects by directly inhibiting GPX4 activity. Knocking out GPX4 Leads to excess intracellular lipid peroxide accumulation and cell death[52]. Therefore, GPX4 is an important target for triggering ferroptosis.

OTHER REGULATORY PATHWAYS

FSP1/CoQ/NADPH pathway

In addition to the above-mentioned metabolic regulatory pathways, other cellular pathways are involved in the regulation of ferroptosis. Bersuker *et al*[18] and Doll *et al* [53] found that the FSP1/coenzyme Q (CoQ)/NADPH pathway also inhibits ferroptosis. FSP1 was previously known as apoptosis-inducing-factor mitochondria-as-2. Both research groups found that FSP1 exhibited CoQ oxidoreductase activity, which mediates NAD(P)H-dependent CoQ10 regeneration. Ubiquinol, the reduced

form of CoQ10, captures free radicals that drive lipid peroxidation, thereby preventing oxidative damage to plasma membranes. FSP1 exerts its cellular protective effect against ferroptosis by catalyzing continuous CoQ10 regeneration and improving the free-radical scavenging capacity within cells. Hence, FSP1 catalyzes the synthesis of lipophilic free-radical scavengers and has a protective effect against ferroptosis caused by GPX4 deletion. It is currently believed that the FSP1/NADPH/CoQ10 pathway is independent and parallel to GPX4. Even in the absence of GPX4, FSP1, CoQ10, NADPH, and GSH serve as important antioxidants and free-radical scavengers that exhibit cellular protective effects against ferroptosis in the body.

Voltage-dependent anion channels

Voltage-dependent anion channels (VDACs) are transmembrane channels located on the outer mitochondrial membrane that transport ions and metabolites. VDACs regulate mitochondrial metabolism and energy production and participate in regulating signaling pathways, leading to both cell survival and death. There are numerous VDAC subtypes including VDAC1, VDAC2, and VDAC3. The open state of VDACs mediates the influx of respiratory substrates, ADP, and phosphoric acid into the mitochondria, while its closure blocks transport across mitochondrial membranes [54]. Tubulin, a globular protein on VDACs, can dynamically regulate mitochondrial metabolism and ion transportation by blocking VDACs[55]. Tubulin-induced VDAC closure restricts metabolite influx into the mitochondria and limits ATP production, leading to attenuated oxidative stress due to the inhibition of mitochondrial metabolism and a relatively low ATP/ADP ratio. Erastin can inhibit the effect of tubulin on VDACs and maintain an open state by preventing free tubulins in the cytoplasm from blocking VDACs. The open VDAC state leads to increased mitochondrial metabolism, decreased glycolysis, and elevated ROS production. Exposure of VDACs to the ferroptosis inducer, erastin, causes increased permeability of outer mitochondrial membranes, membrane ion channel opening, and disrupted cellular homeostasis, which results in dysfunctional mitochondrial metabolism and oxidation, increased ROS production, and enhanced lipid peroxidation, eventually triggering ferroptosis[56]. A previous study showed that inhibiting VDAC2 or VDAC3 expression renders cells insensitive to erastin-induced ferroptosis, but upregulating VDAC2 or VDAC3 expression does not significantly increase cellular sensitivity to erastin-induced ferroptosis. These data suggest that despite being involved in the regulation of ferroptosis, neither VDAC2 nor VDAC3 is a prerequisite of ferroptosis [57]. VDAC1 is closely related to the onset of ferroptosis, as it mainly maintains calcium homeostasis and ROS levels in the mitochondria.

FERROPTOSIS INDUCERS AND INHIBITORS

Common ferroptosis inducers

Ferroptosis inducers can be divided into four categories according to their targets (Table 1): System X_c^-; GPX4; GSH; and iron ions and ROS.

Common ferroptosis inhibitors

Ferroptosis inhibitors can be divided into two categories according to their mechanisms of action (Table 2): reduction of intracellular iron accumulation; and inhibition of lipid peroxidation.

FERROPTOSIS AND GI DISEASES

Numerous studies have demonstrated that ferroptosis leads to cell death in GI tumors (*e.g.*, pancreatic, liver, colorectal, and gastric cancers) and plays an important role in inhibiting tumor growth. Therefore, inducing ferroptosis in tumor cells is expected to become a novel therapeutic strategy. Although only limited *in vitro* and *in vivo* experiments on ferroptosis inducers have been conducted, a few small-molecule ferroptosis inducers have been discovered that display excellent therapeutic or synergistic outcomes against tumors.

Ferroptosis and pancreatic cancer

Pancreatic cancer is a highly malignant GI tumor with a poor prognosis. Although there are drugs available to treat pancreatic cancer, patients receiving pharmaco-

Table 1 Common ferroptosis inducers

Target	Inducer	Function	Ref.
System X_c^-	Erastin	Inhibits the activity of System X_c^- and affects the synthesis of GSH; binds to VDAC2/3 to induce mitochondrial dysfunction	[3,6]
	Erastin analogs, piperazine erastin, imidazole ketone erastin	Inhibits the activity of System X_c^- and affects the synthesis of GSH	[103,104]
	Sulfasalazine	Inhibits the activity of System X_c^- (weaker inhibitory effect than erastin)	[3,105,106]
	Sorafenib	Inhibits the activity of System X_c^- (directly affects the synthesis of GSH in a narrow concentration range)	[107]
	Glutamate	Inhibits the activity of System X_c^-, high extracellular glutamate concentrations prevent cystine import	[3,7]
GPX4	(1S,3R)-RSL3	Covalently binds to the selenocysteine residue of GPX4	[5,26]
	DPI7 (ML162), DPI12, DPI17	Covalently bind to GPX4 (at the same binding site as RSL3)	[7,26]
	DPI10 (ML120), DPI13	Indirectly inhibit GPX4 activity or bind to a site different from RSL3	[26,103]
	FIN56	Induces GPX4 degradation; binds and activates SQS to deplete CoQ10	[108]
	Altretamine	Inhibits the activity of GPX4	[64]
GSH	BSO	GSH depletion	[7,103]
	Cisplatin	Binds to GSH to inactivate GXP4	[109]
	DPI2	Depletes GSH	[7,103]
	Cysteinase	Depletes cysteine, resulting in GSH depletion	[7]
	Piperlongumine	Depletes GSH and inhibits the activity of GXP4	[7,64]
ROS and iron	$FINO_2$	Oxidizes Fe^{2+} ions and promotes intracellular accumulation of ROS; indirectly inactivates GPX4; directly oxidizes PUFAs	[110]
	Ferric ammonium citrate	Increases iron abundance	[7]
	Silica-based nanoparticles	Delivers iron into cells and reduce GSH abundance	[7]
	Heme	Upregulates HMOX1 expression and increases the intracellular level of labile iron	[111]
	ART, DHA	Oxidize Fe^{2+} ions and promote intracellular accumulation of ROS; induce ferritinophagy and the release of labile iron	[7,51,65]
	Siramesine and lapatinib	Downregulate FPN expression and upregulate TRF expression to increase intracellular labile iron levels	[112]

ART: Artesunate; DHA: Dihydroartemisinin; GPX4: Glutathione peroxidase 4; GSH: Glutathione; HMOX1: Heme oxygenase 1; PUFAs: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SQS: Squalene synthase; VDAC: Voltage-dependent anion channel.

therapy rarely survive more than 6 mo. Gemcitabine is the first-line chemotherapeutic agent for pancreatic cancer, but pharmacotherapy and immunotherapy still fail to yield an ideal therapeutic outcome. Therefore, it is imperative to develop new strategies for enhancing the sensitivity of pancreatic cancer to immunotherapy and reducing its resistance to gemcitabine[58]. Tang *et al*[59] utilized public databases to systematically analyze the expression of 43 ferroptosis regulators in 31 cancer types and constructed a highly accurate prognostic prediction model for pancreatic cancer based on ferroptosis regulators. A follow-up investigation on the effect of ferroptosis on the tumor microenvironment revealed that tumors that are highly sensitive to ferroptosis may also be sensitive to immune checkpoint inhibitors and *vice versa*. The authors also found that gemcitabine-resistant cancer cells had increased expression levels of SLC7A11 and SLC3A2, but their effects on ferroptosis sensitivity require further investigation. Zhu *et al*[60] found that heat shock protein family A55 (HSPA5) is closely associated with the prognosis of pancreatic cancer patients who received gemcitabine treatment. Activating the HSPA5-GPX4 pathway in pancreatic cancer cells may lead to gemcitabine resistance that may be reversed by inhibiting HSPA5 or GPX4 expression, which may also induce ferroptosis. Shintoku *et al*[61] demonstrated that erastin and RSL3 can induce pancreatic cancer cell death, and LOXs can increase the sensitivity of tumor cells with mutated RAS to erastin and RSL3. A subsequent study by Kuang *et al*[62] showed that the redox regulator quinazolindione (QD) inhibits

Table 2 Common ferroptosis inhibitors

Target	Inhibitors	Function	Ref.
Lipid peroxidation	Vitamin E, α-tocopherol, trolox, tocotrienols	Block propagation of lipid peroxidation, may inhibit lipoxygenases	[7]
	Deuterated polyunsaturated fatty acids	Block initiation and propagation of lipid peroxidation	[7]
	Butylated hydroxytoluene, butylated hydroxyanisole	Block lipid peroxidation	[7]
	Ferrostatins, liproxstatins	Scavenge ROS and inhibit lipid peroxidation; regulate the expression of oxidation-related proteins	[7]
	CoQ10, idebenone	Block lipid peroxidation	[7]
	Baicalein, PD-146176, AA-861, zileuton	Block lipoxygenase-induced lipid peroxidation	[7]
	Troglitazone	Specifically inhibits ACSL4	[12]
	Zileuton	Specifically inhibits LOX	[113]
	Vildagliptin, alogliptin, linagliptin	Block DPP4-mediated lipid peroxidation	[7, 49]
Iron	Deferoxamine, cyclipirox, deferiprone	Deplete iron and prevent iron-dependent lipid peroxidation	[7]
	Nitrogen oxides	Block the Fenton reaction and inhibit the production of hydroxyl radicals	[114]
	Curcumin	Chelates iron to reduce iron accumulation; activates the Nrf2 signaling pathway	[115]

ACSL4: Acyl-CoA synthase long-chain family member 4; LOX: Lipoxygenase; ROS: Reactive oxygen species.

pancreatic cancer cell proliferation by inducing ferroptosis. Further, the compound QD325 significantly inhibits the growth of transplanted tumors in mice and is well tolerated *in vivo*. Kasukabe *et al*[63] showed that the combination of cotylenin A (CN-A) and phenethyl isothiocyanate significantly inhibits pancreatic cancer cell proliferation by promoting ferroptosis. A study carried out by Yamaguchi *et al*[64] suggested that piperlongumine could synergistically kill human pancreatic cancer cells with CN-A or sulfasalazine *via* ferroptosis. In recent years, some Chinese herbal medicines have also been found to exert antitumor effects by inducing ferroptosis. Previous *in vitro* and *in vivo* assays showed that the antimalarial drug, artesunate, could cause excessive intracellular ROS accumulation by promoting lipid peroxidation and regulating iron metabolism. Additionally, artesunate can specifically induce ferroptosis in pancreatic cancer cells with a mutated *Kras* gene while exerting minimal toxic effects on normal cells[65], primarily by increasing ROS production[51]. A further study revealed that inhibiting glucose regulatory protein 78 expression reverses the resistance of pancreatic cancer cells to ferroptosis and enhances the sensitivity of tumors to artesunate[66]. The animal model constructed by Badgley *et al*[67] showed that therapeutic cysteine depletion can induce ferroptosis in pancreatic tumors in mice with mutated *Kras*/*p53*. However, Dai *et al*[68] recently found that ferroptosis can promote dead cancer cells to release KRAS protein, which will then be packaged into exosomes and taken up by macrophages. Then, the macrophages undergo polarization to M2 macrophages, which promote the malignant growth of pancreatic cancer. These results indicate that ferroptosis may exhibit complicated biological effects in the treatment of pancreatic cancer.

Ferroptosis and liver cancer

Surgery is the most important therapeutic approach for patients with hepatocellular carcinoma (HCC), but the rate of postoperative recurrence and metastasis is relatively high. Sorafenib is a commonly used chemotherapeutic drug against HCC, but it is difficult to clinically determine the prognosis of HCC and reduce sorafenib resistance [69]. Shan *et al*[70] analyzed two different public HCC databases and found that ubiquitin-like modifier activating enzyme 1 (UBA1) can regulate ferroptosis in HCC cells *via* the Nrf2 pathway. The authors subsequently confirmed that silencing UBA1 gene expression inhibits HCC proliferation, migration, and invasion, increasing Fe^{2+} and MDA levels in cancer cells. These results indicate that UBA1 can be used as an independent indicator of liver cancer progression. Liang *et al*[71] systematically analyzed the expression of 60 ferroptosis-associated genes in HCC tumor tissues and their relationships with the overall survival of patients. The authors proposed and validated a prognostic model comprising 10 ferroptosis-associated genes (ACACA,

ACSL3, CISD1, CARS, G6PD, GPX4, NQO1, NFS1, SLC7A11, and SLC1A5). These efforts provided an important approach for elucidating mechanisms underlying HCC development and predicting its prognosis.

Studies on the mechanisms through which sorafenib and erastin induce ferroptosis in HCC have provided new approaches for addressing chemotherapeutic drug resistance. Louandre *et al*[72] showed that sorafenib-treated HCC cells had significantly lower retinoblastoma protein expression than untreated HCC cells, with a mortality rate two to three times higher than that of the untreated group. Subsequently, *in vivo* experiments on mice implanted with HCC cells and *in vitro* experiments on shRb-transfected Huh7 cells clarified the mechanism through which Rb regulates sorafenib-induced ferroptosis in HCC. Sorafenib induces ferroptosis in HCC by enhancing mitochondrial ROS generation, while Rb inactivation aggravates ferroptosis by increasing mitochondrial ROS levels and oxidative stress. Sun *et al*[11] reported that the p62-Keap1-Nrf2 signaling pathway plays an important role in erastin/sorafenib-induced ferroptosis in HCC, where it modulates ferroptosis by regulating the expression of downstream iron- and ROS metabolism-related genes. Interfering with p62 expression can enhance erastin/sorafenib-induced ferroptosis in HCC. Additionally, experiments in Nrf2-shRNA-transfected HCC cells and mice implanted with Nrf2-shRNA-transfected HCC cells showed that Nrf2 knockdown enhances the antitumor activity of erastin/sorafenib against HCC[73]. Qi *et al*[74] found that erastin significantly inhibits the expression of GA binding protein transcription factor subunit β 1 (GABPB1) protein and peroxidase genes in HCC cells, thereby resulting in intracellular ROS and malondialdehyde accumulation, which leads to cell death. Therefore, GABPB1 may be a key molecule that mediates erastin-induced ferroptosis in HCC. Further, ACSL3 and ACSL4 expression is significantly upregulated in HCC[75], and ACSL4 contributes to erastin-induced ferroptosis *via* 5-hydroxyeicosatetraenoic acid-mediated lipotoxicity[14]. Additional studies[76-78] have shown that inhibiting metallothionein 1G and oxidative stress-related protein sigma 1 receptor enhances the sensitivity of liver cancer cells to sorafenib by inducing ferroptosis. Wang *et al*[79] identified and explored branched-chain amino acid aminotransferase 2 (BCAT2), which is involved in System X_c^- inhibitor-induced ferroptosis in liver cancer. In addition, BCAT2 also participates in ferroptosis synergistically induced by sulfasalazine and sorafenib.

Combination therapy may improve the clinical outcomes of patients with liver cancer by partially addressing the issue of drug resistance. Low-density lipoprotein nanoparticles reconstituted with the natural omega-3 PUFA, docosahexaenoic acid (LDL-DHA), can effectively kill liver cancer cells by triggering ferroptosis[80]. The combined treatment of liver cancer cells with erastin, sorafenib, and haloperidol can elevate intracellular iron ion concentrations, which generate excessive ROS *via* the Fenton reaction and increase lipid oxidation, thereby inducing ferroptosis in liver cancer cells[77]. Shang *et al*[81] found that ceruloplasmin (CP) inhibits ferroptosis by regulating iron homeostasis in HCC cells, while inhibiting CP significantly increases intracellular Fe^{2+} and ROS accumulation, thereby promoting erastin- and RSL3-induced ferroptosis in HCC. Li *et al*[82] reported that sorafenib and artesunate synergistically suppress liver cancer by inducing ferroptosis. Further, nanoparticle-based drugs also offer a new direction for *in situ* induction of ferroptosis in liver cancer. Tang *et al*[83] showed that manganese-silica nanodrugs induce ferroptosis in tumor cells by rapidly depleting intracellular GSH. LDL-DHA nanoparticles increase lipid peroxidation in liver cancer cells, reduce GSH levels, and inhibit GPX4 activity, thereby inducing ferroptosis that kills liver cancer cells and inhibits the *in situ* growth of liver tumors in rats[80].

Ferroptosis and gastric cancer

Gastric cancer (GC) is among the most common causes of cancer-related deaths worldwide, with nearly one million cases diagnosed each year and more than 730000 deaths. Conventional treatments for GC include surgery, chemotherapy, and radiotherapy. Chemotherapy, despite being the primary therapeutic approach, causes significant side effects for most patients and often cannot cure patients with advanced GC[84]. Therefore, it is necessary to develop a better therapeutic approach for GC. Lee *et al*[85] found that the sensitivity of GC cells to ferroptosis depends on PUFA biosynthesis. Stearoyl-CoA desaturase 1 (SCD1) promotes tumor growth and makes GC cells resistant to ferroptosis. Notably, GC patients with high SCD1 expression may not have an optimistic prognosis. Taken together, this study provides new insights into the potential of SCD1 as a biomarker and therapeutic target for GC[86]. Hao *et al*[87] found that inhibiting cysteine dioxygenase 1 (CDO1) expression could inhibit ferroptosis in GC by upregulating GPX4 expression and preventing ROS production.

Sun *et al*[88] showed that perilipin2 inhibits ferroptosis in GC by regulating ACSL3 and 15-LOX. Some ingredients of Chinese medicines, such as *Actinidia chinensis* planch [89] and Tanshinone IIA[90] also exhibit anticancer effects against GC by participating in ferroptosis.

Ferroptosis and colorectal cancer

Colorectal cancer (CRC) is the most common malignant GI tumor that poses a major threat to human health. Recently, an increasing trend in CRC incidence and fatality rates has been observed, resulting from improved living standards and dietary changes[91,92]. A previous study showed that the ferroptosis inducer RSL3 triggers ferroptosis in various CRC cell types by affecting GPX4 activity in a dose- and time-dependent manner[93]. Acyl-CoA dehydrogenase, short/branched chain (ACADSB), which belongs to the acyl-CoA dehydrogenase family, reduces GSH concentration by negatively regulating GSH reductase and GPX4 expression. Further, ACADSB affects CRC cell migration, invasion, and proliferation by regulating ferroptosis[94]. Another study on ferroptosis-related mechanisms in CRC laid the foundation for the development of anticancer drugs against CRC. Park *et al*[95] showed that bromelain affects ferroptosis by regulating ACSL4 expression in CRC cells with Kras mutations. Additionally, talaroconvolutin A[96], 2-imino-6-methoxy-2H-chromene-3-carbothioamide[97], and resibufogenin[98] have been found to inhibit CRC cell proliferation and tumorigenesis by modulating ferroptosis in CRC cells. Some studies also found that combination therapy could partially address the issue of CRC resistance to chemotherapeutic drugs *via* ferroptosis. Andrographis enhances the sensitivity of CRC cells to 5-fluorouracil by promoting ferroptosis[99]. The combination therapy using the natural products β-elemene and cetuximab can kill CRC cells with mutated KRAS genes by inducing ferroptosis and inhibiting epithelial-mesenchymal transition[100]. The combination of high-dose vitamin C and cetuximab can improve the drug sensitivity of CRC by triggering ferroptosis, thereby laying the foundation for the treatment of CRC[101].

CONCLUSION

Ferroptosis has received increasing attention since being proposed as a form of RCD by Dixon *et al*[3] in 2012. Numerous in-depth studies have been conducted on the complex molecular mechanisms underlying ferroptosis. These studies facilitate a deeper understanding of the onset and progression of ferroptosis-associated diseases. The further development of relevant targeted drugs has also led to the emergence of a new research field associated with ferroptosis onset and progression for the treatment of GI tumors[59]. Following the discovery of erastin in 2003, numerous ferroptosis inducers and inhibitors have been identified because of the increasing importance of the relationship between ferroptosis and GI tumors[7]. Sorafenib, the sole first-line drug for liver cancer, is believed to kill hepatocytes *via* ferroptosis. Additionally, some *in vitro* and *in vivo* drug trials on pancreatic cancer have provided new theoretical bases and research directions for the pharmacotherapy of pancreatic cancer. Some studies on ferroptosis in GC and CRC indicated that inducing ferroptosis could cause cell death in GI tumors and exert a synergistic effect with other anticancer drugs, thereby enhancing tumor sensitivity to existing treatments. Hence, inducing ferroptosis may have considerable potential for treating GI tumors[102]. However, research on ferroptosis is still at a preliminary stage, and it is of great theoretical and practical significance to continuously explore the mechanisms and roles of ferroptosis in various diseases. These studies will reveal highly effective and targeted therapeutic approaches. For instance, the mechanism and key regulators of ferroptosis as well as its relationships with tumor-associated genes and other RCDs (*e.g.*, autophagy and apoptosis), are potential directions and goals for future studies. Collectively, these studies will facilitate an in-depth understanding of the molecular mechanism through which GI tumors evade cell death and promote the development of novel effective therapeutic strategies. Therefore, further discoveries and investigation of ferroptosis inducers and inhibitors will provide a theoretical foundation and new method for the treatment of GI tumors.

REFERENCES

1 **Galluzzi L**, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH, Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Boya P, Brenner C, Campanella M, Candi E, Carmona-Gutierrez D, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Cohen GM, Conrad M, Cubillos-Ruiz JR, Czabotar PE, D'Angiolella V, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, DeBerardinis RJ, Deshmukh M, Di Daniele N, Di Virgilio F, Dixit VM, Dixon SJ, Duckett CS, Dynlacht BD, El-Deiry WS, Elrod JW, Fimia GM, Fulda S, García-Sáez AJ, Garg AD, Garrido C, Gavathiotis E, Golstein P, Gottlieb E, Green DR, Greene LA, Gronemeyer H, Gross A, Hajnoczky G, Hardwick JM, Harris IS, Hengartner MO, Hetz C, Ichijo H, Jäättelä M, Joseph B, Jost PJ, Juin PP, Kaiser WJ, Karin M, Kaufmann T, Kepp O, Kimchi A, Kitsis RN, Klionsky DJ, Knight RA, Kumar S, Lee SW, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, López-Otín C, Lowe SW, Luedde T, Lugli E, MacFarlane M, Madeo F, Malewicz M, Malorni W, Manic G, Marine JC, Martin SJ, Martinou JC, Medema JP, Mehlen P, Meier P, Melino S, Miao EA, Molkentin JD, Moll UM, Muñoz-Pinedo C, Nagata S, Nuñez G, Oberst A, Oren M, Overholtzer M, Pagano M, Panaretakis T, Pasparakis M, Penninger JM, Pereira DM, Pervaiz S, Peter ME, Piacentini M, Pinton P, Prehn JHM, Puthalakath H, Rabinovich GA, Rehm M, Rizzuto R, Rodrigues CMP, Rubinsztein DC, Rudel T, Ryan KM, Sayan E, Scorrano L, Shao F, Shi Y, Silke J, Simon HU, Sistigu A, Stockwell BR, Strasser A, Szabadkai G, Tait SWG, Tang D, Tavernarakis N, Thorburn A, Tsujimoto Y, Turk B, Vanden Berghe T, Vandenabeele P, Vander Heiden MG, Villunger A, Virgin HW, Vousden KH, Vucic D, Wagner EF, Walczak H, Wallach D, Wang Y, Wells JA, Wood W, Yuan J, Zakeri Z, Zhivotovsky B, Zitvogel L, Melino G, Kroemer G. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* 2018; **25**: 486-541 [PMID: 29362479 DOI: 10.1038/s41418-017-0012-4]

2 **Zheng H**, Jiang J, Xu S, Liu W, Xie Q, Cai X, Zhang J, Liu S, Li R. Nanoparticle-induced ferroptosis: detection methods, mechanisms and applications. *Nanoscale* 2021; **13**: 2266-2285 [PMID: 33480938 DOI: 10.1039/d0nr08478f]

3 **Dixon SJ**, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B 3rd, Stockwell BR. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012; **149**: 1060-1072 [PMID: 22632970 DOI: 10.1016/j.cell.2012.03.042]

4 **Dolma S**, Lessnick SL, Hahn WC, Stockwell BR. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell* 2003; **3**: 285-296 [PMID: 12676586 DOI: 10.1016/s1535-6108(03)00050-3]

5 **Yang WS**, Stockwell BR. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem Biol* 2008; **15**: 234-245 [PMID: 18355723 DOI: 10.1016/j.chembiol.2008.02.010]

6 **Yagoda N**, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, Wolpaw AJ, Smukste I, Peltier JM, Boniface JJ, Smith R, Lessnick SL, Sahasrabudhe S, Stockwell BR. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature* 2007; **447**: 864-868 [PMID: 17568748 DOI: 10.1038/nature05859]

7 **Stockwell BR**, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salnikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* 2017; **171**: 273-285 [PMID: 28985560 DOI: 10.1016/j.cell.2017.09.021]

8 **Tang M**, Chen Z, Wu D, Chen L. Ferritinophagy/ferroptosis: Iron-related newcomers in human diseases. *J Cell Physiol* 2018; **233**: 9179-9190 [PMID: 30076709 DOI: 10.1002/jcp.26954]

9 **Liang C**, Zhang X, Yang M, Dong X. Recent Progress in Ferroptosis Inducers for Cancer Therapy. *Adv Mater* 2019; **31**: e1904197 [PMID: 31595562 DOI: 10.1002/adma.201904197]

10 **Hou W**, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ 3rd, Kang R, Tang D. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 2016; **12**: 1425-1428 [PMID: 27245739 DOI: 10.1080/15548627.2016.1187366]

11 **Sun X**, Ou Z, Chen R, Niu X, Chen D, Kang R, Tang D. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology* 2016; **63**: 173-184 [PMID: 26403645 DOI: 10.1002/hep.28251]

12 **Doll S**, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trümbach D, Mao G, Qu F, Bayir H, Füllekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JP, Conrad M. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 2017; **13**: 91-98 [PMID: 27842070 DOI: 10.1038/nchembio.2239]

13 **Dixon SJ**, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G, Stockwell BR. Human Haploid Cell Genetics Reveals Roles for Lipid Metabolism Genes in Nonapoptotic Cell Death. *ACS Chem Biol* 2015; **10**: 1604-1609 [PMID: 25965523 DOI: 10.1021/acschembio.5b00245]

14 **Anthonymuthu TS**, Kenny EM, Shrivastava I, Tyurina YY, Hier ZE, Ting HC, Dar HH, Tyurin VA, Nesterova A, Amoscato AA, Mikulska-Ruminska K, Rosenbaum JC, Mao G, Zhao J, Conrad M, Kellum JA, Wenzel SE, VanDemark AP, Bahar I, Kagan VE, Bayır H. Empowerment of 15-

Lipoxygenase Catalytic Competence in Selective Oxidation of Membrane ETE-PE to Ferroptotic Death Signals, HpETE-PE. *J Am Chem Soc* 2018; **140**: 17835-17839 [PMID: 30525572 DOI: 10.1021/jacs.8b09913]

15 **Song X**, Zhu S, Chen P, Hou W, Wen Q, Liu J, Xie Y, Klionsky DJ, Kroemer G, Lotze MT, Zeh HJ, Kang R, Tang D. AMPK-Mediated BECN1 Phosphorylation Promotes Ferroptosis by Directly Blocking System X_c^- Activity. *Curr Biol* 2018; **28**: 2388-2399.e5 [PMID: 30057310 DOI: 10.1016/j.cub.2018.05.094]

16 **Hao S**, Liang B, Huang Q, Dong S, Wu Z, He W, Shi M. Metabolic networks in ferroptosis. *Oncol Lett* 2018; **15**: 5405-5411 [PMID: 29556292 DOI: 10.3892/ol.2018.8066]

17 **Cao JY**, Dixon SJ. Mechanisms of ferroptosis. *Cell Mol Life Sci* 2016; **73**: 2195-2209 [PMID: 27048822 DOI: 10.1007/s00018-016-2194-1]

18 **Bersuker K**, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R, Bassik MC, Nomura DK, Dixon SJ, Olzmann JA. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 2019; **575**: 688-692 [PMID: 31634900 DOI: 10.1038/s41586-019-1705-2]

19 **Torti SV**, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; **13**: 342-355 [PMID: 23594855 DOI: 10.1038/nrc3495]

20 **Yang WS**, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. *Trends Cell Biol* 2016; **26**: 165-176 [PMID: 26653790 DOI: 10.1016/j.tcb.2015.10.014]

21 **Wang S**, Luo J, Zhang Z, Dong D, Shen Y, Fang Y, Hu L, Liu M, Dai C, Peng S, Fang Z, Shang P. Iron and magnetic: new research direction of the ferroptosis-based cancer therapy. *Am J Cancer Res* 2018; **8**: 1933-1946 [PMID: 30416846]

22 **Bogdan AR**, Miyazawa M, Hashimoto K, Tsuji Y. Regulators of Iron Homeostasis: New Players in Metabolism, Cell Death, and Disease. *Trends Biochem Sci* 2016; **41**: 274-286 [PMID: 26725301 DOI: 10.1016/j.tibs.2015.11.012]

23 **Manz DH**, Blanchette NL, Paul BT, Torti FM, Torti SV. Iron and cancer: recent insights. *Ann N Y Acad Sci* 2016; **1368**: 149-161 [PMID: 26890363 DOI: 10.1111/nyas.13008]

24 **Kindrat I**, Tryndyak V, de Conti A, Shpyleva S, Mudalige TK, Kobets T, Erstenyuk AM, Beland FA, Pogribny IP. MicroRNA-152-mediated dysregulation of hepatic transferrin receptor 1 in liver carcinogenesis. *Oncotarget* 2016; **7**: 1276-1287 [PMID: 26657500 DOI: 10.18632/oncotarget.6004]

25 **Geng N**, Shi BJ, Li SL, Zhong ZY, Li YC, Xua WL, Zhou H, Cai JH. Knockdown of ferroportin accelerates erastin-induced ferroptosis in neuroblastoma cells. *Eur Rev Med Pharmacol Sci* 2018; **22**: 3826-3836 [PMID: 29949159 DOI: 10.26355/eurrev_201806_15267]

26 **Yang WS**, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc Natl Acad Sci U S A* 2016; **113**: E4966-E4975 [PMID: 27506793 DOI: 10.1073/pnas.1603244113]

27 **Du J**, Wang T, Li Y, Zhou Y, Wang X, Yu X, Ren X, An Y, Wu Y, Sun W, Fan W, Zhu Q, Wang Y, Tong X. DHA inhibits proliferation and induces ferroptosis of leukemia cells through autophagy dependent degradation of ferritin. *Free Radic Biol Med* 2019; **131**: 356-369 [PMID: 30557609 DOI: 10.1016/j.freeradbiomed.2018.12.011]

28 **Zhou B**, Liu J, Kang R, Klionsky DJ, Kroemer G, Tang D. Ferroptosis is a type of autophagy-dependent cell death. *Semin Cancer Biol* 2020; **66**: 89-100 [PMID: 30880243 DOI: 10.1016/j.semcancer.2019.03.002]

29 **Sun X**, Ou Z, Xie M, Kang R, Fan Y, Niu X, Wang H, Cao L, Tang D. HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene* 2015; **34**: 5617-5625 [PMID: 25728673 DOI: 10.1038/onc.2015.32]

30 **Yuan H**, Li X, Zhang X, Kang R, Tang D. CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. *Biochem Biophys Res Commun* 2016; **478**: 838-844 [PMID: 27510639 DOI: 10.1016/j.bbrc.2016.08.034]

31 **Dixon SJ**, Stockwell BR. The role of iron and reactive oxygen species in cell death. *Nat Chem Biol* 2014; **10**: 9-17 [PMID: 24346035 DOI: 10.1038/nchembio.1416]

32 **Parisi LR**, Li N, Atilla-Gokcumen GE. Very Long Chain Fatty Acids Are Functionally Involved in Necroptosis. *Cell Chem Biol* 2017; **24**: 1445-1454.e8 [PMID: 29033315 DOI: 10.1016/j.chembiol.2017.08.026]

33 **Magtanong L**, Ko PJ, To M, Cao JY, Forcina GC, Tarangelo A, Ward CC, Cho K, Patti GJ, Nomura DK, Olzmann JA, Dixon SJ. Exogenous Monounsaturated Fatty Acids Promote a Ferroptosis-Resistant Cell State. *Cell Chem Biol* 2019; **26**: 420-432.e9 [PMID: 30686757 DOI: 10.1016/j.chembiol.2018.11.016]

34 **Conrad M**, Kagan VE, Bayir H, Pagnussat GC, Head B, Traber MG, Stockwell BR. Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev* 2018; **32**: 602-619 [PMID: 29802123 DOI: 10.1101/gad.314674.118]

35 **Zhong H**, Yin H. Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. *Redox Biol* 2015; **4**: 193-199 [PMID: 25598486 DOI: 10.1016/j.redox.2014.12.011]

36 **Shimbara-Matsubayashi S**, Kuwata H, Tanaka N, Kato M, Hara S. Analysis on the Substrate Specificity of Recombinant Human Acyl-CoA Synthetase ACSL4 Variants. *Biol Pharm Bull* 2019; **42**: 850-855 [PMID: 31061331 DOI: 10.1248/bpb.b19-00085]

37 **Imai H**, Matsuoka M, Kumagai T, Sakamoto T, Koumura T. Lipid Peroxidation-Dependent Cell Death Regulated by GPx4 and Ferroptosis. *Curr Top Microbiol Immunol* 2017; **403**: 143-170

[PMID: 28204974 DOI: 10.1007/82_2016_508]
38 **Zhao J**, O'Donnell VB, Balzar S, St Croix CM, Trudeau JB, Wenzel SE. 15-Lipoxygenase 1 interacts with phosphatidylethanolamine-binding protein to regulate MAPK signaling in human airway epithelial cells. *Proc Natl Acad Sci U S A* 2011; **108**: 14246-14251 [PMID: 21831839 DOI: 10.1073/pnas.1018075108]
39 **Forman HJ**, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med* 2009; **30**: 1-12 [PMID: 18796312 DOI: 10.1016/j.mam.2008.08.006]
40 **Lv H**, Zhen C, Liu J, Yang P, Hu L, Shang P. Unraveling the Potential Role of Glutathione in Multiple Forms of Cell Death in Cancer Therapy. *Oxid Med Cell Longev* 2019; **2019**: 3150145 [PMID: 31281572 DOI: 10.1155/2019/3150145]
41 **Liu J**, Xia X, Huang P. xCT: A Critical Molecule That Links Cancer Metabolism to Redox Signaling. *Mol Ther* 2020; **28**: 2358-2366 [PMID: 32931751 DOI: 10.1016/j.ymthe.2020.08.021]
42 **Koppula P**, Zhuang L, Gan B. Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. *Protein Cell* 2021; **12**: 599-620 [PMID: 33000412 DOI: 10.1007/s13238-020-00789-5]
43 **Ottestad-Hansen S**, Hu QX, Follin-Arbelet VV, Bentea E, Sato H, Massie A, Zhou Y, Danbolt NC. The cystine-glutamate exchanger (xCT, Slc7a11) is expressed in significant concentrations in a subpopulation of astrocytes in the mouse brain. *Glia* 2018; **66**: 951-970 [PMID: 29350434 DOI: 10.1002/glia.23294]
44 **Miyamoto K**, Watanabe M, Boku S, Sukeno M, Morita M, Kondo H, Sakaguchi K, Taguchi T, Sakai T. xCT Inhibition Increases Sensitivity to Vorinostat in a ROS-Dependent Manner. *Cancers (Basel)* 2020; **12** [PMID: 32235498 DOI: 10.3390/cancers12040827]
45 **Xie Y**, Li J, Kang R, Tang D. Interplay Between Lipid Metabolism and Autophagy. *Front Cell Dev Biol* 2020; **8**: 431 [PMID: 32582708 DOI: 10.3389/fcell.2020.00431]
46 **Hayano M**, Yang WS, Corn CK, Pagano NC, Stockwell BR. Loss of cysteinyl-tRNA synthetase (CARS) induces the transsulfuration pathway and inhibits ferroptosis induced by cystine deprivation. *Cell Death Differ* 2016; **23**: 270-278 [PMID: 26184909 DOI: 10.1038/cdd.2015.93]
47 **Kang R**, Kroemer G, Tang D. The tumor suppressor protein p53 and the ferroptosis network. *Free Radic Biol Med* 2019; **133**: 162-168 [PMID: 29800655 DOI: 10.1016/j.freeradbiomed.2018.05.074]
48 **Jennis M**, Kung CP, Basu S, Budina-Kolomets A, Leu JI, Khaku S, Scott JP, Cai KQ, Campbell MR, Porter DK, Wang X, Bell DA, Li X, Garlick DS, Liu Q, Hollstein M, George DL, Murphy ME. An African-specific polymorphism in the TP53 gene impairs p53 tumor suppressor function in a mouse model. *Genes Dev* 2016; **30**: 918-930 [PMID: 27034505 DOI: 10.1101/gad.275891.115]
49 **Xie Y**, Zhu S, Song X, Sun X, Fan Y, Liu J, Zhong M, Yuan H, Zhang L, Billiar TR, Lotze MT, Zeh HJ 3rd, Kang R, Kroemer G, Tang D. The Tumor Suppressor p53 Limits Ferroptosis by Blocking DPP4 Activity. *Cell Rep* 2017; **20**: 1692-1704 [PMID: 28813679 DOI: 10.1016/j.celrep.2017.07.055]
50 **Li Q**, Han X, Lan X, Gao Y, Wan J, Durham F, Cheng T, Yang J, Wang Z, Jiang C, Ying M, Koehler RC, Stockwell BR, Wang J. Inhibition of neuronal ferroptosis protects hemorrhagic brain. *JCI Insight* 2017; **2**: e90777 [PMID: 28405617 DOI: 10.1172/jci.insight.90777]
51 **Ooko E**, Saeed ME, Kadioglu O, Sarvi S, Colak M, Elmasaoudi K, Janah R, Greten HJ, Efferth T. Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells. *Phytomedicine* 2015; **22**: 1045-1054 [PMID: 26407947 DOI: 10.1016/j.phymed.2015.08.002]
52 **Gong Y**, Wang N, Liu N, Dong H. Lipid Peroxidation and GPX4 Inhibition Are Common Causes for Myofibroblast Differentiation and Ferroptosis. *DNA Cell Biol* 2019; **38**: 725-733 [PMID: 31140862 DOI: 10.1089/dna.2018.4541]
53 **Doll S**, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, Goya Grocin A, Xavier da Silva TN, Panzilius E, Scheel CH, Mourão A, Buday K, Sato M, Wanninger J, Vignane T, Mohana V, Rehberg M, Flatley A, Schepers A, Kurz A, White D, Sauer M, Sattler M, Tate EW, Schmitz W, Schulze A, O'Donnell V, Proneth B, Popowicz GM, Pratt DA, Angeli JPF, Conrad M. FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 2019; **575**: 693-698 [PMID: 31634899 DOI: 10.1038/s41586-019-1707-0]
54 **Mazure NM**. VDAC in cancer. *Biochim Biophys Acta Bioenerg* 2017; **1858**: 665-673 [PMID: 28283400 DOI: 10.1016/j.bbabio.2017.03.002]
55 **Maldonado EN**. VDAC-Tubulin, an Anti-Warburg Pro-Oxidant Switch. *Front Oncol* 2017; **7**: 4 [PMID: 28168164 DOI: 10.3389/fonc.2017.00004]
56 **Chen Y**, Liu Y, Lan T, Qin W, Zhu Y, Qin K, Gao J, Wang H, Hou X, Chen N, Friedmann Angeli JP, Conrad M, Wang C. Quantitative Profiling of Protein Carbonylations in Ferroptosis by an Aniline-Derived Probe. *J Am Chem Soc* 2018; **140**: 4712-4720 [PMID: 29569437 DOI: 10.1021/jacs.8b01462]
57 **Lemasters JJ**. Evolution of Voltage-Dependent Anion Channel Function: From Molecular Sieve to Governator to Actuator of Ferroptosis. *Front Oncol* 2017; **7**: 303 [PMID: 29312883 DOI: 10.3389/fonc.2017.00303]
58 **Arya N**, Wyse JM, Jayaraman S, Ball CG, Lam E, Paquin SC, Lightfoot P, Sahai AV. A proposal for the ideal algorithm for the diagnosis, staging, and treatment of pancreas masses suspicious for pancreatic adenocarcinoma: Results of a working group of the Canadian Society for Endoscopic Ultrasound. *Endosc Ultrasound* 2020; **9**: 154-161 [PMID: 32584310]
59 **Tang R**, Hua J, Xu J, Liang C, Meng Q, Liu J, Zhang B, Yu X, Shi S. The role of ferroptosis regulators in the prognosis, immune activity and gemcitabine resistance of pancreatic cancer. *Ann*

Transl Med 2020; **8**: 1347 [PMID: 33313092 DOI: 10.21037/atm-20-2554a]

60 **Zhu S**, Zhang Q, Sun X, Zeh HJ 3rd, Lotze MT, Kang R, Tang D. HSPA5 Regulates Ferroptotic Cell Death in Cancer Cells. *Cancer Res* 2017; **77**: 2064-2077 [PMID: 28130223 DOI: 10.1158/0008-5472.CAN-16-1979]

61 **Shintoku R**, Takigawa Y, Yamada K, Kubota C, Yoshimoto Y, Takeuchi T, Koshiishi I, Torii S. Lipoxygenase-mediated generation of lipid peroxides enhances ferroptosis induced by erastin and RSL3. *Cancer Sci* 2017; **108**: 2187-2194 [PMID: 28837253 DOI: 10.1111/cas.13380]

62 **Kuang Y**, Sechi M, Nurra S, Ljungman M, Neamati N. Design and Synthesis of Novel Reactive Oxygen Species Inducers for the Treatment of Pancreatic Ductal Adenocarcinoma. *J Med Chem* 2018; **61**: 1576-1594 [PMID: 29328656 DOI: 10.1021/acs.jmedchem.7b01463]

63 **Kasukabe T**, Honma Y, Okabe-Kado J, Higuchi Y, Kato N, Kumakura S. Combined treatment with cotylenin A and phenethyl isothiocyanate induces strong antitumor activity mainly through the induction of ferroptotic cell death in human pancreatic cancer cells. *Oncol Rep* 2016; **36**: 968-976 [PMID: 27375275 DOI: 10.3892/or.2016.4867]

64 **Yamaguchi Y**, Kasukabe T, Kumakura S. Piperlongumine rapidly induces the death of human pancreatic cancer cells mainly through the induction of ferroptosis. *Int J Oncol* 2018; **52**: 1011-1022 [PMID: 29393418 DOI: 10.3892/ijo.2018.4259]

65 **Eling N**, Reuter L, Hazin J, Hamacher-Brady A, Brady NR. Identification of artesunate as a specific activator of ferroptosis in pancreatic cancer cells. *Oncoscience* 2015; **2**: 517-532 [PMID: 26097885 DOI: 10.18632/oncoscience.160]

66 **Wang K**, Zhang Z, Wang M, Cao X, Qi J, Wang D, Gong A, Zhu H. Role of GRP78 inhibiting artesunate-induced ferroptosis in *KRAS* mutant pancreatic cancer cells. *Drug Des Devel Ther* 2019; **13**: 2135-2144 [PMID: 31456633 DOI: 10.2147/DDDT.S199459]

67 **Badgley MA**, Kremer DM, Maurer HC, DelGiorno KE, Lee HJ, Purohit V, Sagalovskiy IR, Ma A, Kapilian J, Firl CEM, Decker AR, Sastra SA, Palermo CF, Andrade LR, Sajjakulnukit P, Zhang L, Tolstyka ZP, Hirschhorn T, Lamb C, Liu T, Gu W, Seeley ES, Stone E, Georgiou G, Manor U, Iuga A, Wahl GM, Stockwell BR, Lyssiotis CA, Olive KP. Cysteine depletion induces pancreatic tumor ferroptosis in mice. *Science* 2020; **368**: 85-89 [PMID: 32241947 DOI: 10.1126/science.aaw9872]

68 **Dai E**, Han L, Liu J, Xie Y, Kroemer G, Klionsky DJ, Zeh HJ, Kang R, Wang J, Tang D. Autophagy-dependent ferroptosis drives tumor-associated macrophage polarization *via* release and uptake of oncogenic KRAS protein. *Autophagy* 2020; **16**: 2069-2083 [PMID: 31920150 DOI: 10.1080/15548627.2020.1714209]

69 **Mohamed AA**, Omar AAA, El-Awady RR, Hassan SMA, Eitah WMS, Ahmed R, Khater A, Tantawi OMS, Mohamed AA. MiR-155 and MiR-665 Role as Potential Non-invasive Biomarkers for Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C Virus Infection. *J Transl Int Med* 2020; **8**: 32-40 [PMID: 32435610 DOI: 10.2478/jtim-2020-0006]

70 **Shan Y**, Yang G, Huang H, Zhou Y, Hu X, Lu Q, Guo P, Hou J, Cao L, Tian F, Pan Q. Ubiquitin-Like Modifier Activating Enzyme 1 as a Novel Diagnostic and Prognostic Indicator That Correlates With Ferroptosis and the Malignant Phenotypes of Liver Cancer Cells. *Front Oncol* 2020; **10**: 592413 [PMID: 33344241 DOI: 10.3389/fonc.2020.592413]

71 **Liang JY**, Wang DS, Lin HC, Chen XX, Yang H, Zheng Y, Li YH. A Novel Ferroptosis-related Gene Signature for Overall Survival Prediction in Patients with Hepatocellular Carcinoma. *Int J Biol Sci* 2020; **16**: 2430-2441 [PMID: 32760210 DOI: 10.7150/ijbs.45050]

72 **Louandre C**, Marcq I, Bouhlal H, Lachaier E, Godin C, Saidak Z, François C, Chatelain D, Debuysscher V, Barbare JC, Chauffert B, Galmiche A. The retinoblastoma (Rb) protein regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma cells. *Cancer Lett* 2015; **356**: 971-977 [PMID: 25444922 DOI: 10.1016/j.canlet.2014.11.014]

73 **Zhang Z**, Yao Z, Wang L, Ding H, Shao J, Chen A, Zhang F, Zheng S. Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/HuR to regulate ferroptosis in hepatic stellate cells. *Autophagy* 2018; **14**: 2083-2103 [PMID: 30081711 DOI: 10.1080/15548627.2018.1503146]

74 **Qi W**, Li Z, Xia L, Dai J, Zhang Q, Wu C, Xu S. LncRNA GABPB1-AS1 and GABPB1 regulate oxidative stress during erastin-induced ferroptosis in HepG2 hepatocellular carcinoma cells. *Sci Rep* 2019; **9**: 16185 [PMID: 31700067 DOI: 10.1038/s41598-019-52837-8]

75 **Ndiaye H**, Liu JY, Hall A, Minogue S, Morgan MY, Waugh MG. Immunohistochemical staining reveals differential expression of ACSL3 and ACSL4 in hepatocellular carcinoma and hepatic gastrointestinal metastases. *Biosci Rep* 2020; **40** [PMID: 32286604 DOI: 10.1042/bsr20200219]

76 **Sun X**, Niu X, Chen R, He W, Chen D, Kang R, Tang D. Metallothionein-1G facilitates sorafenib resistance through inhibition of ferroptosis. *Hepatology* 2016; **64**: 488-500 [PMID: 27015352 DOI: 10.1002/hep.28574]

77 **Bai T**, Wang S, Zhao Y, Zhu R, Wang W, Sun Y. Haloperidol, a sigma receptor 1 antagonist, promotes ferroptosis in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2017; **491**: 919-925 [PMID: 28756230 DOI: 10.1016/j.bbrc.2017.07.136]

78 **Bai T**, Lei P, Zhou H, Liang R, Zhu R, Wang W, Zhou L, Sun Y. Sigma-1 receptor protects against ferroptosis in hepatocellular carcinoma cells. *J Cell Mol Med* 2019; **23**: 7349-7359 [PMID: 31507082 DOI: 10.1111/jcmm.14594]

79 **Wang K**, Zhang Z, Tsai HI, Liu Y, Gao J, Wang M, Song L, Cao X, Xu Z, Chen H, Gong A, Wang D, Cheng F, Zhu H. Branched-chain amino acid aminotransferase 2 regulates ferroptotic cell death in cancer cells. *Cell Death Differ* 2021; **28**: 1222-1236 [PMID: 33097833 DOI: 10.1038/s41418-020-00644-4]

80 **Ou W**, Mulik RS, Anwar A, McDonald JG, He X, Corbin IR. Low-density lipoprotein docosahexaenoic acid nanoparticles induce ferroptotic cell death in hepatocellular carcinoma. *Free Radic Biol Med* 2017; **112**: 597-607 [PMID: 28893626 DOI: 10.1016/j.freeradbiomed.2017.09.002]

81 **Shang Y**, Luo M, Yao F, Wang S, Yuan Z, Yang Y. Ceruloplasmin suppresses ferroptosis by regulating iron homeostasis in hepatocellular carcinoma cells. *Cell Signal* 2020; **72**: 109633 [PMID: 32283255 DOI: 10.1016/j.cellsig.2020.109633]

82 **Li ZJ**, Dai HQ, Huang XW, Feng J, Deng JH, Wang ZX, Yang XM, Liu YJ, Wu Y, Chen PH, Shi H, Wang JG, Zhou J, Lu GD. Artesunate synergizes with sorafenib to induce ferroptosis in hepatocellular carcinoma. *Acta Pharmacol Sin* 2021; **42**: 301-310 [PMID: 32699265 DOI: 10.1038/s41401-020-0478-3]

83 **Tang H**, Chen D, Li C, Zheng C, Wu X, Zhang Y, Song Q, Fei W. Dual GSH-exhausting sorafenib loaded manganese-silica nanodrugs for inducing the ferroptosis of hepatocellular carcinoma cells. *Int J Pharm* 2019; **572**: 118782 [PMID: 31678528 DOI: 10.1016/j.ijpharm.2019.118782]

84 **Wang G**, Liu X, Wang S, Ge N, Guo J, Sun S. Endoscopic Ultrasound-guided Gastroenterostomy: A Promising Alternative to Surgery. *J Transl Int Med* 2019; **7**: 93-99 [PMID: 31637179 DOI: 10.2478/jtim-2019-0021]

85 **Lee JY**, Nam M, Son HY, Hyun K, Jang SY, Kim JW, Kim MW, Jung Y, Jang E, Yoon SJ, Kim J, Seo J, Min JK, Oh KJ, Han BS, Kim WK, Bae KH, Song J, Huh YM, Hwang GS, Lee EW, Lee SC. Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. *Proc Natl Acad Sci U S A* 2020; **117**: 32433-32442 [PMID: 33288688 DOI: 10.1073/pnas.2006828117]

86 **Wang C**, Shi M, Ji J, Cai Q, Zhao Q, Jiang J, Liu J, Zhang H, Zhu Z, Zhang J. Stearoyl-CoA desaturase 1 (SCD1) facilitates the growth and anti-ferroptosis of gastric cancer cells and predicts poor prognosis of gastric cancer. *Aging (Albany NY)* 2020; **12**: 15374-15391 [PMID: 32726752 DOI: 10.18632/aging.103598]

87 **Hao S**, Yu J, He W, Huang Q, Zhao Y, Liang B, Zhang S, Wen Z, Dong S, Rao J, Liao W, Shi M. Cysteine Dioxygenase 1 Mediates Erastin-Induced Ferroptosis in Human Gastric Cancer Cells. *Neoplasia* 2017; **19**: 1022-1032 [PMID: 29144989 DOI: 10.1016/j.neo.2017.10.005]

88 **Sun X**, Yang S, Feng X, Zheng Y, Zhou J, Wang H, Zhang Y, Sun H, He C. The modification of ferroptosis and abnormal lipometabolism through overexpression and knockdown of potential prognostic biomarker perilipin2 in gastric carcinoma. *Gastric Cancer* 2020; **23**: 241-259 [PMID: 31520166 DOI: 10.1007/s10120-019-01004-z]

89 **Gao Z**, Deng G, Li Y, Huang H, Sun X, Shi H, Yao X, Gao L, Ju Y, Luo M. Actinidia chinensis Planch prevents proliferation and migration of gastric cancer associated with apoptosis, ferroptosis activation and mesenchymal phenotype suppression. *Biomed Pharmacother* 2020; **126**: 110092 [PMID: 32203890 DOI: 10.1016/j.biopha.2020.110092]

90 **Guan Z**, Chen J, Li X, Dong N. Tanshinone IIA induces ferroptosis in gastric cancer cells through p53-mediated SLC7A11 down-regulation. *Biosci Rep* 2020; **40** [PMID: 32776119 DOI: 10.1042/bsr20201807]

91 **Nunes G**, Marques PP, Patita M, Allen M, Gargaté L. EUS-guided recanalization of complete colorectal anastomotic stenosis using a lumen-apposing metal stent. *Endosc Ultrasound* 2019; **8**: 211-212 [PMID: 30785118 DOI: 10.4103/eus.eus_62_18]

92 **Sak K**. A Hypothetical Approach on Gender Differences in Cancer Diagnosis. *J Transl Int Med* 2019; **7**: 90-92 [PMID: 31637178 DOI: 10.2478/jtim-2019-0020]

93 **Sui X**, Zhang R, Liu S, Duan T, Zhai L, Zhang M, Han X, Xiang Y, Huang X, Lin H, Xie T. RSL3 Drives Ferroptosis Through GPX4 Inactivation and ROS Production in Colorectal Cancer. *Front Pharmacol* 2018; **9**: 1371 [PMID: 30524291 DOI: 10.3389/fphar.2018.01371]

94 **Lu D**, Yang Z, Xia Q, Gao S, Sun S, Luo X, Li Z, Zhang X, Li X. ACADSB regulates ferroptosis and affects the migration, invasion, and proliferation of colorectal cancer cells. *Cell Biol Int* 2020; **44**: 2334-2343 [PMID: 32776663 DOI: 10.1002/cbin.11443]

95 **Park S**, Oh J, Kim M, Jin EJ. Bromelain effectively suppresses Kras-mutant colorectal cancer by stimulating ferroptosis. *Anim Cells Syst (Seoul)* 2018; **22**: 334-340 [PMID: 30460115 DOI: 10.1080/19768354.2018.1512521]

96 **Xia Y**, Liu S, Li C, Ai Z, Shen W, Ren W, Yang X. Discovery of a novel ferroptosis inducer-talaroconvolutin A-killing colorectal cancer cells *in vitro* and in vivo. *Cell Death Dis* 2020; **11**: 988 [PMID: 33203867 DOI: 10.1038/s41419-020-03194-2]

97 **Zhang L**, Liu W, Liu F, Wang Q, Song M, Yu Q, Tang K, Teng T, Wu D, Wang X, Han W, Li Y. IMCA Induces Ferroptosis Mediated by SLC7A11 through the AMPK/mTOR Pathway in Colorectal Cancer. *Oxid Med Cell Longev* 2020; **2020**: 1675613 [PMID: 32322334 DOI: 10.1155/2020/1675613]

98 **Shen LD**, Qi WH, Bai JJ, Zuo CY, Bai DL, Gao WD, Zong XL, Hao TT, Ma Y, Cao GC. Resibufogenin inhibited colorectal cancer cell growth and tumorigenesis through triggering ferroptosis and ROS production mediated by GPX4 inactivation. *Anat Rec (Hoboken)* 2021; **304**: 313-322 [PMID: 31961485 DOI: 10.1002/ar.24378]

99 **Sharma P**, Shimura T, Banwait JK, Goel A. Andrographis-mediated chemosensitization through activation of ferroptosis and suppression of β-catenin/Wnt-signaling pathways in colorectal cancer. *Carcinogenesis* 2020; **41**: 1385-1394 [PMID: 32835374 DOI: 10.1093/carcin/bgaa090]

100 **Chen P**, Li X, Zhang R, Liu S, Xiang Y, Zhang M, Chen X, Pan T, Yan L, Feng J, Duan T, Wang D, Chen B, Jin T, Wang W, Chen L, Huang X, Zhang W, Sun Y, Li G, Kong L, Li Y, Yang Z, Zhang

Q, Zhuo L, Sui X, Xie T. Combinative treatment of β-elemene and cetuximab is sensitive to KRAS mutant colorectal cancer cells by inducing ferroptosis and inhibiting epithelial-mesenchymal transformation. *Theranostics* 2020; **10**: 5107-5119 [PMID: 32308771 DOI: 10.7150/thno.44705]

101 **Lorenzato A**, Magrì A, Matafora V, Audrito V, Arcella P, Lazzari L, Montone M, Lamba S, Deaglio S, Siena S, Bertotti A, Trusolino L, Bachi A, Di Nicolantonio F, Bardelli A, Arena S. Vitamin C Restricts the Emergence of Acquired Resistance to EGFR-Targeted Therapies in Colorectal Cancer. *Cancers (Basel)* 2020; **12** [PMID: 32183295 DOI: 10.3390/cancers12030685]

102 **Friedmann Angeli JP**, Krysko DV, Conrad M. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nat Rev Cancer* 2019; **19**: 405-414 [PMID: 31101865 DOI: 10.1038/s41568-019-0149-1]

103 **Yang WS**, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014; **156**: 317-331 [PMID: 24439385 DOI: 10.1016/j.cell.2013.12.010]

104 **Zhang Y**, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, Uchida K, O'Connor OA, Stockwell BR. Imidazole Ketone Erastin Induces Ferroptosis and Slows Tumor Growth in a Mouse Lymphoma Model. *Cell Chem Biol* 2019; **26**: 623-633.e9 [PMID: 30799221 DOI: 10.1016/j.chembiol.2019.01.008]

105 **Gout PW**, Buckley AR, Simms CR, Bruchovsky N. Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x(c)- cystine transporter: a new action for an old drug. *Leukemia* 2001; **15**: 1633-1640 [PMID: 11587223 DOI: 10.1038/sj.leu.2402238]

106 **Kim EH**, Shin D, Lee J, Jung AR, Roh JL. CISD2 inhibition overcomes resistance to sulfasalazine-induced ferroptotic cell death in head and neck cancer. *Cancer Lett* 2018; **432**: 180-190 [PMID: 29928961 DOI: 10.1016/j.canlet.2018.06.018]

107 **Dixon SJ**, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, Gleason CE, Tatonetti NP, Slusher BS, Stockwell BR. Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *Elife* 2014; **3**: e02523 [PMID: 24844246 DOI: 10.7554/eLife.02523]

108 **Shimada K**, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ, Stockwell BR. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat Chem Biol* 2016; **12**: 497-503 [PMID: 27159577 DOI: 10.1038/nchembio.2079]

109 **Guo J**, Xu B, Han Q, Zhou H, Xia Y, Gong C, Dai X, Li Z, Wu G. Ferroptosis: A Novel Anti-tumor Action for Cisplatin. *Cancer Res Treat* 2018; **50**: 445-460 [PMID: 28494534 DOI: 10.4143/crt.2016.572]

110 **Abrams RP**, Carroll WL, Woerpel KA. Five-Membered Ring Peroxide Selectively Initiates Ferroptosis in Cancer Cells. *ACS Chem Biol* 2016; **11**: 1305-1312 [PMID: 26797166 DOI: 10.1021/acschembio.5b00900]

111 **NaveenKumar SK**, SharathBabu BN, Hemshekhar M, Kemparaju K, Girish KS, Mugesh G. The Role of Reactive Oxygen Species and Ferroptosis in Heme-Mediated Activation of Human Platelets. *ACS Chem Biol* 2018; **13**: 1996-2002 [PMID: 29869870 DOI: 10.1021/acschembio.8b00458]

112 **Ma S**, Henson ES, Chen Y, Gibson SB. Ferroptosis is induced following siramesine and lapatinib treatment of breast cancer cells. *Cell Death Dis* 2016; **7**: e2307 [PMID: 27441659 DOI: 10.1038/cddis.2016.208]

113 **Yuan H**, Li X, Zhang X, Kang R, Tang D. Identification of ACSL4 as a biomarker and contributor of ferroptosis. *Biochem Biophys Res Commun* 2016; **478**: 1338-1343 [PMID: 27565726 DOI: 10.1016/j.bbrc.2016.08.124]

114 **Shi F**, Zhang P, Mao Y, Wang C, Zheng M, Zhao Z. The nitroxide Tempo inhibits hydroxyl radical production from the Fenton-like reaction of iron(II)-citrate with hydrogen peroxide. *Biochem Biophys Res Commun* 2017; **483**: 159-164 [PMID: 28042034 DOI: 10.1016/j.bbrc.2016.12.174]

115 **Guerrero-Hue M**, García-Caballero C, Palomino-Antolín A, Rubio-Navarro A, Vázquez-Carballo C, Herencia C, Martín-Sanchez D, Farré-Alins V, Egea J, Cannata P, Praga M, Ortiz A, Egido J, Sanz AB, Moreno JA. Curcumin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis-mediated cell death. *FASEB J* 2019; **33**: 8961-8975 [PMID: 31034781 DOI: 10.1096/fj.201900077R]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 19-37

DOI: 10.4251/wjgo.v14.i1.19

ISSN 1948-5204 (online)

REVIEW

Biomarkers for response to immune checkpoint inhibitors in gastrointestinal cancers

Meng Li, Denis Kaili, Lei Shi

ORCID number: Meng Li 0000-0002-2911-4293; Denis Kaili 0000-0002-4843-9974; Lei Shi 0000-0002-7995-5664.

Author contributions: Li M and Shi L participated in the design of the study, and prepared the tables and figure; Li M, Kaili D, and Shi L participated in writing the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no competing or financial interests for this article.

Supported by Fundamental Research Funds for the Central Universities, No. 2019CDYGYB024.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

Open-Access: This article is an open-access article that was

Meng Li, Lei Shi, School of Life Sciences, Chongqing University, Chongqing 400044, China

Denis Kaili, Department of Surgery, Wexner Medical Center, The Ohio State University, Columbus, OH 43210, United States

Corresponding author: Lei Shi, PhD, Associate Professor, School of Life Sciences, Chongqing University, No. 174 Shazheng Street, Shapingba District, Chongqing 400044, China. shil@cqu.edu.cn

Abstract

Gastrointestinal (GI) cancers account for a large proportion of cancer deaths worldwide and pose a major public health challenge. Immunotherapy is considered to be one of the prominent and successful approaches in cancer treatment in recent years. Among them, immune checkpoint inhibitor (ICI) therapy, has received widespread attention, and many clinical findings support the feasibility of ICIs, with sustained responses and significantly prolonged lifespan observed in a wide range of tumors. However, patients treated with ICIs have not fully benefited, and therefore, the identification and development of biomarkers for predicting ICI treatment response have received further attention and exploration. From tumor genome to molecular interactions in the tumor microenvironment, and further expanding to circulating biomarkers and patient characteristics, the exploration of biomarkers is evolving with high-throughput sequencing as well as bioinformatics. More large-scale prospective and specific studies are needed to explore biomarkers in GI cancers. In this review, we summarize the known biomarkers used in ICI therapy for GI tumors. In addition, some ICI biomarkers applied to other tumors are included to provide insights and further validation for GI tumors. Moreover, we present single-cell analysis and machine learning approaches that have emerged in recent years. Although there are no clear applications yet, it can be expected that these techniques will play an important role in the application of biomarker prediction.

Key Words: Immunotherapy; Immune checkpoint inhibitor; Biomarker; Predictive response; Gastrointestinal cancer

Received: February 22, 2021
Peer-review started: February 22, 2021
First decision: August 19, 2021
Revised: September 8, 2021
Accepted: December 21, 2021
Article in press: December 21, 2021
Published online: January 15, 2022

P-Reviewer: Kocollari A, Pompella L, Serban ED
S-Editor: Fan JR
L-Editor: Wang TQ
P-Editor: Fan JR

Core Tip: Cancer immunotherapy and immune checkpoint inhibitors (ICIs) have recently revolutionized gastrointestinal (GI) cancer treatment, providing unprecedented clinical benefits. However, GI patients treated with ICIs do not fully benefit, and therefore, the identification and development of biomarkers for predicting ICI response have become a pressing issue to be solved now. In this review, we summarize the use of predictive biomarkers for ICI treatment response in GI cancers, and discuss novel biomarkers under development. We also present important biomarkers in other tumors with the aim of providing a cutting-edge reference for GI cancer research.

Citation: Li M, Kaili D, Shi L. Biomarkers for response to immune checkpoint inhibitors in gastrointestinal cancers. *World J Gastrointest Oncol* 2022; 14(1): 19-37
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/19.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.19

INTRODUCTION

Gastrointestinal (GI) cancers are common among all cancer types, and the incidence and mortality rates of GI cancers are increasing year by year, especially in colorectal cancer (CRC), which is also accompanied by a tendency of rejuvenation[1]. GI cancers mainly occur in the GI system and related digestive organs, including the esophagus, stomach, biliary tract system, liver, pancreas, small intestine, rectum, and anus. Among them, hepatocellular carcinoma (HCC) has the highest morbidity and mortality rate. For example, from 2000 to 2016, the mortality rate for HCC increased by 43% (from 7.2 to 10.3 *per* 100000), with a 5-year survival rate of only 18% in the United States[2]. Treatment strategies for GI cancers include surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, among which immunotherapy is a hot topic in recent years.

Immunotherapy is a relatively new therapeutic strategy that has received widespread attention, mainly including immune checkpoint inhibitors (ICIs), tumor vaccines, and immune cell therapy. Among these, ICIs are most widely used[3]. Immune checkpoints are used by normal cells to regulate immune cytotoxic functions, thus avoiding the destruction of normal tissues. However, this mechanism can also be borrowed by tumor cells to escape the body's immune surveillance and clearance[4]. ICIs can eliminate this inhibitory effect, allowing immune cells to be reactivated to a working state and destroy tumor cells.

The better studied ICIs are CTLA-4 inhibitors and programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors. Ipilimumab (anti-CTLA-4) was approved by the FDA in 2011 for the treatment of melanoma, followed by the PD-1 inhibitors pembrolizumab and nivolumab for the treatment of melanoma, metastatic non-small cell lung cancer (NSCLC), and DNA mismatch repair-deficient/microsatellite instability-high (dMMR/MIS-H) tumors[5,6]. Although there are many immune checkpoints, not limited to those mentioned above, they have a relatively similar mechanism of action. For example, PD-1 is able to bind to PD-L1 in tumor cells, disabling the ability of T cells to attack cancer cells. Their binding acts as a co-inhibitory signal for T cells and negatively regulates the body's immune response. In turn, tumor cells can upregulate the expression of PD-L1 to inhibit the activation of T cells. This suppression can be abolished after ICI treatment, and in turn, T cells are able to perform their normal functions[7]. In this regard, immunotherapy is now becoming a prospective treatment for GI cancers.

Although immunotherapy has provided sustained clinical benefits, studies have found limitations in the effectiveness of immunotherapy and it is extremely important to study biomarkers to predict more accurate clinical responses[8]. Biomarkers for predicting ICI response have been extensively explored and developed. A variety of biomarkers for GI malignancies have been clinically applied, which can help patients to choose the appropriate targeted therapeutic options. This review highlights biomarkers for predicting the response to ICIs for the treatment of GI tumors. Some biomarkers applied to other tumors are also presented, intending to provide further reference and validation for GI tumors (Figure 1). In addition, we present some new approaches that have emerged in recent years, such as single-cell analysis and machine learning.

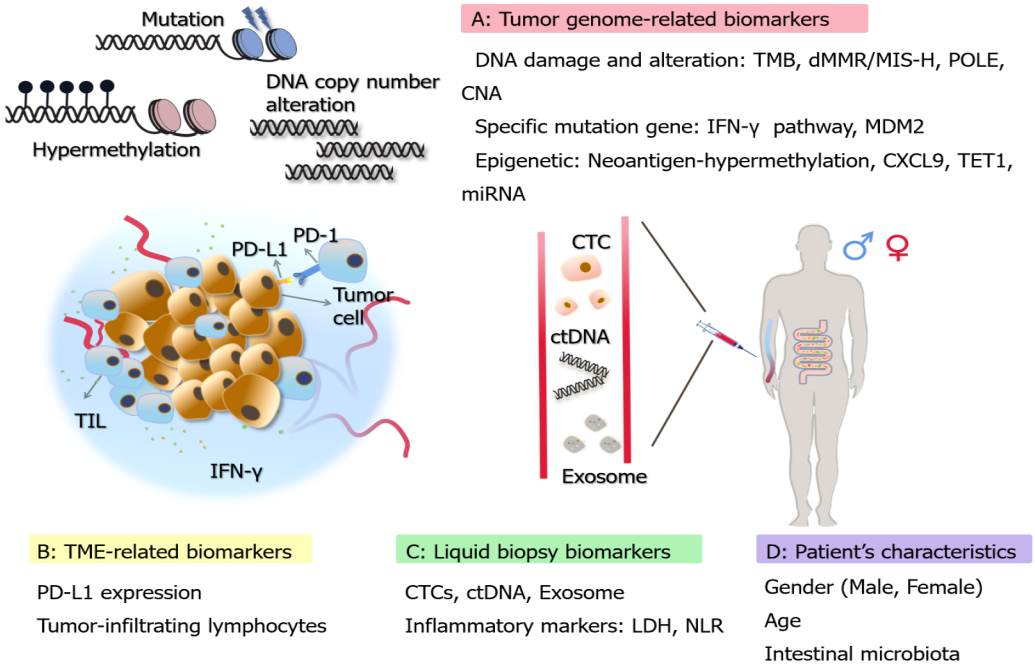


Figure 1 Brief overview of immune checkpoint inhibitor-related biomarkers in gastrointestinal cancers and some novel biomarkers being developed. A: Tumor genome-related biomarkers. The biomarkers in this category are divided into three groups: DNA damage and alteration, including tumor mutation burden, mismatch repair deficiency/high-microsatellite instability (dMMR/MSI-H), POLE, and copy number alteration (CNA); specific mutation genes, including IFN-γ pathway and MDM2; epigenetic alterations, including neoantigen-hypermethylation, CXCL9 epigenetic modification, TET1, and miRNAs; B: TME (tumor immune microenvironment) related biomarkers. PD-L1 expression and tumor infiltrating lymphocytes are involved. In this review, $CD8^+$ and $CD39^+CD8^+$ cells are mainly mentioned; C: Liquid biopsy biomarkers. CTCs, ctDNA, and exosomes are grouped into one group. Inflammatory markers taken from peripheral blood are divided into a separate subcategory, including lactate dehydrogenase and neutrophil-to-lymphocyte ratio; D: Patient's characteristics. The patient's gender, age, and intestinal microbiota are classified in this category. ICI: Immune checkpoint inhibitor; GI: Gastrointestinal; POLE: Polymerase gene epsilon; MDM2: Murine double minute 2; CXCL9: Chemokine (C-X-C motif) ligand 9; TET1: Ten eleven translocation 1; PD-1/L1: Programmed cell death-1/Ligand 1; ctDNA: Circulating tumor DNA; CTC: Circulating tumor cells; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; TIL: Tumor infiltrating lymphocytes.

TUMOR GENOME BIOMARKERS

Tumor mutation burden

The tumor mutation burden (TMB) represents the density of distribution of non-synonymous mutations in the protein-coding region, or simply the number of mutations present in the tumor (Table 1). It is usually defined as the total number of mutations *per* megabase of substitutions and insertions or deletions in the exon coding region of the gene evaluated in the tumor sample and is usually detected as mutations *per* million bases (Mut/Mb)[9]. Traditionally, whole-exome sequencing (WES) has been used to measure TMB, which is considered the standard for TMB determination. However, due to the high cost and relatively slow speed of detection using WES, the accurate determination of TMB by next-generation sequencing (NGS) panels has recently been applied[10]. Quantifying the number of non-synonymous single nucleotide variants (SNVs) by NGS, followed by algorithmic validation and extension to WES, is also one of the feasible approaches in recent years[11].

According to several reports in recent years, increased TMB is associated with the response to ICI therapy, and high TMB was significantly associated with the efficacy of ICIs[12]. There are many data supporting the use of increased TMB as a biomarker for ICI therapy in many pan-cancer treatments. According to a retrospective study that included 27 cancer types, patients with higher TMB were found to have better clinical outcomes and objective response rates (ORR) when treated with PD-1 antibody[13]. In a phase II study of pembrolizumab in Korea, high TMB was defined as more than 400 SNVs in the WES. The results showed that elevated levels of TMB were associated with a high ORR (89%); the moderate TMB group (100-400 SNVs) had an ORR of 20%, while the low TMB group had an ORR of only 7%, indicating a similar positive correlation between high levels of TMB and ICI efficacy, *i.e.*, higher values of TMB represent a higher overall response rate for patients[14].

In another retrospective study, TMB levels of patients with various types of melanoma as well as NSCLC were also classified as low (1-5 Mut/Mb), medium (6-19 Mut/Mb), and high (≥ 20 Mut/Mb). Their analysis indicated that patients with high

Table 1 Summary of biomarkers used or worthwhile in gastrointestinal cancers

Classification	Biomarkers	Tumors	Response	OS	PFS	Others	Ref.
Tumor-genome biomarkers	TMB	Multiple GI	Pos/Neg[1]	14.6/4.0 mo	Unreached/2 mo (CRC)	NA	[19-21]
	dMMR/MSI-H	Multiple GI	Pos	Unreached *vs* 5.0 mo (CRC)	Unreached *vs* 2.2 mo (CRC)	Higher DCB (59.1% *vs* 28.6%, GI tumors)	[30,31]
	CNA	Multiple GI	Neg	Unreached[2]	Over 10 mo	NA	[31]
	IFN-γ-related	Multiple GI	Pos	Positive correlation (GC)	Positive correlation (GC and ESCA)	NA	[40,42]
	MDM2	HCC	Neg	NA	NA	Correlated with HPD	[50]
TME biomarkers	PD-L1	Multiple GI	Pos	NA	NA	NA	[53,54]
	TIL	Multiple GI	Pos	Prolonged OS (ESCA)	NA	3-yr RFS 71.6% *vs* 55.3% (CRC)	[67,78]
Liquid-biopsy biomarkers	ctDNA	Multiple GI	Neg	NA	4.9 mo *vs* 7.4 mo (GC)	2-yr RFS 66% *vs* 100% (CRC)	[73,74]
	Exosome	GC	Neg	Reduced OS	NA	High level Exosome	[78]

[1]In tumor mutation burden (TMB), the Neg means that immune checkpoint inhibitor treatment response of TMB-L patients may be better by epigenetic modifications.

[2]Represent a wide variety of gastrointestinal tumors and do not refer to any particular type.

OS: Overall survival; PFS: Progression-free survival; TMB: Tumor mutation burden; dMMR: Mismatch repair deficiency; MSI-H: Highly microsatellite instability; CNA: Copy number alteration; TIL: Tumor infiltrating lymphocyte; ctDNA: Circulating tumor DNA; GI: Gastrointestinal; GC: Gastric cancer; CRC: Colorectal cancer; HCC: Hepatocellular cancer; ESCA: Esophageal cancer; HPD: Hyperprogressive disease; DCB: Durable clinical benefit; RFS: Recurrence free survival; Pos: Positive; Neg: Negative; NA: Not applicable; ICI: Immune checkpoint inhibitor.

levels of TMB had the highest response rate to ICI treatment, reaching 58%, and also had the longest duration of progression-free survival (PFS) at 12.8 mo. The other two treatment groups had a response rate of only 20% and a PFS of only 3.3 mo[15]. Another study detected TMB (cut-off value of 20 Mut/Mb) in 4064 NSCLC patients and found that patients with high levels of TMB (TMB-H) had a significantly higher overall survival (OS) and disease control rate (DCR) when treated with anti-PD-1/L1 agents compared to patients with low levels of TMB (TMB-L)[16]. Similar results were presented in another study showing significantly better durable clinical benefit (DCB) and PFS in the TMB-H population in a cohort with 78 NSCLC patients treated with anti-PD-1/L1 antibodies[17]. Additionally, in a prospective analysis of KEYNOTE-158, Marabelle *et al*[18] assessed the association of pembrolizumab monotherapy in terms of TMB (tTMB) and clinical outcome across ten different advanced solid tumors types, including anal, biliary, *etc*. The results revealed that in terms of efficacy, the ORR (29% *vs* 6%) was better in the tTMB-high group (defined as ≥ 10 Mut/Mb) than in the tTMB-low group (< 10 Mut/Mb), and the median durable response (follow-up of approximately 3 years) was not reached, while the tTMB-low group only reached 33.1 mo[18].

Data from the above-mentioned studies have demonstrated the significant role of high levels of TMB in predicting ICI efficacy, and the results of TMB in GI cancers are no exception to other tumor types. In a phase I study with the anti-PD-1 antibody toripalimib, patients with metastatic gastric cancer (GC) with high TMB (> 20 Mut/Mb) had a better response in survival compared to those with low TMB (15 mo *vs* 4 mo)[19,20]. In patients with advanced GC, patients with high TMB (≥ 12 Mut/Mb) had significantly better efficiency (33.3% *vs* 7.1%) and OS time (14.6 *vs* 4.0 mo) than patients with low TMB (< 12 Mut/Mb)[20]. In a study of metastatic CRC, none of the TMB-H group had achieved PFS (median follow-up > 18 mo), while the TMB-L group had a PFS of only 2 mo and approximately 66% of TMB-L patients developed further disease[21]. In conclusion, high levels of TMB in ICI therapy represent improved patient treatment efficiency and better prognostic outcomes.

Several studies presented at the 2020 American Society of Clinical Oncology meeting confirmed the predictive value of TMB in immunotherapy or combination therapy, although TMB still has limitations as a biomarker. In addition, several general issues deserve further attention, both in the application of GI cancers and in a wide range of other tumor types. First, there is no clear TMB cut-off value as a criterion to

accurately determine which patients can benefit from ICI treatment[22]. Second, testing at the proteomic level may provide a clear picture of the mutational load on the membrane of tumor cells, as some mutations that cause an immune response may originate from only a small subset of genes[23]. Third, factors such as allele frequency might be considered for further and more accurate prediction of ICI efficacy[24].

dMMR/MSI-H

MSI refers to microsatellite instability and MMR refers to mismatch repair function. They are closely related, *e.g.*, when the MMR functions are in a proficient state (pMMR), MSI can be repaired to maintain stability (MSS). In contrast, when the expression of any of the MMR-related proteins goes wrong and the MMR function is in a deficient state (dMMR), it leads to defects in cellular repair functions, allowing DNA to accumulate mutations during replication, ultimately leading to the development of MSI[25]. MSI can be broadly classified as highly unstable (MSI-H), lowly unstable (MSI-L), and stable (MSS). The dMMR and MSI-H can be roughly equated, as can pMMR and MSS[26].

The dMMR occurs in a variety of tumor types, especially common in GI cancers, including colorectum, stomach, small intestine, prostate, *etc.*[27]. It has been shown that dMMR/MSI-H tumors have a much higher somatic mutation rate compared to pMMR tumors and are thought to express a large number of shift-code peptides that act as neoantigens and enhance the immune response[28]. In 2017, the United States FDA first approved the PD-1 inhibitor pembrolizumab for the treatment of patients with solid dMMR/MSI-H tumors[29]. Several clinical trials, including KEYNOTE-012, 016, 028, and 158, which included multiple tumor types, have shown that pembrolizumab has promising durable outcomes in treating patients with dMMR/MSI-H tumors[24].

In the treatment of GI cancers, especially in CRC, dMMR/MSI-H is considered to be a relatively well-established group of biomarkers. In the KEYNOTE-164 clinical trial study, the efficacy of pembrolizumab was evaluated in three cohorts of 11 dMMR-CRC, 21 pMMR-CRC, and 9 dMMR non-CRC patients. An immune-related ORR of 40% and a 20-wk PFS of 78% were observed in the dMMR-CRC cohort, while an ORR of 0 and a 20-wk PFS of 11% were observed in the pMMR-CRC cohort. Median PFS and OS were not achieved in the dMMR-CRC cohort, but were 2.2 mo and 5.0 mo, respectively, in the pMMR-CRC cohort. These results demonstrated that dMMR patients are favorable candidates for treatment with ICIs[30]. Lu *et al*[31] investigated the clinical benefit of ICIs in GI patients. They indicated that the incidence of DCB was significantly higher in dMMR/MSI-H patients (59.1%) than in MSI-L/MSS/pMMR patients (28.6%). In addition, the median PFS time was significantly longer in dMMR/MSI-H patients (7.24 mo) than in MSI-L/MSS/pMMR patients (2.67 mo)[31]. These data reveal that dMMR/MSI-H patients have a more favorable ICI response than the other groups. The dMMR/MSI-H has reliable clinical data as a well-established biomarker in GI cancers, especially in CRC. Its application in other GI cancers also deserves attention and further exploration.

Copy number alteration

Recently, it has also been shown that copy number alterations (CNA), including copy number gain (CNgain) and copy number loss (CNloss), have a predictive role in ICI therapy. In melanoma patients treated with ICIs, CNLoss was found to be lower in responders[32]. Some ICI-related immune features were also found to be negatively correlated with CNA in GC and CRC of the Cancer Genome Atlas (TCGA) datasets [33]. Detailed data are presented for elaboration in the study by Lu *et al*[31]. In their study, tumor samples from 93 patients with GI cancers treated with ICIs were tested. CNA load included measures of total CNA, CNgain, and CNloss, while CNgain/CNloss was defined as the total number of genes with CNgain/CNloss present in each sample[31]. They found a significant difference in the CNA burden index between DCB and NDB (no durable benefit) patients treated in the GI group, with DCB patients having a significantly lower CNA burden than NDB patients, suggesting that a low CNA burden may be correlated with better ICIs outcomes. DCB rates were more pronounced in the low and high groups with the same low level of CNgain/CNloss. Further exploration of OS and PFS also led to more favorable data in the low burden group. Based on the study, the group with lower CNA showed a longer median OS (not achieved in all cohorts). For PFS, it was also suggested that the lower CNA group had a longer PFS, all at more than 10 mo[31]. Furthermore, a study by Smeet *et al*[34] on CRC treated with bevacizumab combination therapy also illustrated another perspective on the possibility of CNA as a potential biomarker for ICI treatment. Their study, which also defined three CNA groups, showed that tumors in the low-load

CNA group did not benefit from this combination therapy, while in turn confirmed that ICI therapy is the superior choice. Likewise, the potential of low-load CNA as a predictive biomarker for ICIs was also confirmed[34].

As a noteworthy point, considering the combination of TMB and CNA, a significantly higher proportion of patients with DCB were in the TMB-High/CNA-Low subgroup (12/14) compared to the TMB-Low/CNA-High subgroup (1/28). The median OS (not achieved) was also significantly longer in the TMB-high/CNA-low subgroup than in the other three subgroups (TMB-Low/CNA-Low, 17.3 mo; TMB-High/CNA-High, 12.37 mo; TMB-Low/CNA-High, 6.23 mo)[31]. This result suggests that the combined use of these two biomarkers may have a higher accuracy.

IFN-γ signal and MDM2

Alterations within the tumor-associated signaling pathways also affect the efficacy of ICIs, related to the mechanism of checkpoint inhibitor drugs as well as drug resistance [35]. IFN-γ is a cytokine that stimulates the immune response and is one of the key signals for the activation of immune cells. IFN-γ is also able to trigger a series of events leading to tumor cell death by linking to receptors on the cell surface. Moreover, IFN-γ is able to increase the expression of PD-L1 in tumors and increase the expression of MHC, promoting antigen presentation in antigen presenting cells[36].

Grasso *et al*[37] showed that IFN-γ released by T cells contributes to the amplification of nascent anti-tumor immune response[37]. A study by Karachaliou *et al* [38], which included seven NSCLC patients treated with pembrolizumab, showed that high expression of IFN-γ may be associated with a better PFS and OS in NSCLC patients[38]. Higgs *et al*[39] similarly showed that patients with elevated IFN-γ-associated signaling had a longer median OS (18.1-22.7 mo *vs* 6.5-7.7 mo) and better ORR (6-fold higher) in advanced NSCLC[39]. The above results revealed a trend towards the application of IFN-γ in GI cancers.

KEYNOTE-028 is a phase Ib trial of pembrolizumab in patients with 20 different tumor types, including GI cancers. In the esophageal cohort, 23 patients were enrolled and an IFN-γ signature was detected, showing a trend towards predicting response to ICIs[40]. In GC, Epstein-Barr virus (EBV) is involved in approximately 10% of GC progression, and PD-L1 overexpression is presented as a feature of EBV GC. In addition, IFN-γ signaling was also shown to be involved in a study by Sasaki *et al*[41]. Similarly, in the KEYNOTE-012 clinical trial, which included GC patients treated with pembrolizumab, IFN-γ-related genes were shown to be correlated with OS and PFS [42]. Overall, these results provide useful information revealing the role of IFN-γ in predicting the efficacy of ICIs in GI cancers.

Mutations in genes related to the IFN-γ pathway, such as *IFNGR1/2*, *JAK1/2*, and *IRF1*, also lead to poor outcomes and resistance in patients receiving ICI therapy[35, 43]. The JAKs are key kinases in this pathway, and JAK1/2 shift mutations lead to deficient production of IFN-γ. Shin *et al*[44] indicated that JAK1/2 mutations were associated with resistance to anti-PD-1 therapy in CRC patients[44]. These results suggest that mutations in JAK can lead to poor efficacy of ICIs[44,45].

MDM2 is known as the mouse double minute 2 homolog and is an E3 ubiquitin ligase. When MDM2 is overexpressed due to amplification or improper regulation, it inhibits the activation of P53, which in turn accelerates tumor growth and progression [46]. Kato *et al*[47] analyzed the genomic profiles of 155 patients with multiple tumor types and found that six patients with MDM2 amplification have a time to treatment failure (TTF) less than 2 mo. Four of the six cases (all with MDM2 amplification) showed 2.3 to 42.3-fold hyperprogression compared to ICI pre-treatment[47]. A recent study also showed that cell lines with high MDM2 expression were more potent against T cell-mediated tumor killing, and that targeting MDM2 improve the efficacy of ICIs[48]. These imply that there may be a negative correlation between amplified variants of MDM2 and the efficacy of ICIs, allowing tumors to develop hyperprogression after receiving treatment.

Dysfunction of the MDM2-P53 axis is a major contributor to GI cancers. The main risk factors for HCC include chronic viral infections and metabolic diseases, all of which may contribute to HCC through dysfunction of the MDM2-P53 axis[49]. The results by Wu *et al*[50] on prognostic markers for HCC showed that MDM2 was able to directly act on BIRC5 as well as the downstream transcription factors to regulate its expression, thereby reducing the sensitivity and effectiveness of ICI therapy[50]. Based on the association from the available clinical data, MDM2 is expected to be a more specific negative biomarker for predicting ICIs in HCC, although further prospective studies are needed to corroborate this.

TUMOR IMMUNE MICROENVIRONMENT-RELATED BIOMARKERS

PD-L1 expression

PD-L1 is one of the most studied biomarkers with abundant data in clinical studies [51]. The expression of PD-L1 in tumors measured by immunohistochemistry was one of the first biomarkers developed to predict the benefit of ICIs[52]. In GI cancers such as GC, CRC, and HCC, there is a positive correlation between PD-L1 expression and the efficacy of ICIs[53,54]. Many clinical trials have provided data demonstrating the feasibility of PD-L1 (Keynote-059, Keynote-010, Attraction-02, Checkmate-057, Checkmate-012, *etc.*), and the FDA has approved the application of PD-L1 expression as a biomarker for adjuvant or second-line treatment.

Nevertheless, PD-L1 expression remains limited and somewhat controversial as a comprehensive, stand-alone biomarker. In the trials mentioned above, both Keynote-059 and Attraction-02 did show higher response activity in PD-L1-positive patients, but the data equally showed response activity in PD-L1-negative patients[55]. Concerning the limitations of PD-L1 expression, the following points are noteworthy. First, in the tumor microenvironment, PD-L1 expression displays dynamics and diversity with spatial and temporal heterogeneity[56]. PD-L1 expression detected at a single time point cannot be fully used to assess ICI response[57]. Second, PD-L1 detection criteria are not standardized, with no exact positive scores and thresholds to define[56,58]. Issues such as inconsistent antibody usage and inconsistent detection thresholds make it difficult to standardize staining systems as well[59]. At the molecular level, PD-L1 expression has two components: Tumor cell-associated gene variants and PD-L1 expression induced by IFN-γ secreted by infiltrating T cells. The former has constitutive expression, which is not significantly related to the efficacy of ICIs, while the latter is inducible expression, which is concentrated in the region near the T cells of tumor tissues, and is closely related to the efficacy of ICIs. However, these two types of PD-L1 are not strictly differentiated, which can easily lead to the incorrect conclusion that patients with high PD-L1 expression cannot benefit[60]. Third, the detection methods for PD-L1 expression are not sensitive and precise enough. In an analysis of relevant studies, the response rate to ICIs ranged from 36% to 100% for PD-L1 expression-positive tumors, whereas for PD-L1 expression-negative tumors, the response rate ranged from 0% to 17%[52].

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) represent an effective mechanism of adaptive immunity with anti-tumor potential and have been shown to be associated with prognosis and response to immunotherapy in various types of cancer[61]. TILs originate from areas of tumor tissue, have specific recognition of autologous tumors, and have specific MHC-restricted tumor lysis activity[62]. Among the different types of tumor immune infiltration, the relationship between immune inflammation and ICI treatment is more evident.

Immunoinflammation is characterized by the presence of $CD8^+$ and $CD4^+$ T lymphocytes in the tumor parenchyma and is accompanied by the expression of immune checkpoint molecules, revealing that ICI treatment may generate a tumor immune response[63]. Analysis of pre-treatment samples showed a relative abundance of $CD8^+$ T cells at the infiltrative margins of responders, and serial sampling during treatment showed increased infiltration of $CD8^+$ T cells into the tumor parenchyma [64]. Other data showed that patients with high $CD8^+$ TIL density achieve a longer PFS and OS compared to those with low density[65]. Similarly, in a retrospective study of a series of patients including some with GI cancers, TILs in tumor biopsy samples were shown to be associated with improved survival[66]. In a study by Xiao *et al*[67] on CRC liver metastases, patients with high $CD8^+$TIL had a significantly longer recurrence-free survival (RFS) than those with low CD8+TIL (median RFS: Unmet *vs* 55.8 mo, 3-year RFS 71.6% *vs* 55.3%)[67]. And the prognostic value of TILs was demonstrated by the higher accuracy of combining with PD-L1 expression. In addition, in esophageal cancer, a cohort with PD-L1 expression combined with high $CD8^+$ TILs showed a longer OS[68]. In a peripheral blood analysis of a CRC patient treated with pembrolizumab who had a rapid response, high CD39 expression in $CD8^+$ TILs was also found, suggesting that $CD39^+CD8^+$ TILs may be a promising predictive biomarker in GI cancers[69].

LIQUID BIOPSY BIOMARKERS

Circulating tumor DNA, circulating tumor cells, and exosomes

The non-invasive nature of liquid biopsy reduces patient suffering compared to sampling of surgery, while adding advantages that tissue biopsy does not offer. Liquid biopsy overcomes the inevitable heterogeneity of tissue biopsy, allowing for multiple sampling and providing real-time data on tumor changes and relatively more comprehensive results[70]. Circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes are commonly promising biomarkers for liquid biopsy.

ctDNA is mainly released by dead cancer cells, or can also be secreted directly by CTCs, reflecting information about the entire tumor genome, and the variability of its data provides the feasibility of dynamic monitoring of tumor progression throughout the treatment regimen[71]. Several studies have shown that high ctDNA mutations are associated with a poor OS and prognosis in patients with different cancer types treated with ICIs[24]. Lee *et al*[72] showed that melanoma patients with persistently elevated ctDNA during anti-PD-1 therapy exhibited less favorable responses with a shorter PFS and OS[72]. Also for GI cancers, among 25 patients with stages I-III CRC, the 2-year RFS was 66% in ctDNA-positive patients compared with 100% in negative patients. In addition, ctDNA showed a negative tendency of recurrence rates, in agreement with the previous result[73]. In a study of 46 advanced GC patients treated with anti-PD-1, the mutational status of baseline ctDNA affected the PFS of patients with a median of 7.4 mo (undetectable ctDNA) *vs* 4.9 mo (detectable ctDNA)[74]. This suggests that ctDNA may serve as a potential negative biomarker for response to ICI therapy in patients with advanced GC. Recent reports have also linked the detection of CTCs to tumor metastasis. The results showed that PD-L1 was overexpressed in CTCs of patients with advanced head and neck cancers, revealing that combined detection of PD-L1 and CTC may have potential as a biomarker for ICI efficacy prediction[75].

Exosomes are extracellular vesicles carrying tumor-associated proteins, metabolites, RNA, DNA, and lipids, which cover most of the information needed for biopsy and can serve as important biomarkers[76,77]. Zhang *et al*[78] found elevated levels of exosomes in GC patients with liver metastases. Serum exosome levels were higher in GC patients than in healthy subjects, and the number of exosomes in serum was positively correlated with the stage of GC[78]. It has been further revealed that the mRNA expression of *PD-L1* in plasma exosomes correlates with the efficacy of ICIs, which may lead to the suppression of effector lymphocytes involved in antitumor immunity, making ICIs less effective[79]. Still in GC, according to Fan *et al*[80], OS was significantly lower in the high exosomal PD-L1 group than in the low group. In their subgroup analysis, this difference was found to be even more pronounced in early GC, suggesting that high exosomal PD-L1 could be used as a predictor of the early stage of GC[80]. The combination of exosome and PD-L1 assays has informative implications in GI cancers; however, it remains to be noted that exosomes still face challenges as biomarkers, and need to be further explored to accurately measure their quantity and purity.

OTHER BIOMARKERS OF WORTH IN GI CANCERS

The details of the above biomarkers that have been studied or applied in GI cancers are summarized in Table 1. And in addition to the biomarkers mentioned above, here we also discuss and summarize some of the biomarkers that appear more frequently in a variety of other tumors, including patient characteristics, neoantigens, inflammatory indicators, and epigenetics (Table 2). These biomarkers deserve further prospective study and development in ICI-treated GI cancers, and provide new ideas for the identification of novel biomarkers as well.

Factors related to the patient's characteristics

The efficacy of ICI treatment is also highly dependent on patient's characteristics, such as gender, age, and the homeostasis of the body's internal environment. The application of these characteristics in GI cancers is not yet supported by a large amount of data, but the correlation of these characteristics with the efficacy of ICI treatment provides a novel idea for future studies, which can be combined with other markers to improve the predictive accuracy. The first point worth mentioning is the possible correlation between the efficacy of ICIs and the gender of the patient. A meta-analysis including 20 randomized controlled trials conducted by Conforti *et al*[81] reported better efficacy of ICIs in male patients than in females[81]. Schreiber *et al*[82]

Table 2 Summary of biomarkers worthy of further development in gastrointestinal tumors

Classification	Biomarkers	Tumor type	Response to ICI	Ref.
Tumor-genome biomarkers	POLE-mutation	Endometrial carcinoma	Pos	[93]
	Neoantigen	Pulmonary adenocarcinoma	Pos	[94]
Liquid-biopsy biomarkers	LDH	Melanoma	Neg	[99]
	NLR	Advanced solid tumors	Neg	[100]
Epigenetic	TET1-mutation	Multiple tumor types	Pos	[105]
	miRNA	Non-small-cell lung cancer	Pos	[107]
Patient characteristic	Gender	NA	Male: Pos; Female: Neg	[81,82]
	Age	NA	Controversial[1]	[84,115]
	Intestinal microbiota	NA	Pos/Neg	[85-87]

[1]Age as a marker remains controversial, and there are conflicting cases of relevant data.

POLE: Polymerase gene epsilon; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; miRNA: Micro RNA; Pos positive: Neg negative; NA: Not applicable; ICI: Immune checkpoint inhibitor.

suggested that women have more effective immunosurveillance mechanisms compared to men, and this immunosurveillance capacity allows women to be less immunogenic in advanced tumors. They further implied that women may have stronger immune escape mechanisms, and thus they may be more resistant to immunotherapy[82].

Age is also an important marker. There is a relationship between aging and restricted immune function, with significant effects on both innate and acquired immune responses[83]. Nishijima *et al*[84] reported an association with better ORR in patients aged less than 75 years treated with ICIs[84]. In addition, the fraction and diversity of the intestinal microbiota were likewise found to be associated with the efficacy of ICIs, where effective patients tend to have high levels of polyphenism and ruminal cocci family[85]. The intestinal microbiota can influence the process of cancer development and progression by altering the host immune system and regulating metabolism[86]. It was evidenced that patients treated with antibiotics for 2 mo before or after ICI treatment had a significantly lower clinical benefit than those without antibiotics, probably because antibiotics disrupted the homeostasis of gut microbiota and certain dominant intestinal flora in patients[87].

POLE and neoantigen

As mentioned above, TMB and dMMR/MSI-H were biomarkers at the tumor genome level, and correspondingly, another one of interest needs to be presented here, which is POLE. Polymerase ε (encoded by the *POLE* gene) performs error correction during DNA replication, ensuring the accuracy of the replication process[88,89]. Mutations in POLE severely affect the error correction function, leading to the accumulation of a large number of somatic mutations and elevated TMB. $CD8^+$ lymphocyte infiltration in tumors is also significantly increased, promoting the production of tumor-specific neoantigens[90-92]. From a retrospective study conducted by Domingo *et al*[90] including 6517 CRC patients, 66 of them (1.0%) were found to have POLE mutations with the highest mutational burden, all with MSS[90]. However, it is worth mentioning that even patients with the MSS type carry a highly mutated profile. Howitt *et al*[93] reported that POLE mutations in endometrial carcinoma lead to an elevated tumor neoantigen load and PD-1 overexpression in tumor-infiltrating cells [93]. These results indicated that POLE mutations have a role as prognostic markers, and the detection of POLE can also be applied to GI cancers to predict the survival benefit of ICI therapy.

TMB, dMMR/MSI-H, and POLE are all valid indicators as biomarkers, and there is a link between these three. As previously mentioned, mutations in POLE can lead to high levels of TMB[11]. Chalmers *et al*[11] indicated that MSI-H can be usually used as a subset of high TMB, and the vast majority of MSI-H samples also had high levels of TMB (83%), with 97% of them having TMB ≥ 10 Mut/Mb. Nevertheless, it depends on the tumor type, and in GI cancers such as gastric, duodenal, and small intestinal adenocarcinomas, MSI-H and high TMB are found almost simultaneously[11]. Both can be used as combined biomarkers to predict the response to ICIs in GI cancers.

Common to all three biomarkers mentioned above is that they all increase neoantigen generation. Higher levels of TMB may increase the chance of immunogenic neoantigens[94]. High levels of somatic mutations in MSI-H and POLE also lead to an increase in neoantigens[30]. It means that these tumor cells are more likely to be recognized by immune cells, in which case the efficacy of ICIs is also more pronounced. It has been suggested that hypermethylation of the neoantigen gene promoter may be important for immune editing and tumor immune escape[95]. Therefore, neoantigens are also in the scope of exploring ICIs biomarkers for GI cancers. Neoantigens are not only highly specific and strongly immunogenic, but are also ideal targets for immunotherapy. The presentation and recognition of neoantigens largely influence the outcome of ICI treatment, making it undoubtedly an important target for predicting the efficacy of ICIs[96]. Studies have shown that in primary pulmonary adenocarcinoma, clonal neoantigen load is associated with a longer OS [94]. The relationship between neoantigens and the clinical benefit of treating GI cancers needs to be supported by additional and more specific data.

Inflammatory indicators

GI cancers are similar to other types of tumors in that tumor-associated inflammatory processes often establish immune tolerance, promote tumor growth and metastasis, and activate oncogenic signal transduction pathways[97]. Some conventional inflammatory indicators, such as neutrophil-to-lymphocyte ratio (NLR) and lactate dehydrogenase (LDH), have been used as ICI response biomarkers for a variety of tumors, which could also serve as promising markers in GI cancers[98]. In a blood test performed on 66 melanoma patients treated with ICIs, baseline values of serum LDH and changes in LDH during ICI treatment were found to correlate with patient response and survival outcomes, with higher baseline serum LDH values and a 10% increase from baseline during treatment likely indicating inferior ICI efficacy[99]. NLR has also been more established as a biomarker. According to the NLR kinetics study in patients with advanced solid tumors treated with PD-1/L1 inhibitors, the median OS of patients with high NLR was 8.5 mo, while the median OS of patients with low NLR was 19.4 mo[100]. Similar results were found by Jiang *et al*[101], showing that high NLR was associated with a poor OS and PFS[101].

Epigenetic markers

Epigenetic alterations are also an area of interest as potential biomarkers. As mentioned above, high levels of TMB tend to be correlated with a better ICI response, but some tumors with low-level TMB may improve the immunogenicity of their tumor neoplastic antigens through epigenetic modifications, when the efficacy of ICIs is instead better[102]. In GC, alterations in the somatic epigenetic promoter have also been described to be associated with immune editing and tumor escape[103]. It has also been shown that the CC family chemokine ligand 9 (CXCL9) is epigenetically modified to suppress its biological function, ultimately blocking effector T cells from infiltrating into the tumor bed for its immune function[104]. In a report examining the relevance of DNA methylation-regulated genes to ICI response, mutated TET1 was significantly enriched among the 21 related genes studied in patients responding to ICIs. Moreover, mutant TET1 was strongly associated with a higher ORR, longer PFS, and better OS and DCB, which could serve as a novel predictive biomarker across multiple cancer types[105].

In addition to modifications such as methylation, miRNAs are also of interest for further development. In epigenetics, miRNA quantification is one of the most accessible markers. MiRNAs can be direct or indirect regulators of PD-L1 expression, as well as of many other immune checkpoints, such as LAG-3, TIM-3, BTLA, or CTLA-4[106]. A study in NSCLC showed that serum miRNA profiles can discriminate responders to ICIs. In that study, Fan *et al*[107] found that increased expression of miR-93, -200, -27a, -28, -424, and other miRNAs were significantly associated with prognosis, highlighting the predictive value of miRNAs[107]. The emergence of TET1, miRNAs, and other epigenetic examples suggests that there are still more possibilities that need to be further explored in the field of GI tumors.

EMERGING TECHNOLOGIES FOR OPTIMIZING BIOMARKERS

Single-cell sequencing analysis

Moreover, with the evolving concept of precision medicine, biomarker research is facing the same trend. Tumors contain different and evolving cell populations, a

property also known as tumor heterogeneity, which is a major driver of resistance to treatment and tumor metastasis and one of the factors affecting the efficacy of ICIs [108]. It is essential to fully understand heterogeneity, especially in the TME. Analysis of TME heterogeneity and the phenotypes of various cell types by single-cell analysis techniques can help optimize existing therapeutic strategies or discover new ones, and improve the efficacy of the currently used biomarkers, although some limitations remain. In uveal melanoma, the single-cell analysis revealed that $CD8^+$ T cells predominantly express LAG3 rather than conventional PD-L1, revealing the limited availability of ICIs for treating this type of tumor[109]. It illustrates that the selection of biomarkers in different tumor contexts should be further categorized and considered. In GI cancers, single-cell analysis techniques have also made a notable impact. In the study of GI stromal tumors, Mao *et al*[110] applied single-cell transcriptome analysis to reveal their heterogeneity. They also observed that tumor cell related signatures with high proliferation rates were associated with a high risk of tumor malignancy and metastasis, suggesting that this may serve as a prognostic marker or complement [110]. In a study of CRC by Di *et al*[111], T-cell phenotypes were mapped by single-cell mass cytometry. They identified increased heterogeneity of T cells and immunosuppressive T-cell phenotypes in tumor lesions. Altering this immunosuppressive TME is important to improve the ICI response, and single-cell analysis provides very valuable information to improve the immune response in CRC[111].

Apart from the transcriptomics mentioned above, multi-omics is more noteworthy in single-cell analysis. In a study by Zhou *et al*[112], the percentage of fibroblasts with altered somatic copy number was found to be much higher in CRC than in adjacent normal tissues by using single-cell multi-omics sequencing. Five genes (*BGN, RCN3, TAGLN, MYL9,* and *TPM2*) were also identified as fibroblast-specific biomarkers of poorer prognosis in CRC[112]. This study further explored new CAN-based biomarkers, of which single-cell multi-omics analysis is an essential and important part, which also provides us with new ideas in studying ICI response biomarkers in GI cancers as well.

Machine learning

Along with the growing development of bioinformatics, machine learning, and artificial intelligence, biomarkers will be further improved. For example, in the work of Lu *et al*[113], tumor samples from patients with metastatic GI cancers treated with ICIs were sequenced for immuno-oncology (IO)-related gene targets and combined with the application of linear support vector machine learning strategy to construct an RNA signature (IO score) as a predictive model. Notably, its overall accuracy in discriminating DCB and NDB reached 94% and 83%, respectively, and the IO-score showed superior predictive value with higher odds ratio than the traditional biomarker[113].

CONCLUSION

Research in the field of ICIs has been steadily increasing. In GI cancers, ICI-related studies have also been emerging, addressing the importance of ICIs in tumor immunotherapy from different perspectives. Many recent ongoing studies in GI cancers also highlight the potential for diversification of ICIs, particularly in combination or neoadjuvant therapy, where the utility of ICIs has been further investigated. By combining chemotherapy and targeted agents, these studies provide insight into eradicating micrometastatic GI cancers, overcoming resistance to ICIs, and improving ICI treatment. We summarize in Table 3 a number of clinical studies that are currently ongoing to provide a valuable reference for this purpose. However, it needs to be noticed that these ongoing clinical trials do not specifically target one or more biomarkers to predict response to ICIs. Rather, it is more about the combination of ICI therapy with other therapies, which may have little relevance to our topic. Nonetheless, these clinical trials can provide us with a wealth of useful information that we can use in subsequent data analysis for biomarker identification.

Although many new biomarkers have been identified in GI cancers, there is a relative lack of research compared to other tumor types such as melanoma and NSCLC, and validation from clinical trials is still lacking. In this review, we summarize not only biomarkers that are supported by studies in GI cancers, but also biomarkers that are informed in other tumors, in terms of tumor genomic information, TME, liquid biopsies, and epigenetic and patients' characteristics in relation to ICI response. Among these markers, studies on TMB and PD-L1 need to be further improved, and the delineation of cut-off values is not sufficiently clear, especially for

Table 3 Clinical trials on combination therapy or neoadjuvant therapy being conducted in the immune checkpoint inhibitor treatment of gastrointestinal tumors

Clinical trial ID	Cancer type	Study type	Phase	Number	Strategy	Ref.
NCT02918162	GC; Adenocarcinoma of the GE junction	Interventional	2	40	Pembrolizumab combined with stand of care chemotherapy regimen	[116]
NCT04948125	GC	Interventional	2	20	Camrelizumab combined with Apatinib Mesylate	[117]
NCT04196465	GC, ESCA, HCC	Interventional	2	48	IMC-001 as neoadjuvant therapy	[118]
NCT03841110	GC, CRC	Interventional	1	76	FT500 combined with Nivolumab/Pembrolizumab/Atezulizumab	[119]
NCT02903914	GC, CRC	Interventional	1/2	260	Pembrolizumab combined with Arginase Inhibitor INCB001158	[120]
NCT03259867	HCC, GC, CRC (All have liver lesions)	Interventional	2	80	Pembrolizumab/Nivolumab combined with TATE	[121]
NCT04822103	ESCA	Observational	NA	150	ICIs combined with Neoadjuvant chemotherapy	[122]

GI: Gastrointestinal; GC: Gastric cancer; ESCA: Esophageal cancer; GE: Gastroesophageal; HCC: Hepatocellular cancer; CRC: Colorectal cancer; TATE: Transarterial Tirapazamine Embolization; NA: Not Applicable; ICI: Immune checkpoint inhibitor.

PD-L1 expression, which has been shown in a number of studies to respond to ICIs in PD-L1-negative patients[52]. As a stand-alone biomarker, PD-L1 is still considered to be controversial. In addition, markers associated with patient characteristics also have conflicting data, and current studies are not systematic and not clear enough and need to be confirmed by some large-scale prospective studies[114,115]. Another key point that needs attention is that the current ICI predictive biomarkers for GI cancers are mostly focused on CRC cases, while they have relatively little application in other GI cancers such as GC and HCC, and more research investment is needed.

For the future trend of biomarkers, considering that a single biomarker is mostly insufficient, the strategy of combining two or more biomarkers is noteworthy, such as combining information from epigenetics and tumor genome, TMB and CNA in subgroup analysis, *etc*. The integration of multiple factors is necessary to improve accuracy. And along with the continuous research on ICIs therapy, biomarkers for combination therapy or neoadjuvant therapy also need to keep pace with the development to further promote precision therapy. Meanwhile, with the development of big data and bioinformatics, an increasing number of cutting-edge technologies such as machine learning, artificial intelligence, and single-cell analysis will also be applied for further optimization and refinement, making the efficacy of tumor immunotherapy steadily improved. For the current research, more prospective studies are needed, and more data will help to optimize these computational models. From this point of view, the identification of biomarkers that can be used to accurately predict ICI is just beginning, and much more remains to be done, which could become a major trend and focus in the future.

REFERENCES

1 **Arnold M**, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, Bray F. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology* 2020; **159**: 335-349.e15 [PMID: 32247694 DOI: 10.1053/j.gastro.2020.02.068]

2 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]

3 **Abbott M**, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* 2019; **35**: 150923 [PMID: 31526550 DOI: 10.1016/j.soncn.2019.08.002]

4 **Topalian SL**, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 2015; **27**: 450-461 [PMID: 25858804 DOI: 10.1016/j.ccell.2015.03.001]

5 **Hazarika M**, Chuk MK, Theoret MR, Mushti S, He K, Weis SL, Putman AH, Helms WS, Cao X, Li H, Zhao H, Zhao L, Welch J, Graham L, Libeg M, Sridhara R, Keegan P, Pazdur R. U.S. FDA Approval Summary: Nivolumab for Treatment of Unresectable or Metastatic Melanoma Following Progression on Ipilimumab. *Clin Cancer Res* 2017; **23**: 3484-3488 [PMID: 28087644 DOI: 10.1158/1078-0432.CCR-16-0712]

6 **Ramagopal UA**, Liu W, Garrett-Thomson SC, Bonanno JB, Yan Q, Srinivasan M, Wong SC, Bell A, Mankikar S, Rangan VS, Deshpande S, Korman AJ, Almo SC. Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab. *Proc Natl Acad Sci U S A* 2017; **114**: E4223-E4232 [PMID: 28484017 DOI: 10.1073/pnas.1617941114]

7 **Postow MA**, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol* 2015; **33**: 1974-1982 [PMID: 25605845 DOI: 10.1200/JCO.2014.59.4358]

8 **Couzin-Frankel J**. Breakthrough of the year 2013. Cancer immunotherapy. *Science* 2013; **342**: 1432-1433 [PMID: 24357284 DOI: 10.1126/science.342.6165.1432]

9 **Frampton GM**, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, Schnall-Levin M, White J, Sanford EM, An P, Sun J, Juhn F, Brennan K, Iwanik K, Maillet A, Buell J, White E, Zhao M, Balasubramanian S, Terzic S, Richards T, Banning V, Garcia L, Mahoney K, Zwirko Z, Donahue A, Beltran H, Mosquera JM, Rubin MA, Dogan S, Hedvat CV, Berger MF, Pusztai L, Lechner M, Boshoff C, Jarosz M, Vietz C, Parker A, Miller VA, Ross JS, Curran J, Cronin MT, Stephens PJ, Lipson D, Yelensky R. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 2013; **31**: 1023-1031 [PMID: 24142049 DOI: 10.1038/nbt.2696]

10 **Rizvi H**, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, Plodkowski A, Long N, Sauter JL, Rekhtman N, Hollmann T, Schalper KA, Gainor JF, Shen R, Ni A, Arbour KC, Merghoub T, Wolchok J, Snyder A, Chaft JE, Kris MG, Rudin CM, Socci ND, Berger MF, Taylor BS, Zehir A, Solit DB, Arcila ME, Ladanyi M, Riely GJ, Schultz N, Hellmann MD. Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing. *J Clin Oncol* 2018; **36**: 633-641 [PMID: 29337640 DOI: 10.1200/JCO.2017.75.3384]

11 **Chalmers ZR**, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, Huang F, He Y, Sun J, Tabori U, Kennedy M, Lieber DS, Roels S, White J, Otto GA, Ross JS, Garraway L, Miller VA, Stephens PJ, Frampton GM. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 2017; **9**: 34 [PMID: 28420421 DOI: 10.1186/s13073-017-0424-2]

12 **Sholl LM**, Hirsch FR, Hwang D, Botling J, Lopez-Rios F, Bubendorf L, Mino-Kenudson M, Roden AC, Beasley MB, Borczuk A, Brambilla E, Chen G, Chou TY, Chung JH, Cooper WA, Dacic S, Lantuejoul S, Jain D, Lin D, Minami Y, Moreira A, Nicholson AG, Noguchi M, Papotti M, Pelosi G, Poleri C, Rekhtman N, Tsao MS, Thunnissen E, Travis W, Yatabe Y, Yoshida A, Daigneault JB, Zehir A, Peters S, Wistuba II, Kerr KM, Longshore JW. The Promises and Challenges of Tumor Mutation Burden as an Immunotherapy Biomarker: A Perspective from the International Association for the Study of Lung Cancer Pathology Committee. *J Thorac Oncol* 2020; **15**: 1409-1424 [PMID: 32522712 DOI: 10.1016/j.jtho.2020.05.019]

13 **Yarchoan M**, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med* 2017; **377**: 2500-2501 [PMID: 29262275 DOI: 10.1056/NEJMc1713444]

14 **Kim ST**, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, Park YS, Lim HY, Lee H, Choi M, Talasaz A, Kang PS, Cheng J, Loboda A, Lee J, Kang WK. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018; **24**: 1449-1458 [PMID: 30013197 DOI: 10.1038/s41591-018-0101-z]

15 **Goodman AM**, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA, Kurzrock R. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther* 2017; **16**: 2598-2608 [PMID: 28835386 DOI: 10.1158/1535-7163.MCT-17-0386]

16 **Singal G**, Miller PG, Agarwala V, Li G, Kaushik G, Backenroth D, Gossai A, Frampton GM, Torres AZ, Lehnert EM, Bourque D, O'Connell C, Bowser B, Caron T, Baydur E, Seidl-Rathkopf K, Ivanov I, Alpha-Cobb G, Guria A, He J, Frank S, Nunnally AC, Bailey M, Jaskiw A, Feuchtbaum D, Nussbaum N, Abernethy AP, Miller VA. Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database. *JAMA* 2019; **321**: 1391-1399 [PMID: 30964529 DOI: 10.1001/jama.2019.3241]

17 **Fang W**, Ma Y, Yin JC, Hong S, Zhou H, Wang A, Wang F, Bao H, Wu X, Yang Y, Huang Y, Zhao H, Shao YW, Zhang L. Comprehensive Genomic Profiling Identifies Novel Genetic Predictors of Response to Anti-PD-(L)1 Therapies in Non-Small Cell Lung Cancer. *Clin Cancer Res* 2019; **25**: 5015-5026 [PMID: 31085721 DOI: 10.1158/1078-0432.CCR-19-0585]

18 **Marabelle A**, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, Chung H C, Kindler H L, Lopez-Martin J A, Miller W H, Jr. , Italiano A, Kao S, Piha-Paul S A, Delord J P, McWilliams R R, Fabrizio D A, Aurora-Garg D, Xu L, Jin F, Norwood K, Bang Y J. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: Prospective biomarker analysis of the multicohort, open-label, phase 2 keynote-158 study. *Lancet Oncol* 2020; **21**: 1353-1365 [DOI: 10.1016/s1470-2045(20)30445-9]

19 **Xu R-H**, Wang F, Wei X-L, Xu N, Shen L, Dai G, Yuan X, Chen Y, Yang S, Shi J, Hu X-C, Lin X, Zhang Q, Feng J F, Ba Y, Liu Y, Wu H, Feng H, Yao S. Tumor mutational burden identifies chemorefractory gastric cancer with overall survival advantage after receiving toripalimab, a pd-1 antibody. *J Clin Oncol* 2019; **37**: 4021-4021 [DOI: 10.1200/jco.2019.37.15_suppl.4021]

20 **Wang F**, Wei XL, Wang FH, Xu N, Shen L, Dai GH, Yuan XL, Chen Y, Yang SJ, Shi JH, Hu XC,

Lin XY, Zhang QY, Feng JF, Ba Y, Liu YP, Li W, Shu YQ, Jiang Y, Li Q, Wang JW, Wu H, Feng H, Yao S, Xu RH. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. *Ann Oncol* 2019; **30**: 1479-1486 [PMID: 31236579 DOI: 10.1093/annonc/mdz197]

21 **Schrock AB**, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, Miller VA, Lim D, Amanam I, Chao J, Catenacci D, Cho M, Braiteh F, Klempner SJ, Ali SM, Fakih M. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Ann Oncol* 2019; **30**: 1096-1103 [PMID: 31038663 DOI: 10.1093/annonc/mdz134]

22 **Addeo A**, Friedlaender A, Banna GL, Weiss GJ. TMB or not TMB as a biomarker: That is the question. *Crit Rev Oncol Hematol* 2021; **163**: 103374 [PMID: 34087341 DOI: 10.1016/j.critrevonc.2021.103374]

23 **Topalian SL**, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* 2016; **16**: 275-287 [PMID: 27079802 DOI: 10.1038/nrc.2016.36]

24 **Bai R**, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. *Biomark Res* 2020; **8**: 34 [PMID: 32864131 DOI: 10.1186/s40364-020-00209-0]

25 **Svrcek M**, Lascols O, Cohen R, Collura A, Jonchère V, Fléjou JF, Buhard O, Duval A. MSI/MMR-deficient tumor diagnosis: Which standard for screening and for diagnosis? *Bull Cancer* 2019; **106**: 119-128 [PMID: 30713006 DOI: 10.1016/j.bulcan.2018.12.008]

26 **Li K**, Luo H, Huang L, Zhu X. Microsatellite instability: a review of what the oncologist should know. *Cancer Cell Int* 2020; **20**: 16 [PMID: 31956294 DOI: 10.1186/s12935-019-1091-8]

27 **Salem ME**, Puccini A, Grothey A, Raghavan D, Goldberg RM, Xiu J, Korn WM, Weinberg BA, Hwang JJ, Shields AF, Marshall JL, Philip PA, Lenz HJ. Landscape of Tumor Mutation Load, Mismatch Repair Deficiency, and PD-L1 Expression in a Large Patient Cohort of Gastrointestinal Cancers. *Mol Cancer Res* 2018; **16**: 805-812 [PMID: 29523759 DOI: 10.1158/1541-7786.MCR-17-0735]

28 **Schwitalle Y**, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A, von Knebel Doeberitz M. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* 2008; **134**: 988-997 [PMID: 18395080 DOI: 10.1053/j.gastro.2008.01.015]

29 **Solomon BL**, Garrido-Laguna I. Upper gastrointestinal malignancies in 2017: current perspectives and future approaches. *Future Oncol* 2018; **14**: 947-962 [PMID: 29542354 DOI: 10.2217/fon-2017-0597]

30 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]

31 **Lu Z**, Chen H, Li S, Gong J, Li J, Zou J, Wu L, Yu J, Han W, Sun H, Jiao X, Zhang X, Peng Z, Lu M, Wang Z, Zhang H, Shen L. Tumor copy-number alterations predict response to immune-checkpoint-blockade in gastrointestinal cancer. *J Immunother Cancer* 2020; **8** [PMID: 32792358 DOI: 10.1136/jitc-2019-000374]

32 **Roh W**, Chen PL, Reuben A, Spencer CN, Prieto PA, Miller JP, Gopalakrishnan V, Wang F, Cooper ZA, Reddy SM, Gumbs C, Little L, Chang Q, Chen WS, Wani K, De Macedo MP, Chen E, Austin-Breneman JL, Jiang H, Roszik J, Tetzlaff MT, Davies MA, Gershenwald JE, Tawbi H, Lazar AJ, Hwu P, Hwu WJ, Diab A, Glitza IC, Patel SP, Woodman SE, Amaria RN, Prieto VG, Hu J, Sharma P, Allison JP, Chin L, Zhang J, Wargo JA, Futreal PA. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. *Sci Transl Med* 2017; **9** [PMID: 28251903 DOI: 10.1126/scitranslmed.aah3560]

33 **Budczies J**, Seidel A, Christopoulos P, Endris V, Kloor M, Győrffy B, Seliger B, Schirmacher P, Stenzinger A, Denkert C. Integrated analysis of the immunological and genetic status in and across cancer types: impact of mutational signatures beyond tumor mutational burden. *Oncoimmunology* 2018; **7**: e1526613 [PMID: 30524909 DOI: 10.1080/2162402X.2018.1526613]

34 **Smeets D**, Miller IS, O'Connor DP, Das S, Moran B, Boeckx B, Gaiser T, Betge J, Barat A, Klinger R, van Grieken NCT, Cremolini C, Prenen H, Mazzone M, Depreeuw J, Bacon O, Fender B, Brady J, Hennessy BT, McNamara DA, Kay E, Verheul HM, Maarten N, Gallagher WM, Murphy V, Prehn JHM, Koopman M, Punt CJA, Loupakis F, Ebert MPA, Ylstra B, Lambrechts D, Byrne AT. Copy number load predicts outcome of metastatic colorectal cancer patients receiving bevacizumab combination therapy. *Nat Commun* 2018; **9**: 4112 [PMID: 30291241 DOI: 10.1038/s41467-018-06567-6]

35 **Possick JD**. Pulmonary Toxicities from Checkpoint Immunotherapy for Malignancy. *Clin Chest Med* 2017; **38**: 223-232 [PMID: 28477635 DOI: 10.1016/j.ccm.2016.12.012]

36 **Garcia-Diaz A**, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, Zaretsky JM, Sun L, Hugo W, Wang X, Parisi G, Saus CP, Torrejon DY, Graeber TG, Comin-Anduix B, Hu-Lieskovan S, Damoiseaux R, Lo RS, Ribas A. Interferon Receptor Signaling Pathways Regulating PD-L1 and PD-L2 Expression. *Cell Rep* 2017; **19**: 1189-1201 [PMID: 28494868 DOI: 10.1016/j.celrep.2017.04.031]

37 **Grasso CS**, Tsoi J, Onyshchenko M, Abril-Rodriguez G, Ross-Macdonald P, Wind-Rotolo M, Champhekar A, Medina E, Torrejon DY, Shin DS, Tran P, Kim YJ, Puig-Saus C, Campbell K, Vega-Crespo A, Quist M, Martignier C, Luke JJ, Wolchok JD, Johnson DB, Chmielowski B, Hodi FS, Bhatia S, Sharfman W, Urba WJ, Slingluff CL, Jr Diab A, Haanen J, Algarra SM, Pardoll DM, Anagnostou V, Topalian SL, Velculescu VE, Speiser DE, Kalbasi A, Ribas A. Conserved interferon-γ signaling drives clinical response to immune checkpoint blockade therapy in melanoma. *Cancer Cell* 2020; **38**: 500-515.e503 [DOI: 10.1016/j.ccell.2020.11.015]

38 **Karachaliou N**, Gonzalez-Cao M, Crespo G, Drozdowskyj A, Aldeguer E, Gimenez-Capitan A, Teixido C, Molina-Vila MA, Viteri S, De Los Llanos Gil M, Algarra SM, Perez-Ruiz E, Marquez-Rodas I, Rodriguez-Abreu D, Blanco R, Puertolas T, Royo MA, Rosell R. Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. *Ther Adv Med Oncol* 2018; **10**: 1758834017749748 [PMID: 29383037 DOI: 10.1177/1758834017749748]

39 **Higgs BW**, Morehouse CA, Streicher K, Brohawn PZ, Pilataxi F, Gupta A, Ranade K. Interferon Gamma Messenger RNA Signature in Tumor Biopsies Predicts Outcomes in Patients with Non-Small Cell Lung Carcinoma or Urothelial Cancer Treated with Durvalumab. *Clin Cancer Res* 2018; **24**: 3857-3866 [PMID: 29716923 DOI: 10.1158/1078-0432.CCR-17-3451]

40 **Doi T**, Piha-Paul SA, Jalal SI, Saraf S, Lunceford J, Koshiji M, Bennouna J. Safety and Antitumor Activity of the Anti-Programmed Death-1 Antibody Pembrolizumab in Patients With Advanced Esophageal Carcinoma. *J Clin Oncol* 2018; **36**: 61-67 [PMID: 29116900 DOI: 10.1200/JCO.2017.74.9846]

41 **Sasaki S**, Nishikawa J, Sakai K, Iizasa H, Yoshiyama H, Yanagihara M, Shuto T, Shimokuri K, Kanda T, Suehiro Y, Yamasaki T, Sakaida I. EBV-associated gastric cancer evades T-cell immunity by PD-1/PD-L1 interactions. *Gastric Cancer* 2019; **22**: 486-496 [PMID: 30264329 DOI: 10.1007/s10120-018-0880-4]

42 **Muro K**, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang YJ. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; **17**: 717-726 [PMID: 27157491 DOI: 10.1016/S1470-2045(16)00175-3]

43 **Darnell JE**, Kerr IM, Stark GR. Jak-stat pathways and transcriptional activation in response to ifns and other extracellular signaling proteins. *Science* 1994; **264**: 1415-1421 [DOI: 10.1126/science.8197455]

44 **Shin D S**, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, Grasso CS, Hugo W, Sandoval S, Torrejon DY, Palaskas N, Rodriguez GA, Parisi G, Azhdam A, Chmielowski B, Cherry G, Seja E, Berent-Maoz B, Shintaku IP, Le DT, Pardoll DM, Diaz LA, Jr Tumeh PC, Graeber TG, Lo RS, Comin-Anduix B, Ribas A. Primary resistance to pd-1 blockade mediated by jak1/2 mutations. *Cancer Discov* 2017; **7**: 188-201 [DOI: 10.1158/2159-8290.cd-16-1223]

45 **Albacker LA**, Wu J, Smith P, Warmuth M, Stephens PJ, Zhu P, Yu L, Chmielecki J. Loss of function JAK1 mutations occur at high frequency in cancers with microsatellite instability and are suggestive of immune evasion. *PLoS One* 2017; **12**: e0176181 [PMID: 29121062 DOI: 10.1371/journal.pone.0176181]

46 **Gudikote JP**, Cascone T, Poteete A, Sitthideatphaiboon P, Wu Q, Morikawa N, Zhang F, Peng S, Tong P, Li L, Shen L, Nilsson M, Jones P, Sulman EP, Wang J, Bourdon JC, Johnson FM, Heymach JV. Inhibition of nonsense-mediated decay rescues p53β/γ isoform expression and activates the p53 pathway in MDM2-overexpressing and select p53-mutant cancers. *J Biol Chem* 2021; **297**: 101163 [PMID: 34481841 DOI: 10.1016/j.jbc.2021.101163]

47 **Kato S**, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after Immunotherapy: Analysis of Genomic Alterations Associated with Accelerated Growth Rate. *Clin Cancer Res* 2017; **23**: 4242-4250 [PMID: 28351930 DOI: 10.1158/1078-0432.CCR-16-3133]

48 **Sahin I**, Zhang S, Navaraj A, Zhou L, Dizon D, Safran H, El-Deiry WS. AMG-232 sensitizes high MDM2-expressing tumor cells to T-cell-mediated killing. *Cell Death Discov* 2020; **6**: 57 [PMID: 32655895 DOI: 10.1038/s41420-020-0292-1]

49 **Cao H**, Chen X, Wang Z, Wang L, Xia Q, Zhang W. The role of MDM2-p53 axis dysfunction in the hepatocellular carcinoma transformation. *Cell Death Discov* 2020; **6**: 53 [PMID: 32595984 DOI: 10.1038/s41420-020-0287-y]

50 **Wu L**, Quan W, Luo Q, Pan Y, Peng D, Zhang G. Identification of an Immune-Related Prognostic Predictor in Hepatocellular Carcinoma. *Front Mol Biosci* 2020; **7**: 567950 [PMID: 33195412 DOI: 10.3389/fmolb.2020.567950]

51 **Doroshow DB**, Bhalla S, Beasley MB, Sholl LM, Kerr KM, Gnjatic S, Wistuba II, Rimm DL, Tsao MS, Hirsch FR. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. *Nat Rev Clin Oncol* 2021; **18**: 345-362 [PMID: 33580222 DOI: 10.1038/s41571-021-00473-5]

52 **Patel SP**, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. *Mol Cancer Ther* 2015; **14**: 847-856 [PMID: 25695955 DOI: 10.1158/1535-7163.MCT-14-0983]

53 **Cui C**, Yu B, Jiang Q, Li X, Shi K, Yang Z. The roles of PD-1/PD-L1 and its signalling pathway in gastrointestinal tract cancers. *Clin Exp Pharmacol Physiol* 2019; **46**: 3-10 [PMID: 30161295 DOI: 10.1111/1440-1681.13028]

54 **Das S**, Cimino S, Davis S, Ciombor K. All in the Levels-Programmed Death-Ligand 1 Expression as a Biomarker for Immune Checkpoint Inhibitor Response in Patients with Gastrointestinal Cancer.

Oncologist 2021; **26**: e186-e188 [PMID: 32945067 DOI: 10.1002/onco.13526]
55 **Stein A**, Moehler M, Trojan J, Goekkurt E, Vogel A. Immuno-oncology in GI tumours: Clinical evidence and emerging trials of PD-1/PD-L1 antagonists. *Crit Rev Oncol Hematol* 2018; **130**: 13-26 [PMID: 30196908 DOI: 10.1016/j.critrevonc.2018.07.001]
56 **Nishino M**, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. *Nat Rev Clin Oncol* 2017; **14**: 655-668 [PMID: 28653677 DOI: 10.1038/nrclinonc.2017.88]
57 **Meng X**, Huang Z, Teng F, Xing L, Yu J. Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy. *Cancer Treat Rev* 2015; **41**: 868-876 [PMID: 26589760 DOI: 10.1016/j.ctrv.2015.11.001]
58 **Sacher AG**, Gandhi L. Biomarkers for the Clinical Use of PD-1/PD-L1 Inhibitors in Non-Small-Cell Lung Cancer: A Review. *JAMA Oncol* 2016; **2**: 1217-1222 [PMID: 27310809 DOI: 10.1001/jamaoncol.2016.0639]
59 **Hutarew G**. PD-L1 testing, fit for routine evaluation? *Memo* 2016; **9**: 201-206 [PMID: 28058063 DOI: 10.1007/s12254-016-0292-2]
60 **Ribas A**, Hu-Lieskovan S. What does PD-L1 positive or negative mean? *J Exp Med* 2016; **213**: 2835-2840 [PMID: 27903604 DOI: 10.1084/jem.20161462]
61 **Garber K**. Pursuit of tumor-infiltrating lymphocyte immunotherapy speeds up. *Nat Biotechnol* 2019; **37**: 969-971 [PMID: 31485043 DOI: 10.1038/d41587-019-00023-6]
62 **Halapi E**. Oligoclonal T cells in human cancer. *Med Oncol* 1998; **15**: 203-211 [PMID: 9951682 DOI: 10.1007/BF02787202]
63 **Llosa NJ**, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; **5**: 43-51 [PMID: 25358689 DOI: 10.1158/2159-8290.CD-14-0863]
64 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505 DOI: 10.1038/nature13954]
65 **Tokito T**, Azuma K, Kawahara A, Ishii H, Yamada K, Matsuo N, Kinoshita T, Mizukami N, Ono H, Kage M, Hoshino T. Predictive relevance of PD-L1 expression combined with CD8+ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy. *Eur J Cancer* 2016; **55**: 7-14 [PMID: 26771872 DOI: 10.1016/j.ejca.2015.11.020]
66 **Gibney GT**, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* 2016; **17**: e542-e551 [PMID: 27924752 DOI: 10.1016/S1470-2045(16)30406-5]
67 **Xiao B**, Peng J, Wang Y, Deng Y, Ou Q, Wu X, Lin J, Pan Z, Zhang L. Prognostic value of tumor infiltrating lymphocytes combined with PD-L1 expression for patients with solitary colorectal cancer liver metastasis. *Ann Transl Med* 2020; **8**: 1221 [PMID: 33178753 DOI: 10.21037/atm-20-2762a]
68 **Yagi T**, Baba Y, Ishimoto T, Iwatsuki M, Miyamoto Y, Yoshida N, Watanabe M, Baba H. PD-L1 Expression, Tumor-infiltrating Lymphocytes, and Clinical Outcome in Patients With Surgically Resected Esophageal Cancer. *Ann Surg* 2019; **269**: 471-478 [PMID: 29206673 DOI: 10.1097/SLA.0000000000002616]
69 **Simoni Y**, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, Yeong JPS, Nahar R, Zhang T, Kared H, Duan K, Ang N, Poidinger M, Lee YY, Larbi A, Khng AJ, Tan E, Fu C, Mathew R, Teo M, Lim WT, Toh CK, Ong BH, Koh T, Hillmer AM, Takano A, Lim TKH, Tan EH, Zhai W, Tan DSW, Tan IB, Newell EW. Bystander CD8$^+$ T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* 2018; **557**: 575-579 [PMID: 29769722 DOI: 10.1038/s41586-018-0130-2]
70 **Poulet G**, Massias J, Taly V. Liquid Biopsy: General Concepts. *Acta Cytol* 2019; **63**: 449-455 [PMID: 31091522 DOI: 10.1159/000499337]
71 **Wu X**, Li J, Gassa A, Buchner D, Alakus H, Dong Q, Ren N, Liu M, Odenthal M, Stippel D, Bruns C, Zhao Y, Wahba R. Circulating tumor DNA as an emerging liquid biopsy biomarker for early diagnosis and therapeutic monitoring in hepatocellular carcinoma. *Int J Biol Sci* 2020; **16**: 1551-1562 [PMID: 32226301 DOI: 10.7150/ijbs.44024]
72 **Lee JH**, Long GV, Boyd S, Lo S, Menzies AM, Tembe V, Guminski A, Jakrot V, Scolyer RA, Mann GJ, Kefford RF, Carlino MS, Rizos H. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. *Ann Oncol* 2017; **28**: 1130-1136 [PMID: 28327969 DOI: 10.1093/annonc/mdx026]
73 **Lecomte T**, Berger A, Zinzindohoué F, Micard S, Landi B, Blons H, Beaune P, Cugnenc PH, Laurent-Puig P. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. *Int J Cancer* 2002; **100**: 542-548 [PMID: 12124803 DOI: 10.1002/ijc.10526]
74 **Jin Y**, Chen DL, Wang F, Yang CP, Chen XX, You JQ, Huang JS, Shao Y, Zhu DQ, Ouyang YM, Luo HY, Wang ZQ, Wang FH, Li YH, Xu RH, Zhang DS. The predicting role of circulating tumor DNA landscape in gastric cancer patients treated with immune checkpoint inhibitors. *Mol Cancer* 2020; **19**: 154 [PMID: 33126883 DOI: 10.1186/s12943-020-01274-7]

75 **Kulasinghe A**, Perry C, Kenny L, Warkiani ME, Nelson C, Punyadeera C. PD-L1 expressing circulating tumour cells in head and neck cancers. *BMC Cancer* 2017; **17**: 333 [PMID: 28511705 DOI: 10.1186/s12885-017-3316-3]

76 **Bobrie A**, Colombo M, Raposo G, Théry C. Exosome secretion: molecular mechanisms and roles in immune responses. *Traffic* 2011; **12**: 1659-1668 [PMID: 21645191 DOI: 10.1111/j.1600-0854.2011.01225.x]

77 **Colombo M**, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014; **30**: 255-289 [PMID: 25288114 DOI: 10.1146/annurev-cellbio-101512-122326]

78 **Zhang H**, Deng T, Liu R, Bai M, Zhou L, Wang X, Li S, Yang H, Li J, Ning T, Huang D, Li H, Zhang L, Ying G, Ba Y. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. *Nat Commun* 2017; **8**: 15016 [PMID: 28393839 DOI: 10.1038/ncomms15016]

79 **Del Re M**, Marconcini R, Pasquini G, Rofi E, Vivaldi C, Bloise F, Restante G, Arrigoni E, Caparello C, Bianco MG, Crucitta S, Petrini I, Vasile E, Falcone A, Danesi R. PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br J Cancer* 2018; **118**: 820-824 [PMID: 29509748 DOI: 10.1038/bjc.2018.9]

80 **Fan Y**, Che X, Qu J, Hou K, Wen T, Li Z, Li C, Wang S, Xu L, Liu Y, Qu X. Exosomal PD-L1 Retains Immunosuppressive Activity and is Associated with Gastric Cancer Prognosis. *Ann Surg Oncol* 2019; **26**: 3745-3755 [PMID: 31087180 DOI: 10.1245/s10434-019-07431-7]

81 **Conforti F**, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD, Goldhirsch A. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol* 2018; **19**: 737-746 [PMID: 29778737 DOI: 10.1016/S1470-2045(18)30261-4]

82 **Schreiber RD**, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011; **331**: 1565-1570 [PMID: 21436444 DOI: 10.1126/science.1203486]

83 **Fulop T**, Larbi A, Kotb R, de Angelis F, Pawelec G. Aging, immunity, and cancer. *Discov Med* 2011; **11**: 537-550 [PMID: 21712020]

84 **Nishijima TF**, Muss HB, Shachar SS, Moschos SJ. Comparison of efficacy of immune checkpoint inhibitors (ICIs) between younger and older patients: A systematic review and meta-analysis. *Cancer Treat Rev* 2016; **45**: 30-37 [PMID: 26946217 DOI: 10.1016/j.ctrv.2016.02.006]

85 **Gopalakrishnan V**, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, Cogdill AP, Zhao L, Hudgens CW, Hutchinson DS, Manzo T, Petaccia de Macedo M, Cotechini T, Kumar T, Chen WS, Reddy SM, Szczepaniak Sloane R, Galloway-Pena J, Jiang H, Chen PL, Shpall EJ, Rezvani K, Alousi AM, Chemaly RF, Shelburne S, Vence LM, Okhuysen PC, Jensen VB, Swennes AG, McAllister F, Marcelo Riquelme Sanchez E, Zhang Y, Le Chatelier E, Zitvogel L, Pons N, Austin-Breneman JL, Haydu LE, Burton EM, Gardner JM, Sirmans E, Hu J, Lazar AJ, Tsujikawa T, Diab A, Tawbi H, Glitza IC, Hwu WJ, Patel SP, Woodman SE, Amaria RN, Davies MA, Gershenwald JE, Hwu P, Lee JE, Zhang J, Coussens LM, Cooper ZA, Futreal PA, Daniel CR, Ajami NJ, Petrosino JF, Tetzlaff MT, Sharma P, Allison JP, Jenq RR, Wargo JA. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018; **359**: 97-103 [PMID: 29097493 DOI: 10.1126/science.aan4236]

86 **Tanaka Y**, Shimizu S, Shirotani M, Yorozu K, Kitamura K, Oehorumu M, Kawai Y, Fukuzawa Y. Nutrition and Cancer Risk from the Viewpoint of the Intestinal Microbiome. *Nutrients* 2021; **13** [PMID: 34684330 DOI: 10.3390/nu13103326]

87 **Routy B**, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, Fidelle M, Flament C, Poirier-Colame V, Opolon P, Klein C, Iribarren K, Mondragón L, Jacquelot N, Qu B, Ferrere G, Clémenson C, Mezquita L, Masip JR, Naltet C, Brosseau S, Kaderbhai C, Richard C, Rizvi H, Levenez F, Galleron N, Quinquis B, Pons N, Ryffel B, Minard-Colin V, Gonin P, Soria JC, Deutsch E, Loriot Y, Ghiringhelli F, Zalcman G, Goldwasser F, Escudier B, Hellmann MD, Eggermont A, Raoult D, Albiges L, Kroemer G, Zitvogel L. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; **359**: 91-97 [PMID: 29097494 DOI: 10.1126/science.aan3706]

88 **Bourdais R**, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, Baumgaertner I, Neuzillet C, Tournigand C. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. *Crit Rev Oncol Hematol* 2017; **113**: 242-248 [PMID: 28427513 DOI: 10.1016/j.critrevonc.2017.03.027]

89 **Nebot-Bral L**, Brandao D, Verlingue L, Rouleau E, Caron O, Despras E, El-Dakdouki Y, Champiat S, Aoufouchi S, Leary A, Marabelle A, Malka D, Chaput N, Kannouche PL. Hypermutated tumours in the era of immunotherapy: The paradigm of personalised medicine. *Eur J Cancer* 2017; **84**: 290-303 [PMID: 28846956 DOI: 10.1016/j.ejca.2017.07.026]

90 **Domingo E**, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, Fessler E, Medema JP, Boot A, Morreau H, van Wezel T, Liefers GJ, Lothe RA, Danielsen SA, Sveen A, Nesbakken A, Zlobec I, Lugli A, Koelzer VH, Berger MD, Castellví-Bel S, Muñoz J; Epicolon consortium, de Bruyn M, Nijman HW, Novelli M, Lawson K, Oukrif D, Frangou E, Dutton P, Tejpar S, Delorenzi M, Kerr R, Kerr D, Tomlinson I, Church DN. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol* 2016; **1**: 207-216 [PMID: 28404093 DOI: 10.1016/S2468-1253(16)30014-0]

91 **Rayner E**, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I, Church DN. A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat Rev Cancer* 2016; **16**: 71-81 [PMID: 26822575 DOI: 10.1038/nrc.2015.12]

92 **Shlien A**, Campbell BB, de Borja R, Alexandrov LB, Merico D, Wedge D, Van Loo P, Tarpey PS, Coupland P, Behjati S, Pollett A, Lipman T, Heidari A, Deshmukh S, Avitzur N, Meier B, Gerstung M, Hong Y, Merino DM, Ramakrishna M, Remke M, Arnold R, Panigrahi GB, Thakkar NP, Hodel KP, Henninger EE, Göksenin AY, Bakry D, Charames GS, Druker H, Lerner-Ellis J, Mistry M, Dvir R, Grant R, Elhasid R, Farah R, Taylor GP, Nathan PC, Alexander S, Ben-Shachar S, Ling SC, Gallinger S, Constantini S, Dirks P, Huang A, Scherer SW, Grundy RG, Durno C, Aronson M, Gartner A, Meyn MS, Taylor MD, Pursell ZF, Pearson CE, Malkin D, Futreal PA, Stratton MR, Bouffet E, Hawkins C, Campbell PJ, Tabori U; Biallelic Mismatch Repair Deficiency Consortium. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. *Nat Genet* 2015; **47**: 257-262 [PMID: 25642631 DOI: 10.1038/ng.3202]

93 **Howitt BE**, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, Stover E, Strickland KC, D'Andrea AD, Wu CJ, Matulonis UA, Konstantinopoulos PA. Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. *JAMA Oncol* 2015; **1**: 1319-1323 [PMID: 26181000 DOI: 10.1001/jamaoncol.2015.2151]

94 **McGranahan N**, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, Watkins TB, Shafi S, Murugaesu N, Mitter R, Akarca AU, Linares J, Marafioti T, Henry JY, Van Allen EM, Miao D, Schilling B, Schadendorf D, Garraway LA, Makarov V, Rizvi NA, Snyder A, Hellmann MD, Merghoub T, Wolchok JD, Shukla SA, Wu CJ, Peggs KS, Chan TA, Hadrup SR, Quezada SA, Swanton C. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016; **351**: 1463-1469 [PMID: 26940869 DOI: 10.1126/science.aaf1490]

95 **Yi M**, Dong B, Chu Q, Wu K. Immune pressures drive the promoter hypermethylation of neoantigen genes. *Exp Hematol Oncol* 2019; **8**: 32 [PMID: 31799063 DOI: 10.1186/s40164-019-0156-7]

96 **Ma W**, Pham B, Li T. Cancer neoantigens as potential targets for immunotherapy. *Clin Exp Metastasis* 2021; 1-10 [PMID: 33950415 DOI: 10.1007/s10585-021-10091-1]

97 **Li L**, Yu R, Cai T, Chen Z, Lan M, Zou T, Wang B, Wang Q, Zhao Y, Cai Y. Effects of immune cells and cytokines on inflammation and immunosuppression in the tumor microenvironment. *Int Immunopharmacol* 2020; **88**: 106939 [PMID: 33182039 DOI: 10.1016/j.intimp.2020.106939]

98 **Minami S**, Ihara S, Ikuta S, Komuta K. Gustave Roussy Immune Score and Royal Marsden Hospital Prognostic Score Are Biomarkers of Immune-Checkpoint Inhibitor for Non-Small Cell Lung Cancer. *World J Oncol* 2019; **10**: 90-100 [PMID: 31068989 DOI: 10.14740/wjon1193]

99 **Diem S**, Kasenda B, Spain L, Martin-Liberal J, Marconcini R, Gore M, Larkin J. Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma. *Br J Cancer* 2016; **114**: 256-261 [PMID: 26794281 DOI: 10.1038/bjc.2015.467]

100 **Ameratunga M**, Chénard-Poirier M, Moreno Candilejo I, Pedregal M, Lui A, Dolling D, Aversa C, Ingles Garces A, Ang JE, Banerji U, Kaye S, Gan H, Doger B, Moreno V, de Bono J, Lopez J. Neutrophil-lymphocyte ratio kinetics in patients with advanced solid tumours on phase I trials of PD-1/PD-L1 inhibitors. *Eur J Cancer* 2018; **89**: 56-63 [PMID: 29227818 DOI: 10.1016/j.ejca.2017.11.012]

101 **Jiang T**, Qiao M, Zhao C, Li X, Gao G, Su C, Ren S, Zhou C. Pretreatment neutrophil-to-lymphocyte ratio is associated with outcome of advanced-stage cancer patients treated with immunotherapy: a meta-analysis. *Cancer Immunol Immunother* 2018; **67**: 713-727 [PMID: 29423649 DOI: 10.1007/s00262-018-2126-z]

102 **Arenas-Ramirez N**, Sahin D, Boyman O. Epigenetic mechanisms of tumor resistance to immunotherapy. *Cell Mol Life Sci* 2018; **75**: 4163-4176 [PMID: 30140960 DOI: 10.1007/s00018-018-2908-7]

103 **Qamra A**, Xing M, Padmanabhan N, Kwok JJT, Zhang S, Xu C, Leong YS, Lee Lim AP, Tang Q, Ooi WF, Suling Lin J, Nandi T, Yao X, Ong X, Lee M, Tay ST, Keng ATL, Gondo Santoso E, Ng CCY, Ng A, Jusakul A, Smoot D, Ashktorab H, Rha SY, Yeoh KG, Peng Yong W, Chow PKH, Chan WH, Ong HS, Soo KC, Kim KM, Wong WK, Rozen SG, Teh BT, Kappei D, Lee J, Connolly J, Tan P. Epigenomic Promoter Alterations Amplify Gene Isoform and Immunogenic Diversity in Gastric Adenocarcinoma. *Cancer Discov* 2017; **7**: 630-651 [PMID: 28320776 DOI: 10.1158/2159-8290.CD-16-1022]

104 **Herbst RS**, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515**: 563-567 [PMID: 25428504 DOI: 10.1038/nature14011]

105 **Wu HX**, Chen YX, Wang ZX, Zhao Q, He MM, Wang YN, Wang F, Xu RH. Alteration in TET1 as potential biomarker for immune checkpoint blockade in multiple cancers. *J Immunother Cancer* 2019; **7**: 264 [PMID: 31623662 DOI: 10.1186/s40425-019-0737-3]

106 **Perrier A**, Didelot A, Laurent-Puig P, Blons H, Garinet S. Epigenetic Mechanisms of Resistance to Immune Checkpoint Inhibitors. *Biomolecules* 2020; **10** [PMID: 32708698 DOI: 10.3390/biom10071061]

107 **Fan J**, Yin Z, Xu J, Wu F, Huang Q, Yang L, Jin Y, Yang G. Circulating microRNAs predict the response to anti-PD-1 therapy in non-small cell lung cancer. *Genomics* 2020; **112**: 2063-2071 [PMID: 31786291 DOI: 10.1016/j.ygeno.2019.11.019]

108 **González-Silva L**, Quevedo L, Varela I. Tumor Functional Heterogeneity Unraveled by scRNA-seq Technologies. *Trends Cancer* 2020; **6**: 13-19 [PMID: 31952776 DOI: 10.1016/j.trecan.2019.11.010]

109 **Durante MA**, Rodriguez DA, Kurtenbach S, Kuznetsov JN, Sanchez MI, Decatur CL, Snyder H, Feun LG, Livingstone AS, Harbour JW. Single-cell analysis reveals new evolutionary complexity in uveal melanoma. *Nat Commun* 2020; **11**: 496 [PMID: 31980621 DOI: 10.1038/s41467-019-14256-1]

110 **Mao X**, Yang X, Chen X, Yu S, Zhang B, Ji Y, Chen Y, Ouyang Y, Luo W. Single-cell transcriptome analysis revealed the heterogeneity and microenvironment of gastrointestinal stromal tumors. *Cancer Sci* 2021; **112**: 1262-1274 [PMID: 33393143 DOI: 10.1111/cas.14795]

111 **Di J**, Liu M, Fan Y, Gao P, Wang Z, Jiang B, Su X. Phenotype molding of T cells in colorectal cancer by single-cell analysis. *Int J Cancer* 2020; **146**: 2281-2295 [PMID: 31901134 DOI: 10.1002/ijc.32856]

112 **Zhou Y**, Bian S, Zhou X, Cui Y, Wang W, Wen L, Guo L, Fu W, Tang F. Single-Cell Multiomics Sequencing Reveals Prevalent Genomic Alterations in Tumor Stromal Cells of Human Colorectal Cancer. *Cancer Cell* 2020; **38**: 818-828.e5 [PMID: 33096021 DOI: 10.1016/j.ccell.2020.09.015]

113 **Lu Z**, Chen H, Jiao X, Zhou W, Han W, Li S, Liu C, Gong J, Li J, Zhang X, Wang X, Peng Z, Qi C, Wang Z, Li Y, Brock M, Zhang H, Shen L. Prediction of immune checkpoint inhibition with immune oncology-related gene expression in gastrointestinal cancer using a machine learning classifier. *J Immunother Cancer* 2020; **8** [PMID: 32792359 DOI: 10.1136/jitc-2020-000631]

114 **Conforti F**, Pala L, Pagan E, Corti C, Bagnardi V, Queirolo P, Catania C, De Pas T, Giaccone G. Sex-based differences in response to anti-PD-1 or PD-L1 treatment in patients with non-small-cell lung cancer expressing high PD-L1 Levels. A systematic review and meta-analysis of randomized clinical trials. *ESMO Open* 2021; **6**: 100251 [PMID: 34455288 DOI: 10.1016/j.esmoop.2021.100251]

115 **Elias R**, Karantanos T, Sira E, Hartshorn KL. Immunotherapy comes of age: Immune aging & checkpoint inhibitors. *J Geriatr Oncol* 2017; **8**: 229-235 [PMID: 28223073 DOI: 10.1016/j.jgo.2017.02.001]

116 **Manji G**. Perioperative Chemo and Pembrolizumab in Gastric Cancer. [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT02918162

117 **Ying L**. Camrelizumab Combined with Apatinib for Advanced Gastric or Esophagogastric Adenocarcinoma [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT04948125

118 **Park RS**. Phase II Study of Neoadjuvant Immune Checkpoint Inhibitor in Patients with resectable Gastrointestinal Cancers (NeoChance). [cited 8 September 2021]. Available from: ClinicalTrials.gov

119 **Fate Therapeutics**. FT500 as Monotherapy and in Combination With Immune Checkpoint Inhibitors in Subjects With Advanced Solid Tumors. [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT03841110

120 Incyte Corporations. Arginase Inhibitor INCB001158 as a Single Agent and in Combination with Immune Checkpoint Therapy in Patients With Advanced/Metastatic Solid Tumors. [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT02903914

121 **Teclison Ltd**. Combination of TATE and PD-1 Inhibitor in Liver Cancer (TATE-PD1) [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT03259867

122 **Guangdong Provincial People's Hospital**. A Real-World Study of Immune Checkpoint Inhibitors and Chemotherapy for Advanced Esophageal Cancer. [cited 8 September 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT04822103

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 38-54

DOI: 10.4251/wjgo.v14.i1.38

ISSN 1948-5204 (online)

REVIEW

Proteasome regulators in pancreatic cancer

Nirosha J Murugan, Ioannis A Voutsadakis

ORCID number: Nirosha J Murugan 0000-0001-7260-9956; Ioannis A Voutsadakis 0000-0002-9301-5951.

Author contributions: Voutsadakis IA conceived and designed the study, both authors reviewed the literature, wrote, revised and approved the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest regarding this manuscript.

Country/Territory of origin: Canada

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Nirosha J Murugan, Department of Biology, Algoma University, Sault Sainte Marie P6A3T6, ON, Canada

Ioannis A Voutsadakis, Department of Medical Oncology, Sault Area Hospital, Sault Sainte Marie P6A3T6, ON, Canada

Corresponding author: Ioannis A Voutsadakis, MD, PhD, Associate Professor, Doctor, Department of Medical Oncology, Sault Area Hospital, 750 Great Northern Road, Sault Sainte Marie P6A3T6, ON, Canada. ivoutsadakis@yahoo.com

Abstract

Pancreatic adenocarcinoma is one of the most lethal cancers with rising incidence. Despite progress in its treatment, with the introduction of more effective chemotherapy regimens in the last decade, prognosis of metastatic disease remains inferior to other cancers with long term survival being the exception. Molecular characterization of pancreatic cancer has elucidated the landscape of the disease and has revealed common lesions that contribute to pancreatic carcinogenesis. Regulation of proteostasis is critical in cancers due to increased protein turnover required to support the intense metabolism of cancer cells. The proteasome is an integral part of this regulation and is regulated, in its turn, by key transcription factors, which induce transcription of proteasome structural units. These include FOXO family transcription factors, NFE2L2, hHSF1 and hHSF2, and NF-Y. Networks that encompass proteasome regulators and transduction pathways dysregulated in pancreatic cancer such as the KRAS/BRAF/MAPK and the Transforming growth factor beta/SMAD pathway contribute to pancreatic cancer progression. This review discusses the proteasome and its transcription factors within the pancreatic cancer cellular micro-environment. We also consider the role of stemness in carcinogenesis and the use of proteasome inhibitors as therapeutic agents.

Key Words: Pancreatic adenocarcinoma; Proteasome; Transcription factors; Regulation; Unfolded protein response; Cancer stem cells

Core Tip: Pancreatic adenocarcinoma is a gastrointestinal cancer with high incidence and bleak outcomes. The molecular pathways involved in the pathogenesis of the

Received: February 23, 2021
Peer-review started: February 23, 2021
First decision: March 29, 2021
Revised: April 14, 2021
Accepted: December 2, 2021
Article in press: December 2, 2021
Published online: January 15, 2022

P-Reviewer: Jiang W
S-Editor: Wu YXJ
L-Editor: A
P-Editor: Wu YXJ

disease have been increasingly clarified in recent years. This article reviews the role of proteostasis regulation through the proteasome in pancreatic cancer. Major molecular pathways affected in pancreatic cancer closely interconnect with regulators of the proteasome.

Citation: Murugan NJ, Voutsadakis IA. Proteasome regulators in pancreatic cancer. *World J Gastrointest Oncol* 2022; 14(1): 38-54
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/38.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.38

INTRODUCTION

Pancreatic cancer (PC) is a prevalent digestive system malignancy with a 5-year survival rate of less than 5%, a median survival period of 6 mo and, in 2019, accounted for 45750 deaths in the United States alone[1]. Despite representing the thirteenth most prevalent cancer, accounting for 458000 cases worldwide, it is the seventh most deadly cancer[2], and affects men slightly more than women. Unfortunately, PC tends to exhibit a distinct resistance to radiotherapy and chemotherapy, contributing to increased mortality. Understanding how particular lesions can induce and maintain pancreatic carcinogenesis, as well as how the molecular mechanisms both upstream and downstream of the initial pathology contribute to morbidity, treatment resistance, and mortality will ultimately inform clinical strategies to combat this deadly disease.

Pancreatic adenocarcinoma is a cancer with low overall tumor mutation burden (TMB). The majority of pancreatic cancer cases in The Cancer Genome Atlas series display less than 50 mutations, and only few cancers have 50 to 80 mutations and even fewer cases have a TMB above 80 mutations[3]. Few cancer-associated genes display recurrent mutations in pancreatic adenocarcinoma and include activating mutations in the oncogene *KRAS* which are observed in most cases of these cancers, and mutations in tumor suppressor *TP53* encoding for the p53 protein which are observed in about two thirds of cases. Two additional tumor suppressors, cell cycle inhibitor p16 and the Transforming growth factor beta (TGFβ) pathway signal transducer SMAD4, are mutated in about 20% of cases in pancreatic cancers[3,4]. p16 is encoded by gene *CDKN2A* which is located at chromosome 9p. The same locus also encodes for the p53 positive regulator $p14^{ARF}$. The locus is deleted in 10% to 25% of pancreatic cancer cases. Besides these four recurrent gene abnormalities no other oncogenes or tumor suppressors are commonly altered in pancreatic cancers. Studies of genomic profiles of pancreatic cancers have shown that the disease is heterogeneous and different subtypes exist, similar with other cancers. A most recent genomic classification, for example, assigns pancreatic cancer in four types: squamous, pancreatic progenitor, immunogenic and aberrant differentiation endocrine-exocrine[5].

Cancer cells rely on proteasome activity for regulation of their increased metabolism [6,7]. In addition, specific cancer-associated pathways are dependent on proteasome degradation of tumor suppressors or for neutralization of inhibitors of oncogenes. As examples, activation of the NF-κB pathway requires proteasome degradation of inhibiting I-κB protein and tumor suppressor p53 is tightly controlled through ubiquitination by ubiquitin ligase MDM2 and other ligases for proteasome degradation[8]. Another example is provided by kinase GSK3β, which phosphorylates substrates such as oncogenic β-catenin for subsequent ubiquitination and proteasome degradation. This process is frequently debilitated in cancers[9]. Pharmacologic proteasome inhibition leads to pancreatic cancer cells growth arrest and decreased viability *in vitro* and in vivo, especially in combination therapies[10]. In contrast, pancreatic cancer cells that acquire the ability to shut down protein translation in conditions of proteasome inhibition are resistant to proteasome inhibitors[11].

Given the importance of proteostasis regulation in cancer and the increased metabolism of cancer cells, proteasome sub-unit production and availability as building blocks of the organelle are regulated at the transcriptional level by several factors[12]. The current paper will discuss the regulation of proteasome by these factors in cancer with a focus on pancreatic cancer.

PROTEASOME

Proteasomes are multi-subunit proteolytic organelles that recognize, unfold, and digest unneeded or damaged proteins within the cell. In mammals, the most common variant is the cytosolic 26S proteosome (2-MDa) that consists of a core protease (750 kDa) with a Svedberg sedimentation coefficient (rate under acceleration) or S-value of 20 (or 20 × 10^8 cm^2/s). The core is flanked by two, ATP-dependent 19 S regulatory subunits (700 kDa, called PA700) that unfold substrates and direct them toward the core for degradation[13]. Together, they form a tube-like structure through which linearized proteins pass and get cleaved within the center-most lumen of the complex. Unlike lysosomes, which are primarily activated under stress conditions, proteasomes (which can also become activated under stress conditions) regulate normative protein turnover associated with basal metabolic conditions. The process is highly selective, owing to the identification of targets by a three-step ubiquitination process. Briefly, E1 ubiquitin-activating enzymes capture ubiquitin which are then transferred to E2 ubiquitin-conjugating enzymes which then, in coordination with E3 ubiquitin ligases, catalyze bonds between the C-terminal glycine of the ubiquitin molecule and a lysine molecule within the substrate protein. The net result of the E1-E2-E3 cascade is the addition of one or more ubiquitin molecules to the substrate protein which are then recognized by ubiquitin-binding sites located at the 19S caps of the proteosome. Proteasome recognition requires a chain of at least four ubiquitin molecules tagged to a target protein, usually through lysines at position 48 of each ubiquitin molecule[14]. Once ubiquitinated proteins are unfolded and directed toward the central protease by the 19S subunits, proteolysis proceeds whereby the inner β subunit rings of the core trigger a threonine-dependent nucleophilic attack[15]. Specifically, β1, β2, and β5 subunits within the inner-most domains of the core generate caspase-like, trypsin-like, and chymotrypsin-like activities, respectively. As proteins pass through the core, they are cleaved into short polypeptides typically consisting of 3 to 15 amino-acid residues each which are then recycled by hydrolysis.

The ubiquitin-proteasome proteolytic pathway has, unsurprisingly, been identified as a conserved cell function that can be exploited to combat cancer. The discovery that caspases–a family of proteases–were the chief executors of apoptosis spurred initial interest in the proteasome as a potential mediator of similar functions relevant to the control of programmed cell death. Further, its role as a regulator of the tumor suppressor protein p53, Bcl-2 family apoptosis inhibitors, and cyclin-dependent kinase (CDK) inhibitors reflected its potential as a target for cancer therapy[16,17]. Indeed, proteasome inhibitors, either alone or in combination with other anti-cancer therapies, have confirmed anti-tumor properties in hematologic cancers[18,19]. Antineoplastic activity derives from diverse actions such as blocking antiapoptotic genes, down-regulating survival signals, preventing efflux of cytotoxic agents, promoting DNA stabilization for cleaving, and by other mechanisms. The potential of proteasome inhibition has been translated to successful therapies in hematologic malignancies including multiple myeloma and mantle cell lymphoma[20,21]. However, in solid tumors, proteasome inhibition has not met with similar success and no benefit has been shown, despite extensive clinical investigations[22,23]. Development, on most occasions, has followed a one-size-fit-all model with no attempt to tailor treatment to sub-sets with possible sensitivity, despite pre-clinical evidence for the existence of such molecular sub-sets in various cancers[24]. Thus, a targeted development of proteasome inhibitors based on biomarkers deserves further investigation in solid tumors, including pancreatic cancer.

REGULATORS OF PROTEASOME WITH FOCUS ON PANCREATIC CANCER

Regulation of proteostasis is an important part of metabolism in active cells including cancer cells which possess an increased proteasome activity[25]. Proteasome regulation at the transcriptional level is effectuated in various cellular contexts by coordinated transcription of the proteasome multiple sub-units by a panel of transcription factors that include FOXO family members, NFE2L2 (also called NRF2), heat shock factors 1 and 2 (hHSF1 and hHSF2) and NF-Y (Figure 1). Activity of these factors vary in cancer cells but results in the overall increased proteasome availability and function that is necessary for their increased protein turn-over associated with cellular processes. Increased proteasome activity in bulk cancer cells contrast with reduced activity in cancer stem cells which are metabolically less active or quiescent

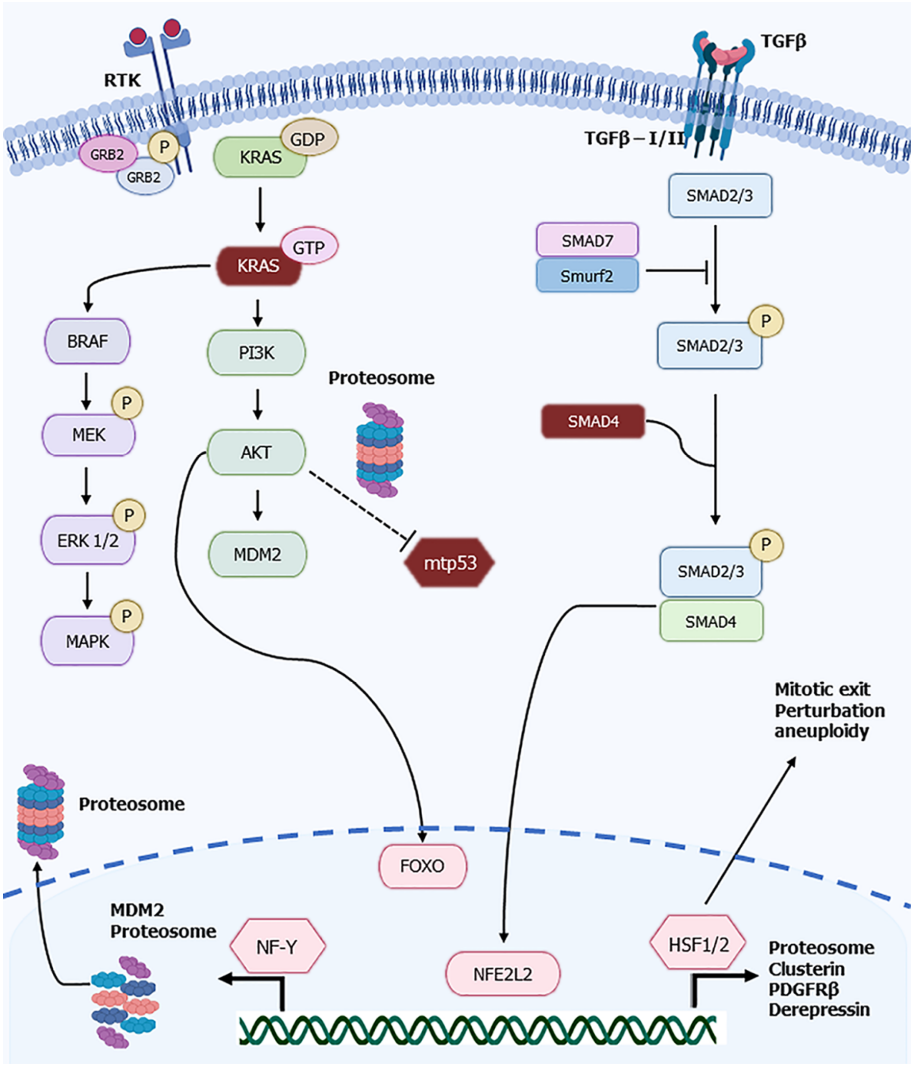


Figure 1 Schematic of receptor tyrosine kinases/KRAS and transforming growth factor beta/SMAD signaling pathways and downstream key transcription factors regulating the proteosome including FOXO, NF-Y, NFE2L2, and heat shock factors 1/2. Stabilized mutant p53 is involved in proteostasis transcription factors deregulation. For details see text. RTK: Receptor tyrosine kinases; TGFβ: Transforming growth factor beta; hHSF1/2: Heat shock factors 1/2; mtp53: Mutant p53.

[12,26]. Despite lower proteasome activity, cancer stem cells still depend on proteostatic controls for their basic functions and are sensitive to proteasome inhibition. Proteasome inhibitor, carfilzomib sensitivity has been described in the squamous/cornified subtype of pancreatic cancer[27]. This subtype presents with down-regulated epithelial mesenchymal transition (EMT) genes which are associated with cancer stem characteristics, suggesting a lower abundance of stem cell sub-population. Although no correlation with proteasome activity was found in squamous/cornified pancreatic cancers, a correlation with unfolded protein response genes ATF4 and CHOP expression and de novo RNA and protein synthesis was discerned, confirming the role of proteostasis[27]. The following sub-sections will discuss the regulation of the proteasome by the major transcription factors that govern their sub-units' transcription.

FOXO

FOXO family transcription factors regulate key carcinogenesis processes such as apoptosis and proliferation, acting as tumor suppressors. Thus, suppression of FOXO factors is important during carcinogenesis and is accomplished through activation of kinase Akt, the major negative regulator of FOXOs, which is commonly activated down-stream of receptor tyrosine kinases, by KRAS and PI3K kinase cascades in various cancers[28] (Figure 1). FOXO transcription factors have a positive effect in proteasome regulation through activation of transcription of proteasome sub-units [12]. A pathophysiologic role of FOXO transcription factors in muscle atrophy through

proteasome genes induction, and reversal of atrophy by protein kinase A inhibition of FOXO members FOXO1 and FOXO3 has been described[29]. Transcription co-activator Peroxisome Proliferator Activated Receptor gamma Co-activator 1alpha (PGC-1α) counteracts the atrophy promoting effects of FOXO3 and reduces denervation-induced muscle atrophy and cancer cachexia in mice[30]. In pancreatic cancer, PGC-1α, acting in concert with the nuclear receptor family transcription factors PPARγ and RXRα, has a tumor suppressing effect through induction of phosphatase PTEN and inhibition of Akt[31]. Given that p53 is a negative regulator of PCG-1α, PCG-1α may be up-regulated in pancreatic cancers with TP53 mutations[32]. Whether PCG-1α negatively regulates FOXO activity in pancreatic cancer, similarly to the effects in muscle remains unstudied. Such a negative regulation would synergize with the activity of the mutant KRAS/ PI3K/ Akt cascade.

The family member FOXO3 has an indirect negative effect on proteasome regulation through induction of protein Keap1, a negative regulator of NFE2L2[33]. Moreover, FOXO3 activated by cGMP has a role in maintenance of pancreatic cancer stem cells with CD44+ phenotype[34]. Keap1 induction due to FOXO3 activation would suppress proteasome transcription in these cells through NFE2L2 down-regulation. Cancer stem cells are, in general, less active metabolically and present a lower proteasome activity [12]. Also relevant for cancer stem cells, FOXO activity in proteasome regulation is counter-acted by transcription regulator ZEB1[35]. ZEB1 is a zinc finger protein that belongs to the core regulators of EMT, a process involved in metastasis and associated with stemness[36,37]. The dual role, direct and indirect, of FOXO family members in the transcription of proteasome sub-units may decrease the importance of FOXO transcriptional activity for maintenance of proteasome function in cancer cells. Suppression of FOXO family activity through upstream overactivated KRAS and PI3K/ Akt signaling, in addition to other pro-carcinogenic effects, down-regulates Keap1 leading to an eventual stabilization and increased activity of NFE2L2, which is a key factor for proteasome regulation and reactive oxygen species detoxification[38].

A reciprocal regulation of FOXO transcription factors by the proteasome is mediated by ubiquitination and proteasome degradation through the action of ubiquitin ligase MDM2, which also ubiquitinates p53 and the other proteasome regulating transcription factor NFE2L2[39].

NFE2L2 (NRF2)

NFE2L2 is a transcription factor that serves as a bulwark against oxidative damage following injury or inflammation by regulating the expression of antioxidant proteins [40]. It has also been implicated as a major participant in regulatory networks that control cell metabolism, autophagy, mitochondrial function, and proteolysis, including upregulated expression of the catalytic subunits of the 26S proteasome[41]. As the accumulation of oxidated and polyubiquitinated protein aggregates are major correlates of aging and senescence[42,43], regulation of NFE2L2 demonstrates therapeutic potential for neurodegenerative and cardiac disease as well as cancer[41].

NFE2L2 is overexpressed and predicts anti-cancer drug resistance[44,45]. The neoplastic progression to pancreatic ductal adenocarcinoma is induced by a signaling pathway that is potentiated by stress involving NFE2L2, E3 ubiquitin-protein ligase MDM2 (a negative regulator of p53), and ubiquitin-binding protein p62[46]. The mechanism is thought to involve the accumulation of p62 which triggers NFE2L2-MDM2 to modulate p53 and the morphogen Notch which induce conversions of acinar cells to progenitor-like cells, which then accelerate lesions toward malignant phenotypes. Pancreatic carcinogenesis can be experimentally suppressed by selective deletion of NFE2L2 in a mutant K-ras and p53 model[47], highlighting the intrinsic links between oxidative stress, NFE2L2, proteostasis, and cancer. Mice with mutations in K-ras and p53 display decreased pancreatic tumor formation and progression when NFE2L2 is lost compared with animals with intact NFE2L2. Loss of NFE2L2 results in down-regulation of several oxidative detoxification enzymes, such as glutathione S-transferases and UDP glucuronosyltransferases. In addition, transporters of the ABC family are down-regulated leading to increased gemcitabine sensitivity[48]. However, the pro-carcinogenic effect of NFE2L2 is tumor environment-specific, given that in the absence of p53 mutations animals with K-ras mutations and NFE2L2 activation develop pancreatic parenchyma atrophy[47].

hHSF1 and hHSF2

hHSF1 belongs, together with hHSF2, hHSF3 and hHSF4, to a family of transcription factors involved in proteostasis and is upregulated after proteasome inhibition and under other stress conditions[34,49]. hHSF1 is the founding member of the family and is homologous to the fly heat shock factor. In this organism only one HSF protein

exists[50]. Following hyperthermia, oxidative stress or proteasome inhibition hHSF1 dissociates from chaperone HSP90 and trimerizes. The trimeric form is capable of DNA binding to Heat Shock Elements (HSE), the target sequence of Heat Shock Factors and initiation of target gene transcription, that include chaperone proteins, ubiquitin and proteasome sub-nits[50,51]. hHSF2 binds DNA as a homotrimer or heterotrimer with hHSF1, with the activation capacity depending on the consistency of the trimer[52]. Cells that lack HSF2 display decreased expression of proteasome subunits and display p53 stabilization due to decreased proteasome activity[53]. In addition, hHSF1 and hHSF2 participate in proteotoxic stress response by up-regulating transcription of chaperone protein clusterin (also called apolipoprotein J), which possesses a noncanonical HSE in its promoter[54,55]. Thus, HSFs constitute part of a feedback proteostasis response whence proteostasis perturbations lead to up-regulation of these factors that help re-establish the balance of cellular proteins metabolism[56].

Besides the proteostasis response, hHSF1 and hHSF2 have a broader role in cancer that derives from additional functions of these transcription factors in cancer-associated processes, such as proliferation, inhibition of apoptosis and metastasis[51]. hHSF1 promotes the cell cycle through a direct interaction with ubiquitin ligase APC/C co-factor cdc20, which leads to inhibition of cyclin B and securin degradation [57]. Phosphorylation of hHSF1 by mitotic kinase PLK1 is required for mitotic exit inhibition and is observed in cancer cells with mutated p53, which have defective PLK1 function regulation[58]. As a result of mitotic perturbation, aneuploidy ensues with micronuclei formation, a hallmark of chromosomal instability. The role of hHSF1 in metastasis is exemplified by induction of EMT core transcription regulator Slug in breast cancer cells that depends on hHSF1 activation by kinase Akt[59]. hHSF2 deficiency affects cell-cell adhesion cadherins, whose downregulation leads to loss of cellular adhesions and cell demise, possibly due to anoikis[60]. Absence of cell-cell adhesion is associated with intolerance of prolonged proteotoxic stress[60]. This could explain the association of cancer stemness and stem cells, which have decreased proteasome activity with the ability to undergo EMT, which requires dissolving cell adhesions[12]. Indeed, hHSF1 activity endows cancer cells with stem cell properties, at least in the case of breast cancer[61]. hHSF1 induction in breast cancer cells increases cells with the stem cell phenotype and chemotherapy resistance while knockdown of hHSF1 reduces stem cells.

Interestingly, hHSF1 shares with FOXO transcription factors a role in longevity[50]. This could be the result of their also shared role in proteostasis, as improved protein handling provides a benefit in cellular function which would be expected to provide a cell survival advantage. The benefit is usurped by cancer cells, the ultimate immortal cells. A similar dual effect in pancreatic cancer is at play for another proteostatic mechanism related to longevity, autophagy, which is beneficial in established pancreatic cancers[62]. Increased autophagic flux is beneficial and promotes longevity in cells where the mitochondrial membrane potential is preserved through a closed mitochondrial permeability transition pore (mPTP), while apoptosis ensues in cells with high autophagic flux but an open mPTP[63]. KRAS mutations as observed in pancreatic cancer sensitize cells to mitochondrial membrane potential destabilization [64].

NF-Y

The CCAAT-binding factor, also known as NF-Y, is a transcription factor with well-established gene regulatory properties and serves as a safeguard against abnormal translation by maintaining nucleosome-depleted regions at gene promoters[65]. NF-Y is a trimeric complex consisting of NF-YA, NF-YB and NF-YC[66]. The NF-YA subunit is the DNA binding partner and mutations in the DNA binding domain in its carboxyterminus lead to inability of the trimer to bind promoters of target genes. NF-Y has been shown to regulate transcription of cell cycle-related genes including CDKs, embryonic differentiation and morphogenesis by activation of SOX genes, and cancer-related genes including the tumor suppressor TβRII among others[67]. Indeed, the integrity of CCAAT boxes and functional NF-Y complexes are vital to cell cycle progression as a response to DNA damage[68,69]. Several proteasome genes carry CCAAT boxes in their promoters and are regulated by NF-Y[70]. Interestingly, CCAAT boxes are disproportionately represented in gene promoters that are overexpressed in cancers and NF-Y activity is critical to both cell transformation and proliferation by way of interactions with p53[66]. In addition, there is a strong metabolic component to the NF-Y regulome, with de novo biosynthesis of lipids, purines, and polyamines, as well as glycolysis and activation of glutamine pathway[71]. NF-Y therefore appears to represent a watershed factor at the intersection of cell transfor-

mation, proliferation, and metabolism–a deadly combination when co-opted by cancer. In pancreatic cancer, disruption by mutant p53 of the p73/NF-Y complex initiates the transcription of platelet-derived growth factor receptor β (PDGFRβ) and potentiates invasion and metastasis[72]. Thus, NF-Y has been identified as a promising target for anti-cancer therapies. For example, indirubin derivatives, which are known to exert anti-tumor effects by inhibiting CDKs, also act to inhibit binding of NF-Y to DNA[73]. Computational drug repositioning techniques indicate that other candidate anti-cancer drugs are likely to impact NF-Y transcription[74].

REGULATION OF PROTEASOME REGULATORS BY MOLECULAR LESIONS OF PANCREATIC CANCER

As mentioned above, only a few recurrent molecular abnormalities in oncogenes and tumor suppressors are present in pancreatic cancer[3]. Up to 80%-90% of human pancreatic adenocarcinomas bear classic activating mutations at codons 12, 13 or 61 in oncogene KRAS and about two thirds of pancreatic cancers display mutations in tumor suppressor p53. Two other tumor suppressors, SMAD4 and p16 are mutated in a sizeable minority of pancreatic cancers. Lesions in these proteins that affect important molecular pathways of pancreatic carcinogenesis also have repercussions for proteasome master regulators and their function and they will be discussed in this section. Moreover, transcription factors-regulators of the proteasome expression are involved in pathways regulated by common pancreatic cancer molecular lesions and reciprocal relationships exist, constituting an elaborate network.

KRAS

RAS proteins are single-subunit small GTPases that are fundamental to cell signaling pathways, regulating proliferation, cell adhesion, apoptosis, migration, and differentiation. Perhaps the most well-studied and indispensable pathway is the mitogen-activated protein kinase (MAPK) cascade, which potentiates downstream gene transcription involved in proliferation and growth. RAS signals also through the PI3K Akt pathway that regulates metabolism, cell growth and survival[75]. The human genome contains 3 Ras gene isoforms-HRAS, KRAS, and NRAS-which are notably the most predominant oncogenes involved in cancer. KRAS mutations are found in almost all (95%) cases of pancreatic ductal adenocarcinoma, dwarfing the mutation frequencies of HRAS (< 1%) and NRAS (< 1%)[76]. Incidentally, the same pattern of KRAS mutation frequency dominance over other RAS isoforms is observed in colorectal cancer, renal cell carcinoma, and stomach cancer, though to lesser degrees. In lung cancer, polymorphisms associated with the NF-Y binding site within the KRAS gene may increase the likelihood of developing the malignancy[77]. The KRAS oncogene mutation was found to abnormally activate several pathways including the PI3K-AKT-mTOR and Ras-MAPK pathways in both human pancreatic cancers and mouse models of pancreatic adenocarcinoma[78]. Using a mouse model of pancreatic adenocarcinoma, LSL-KrasG12D/+, that recapitulates the development of pancreatic cancer, it was recently demonstrated that the heat shock factor 1 (HSF1) and epidermal growth factor receptor (EGFR) pathway is a major determinant of progression[79]. HSF1 is an important regulator of proteostasis in cancers, exerting control over metabolism and cancer-promoting signals[80,81]. Thus, increased activity emanating from a mutated KRAS has the potential to increase proteasome transcription through regulation of several core regulators including NFE2L2, FOXO family members and hHSF1.

Mutant p53

Proteasome plays a key role in regulation of the physiologic function of wild type p53, as the tumor suppressor turnover needs to be tightly controlled to avoid untimely cell cycle arrest or cell demise from its accumulation in normal cells[9]. Pharmacologic inhibition of the proteasome with bortezomib results in cell death associated with p53 accumulation[82]. The oncogene c-Myc also accumulates in this model which may also result in indirect induction of p53 through p14ARF activation. c-Myc activation in normal cells in stress conditions leads to cell cycle arrest and apoptosis through activation of the p14ARF/ p53 axis and thus, in cancer, neutralization of this axis is a prerequisite for tolerance of c-Myc activation, which is present in a sub-set of pancreatic adenocarcinomas[83].Pancreatic cancers with mutations in TP53 rely less on proteasome activity to neutralize p53 activity, while the sub-set with intact TP53 may

particularly benefit from the increased proteasome activity that results from up-regulation of proteasome sub-units. In addition, mutant TP53 pancreatic cancers carry more often than TP53 wild-type cancers lesions of the CDKN2A locus, encoding for p14ARF and p16, which may impair further residual proteasomal degradation of p53 due to impaired ubiquitination by MDM2[3].

Mutated p53 may play a role in stabilization of NFE2L2 through an interplay between HSP90 that provides a feedback antioxidant response, involving the interaction with p62/sequestrome and stabilization of NFE2L2[84]. This response protected pancreatic cancer cells from excess reactive oxygen species through up-regulation of antioxidant enzymes, in vitro. However, whether proteasome was up-regulated in these cells was not examined in this study[84]. Mutated p53 co-operates with NFE2L2 in transcription of targets genes with ARE sequences in their promoters such as the thioredoxin gene[85]. In addition, the mutant p53 transcriptional program included proteasome genes and induced proteasome inhibitor resistance in another study[86].

Another gain of function of mutated p53 involves promotion of the transcriptional program of transcription factor hHSF1 by interacting directly with hHSF1 phosphorylated at S326[87]. This interaction favors binding of hHSF1 to HSE DNA target sequences and induces cancer cell resistance to proteotoxic stress, by upregulation of genes such as the chaperone HSP90. Increased chaperone activity favors stability of mutant oncoproteins, including mutated p53 itself, in a positive feed-forward loop. Besides chaperone stabilizing activity for mutated p53, HSP90 interacts and interferes with the function of ligase MDM2 and another p53 ligase, CHIP (Carboxyterminus of HSP70 Interacting Protein), preventing mutated p53 ubiquitination and degradation by the proteasome[88]. Thus, chaperoning of mutated p53 by HSP90 instead of HSP70, that interacts with CHIP promotes stability of the mutant protein altering its fate of degradation[89]. In addition, hHSF1 facilitates nuclear localization of p53, where the two factors can continue their co-operation on the transcription from HSE containing promoters[90]. Phosphorylation of hHSF1 at the serine of position 326 is executed by activation of MAPK and PI3K cascades and thus may be a direct effect downstream of activated KRAS[90].

Similar to the interaction with hHSF1, mutant p53 binds to the proteasome regulator NF-Y on CCAAT target sequences and alters the transactivation capability of this regulator[91]. Following exposure to DNA damaging agents, NF-Y bound to mutant p53 interacts with acetyltransferase p300 and activates cell cycle genes, instead of its interaction with HDACs when bound to wild-type p53, which leads to gene suppression and cell cycle arrest[91,92]. In pancreatic cancer, mutant p53 replaces the family member p73 from complexes with NF-Y factors resulting in derepression of the promoter of receptor tyrosine kinase PDGFRβ gene[72]. PDGFRβ signaling contributes to increased metastatic potential in this model. Mutant p53 could contribute also to up-regulation of proteasome genes which are targets of NF-Y.

TGFβ / SMAD

TGFβ is one of three cytokines belonging to the transforming growth factor family. TGFβ is most notably involved in immunosuppression, angiogenesis, metabolic activity, and cell-cycle control. Upon interaction with the TGFβ receptor (type II), this cytokine triggers a signaling cascade which phosphorylates receptor-activated Smad proteins which in turn form complexes, translocate to the nucleus, and induce gene transcription. Inactivation of SMAD4, which mediates pancreatic cell apoptosis and proliferation, is observed in half of advanced pancreatic cancers[93]. While SMAD4-regulated genes associated with the TGFβ pathway are normally tumor-suppressive in pancreatic epithelial cells[94], pancreatic tumors often display increased expression of TGFβ which promotes a tissue microenvironment of paracrine-like signaling that becomes tumorigenic when SMAD4 incurs mutations or complete deletion[95,96]. Further, TGFβ signaling can become deregulated by the genetic state of KRAS, becoming pro-carcinogenic[97]. In addition, specific SMAD4 mutants encountered in pancreatic and colon cancers show an increased phosphorylation by GSK3β kinase and MAPK and subsequent ubiquitination and proteasome degradation[98]. Both GSK3β and MAPK activities are modulated by activated KRAS cascades. Interestingly, pancreatic cancer development is tied to the negative regulation of E-cadherin expression by ZEB2 (also known as SIP1- Smad-interacting protein 1) and is associated with migration and invasiveness[99]. TGFβ's dual, context-dependent relationship as a tumor suppressor or promoter is further complicated when considering that during pancreatic carcinogenesis there is crosstalk with NFE2L2, contributing to malignant transformation[100]. In pancreatic cancer cells, TGFβ signaling up-regulates NFE2L2 and suppresses E-cadherin expression, contributing to invasion (Figure 1). In contrast,

in premalignant human pancreatic duct cells, TGFβ is not able to affect NFE2L2. Knockdown of NFE2L2 decreases the potential of TGFβ to induce invasion of pancreatic cancer cells[100]. TGFβ signaling regulates also nuclear localization of the NF-YA sub-unit of NF-Y factor[101]. In cells exposed to TGFβ, NF-YA localizes to the nucleus and binds target gene promoters in a manner that depends on the activity of MAPK kinases but is independent of the SMAD factors. The baseline activity of MAPK kinases in various cell types affects the kinetics of NF-YA nuclear localization and activity[101]. The intermediate signal from TGFβ to MAPK for the activation of NF-Y may be carried by MAPKKK TGFβ Activated Kinase 1 (TAK1)[102]. These data suggest that the micro-environment of cancer cells rewires TGFβ transduction that may underline proteasome levels and activity[103].

p16 mutations and deep deletions of the p16/ p14 locus

p16 (INK4a) is a cyclin dependent kinase inhibitor that inhibits the Cyclin Dependent Kinase 4 and 6 (CDK4/6)/Cyclin D complex activity resulting in cell cycle arrest[104]. Cyclin D is a proteasome substrate and is degraded when p16 prevents interaction of the CDK4/Cyclin D complex with the Retinoblastoma (Rb) protein. Cells with activated KRAS/PI3K/AKT pathway and with increased KRAS/BRAF/MEK activity undergo oncogene induced senescence through up-regulation of p16 and the related cyclin dependent kinase inhibitor p15 and thus, derive benefit from absence of p16 [105,106]. The benefit of inactivating p16 in KRAS mutated cancers is also observed in colorectal cancers where the mechanism is promoter methylation at the CDKN2A gene locus that encodes for p16[107]. Inactivation of the fail-safe mechanism of senescence induction in KRAS mutant cells may be accomplished in different cancers by different mechanisms such as mutations, deletion of the locus and promoter methylation[108]. A subset of pancreatic cancers bears mutations in the CDKN2A gene. Other pancreatic cancer cases display deletions of the CDKN2A locus, leading, besides the absence of p16, to absence of expression of p14ARF protein, a positive regulator of p53. p14ARF protein is transcribed from the same locus with an overlapping sequence but an alternative reading frame. Absence of p14ARF allows MDM2 to ubiquitinate wild type p53 for proteasomal degradation. In pancreatic cancers with p16 mutations or deletions, the absence of p16 function allows proliferation despite high proteasome activity[109]. In addition, p14ARF has regulatory activities beyond induction of p53, among which is a negative regulation of NFE2L2[110]. p14ARF prevents NFE2L2 from activating targets genes such as SLC7A11. Thus, deletion of p14ARF in pancreatic cancer may contribute to NFE2L2 activation and increased target gene expression, including proteasome component genes. On the other hand, cell cycle inhibitor p16 is a target gene of NFE2L2 and as a result deletion of the locus prevents up-regulation that would be an effect of NFE2L2 activation[6].

p16 also regulates the transcriptional activity of transcription factor NF-Y through inhibition of cyclin dependent kinases[111]. The transcription of human thymidine kinase gene which possesses a CAATT boxes in its promoter and is activated by NF-Y is reduced in cervical cancer cells transfected with p16. Thus, p16 has a broad effect in proteasome factors regulation and its absence or deregulation in pancreatic cancer may contribute to increased proteasomal activity.

The tumor suppressor role of p16 relates to cell cycle inhibition and permanent exit from cycling, associated with induction of senescence[112]. Hence the frequent occurrence of neutralization of p16 in cancers offers proliferation advantage. In normal cells, protracted activation of p16 also induces senescence and contributes to aging. A related effect of p16 is in prevention of reprogramming and pluripotency. p16 is a major roadblock in the induction of stem cells from adult differentiated cells. In contrast, p16 inhibition favors reprogramming[113]. In pancreatic cancer, expression of p16 by immunohistochemistry, suggesting an intact protein, was associated with an improved prognosis in patients who had undergone surgical resection[114]. Moreover, patients with absence of lesions in any of the other three common molecular alterations of pancreatic cancer had better survival outcomes. This associations together with the roles of common pancreatic cancer-related pathways in proteasome expression and function further argues for the important role of the proteasome in the disease.

THE PROTEASOME AND ITS REGULATION IN EMT AND STEMNESS AND THERAPEUTIC IMPLICATIONS IN PANCREATIC CANCER

Proteasome as a major regulator of proteostasis is important for the function of any cell, including cancer cells. Regulation of proteasomes is critical for the normal function of cells and needs to be tightly modulated to reflect the metabolic needs of host cells. During embryonic development, the proteasome is fundamental for embryonic cell proliferation and regulated apoptosis, both co-operating in organismal morphogenesis. Up-regulation of proteasome production induced by transcription factor NFE2L2 and high levels of proteasome activity are present in human embryonic stem cells, which confers structural and functional plasticity[115]. Similarly, cancer cells are characterized by significant intratumoral plasticity that is integral to their neoplastic state and derives from the inherent instability of their genomes[116]. Indeed, pancreatic adenocarcinomas and several other cancers contain, for example, inactivating mutations of the guardian of the genome p53. Further, cancers contain cells with variable proliferation status ranging from a usually smaller subset with lower proliferation and stem cell characteristics and a bulk cancer cell component that are more differentiated and possess higher proliferative activity. Between these extremes, a cell compartment with variable differentiation and proliferation states fills the spectrum. These compartments are not static and cells transition between different proliferative and differentiation states, which is facilitated by their genomic instability. Thus, the stemness of cancer, although it shares characteristics with development, is more fluid than the embryonic stem cell state where the directionality is solely towards differentiation, and typically an ontogenetic goal state set by the body plan. In addition, the cancer stem cell state is less proliferative than embryonic stem cells, where the basic functional output is to maintain cancer cell supply and protect the tumor from external toxins, such as chemotherapy. In contrast, the primary functional output of embryonic stem cells is exactly timed and drives the development of a whole organism from one to several cells. Thus, in contrast to embryonic stem cells, cancer stem cells are for protracted periods quiescent and have low metabolic activity. As a result, in contrast to embryonic stem cells and to bulk cancer cells, their proteasome activity is low[26].

The plasticity of cancer stemness endows cancer stem cells with another property derived from embryonic development, EMT and the reverse process, MET. Cancer stem cells have the ability to access both an epithelial and a mesenchymal state using the two processes appropriated from development. When directed towards differentiation stem cells lose this ability and are locked, albeit not irreversibly, into the epithelial state of the bulk stem cells. Notably, mesenchymal stem cell function declines with senescence, which is linked to proteasome dysfunction, where stemness can be enhanced by core subunit β5-overexpression-induced proteasomal re-activation [117]. Fluctuations of proteasome levels and function follow and are regulated by the state of the cancer cell with low levels being adequate in cancer stem cells, but high levels required in proliferating bulk cancer cells with high metabolic and protein turn over. Thus, tight regulation of proteasome levels is expected to be critical for cancer cells and fluctuations of proteasome availability are integrated in the cancer signaling programs with key players being parts of major cancer pathways as detailed in the previous paragraphs. Unsurprisingly, proteasomal regulators such as the deubiquitinating enzyme USP21, which is frequently amplified in 22% of pancreatic adenocarcinomas, when overexpressed, promotes stemness in cancer cells, enhances tumor growth, and drives progression from the precursor pancreatic intraepithelial neoplasia (PanIN) to pancreatic adenocarcinoma[118].

Despite the critical role of proteasome in cancer, therapeutic exploitation of proteasome inhibition has not been successful in solid tumors, in stark contrast to specific hematologic malignancies, such as multiple myeloma where proteasome inhibitors are successfully integrated in the therapeutic armamentarium[119]. In pancreatic cancer, proteasome inhibitors have produced disappointing results in clinical trials and development is not actively in pursuit[120]. However, it is clear from pre-clinical investigations that subsets of pancreatic cancer cells are sensitive to proteasome inhibition. Pancreatic cancers with oncogene c-Myc constitute a subset with such vulnerability[121]. Although c-Myc remains not directly targetable, high activity confers sensitivity to proteotoxic stress and pharmacologic proteasome inhibition. Interestingly, c-Myc amplifications in pancreatic cancer are observed exclusively in cases with p53 mutations, consistent with the induction of oncogenic stress-induced apoptosis if p53 is intact.

Differential regulation of protein translation may also confer proteasome inhibition sensitivity in subsets of pancreatic adenocarcinomas. Pancreatic cancer cells exposed to bortezomib up-regulated the kinase EIF2AK1 (eukaryotic translation Initiation Factor 2 alpha Kinase 1, also known as HRI- Heme Regulated Inhibitor) and are resistant to the drug[11]. Knockdown of EIF2AK1 led to increased protein translation and accumulation resulting to cell death after bortezomib exposure. Another study in pancreatic cancer cell lines that were resistant to 5-FU chemotherapy showed that these lines displayed stem cell and EMT markers expression and high activity of the proteasome master regulator NFE2L2[122]. Knockdown of NFE2L2 in this model sensitized cells to 5-FU.

mTOR inhibitors are drugs that directly affect proteostasis by interfering with protein translation through inhibition of the translation initiation complex[123]. Everolimus, an mTOR inhibitor, provides an example of the challenges and disappointments the pancreatic cancer therapeutics field has faced. The drug is successfully used in combination with hormonal therapy for the treatment of metastatic Estrogen Receptor positive breast cancer[124]. Everolimus has been investigated in pancreatic adenocarcinoma both alone and in combination with various other drugs with the aim to inhibit protein production in proliferating cancer cells. Clinical translation of initial positive pre-clinical data has provided disappointing results[125,126]. As monotherapy, everolimus produces no responses and adding it to chemotherapy or targeted therapies has not provided any benefit. However, the clinical development of the drug has followed the usual chemotherapy development paradigm without any attempt to identify and include sub-sets of pancreatic cancer patients with probable sensitivity to everolimus based on underlying molecular defects. In addition, inhibition of protein translation may not be a good target in pancreatic cancer altogether, based on the above discussion, given that protein production shut-down may alleviate proteostatic stress and could counter-intuitively promote the ability of cancer cells to cope with this stress. In addition, decreased protein turn over could allow for a decreased proteasome activity compatible with acquiring a cancer stem cell phenotype and drug resistance. Other studies have shown that resistance to everolimus results from a feed-back activation of up-stream receptor tyrosine kinases. Nevertheless, attempts to address this resistance mechanism with combinations of everolimus and EGFR kinase inhibitor erlotinib were not met with success[127].

CONCLUSION

It is evident that challenges in pancreatic cancer therapeutics remain. However, targeted exploitation of pancreatic cancer vulnerabilities stemming from proteostasis dysregulation is possible and could promote therapeutics in well-defined molecular sub-sets of patients. Further development of proteasome inhibitors, possibly in combination with other molecularly defined targeted therapies, relies on the discovery of synthetic vulnerabilities. A strategy for drug development of proteasome inhibitors in pancreatic cancer should identify vulnerable cell lines in vitro, examine their molecular make-up and subsequently examine whether patient-derived xenografts with similar molecular lesions are indeed sensitive to these drugs or their combinations with other candidate drugs, before testing the drug(s) in pancreatic cancer patients bearing tumors with the same underlying driver molecular defects.

REFERENCES

1 **Miller KD**, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. *CA Cancer J Clin* 2016; **66**: 271-289 [PMID: 27253694 DOI: 10.3322/caac.21349]

2 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

3 **Cancer Genome Atlas Research Network**. Cancer Genome Atlas Research Network. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. *Cancer Cell* 2017; **32**: 185-203.e13 [PMID: 28810144 DOI: 10.1016/j.ccell.2017.07.007]

4 **Zehir A**, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J,

Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen HW, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M, Berger MF. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; **23**: 703-713 [PMID: 28481359 DOI: 10.1038/nm.4333]

5 **Bailey P**, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM; Australian Pancreatic Cancer Genome Initiative, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; **531**: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

6 **Hashimoto-Hachiya A**, Tsuji G, Furue M. Antioxidants cinnamaldehyde and Galactomyces fermentation filtrate downregulate senescence marker CDKN2A/p16INK4A *via* NRF2 activation in keratinocytes. *J Dermatol Sci* 2019; **96**: 53-56 [PMID: 31606066 DOI: 10.1016/j.jdermsci.2019.09.002]

7 **Shirazi F**, Jones RJ, Singh RK, Zou J, Kuiatse I, Berkova Z, Wang H, Lee HC, Hong S, Dick L, Chattopadhyay N, Orlowski RZ. Activating *KRAS*, *NRAS*, and *BRAF* mutants enhance proteasome capacity and reduce endoplasmic reticulum stress in multiple myeloma. *Proc Natl Acad Sci U S A* 2020; **117**: 20004-20014 [PMID: 32747568 DOI: 10.1073/pnas.2005052117]

8 **Voutsadakis IA**. Pathogenesis of colorectal carcinoma and therapeutic implications: the roles of the ubiquitin-proteasome system and Cox-2. *J Cell Mol Med* 2007; **11**: 252-285 [PMID: 17488476 DOI: 10.1111/j.1582-4934.2007.00032.x]

9 **Voutsadakis IA**. The ubiquitin-proteasome system in colorectal cancer. *Biochim Biophys Acta* 2008; **1782**: 800-808 [PMID: 18619533 DOI: 10.1016/j.bbadis.2008.06.007]

10 **Belalcazar A**, Shaib WL, Farren MR, Zhang C, Chen Z, Yang L, Lesinski GB, El-Rayes BF, Nagaraju GP. Inhibiting heat shock protein 90 and the ubiquitin-proteasome pathway impairs metabolic homeostasis and leads to cell death in human pancreatic cancer cells. *Cancer* 2017; **123**: 4924-4933 [PMID: 28841232 DOI: 10.1002/cncr.30944]

11 **White MC**, Schroeder RD, Zhu K, Xiong K, McConkey DJ. HRI-mediated translational repression reduces proteotoxicity and sensitivity to bortezomib in human pancreatic cancer cells. *Oncogene* 2018; **37**: 4413-4427 [PMID: 29720726 DOI: 10.1038/s41388-018-0227-y]

12 **Voutsadakis IA**. Proteasome expression and activity in cancer and cancer stem cells. *Tumour Biol* 2017; **39**: 1010428317692248 [PMID: 28345458 DOI: 10.1177/1010428317692248]

13 **Tanaka K**, Yoshimura T, Kumatori A, Ichihara A, Ikai A, Nishigai M, Kameyama K, Takagi T. Proteasomes (multi-protease complexes) as 20 S ring-shaped particles in a variety of eukaryotic cells. *J Biol Chem* 1988; **263**: 16209-16217 [PMID: 3141402]

14 **Grice GL**, Nathan JA. The recognition of ubiquitinated proteins by the proteasome. *Cell Mol Life Sci* 2016; **73**: 3497-3506 [PMID: 27137187 DOI: 10.1007/s00018-016-2255-5]

15 **Tanaka K**. The proteasome: overview of structure and functions. *Proc Jpn Acad Ser B Phys Biol Sci* 2009; **85**: 12-36 [PMID: 19145068 DOI: 10.2183/pjab.85.12]

16 **Almond JB**, Cohen GM. The proteasome: a novel target for cancer chemotherapy. *Leukemia* 2002; **16**: 433-443 [PMID: 11960320 DOI: 10.1038/sj.leu.2402417]

17 **Ciznadija D**, Zhu XH, Koff A. Hdm2- and proteasome-dependent turnover limits p21 accumulation during S phase. *Cell Cycle* 2011; **10**: 2714-2723 [PMID: 21768776 DOI: 10.4161/cc.10.16.16725]

18 **Fujita E**, Mukasa T, Tsukahara T, Arahata K, Omura S, Momoi T. Enhancement of CPP32-like activity in the TNF-treated U937 cells by the proteasome inhibitors. *Biochem Biophys Res Commun* 1996; **224**: 74-79 [PMID: 8694836 DOI: 10.1006/bbrc.1996.0986]

19 **Imajoh-Ohmi S**, Kawaguchi T, Sugiyama S, Tanaka K, Omura S, Kikuchi H. Lactacystin, a specific inhibitor of the proteasome, induces apoptosis in human monoblast U937 cells. *Biochem Biophys Res Commun* 1995; **217**: 1070-1077 [PMID: 8554559 DOI: 10.1006/bbrc.1995.2878]

20 **Lee HC**, Cerchione C. How I treat relapsed and/or refractory multiple myeloma. *Hematol Rep* 2020; **12**: 8955 [PMID: 33042504 DOI: 10.4081/hr.2020.8955]

21 **Scott K**, Hayden PJ, Will A, Wheatley K, Coyne I. Bortezomib for the treatment of multiple myeloma. Cochrane Database of Systematic Reviews, 2013, John Wiley & Sons, Ltd [DOI: 10.1002/14651858.cd010816]

22 **Infante JR**, Mendelson DS, Burris HA 3rd, Bendell JC, Tolcher AW, Gordon MS, Gillenwater HH, Arastu-Kapur S, Wong HL, Papadopoulos KP. A first-in-human dose-escalation study of the oral proteasome inhibitor oprozomib in patients with advanced solid tumors. *Invest New Drugs* 2016; **34**: 216-224 [PMID: 26924128 DOI: 10.1007/s10637-016-0327-x]

23 **Roth P**, Mason WP, Richardson PG, Weller M. Proteasome inhibition for the treatment of glioblastoma. *Expert Opin Investig Drugs* 2020; **29**: 1133-1141 [PMID: 32746640 DOI: 10.1080/13543784.2020.1803827]

24 **Chattopadhyay N**, Berger AJ, Koenig E, Bannerman B, Garnsey J, Bernard H, Hales P, Maldonado Lopez A, Yang Y, Donelan J, Jordan K, Tirrell S, Stringer B, Xia C, Hather G, Galvin K, Manfredi M, Rhodes N, Amidon B. KRAS Genotype Correlates with Proteasome Inhibitor Ixazomib Activity in Preclinical In Vivo Models of Colon and Non-Small Cell Lung Cancer: Potential Role of Tumor Metabolism. *PLoS One* 2015; **10**: e0144825 [PMID: 26709701 DOI: 10.1371/journal.pone.0144825]

25 **Vlashi E**, Lagadec C, Chan M, Frohnen P, McDonald AJ, Pajonk F. Targeted elimination of breast cancer cells with low proteasome activity is sufficient for tumor regression. *Breast Cancer Res Treat* 2013; **141**: 197-203 [PMID: 24013708 DOI: 10.1007/s10549-013-2688-6]

26 **Vlashi E**, Kim K, Lagadec C, Donna LD, McDonald JT, Eghbali M, Sayre JW, Stefani E, McBride W, Pajonk F. In vivo imaging, tracking, and targeting of cancer stem cells. *J Natl Cancer Inst* 2009; **101**: 350-359 [PMID: 19244169 DOI: 10.1093/jnci/djn509]

27 **Fraunhoffer NA**, Abuelafia AM, Bigonnet M, Gayet O, Roques J, Telle E, Santofimia-Castaño P, Borrello MT, Chuluyan E, Dusetti N, Iovanna J. Evidencing a Pancreatic Ductal Adenocarcinoma Subpopulation Sensitive to the Proteasome Inhibitor Carfilzomib. *Clin Cancer Res* 2020; **26**: 5506-5519 [PMID: 32669378 DOI: 10.1158/1078-0432.CCR-20-1232]

28 **Alessi DR**, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* 1997; **7**: 261-269 [PMID: 9094314 DOI: 10.1016/s0960-9822(06)00122-9]

29 **Silveira WA**, Gonçalves DA, Machado J, Lautherbach N, Lustrino D, Paula-Gomes S, Pereira MG, Miyabara EH, Sandri M, Kettelhut IC, Navegantes LC. cAMP-dependent protein kinase inhibits FoxO activity and regulates skeletal muscle plasticity in mice. *FASEB J* 2020; **34**: 12946-12962 [PMID: 32772437 DOI: 10.1096/fj.201902102RR]

30 **Sandri M**, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM. PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc Natl Acad Sci U S A* 2006; **103**: 16260-16265 [PMID: 17053067 DOI: 10.1073/pnas.0607795103]

31 **Farrow B**, Evers BM. Activation of PPARgamma increases PTEN expression in pancreatic cancer cells. *Biochem Biophys Res Commun* 2003; **301**: 50-53 [PMID: 12535639]

32 **Deng X**, Li Y, Gu S, Chen Y, Yu B, Su J, Sun L, Liu Y. p53 Affects PGC1α Stability Through AKT/GSK-3β to Enhance Cisplatin Sensitivity in Non-Small Cell Lung Cancer. *Front Oncol* 2020; **10**: 1252 [PMID: 32974127 DOI: 10.3389/fonc.2020.01252]

33 **Guan L**, Zhang L, Gong Z, Hou X, Xu Y, Feng X, Wang H, You H. FoxO3 inactivation promotes human cholangiocarcinoma tumorigenesis and chemoresistance through Keap1-Nrf2 signaling. *Hepatology* 2016; **63**: 1914-1927 [PMID: 26857210 DOI: 10.1002/hep.28496]

34 **Kawazoe Y**, Nakai A, Tanabe M, Nagata K. Proteasome inhibition leads to the activation of all members of the heat-shock-factor family. *Eur J Biochem* 1998; **255**: 356-362 [PMID: 9716376 DOI: 10.1046/j.1432-1327.1998.2550356.x]

35 **Ninfali C**, Siles L, Darling DS, Postigo A. Regulation of muscle atrophy-related genes by the opposing transcriptional activities of ZEB1/CtBP and FOXO3. *Nucleic Acids Res* 2018; **46**: 10697-10708 [PMID: 30304480 DOI: 10.1093/nar/gky835]

36 **Battula VL**, Evans KW, Hollier BG, Shi Y, Marini FC, Ayyanan A, Wang RY, Brisken C, Guerra R, Andreeff M, Mani SA. Epithelial-mesenchymal transition-derived cells exhibit multilineage differentiation potential similar to mesenchymal stem cells. *Stem Cells* 2010; **28**: 1435-1445 [PMID: 20572012 DOI: 10.1002/stem.467]

37 **Morel AP**, Lièvre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS One* 2008; **3**: e2888 [PMID: 18682804 DOI: 10.1371/journal.pone.0002888]

38 **Roy SK**, Srivastava RK, Shankar S. Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer. *J Mol Signal* 2010; **5**: 10 [PMID: 20642839 DOI: 10.1186/1750-2187-5-10]

39 **Fu W**, Ma Q, Chen L, Li P, Zhang M, Ramamoorthy S, Nawaz Z, Shimojima T, Wang H, Yang Y, Shen Z, Zhang Y, Zhang X, Nicosia SV, Pledger JW, Chen J, Bai W. MDM2 acts downstream of p53 as an E3 ligase to promote FOXO ubiquitination and degradation. *J Biol Chem* 2009; **284**: 13987-14000 [PMID: 19321440 DOI: 10.1074/jbc.M901758200]

40 **He F**, Ru X, Wen T. NRF2, a Transcription Factor for Stress Response and Beyond. *Int J Mol Sci* 2020; **21** [PMID: 32640524 DOI: 10.3390/ijms21134777]

41 **Dodson M**, de la Vega MR, Cholanians AB, Schmidlin CJ, Chapman E, Zhang DD. Modulating NRF2 in Disease: Timing Is Everything. *Annu Rev Pharmacol Toxicol* 2019; **59**: 555-575 [PMID: 30256716 DOI: 10.1146/annurev-pharmtox-010818-021856]

42 **Pan JX**, Short SR, Goff SA, Dice JF. Ubiquitin pools, ubiquitin mRNA levels, and ubiquitin-mediated proteolysis in aging human fibroblasts. *Exp Gerontol* 1993; **28**: 39-49 [PMID: 8382166 DOI: 10.1016/0531-5565(93)90018-9]

43 **Pickering AM**, Davies KJ. Degradation of damaged proteins: the main function of the 20S proteasome. *Prog Mol Biol Transl Sci* 2012; **109**: 227-248 [PMID: 22727423 DOI: 10.1016/B978-0-12-397863-9.00006-7]

44 **Hong YB**, Kang HJ, Kwon SY, Kim HJ, Kwon KY, Cho CH, Lee JM, Kallakury BV, Bae I. Nuclear factor (erythroid-derived 2)-like 2 regulates drug resistance in pancreatic cancer cells. *Pancreas* 2010; **39**: 463-472 [PMID: 20118824 DOI: 10.1097/MPA.0b013e3181c31314]

45 **Lister A**, Nedjadi T, Kitteringham NR, Campbell F, Costello E, Lloyd B, Copple IM, Williams S, Owen A, Neoptolemos JP, Goldring CE, Park BK. Nrf2 is overexpressed in pancreatic cancer: implications for cell proliferation and therapy. *Mol Cancer* 2011; **10**: 37 [PMID: 21489257 DOI: 10.1186/1476-4598-10-37]

46 **You A**, Nam CW, Wakabayashi N, Yamamoto M, Kensler TW, Kwak MK. Transcription factor Nrf2 maintains the basal expression of Mdm2: An implication of the regulation of p53 signaling by Nrf2. *Arch Biochem Biophys* 2011; **507**: 356-364 [PMID: 21211512 DOI: 10.1016/j.abb.2010.12.034]

47 **Hamada S**, Shimosegawa T, Taguchi K, Nabeshima T, Yamamoto M, Masamune A. Simultaneous K-ras activation and Keap1 deletion cause atrophy of pancreatic parenchyma. *Am J Physiol Gastrointest Liver Physiol* 2018; **314**: G65-G74 [PMID: 28971839 DOI: 10.1152/ajpgi.00228.2017]

48 **Hamada S**, Taguchi K, Masamune A, Yamamoto M, Shimosegawa T. Nrf2 promotes mutant K-ras/p53-driven pancreatic carcinogenesis. *Carcinogenesis* 2017; **38**: 661-670 [PMID: 29240881 DOI: 10.1093/carcin/bgx043]

49 **Mathew A**, Mathur SK, Morimoto RI. Heat shock response and protein degradation: regulation of HSF2 by the ubiquitin-proteasome pathway. *Mol Cell Biol* 1998; **18**: 5091-5098 [PMID: 9710593 DOI: 10.1128/MCB.18.9.5091]

50 **Anckar J**, Sistonen L. Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu Rev Biochem* 2011; **80**: 1089-1115 [PMID: 21417720 DOI: 10.1146/annurev-biochem-060809-095203]

51 **Dong B**, Jaeger AM, Thiele DJ. Inhibiting Heat Shock Factor 1 in Cancer: A Unique Therapeutic Opportunity. *Trends Pharmacol Sci* 2019; **40**: 986-1005 [PMID: 31727393 DOI: 10.1016/j.tips.2019.10.008]

52 **Puustinen MC**, Sistonen L. Molecular Mechanisms of Heat Shock Factors in Cancer. *Cells* 2020; **9** [PMID: 32408596 DOI: 10.3390/cells9051202]

53 **Pincus D**. Regulation of Hsf1 and the Heat Shock Response. *Adv Exp Med Biol* 2020; **1243**: 41-50 [PMID: 32297210 DOI: 10.1007/978-3-030-40204-4_3]

54 **Björk JK**, Sistonen L. Clustering of heat-shock factors. *Biochem J* 2006; **395**: e5-e6 [PMID: 16541486 DOI: 10.1042/bj20060071]

55 **Loison F**, Debure L, Nizard P, le Goff P, Michel D, le Dréan Y. Up-regulation of the clusterin gene after proteotoxic stress: implication of HSF1-HSF2 heterocomplexes. *Biochem J* 2006; **395**: 223-231 [PMID: 16336210 DOI: 10.1042/BJ20051190]

56 **Santopolo S**, Riccio A, Rossi A, Santoro MG. The proteostasis guardian HSF1 directs the transcription of its paralog and interactor HSF2 during proteasome dysfunction. *Cell Mol Life Sci* 2021; **78**: 1113-1129 [PMID: 32607595 DOI: 10.1007/s00018-020-03568-x]

57 **Lee YJ**, Kim EH, Lee JS, Jeoung D, Bae S, Kwon SH, Lee YS. HSF1 as a mitotic regulator: phosphorylation of HSF1 by Plk1 is essential for mitotic progression. *Cancer Res* 2008; **68**: 7550-7560 [PMID: 18794143 DOI: 10.1158/0008-5472.CAN-08-0129]

58 **Kim EH**, Lee YJ, Bae S, Lee JS, Kim J, Lee YS. Heat shock factor 1-mediated aneuploidy requires a defective function of p53. *Cancer Res* 2009; **69**: 9404-9412 [PMID: 19934326 DOI: 10.1158/0008-5472.CAN-09-1411]

59 **Frezzato F**, Raggi F, Martini V, Severin F, Trimarco V, Visentin A, Scomazzon E, Accordi B, Bresolin S, Piazza F, Facco M, Basso G, Semenzato G, Trentin L. HSP70/HSF1 axis, regulated *via* a PI3K/AKT pathway, is a druggable target in chronic lymphocytic leukemia. *Int J Cancer* 2019; **145**: 3089-3100 [PMID: 31044428 DOI: 10.1002/ijc.32383]

60 **Joutsen J**, Da Silva AJ, Luoto JC, Budzynski MA, Nylund AS, de Thonel A, Concordet JP, Mezger V, Sabéran-Djoneidi D, Henriksson E, Sistonen L. Heat Shock Factor 2 Protects against Proteotoxicity by Maintaining Cell-Cell Adhesion. *Cell Rep* 2020; **30**: 583-597.e6 [PMID: 31940498 DOI: 10.1016/j.celrep.2019.12.037]

61 **Wang B**, Lee CW, Witt A, Thakkar A, Ince TA. Heat shock factor 1 induces cancer stem cell phenotype in breast cancer cell lines. *Breast Cancer Res Treat* 2015; **153**: 57-66 [PMID: 26223813 DOI: 10.1007/s10549-015-3521-1]

62 **Ma S**, Kong D, Fu X, Liu L, Liu Y, Xue C, Tian Z, Li L, Liu X. p53-Induced Autophagy Regulates Chemotherapy and Radiotherapy Resistance in Multidrug Resistance Cancer Cells. *Dose Response* 2021; **19**: 15593258211048046 [PMID: 34646092 DOI: 10.1177/15593258211048046]

63 **Kaushik S**, Tasset I, Arias E, Pampliega O, Wong E, Martinez-Vicente M, Cuervo AM. Autophagy and the hallmarks of aging. *Ageing Res Rev* 2021; **72**: 101468 [PMID: 34563704 DOI: 10.1016/j.arr.2021.101468]

64 **Wu PK**, Hong SK, Starenki D, Oshima K, Shao H, Gestwicki JE, Tsai S, Park JI. Mortalin/HSPA9 targeting selectively induces KRAS tumor cell death by perturbing mitochondrial membrane permeability. *Oncogene* 2020; **39**: 4257-4270 [PMID: 32291414 DOI: 10.1038/s41388-020-1285-5]

65 **Oldfield AJ**, Henriques T, Kumar D, Burkholder AB, Cinghu S, Paulet D, Bennett BD, Yang P, Scruggs BS, Lavender CA, Rivals E, Adelman K, Jothi R. NF-Y controls fidelity of transcription

initiation at gene promoters through maintenance of the nucleosome-depleted region. *Nat Commun* 2019; **10**: 3072 [PMID: 31296853 DOI: 10.1038/s41467-019-10905-7]

66 **Gurtner A**, Manni I, Piaggio G. NF-Y in cancer: Impact on cell transformation of a gene essential for proliferation. *Biochim Biophys Acta Gene Regul Mech* 2017; **1860**: 604-616 [PMID: 27939755 DOI: 10.1016/j.bbagrm.2016.12.005]

67 **Ly LL**, Yoshida H, Yamaguchi M. Nuclear transcription factor Y and its roles in cellular processes related to human disease. *Am J Cancer Res* 2013; **3**: 339-346 [PMID: 23977444]

68 **Chae HD**, Kim J, Shin DY. NF-Y binds to both G1- and G2-specific cyclin promoters; a possible role in linking CDK2/Cyclin A to CDK1/Cyclin B. *BMB Rep* 2011; **44**: 553-557 [PMID: 21871181 DOI: 10.5483/bmbrep.2011.44.8.553]

69 **Yun J**, Chae HD, Choy HE, Chung J, Yoo HS, Han MH, Shin DY. p53 negatively regulates cdc2 transcription *via* the CCAAT-binding NF-Y transcription factor. *J Biol Chem* 1999; **274**: 29677-29682 [PMID: 10514438 DOI: 10.1074/jbc.274.42.29677]

70 **Xu H**, Fu J, Ha SW, Ju D, Zheng J, Li L, Xie Y. The CCAAT box-binding transcription factor NF-Y regulates basal expression of human proteasome genes. *Biochim Biophys Acta* 2012; **1823**: 818-825 [PMID: 22285817 DOI: 10.1016/j.bbamcr.2012.01.002]

71 **Benatti P**, Chiaramonte ML, Lorenzo M, Hartley JA, Hochhauser D, Gnesutta N, Mantovani R, Imbriano C, Dolfini D. NF-Y activates genes of metabolic pathways altered in cancer cells. *Oncotarget* 2016; **7**: 1633-1650 [PMID: 26646448 DOI: 10.18632/oncotarget.6453]

72 **Weissmueller S**, Manchado E, Saborowski M, Morris JP 4th, Wagenblast E, Davis CA, Moon SH, Pfister NT, Tschaharganeh DF, Kitzing T, Aust D, Markert EK, Wu J, Grimmond SM, Pilarsky C, Prives C, Biankin AV, Lowe SW. Mutant p53 drives pancreatic cancer metastasis through cell-autonomous PDGF receptor β signaling. *Cell* 2014; **157**: 382-394 [PMID: 24725405 DOI: 10.1016/j.cell.2014.01.066]

73 **Tanaka T**, Ohashi S, Saito H, Higuchi T, Tabata K, Kosuge Y, Suzuki T, Miyairi S, Kobayashi S. Indirubin derivatives alter DNA binding activity of the transcription factor NF-Y and inhibit MDR1 gene promoter. *Eur J Pharmacol* 2014; **741**: 83-89 [PMID: 25066113 DOI: 10.1016/j.ejphar.2014.07.035]

74 **Gayvert KM**, Dardenne E, Cheung C, Boland MR, Lorberbaum T, Wanjala J, Chen Y, Rubin MA, Tatonetti NP, Rickman DS, Elemento O. A Computational Drug Repositioning Approach for Targeting Oncogenic Transcription Factors. *Cell Rep* 2016; **15**: 2348-2356 [PMID: 27264179 DOI: 10.1016/j.celrep.2016.05.037]

75 **Fruman DA**, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K Pathway in Human Disease. *Cell* 2017; **170**: 605-635 [PMID: 28802037 DOI: 10.1016/j.cell.2017.07.029]

76 **Lanfredini S**, Thapa A, O'Neill E. RAS in pancreatic cancer. *Biochem Soc Trans* 2019; **47**: 961-972 [PMID: 31341034 DOI: 10.1042/BST20170521]

77 **Antontseva EV**, Matveeva MY, Bondar NP, Kashina EV, Leberfarb EY, Bryzgalov LO, Gervas PA, Ponomareva AA, Cherdyntseva NV, Orlov YL, Merkulova TI. Regulatory single nucleotide polymorphisms at the beginning of intron 2 of the human KRAS gene. *J Biosci* 2015; **40**: 873-883 [PMID: 26648033 DOI: 10.1007/s12038-015-9567-8]

78 **Mann KM**, Ying H, Juan J, Jenkins NA, Copeland NG. KRAS-related proteins in pancreatic cancer. *Pharmacol Ther* 2016; **168**: 29-42 [PMID: 27595930 DOI: 10.1016/j.pharmthera.2016.09.003]

79 **Qian W**, Chen K, Qin T, Xiao Y, Li J, Yue Y, Zhou C, Ma J, Duan W, Lei J, Han L, Li L, Shen X, Wu Z, Ma Q, Wang Z. The EGFR-HSF1 axis accelerates the tumorigenesis of pancreatic cancer. Journal of experimental & clinical cancer research : CR, 2021; 40: 25-25 [DOI: 10.21203/rs.3.rs-53955/v2]

80 **Ciocca DR**, Arrigo AP, Calderwood SK. Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. *Arch Toxicol* 2013; **87**: 19-48 [PMID: 22885793 DOI: 10.1007/s00204-012-0918-z]

81 **Tang Z**, Dai S, He Y, Doty RA, Shultz LD, Sampson SB, Dai C. MEK guards proteome stability and inhibits tumor-suppressive amyloidogenesis *via* HSF1. *Cell* 2015; **160**: 729-744 [PMID: 25679764 DOI: 10.1016/j.cell.2015.01.028]

82 **Chen S**, Blank JL, Peters T, Liu XJ, Rappoli DM, Pickard MD, Menon S, Yu J, Driscoll DL, Lingaraj T, Burkhardt AL, Chen W, Garcia K, Sappal DS, Gray J, Hales P, Leroy PJ, Ringeling J, Rabino C, Spelman JJ, Morgenstern JP, Lightcap ES. Genome-wide siRNA screen for modulators of cell death induced by proteasome inhibitor bortezomib. *Cancer Res* 2010; **70**: 4318-4326 [PMID: 20460535 DOI: 10.1158/0008-5472.CAN-09-4428]

83 **Campaner S**, Doni M, Hydbring P, Verrecchia A, Bianchi L, Sardella D, Schleker T, Perna D, Tronnersjö S, Murga M, Fernandez-Capetillo O, Barbacid M, Larsson LG, Amati B. Cdk2 suppresses cellular senescence induced by the c-myc oncogene. *Nat Cell Biol* 2010; **12**: 54-9; sup pp 1 [PMID: 20010815 DOI: 10.1038/ncb2004]

84 **Gilardini Montani MS**, Cecere N, Granato M, Romeo MA, Falcinelli L, Ciciarelli U, D'Orazi G, Faggioni A, Cirone M. Mutant p53, Stabilized by Its Interplay with HSP90, Activates a Positive Feed-Back Loop Between NRF2 and p62 that Induces Chemo-Resistance to Apigenin in Pancreatic Cancer Cells. *Cancers (Basel)* 2019; **11** [PMID: 31121848 DOI: 10.3390/cancers11050703]

85 **Lisek K**, Campaner E, Ciani Y, Walerych D, Del Sal G. Mutant p53 tunes the NRF2-dependent antioxidant response to support survival of cancer cells. *Oncotarget* 2018; **9**: 20508-20523 [PMID: 29755668 DOI: 10.18632/oncotarget.24974]

86 **Walerych D**, Lisek K, Sommaggio R, Piazza S, Ciani Y, Dalla E, Rajkowska K, Gaweda-Walerych

K, Ingallina E, Tonelli C, Morelli MJ, Amato A, Eterno V, Zambelli A, Rosato A, Amati B, Wiśniewski JR, Del Sal G. Proteasome machinery is instrumental in a common gain-of-function program of the p53 missense mutants in cancer. *Nat Cell Biol* 2016; **18**: 897-909 [PMID: 27347849 DOI: 10.1038/ncb3380]

87 **Li D**, Yallowitz A, Ozog L, Marchenko N. A gain-of-function mutant p53-HSF1 feed forward circuit governs adaptation of cancer cells to proteotoxic stress. *Cell Death Dis* 2014; **5**: e1194 [PMID: 24763051 DOI: 10.1038/cddis.2014.158]

88 **Li D**, Marchenko ND, Schulz R, Fischer V, Velasco-Hernandez T, Talos F, Moll UM. Functional inactivation of endogenous MDM2 and CHIP by HSP90 causes aberrant stabilization of mutant p53 in human cancer cells. *Mol Cancer Res* 2011; **9**: 577-588 [PMID: 21478269 DOI: 10.1158/1541-7786.MCR-10-0534]

89 **Li Q**, Feldman RA, Radhakrishnan VM, Carey S, Martinez JD. Hsf1 is required for the nuclear translocation of p53 tumor suppressor. *Neoplasia* 2008; **10**: 1138-1145 [PMID: 18813348 DOI: 10.1593/neo.08430]

90 **Toma-Jonik A**, Vydra N, Janus P, Widłak W. Interplay between HSF1 and p53 signaling pathways in cancer initiation and progression: non-oncogene and oncogene addiction. *Cell Oncol (Dordr)* 2019; **42**: 579-589 [PMID: 31183804 DOI: 10.1007/s13402-019-00452-0]

91 **Di Agostino S**, Strano S, Emiliozzi V, Zerbini V, Mottolese M, Sacchi A, Blandino G, Piaggio G. Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell* 2006; **10**: 191-202 [PMID: 16959611 DOI: 10.1016/j.ccr.2006.08.013]

92 **Peart MJ**, Prives C. Mutant p53 gain of function: the NF-Y connection. *Cancer Cell* 2006; **10**: 173-174 [PMID: 16959607 DOI: 10.1016/j.ccr.2006.08.014]

93 **Xia X**, Wu W, Huang C, Cen G, Jiang T, Cao J, Huang K, Qiu Z. SMAD4 and its role in pancreatic cancer. *Tumour Biol* 2015; **36**: 111-119 [PMID: 25464861 DOI: 10.1007/s13277-014-2883-z]

94 **Saiki Y**, Horii A. Molecular pathology of pancreatic cancer. *Pathol Int* 2014; **64**: 10-19 [PMID: 24471965 DOI: 10.1111/pin.12114]

95 **Biswas S**, Criswell TL, Wang SE, Arteaga CL. Inhibition of transforming growth factor-beta signaling in human cancer: targeting a tumor suppressor network as a therapeutic strategy. *Clin Cancer Res* 2006; **12**: 4142-4146 [PMID: 16857784 DOI: 10.1158/1078-0432.CCR-06-0952]

96 **Jakowlew SB**. Transforming growth factor-beta in cancer and metastasis. *Cancer Metastasis Rev* 2006; **25**: 435-457 [PMID: 16951986 DOI: 10.1007/s10555-006-9006-2]

97 **Vivekanandhan S**, Mukhopadhyay D. Genetic status of KRAS influences Transforming Growth Factor-beta (TGF-β) signaling: An insight into Neuropilin-1 (NRP1) mediated tumorigenesis. *Semin Cancer Biol* 2019; **54**: 72-79 [PMID: 29409705 DOI: 10.1016/j.semcancer.2018.01.014]

98 **Demagny H**, De Robertis EM. Point mutations in the tumor suppressor Smad4/DPC4 enhance its phosphorylation by GSK3 and reversibly inactivate TGF-β signaling. *Mol Cell Oncol* 2016; **3**: e1025181 [PMID: 27308538 DOI: 10.1080/23723556.2015.1025181]

99 **Imamichi Y**, Menke A. Signaling pathways involved in collagen-induced disruption of the E-cadherin complex during epithelial-mesenchymal transition. *Cells Tissues Organs* 2007; **185**: 180-190 [PMID: 17587824 DOI: 10.1159/000101319]

100 **Arfmann-Knübel S**, Struck B, Genrich G, Helm O, Sipos B, Sebens S, Schäfer H. The Crosstalk between Nrf2 and TGF-β1 in the Epithelial-Mesenchymal Transition of Pancreatic Duct Epithelial Cells. *PLoS One* 2015; **10**: e0132978 [PMID: 26226105 DOI: 10.1371/journal.pone.0132978]

101 **Roy SK**, Wang J, Yang P. Dexamethasone inhibits transforming growth factor-beta receptor (Tbeta R) messenger RNA expression in hamster preantral follicles: possible association with NF-YA. *Biol Reprod* 2003; **68**: 2180-2188 [PMID: 12606399 DOI: 10.1095/biolreprod.102.013011]

102 **Eggen BJ**, Benus GF, Folkertsma S, Jonk LJ, Kruijer W. TAK1 activation of the mouse JunB promoter is mediated through a CCAAT box and NF-Y. *FEBS Lett* 2001; **506**: 267-271 [PMID: 11602259 DOI: 10.1016/s0014-5793(01)02928-3]

103 **Dardare J**, Witz A, Merlin JL, Gilson P, Harlé A. SMAD4 and the TGFβ Pathway in Patients with Pancreatic Ductal Adenocarcinoma. *Int J Mol Sci* 2020; **21** [PMID: 32429474 DOI: 10.3390/ijms21103534]

104 **Voutsadakis IA**. Ubiquitin, ubiquitination and the ubiquitin-proteasome system in cancer. Atlas of Genetics and Cytogenetics in Oncology and Haematology 2011 [DOI: 10.4267/2042/44905]

105 **Cisowski J**, Sayin VI, Liu M, Karlsson C, Bergo MO. Oncogene-induced senescence underlies the mutual exclusive nature of oncogenic KRAS and BRAF. *Oncogene* 2016; **35**: 1328-1333 [PMID: 26028035 DOI: 10.1038/onc.2015.186]

106 **El Maï M**, Marzullo M, de Castro IP, Ferreira MG. Opposing p53 and mTOR/AKT promote an in vivo switch from apoptosis to senescence upon telomere shortening in zebrafish. *Elife* 2020; **9** [PMID: 32427102 DOI: 10.7554/eLife.54935]

107 **Kohonen-Corish MR**, Tseung J, Chan C, Currey N, Dent OF, Clarke S, Bokey L, Chapuis PH. KRAS mutations and CDKN2A promoter methylation show an interactive adverse effect on survival and predict recurrence of rectal cancer. *Int J Cancer* 2014; **134**: 2820-2828 [PMID: 24259266 DOI: 10.1002/ijc.28619]

108 **Singh SK**, Ellenrieder V. Senescence in pancreatic carcinogenesis: from signalling to chromatin remodelling and epigenetics. *Gut* 2013; **62**: 1364-1372 [PMID: 23408353 DOI: 10.1136/gutjnl-2012-302793]

109 **Qie S**, Diehl JA. Cyclin D degradation by E3 ligases in cancer progression and treatment. *Semin*

Cancer Biol 2020; **67**: 159-170 [PMID: 32006569 DOI: 10.1016/j.semcancer.2020.01.012]

110 **Chen D**, Tavana O, Chu B, Erber L, Chen Y, Baer R, Gu W. NRF2 Is a Major Target of ARF in p53-Independent Tumor Suppression. *Mol Cell* 2017; **68**: 224-232.e4 [PMID: 28985506 DOI: 10.1016/j.molcel.2017.09.009]

111 **Chang ZF**, Huang DY, Hu SF. NF-Y-mediated trans-activation of the human thymidine kinase promoter is closely linked to activation of cyclin-dependent kinase. *J Cell Biochem* 1999; **75**: 300-309 [PMID: 10502302]

112 **LaPak KM**, Burd CE. The molecular balancing act of p16(INK4a) in cancer and aging. *Mol Cancer Res* 2014; **12**: 167-183 [PMID: 24136988 DOI: 10.1158/1541-7786.MCR-13-0350]

113 **Hayashi Y**, Hsiao EC, Sami S, Lancero M, Schlieve CR, Nguyen T, Yano K, Nagahashi A, Ikeya M, Matsumoto Y, Nishimura K, Fukuda A, Hisatake K, Tomoda K, Asaka I, Toguchida J, Conklin BR, Yamanaka S. BMP-SMAD-ID promotes reprogramming to pluripotency by inhibiting p16/INK4A-dependent senescence. *Proc Natl Acad Sci U S A* 2016; **113**: 13057-13062 [PMID: 27794120 DOI: 10.1073/pnas.1603668113]

114 **Schlitter AM**, Segler A, Steiger K, Michalski CW, Jäger C, Konukiewitz B, Pfarr N, Endris V, Bettstetter M, Kong B, Regel I, Kleeff J, Klöppel G, Esposito I. Molecular, morphological and survival analysis of 177 resected pancreatic ductal adenocarcinomas (PDACs): Identification of prognostic subtypes. *Sci Rep* 2017; **7**: 41064 [PMID: 28145465 DOI: 10.1038/srep41064]

115 **Kneissig M**, Bernhard S, Storchova Z. Modelling chromosome structural and copy number changes to understand cancer genomes. *Curr Opin Genet Dev* 2019; **54**: 25-32 [PMID: 30921673 DOI: 10.1016/j.gde.2019.02.005]

116 **Jang J**, Wang Y, Kim HS, Lalli MA, Kosik KS. Nrf2, a regulator of the proteasome, controls self-renewal and pluripotency in human embryonic stem cells. *Stem Cells* 2014; **32**: 2616-2625 [PMID: 24895273 DOI: 10.1002/stem.1764]

117 **McKenna DJ**, Nazarali AJ, Himeno A, Saavedra JM. Chronic treatment with (+/-)DOI, a psychotomimetic 5-HT2 agonist, downregulates 5-HT2 receptors in rat brain. *Neuropsychopharmacology* 1989; **2**: 81-87 [PMID: 2803482 DOI: 10.1016/0893-133x(89)90010-9]

118 **Hou P**, Ma X, Zhang Q, Wu CJ, Liao W, Li J, Wang H, Zhao J, Zhou X, Guan C, Ackroyd J, Jiang S, Zhang J, Spring DJ, Wang YA, DePinho RA. USP21 deubiquitinase promotes pancreas cancer cell stemness *via* Wnt pathway activation. *Genes Dev* 2019; **33**: 1361-1366 [PMID: 31488580 DOI: 10.1101/gad.326314.119]

119 **Kumar SK**, Callander NS, Hillengass J, Liedtke M, Baljevic M, Campagnaro E, Castillo JJ, Chandler JC, Cornell RF, Costello C, Efebera Y, Faiman M, Garfall A, Godby K, Holmberg L, Htut M, Huff CA, Kang Y, Landgren O, Malek E, Martin T, Omel J, Raje N, Sborov D, Singhal S, Stockerl-Goldstein K, Tan C, Weber D, Johnson-Chilla A, Keller J, Kumar R. NCCN Guidelines Insights: Multiple Myeloma, Version 1.2020. *J Natl Compr Canc Netw* 2019; **17**: 1154-1165 [PMID: 31590151 DOI: 10.6004/jnccn.2019.0049]

120 **Alberts SR**, Foster NR, Morton RF, Kugler J, Schaefer P, Wiesenfeld M, Fitch TR, Steen P, Kim GP, Gill S. PS-341 and gemcitabine in patients with metastatic pancreatic adenocarcinoma: a North Central Cancer Treatment Group (NCCTG) randomized phase II study. *Ann Oncol* 2005; **16**: 1654-1661 [PMID: 16085692 DOI: 10.1093/annonc/mdi324]

121 **Lankes K**, Hassan Z, Doffo MJ, Schneeweis C, Lier S, Öllinger R, Rad R, Krämer OH, Keller U, Saur D, Reichert M, Schneider G, Wirth M. Targeting the ubiquitin-proteasome system in a pancreatic cancer subtype with hyperactive MYC. *Mol Oncol* 2020; **14**: 3048-3064 [PMID: 33099868 DOI: 10.1002/1878-0261.12835]

122 **Kim EJ**, Kim YJ, Lee HI, Jeong SH, Nam HJ, Cho JH. NRF2 Knockdown Resensitizes 5-Fluorouracil-Resistant Pancreatic Cancer Cells by Suppressing HO-1 and ABCG2 Expression. *Int J Mol Sci* 2020; **21** [PMID: 32629871 DOI: 10.3390/ijms21134646]

123 **Babiker HM**, Karass M, Recio-Boiles A, Chandana SR, McBride A, Mahadevan D. Everolimus for the treatment of advanced pancreatic ductal adenocarcinoma (PDAC). *Expert Opin Investig Drugs* 2019; **28**: 583-592 [PMID: 31215251 DOI: 10.1080/13543784.2019.1632289]

124 **Royce M**, Bachelot T, Villanueva C, Özgüroglu M, Azevedo SJ, Cruz FM, Debled M, Hegg R, Toyama T, Falkson C, Jeong J, Srimuninnimit V, Gradishar WJ, Arce C, Ridolfi A, Lin C, Cardoso F. Everolimus Plus Endocrine Therapy for Postmenopausal Women With Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: A Clinical Trial. *JAMA Oncol* 2018; **4**: 977-984 [PMID: 29566104 DOI: 10.1001/jamaoncol.2018.0060]

125 **Wolpin BM**, Hezel AF, Abrams T, Blaszkowsky LS, Meyerhardt JA, Chan JA, Enzinger PC, Allen B, Clark JW, Ryan DP, Fuchs CS. Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 193-198 [PMID: 19047305 DOI: 10.1200/JCO.2008.18.9514]

126 **Kordes S**, Richel DJ, Klümpen HJ, Weterman MJ, Stevens AJ, Wilmink JW. A phase I/II, non-randomized, feasibility/safety and efficacy study of the combination of everolimus, cetuximab and capecitabine in patients with advanced pancreatic cancer. *Invest New Drugs* 2013; **31**: 85-91 [PMID: 22367239 DOI: 10.1007/s10637-012-9802-1]

127 **Javle MM**, Shroff RT, Xiong H, Varadhachary GA, Fogelman D, Reddy SA, Davis D, Zhang Y, Wolff RA, Abbruzzese JL. Inhibition of the mammalian target of rapamycin (mTOR) in advanced pancreatic cancer: results of two phase II studies. *BMC Cancer* 2010; **10**: 368 [PMID: 20630061 DOI: 10.1186/1471-2407-10-368]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 55-74

DOI: 10.4251/wjgo.v14.i1.55

ISSN 1948-5204 (online)

REVIEW

Effects of *Helicobacter pylori* infection in gastrointestinal tract malignant diseases: From the oral cavity to rectum

Yang-Che Kuo, Lo-Yip Yu, Horng-Yuan Wang, Ming-Jen Chen, Ming-Shiang Wu, Chun-Jen Liu, Ying-Chun Lin, Shou-Chuan Shih, Kuang-Chun Hu

ORCID number: Yang-Che Kuo 0000-0003-0919-5804; Lo-Yip Yu 0000-0003-2394-8200; Horng-Yuan Wang 0000-0003-3313-5879; Ming-Jen Chen 0000-0001-5849-4259; Ming-Shiang Wu 0000-0002-1940-6428; Chun-Jen Liu 0000-0002-6202-0993; Ying-Chun Lin 0000-0002-8629-5449; Shou-Chuan Shih 0000-0002-3826-6678; Kuang-Chun Hu 0000-0001-7127-4015.

Author contributions: Wang HY, Chen MJ, Wu MS, Liu CJ, Lin YC and Shih SC performed literature research; Kuo YC, Yu LY, Lin YC and Hu KC wrote the manuscript and performed the revision and approval of the final version; Hu KC designed research, coordinated and corrected the writing of the paper.

Conflict-of-interest statement: The authors deny any conflict of interest.

Supported by 108DMH0100146 and 108WHK0910031.

Country/Territory of origin: Taiwan

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Yang-Che Kuo, Horng-Yuan Wang, Ming-Jen Chen, Division of Gastroenterology, Department of Internal Medicine, MacKay Memorial Hospital, Taipei 10449, Taiwan

Lo-Yip Yu, Department of Internal Medicine, Healthy Evaluation Center, Mackay Memorial Hospital, Taipei 10449, Taiwan

Ming-Shiang Wu, Department of Internal Medicine, National Taiwan University Hospital, Taipei 10051, Taiwan

Chun-Jen Liu, Department of Internal Medicine, Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei 10051, Taiwan

Ying-Chun Lin, Department of Anesthesia, MacKay Memorial Hospital, Taipei 10449, Taiwan

Shou-Chuan Shih, Division of Gastroenterology, Department of Internal Medicine, Health Evaluate Center, Mackay Memorial Hospital, Taipei 10449, Taiwan

Kuang-Chun Hu, Department of Internal Medicine, Healthy Evaluation Center, Mackay Memorial Hospital, MacKay Junior College of Medicine, Nursing, and Management, Taipei 10038, Taiwan

Corresponding author: Kuang-Chun Hu, MD, PhD, Attending Doctor, Department of Internal Medicine, Healthy Evaluation Center, Mackay Memorial Hospital, MacKay Junior College of Medicine, Nursing, and Management, No. 92, Sec. 2, Chung-Shan North Road, Taipei 10038, Taiwan. mimiandbear2001@yahoo.com.tw

Abstract

Helicobacter pylori (*H. pylori*) has infected approximately fifty percent of humans for a long period of time. However, improvements in the public health environment have led to a decreased chance of *H. pylori* infection. However, a high infection rate is noted in populations with a high incidence rate of gastric cancer (GC). The worldwide fraction of GC attributable to *H. pylori* is greater than 85%, and a high *H. pylori* prevalence is noted in gastric mucosa-associated lymphoid tissue lymphoma patients. These results indicate that the majority of GC cases can be prevented if *H. pylori* infection is eliminated. Because *H. pylori* exhibits oral-oral or fecal-oral transmission, the relationship between this microorganism and other digestive tract malignant diseases has also attracted attention. This review article provides an overview of *H. pylori* and the condition of the whole gastrointestinal

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 7, 2021
Peer-review started: March 7, 2021
First decision: April 19, 2021
Revised: May 3, 2021
Accepted: December 9, 2021
Article in press: December 9, 2021
Published online: January 15, 2022

P-Reviewer: Pichon M
S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

tract environment to further understand the correlation between the pathogen and the host, thus allowing improved realization of disease presentation.

Key Words: *Helicobacter pylori*; Gastrointestinal tract; Malignant disease

Core Tip: *Helicobacter pylori* (*H. pylori*) has infected approximately fifty percent of humans for a long period of time. However, improvements in the public health environment have led to a decreased chance of *H. pylori* infection. This review article provides an overview of the correlation of *H. pylori* infection and gastrointestinal tract malignant diseases. Based on data on *H. pylori*, we believe that the digestive tract microenvironment and *H. pylori* motility affect the risk of cancer formation by *H. pylori* infection.

Citation: Kuo YC, Yu LY, Wang HY, Chen MJ, Wu MS, Liu CJ, Lin YC, Shih SC, Hu KC. Effects of *Helicobacter pylori* infection in gastrointestinal tract malignant diseases: From the oral cavity to rectum. *World J Gastrointest Oncol* 2022; 14(1): 55-74
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/55.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.55

INTRODUCTION

According to 2020 Global Cancer Observatory data, seven of the top twenty cancers with the highest cumulative risk of incidence affect the gastrointestinal system, including oral cavity, laryngeal-pharynx, esophagus, stomach, pancreas, liver and colorectal cancers (CRC). In 2020, gastrointestinal oncology diseases accounted for greater than 26% of all cancers worldwide. With the exception of pancreatic cancer and liver cancer, other cancer tracts were interlinked (Figure 1)[1]. Three major risk factors are thought to be related to cancers: Obesity, infection and ultraviolet radiation. Several infections are considered to be related to cancer formation, including infection by *Helicobacter pylori* (*H. pylori*), human papillomavirus (HPV), and hepatitis B and C viruses, and these different infectious agents account for greater than 90% of infection-related cancers worldwide[2]. *H. pylori* was identified as an origin of peptic ulcer disease and has become an important public health issue worldwide since 1982. With different geographic areas, ages, ethnicities and socioeconomic statuses, the prevalence rates of *H. pylori* infection are also different[3,4]. Approximately 50% of people worldwide are infected by this bacterium. At the beginning of the 21st century, the prevalence was reduced in highly industrialized countries of the Western world. In contrast, the prevalence remains high in developing and newly industrialized countries. The discrepancy in prevalence may result from the degree of urbanization, sanitation, access to clean water, and socioeconomic status[5].

According to the International Agency for Research on Cancer, *H. pylori* is a human carcinogen highly correlated with gastric cancer (GC)[6]. *H. pylori* also accounted for approximately 810000 infection-related cancer cases, which is greater than that reported for any other microorganism, in 2018[2].

Because *H. pylori* exhibits oral-oral or fecal-oral transmission and the whole gastrointestinal tract is connected, we further surveyed the role of *H. pylori* in different gastrointestinal malignant diseases to provide a better understanding of the relationship between *H. pylori* infection and malignant diseases. GC was highly correlated to *H. pylori* infection, but the relationship between *H. pylori* and cancer formation in other gastrointestinal tract malignant diseases, such as oral cavity cancer, laryngeal cancer, esophageal cancer, and colon cancer, has not been completely studied. Recent studies have shown some association between GC and *H. pylori* infection but lack a further overview of *H. pylori* infection and malignant diseases of the whole gastrointestinal tract. Past studies used the host viewpoint and examined which pathogen could cause disease in the host. This review article used the viewpoint of microorganisms and focused on the effects of *H. pylori* infection in gastrointestinal tract malignant diseases from the oral cavity to the rectum to further realize the

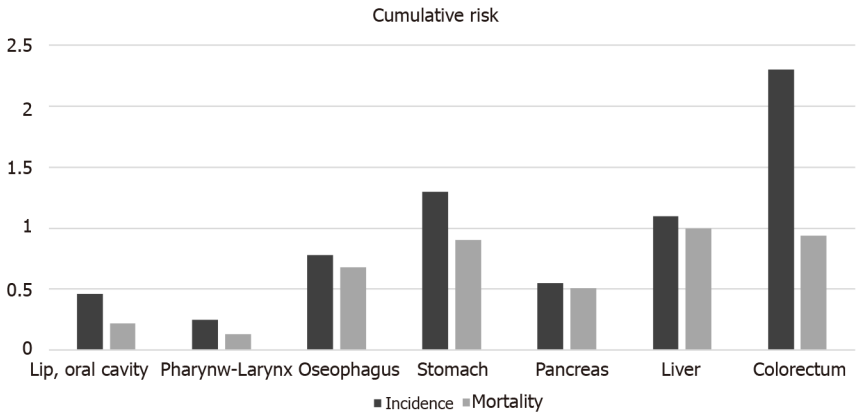


Figure 1 Estimated cumulative risk of incidence and mortality of gastroenterology tract malignancy disease in 2020, both sexes, ages 0-74 (reproduced from http://globocan.iarc.fr/).

connection between infectious and malignant diseases.

ORAL CANCER

Epidemiology of oral cancer

Oral cancer represents approximately 3% of all cancers worldwide and is the 6th most common cancer globally. As a popular habit in Asian countries, betel quid chewing is associated with periodontal disease, oral submucous fibrosis, and oral cancer[7,8]. Compared with other tumors in the oral cavity, oral squamous cell carcinoma (OSCC) tends to exhibit local invasion and metastasis[9]. In addition, OSCC occurs more frequently in middle-aged and older populations, particularly in men[10].

Pathological differences in oral cancer

Constituting 94% of oral malignancies, OSCC is far more common than the remaining malignancies, including salivary gland cancer, soft tissue sarcoma, jaw osteosarcoma, non-Hodgkin's lymphoma, melanomas and metastatic tumors, in the oral cavity[11, 12].

Role of H. pylori in oral cancer

As a Class I carcinogen, the role of *H. pylori* in oral cancer is not yet clear. Whether the colonization of *H. pylori* is facilitated by betel chewing-related lesions or the resulting chemical changes in the oral cavity remains an important issue to be studied. By comparing the prevalence of *H. pylori* in patients with oral cancer and healthy controls with different betel chewing statuses (Table 1), Fernando *et al*[13] noticed a significantly higher rate of infection among betel chewers regardless of the cancer status. Thus, betel chewing, not oral cancer, is a potential contributing factor to *H. pylori* infection.

Few studies have shown the association between *H. pylori* and oral cancer. Grandis *et al*[14] reported a similar seroprevalence of *H. pylori* in 21 patients with oral cancer and 21 controls; thus, the association could not be proven. Another study adopted polymerase chain reaction (PCR) and culture techniques to identify the existence of *H. pylori* in serum and tissue samples and reported insignificant differences in the prevalence of *H. pylori* between patients with oral cancer and controls. Nevertheless, the odds ratio (OR) was 3.0 [95% confidence interval (CI): 0.34-26.4] by culture and 1.5 (95%CI: 0.28-8.0) by PCR[15]. Only a few studies have attempted to examine the presence of *H. pylori* in OSCC[16]. Due to conflicting results, the relationship between *H. pylori* and OSCC cannot be concluded. The variable results may be caused by differences in methodology, specifically the disparity in the sensitivity and specificity of diagnostic methods. Using the three detection methods [*H. pylori* immunoglobin (Ig) G antibodies, PCR, and histochemical staining], Meng *et al*[17] suggested an inverse association between *H. pylori* infection and OSCC in the subgroup of individuals over 60 years of age according to the prevalence (35.3% *vs* 54.8%, P = 0.012), stratification analysis (P = 0.037) and Spearman's correlation (coef. = -0.191, P = 0.012). Regardless of race, lifestyle and habitual risk factors, the absence of *H. pylori* in the available OSCC

Table 1 Association with *Helicobacter pylori* infection and oral squamous cell carcinoma

	Prevalence of *H. pylori*	Diagnostic tool of *H. pylori*	Study design	*P* value
Fernando *et al* [13]	Betel Chewers (20/104; 19.2%) and non-betel chewers (4/69; 5.8%)	Serology	Case- control study	< 0.05
Grandis *et al*[14]	Case 57% *vs* controls 62%	Serology	Case- control study	> 0.05
Dayama *et al* [15]	OR: 3.0; 95%CI: 0.34-26.4	Serum and tissue samples (PCR and culture)	Case- control study	NA
Gupta *et al*[16]	OR: 2.29; 95%CI: 0.61-8.68	Serology, PCR, culture	Meta-analysis	NA
Meng *et al*[17]	Case 35.3% *vs* controls 54.8%	Serology, PCR, histochemical staining	Case- control study	0.012

H. pylori: *Helicobacter pylori*; OR: Odds ratio; CI: Confidence interval; PCR: Polymerase chain reaction; NA: Not available.

cohorts indicates that *H. pylori* is unlikely to contribute to OSCC pathogenesis[18].

Role of the host effect in oral cancer

It is well known that *H. pylori* modifies the host's immune response, resulting in GC. A similar mechanism might contribute to oral carcinoma; however, this relationship has not been revealed to date. To illustrate the potential relationship between *H. pylori* and oral cancer, a prospective cohort should be conducted in the future. Other risk factors, such as smoking, alcohol consumption, fungi (candidiasis) and viruses (Epstein-Barr virus and HPV), have already been extensively studied[19].

Summary

According to currently available studies, the relationship between *H. pylori* and oral malignancy cannot be made at present (Tables 1 and 2). Results varied among the studies due to the use of different diagnostic methods (culture, immunohistochemistry, enzyme-linked immunosorbent assay, PCR) adopted for *H. pylori* identification. Overall, the meta-analysis revealed a nonsignificant association between the bacterium and OSCC.

PHARYNGEAL-LARYNGEAL CANCER

Epidemiology of pharyngeal-laryngeal cancer

Pharyngeal-laryngeal cancer is a common malignancy of the upper aerodigestive tract. The prevalence is greater in people over the age of 60 and in males (5.8 cases per 100000 in males *vs* 1.2 per 100000 in females)[20]. Pharyngeal-laryngeal cancer comprises 2%-3% of the malignancies of the whole body and constitutes 25% of head and neck cancers[21]. In addition, racial differences were noticed with a younger age and a higher incidence and mortality in African Americans than in Caucasians[22,23]. Moreover, the younger (< 40 years old) the patients were diagnosed, the more aggressive and the poorer the survival rate[24]. Major risk factors for pharyngeal-laryngeal cancer include cigarette smoking and alcohol consumption. A study examining the effect of alcohol consumption and smoking in laryngeal cancer reported that the adjusted odds ratios for nonsmoking heavy drinkers (defined as > 8 drinks per day) and for nondrinking smokers were 2.46 and 9.38, respectively[25]. Microbes, viruses, occupational exposures, gastroesophageal reflux, and genetic inheritance, for example, were also linked to malignancy[26].

Pathological differences in pharyngeal-laryngeal cancer

Most of these cancers are squamous cell carcinoma, accounting for 85%-95% of pharyngeal-laryngeal malignancies[27].

Role of H. pylori in pharyngeal-laryngeal cancer

H. pylori has been detected in tooth plaque, saliva, nasal sinuses, and the middle ear [28,29]. The association between *H. pylori* infection and pharyngeal-laryngeal cancer has been described by Zhou *et al*[30]. Eleven studies were included in a meta-analysis

Table 2 *Helicobacter pylori* infection effect in whole gastroenterology tract malignant diseases

Site	Malignant cell type	*H. pylori* effect	Odds ratio	95%CI	*P* value	Gastrointestinal transit time
Oral cavity	Squamous cell carcinoma	Non related				1 min
Pharynx-larynx	Squamous cell carcinoma	Increased risk[1]	2.87	1.71-4.84	< 0.05	1 s
Oesophagus	Squamous cell carcinoma	Non related				4-8 s
	Adenocarcinoma	Protected effect	0.56	0.46-0.68	< 0.05	
Stomach	Adenocarcinoma	Cause-effect	5.9	3.4-10.3	< 0.05	2-4 h
	MALT lymphoma	Cause-effect	1.96	1.0-3.9	< 0.05	
Small intestine	Lymphoma	Non related				6 h
Colorectum	Adenocarcinoma	Partial cause-effect	1.7	1.64-1.76	< 0.05	10 h to days
	Lymphoma	Non related				

[1]Influence of smoking and alcohol consumption on *Helicobacter pylori* and laryngeal carcinoma was not removed from their study. CI: Confidence interval; MALT: Mucosa-associated lymphoid tissue; *H. pylori*: *Helicobacter pylori*.

that demonstrated a significantly higher rate of *H. pylori* infection in patients with pharyngeal-laryngeal cancer compared with healthy controls (OR = 2.87, 95%CI: 1.71-4.84; $P < 0.0001$). Furthermore, the ORs for laryngeal carcinoma were greater than those for pharyngeal cancer [(OR: 3.28, 95%CI: 1.91-5.63) *vs* (OR: 1.35, 95%CI: 0.86-2.12), respectively]. On the basis of the study results, a relationship between *H. pylori* infection and laryngeal carcinoma but not pharyngeal cancer was suggested. This association may result from the direct exposure of the larynx to known carcinogens (*e.g.*, alcohol and tobacco), whereas mucosal and immune barriers were broken down after *H. pylori* infected the larynx. In patients with either benign or malignant laryngeal diseases, *H. pylori* was detected in greater than one-third (38.8%) of the biopsy samples from the larynx. The infection rate of *H. pylori* was highest in patients with laryngeal cancer (46.2%) and chronic laryngitis (45.5%) and was significantly lower in controls (9.1%)[31]. Based on the results of a meta-analysis, *H. pylori* infection increases the risk of laryngeal cancer by twofold compared to controls[32].

Burduk *et al*[33] showed a correlation of a high incidence of positivity for the *H. pylori* cytotoxin-associated gene A (*CagA*) gene in laryngeal cancer tissue (46.7% to 49.3%) and a reduced survival rate. However, several studies failed to demonstrate the direct correlation between *H. pylori* and laryngeal cancer. A study found a significantly higher frequency of *H. pylori* colonization at the antrum compared with the gastric body in patients with laryngeal cancer. It was hypothesized that *H. pylori* in the antrum reduces gastric acid when colonizing the body and increases by G cell hyperplasia, thus leading to laryngeal cancer through gastric reflux[34]. *H. pylori* has been identified in some laryngeal diseases. Given the lack of reliable research, the role of *H. pylori* in the larynx remains unclear.

In addition, acting as confounders, smoking cigarettes and alcohol consumption could mask the true relationship between laryngeal cancer and *H. pylori* infection, and well examined evidence supports the role of these confounders in the development of laryngeal cancer. Zhou *et al*[30] declared that no adjustment was made to eliminate the influence of tobacco and alcohol in their study. More concrete evidence is needed to determine whether *H. pylori* infection is simply associated with or has a causal relation with smoking and drinking among patients with laryngeal-pharyngeal cancer. Furthermore, given the lack of the temporality between laryngeal cancer and *H. pylori* infection, the causal relation cannot be defined by these studies. Finally, almost all these studies were case-control studies with potential recall and selection biases that potentially influenced the outcomes of the present research.

Summary

Current studies demonstrate the existence of *H. pylori* in the laryngeal mucosa (Tables 2 and 3) and support a possible connection between *H. pylori* infection and laryngeal cancer, but this relationship is not noted in pharyngeal cancer. The etiological mechanism of *H. pylori*-induced laryngeal squamous cell carcinoma is unclear, and related studies are lacking. Further evaluation of the cause-effect of *H. pylori* infection

Table 3 Association with *Helicobacter pylori* infection and pharyngeal-laryngeal cancer

	Prevalence of *H. pylori*	Diagnostic tool of *H. pylori*	Study design	*P* value
Zhou *et al*[30]	Laryngeal CA: OR: 3.28; 95%CI: 1.91-5.63	Histochemical, PCR, rapid urease test	Meta-analysis	< 0.0001
	Pharyngeal CA: OR: 1.35; 95%CI: 0.86-2.12			= 0.188
Siupsinskiene *et al*[31]	Laryngeal CA: Case 46.2% and controls 9.1%	Rapid urease test	Case- control study	< 0.05
Zhou *et al*[32]	Laryngeal CA: OR: 2.3 95%CI: 1.28-3.23	Serology, histopathological methods	Meta-analysis	< 0.01
Pirzadeh *et al*[34]	Laryngeal CA: Case 49.2% and controls 40%	Rapid urease test	Case- control study	NA

OR: Odds ratio; CI: Confidence interval; PCR: Polymerase chain reaction; *H. pylori*: *Helicobacter pylori*; CA: Cancer; NA: Not available.

and pharyngeal-laryngeal cancer is required.

ESOPHAGEAL CANCER

Epidemiology of esophageal cancer

Esophageal cancer constitutes 5.3% of all global cancer deaths and affects greater than 570000 people worldwide. However, the incidence rate varies across regions and populations[35]. Esophageal cancer can be categorized into two main subtypes: Esophageal squamous-cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). In past years, ESCC accounted for 70% of all esophageal cancer cases, and EAC has observed a significant and sustained rise in Western industrialized countries[36]. ESCC exhibits severe geographic distribution differences: The incidence rate is highest in Eastern to Central Asia followed by the Indian Ocean coast and can exhibit greater than tenfold differences among countries. On the other hand, the prevalence of EAC increased in several regions, such as North America and Europe[36,37]. In addition, the global incidence of esophageal cancer in men is 70%, and the cumulative risk from birth to 74 years of age is also higher in men compared with women (1.15% *vs* 0.43%, respectively)[35]. Regarding subtypes, men have a higher risk for developing both ESCC and EAC than women with three- to fourfold and seven- to tenfold differences for each type[38]. The incidence of esophageal carcinoma increases with age, peaks in the seventh and eighth decades of life, and is rare in younger people[37].

Pathological and etiological differences in esophageal cancer

ESCC and EAC exhibit very different biological presentations. ESCC is primarily found in the middle third of the esophagus, whereas EAC is located more often in the distal third of the esophagus[39]. Several dietary habits are related to both types of esophageal cancer. For example, a high intake of red meats, fats, and processed foods is linked to an increased risk, whereas a high intake of fiber, fresh fruits, and vegetables is associated with a lower risk[37]. Other major risk factors differ in these two types of esophageal cancer. ESCC is three to five times as likely to occur in people who consume alcohol (three or more drinks daily)[37]. Smoking or betel quid chewing also increase the risk of ESCC. In addition, the combination of alcohol intake and smoking has a synergistic effect in increasing ESCC risk[37,40]. The absolute risk of EAC developing in an individual 50 years of age or older is approximately 0.04% per year, and that risk is approximately twice as high among current smokers as it is among people who have never smoked[37,41]. The first risk factor reported for EAC was gastroesophageal reflux disease (GERD), which was identified in the 1990s[42]. Several significant associations between two of the common GERD symptoms, *i.e.*, heartburn sensation and acid regurgitation, and the risk of EAC have been demon-strated by several studies. When heartburn symptoms presented for at least 30 years, the risk of EAC was 6.2-fold greater than that in individuals without heartburn[43]. The increasing prevalence of GERD combined with the declining prevalence of *H. pylori* infection has been hypothesized to be related to the increasing incidence of EAC.

Role of H. pylori in esophageal cancer

Rokkas *et al*[44] showed no consistent association between *H. pylori* infection and ESCC. Unlike ESCC, several studies have found that *H. pylori* infection is prevalent and leads to a reduced risk of EAC (OR: 0.50-0.57)[44-46]. Xie *et al*[47] showed that the

risk of adenocarcinoma decreased by 41% among persons with *H. pylori* infection. Since the middle of the twentieth century, the prevalence of *H. pylori* infection has decreased in Western populations, and an increasing incidence of EAC has occurred. Scientists have proposed that the elevated incidence of EAC might result from the decreased *H. pylori* infection rate in these populations[48,49]. The possible mechanism of this bacterial infection effect might involve *H. pylori* infection-induced host atrophic gastritis formation followed by reduced volume and acidity of gastric juice. Finally, this situation could counteract GERD and thereby reduce the risk of EAC[50]. Further meta-analysis studies also supported the notion of a decreased risk of EAC up to 40%-60% and an OR of 0.56 for *H. pylori* infection (95%CI: 0.46-0.68, $P < 0.05$)[46,47].

Role of host genetic effects in esophageal cancer

Past studies have shown that esophageal cancer might not be associated with family history. However, in China, studies have demonstrated an approximately two-fold increased risk of ESCC in patients with first-degree relatives who have ESCC[51,52]. This situation might be explained by family members sharing some habitual factors, such as diet, obesity, alcohol and smoking. Several genetic disorders have been thought to be related to ESCC. For example, the concentrations of acetaldehyde after alcohol consumption are higher in persons with particular variants in the acetaldehyde dehydrogenase gene and the aldehyde dehydrogenase 2 family gene. If patients had these polymorphic variants, the risk of ESCC was increased up to 43- to 73-fold[53]. In all ESCC individuals, 83% had TP53 mutations, 76% exhibited EGFR overexpression, 46% harbored CCND1 mutations and 24% had CDK4/CDK6 mutations[40]. In EAC patients, 19% exhibited CCNE1 amplification, and 17% harbored cyclin E and MGST1 mutations[40]. These genetic studies might help us to detect esophageal cancer in earlier stages[54]. In addition to the above description, there are several known risk factors related to esophageal cancer. Excess intake of processed foods, hot foods and red meat was associated with an increased risk of both ESCC and EAC, and an increased intake of fresh fruits, vegetables and fiber was associated with a lower risk [37]. Obesity and increased body mass index (BMI) were also thought to be associated with EAC. In particular, if the increase in BMI began in childhood or adolescence, the EAC risk seemed to be stronger than if the increase in BMI began in adulthood[55].

Summary

Unlike other gastrointestinal tract malignancy diseases, *H. pylori* infection might indicate a decreased risk of EAC and be unrelated to ESCC. The OR of EAC in *H. pylori* -infected participants was 0.56 (95%CI: 0.46-0.68, $P < 0.05$) (Tables 2 and 4). *H. pylori* infection might increase atrophic gastritis in the host and decrease gastric acid formation, leading to a decrease in GERD and the probability of EAC. To prevent esophageal cancer, the elimination of smoking and alcohol and very hot food or drink consumption and the practice of healthy dietary habits are beneficial.

GASTRIC ADENOCARCINOMA

Epidemiology and etiology of GC

Although it is steadily decreasing in incidence, GC remains one of the most common malignant diseases worldwide[35]. According to GLOBOCAN 2020 data, GC is the sixth most commonly diagnosed cancer and the fifth leading cause of cancer mortality in the world, following lung, breast, colorectal and liver cancer. A Global Cancer Observatory report in 2018 noted that the cumulative risk of GC was higher in men than in women (1.87% and 0.79%)[1]. Compared to North and East Africa and North America, the incidence of GC was higher in East and Central Asia. In East Asia, the average incidence of GC for men and women is 3.21 and 1.32 per individual, respectively, whereas the incidence is 0.56 per million individuals in North America. The risk varies from six- to fifteen-fold between areas with the highest and the lowest incidence. The cause of this difference might be related to region and culture[56]. Ninety-five percent of GCs are adenocarcinomas followed by primary gastric lymphoma, and we focus on reviewing adenocarcinoma in this section. According to the anatomical site, gastric adenocarcinomas can be classified into cardia GCs and non-cardia GCs. The pathogenesis of cardia GCs might be related to GERD or EAC. Non-cardia GCs are caused by *H. pylori*-related atrophic gastritis and a variety of environmental factors, such as diet, alcohol, and smoking[57].

Table 4 Association with *Helicobacter pylori* infection and Esophageal cancer

	Prevalence of *H. pylori*	Diagnostic tool of *H. pylori*	Study design	*P* value
Rokkas *et al*[44]	EAC: 0.52 (95%CI: 0.37-0.73)	Serology and/or histology	Meta-analysis	EAC: $P < 0.001$
	ESCC: 0.85 (95%CI: 0.55-1.33)			ESCC: $P = 0.48$
Islami *et al*[45]	EAC: 0.56 (95%CI: 0.46-0.68)	Serology and/or histology	Meta-analysis	NA
	ESCC: 1.10 (95%CI: 0.78-1.55)			
Nie *et al*[46]	EAC: 0.57 (95%CI: 0.44-0.73)	Serology and/or histology; rapid urease test	Meta-analysis	NA
	ESCC:1.16 (95%CI: 0.8.-1.60)			
Xie *et al*[47]	EAC: 0.59 (95%CI: 0.51-0.68)	Serology and/or histology; rapid urease test	Meta-analysis	NA
	ESCC: 0.97 (95%CI: 0.76-1.24)			
	ESCC in Eastern: 0.66 (95%CI: 0.43-0.89)			

CI: Confidence interval; *H. pylori*: *Helicobacter pylori*; EAC: Esophageal adenocarcinoma; ESCC: Esophageal squamous-cell carcinoma; NA: Not available.

In the past fifty years, the incidence of GC has steadily declined. This trend was more significant in East Asia and might be due to a successful reduction in the number of *H. pylori* infections. Approximately 90% of cases of non-cardia GCs are attributable to *H. pylori* infection. Given *H. pylori* eradication and reduced infection rates, the incidence of non-cardia GCs is also declining[58]. In addition to *H. pylori* eradication, improved food conservation, higher standards of hygiene, and high intake of fresh fruits and vegetables could explain the reduced incidence of GCs[59].

Role of H. pylori in GC

In 1982, Warren and Marshal[60] found a connection between *H. pylori* and gastric ulcer disease, and since then, this bacterium has become a topic of study in the gastroenterology field. Twelve years later, the International Agency for Research on Cancer recognized *H. pylori* as a class I carcinogen[61]. For general microorganisms, the stomach environment is not suitable for survival because the gastric acid and pH level is less than 0.3-2.9[62]. However, with the assistance of urease-derived ammonia, *H. pylori* can buffer cytosolic, periplasmic and surface acidity in such an extreme environment of the stomach[63]. This environment might induce *H. pylori* to become the predominant microorganism in the stomach. In addition, the gastric transit time was greater than 2-4 h (Table 2), giving *H. pylori* more chances to attach to the stomach. When *H. pylori* strains carry the cag pathogenicity island (*cag*PAI), the risk of peptic ulcer disease or GC increases. With a size of 40 kb, *cag*PAI contains 30 genes, including *cagA*[64]. A previous study showed that *H. pylori*-infected people had an approximately sixfold increased risk of developing non-cardia GCs (OR: 5.9; 95%CI: 3.4-10.3) compared with uninfected individuals[65]. Furthermore, compared to infection with *cagA*-negative strains, a 1.64-fold (95%CI: 1.21-2.24) increased risk of GC was found for *cagA*-positive strains[66]. In gastric epithelial cells, a cell scattering effect caused by cytoskeletal modifications and proinflammatory responses triggered by the transcription factor NF-κB were observed when a functional *cag*PAI was present in *H. pylori*[67,68]. Activation of growth factor receptors, cell proliferation, inhibition of apoptosis, invasion and angiogenesis occurred through *cag*A[69].

The connection between *H. pylori* infection and GC was most significant in whole gastrointestinal tract cancer. *H. pylori* infection increases GC incidence, but GC incidence is decreased after *H. pylori* eradication. Lee *et al*[70] demonstrated an association of *H. pylori* infection eradication with a reduced incidence of GC in a meta-analysis study. After adjustment for baseline GC incidence, the pooled incidence rate for individuals receiving *H. pylori* eradication treatment was 0.53 (95%CI: 0.44-0.64). Recently, the long-term benefits of eradication were confirmed by Chiang *et al*[71], revealing a significant reduction in the occurrence of GC by 53% for a high-risk Taiwanese population. From 2004 to 2018, a mass eradication program was conducted in patients older than 30 years old on the Matsu Islands, where *H. pylori* infection was prevalent. After *H. pylori* eradication, the infection rates declined from 64% to 15%. GC incidence and mortality after the chemoprevention period were reduced to 53% (95%CI: 0.3-0.69) and 25% (95%CI: 0.14-0.51), respectively. The 2020 Taipei global consensus supported that "eradication therapy should be offered to all individuals

infected with *H. pylori*" and suggested that screen-and-treat is a cost-effective strategy for young adults in GC high incidence areas at the general population level[72].

Role of host genetic effects in GC

In addition to *H. pylori* infection, dietary habits, lifestyle, family history and occupational exposure are also risk factors for GC. Fresh fruits and vegetables are protective against GC. Compared to individuals who intake less than one serving fruit and vegetable *per* day, participants who ate 2-5 servings had a hazard ratio (HR) of 0.56 (95%CI: 0.34-0.93)[73]. Some scientists have suggested that this might be related to an increase in vitamin C in fresh fruits and vegetables[74]. On the other hand, pickled vegetables, dried fish, and salted fish were associated with an increased incidence of GC[75]. High dietary salt intake was also associated with an increased risk of GC when salt intake was more than 10 g per day[76]. Regarding lifestyle, alcohol intake and smoking were thought to increase GC incidence. Duell *et al*[77] found that modest alcohol intake of greater 60 grams per day would increase the risk of GC to 1.65 (95%CI: 1.06-2.58). The meta-analysis conducted by Ladeiras-Lopes *et al*[78] included 42 studies from Asia, Europe and the United States and reported a relative risk of 1.53 for smokers (1.62 males and 1.2 females). Smoking not only increased GC risk but also affected GC recurrence and survival. As an independent risk factor, smokers had a significantly worse 5-year disease-free survival (HR: 1.46, P = 0.007) and overall survival (HR: 1.48, P = 0.003) than nonsmokers[79]. The GC risk was increased two- to threefold in first-degree relatives of patients with this disease. This finding might be due to the familial clustering trend of *H. pylori* infection[80]. Occupational exposures to dust and heat, such as those experienced by chefs, wood processing plant operators, food processing and related trade workers, and machine operators, was linked to a significantly raising risk of diffuse GC[67].

Summary

In the whole gastrointestinal tract, GC was most related to *H. pylori* infection, especially in non-cardia GCs. The OR of GC in *H. pylori*-infected participants was 5.9 (95%CI: 3.4-10.3, P < 0.05) (Tables 2 and 5). The host organ environment and pathogen characteristics might explain this result. The very low pH level in the stomach allows *H. pylori* to predominate in this niche, and adequate gastric transit time provides this bacterium with a greater chance of colonization in the stomach. *H. pylori* strains with a functional *cag*PAI further increased the risk for GC by 1.64-fold. Based on the 2020 Taipei global consensus, mass screening and eradication of *H. pylori* are necessary to prevent GC in high-risk populations.

GASTROINTESTINAL TRACT LYMPHOMA

Epidemiology of gastrointestinal tract lymphoma

As the most frequent location for extranodal lymphoma, the gastrointestinal tract represents 5%-20% of all cases[81]. However, primary gastrointestinal lymphoma is very rare. It only constitutes approximately 1%-4% of all gastrointestinal cancers. It is slightly male predominant with a men-women ratio of 3:2. Lymphoma incidence exhibits a double peak: One in patients younger than 10 years old and another in those with a mean age of 53 years[82].

The prevalence of lymphoma among different gastrointestinal locations is highest for the stomach (60%-75%) followed by the small intestine, ileocecal region and rectum [83]. With an elevated incidence worldwide, non-Hodgkin's lymphomas (NHLs) are the most common primary gastric lymphomas, accounting for 5% of gastric malignancies[84]. Primary small intestinal lymphoma occurrence is comparatively rare, constituting 19%-38% of small intestine cancers[85], 20%-30% of primary gut lymphomas[86], and 4%-12% of all NHLs[87]. The most frequent location of small intestine lymphoma involvement is the ileum (60%-65%) followed by the jejunum (20%-25%) and duodenum (6%-8%)[88].

Colorectal lymphoma constitutes 6%-12% of all gastrointestinal lymphomas. Simply contributing 0.2% of all cancers, it is very rare for primary colorectal lymphoma[89]. The most common sites of tumor growth are the cecum (71.5%), rectum (16.9%), and ascending colon (6.2%), whereas the sigmoid colon is rarely involved[90]. Primarily occurring from the fourth to the seventh decades of life, primary colorectal lymphomas are diagnosed at an average age of 50 years. Males are affected approximately twofold more frequently than females[91].

Table 5 Association with *Helicobacter pylori* infection and gastric cancer

	Prevalence of *H. pylori*	Diagnostic tool of *H. pylori*	Study design	*P* value
Helicobacter and Cancer Collaborative Group[68]	Non-cardia GC: OR: 5.9; 95%CI: 3.4-10.3	Serology and/or histology	Meta-analysis	$P = 0.002$
Huang *et al*[66]	For cagA-positive OR: 1.64; 95%CI: 1.21-2.24[1]	Serology and/or histology	Meta-analysis	NA
Gastric cancer incidence decreased after *H. pylori* eradication				
Lee *et al*[70]	Incidence rate ratio = 0.53; 95%CI: 0.44-0.64	Serology and/or histology; rapid urease test	Meta-analysis	NA
Chiang *et al*[71]	Reducing GC incidence of 0.53; 95%CI :0.3-0.69	Rapid urease test	Prospective study	$P < 0.001$

[1]In *Helicobacter pylori*-infected populations, cagA-positive strains further increased the risk for gastric cancer by 1.64-fold. GC: Gastric cancer; OR: Odds ratio; CI: Confidence interval; *H. pylori*: *Helicobacter pylori*; NA: Not available.

Pathological differences in gastrointestinal tract lymphoma

Histopathologically, approximately 90% of primary gastrointestinal lymphomas are of the B cell lineage. Among them, over 90% are mucosa-associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL). Notably, MALT lymphoma constitutes half of all primary lymphomas with gastric involvement[92].

Primary small intestine lymphomas that are more heterogeneous than those in the stomach include MALT lymphoma, DLBCL, enteropathy-associated T-cell lymphoma, mantle cell lymphoma (MCL), follicular lymphoma and immunoproliferative lymphoma[93].

Primary colorectal lymphomas include MALT-related low-grade B-cell lymphoma, MCL, and peripheral T-cell lymphoma. Manifesting as multiple polyps, MCL is aggressive. In contrast, low-grade B-cell lymphoma derived from MALT is indolent and occasionally appears as multiple polyps. Colonic peripheral T-cell lymphoma expresses as either a diffuse or a focal segmental lesion with extensive mucosal ulceration[94].

Role of H. pylori in gastrointestinal tract lymphoma

A previous large population-based study, in which the seroprevalence of *H. pylori* was higher in patients with gastric lymphoma than in matched controls, confirmed the relationship between *H. pylori*-related chronic gastritis and MALT lymphoma[95]. Gastric MALT lymphoma is highly correlated with *H. pylori* in 72%-98% of low-grade cases[96]. In a retrospective study conducted by Parsonnet *et al*[95], *H. pylori* seropositivity preceded the diagnosis of gastric NHL for years (OR: 6.3; 95%CI: 2.0-19.9). MALT lymphoma was positively correlated with *H. pylori* infection (OR: 1.96; 95%CI: 1.0-3.9)[97]. The regression of low-grade gastric MALT lymphoma after the eradication of *H. pylori* has been described by some recent studies[98]. Epidemiological and experimental data support the hypothesis that *H. pylori* can serve as an antigenic stimulus supporting the growth of gastric lymphoma. Polymorphisms in host genes regulating the inflammatory response and antioxidative mechanisms in gastric MALT lymphoma patients suggest a correlation with the capacity to neutralize free radicals, and individual variations in the inflammatory response to *H. pylori* have been observed in recent research[99]. Expression of the CagA protein by *H. pylori* strains induced severe gastritis or even peptic ulcerations. The hypothesis that CagA+ *H. pylori* strains are linked to the development of gastric MALT lymphomas is observed in nearly all cases of patients in whom anti-CagA antibodies are present at a higher rate compared with inactive gastritis cases[100].

Parsonnet *et al*[95] failed to demonstrate a correlation between non-gastric NHL and prior *H. pylori* infection (OR: 1.2; 95%CI: 0.5-3.0). Several cases of colorectal MALT lymphoma that disappeared completely after *H. pylori* eradication were presented in 1998[101]. Unlike gastric MALT lymphomas, which can be successfully treated by *H. pylori* eradication alone, colorectal MALT lymphomas, which have different relationships with *H. pylori* infection, act and are viewed as a distinct clinical entity. However, antibiotic treatment against *H. pylori* is effective for colonic MALT lymphoma, and this treatment even influences *H. pylori*-negative patients[102].

Role of the host effect in gastrointestinal tract lymphoma

Sixty-five percent of gastric MALT lymphomas present with chromosomal translocations, including the t(14;18)(q32;q21) translocation, which causes deregulation of MALT1; the t(11;18)(q21;q21) translocation, which causes the formation of the chimeric fusion gene AP12-MALT1; and the t(1;14)(p22;q32) translocation, which causes deregulation of BCL10. Through the regulation of different genes, these translocations are involved in immunity, inflammation and apoptosis[103].

Polymorphisms of specific cytokines have been researched in the context of MALT lymphoma. Upregulation of IL-1 production is typically noted in the presence of *H. pylori*[104]. High IL-1 levels favor a proinflammatory response. In combination with the inhibition of gastric acid, extensive *H. pylori* colonization is facilitated, and MALT growth is promoted[99].

Acting on the signaling pathway, tumor necrosis factor (TNF) and its receptors greatly influence the immune response. TNF may accelerate the growth of lymphoid cells *in vitro*, and high concentrations of TNF were detected in patients with malignant lymphoma[105].

A well-known oncogene, Bcl-6, which is located on the long arm of chromosome 3, is found in most extranodal high-grade lymphomas. Its overexpression was also reported in gastric DLBCL[106].

Summary

Gastrointestinal lymphoma is a relatively rare disease with a diverse clinical presentation. The epidemiology and histopathologic subtypes as well as their relationship with *H. pylori* infection, are highlighted in this review. For gastric MALT lymphoma, a positive association with *H. pylori* infection was found (OR: 1.96; 95%CI: 1.0-3.9, $P < 0.05$) (Tables 2 and 6). Other non-gastric MALT lymphomas did not show this association.

CRC

Epidemiology of CRC

CRC is the third most commonly diagnosed cancer in males and the second most commonly diagnosed cancer in females worldwide[107]. In the United States, CRC ranks as the second leading cause of cancer mortality in the population. This trend was similar in Europe, Australia and New Zealand, and these countries showed higher CRC incidence rates[108]. Japan, Thailand, Saudi Arabia and Iran have suffered rapid increases in CRC incidence over the past 30 years[109-111]. However, the age-standardized incidence rates vary in different countries. The country with the highest incidence rate was hungary, which had 51.2 cases per 100000 persons per year, and the country with the lowest incidence rate was Gambia with 1.1 cases per 100000 persons per year. The cause of this variation might be due to several factors, such as lifestyle, genetics, economic status (for example, meat consumption) and life expectancy (for example, some underdeveloped countries had lower CRC incidence rates because fewer people reach ages over 65 years, when most CRC is diagnosed)[107,112]. It is worth noting that some countries had a low CRC risk regardless of a high prevalence of *H. pylori*. This finding challenges the connection between *H. pylori* and CRC development.

This result might be explained by the fact that CRC has multiple contributing factors and *H. pylori* infection is one of them. For example, together with hyperglycemia, *H. pylori* infection has a synergistic effect on the risk of colon adenoma[113]. Areas with a higher prevalence of *H. pylori* infection but lower incidence of CRC, including Asia, some eastern European countries, and specific countries in South America, exhibit a lower diabetes prevalence[114]. This finding indicates that if the DM prevalence increases, the CRC prevalence might be elevated, which leads to areas with a higher prevalence of *H. pylori* infection but lower CRC incidence rates.

Pathological differences in CRC

There are three major pathologic pathways of CRC: The adenoma-carcinoma sequence, the serrated pathway and the inflammatory pathway. An estimated 85%-90% of sporadic CRC cases are derived from the adenoma-carcinoma sequence. In this pathway, several stepwise accumulations of genetic and epigenetic alterations drive the transformation of normal colon mucosal cells into an adenoma. First, the inactivated tumor suppressor gene *APC* is regarded as the gatekeeper against

Table 6 Association with *Helicobacter pylori* infection and gastrointestinal tract lymphoma

	Prevalence of *H. pylori*	Diagnostic tool for *H. pylori*	Study design	*P* value
Parsonnet *et al*[95] (Gastric NHL)	OR: 6.3; 95%CI: 2.0-19.9	Serology	Case- control study	NA
Ishikura *et al*[97] (Gastric lymphoma overall)	OR: 2.14; 95%CI: 1.3-3.5	Serology	Case- control study	$P = 0.003$
Ishikura *et al*[97] (Gastric MALT)	OR: 1.96; 95%CI: 1.0-3.9	Serology	Case- control study	$P = 0.051$
Ishikura *et al*[97] (Gastric DLBCL)	OR: 1.92; 95%CI: 0.74-4.95	Serology	Case- control study	$P = 0.178$
Parsonnet *et al*[95] (Non-gastric NHL)	OR: 1.2; 95%CI: 0.5-3.0	Serology	Case- control study	NA

NHL: Non-Hodgkin's lymphomas; MALT: Mucosa-associated lymphoid tissue; DLBCL: Diffuse large B-cell lymphoma; OR: Odds ratio; CI: Confidence interval; *H. pylori*: *Helicobacter pylori*; NA: Not available.

Table 7 Association with *Helicobacter pylori* infection and colorectal adenoma/ cancer

	Prevalence of *H. pylori*	Diagnostic tool of *H. pylori*	Study design	*P* value
Hu *et al*[113]	OR: 1.44; 95%CI: 1.2-1.73	Rapid urease test	Retrospective	$P < 0.001$
Sonnenberg *et al*[122]	OR: 1.52; 95%CI: 1.46-1.57	Histology	Retrospective	NA
Liou *et al*[124]	Case 14.2% *vs* controls 11.8%	^{13}C-UBT	Case-control study	$P = 0.513$
Choi *et al*[126]	OR: 1.49; 95%CI: 1.37-1.62	Serology, histology, rapid urease test and ^{13}C-UBT	Meta-analysis	$P < 0.001$
Zuo *et al*[127][1]	OR: 1.70; 95%CI: 1.64-1.76	Serology, histology and rapid urease test	Meta-analysis	NA
Colorectal adenoma incidence decreased after *H. pylori* eradication				
Hu *et al*[133][2]	HR: 3.04; 95%CI: 1.754-5.280	Rapid urease test	Retrospective cohort	$P < 0.001$

[1]For colorectal cancer.
[2]Second rapid urease test (+) *vs* (-). ^{13}C-UBT: ^{13}C-urea breath test; OR: Odds ratio; CI: Confidence interval; *H. pylori*: *Helicobacter pylori*; HR: Hazard ratio; NA: Not available.

colorectal neoplasms. Second, *KRAS*, an oncogene mutation, facilitates adenoma growth. Then, inactivation of the tumor suppressor gene (*i.e.*, *TP53*) promotes CRC progression[115,116]. Approximately 10%-15% of sporadic CRC is caused by the serrated pathway. This pathway includes several gene mutations. Oncogene *BRAF* mutations induce uncontrolled cell proliferation and contribute to the formation of hyperplastic polyps through constitutive activation of the MAPK pathway[117]. Then, hypermethylation at repetitive CG dinucleotides CpG island methylator phenotype (CIMP) results in mutations in the promoter regions of tumor suppressor genes. CIMP presents cell progression to sessile serrated adenoma and CRC. Approximately 75% of sessile serrated adenomas and 90% of serrated adenocarcinomas had CIMP-positive presentations[118,119]. Less than 2% of all CRC is caused by the inflammatory pathway. In this path-way, normal colon mucosal cells progress from indefinite dysplasia to low-grade dysplasia, high-grade dysplasia and cancer due to chronic inflammation[107].

Role of H. pylori in CRC

Since the 1990s, the connection between *H. pylori* and colorectal neoplasm formation has been widely discussed by scientists. Most reports demonstrated that *H. pylori* was linked to both benign and malignant colon lesions. For instance, *H. pylori* contributes to an elevated risk of 1.3- to 1.97-fold for colon adenoma with or without high-grade dysplasia[113,120-123]. Some scientists did not agree because their data revealed an insignificant increase in colon adenoma in combination with *H. pylori* infection[124, 125]. Nonetheless, two recent meta-analysis studies uncovered a significant and positive correlation between *H. pylori* infection and the risk of colorectal adenoma (OR: 1.49, 95%CI: 1.37-1.62)[126] and CRC (OR: 1.70; 95%CI: 1.64-1.76, $I^2 = 97\%$)[127]. The potential mechanisms for *H. pylori*-induced colorectal neoplasms might include direct and/or indirect effects. However, a few studies have shown positive *H. pylori* PCR histology in colon tumors and found *H. pylori* in 22%-27% of colorectal polyps or

cancers[128,129]. Recent studies favored the associations between CRC and bloodstream infections caused by *Streptococcus gallolyticus* (*S. gallolyticus*), *Bacteroides fragilis* (*B. fragilis*) and *Fusobacterium nucleatum* (*F. nucleatum*)[130]. Thus, *S. gallolyticus, B. fragilis* and *F. nucleatum* could have direct effects on the formation of colon neoplasms or cancer.

H. pylori might affect colorectal tumors through indirect effects. In the whole gastrointestinal tract, the colonic transit time is the longest[131] (Table 2). The long transit time offers more opportunities for *H. pylori* to alter the colonization of the colon, in which other bacteria might promote the development of neoplasms. Additionally, *H. pylori* enhances the release of gastrin, which contributes to colorectal carcinogenesis, possibly through its mitogen activity. *H. pylori* also appears to be associated with metabolic diseases with established connections with CRC. Finally, systemic inflammatory responses triggered by *H. pylori*-induced chronic inflammation of the gastric epithelium may increase the risk of CRC[132]. Although the possible mechanism of *H. pylori*-induced CRC was indirect, our previous study demonstrated a reduced risk of colorectal adenoma after successful eradication therapy[133]. This result implies that *H. pylori* is related to colon neoplasm formation by being a "biomarker" or "indicator organism", reflecting exposure to immune-stimulating carcinogenic bacteria or antigens.

Role of the host effect in CRC

In addition to *H. pylori*, several host factors and other environmental factors potentially contribute to the pathogenesis of CRC. Risk factors for colorectal neoplasms included age 60 years or older, male sex, obesity, diet, dyslipidemia, impaired glucose tolerance, a family history of CRC, alcohol intake, tobacco use, and sedentary lifestyle[134-136]. Most of these risk factors were associated with metabolic syndrome. Compared with healthy individuals, patients with hyperglycemia have a higher prevalence of colonic neoplasms (26.6% *vs* 16.5%, $P < 0.001$)[137]. Waist circumference, one of the components of metabolic syndrome, was an independent risk factor for colorectal adenoma, and diabetes mellitus type 2 had an OR of 1.38 for CRC[138]. Compared to non- or occasional drinkers, people who consume four more drinks per day have a 72% increased risk of developing CRC. Cigarette smoking increased the risk of CRC approximately two- to threefold compared with nonsmokers[139].

Summary

H. pylori infection might indicate an increased risk of CRC. The OR of CRC in *H. pylori*-infected participants was 1.70 (95%CI: 1.64-1.76, $P < 0.05$) (Tables 2 and 7)[127]. Although *H. pylori* infection might have an indirect effect on the formation of CRC, the presence or absence of this bacterium could remind clinicians of the possibility of CRC. *H. pylori* eradication therapy benefits both gastric malignancies and colorectal neoplasms by reducing their occurrence.

CONCLUSION

Given that *H. pylori* infection is an important infectious disease worldwide and affects human health through correlation with several diseases, such as gastric ulcers, GC and gastric MALT lymphoma, further realization of the effects of this bacterium in other gastrointestinal tract diseases is necessary. *H. pylori* infection induces chronic inflammatory changes in the human body and then increases GC, gastric MALT lymphoma and colorectal adenoma formation. In addition, an inverse relationship between *H. pylori* infection and EAC formation was observed due to atrophic gastritis and decreased gastric acid formation. From a microorganism viewpoint, the host gastroenterological microenvironment and motility status might play an important role in deciding which bacteria could colonize organs and subsequently induce chronic inflammatory and malignant changes in host organs. Further evaluation of human and bacterial interactions might allow us to better understand disease treatment.

REFERENCES

1 **Ferlay J**, Ervik M, Lam F, et al Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. [cited 28 January 2021]. Available from: https://gco.iarc.fr/today

2 **de Martel C**, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health* 2020; **8**: e180-e190 [PMID: 31862245 DOI: 10.1016/S2214-109X(19)30488-7]
3 **Mandeville KL**, Krabshuis J, Ladep NG, Mulder CJ, Quigley EM, Khan SA. Gastroenterology in developing countries: issues and advances. *World J Gastroenterol* 2009; **15**: 2839-2854 [PMID: 19533805 DOI: 10.3748/wjg.15.2839]
4 **Go MF**. Review article: natural history and epidemiology of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; **16** Suppl 1: 3-15 [PMID: 11849122 DOI: 10.1046/j.1365-2036.2002.0160s1003.x]
5 **Hooi JKY**, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JJY, Kaplan GG, Ng SC. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017; **153**: 420-429 [PMID: 28456631 DOI: 10.1053/j.gastro.2017.04.022]
6 Infection with Helicobacter pylori. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 177-240 [PMID: 7715070]
7 Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines. IARC Working Group. Lyon, 23-30 October 1984. *IARC Monogr Eval Carcinog Risk Chem Hum* 1985; **37**: 1-268 [PMID: 3866741]
8 **MEHTA FS**, SANJANA MK, BARRETTO MA. Relation of betel leaf chewing to periodontal disease. *J Am Dent Assoc* 1955; **50**: 531-536 [PMID: 14366931 DOI: 10.14219/jada.archive.1955.0098]
9 **Agni NA**, Prasad G, Borle RM, Shukla S, Grover S, Korde S. Assessment of perineural infiltration and spread of oral squamous cell carcinoma: a clinicohistopathologic study. *Indian J Cancer* 2010; **47**: 199-205 [PMID: 20448387 DOI: 10.4103/0019-509X.63024]
10 **Müller S**, Pan Y, Li R, Chi AC. Changing trends in oral squamous cell carcinoma with particular reference to young patients: 1971-2006. The Emory University experience. *Head Neck Pathol* 2008; **2**: 60-66 [PMID: 20614324 DOI: 10.1007/s12105-008-0054-5]
11 **Mohtasham N**, Babakoohi S, Salehinejad J, Montaser-Kouhsari L, Shakeri MT, Shojaee S, Sistani NS, Firooz A. Mast cell density and angiogenesis in oral dysplastic epithelium and low- and high-grade oral squamous cell carcinoma. *Acta Odontol Scand* 2010; **68**: 300-304 [PMID: 20586672 DOI: 10.3109/00016357.2010.494622]
12 **van der Waal R**, van der Waal I. Oral non-squamous malignant tumors; diagnosis and treatment. *Med Oral Patol Oral Cir Bucal* 2007; **12**: E486-E491 [PMID: 17978771]
13 **Fernando N**, Jayakumar G, Perera N, Amarasingha I, Meedin F, Holton J. Presence of Helicobacter pylori in betel chewers and non betel chewers with and without oral cancers. *BMC Oral Health* 2009; **9**: 23 [PMID: 19772630 DOI: 10.1186/1472-6831-9-23]
14 **Grandis JR**, Perez-Perez GI, Yu VL, Johnson JT, Blaser MJ. Lack of serologic evidence for Helicobacter pylori infection in head and neck cancer. *Head Neck* 1997; **19**: 216-218 [PMID: 9142522 DOI: 10.1002/(sici)1097-0347(199705)19:3<216::aid-hed9>3.0.co;2-5]
15 **Dayama A**, Srivastava V, Shukla M, Singh R, Pandey M. Helicobacter pylori and oral cancer: possible association in a preliminary case control study. *Asian Pac J Cancer Prev* 2011; **12**: 1333-1336 [PMID: 21875292]
16 **Gupta AA**, Kheur S, Raj AT, Mahajan P. Association of Helicobacter pylori with oral potentially malignant disorders and oral squamous cell carcinoma-a systematic review and meta-analysis. *Clin Oral Investig* 2020; **24**: 13-23 [PMID: 31707627 DOI: 10.1007/s00784-019-03125-2]
17 **Meng X**, Wang Q, He C, Chen M, Liu J, Liu W, Yuan Y. An inverse association of Helicobacter pylori infection with oral squamous cell carcinoma. *J Oral Pathol Med* 2016; **45**: 17-22 [PMID: 25899621 DOI: 10.1111/jop.12324]
18 **Pandey S**, Follin-Arbelet B, Pun CB, Gautam DK, Johannessen AC, Petersen FC, Costea DE, Sapkota D. Helicobacter pylori was not detected in oral squamous cell carcinomas from cohorts of Norwegian and Nepalese patients. *Sci Rep* 2020; **10**: 8737 [PMID: 32457404 DOI: 10.1038/s41598-020-65694-7]
19 **Waerhaug J**. Prevalence of periodontal disease in Ceylon. Association with age, sex, oral hygiene, socio-economic factors, vitamin deficiencies, malnutrition, betel and tobacco consumption and ethnic group. Final report. *Acta Odontol Scand* 1967; **25**: 205-231 [PMID: 5233925 DOI: 10.3109/00016356709028749]
20 **Baselga J**. Why the epidermal growth factor receptor? *Oncologist* 2002; **7** Suppl 4: 2-8 [PMID: 12202782 DOI: 10.1634/theoncologist.7-suppl_4-2]
21 **Koufman JA**, Burke AJ. The etiology and pathogenesis of laryngeal carcinoma. *Otolaryngol Clin North Am* 1997; **30**: 1-19 [PMID: 8995133]
22 **Goodwin WJ**, Thomas GR, Parker DF, Joseph D, Levis S, Franzmann E, Anello C, Hu JJ. Unequal burden of head and neck cancer in the United States. *Head Neck* 2008; **30**: 358-371 [PMID: 17972309 DOI: 10.1002/hed.20710]
23 **Shin JY**, Truong MT. Racial disparities in laryngeal cancer treatment and outcome: A population-based analysis of 24,069 patients. *Laryngoscope* 2015; **125**: 1667-1674 [PMID: 25694265 DOI: 10.1002/lary.25212]
24 **Li R**, Yu S, Zhu W, Wang S, Yan L. Studying the impact of young age on prognosis and treatment in laryngeal squamous cell carcinomas using the SEER database. *PeerJ* 2019; **7**: e7368 [PMID: 31380154 DOI: 10.7717/peerj.7368]

25 **Bosetti C**, Gallus S, Franceschi S, Levi F, Bertuzzi M, Negri E, Talamini R, La Vecchia C. Cancer of the larynx in non-smoking alcohol drinkers and in non-drinking tobacco smokers. *Br J Cancer* 2002; **87**: 516-518 [PMID: 12189548 DOI: 10.1038/sj.bjc.6600469]

26 **Tutar H**, Erdamar H, Köybaşioğlu A, Dinç AE, Ceylan A, Uslu S. Can bile acids be an etiological factor for laryngeal carcinoma? *ORL J Otorhinolaryngol Relat Spec* 2011; **73**: 156-161 [PMID: 21508656 DOI: 10.1159/000327521]

27 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]

28 **Sudhoff H**, Rajagopal S, Baguley DM, Ebmeyer J, Schmelzer A, Schreiber S, Moffat DA. A critical evaluation of the evidence on a causal relationship between Helicobacter pylori and otitis media with effusion. *J Laryngol Otol* 2008; **122**: 905-911 [PMID: 18036278 DOI: 10.1017/S0022215107000989]

29 **Ozdek A**, Cirak MY, Samim E, Bayiz U, Safak MA, Turet S. A possible role of Helicobacter pylori in chronic rhinosinusitis: a preliminary report. *Laryngoscope* 2003; **113**: 679-682 [PMID: 12671428 DOI: 10.1097/00005537-200304000-00018]

30 **Zhou J**, Zhang D, Yang Y, Zhou L, Tao L. Association between helicobacter pylori infection and carcinoma of the larynx or pharynx. *Head Neck* 2016; **38** Suppl 1: E2291-E2296 [PMID: 26316145 DOI: 10.1002/hed.24214]

31 **Siupsinskiene N**, Jurgutaviciute V, Katutiene I, Janciauskas D, Vaitkus S, Adamonis K. Helicobacter pylori infection in laryngeal diseases. *Eur Arch Otorhinolaryngol* 2013; **270**: 2283-2288 [PMID: 23572292 DOI: 10.1007/s00405-013-2475-3]

32 **Zhuo XL**, Wang Y, Zhuo WL, Zhang XY. Possible association of Helicobacter pylori infection with laryngeal cancer risk: an evidence-based meta-analysis. *Arch Med Res* 2008; **39**: 625-628 [PMID: 18662596 DOI: 10.1016/j.arcmed.2008.04.008]

33 **Burduk PK**. Association between infection of virulence cagA gene Helicobacter pylori and laryngeal squamous cell carcinoma. *Med Sci Monit* 2013; **19**: 584-591 [PMID: 23860397 DOI: 10.12659/MSM.889011]

34 **Pirzadeh A**, Doustmohammadian N, Khoshbaten M, Doustmohammadion S. Is there any association between Helicobacter Pylori infection and laryngeal carcinoma? *Asian Pac J Cancer Prev* 2011; **12**: 897-900 [PMID: 21790222]

35 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

36 **Arnold M**, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. *Gut* 2015; **64**: 381-387 [PMID: 25320104 DOI: 10.1136/gutjnl-2014-308124]

37 **Rustgi AK**, El-Serag HB. Esophageal carcinoma. *N Engl J Med* 2014; **371**: 2499-2509 [PMID: 25539106 DOI: 10.1056/NEJMra1314530]

38 **Uhlenhopp DJ**, Then EO, Sunkara T, Gaduputi V. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. *Clin J Gastroenterol* 2020; **13**: 1010-1021 [PMID: 32965635 DOI: 10.1007/s12328-020-01237-x]

39 **Pickens A**, Orringer MB. Geographical distribution and racial disparity in esophageal cancer. *Ann Thorac Surg* 2003; **76**: S1367-S1369 [PMID: 14530066 DOI: 10.1016/s0003-4975(03)01202-5]

40 **Huang FL**, Yu SJ. Esophageal cancer: Risk factors, genetic association, and treatment. *Asian J Surg* 2018; **41**: 210-215 [PMID: 27986415 DOI: 10.1016/j.asjsur.2016.10.005]

41 **Tramacere I**, La Vecchia C, Negri E. Tobacco smoking and esophageal and gastric cardia adenocarcinoma: a meta-analysis. *Epidemiology* 2011; **22**: 344-349 [PMID: 21330928 DOI: 10.1097/EDE.0b013e31821092cd]

42 **Chow WH**, Finkle WD, McLaughlin JK, Frankl H, Ziel HK, Fraumeni JF Jr. The relation of gastroesophageal reflux disease and its treatment to adenocarcinomas of the esophagus and gastric cardia. *JAMA* 1995; **274**: 474-477 [PMID: 7629956]

43 **Cook MB**, Corley DA, Murray LJ, Liao LM, Kamangar F, Ye W, Gammon MD, Risch HA, Casson AG, Freedman ND, Chow WH, Wu AH, Bernstein L, Nyrén O, Pandeya N, Whiteman DC, Vaughan TL. Gastroesophageal reflux in relation to adenocarcinomas of the esophagus: a pooled analysis from the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON). *PLoS One* 2014; **9**: e103508 [PMID: 25075959 DOI: 10.1371/journal.pone.0103508]

44 **Rokkas T**, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between Helicobacter pylori infection and esophageal neoplasia: a meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1413-1417, 1417.e1 [PMID: 17997357 DOI: 10.1016/j.cgh.2007.08.010]

45 **Islami F**, Kamangar F. Helicobacter pylori and esophageal cancer risk: a meta-analysis. *Cancer Prev Res (Phila)* 2008; **1**: 329-338 [PMID: 19138977 DOI: 10.1158/1940-6207.CAPR-08-0109]

46 **Nie S**, Chen T, Yang X, Huai P, Lu M. Association of Helicobacter pylori infection with esophageal adenocarcinoma and squamous cell carcinoma: a meta-analysis. *Dis Esophagus* 2014; **27**: 645-653 [PMID: 24635571 DOI: 10.1111/dote.12194]

47 **Xie FJ**, Zhang YP, Zheng QQ, Jin HC, Wang FL, Chen M, Shao L, Zou DH, Yu XM, Mao WM. Helicobacter pylori infection and esophageal cancer risk: an updated meta-analysis. *World J Gastroenterol* 2013; **19**: 6098-6107 [PMID: 24106412 DOI: 10.3748/wjg.v19.i36.6098]

48 **Banatvala N**, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. *J Infect Dis* 1993; **168**: 219-221 [PMID: 8515114 DOI:

10.1093/infdis/168.1.219]
49 **Rehnberg-Laiho L**, Rautelin H, Koskela P, Sarna S, Pukkala E, Aromaa A, Knekt P, Kosunen TU. Decreasing prevalence of helicobacter antibodies in Finland, with reference to the decreasing incidence of gastric cancer. *Epidemiol Infect* 2001; **126**: 37-42 [PMID: 11293681]
50 **Atherton JC**, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. *J Clin Invest* 2009; **119**: 2475-2487 [PMID: 19729845 DOI: 10.1172/JCI38605]
51 **Turati F**, Edefonti V, Bosetti C, Ferraroni M, Malvezzi M, Franceschi S, Talamini R, Montella M, Levi F, Dal Maso L, Serraino D, Polesel J, Negri E, Decarli A, La Vecchia C. Family history of cancer and the risk of cancer: a network of case-control studies. *Ann Oncol* 2013; **24**: 2651-2656 [PMID: 23884440 DOI: 10.1093/annonc/mdt280]
52 **Su Z**, Zou GR, Mao YP, OuYang PY, Cao XL, Xie FY, Li Q. Prognostic impact of family history of cancer in Southern Chinese patients with esophageal squamous cell cancer. *J Cancer* 2019; **10**: 1349-1357 [PMID: 31031844 DOI: 10.7150/jca.26511]
53 **Yokoyama T**, Yokoyama A, Kato H, Tsujinaka T, Muto M, Omori T, Haneda T, Kumagai Y, Igaki H, Yokoyama M, Watanabe H, Yoshimizu H. Alcohol flushing, alcohol and aldehyde dehydrogenase genotypes, and risk for esophageal squamous cell carcinoma in Japanese men. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1227-1233 [PMID: 14652286]
54 **Yokoyama T**, Yokoyama A, Kumagai Y, Omori T, Kato H, Igaki H, Tsujinaka T, Muto M, Yokoyama M, Watanabe H. Health risk appraisal models for mass screening of esophageal cancer in Japanese men. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 2846-2854 [PMID: 18843030 DOI: 10.1158/1055-9965.EPI-08-0397]
55 **Petrick JL**, Kelly SP, Liao LM, Freedman ND, Graubard BI, Cook MB. Body weight trajectories and risk of oesophageal and gastric cardia adenocarcinomas: a pooled analysis of NIH-AARP and PLCO Studies. *Br J Cancer* 2017; **116**: 951-959 [PMID: 28196067 DOI: 10.1038/bjc.2017.29]
56 **Rawla P**, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol* 2019; **14**: 26-38 [PMID: 30944675 DOI: 10.5114/pg.2018.80001]
57 **Mukaisho K**, Nakayama T, Hagiwara T, Hattori T, Sugihara H. Two distinct etiologies of gastric cardia adenocarcinoma: interactions among pH, Helicobacter pylori, and bile acids. *Front Microbiol* 2015; **6**: 412 [PMID: 26029176 DOI: 10.3389/fmicb.2015.00412]
58 **Balakrishnan M**, George R, Sharma A, Graham DY. Changing Trends in Stomach Cancer Throughout the World. *Curr Gastroenterol Rep* 2017; **19**: 36 [PMID: 28730504 DOI: 10.1007/s11894-017-0575-8]
59 **Muñoz N**, Franceschi S. Epidemiology of gastric cancer and perspectives for prevention. *Salud Publica Mex* 1997; **39**: 318-330 [PMID: 9337564 DOI: 10.1590/s0036-36341997000400010]
60 **Warren JR**, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275 [PMID: 6134060]
61 **Møller H**, Heseltine E, Vainio H. Working group report on schistosomes, liver flukes and Helicobacter pylori. *Int J Cancer* 1995; **60**: 587-589 [PMID: 7860130 DOI: 10.1002/ijc.2910600502]
62 **Ayazi S**, Leers JM, Oezcelik A, Abate E, Peyre CG, Hagen JA, DeMeester SR, Banki F, Lipham JC, DeMeester TR, Crookes PF. Measurement of gastric pH in ambulatory esophageal pH monitoring. *Surg Endosc* 2009; **23**: 1968-1973 [PMID: 19067071 DOI: 10.1007/s00464-008-0218-0]
63 **Montecucco C**, Rappuoli R. Living dangerously: how Helicobacter pylori survives in the human stomach. *Nat Rev Mol Cell Biol* 2001; **2**: 457-466 [PMID: 11389469 DOI: 10.1038/35073084]
64 **Nagini S**. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. *World J Gastrointest Oncol* 2012; **4**: 156-169 [PMID: 22844547 DOI: 10.4251/wjgo.v4.i7.156]
65 **Helicobacter and Cancer Collaborative Group**. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**: 347-353 [PMID: 11511555 DOI: 10.1136/gut.49.3.347]
66 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644 [PMID: 14724815 DOI: 10.1053/j.gastro.2003.08.033]
67 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115 [PMID: 7743510]
68 **Fischer W**, Püls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R. Systematic mutagenesis of the Helicobacter pylori cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol* 2001; **42**: 1337-1348 [PMID: 11886563 DOI: 10.1046/j.1365-2958.2001.02714.x]
69 **Yang ZM**, Chen WW, Wang YF. Gene expression profiling in gastric mucosa from Helicobacter pylori-infected and uninfected patients undergoing chronic superficial gastritis. *PLoS One* 2012; **7**: e33030 [PMID: 22438889 DOI: 10.1371/journal.pone.0033030]
70 **Lee YC**, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, Graham DY. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. *Gastroenterology* 2016; **150**: 1113-1124.e5 [PMID: 26836587 DOI: 10.1053/j.gastro.2016.01.028]
71 **Chiang TH**, Chang WJ, Chen SL, Yen AM, Fann JC, Chiu SY, Chen YR, Chuang SL, Shieh CF,

Liu CY, Chiu HM, Chiang H, Shun CT, Lin MW, Wu MS, Lin JT, Chan CC, Graham DY, Chen HH, Lee YC. Mass eradication of *Helicobacter pylori* to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands. *Gut* 2021; **70**: 243-250 [PMID: 32792335 DOI: 10.1136/gutjnl-2020-322200]

72 **Liou JM**, Malfertheiner P, Lee YC, Sheu BS, Sugano K, Cheng HC, Yeoh KG, Hsu PI, Goh KL, Mahachai V, Gotoda T, Chang WL, Chen MJ, Chiang TH, Chen CC, Wu CY, Leow AH, Wu JY, Wu DC, Hong TC, Lu H, Yamaoka Y, Megraud F, Chan FKL, Sung JJ, Lin JT, Graham DY, Wu MS, El-Omar EM; Asian Pacific Alliance on Helicobacter and Microbiota (APAHAM). Screening and eradication of *Helicobacter pylori* for gastric cancer prevention: the Taipei global consensus. *Gut* 2020; **69**: 2093-2112 [PMID: 33004546 DOI: 10.1136/gutjnl-2020-322368]

73 **Larsson SC**, Bergkvist L, Wolk A. Fruit and vegetable consumption and incidence of gastric cancer: a prospective study. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1998-2001 [PMID: 17035412 DOI: 10.1158/1055-9965.EPI-06-0402]

74 **Neugut AI**, Hayek M, Howe G. Epidemiology of gastric cancer. *Semin Oncol* 1996; **23**: 281-291 [PMID: 8658212]

75 **Tsugane S**. Salt, salted food intake, and risk of gastric cancer: epidemiologic evidence. *Cancer Sci* 2005; **96**: 1-6 [PMID: 15649247 DOI: 10.1111/j.1349-7006.2005.00006.x]

76 **Shikata K**, Kiyohara Y, Kubo M, Yonemoto K, Ninomiya T, Shirota T, Tanizaki Y, Doi Y, Tanaka K, Oishi Y, Matsumoto T, Iida M. A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: the Hisayama study. *Int J Cancer* 2006; **119**: 196-201 [PMID: 16450397 DOI: 10.1002/ijc.21822]

77 **Duell EJ**, Travier N, Lujan-Barroso L, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Palli D, Krogh V, Panico S, Tumino R, Sacerdote C, Quirós JR, Sánchez-Cantalejo E, Navarro C, Gurrea AB, Dorronsoro M, Khaw KT, Allen NE, Key TJ, Bueno-de-Mesquita HB, Ros MM, Numans ME, Peeters PH, Trichopoulou A, Naska A, Dilis V, Teucher B, Kaaks R, Boeing H, Schütze M, Regner S, Lindkvist B, Johansson I, Hallmans G, Overvad K, Egeberg R, Tjønneland A, Lund E, Weiderpass E, Braaten T, Romieu I, Ferrari P, Jenab M, Stenling R, Aune D, Norat T, Riboli E, González CA. Alcohol consumption and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Am J Clin Nutr* 2011; **94**: 1266-1275 [PMID: 21993435 DOI: 10.3945/ajcn.111.012351]

78 **Ladeiras-Lopes R**, Pereira AK, Nogueira A, Pinheiro-Torres T, Pinto I, Santos-Pereira R, Lunet N. Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. *Cancer Causes Control* 2008; **19**: 689-701 [PMID: 18293090 DOI: 10.1007/s10552-008-9132-y]

79 **Smyth EC**, Capanu M, Janjigian YY, Kelsen DK, Coit D, Strong VE, Shah MA. Tobacco use is associated with increased recurrence and death from gastric cancer. *Ann Surg Oncol* 2012; **19**: 2088-2094 [PMID: 22395977 DOI: 10.1245/s10434-012-2230-9]

80 **Lissowska J**, Groves FD, Sobin LH, Fraumeni JF Jr, Nasierowska-Guttmejer A, Radziszewski J, Regula J, Hsing AW, Zatonski W, Blot WJ, Chow WH. Family history and risk of stomach cancer in Warsaw, Poland. *Eur J Cancer Prev* 1999; **8**: 223-227 [PMID: 10443951 DOI: 10.1097/00008469-199906000-00010]

81 **Freeman C**, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972; **29**: 252-260 [PMID: 5007387 DOI: 10.1002/1097-0142(197201)29:1<252::aid-cncr2820290138>3.0.co;2-#]

82 **Dodd GD**. Lymphoma of the hollow abdominal viscera. *Radiol Clin North Am* 1990; **28**: 771-783 [PMID: 2190270]

83 **Herrmann R**, Panahon AM, Barcos MP, Walsh D, Stutzman L. Gastrointestinal involvement in non-Hodgkin's lymphoma. *Cancer* 1980; **46**: 215-222 [PMID: 7388763 DOI: 10.1002/1097-0142(19800701)46:1<215::aid-cncr2820460136>3.0.co;2-6]

84 **Ferrucci PF**, Zucca E. Primary gastric lymphoma pathogenesis and treatment: what has changed over the past 10 years? *Br J Haematol* 2007; **136**: 521-538 [PMID: 17156403 DOI: 10.1111/j.1365-2141.2006.06444.x]

85 **DARLING RC**, WELCH CE. Tumors of the small intestine. *N Engl J Med* 1959; **260**: 397-408 [PMID: 13632900 DOI: 10.1056/NEJM195902262600901]

86 **Naqvi MS**, Burrows L, Kark AE. Lymphoma of the gastrointestinal tract: prognostic guides based on 162 cases. *Ann Surg* 1969; **170**: 221-231 [PMID: 5819579 DOI: 10.1097/00000658-196908000-00010]

87 **Bush RS**. Primary lymphoma of the gastrointestinal tract. *JAMA* 1974; **228**: 1291-1294 [PMID: 4406525]

88 **Schottenfeld D**, Beebe-Dimmer JL, Vigneau FD. The epidemiology and pathogenesis of neoplasia in the small intestine. *Ann Epidemiol* 2009; **19**: 58-69 [PMID: 19064190 DOI: 10.1016/j.annepidem.2008.10.004]

89 **Dionigi G**, Annoni M, Rovera F, Boni L, Villa F, Castano P, Bianchi V, Dionigi R. Primary colorectal lymphomas: review of the literature. *Surg Oncol* 2007; **16** Suppl 1: S169-S171 [PMID: 18024019 DOI: 10.1016/j.suronc.2007.10.021]

90 **Jinnai D**, Iwasa Z, Watanuki T. Malignant lymphoma of the large intestine--operative results in Japan. *Jpn J Surg* 1983; **13**: 331-336 [PMID: 6645123 DOI: 10.1007/BF02469515]

91 **Kim YH**, Lee JH, Yang SK, Kim TI, Kim JS, Kim HJ, Kim JI, Kim SW, Kim JO, Jung IK, Jung SA, Jung MK, Kim HS, Myung SJ, Kim WH, Rhee JC, Choi KY, Song IS, Hyun JH, Min YI. Primary colon lymphoma in Korea: a KASID (Korean Association for the Study of Intestinal

Diseases) Study. *Dig Dis Sci* 2005; **50**: 2243-2247 [PMID: 16416168 DOI: 10.1007/s10620-005-3041-7]
92 **Ghimire P**, Wu GY, Zhu L. Primary gastrointestinal lymphoma. *World J Gastroenterol* 2011; **17**: 697-707 [PMID: 21390139 DOI: 10.3748/wjg.v17.i6.697]
93 **Jaffe ES**, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood* 2008; **112**: 4384-4399 [PMID: 19029456 DOI: 10.1182/blood-2008-07-077982]
94 **Lee HJ**, Han JK, Kim TK, Kim YH, Kim AY, Kim KW, Choi JY, Choi BI. Primary colorectal lymphoma: spectrum of imaging findings with pathologic correlation. *Eur Radiol* 2002; **12**: 2242-2249 [PMID: 12195476 DOI: 10.1007/s00330-002-1307-4]
95 **Parsonnet J**, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med* 1994; **330**: 1267-1271 [PMID: 8145781 DOI: 10.1056/NEJM199405053301803]
96 **Wotherspoon AC**, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; **338**: 1175-1176 [PMID: 1682595 DOI: 10.1016/0140-6736(91)92035-z]
97 **Ishikura N**, Usui Y, Ito H, Kasugai Y, Oze I, Kato S, Yatabe Y, Nakamura S, Matsuo K. Helicobacter pylori (HP) infection alone, but not HP-induced atrophic gastritis, increases the risk of gastric lymphoma: a case-control study in Japan. *Ann Hematol* 2019; **98**: 1981-1987 [PMID: 31177299 DOI: 10.1007/s00277-019-03721-y]
98 **Roggero E**, Zucca E, Pinotti G, Pascarella A, Capella C, Savio A, Pedrinis E, Paterlini A, Venco A, Cavalli F. Eradication of Helicobacter pylori infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *Ann Intern Med* 1995; **122**: 767-769 [PMID: 7717599 DOI: 10.7326/0003-4819-122-10-199505150-00006]
99 **Rollinson S**, Levene AP, Mensah FK, Roddam PL, Allan JM, Diss TC, Roman E, Jack A, MacLennan K, Dixon MF, Morgan GJ. Gastric marginal zone lymphoma is associated with polymorphisms in genes involved in inflammatory response and antioxidative capacity. *Blood* 2003; **102**: 1007-1011 [PMID: 12676777 DOI: 10.1182/blood-2002-12-3803]
100 **Ahmed N**, Sechi LA. Helicobacter pylori and gastroduodenal pathology: new threats of the old friend. *Ann Clin Microbiol Antimicrob* 2005; **4**: 1 [PMID: 15634357 DOI: 10.1186/1476-0711-4-1]
101 **Matsumoto T**, Shimizu M, Iida M, Amano K, Nakamura S, Fujishima M. Primary low-grade, B-cell, mucosa-associated lymphoid tissue lymphoma of the colorectum: clinical and colonoscopic features in six cases. *Gastrointest Endosc* 1998; **48**: 501-508 [PMID: 9831839 DOI: 10.1016/s0016-5107(98)70092-6]
102 **Grünberger B**, Wöhrer S, Streubel B, Formanek M, Petkov V, Puespoek A, Haefner M, Hejna M, Jaeger U, Chott A, Raderer M. Antibiotic treatment is not effective in patients infected with Helicobacter pylori suffering from extragastric MALT lymphoma. *J Clin Oncol* 2006; **24**: 1370-1375 [PMID: 16549831 DOI: 10.1200/JCO.2005.02.9025]
103 **Cavalli F**, Isaacson PG, Gascoyne RD, Zucca E. MALT Lymphomas. *Hematology Am Soc Hematol Educ Program* 2001; 241-258 [PMID: 11722987 DOI: 10.1182/asheducation-2001.1.241]
104 **Dinarello CA**. Biologic basis for interleukin-1 in disease. *Blood* 1996; **87**: 2095-2147 [PMID: 8630372]
105 **Locksley RM**, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001; **104**: 487-501 [PMID: 11239407 DOI: 10.1016/s0092-8674(01)00237-9]
106 **Martelli M**, Ferreri AJ, Agostinelli C, Di Rocco A, Pfreundschuh M, Pileri SA. Diffuse large B-cell lymphoma. *Crit Rev Oncol Hematol* 2013; **87**: 146-171 [PMID: 23375551 DOI: 10.1016/j.critrevonc.2012.12.009]
107 **Keum N**, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 713-732 [PMID: 31455888 DOI: 10.1038/s41575-019-0189-8]
108 **Siegel RL**, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 145-164 [PMID: 32133645 DOI: 10.3322/caac.21601]
109 **Khuhaprema T**, Srivatanakul P. Colon and rectum cancer in Thailand: an overview. *Jpn J Clin Oncol* 2008; **38**: 237-243 [PMID: 18356191 DOI: 10.1093/jjco/hyn020]
110 **Dolatkhah R**, Somi MH, Bonyadi MJ, Asvadi Kermani I, Farassati F, Dastgiri S. Colorectal cancer in iran: molecular epidemiology and screening strategies. *J Cancer Epidemiol* 2015; **2015**: 643020 [PMID: 25685149 DOI: 10.1155/2015/643020]
111 **Ibrahim EM**, Zeeneldin AA, El-Khodary TR, Al-Gahmi AM, Bin Sadiq BM. Past, present and future of colorectal cancer in the Kingdom of Saudi Arabia. *Saudi J Gastroenterol* 2008; **14**: 178-182 [PMID: 19568534 DOI: 10.4103/1319-3767.43275]
112 International Agency for Research on Cancer. Globocan 2018: Cancer Fact Sheets — Colorectal Cancer. IARC. [cited 3 March 2021]. Available from: http://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf
113 **Hu KC**, Wu MS, Chu CH, Wang HY, Lin SC, Liu SC, Liu CC, Su TH, Chen CL, Liu CJ, Shih SC. Synergistic Effect of Hyperglycemia and Helicobacterpylori Infection Status on Colorectal Adenoma Risk. *J Clin Endocrinol Metab* 2017; **102**: 2744-2750 [PMID: 28475740 DOI: 10.1210/jc.2017-00257]

114 **NCD Risk Factor Collaboration (NCD-RisC)**. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**: 1513-1530 [PMID: 27061677 DOI: 10.1016/S0140-6736(16)00618-8]

115 **Dow LE**, O'Rourke KP, Simon J, Tschaharganeh DF, van Es JH, Clevers H, Lowe SW. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. *Cell* 2015; **161**: 1539-1552 [PMID: 26091037 DOI: 10.1016/j.cell.2015.05.033]

116 **Armaghany T**, Wilson JD, Chu Q, Mills G. Genetic alterations in colorectal cancer. *Gastrointest Cancer Res* 2012; **5**: 19-27 [PMID: 22574233]

117 **Kedrin D**, Gala MK. Genetics of the serrated pathway to colorectal cancer. *Clin Transl Gastroenterol* 2015; **6**: e84 [PMID: 25856207 DOI: 10.1038/ctg.2015.12]

118 **O'Brien MJ**, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006; **30**: 1491-1501 [PMID: 17122504 DOI: 10.1097/01.pas.0000213313.36306.85]

119 **Kim KM**, Lee EJ, Ha S, Kang SY, Jang KT, Park CK, Kim JY, Kim YH, Chang DK, Odze RD. Molecular features of colorectal hyperplastic polyps and sessile serrated adenoma/polyps from Korea. *Am J Surg Pathol* 2011; **35**: 1274-1286 [PMID: 21836485 DOI: 10.1097/PAS.0b013e318224cd2e]

120 **Meucci G**, Tatarella M, Vecchi M, Ranzi ML, Biguzzi E, Beccari G, Clerici E, de Franchis R. High prevalence of Helicobacter pylori infection in patients with colonic adenomas and carcinomas. *J Clin Gastroenterol* 1997; **25**: 605-607 [PMID: 9451672 DOI: 10.1097/00004836-199712000-00011]

121 **Breuer-Katschinski B**, Nemes K, Marr A, Rump B, Leiendecker B, Breuer N, Goebell H. Helicobacter pylori and the risk of colonic adenomas. Colorectal Adenoma Study Group. *Digestion* 1999; **60**: 210-215 [PMID: 10343134 DOI: 10.1159/000007661]

122 **Sonnenberg A**, Genta RM. Helicobacter pylori is a risk factor for colonic neoplasms. *Am J Gastroenterol* 2013; **108**: 208-215 [PMID: 23208272 DOI: 10.1038/ajg.2012.407]

123 **Rokkas T**, Sechopoulos P, Pistiolas D, Kothonas F, Margantinis G, Koukoulis G. The relationship of Helicobacter pylori infection and colon neoplasia, on the basis of meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 1286-1294 [PMID: 23820245 DOI: 10.1097/MEG.0b013e328363d3cd]

124 **Liou JM**, Lin JW, Huang SP, Lin JT, Wu MS. Helicobacter pylori infection is not associated with increased risk of colorectal polyps in Taiwanese. *Int J Cancer* 2006; **119**: 1999-2000 [PMID: 16708392 DOI: 10.1002/ijc.22050]

125 **Machida-Montani A**, Sasazuki S, Inoue M, Natsukawa S, Shaura K, Koizumi Y, Kasuga Y, Hanaoka T, Tsugane S. Atrophic gastritis, Helicobacter pylori, and colorectal cancer risk: a case-control study. *Helicobacter* 2007; **12**: 328-332 [PMID: 17669106 DOI: 10.1111/j.1523-5378.2007.00513.x]

126 **Choi DS**, Seo SI, Shin WG, Park CH. Risk for Colorectal Neoplasia in Patients With Helicobacter pylori Infection: A Systematic Review and Meta-analysis. *Clin Transl Gastroenterol* 2020; **11**: e00127 [PMID: 32032128 DOI: 10.14309/ctg.0000000000000127]

127 **Zuo Y**, Jing Z, Bie M, Xu C, Hao X, Wang B. Association between Helicobacter pylori infection and the risk of colorectal cancer: A systematic review and meta-analysis. *Medicine (Baltimore)* 2020; **99**: e21832 [PMID: 32925719 DOI: 10.1097/MD.0000000000021832]

128 **Grahn N**, Hmani-Aifa M, Fransén K, Söderkvist P, Monstein HJ. Molecular identification of Helicobacter DNA present in human colorectal adenocarcinomas by 16S rDNA PCR amplification and pyrosequencing analysis. *J Med Microbiol* 2005; **54**: 1031-1035 [PMID: 16192433 DOI: 10.1099/jmm.0.46122-0]

129 **Soylu A**, Ozkara S, Alis H, Dolay K, Kalayci M, Yasar N, Kumbasar AB. Immunohistochemical testing for Helicobacter Pylori existence in neoplasms of the colon. *BMC Gastroenterol* 2008; **8**: 35 [PMID: 18702825 DOI: 10.1186/1471-230X-8-35]

130 **Wong SH**, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 690-704 [PMID: 31554963 DOI: 10.1038/s41575-019-0209-8]

131 **Hua S**, Marks E, Schneider JJ, Keely S. Advances in oral nano-delivery systems for colon targeted drug delivery in inflammatory bowel disease: selective targeting to diseased versus healthy tissue. *Nanomedicine* 2015; **11**: 1117-1132 [PMID: 25784453 DOI: 10.1016/j.nano.2015.02.018]

132 **Butt J**, Epplein M. Helicobacter pylori and colorectal cancer-A bacterium going abroad? *PLoS Pathog* 2019; **15**: e1007861 [PMID: 31393968 DOI: 10.1371/journal.ppat.1007861]

133 **Hu KC**, Wu MS, Chu CH, Wang HY, Lin SC, Liu CC, Su TH, Liao WC, Chen CL, Liu CJ, Shih SC. Decreased Colorectal Adenoma Risk After Helicobacter pylori Eradication: A Retrospective Cohort Study. *Clin Infect Dis* 2019; **68**: 2105-2113 [PMID: 30566695 DOI: 10.1093/cid/ciy591]

134 **Ahmed RL**, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. *Cancer* 2006; **107**: 28-36 [PMID: 16721800 DOI: 10.1002/cncr.21950]

135 **Haggar FA**, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* 2009; **22**: 191-197 [PMID: 21037809 DOI: 10.1055/s-0029-1242458]

136 **Lieberman DA**, Prindiville S, Weiss DG, Willett W; VA Cooperative Study Group 380. Risk

factors for advanced colonic neoplasia and hyperplastic polyps in asymptomatic individuals. *JAMA* 2003; **290**: 2959-2967 [PMID: 14665657 DOI: 10.1001/jama.290.22.2959]

137 **Tseng PH**, Lee YC, Chiu HM, Chen CC, Liao WC, Tu CH, Yang WS, Wu MS. Association of diabetes and HbA1c levels with gastrointestinal manifestations. *Diabetes Care* 2012; **35**: 1053-1060 [PMID: 22410812 DOI: 10.2337/dc11-1596]

138 **Yuhara H**, Steinmaus C, Cohen SE, Corley DA, Tei Y, Buffler PA. Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? *Am J Gastroenterol* 2011; **106**: 1911-21; quiz 1922 [PMID: 21912438 DOI: 10.1038/ajg.2011.301]

139 **Tsong WH**, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. *Br J Cancer* 2007; **96**: 821-827 [PMID: 17311023 DOI: 10.1038/sj.bjc.6603623]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 75-89

DOI: 10.4251/wjgo.v14.i1.75

ISSN 1948-5204 (online)

REVIEW

Bromodomain and extra-terminal inhibitors emerge as potential therapeutic avenues for gastrointestinal cancers

Hui-Yan Sun, Song-Tao Du, Ya-Yun Li, Guang-Tong Deng, Fu-Rong Zeng

ORCID number: Hui-Yan Sun 0000-0002-5697-933X; Song-Tao Du 0000-0001-5536-3632; Ya-Yun Li 0000-0002-4799-663X; Guang-Tong Deng 0000-0002-4424-9727; Fu-Rong Zeng 0000-0001-6621-8131.

Author contributions: Zeng FR, Deng GT and Sun HY designed the study; Sun HY and Deng GT wrote the manuscript; Du ST, Li YY helped to revise the manuscript; all the authors supported the study.

Conflict-of-interest statement: No conflict of interest.

Supported by Fellowship of the China Postdoctoral Science Foundation, No. 2020M682594, and No. 2021T140748.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Open-Access: This article is an

Hui-Yan Sun, Song-Tao Du, Ya-Yun Li, Guang-Tong Deng, Fu-Rong Zeng, Department of Dermatology, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Hui-Yan Sun, Fu-Rong Zeng, Department of Oncology, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Hui-Yan Sun, Song-Tao Du, Ya-Yun Li, Guang-Tong Deng, Fu-Rong Zeng, Hunan Engineering Research Center of Skin Health and Disease, Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Hui-Yan Sun, Song-Tao Du, Ya-Yun Li, Guang-Tong Deng, Fu-Rong Zeng, National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Song-Tao Du, Department of Colorectal Surgical Oncology, Harbin Medical University Cancer Hospital, Harbin 150081, Heilongjiang Province, China

Corresponding author: Fu-Rong Zeng, MD, Doctor, Department of Oncology, Xiangya Hospital, Central South University, No. 87 Xiangya Road, Changsha 410008, Hunan Province, China. zengflorachn@hotmail.com

Abstract

Gastrointestinal (GI) cancers, including colorectal cancer, pancreatic cancer, liver cancer and gastric cancer, are severe social burdens due to high incidence and mortality rates. Bromodomain and extra-terminal (BET) proteins are epigenetic readers consisting of four conserved members (BRD2, BRD3, BRD4 and BRDT). BET family perform pivotal roles in tumorigenesis through transcriptional regulation, thereby emerging as potential therapeutic targets. BET inhibitors, disrupting the interaction between BET proteins and acetylated lysines, have been reported to suppress tumor initiation and progression in most of GI cancers. In this review, we will demonstrate how BET proteins participate in the GI cancers progression and highlight the therapeutic potential of targeting BET proteins for GI cancers treatment.

Key Words: Gastrointestinal cancer; Bromodomain and extra-terminal proteins; Bromodomain and extra-terminal inhibitors; Acetylated lysines

Received: March 17, 2021
Peer-review started: March 17, 2021
First decision: July 27, 2021
Revised: August 11, 2021
Accepted: November 30, 2021
Article in press: November 30, 2021
Published online: January 15, 2022

P-Reviewer: Biondi A
S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

Core Tip: Bromodomain and extra-terminal (BET) inhibitors, as promising targeted agents, emerge as a new therapeutic avenue for gastrointestinal (GI) cancers. Based on preclinical evidence, BET inhibitors, alone or in combination with other therapies, were effective to suppress the progression of GI cancers.

Citation: Sun HY, Du ST, Li YY, Deng GT, Zeng FR. Bromodomain and extra-terminal inhibitors emerge as potential therapeutic avenues for gastrointestinal cancers. *World J Gastrointest Oncol* 2022; 14(1): 75-89
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/75.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.75

INTRODUCTION

Gastrointestinal (GI) cancers, including colorectal cancer (CRC), liver cancer, gastric cancer (GC) and pancreatic cancer, are among the most common malignancies worldwide with high incidence and mortality rates. In the latest global cancer data of 2020, CRC is the second leading cause of cancer death (9.4% of the total cancer deaths), followed by stomach cancer (8.3%), liver cancer (7.7%) and pancreatic cancer (4.6%) [1]. Surgery still remains the only curative treatment for GI cancers[2]. However, most patients are diagnosed as GI cancer at advanced stages or metastases, and thus lose the chance of surgery. Several therapies including chemotherapy[3,4], radiotherapy[5], chemoradiotherapy[6] and immunotherapy[7,8], have been developed for those GI cancers patients who are intolerable to operation. Unfortunately, inevitable toxicity[9], innate or acquired chemo-resistance[10] and low response[11] limit the clinical use of these treatments, highlighting the need for developing new therapeutic strategies.

Bromodomain and extra-terminal (BET) protein inhibitors emerge as a new therapeutic avenue for multiple cancers, including GI cancers. BET inhibitors exert anti-cancer activities by competitively binding to BET proteins and disrupting the interaction between BET proteins and acetylated lysines. Increasing studies have reported that upregulation of BET proteins leads to abnormal transcriptional regulation[12], which facilitates tumor initiation and progression. Down-regulation of BET proteins expression and inactivation of their function represent a possible mechanism of anti-tumor effect of BET inhibitors. Therefore, BET inhibitors present to be a rational strategy for the sake of GI cancers treatment. Several BET inhibitors targeting the BET bromodomains (BD) are currently under clinical investigations and preclinical data provides rationale for the use of BET inhibitors in treating GI cancers.

In this review, we will briefly describe the structure and inhibition mechanism of BET proteins and illustrate the role of BET proteins in the initiation and progression of human GI cancers. Then, we will identify whether targeting BET proteins, alone or in combination with other therapies, exhibits potential benefits in GI cancers through preclinical evidence. Finally, we will speculate the outlook of the translation of BET inhibitors into clinic.

BET PROTEINS: STRUCTURE AND INHIBITION MECHANISM

BET family proteins include four subtypes: BRD2 (also known as FSRG1, RING3, RNF3, FSH, or D6S113E), BRD3 (also known as ORFX or RING3L), BRD4 (also known as MCAP or HUNK1) and BRDT (also known as BRD6, CT9, or SPGF21)[13,14]. Each of the BET proteins has a highly conversed structure including two tandem ~110 amino acid bromodomains (BD1 and BD2) with direct specificity for acetylated lysines, followed by an extra-terminal (ET) protein-protein interaction domain[15]. Notably, BRD4 and BRDT comprise a C-terminal domain, which functionally recruits transcriptional regulators, like the positive transcription elongation factor b (P-TEFb)[16,17] (Figure 1). The similarity and difference in structure among BET proteins may partly interpret the parallel and differential function in human disease, especially in cancer.

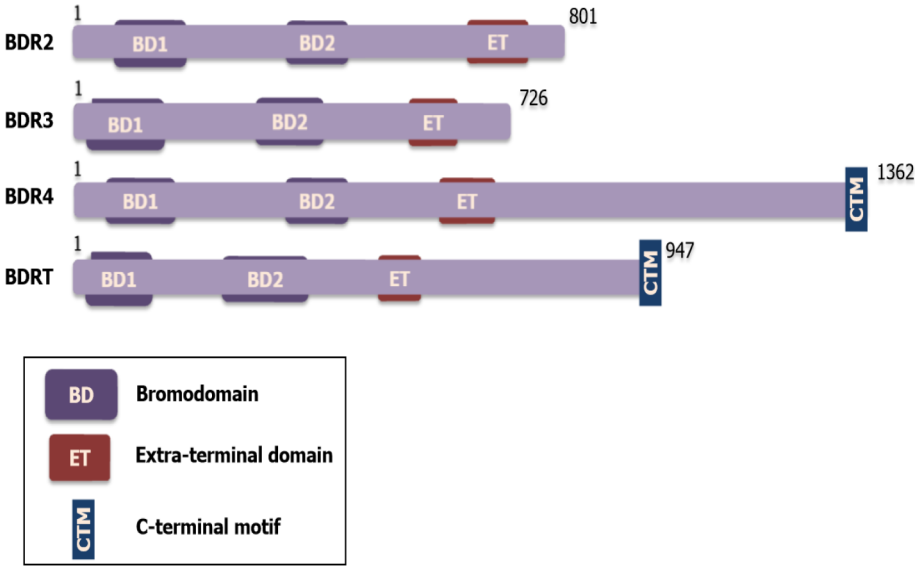


Figure 1 Schematic of basic domain structure of Bromodomain and extra-terminal protein family; BRD2, BRD3, BRD4, and BRDT. Each Bromodomain and extra-terminal protein has two bromodomains (BD1 and BD2) and one extra-terminal domain. And BRD4 and BRDT specially contain a C-terminal motif. ET: Extra-terminal; BD: Bromodomain.

BET proteins have two BDs with the acetylated lysine binding pocket. Compared with acetylated histones, BDs have a higher affinity for small molecules, which provide new possibilities for the development of inhibitors[18]. By occupying the BD pockets, BET inhibitors, such as JQ-1, mimic the binding mode and competitively inhibit binding between acetylated lysines and BDs, resulting in disrupting oncogenic rearrangement and inhibiting the development of some aggressive types of cancer (Figure 2).

BET PROTEINS IN GI CANCERS

Oncogenic roles of BET proteins family were firstly revealed in the NUT carcinoma. BRD4 and BRD3 are involved in the chromosomal rearrangements of NUT carcinoma by forming BET-NUT fusion protein[19]. The inspirational discovery that BET proteins serve as potential cancer therapeutic targets encourages researchers to look for possible functions of BET proteins in other cancers, including GI cancers. Strikingly, BET proteins (BRD2, BRD4) are overexpressed in GI cancers and have been reported to promote GI cancers progression *via* multiple mechanisms.

BRD2 was firstly defined as a non-canonical protein kinase[20], which could promote the GI cancers progression by recruiting transcriptional factors and initiating transcriptional regulation. Recent studies demonstrated that BRD2 promoted the progression of CRC, pancreatic ductal adenocarcinoma (PDAC) and GC[21]. Specifically, BRD2 forms a complex with transcription factor ELK4 by recognizing its K125 acetyl-lysine, and then activates transcription of LAMB3 in CRC, leading to tumor growth and metastasis[22]. Moreover, BRD2 drives a fibroinflammatory stromal reaction in PDAC by initiating the transcription of oncogene cellular-myelocytomatosis (c-MYC) and other stroma-inducible genes[23]. Huang *et al*[24] illustrated a different pathway that BRD2 could activate the transcriptional factor GLI, which regulated the pancreatic cancer microenvironment. These findings suggest that BRD2 is a poor prognostic predictor of GI cancers.

BRD3 was rarely studied in GI cancers. However, recently, some frameshift mutations of BRD3 have been found in GC[25]. Also, Tan *et al*[26] found that BRD3 was among the top six driver genes for familial aggregation of PDAC through whole-genome sequencing. That means unlike BRD2/4, BRD3 may function in GI cancer through a different mechanism.

BRD4 is the most extensively studied BET proteins in GI cancers which is highly expressed in cancer tissues and cell lines, including CRC[27], pancreatic cancer[28], liver cancer[29], and GC[30]. The overexpression of BRD4 promotes GI cancer cell growth, differentiation and metastasis, and correlates with poor outcome of GI cancers patients[31,32]. On one hand, BRD4 could directly bind to the promoter region of

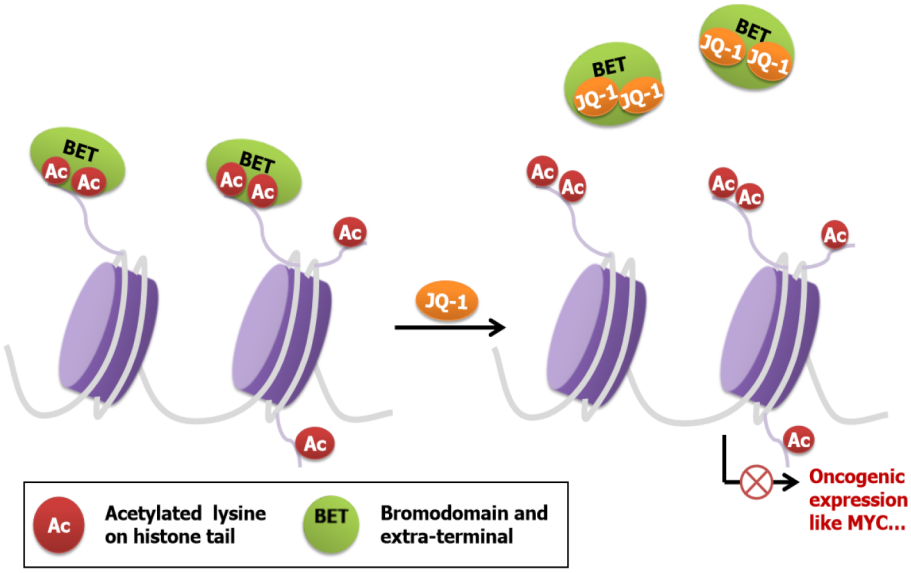


Figure 2 Schematic of the mechanism of the action of Bromodomain and extra-terminal inhibitors. Upon Bromodomain and extra-terminal (BET) inhibitors binding to Bromodomains, BET proteins are displaced from chromatin. Lacking domains directly interacting with chromatin, BET proteins fail to activate oncogenes, and thus BET inhibitors exert cytotoxic effects on cancer cells. BET: Bromodomain and extra-terminal.

oncogenes and induce their overexpression, including c-MYC[33], E2F2[34], caveolin-2 [28], PES1[35] and CD276[36]. On the other hand, BRD4 could recognize acetylated lysines on epithelial-to-mesenchymal transition (EMT)-activating transcriptional factors like Twist or Snail, the activation of which facilitated the differentiation and survival of EMT cells and promoted metastatic growth in GI cancers[27,37,38]. Additionally, BRD4 was reported to be recruited to senescence-activated super-enhancers to mediate cellular senescence[39]. The senescent cancer cells induced the secretion of various cytokines and increased CRC cells migration and invasion abilities [40]. In addition to the direct induction of tumorigenesis, BRD4 was also involved in the crosstalk between cancer and cancer-associated fibroblasts. Inhibiting the BRD4 protein changed both transcription and structure of matrisome in PDAC and resulted in better patients' survival[41]. Moreover, Yasukawa *et al*[42] also described that BRD4 played an important role in cancer associated fibroblasts in GC[42]. These oncogenic functions suggest that BRD4 is an important molecular target for GI cancers.

BET INHIBITORS IN GI CANCERS

Given that BET proteins are important regulators in GI cancer, targeting BET proteins will be a good therapeutic strategy for GI cancers treatment. A series of compounds have been reported as potential therapeutic avenues for GI cancers by targeting BET proteins (Table 1). BET inhibitors share the similar mechanism by displacing BET proteins from chromatin and regulating transcriptional factors. By mediating cell cycle arrest, facilitating apoptosis, and inducing senescence, BET inhibitors functionally inhibit cell proliferation, invasion and migration in most GI cancers including CRC, pancreatic cancer, liver cancer and GC[43]. Mechanically, BET inhibitors exert anti-tumor activity in c-MYC dependent, as well as c-MYC independent manners[44]. BET inhibitors have been widely used in preclinical models, but BET inhibitors alone exhibit limited-single agent activity confronting drug resistance. Combinational therapy with chemotherapy, immunotherapy or other small molecule inhibitors may amplify the clinical outcomes in GI cancers. Herein, we review the application of BET inhibitors in GI cancers.

CRC

Preclinical data demonstrated that BET inhibitors alone had exhibited efficacy against CRC by inhibiting tumor growth and inducing apoptosis *in vivo* and *vitro*[27,45]. However, resistance to BET inhibitors was the major obstacle to CRC treatment. Wang *et al*[46] raised one possible mechanism that the interaction of STAT3 through BRD4 phosphorylation might result in the resistance of BET inhibitors in CRC. Combining BET inhibitors and other targeted therapies could help to overcome resistance and

Table 1 Preclinical models of Bromodomain and extra-terminal inhibitors in gastrointestinal cancers

GI cancers models	BET inhibitors	Combination with	Targets	Pathway/mechanism	Ref.
CRC	JQ-1	5-FU	DR5	Apoptosis	[49]
	JQ-1	Bortezomib	MYC, FOXM1	G2/M arrest	[47]
	JQ-1	-	HGF, MET	Cancer-associated fibroblasts	[98]
	Apabetalone	-	APOA1	Intracellular cholesterol metabolism	[99]
	JQ-1	BEZ235 (PI3K/mTOR inhibitor)	RTKs	Overcome resistance to PI3K/mTOR inhibition	[40]
	JQ-1	Sulforaphane (HDAC3 inhibitor)	ERCC2	Nucleotide excision repair pathway	[48]
	I-BET151, bromosporine	-	BRD4, SNAIL, SLUG	EMT	[100]
SMAD4-defificient CRC	OTX-015	-	MYC	MYC-p21 axis, G1 cell cycle arrest	[54]
Colon cancer	JQ-1	-	Nkd2, β-catenin, miR-21	Wnt/β-catenin signaling, apoptosis	[45]
Gastric and colon cancer	JQ-1	Arsenic sulfide	NFATs, c-MYC	Mitochondrial pathway induced cell apoptosis	[51]
PDAC	JQ-1	-	HMGA2	Block growth of chemoresistant cells	[55]
	JQ-1	Olaparib (PARP inhibitor)	BRD2/4, Ku80, RAD51	DNA damage	[60]
	JQ-1	SAHA (HDAC inhibitor)	p57	Cell death	[61]
	JQ-1	Gemcitabine	HMGCS2, APOC1	DNA damage and apoptosis	[62]
	CPI203	-	MYC, GLI, SHH	SHH-GLI signaling pathway, cell cycle progression	[24]
Pancreatic cancer	JQ-1, OTX-015	Quercetin	BRD4(JQ-1) and hnRNPA1(Quercetin)	Apoptosis	[63]
KDM6A null pancreatic cancer	JQ-1	-	MYC, p63, RUNX3	Reverse squamous differentiation	[101, 102]
Liver cancer	JQ-1	-	BRD4, E2F2	BRD4-E2F2-cell cycle regulation axis,	[34]
	JQ-1	-	PD-L1, PD-L2	PD-1/PD-L1 signaling	[71]
HCC	JQ-1, I-BET762	Anti-PD-L1 Ab	BRD4, C/EBPβ, p300	Suppress M-MDSCs, enhance PD-L1 blockade efficacy	[73]
	JQ-1	-	MYC	Impair mitochondrial respiration and glycolysis, induce apoptosis	[66]
	Hjp-6-171	GSK3β inhibitor (CHIR-98014)	β-catenin, NOTUM	WNT pathway	[68]
	SF1126 (Pan PI3K/BRD4 Inhibitor)	Sorafenib	BRD4, c-MYC	Ras/Raf/MAPK, PI3K/AKT/mTOR pathways	[90]
	JQ-1	-	PES1	Cell proliferation, glycolysis	[35]
	JQ-1	Flavopiridol	Mcl-1	Apoptosis	[67]
	JQ-1, OTX-015	-	SMARCA4	Down-regulate migration related genes	[65]
CCA2	JQ-1	PI3K/mTOR inhibitors	c-Myc, YAP	Overcome resistance to PI3K/mTOR inhibition	[64]
Gastric cancer	JQ-1	-	BRD4, E2F	E2F/miR-106b-5p/p21 axis, cellular senescence	[32]
	JQ-1	-	RUNX2	RUNX2/NID1 signaling, site-specific chromatinremodeling	[75]
	JQ1, PNZ5	-	c-MYC	Apoptosis	[33, 74]

	iBET-151	Paclitaxel	RTK	G1 cell cycle arrest	[79]
	AZD5153	-	Sirt5, Mus81	Sirt5/Mus81/ZEB1 axis, inhibit metastasis	[76]
GAC	JQ-1	CA3 (YAP inhibitor)	c-MYC	Gal3/RalA/YAP1/c-MYC axis	[78]

CRC: Colorectal cancer; PDAC: Pancreatic ductal adenocarcinoma; HCC: Hepatocellular carcinoma; CCA: Cholangiocarcinoma; GAC: Gastric adenocarcinoma; 5-FU: 5-fluorouracil; c-MYC: Cellular-myelocytomatosis.

render CRC more sensitive to BET inhibitors. For example, nuclear factor-kappa B inhibitors[47], PI3K/mTOR inhibitors[40], HDAC3 inhibitor[48] have been reported to sensitize GI cancers to BET inhibitors, and finally achieve synergistical effects.

Moreover, BET inhibition could be used in combination with chemotherapy to enhance chemotherapy effect *via* increasing the apoptosis induction[49]. For example, BET inhibitors could increase the sensitivity of CRC cells to 5-fluorouracil[50] and Arsenic sulfide[51,52] (Figure 3). More importantly, this combination therapy could decrease the side effect of chemotherapeutic drugs[53]. Moreover, BET inhibitors conferred a synthetic lethality with loss of SMAD4 in CRC cells by restoring the loss of c-MYC repression[54], suggesting that BET inhibitors were essential for the treatment of SMAD4-deficient CRC.

Pancreatic cancer

BET inhibitors not only effectively inhibited PDAC cell growth in three-dimensional collagen partly by repressing c-MYC expression, but also conducted its efficacy in a MYC-independent way by repressing the expression of FOSL1[55]. However, clinical studies suggested that BET inhibitors monotherapies were not effective revenues for PDAC treatment[56]. Drug resistance assumed the major responsibility for treatment failure. The main mechanism of resistance was associated with either up-regulating or stabilizing c-MYC expression. Loss of FBP1[57] , aberrant expression of ADAR1[58], high levels of GLI[24] and overexpression of PES1[59] could explain the up-regulation of c-MYC in pancreatic cancer.

To improve the efficacy of BET inhibitor on PDAC, several studies evaluated the efficiency of BET inhibitors in combination with other agents. Encouragingly, BET inhibitors could synergize with other target therapy in preclinical PDAC models. For example, BET inhibitor attenuated the DNA repair through decreasing Ku80 and RAD51 proteins, and sensitized the PDAC to PARP inhibitors[60]. Another team also illustrated that BET inhibitors synergizing with HDAC inhibitors enhanced the efficacy of inducing cell death *via* de-repressing p57[61]. In addition to being combined with target therapies, BET augmented the efficiency of chemotherapeutic drugs like Gemcitabine by increasing DNA damage and apoptosis[62]. Besides, BET inhibitors combined with Quercetin suppress hnRNPA1 leading to better therapeutic effect compared with monotherapy[63].

Liver cancer

BET inhibitors exhibit anti-tumorigenic effects on both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), but in different manners. JQ-1 inhibited CCA growth in a MYC-dependent way[64], while JQ-1 played its anti-tumor role in HCC by suppressing E2F2-cell cycle regulation circuit[34] or the expression of SMARCA4[65]. Notably, Yin *et al*[66] stated that JQ-1 exerted more cytotoxicity on MYC-positive HCC cells than sorafenib (first-line drug for advanced HCC) by inducing more apoptosis. This team further demonstrated that EGFR signaling contributed to the JQ1 resistance by stabilizing MYC. Zhang *et al*[67] arrived at a different resistance mechanism that upregulation of Mcl-1 was a major contributor to the resistance to BET inhibitor in HCC cells. They further found that BET inhibitors, in combination with other drugs capable of down-regulating Mcl-1 had a synergic effect in human HCC. Liu *et al*[68] reported another resistance mechanism and the reactivation of WNT pathway in liver cancer cells could increase the sensitivity of HCC to BET inhibitor[68].

BET inhibitor were also reported to impact the immunotherapy efficacy in HCC (Figure 4). Several studies had shown that BET inhibition could enhance anti-tumor immunity *via* modulating programmed cell death-ligand 1 (PD-L1) expression[69,70]. Liu *et al*[71] demonstrated that JQ-1 could decrease the total mRNA and protein levels of PD-L1 in liver cancer cell lines. However, Liu *et al*[72] reported that JQ1 upregulated the expression of PD-L1 on the plasma membrane *in vivo* and *in vitro*, but did not change the total levels of PD-L1 mRNA and protein. Another study conducted by Cheng and his colleague[73] reported that I-BET762, exerted a synergistic effect with

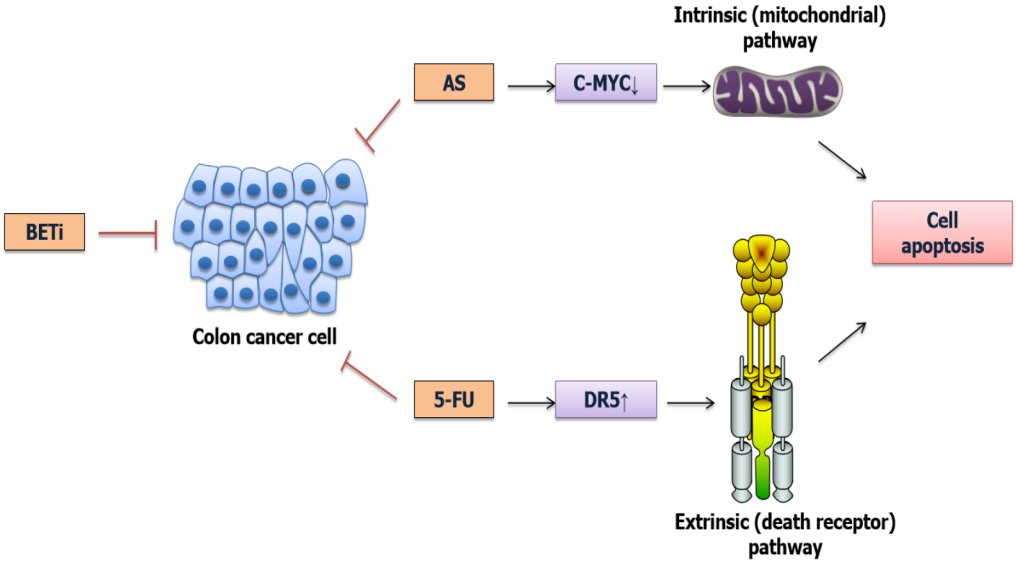


Figure 3 Schematic of Bromodomain and extra-terminal inhibitors enhancing chemotherapy effect through apoptosis induction. Bromodomain and extra-terminal (BET) inhibitors and arsenic sulfide exert synergistic cytotoxicity *via* down-regulating c-MYC and induce cell apoptosis in an intrinsic (mitochondrial) pathway; while BET inhibitors in combination with 5-Fluorouracil mediate apoptosis in a death receptor 5-depedent manner which is regulated in extrinsic(death receptor) pathway. BET: Bromodomain and extra-terminal; AS: Arsenic sulfide; 5-FU: 5-fluorouracil; DR5: Death receptor 5; c-MYC: Cellular-myelocytomatosis.

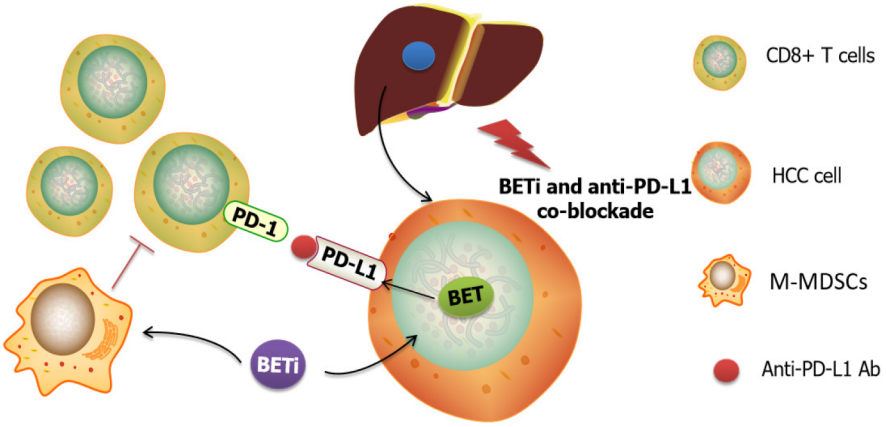


Figure 4 Schematic of Bromodomain and extra-terminal inhibitors combined with anti-programmed death-1-ligand-1 Ab therapeutic effects. Bromodomain and extra-terminal (BET) inhibitors treatment impacts programmed death-1-ligand-1 (PD-L1) expression, resulting in sensitizing the liver response to anti-PD-L1 blockade. Also, the co-inhibition can inhibit liver-infiltrating monocytic myeloid-derived suppressor cells and enhance tumor-infiltrating CD8+ T cells, which contributes to the elimination of drug resistance. BET: Bromodomain and extra-terminal; HCC: Hepatocellular carcinoma; M-MDSCs: Monocytic myeloid-derived suppressor cells; PD-L1: Programmed death-1-ligand-1.

anti-PD-L1 in the HCC model leading to augment tumor infiltrating lymphocytes. Altogether, the mechanism by which BET inhibitors modulate immunotherapy is different, but the phenotypic enhancement of immunotherapy by BET inhibitors is assured.

GC

JQ-1 exerts an anti-cancer effect on GC as well. Interestingly, JQ-1 has race specificity on GC that Asians rendered more resistance to BET inhibitors than Brazilians[74]. Recently, Zhou *et al*[75] noted that JQ-1 suppressed proliferation, migration and invasion of GC cells *via* targeting RUNX2/NID1 axis, while BET inhibitor AZD5153 inhibited GC metastasis by regulating Mus81 at both RNA and protein levels[76]. Kim *et al*[77] revealed new BRD4 inhibitor that showed efficiency in I-BET762 resistant GC cell lines[77]. Additionally, through blocking the expression of c-MYC and YAP1, JQ-1 reduced gastric adenocarcinoma cell growth induced by Gal-3, and the anti-cancer activity could be improved in combination with YAP inhibitors[78]. Other combination strategies with chemotherapy drugs have also been reported. The combination of

I-BET151 and paclitaxel increased the anti-GC tumor effect than single-treatment[79]. Also, JQ-1 synergized with arsenic sulfide targeting c-MYC, exhibits an increasing cytotoxic activity in both gastric and colon cells[52].

NEW BET INHIBITORS USING PROTAC TECHNOLOGY

Though exhibiting promising outcomes in GI cancers, BET inhibitors showed therapeutic limitations due to their reversibility, often followed by re-accumulating BET proteins and removing inhibition of c-MYC[19]. This motivated new BET targeting molecules using Proteolysis Targeting Chimeras (PROTACs) technology to be invented like ARV-825 and A1874. These molecules, also called BRD4-degrading PROTACs, are heterobifunctional compounds that contain two binders with one recruiting an E3 ubiquitin ligase cereblon (CRBN) and the other targeting BRD4 proteins based on BET inhibitors. Data has shown that these molecules induce effective and selective degradation of BRD4[80] (Figure 5). The approach to target BRD4 degradation instead of inhibition resulted in more potent suppression of c-MYC as well as c-MYC-dependent genes and led to a longer-lasting effect in GI cancers. For example, Lu *et al*[81] stated that ARV-825 was superior to OTX-015 and JQ-1 in the suppression of c-MYC expression in CCA and thus exerted more inhibition on CCA cell proliferation and apoptosis. Minko[82] reported a similar anticancer activity of ARV-825 in pancreatic cancer and this activity exhibited in both 2D cell culture and 3D multicellular tumor spheroid models. Additionally, Qin *et al*[83] showed that A1874 down-regulated c-MYC, Bcl-2, and cyclin D1 in colon cancer cells and had an anti-colon cancer activity by inhibiting cell proliferation, invasion and migration. Strikingly, A1874 presented to be much more effective than other BET inhibitors including JQ1 and I-BET151. However, after long-term exposure to BRD4-degrading PROTACs, resistance exists[84]. Downregulating the expression of CRBN is a common mechanism of resistance. In terms of this issue, Otto *et al*[85] proposed an alternative avenue to prevent the development of resistance, which might be the use of several PROTACs to recruit different E3 Ligases.

CLINICAL LANDSCAPE

BET inhibitors, including I-BET762 (NCT01587703), INCB057643 (NCT02711137), INCB054329 (NCT02431260), AZD5153 (NCT03205176) and OTX-015(NCT02698176) have entered Clinical Trial for diverse cancers[86], but the majority of them remain in the Phase I/II. Here, we are concentrating on the trials of BET inhibitors alone or in combination with other inhibitors in GI cancers (Table 2).

I-BET762 (Molibresib) is a pan-BET inhibitor that remarkably inhibits the PDAC cell proliferation by down-regulating c-MYC and reducing protein levels of ERK1/2. Remarkably, the anti-tumor effect can be enhanced combined with gemcitabine[87]. NCT03925428 is a phase I clinical trial that tests the side effects and best dose of I-BET 762 combined with entinostat in solid tumors or lymphomas advanced or refractory, including PDAC. However, the study was withdrawn because other protocol moved to disapprove.

INCB054329 and INCB057643 are two small-molecule BET inhibitors which exhibit anti-cancer activity by reducing the expression level of c-MYC[88,89]. Phase I/II dose-escalation, safety and tolerability studies of INCB054329 and INCB057643 were conducted in subjects with advanced malignancies including GI cancers. INCB054329 was terminated due to an unfavorable clinical Pharmacokinetic (PK) profile (NCT02431260). INCB057643 compared with INCB054329 has a longer half-life and a shorter PK variability. However, patients received INCB057643 resulted in treatment discontinuance or dose interruption or dose reduction due to TRAEs and the study ultimately terminated in 2020 (NCT02711137).

AZD5153 is a novel BRD4 inhibitor, effecting Mus81 down-regulation and suppressing tumor migration in GC[76]. A Phase I study was initiated to evaluate the safety, pharmacokinetics, and pharmacodynamics of AZD5253 alone or in combination with Olaparib in patients with malignant solid tumors, including pancreatic cancer. The recruiting status of this study remains active, not recruiting (NCT03205176).

Dual PI3K/BRD4 Inhibitor SF1126 blocks both the Ras/Raf/MAPK and PI3K/AKT/mTOR pathways and disrupts c-MYC expression as well[90]. And a Phase I clinical trial of SF1126 has completed in humans with well toleration and efficacy in

Table 2 Clinical trials of Bromodomain and extra-terminal inhibitors in gastrointestinal cancers (Trial ID on www.clinicaltrials.gov)

Drug	Combination with	Condition	Status	Clinical phase	Trial ID
INCB054329	-	Solid Tumors and Hematologic Malignancy (CRPC, BC, HGSC, CRC, Ewing sarcoma, Pancreatic adenocarcinoma, AML, MDS, MF, MM)	Terminated due to PK variability	Phase I/II	NCT02431260
INCB057643	Gemcitabine; Paclitaxel; Rucaparib; Abiraterone; Ruxolitinib; Azacitidine	Solid Tumors (CRPC, BC, HGSC, CRC, Glioblastoma multiforme, Ewing sarcoma, Pancreatic adenocarcinoma, AML, MDS)	Terminated due to safety issues	Phase I/II	NCT02711137
AZD5153	Olaparib	Malignant Solid Tumors, Lymphoma, Ovarian Cancer, Breast Cancer, Pancreatic Cancer, Prostate Cancer	Active, not recruiting	Phase I	NCT03205176
I-BET762 (Molibresib, GSK525762)	Entinostat	Solid tumors (Advanced Malignant Solid Neoplasm, Refractory Malignant Solid Neoplasm, Refractory Pancreatic Carcinoma, Stage II/IIA/IIB/III/IV Pancreatic cancer AJCC v8, Unresectable Pancreatic Carcinoma) or Lymphomas	Withdrawn (Other-Protocol moved to Disapprove)	Phase I	NCT03925428
SF1126	-	Advanced Hepatocellular Carcinoma	Active, not recruiting	Phase I	NCT03059147

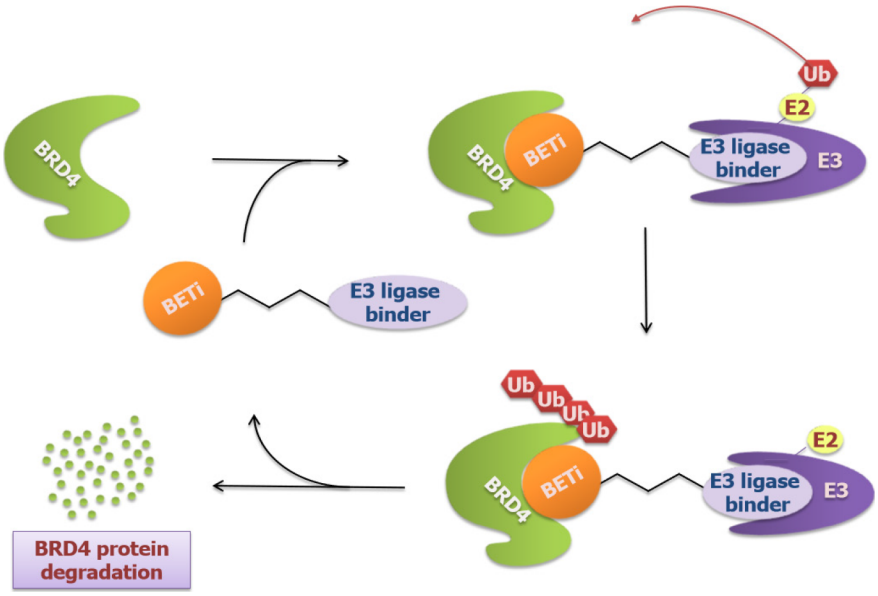


Figure 5 Schematic of new Bromodomain and extra-terminal molecules targeting Bromodomain-containing protein 4 using PROTACs technology. The bifunctional molecules contain two binders with one (usually bromodomain and extra-terminal inhibitors like JQ-1 or OTX015) targeting Bromodomain-containing protein 4 (BRD4) and the other binding E3 Ligase, which triggers the ubiquitination and degradation of BRD4. BRD4: Bromodomain-containing protein 4.

solid tumor including CRC[91]. Recently, SF1126 is being tested in combination with Nivolumab in patients with advanced HCC and this study is expected to be completed by October 2022 (NCT03059147).

With high bioavailability and biosafety, SF1126 has completed a Phase I clinical study and steps into a Phase II study in advanced HCC. And AZD5153 shows an optimistic preclinical result in GC treatment. All these evidences demonstrate that BET inhibitors constitute a promising field of clinical research in GI cancers. Continued progresses are required especially in exploring rational combinations to open new possibilities for BET inhibitors as anti-GI cancers agents.

CONCLUSION

BET inhibitors have emerged as a new possible strategy for the treatment of GI cancers in recent years. However, either nondurable cytotoxic effects, such as thrombocytopenia and GI disorders[92] or drug resistance make BET inhibitors fail to be adminis-

trated as single agents by far. To achieve better selectivity and reduce unwanted toxicities, BET inhibitors continue to be updated, increasing their potential in cancer treatment.

The first-generation pan-BET inhibitors have been identified to suppress GI cancer in preclinical results, however, the inevitable side effects limit their clinical applications. Hence, drug discovery efforts concentrate on selectively inhibiting BET proteins[93]. Selective BD inhibitors achieved almost equally efficiency in cancer to the pan-BET inhibitors[94] and showed less toxicity[95]. A set of selective BD inhibitors help to understand the role of BD in cancers and further focusing on specific BD perturbations may provide more efficiency and tolerability in GI cancers treatment.

Another approach to acquire selective inhibition is to target each BET family members. Since BRD4 is the predominant BET protein that mediates the development of GI cancers, selective BRD4 inhibition may have a better outlook. New BRD4 degraders ARV-825 and A1874 that have already shown their antitumor efficiency in preclinical results support further clinical development of BET inhibitors in GI cancers.

Other strategy to improve the efficacy and pharmacokinetic property of BET inhibitors is *via* modulating their structure. After modification, these major clinical stage BET inhibitors acquire better tumor killing capacity with minimal IC_{50} in multiple solid tumors[96]. The optimistic preclinical result makes it possible to treat GI cancer with single agents.

Additionally, synergistic inhibition provides an optimistic prospect for increasing the efficacy of BET inhibitors. The preclinical and clinical results verify high potential in combinational therapy. The resistance to BET inhibitors will be overcome if combined with drugs targeting the pathways that cause resistance[47]. Besides, the dosage will be decreased dramatically if combined with drugs rendering GI cancers more sensitive to BET inhibitors[97]. Without a doubt, BET inhibitors emerge as a promising avenue for the GI cancers treatment.

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Stoica AF**, Chang CH, Pauklin S. Molecular Therapeutics of Pancreatic Ductal Adenocarcinoma: Targeted Pathways and the Role of Cancer Stem Cells. *Trends Pharmacol Sci* 2020; **41**: 977-993 [PMID: 33092892 DOI: 10.1016/j.tips.2020.09.008]

3 **Moertel CG**. Chemotherapy of gastrointestinal cancer. *N Engl J Med* 1978; **299**: 1049-1052 [PMID: 360064 DOI: 10.1056/NEJM197811092991906]

4 **Chen X**, Zeh HJ, Kang R, Kroemer G, Tang D. Cell death in pancreatic cancer: from pathogenesis to therapy. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 804-823 [PMID: 34331036 DOI: 10.1038/s41575-021-00486-6]

5 **Debenham BJ**, Hu KS, Harrison LB. Present status and future directions of intraoperative radiotherapy. *Lancet Oncol* 2013; **14**: e457-e464 [PMID: 24079873 DOI: 10.1016/S1470-2045(13)70270-5]

6 **Joshi SS**, Badgwell BD. Current treatment and recent progress in gastric cancer. *CA Cancer J Clin* 2021; **71**: 264-279 [PMID: 33592120 DOI: 10.3322/caac.21657]

7 **Long J**, Lin J, Wang A, Wu L, Zheng Y, Yang X, Wan X, Xu H, Chen S, Zhao H. PD-1/PD-L blockade in gastrointestinal cancers: lessons learned and the road toward precision immunotherapy. *J Hematol Oncol* 2017; **10**: 146 [PMID: 28774337 DOI: 10.1186/s13045-017-0511-2]

8 **Golan T**, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, Kindler HL. Maintenance Olaparib for Germline *BRCA*-Mutated Metastatic Pancreatic Cancer. *N Engl J Med* 2019; **381**: 317-327 [PMID: 31157963 DOI: 10.1056/NEJMoa1903387]

9 **Dunn C**, Hong W, Gibbs P, Ackland S, Sjoquist K, Tebbutt NC, Price T, Burge M. Personalizing First-Line Systemic Therapy in Metastatic Colorectal Cancer: Is There a Role for Initial Low-Intensity Therapy in 2021 and Beyond? *Clin Colorectal Cancer* 2021; **20**: 245-255 [PMID: 34103264 DOI: 10.1016/j.clcc.2021.05.001]

10 **Zhang N**, Ng AS, Cai S, Li Q, Yang L, Kerr D. Novel therapeutic strategies: targeting epithelial-mesenchymal transition in colorectal cancer. *Lancet Oncol* 2021; **22**: e358-e368 [PMID: 34339656 DOI: 10.1016/S1470-2045(21)00343-0]

11 **Nussbaum YI**, Manjunath Y, Suvilesh KN, Warren WC, Shyu CR, Kaifi JT, Ciorba MA, Mitchem JB. Current and Prospective Methods for Assessing Anti-Tumor Immunity in Colorectal Cancer. *Int J Mol Sci* 2021; **22** [PMID: 33946558 DOI: 10.3390/ijms22094802]

12 **Doroshow DB**, Eder JP, LoRusso PM. BET inhibitors: a novel epigenetic approach. *Ann Oncol* 2017; **28**: 1776-1787 [PMID: 28838216 DOI: 10.1093/annonc/mdx157]

13 **Florence B**, Faller DV. You bet-cha: a novel family of transcriptional regulators. *Front Biosci* 2001; **6**: D1008-D1018 [PMID: 11487468 DOI: 10.2741/florence]

14 **Wang N**, Wu R, Tang D, Kang R. The BET family in immunity and disease. *Signal Transduct Target Ther* 2021; **6**: 23 [PMID: 33462181 DOI: 10.1038/s41392-020-00384-4]

15 **Dhalluin C**, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM. Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 1999; **399**: 491-496 [PMID: 10365964 DOI: 10.1038/20974]

16 **Itzen F**, Greifenberg AK, Bösken CA, Geyer M. Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation. *Nucleic Acids Res* 2014; **42**: 7577-7590 [PMID: 24860166 DOI: 10.1093/nar/gku449]

17 **Wu SY**, Chiang CM. The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. *J Biol Chem* 2007; **282**: 13141-13145 [PMID: 17329240 DOI: 10.1074/jbc.R700001200]

18 **Filippakopoulos P**, Knapp S. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat Rev Drug Discov* 2014; **13**: 337-356 [PMID: 24751816 DOI: 10.1038/nrd4286]

19 **Stathis A**, Bertoni F. BET Proteins as Targets for Anticancer Treatment. *Cancer Discov* 2018; **8**: 24-36 [PMID: 29263030 DOI: 10.1158/2159-8290.CD-17-0605]

20 **Belkina AC**, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. *Nat Rev Cancer* 2012; **12**: 465-477 [PMID: 22722403 DOI: 10.1038/nrc3256]

21 **Chen Z**, Li Z, Soutto M, Wang W, Piazuelo MB, Zhu S, Guo Y, Maturana MJ, Corvalan AH, Chen X, Xu Z, El-Rifai WM. Integrated Analysis of Mouse and Human Gastric Neoplasms Identifies Conserved microRNA Networks in Gastric Carcinogenesis. *Gastroenterology* 2019; **156**: 1127-1139.e8 [PMID: 30502323 DOI: 10.1053/j.gastro.2018.11.052]

22 **Zhu Z**, Song J, Guo Y, Huang Z, Chen X, Dang X, Huang Y, Wang Y, Ou W, Yang Y, Yu W, Liu CY, Cui L. LAMB3 promotes tumour progression through the AKT-FOXO3/4 axis and is transcriptionally regulated by the BRD2/acetylated ELK4 complex in colorectal cancer. *Oncogene* 2020; **39**: 4666-4680 [PMID: 32398865 DOI: 10.1038/s41388-020-1321-5]

23 **Sherman MH**, Yu RT, Tseng TW, Sousa CM, Liu S, Truitt ML, He N, Ding N, Liddle C, Atkins AR, Leblanc M, Collisson EA, Asara JM, Kimmelman AC, Downes M, Evans RM. Stromal cues regulate the pancreatic cancer epigenome and metabolome. *Proc Natl Acad Sci U S A* 2017; **114**: 1129-1134 [PMID: 28096419 DOI: 10.1073/pnas.1620164114]

24 **Huang Y**, Nahar S, Nakagawa A, Fernandez-Barrena MG, Mertz JA, Bryant BM, Adams CE, Mino-Kenudson M, Von Alt KN, Chang K, Conery AR, Hatton C, Sims RJ 3rd, Fernandez-Zapico ME, Wang X, Lillemoe KD, Fernández-Del Castillo C, Warshaw AL, Thayer SP, Liss AS. Regulation of GLI Underlies a Role for BET Bromodomains in Pancreatic Cancer Growth and the Tumor Microenvironment. *Clin Cancer Res* 2016; **22**: 4259-4270 [PMID: 27169995 DOI: 10.1158/1078-0432.CCR-15-2068]

25 **Cho J**, Chang YH, Heo YJ, Kim S, Kim NK, Park JO, Kang WK, Lee J, Kim KM. Four distinct immune microenvironment subtypes in gastric adenocarcinoma with special reference to microsatellite instability. *ESMO Open* 2018; **3**: e000326 [PMID: 29636988 DOI: 10.1136/esmoopen-2018-000326]

26 **Tan M**, Brusgaard K, Gerdes AM, Mortensen MB, Detlefsen S, Schaffalitzky de Muckadell OB, Joergensen MT. Whole genome sequencing identifies rare germline variants enriched in cancer related genes in first degree relatives of familial pancreatic cancer patients. *Clin Genet* 2021; **100**: 551-562 [PMID: 34313325 DOI: 10.1111/cge.14038]

27 **Hu Y**, Zhou J, Ye F, Xiong H, Peng L, Zheng Z, Xu F, Cui M, Wei C, Wang X, Wang Z, Zhu H, Lee P, Zhou M, Jiang B, Zhang DY. BRD4 inhibitor inhibits colorectal cancer growth and metastasis. *Int J Mol Sci* 2015; **16**: 1928-1948 [PMID: 25603177 DOI: 10.3390/ijms16011928]

28 **Jiao F**, Han T, Yuan C, Liang Y, Cui J, Zhuo M, Wang L. Caveolin-2 is regulated by BRD4 and contributes to cell growth in pancreatic cancer. *Cancer Cell Int* 2020; **20**: 55 [PMID: 32099528 DOI: 10.1186/s12935-020-1135-0]

29 **Niu X**, Wang W, Liang T, Li S, Yang C, Xu X, Li L, Liu S. CPI-203 improves the efficacy of anti-PD-1 therapy by inhibiting the induced PD-L1 overexpression in liver cancer. *Cancer Sci* 2021 [PMID: 34727389 DOI: 10.1111/cas.15190]

30 **Zhu Y**, Yang W, Ji G, Lin N, Wu W, Xiong P, Zheng C, Yan L, Wan P, Wang Y. Bromodomain protein 4 is a novel predictor of survival for gastric carcinoma. *Oncotarget* 2017; **8**: 31092-31100 [PMID: 28415703 DOI: 10.18632/oncotarget.16087]

31 **Zhang P**, Dong Z, Cai J, Zhang C, Shen Z, Ke A, Gao D, Fan J, Shi G. BRD4 promotes tumor growth and epithelial-mesenchymal transition in hepatocellular carcinoma. *Int J Immunopathol Pharmacol* 2015; **28**: 36-44 [PMID: 25816404 DOI: 10.1177/0394632015572070]

32 **Dong X**, Hu X, Chen J, Hu D, Chen LF. BRD4 regulates cellular senescence in gastric cancer cells *via* E2F/miR-106b/p21 axis. *Cell Death Dis* 2018; **9**: 203 [PMID: 29434197 DOI: 10.1038/s41419-017-0181-6]

33 **Ba M**, Long H, Yan Z, Wang S, Wu Y, Tu Y, Gong Y, Cui S. BRD4 promotes gastric cancer progression through the transcriptional and epigenetic regulation of c-MYC. *J Cell Biochem* 2018; **119**: 973-982 [PMID: 28681984 DOI: 10.1002/jcb.26264]

34 **Hong SH**, Eun JW, Choi SK, Shen Q, Choi WS, Han JW, Nam SW, You JS. Epigenetic reader BRD4 inhibition as a therapeutic strategy to suppress E2F2-cell cycle regulation circuit in liver cancer. *Oncotarget* 2016; **7**: 32628-32640 [PMID: 27081696 DOI: 10.18632/oncotarget.8701]

35 **Fan P**, Wang B, Meng Z, Zhao J, Jin X. PES1 is transcriptionally regulated by BRD4 and promotes

cell proliferation and glycolysis in hepatocellular carcinoma. *Int J Biochem Cell Biol* 2018; **104**: 1-8 [PMID: 30172011 DOI: 10.1016/j.biocel.2018.08.014]

36 **Zhao J**, Meng Z, Xie C, Yang C, Liu Z, Wu S, Wang B, Fan P, Jin X, Wu H. B7-H3 is regulated by BRD4 and promotes TLR4 expression in pancreatic ductal adenocarcinoma. *Int J Biochem Cell Biol* 2019; **108**: 84-91 [PMID: 30664982 DOI: 10.1016/j.biocel.2019.01.011]

37 **Wang LT**, Wang SN, Chiou SS, Liu KY, Chai CY, Chiang CM, Huang SK, Yokoyama KK, Hsu SH. TIP60-dependent acetylation of the SPZ1-TWIST complex promotes epithelial-mesenchymal transition and metastasis in liver cancer. *Oncogene* 2019; **38**: 518-532 [PMID: 30154425 DOI: 10.1038/s41388-018-0457-z]

38 **Qin ZY**, Wang T, Su S, Shen LT, Zhu GX, Liu Q, Zhang L, Liu KW, Zhang Y, Zhou ZH, Zhang XN, Wen LZ, Yao YL, Sun WJ, Guo Y, Liu KJ, Liu L, Wang XW, Wei YL, Wang J, Xiao HL, Liu P, Bian XW, Chen DF, Wang B. BRD4 Promotes Gastric Cancer Progression and Metastasis through Acetylation-Dependent Stabilization of Snail. *Cancer Res* 2019; **79**: 4869-4881 [PMID: 31311807 DOI: 10.1158/0008-5472.CAN-19-0442]

39 **Tasdemir N**, Banito A, Roe JS, Alonso-Curbelo D, Camiolo M, Tschaharganeh DF, Huang CH, Aksoy O, Bolden JE, Chen CC, Fennell M, Thapar V, Chicas A, Vakoc CR, Lowe SW. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. *Cancer Discov* 2016; **6**: 612-629 [PMID: 27099234 DOI: 10.1158/2159-8290.CD-16-0217]

40 **Lee HS**, Lee S, Cho KH. Cotargeting BET proteins overcomes resistance arising from PI3K/mTOR blockade-induced protumorigenic senescence in colorectal cancer. *Int J Cancer* 2020; **147**: 2824-2837 [PMID: 32599680 DOI: 10.1002/ijc.33047]

41 **Honselmann KC**, Finetti P, Birnbaum DJ, Monsalve CS, Wellner UF, Begg SKS, Nakagawa A, Hank T, Li A, Goldsworthy MA, Sharma H, Bertucci F, Birnbaum D, Tai E, Ligorio M, Ting DT, Schilling O, Biniossek ML, Bronsert P, Ferrone CR, Keck T, Mino-Kenudson M, Lillemoe KD, Warshaw AL, Fernández-Del Castillo C, Liss AS. Neoplastic-Stromal Cell Cross-talk Regulates Matrisome Expression in Pancreatic Cancer. *Mol Cancer Res* 2020; **18**: 1889-1902 [PMID: 32873625 DOI: 10.1158/1541-7786.MCR-20-0439]

42 **Yasukawa Y**, Hattori N, Iida N, Takeshima H, Maeda M, Kiyono T, Sekine S, Seto Y, Ushijima T. SAA1 is upregulated in gastric cancer-associated fibroblasts possibly by its enhancer activation. *Carcinogenesis* 2021; **42**: 180-189 [PMID: 33284950 DOI: 10.1093/carcin/bgaa131]

43 **Filippakopoulos P**, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. Selective inhibition of BET bromodomains. *Nature* 2010; **468**: 1067-1073 [PMID: 20871596 DOI: 10.1038/nature09504]

44 **Delmore JE**, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 2011; **146**: 904-917 [PMID: 21889194 DOI: 10.1016/j.cell.2011.08.017]

45 **Zhang Y**, Tian S, Xiong J, Zhou Y, Song H, Liu C. JQ-1 Inhibits Colon Cancer Proliferation *via* Suppressing Wnt/β-Catenin Signaling and miR-21. *Chem Res Toxicol* 2018; **31**: 302-307 [PMID: 29600711 DOI: 10.1021/acs.chemrestox.7b00346]

46 **Wang W**, Tang YA, Xiao Q, Lee WC, Cheng B, Niu Z, Oguz G, Feng M, Lee PL, Li B, Yang ZH, Chen YF, Lan P, Wu XJ, Yu Q. Stromal induction of BRD4 phosphorylation Results in Chromatin Remodeling and BET inhibitor Resistance in Colorectal Cancer. *Nat Commun* 2021; **12**: 4441 [PMID: 34290255 DOI: 10.1038/s41467-021-24687-4]

47 **Wu T**, Wang G, Chen W, Zhu Z, Liu Y, Huang Z, Huang Y, Du P, Yang Y, Liu CY, Cui L. Co-inhibition of BET proteins and NF-κB as a potential therapy for colorectal cancer through synergistic inhibiting MYC and FOXM1 expressions. *Cell Death Dis* 2018; **9**: 315 [PMID: 29472532 DOI: 10.1038/s41419-018-0354-y]

48 **Kapoor S**, Gustafson T, Zhang M, Chen YS, Li J, Nguyen N, Perez JET, Dashwood WM, Rajendran P, Dashwood RH. Deacetylase Plus Bromodomain Inhibition Downregulates ERCC2 and Suppresses the Growth of Metastatic Colon Cancer Cells. *Cancers (Basel)* 2021; **13** [PMID: 33809839 DOI: 10.3390/cancers13061438]

49 **Tan X**, Tong J, Wang YJ, Fletcher R, Schoen RE, Yu J, Shen L, Zhang L. BET Inhibitors Potentiate Chemotherapy and Killing of *SPOP*-Mutant Colon Cancer Cells *via* Induction of DR5. *Cancer Res* 2019; **79**: 1191-1203 [PMID: 30674532 DOI: 10.1158/0008-5472.CAN-18-3223]

50 **Cheng X**, Huang Z, Long D, Jin W. BET inhibitor bromosporine enhances 5-FU effect in colorectal cancer cells. *Biochem Biophys Res Commun* 2020; **521**: 840-845 [PMID: 31708100 DOI: 10.1016/j.bbrc.2019.11.009]

51 **Tan Z**, Zhang X, Kang T, Zhang L, Chen S. Arsenic sulfide amplifies JQ1 toxicity *via* mitochondrial pathway in gastric and colon cancer cells. *Drug Des Devel Ther* 2018; **12**: 3913-3927 [PMID: 30532520 DOI: 10.2147/DDDT.S180976]

52 **Zhang L**, Tong Y, Zhang X, Pan M, Chen S. Arsenic sulfide combined with JQ1, chemotherapy agents, or celecoxib inhibit gastric and colon cancer cell growth. *Drug Des Devel Ther* 2015; **9**: 5851-5862 [PMID: 26586936 DOI: 10.2147/DDDT.S92943]

53 **Lei L**, Xie X, He L, Chen K, Lv Z, Zhou B, Li Y, Hu W, Zhou Z. The bromodomain and extra-terminal domain inhibitor JQ1 synergistically sensitizes human colorectal cancer cells to

topoisomerase I inhibitors through repression of Mre11-mediated DNA repair pathway. *Invest New Drugs* 2021; **39**: 362-376 [PMID: 32981006 DOI: 10.1007/s10637-020-01014-0]

54 **Shi C**, Yang EJ, Liu Y, Mou PK, Ren G, Shim JS. Bromodomain and extra-terminal motif (BET) inhibition is synthetic lethal with loss of SMAD4 in colorectal cancer cells *via* restoring the loss of MYC repression. *Oncogene* 2021; **40**: 937-950 [PMID: 33293694 DOI: 10.1038/s41388-020-01580-w]

55 **Sahai V**, Kumar K, Knab LM, Chow CR, Raza SS, Bentrem DJ, Ebine K, Munshi HG. BET bromodomain inhibitors block growth of pancreatic cancer cells in three-dimensional collagen. *Mol Cancer Ther* 2014; **13**: 1907-1917 [PMID: 24807963 DOI: 10.1158/1535-7163.MCT-13-0925]

56 **Hessmann E**, Johnsen SA, Siveke JT, Ellenrieder V. Epigenetic treatment of pancreatic cancer: is there a therapeutic perspective on the horizon? *Gut* 2017; **66**: 168-179 [PMID: 27811314 DOI: 10.1136/gutjnl-2016-312539]

57 **Wang B**, Fan P, Zhao J, Wu H, Jin X. FBP1 Loss contributes to BET inhibitors resistance by undermining c-Myc expression in pancreatic ductal adenocarcinoma. *J Exp Clin Cancer Res* 2018; **37**: 224 [PMID: 30201002 DOI: 10.1186/s13046-018-0888-y]

58 **Sun Y**, Fan J, Wang B, Meng Z, Ren D, Zhao J, Liu Z, Li D, Jin X, Wu H. The aberrant expression of ADAR1 promotes resistance to BET inhibitors in pancreatic cancer by stabilizing c-Myc. *Am J Cancer Res* 2020; **10**: 148-163 [PMID: 32064158]

59 **Jin X**, Fang R, Fan P, Zeng L, Zhang B, Lu X, Liu T. PES1 promotes BET inhibitors resistance and cells proliferation through increasing c-Myc expression in pancreatic cancer. *J Exp Clin Cancer Res* 2019; **38**: 463 [PMID: 31718704 DOI: 10.1186/s13046-019-1466-7]

60 **Miller AL**, Fehling SC, Garcia PL, Gamblin TL, Council LN, van Waardenburg RCAM, Yang ES, Bradner JE, Yoon KJ. The BET inhibitor JQ1 attenuates double-strand break repair and sensitizes models of pancreatic ductal adenocarcinoma to PARP inhibitors. *EBioMedicine* 2019; **44**: 419-430 [PMID: 31126889 DOI: 10.1016/j.ebiom.2019.05.035]

61 **Mazur PK**, Herner A, Mello SS, Wirth M, Hausmann S, Sánchez-Rivera FJ, Lofgren SM, Kuschma T, Hahn SA, Vangala D, Trajkovic-Arsic M, Gupta A, Heid I, Noël PB, Braren R, Erkan M, Kleeff J, Sipos B, Sayles LC, Heikenwalder M, Heßmann E, Ellenrieder V, Esposito I, Jacks T, Bradner JE, Khatri P, Sweet-Cordero EA, Attardi LD, Schmid RM, Schneider G, Sage J, Siveke JT. Combined inhibition of BET family proteins and histone deacetylases as a potential epigenetics-based therapy for pancreatic ductal adenocarcinoma. *Nat Med* 2015; **21**: 1163-1171 [PMID: 26390243 DOI: 10.1038/nm.3952]

62 **Miller AL**, Garcia PL, Fehling SC, Gamblin TL, Vance RB, Council LN, Chen D, Yang ES, van Waardenburg RCAM, Yoon KJ. The BET Inhibitor JQ1 Augments the Antitumor Efficacy of Gemcitabine in Preclinical Models of Pancreatic Cancer. *Cancers (Basel)* 2021; **13** [PMID: 34298684 DOI: 10.3390/cancers13143470]

63 **Pham TND**, Stempel S, Shields MA, Spaulding C, Kumar K, Bentrem DJ, Matsangou M, Munshi HG. Quercetin Enhances the Anti-Tumor Effects of BET Inhibitors by Suppressing hnRNPA1. *Int J Mol Sci* 2019; **20** [PMID: 31480735 DOI: 10.3390/ijms20174293]

64 **Miao X**, Liu C, Jiang Y, Wang Y, Kong D, Wu Z, Wang X, Tian R, Yu X, Zhu X, Gong W. BET protein inhibition evidently enhances sensitivity to PI3K/mTOR dual inhibition in intrahepatic cholangiocarcinoma. *Cell Death Dis* 2021; **12**: 1020 [PMID: 34716294 DOI: 10.1038/s41419-021-04305-3]

65 **Choi HI**, An GY, Baek M, Yoo E, Chai JC, Lee YS, Jung KH, Chai YG. BET inhibitor suppresses migration of human hepatocellular carcinoma by inhibiting SMARCA4. *Sci Rep* 2021; **11**: 11799 [PMID: 34083693 DOI: 10.1038/s41598-021-91284-2]

66 **Yin Y**, Sun M, Zhan X, Wu C, Geng P, Sun X, Wu Y, Zhang S, Qin J, Zhuang Z, Liu Y. EGFR signaling confers resistance to BET inhibition in hepatocellular carcinoma through stabilizing oncogenic MYC. *J Exp Clin Cancer Res* 2019; **38**: 83 [PMID: 30770740 DOI: 10.1186/s13046-019-1082-6]

67 **Zhang HP**, Li GQ, Zhang Y, Guo WZ, Zhang JK, Li J, Lv JF, Zhang SJ. Upregulation of Mcl-1 inhibits JQ1-triggered anticancer activity in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2018; **495**: 2456-2461 [PMID: 29287727 DOI: 10.1016/j.bbrc.2017.12.153]

68 **Liu Y**, Xue M, Cao D, Qin L, Wang Y, Miao Z, Wang P, Hu X, Shen J, Xiong B. Multi-omics characterization of WNT pathway reactivation to ameliorate BET inhibitor resistance in liver cancer cells. *Genomics* 2021; **113**: 1057-1069 [PMID: 33667649 DOI: 10.1016/j.ygeno.2021.02.017]

69 **Zhu H**, Bengsch F, Svoronos N, Rutkowski MR, Bitler BG, Allegrezza MJ, Yokoyama Y, Kossenkov AV, Bradner JE, Conejo-Garcia JR, Zhang R. BET Bromodomain Inhibition Promotes Anti-tumor Immunity by Suppressing PD-L1 Expression. *Cell Rep* 2016; **16**: 2829-2837 [PMID: 27626654 DOI: 10.1016/j.celrep.2016.08.032]

70 **Hogg SJ**, Vervoort SJ, Deswal S, Ott CJ, Li J, Cluse LA, Beavis PA, Darcy PK, Martin BP, Spencer A, Traunbauer AK, Sadovnik I, Bauer K, Valent P, Bradner JE, Zuber J, Shortt J, Johnstone RW. BET-Bromodomain Inhibitors Engage the Host Immune System and Regulate Expression of the Immune Checkpoint Ligand PD-L1. *Cell Rep* 2017; **18**: 2162-2174 [PMID: 28249162 DOI: 10.1016/j.celrep.2017.02.011]

71 **Liu K**, Zhou Z, Gao H, Yang F, Qian Y, Jin H, Guo Y, Liu Y, Li H, Zhang C, Guo J, Wan Y, Chen R. JQ1, a BET-bromodomain inhibitor, inhibits human cancer growth and suppresses PD-L1 expression. *Cell Biol Int* 2019; **43**: 642-650 [PMID: 30958600 DOI: 10.1002/cbin.11139]

72 **Liu C**, Miao X, Wang Y, Wen L, Cheng X, Kong D, Zhao P, Song D, Wang X, Ding X, Xia H,

Wang W, Sun Q, Gong W. Bromo- and extraterminal domain protein inhibition improves immunotherapy efficacy in hepatocellular carcinoma. *Cancer Sci* 2020; **111**: 3503-3515 [PMID: 32726482 DOI: 10.1111/cas.14588]

73 **Liu M**, Zhou J, Liu X, Feng Y, Yang W, Wu F, Cheung OK, Sun H, Zeng X, Tang W, Mok MTS, Wong J, Yeung PC, Lai PBS, Chen Z, Jin H, Chen J, Chan SL, Chan AWH, To KF, Sung JJY, Chen M, Cheng AS. Targeting monocyte-intrinsic enhancer reprogramming improves immunotherapy efficacy in hepatocellular carcinoma. *Gut* 2020; **69**: 365-379 [PMID: 31076403 DOI: 10.1136/gutjnl-2018-317257]

74 **Montenegro RC**, Clark PG, Howarth A, Wan X, Ceroni A, Siejka P, Nunez-Alonso GA, Monteiro O, Rogers C, Gamble V, Burbano R, Brennan PE, Tallant C, Ebner D, Fedorov O, O'Neill E, Knapp S, Dixon D, Müller S. BET inhibition as a new strategy for the treatment of gastric cancer. *Oncotarget* 2016; **7**: 43997-44012 [PMID: 27259267 DOI: 10.18632/oncotarget.9766]

75 **Zhou S**, Zhang S, Wang L, Huang S, Yuan Y, Yang J, Wang H, Li X, Wang P, Zhou L, Xu Y, Gao H, Zhang Y, Lv Y, Zou X. BET protein inhibitor JQ1 downregulates chromatin accessibility and suppresses metastasis of gastric cancer *via* inactivating RUNX2/NID1 signaling. *Oncogenesis* 2020; **9**: 33 [PMID: 32157097 DOI: 10.1038/s41389-020-0218-z]

76 **Yin Y**, Liu W, Shen Q, Zhang P, Wang L, Tao R, Li H, Ma X, Zeng X, Cheong JH, Song S, Ajani JA, Mills GB, Tao K, Peng G. The DNA Endonuclease Mus81 Regulates ZEB1 Expression and Serves as a Target of BET4 Inhibitors in Gastric Cancer. *Mol Cancer Ther* 2019; **18**: 1439-1450 [PMID: 31142662 DOI: 10.1158/1535-7163.MCT-18-0833]

77 **Kim YH**, Kim M, Kim JE, Yoo M, Lee HK, Lee CO, Jung KY, Kim Y, Choi SU, Park CH. Novel brd4 inhibitors with a unique scaffold exhibit antitumor effects. *Oncol Lett* 2021; **21**: 473 [PMID: 33907583 DOI: 10.3892/ol.2021.12734]

78 **Ajani JA**, Estrella JS, Chen Q, Correa AM, Ma L, Scott AW, Jin J, Liu B, Xie M, Sudo K, Shiozaki H, Badgwell B, Weston B, Lee JH, Bhutani MS, Onodera H, Suzuki K, Suzuki A, Ding S, Hofstetter WL, Johnson RL, Bresalier RS, Song S. Galectin-3 expression is prognostic in diffuse type gastric adenocarcinoma, confers aggressive phenotype, and can be targeted by YAP1/BET inhibitors. *Br J Cancer* 2018; **118**: 52-61 [PMID: 29136404 DOI: 10.1038/bjc.2017.388]

79 **Kang SK**, Bae HJ, Kwon WS, Che J, Kim TS, Chung HC, Rha SY. Transcriptome analysis of iBET-151, a BET inhibitor alone and in combination with paclitaxel in gastric cancer cells. *Genomics Inform* 2020; **18**: e37 [PMID: 33412753 DOI: 10.5808/GI.2020.18.4.e37]

80 **Zengerle M**, Chan KH, Ciulli A. Selective Small Molecule Induced Degradation of the BET Bromodomain Protein BRD4. *ACS Chem Biol* 2015; **10**: 1770-1777 [PMID: 26035625 DOI: 10.1021/acschembio.5b00216]

81 **Lu Q**, Ding X, Huang T, Zhang S, Li Y, Xu L, Chen G, Ying Y, Wang Y, Feng Z, Wang L, Zou X. BRD4 degrader ARV-825 produces long-lasting loss of BRD4 protein and exhibits potent efficacy against cholangiocarcinoma cells. *Am J Transl Res* 2019; **11**: 5728-5739 [PMID: 31632543]

82 **Minko T**. Nanoformulation of BRD4-Degrading PROTAC: Improving Druggability To Target the 'Undruggable' MYC in Pancreatic Cancer. *Trends Pharmacol Sci* 2020; **41**: 684-686 [PMID: 32893006 DOI: 10.1016/j.tips.2020.08.008]

83 **Qin AC**, Jin H, Song Y, Gao Y, Chen YF, Zhou LN, Wang SS, Lu XS. The therapeutic effect of the BRD4-degrading PROTAC A1874 in human colon cancer cells. *Cell Death Dis* 2020; **11**: 805 [DOI: 10.1038/s41419-020-03015-6]

84 **Shirasaki R**, Matthews GM, Gandolfi S, de Matos Simoes R, Buckley DL, Raja Vora J, Sievers QL, Brüggenthies JB, Dashevsky O, Poarch H, Tang H, Bariteau MA, Sheffer M, Hu Y, Downey-Kopyscinski SL, Hengeveld PJ, Glassner BJ, Dhimolea E, Ott CJ, Zhang T, Kwiatkowski NP, Laubach JP, Schlossman RL, Richardson PG, Culhane AC, Groen RWJ, Fischer ES, Vazquez F, Tsherniak A, Hahn WC, Levy J, Auclair D, Licht JD, Keats JJ, Boise LH, Ebert BL, Bradner JE, Gray NS, Mitsiades CS. Functional Genomics Identify Distinct and Overlapping Genes Mediating Resistance to Different Classes of Heterobifunctional Degraders of Oncoproteins. *Cell Rep* 2021; **34**: 108532 [PMID: 33406420 DOI: 10.1016/j.celrep.2020.108532]

85 **Otto C**, Schmidt S, Kastner C, Denk S, Kettler J, Müller N, Germer CT, Wolf E, Gallant P, Wiegering A. Targeting bromodomain-containing protein 4 (BRD4) inhibits MYC expression in colorectal cancer cells. *Neoplasia* 2019; **21**: 1110-1120 [PMID: 31734632 DOI: 10.1016/j.neo.2019.10.003]

86 **Alqahtani A**, Choucair K, Ashraf M, Hammouda DM, Alloghbi A, Khan T, Senzer N, Nemunaitis J. Bromodomain and extra-terminal motif inhibitors: a review of preclinical and clinical advances in cancer therapy. *Future Sci OA* 2019; **5**: FSO372 [PMID: 30906568 DOI: 10.4155/fsoa-2018-0115]

87 **Xie F**, Huang M, Lin X, Liu C, Liu Z, Meng F, Wang C, Huang Q. The BET inhibitor I-BET762 inhibits pancreatic ductal adenocarcinoma cell proliferation and enhances the therapeutic effect of gemcitabine. *Sci Rep* 2018; **8**: 8102 [PMID: 29802402 DOI: 10.1038/s41598-018-26496-0]

88 **Falchook G**, Rosen S, LoRusso P, Watts J, Gupta S, Coombs CC, Talpaz M, Kurzrock R, Mita M, Cassaday R, Harb W, Peguero J, Smith DC, Piha-Paul SA, Szmulewitz R, Noel MS, Yeleswaram S, Liu P, Switzky J, Zhou G, Zheng F, Mehta A. Development of 2 Bromodomain and Extraterminal Inhibitors With Distinct Pharmacokinetic and Pharmacodynamic Profiles for the Treatment of Advanced Malignancies. *Clin Cancer Res* 2020; **26**: 1247-1257 [PMID: 31527168 DOI: 10.1158/1078-0432.CCR-18-4071]

89 **Leal AS**, Liu P, Krieger-Burke T, Ruggeri B, Liby KT. The Bromodomain Inhibitor, INCB057643, Targets Both Cancer Cells and the Tumor Microenvironment in Two Preclinical Models of

Pancreatic Cancer. *Cancers (Basel)* 2020; **13** [PMID: 33396954 DOI: 10.3390/cancers13010096]

90 **Singh AR**, Joshi S, Burgoyne AM, Sicklick JK, Ikeda S, Kono Y, Garlich JR, Morales GA, Durden DL. Single Agent and Synergistic Activity of the "First-in-Class" Dual PI3K/BRD4 Inhibitor SF1126 with Sorafenib in Hepatocellular Carcinoma. *Mol Cancer Ther* 2016; **15**: 2553-2562 [PMID: 27496136 DOI: 10.1158/1535-7163.MCT-15-0976]

91 **Mahadevan D**, Chiorean EG, Harris WB, Von Hoff DD, Stejskal-Barnett A, Qi W, Anthony SP, Younger AE, Rensvold DM, Cordova F, Shelton CF, Becker MD, Garlich JR, Durden DL, Ramanathan RK. Phase I pharmacokinetic and pharmacodynamic study of the pan-PI3K/mTORC vascular targeted pro-drug SF1126 in patients with advanced solid tumours and B-cell malignancies. *Eur J Cancer* 2012; **48**: 3319-3327 [PMID: 22921184 DOI: 10.1016/j.ejca.2012.06.027]

92 **Halder TG**, Soldi R, Sharma S. Bromodomain and extraterminal domain protein bromodomain inhibitor based cancer therapeutics. *Curr Opin Oncol* 2021; **33**: 526-531 [PMID: 34280171 DOI: 10.1097/CCO.0000000000000763]

93 **Petretich M**, Demont EH, Grandi P. Domain-selective targeting of BET proteins in cancer and immunological diseases. *Curr Opin Chem Biol* 2020; **57**: 184-193 [PMID: 32741705 DOI: 10.1016/j.cbpa.2020.02.003]

94 **Gilan O**, Rioja I, Knezevic K, Bell MJ, Yeung MM, Harker NR, Lam EYN, Chung CW, Bamborough P, Petretich M, Urh M, Atkinson SJ, Bassil AK, Roberts EJ, Vassiliadis D, Burr ML, Preston AGS, Wellaway C, Werner T, Gray JR, Michon AM, Gobbetti T, Kumar V, Soden PE, Haynes A, Vappiani J, Tough DF, Taylor S, Dawson SJ, Bantscheff M, Lindon M, Drewes G, Demont EH, Daniels DL, Grandi P, Prinjha RK, Dawson MA. Selective targeting of BD1 and BD2 of the BET proteins in cancer and immunoinflammation. *Science* 2020; **368**: 387-394 [PMID: 32193360 DOI: 10.1126/science.aaz8455]

95 **Faivre EJ**, McDaniel KF, Albert DH, Mantena SR, Plotnik JP, Wilcox D, Zhang L, Bui MH, Sheppard GS, Wang L, Sehgal V, Lin X, Huang X, Lu X, Uziel T, Hessler P, Lam LT, Bellin RJ, Mehta G, Fidanze S, Pratt JK, Liu D, Hasvold LA, Sun C, Panchal SC, Nicolette JJ, Fossey SL, Park CH, Longenecker K, Bigelow L, Torrent M, Rosenberg SH, Kati WM, Shen Y. Selective inhibition of the BD2 bromodomain of BET proteins in prostate cancer. *Nature* 2020; **578**: 306-310 [PMID: 31969702 DOI: 10.1038/s41586-020-1930-8]

96 **Yin M**, Guo Y, Hu R, Cai WL, Li Y, Pei S, Sun H, Peng C, Li J, Ye R, Yang Q, Wang N, Tao Y, Chen X, Yan Q. Potent BRD4 inhibitor suppresses cancer cell-macrophage interaction. *Nat Commun* 2020; **11**: 1833 [PMID: 32286255 DOI: 10.1038/s41467-020-15290-0]

97 **Bechter O**, Schöffski P. Make your best BET: The emerging role of BET inhibitor treatment in malignant tumors. *Pharmacol Ther* 2020; **208**: 107479 [PMID: 31931101 DOI: 10.1016/j.pharmthera.2020.107479]

98 **Wen D**, Wang Y, Zhu Z, Huang Z, Cui L, Wu T, Liu CY. Bromodomain and Extraterminal (BET) protein inhibition suppresses tumor progression and inhibits HGF-MET signaling through targeting cancer-associated fibroblasts in colorectal cancer. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165923 [PMID: 32800944 DOI: 10.1016/j.bbadis.2020.165923]

99 **Aguirre-Portolés C**, Feliu J, Reglero G, Ramírez de Molina A. ABCA1 overexpression worsens colorectal cancer prognosis by facilitating tumour growth and caveolin-1-dependent invasiveness, and these effects can be ameliorated using the BET inhibitor apabetalone. *Mol Oncol* 2018; **12**: 1735-1752 [PMID: 30098223 DOI: 10.1002/1878-0261.12367]

100 **Kato Y**, Kondo S, Itakura T, Tokunaga M, Hatayama S, Katayama K, Sugimoto Y. SNAIL- and SLUG-induced side population phenotype of HCT116 human colorectal cancer cells and its regulation by BET inhibitors. *Biochem Biophys Res Commun* 2020; **521**: 152-157 [PMID: 31653342 DOI: 10.1016/j.bbrc.2019.10.094]

101 **Andricovich J**, Perkail S, Kai Y, Casasanta N, Peng W, Tzatsos A. Loss of KDM6A Activates Super-Enhancers to Induce Gender-Specific Squamous-like Pancreatic Cancer and Confers Sensitivity to BET Inhibitors. *Cancer Cell* 2018; **33**: 512-526.e8 [PMID: 29533787 DOI: 10.1016/j.ccell.2018.02.003]

102 **Garcia PL**, Miller AL, Gamblin TL, Council LN, Christein JD, Arnoletti JP, Heslin MJ, Reddy S, Richardson JH, Cui X, van Waardenburg RCAM, Bradner JE, Yang ES, Yoon KJ. JQ1 Induces DNA Damage and Apoptosis, and Inhibits Tumor Growth in a Patient-Derived Xenograft Model of Cholangiocarcinoma. *Mol Cancer Ther* 2018; **17**: 107-118 [DOI: 10.1158/1535-7163.MCT-16-0922]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 90-109

DOI: 10.4251/wjgo.v14.i1.90

ISSN 1948-5204 (online)

REVIEW

Gastric cancer: An epigenetic view

Si-Yuan Tang, Pei-Jun Zhou, Yu Meng, Fu-Rong Zeng, Guang-Tong Deng

ORCID number: Si-Yuan Tang 0000-0003-0368-5108; Pei-Jun Zhou 0000-0001-6897-8812; Yu Meng 0000-0002-2713-4098; Fu-Rong Zeng 0000-0001-6621-8131; Guang-Tong Deng 0000-0002-4424-9727.

Author contributions: Deng GT, Zeng FR, and Tang SY designed the study; Tang SY and Zeng FR wrote the manuscript; Zhou PJ and Meng Y revised the manuscript; All the authors supported the study.

Conflict-of-interest statement: All authors have no any conflicts of interest.

Supported by The fellowship of the China Postdoctoral Science Foundation, No. 2020M682594.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Si-Yuan Tang, Department of Gastroenterology, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Pei-Jun Zhou, Cancer Research Institute, School of Basic Medicine Science, Central South University, School of Basic Medicine Science, Central South University 410008, Hunan Province, China

Yu Meng, Fu-Rong Zeng, Guang-Tong Deng, Department of Dermatology, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Corresponding author: Guang-Tong Deng, MD, Doctor, Department of Dermatology, Xiangya Hospital, Central South University, No. 87 Xiangya Road, Kaifu District, Changsha 410008, Hunan Province, China. dengguangtong@outlook.com

Abstract

Gastric cancer (GC) poses a serious threat worldwide with unfavorable prognosis mainly due to late diagnosis and limited therapies. Therefore, precise molecular classification and search for potential targets are required for diagnosis and treatment, as GC is complicated and heterogeneous in nature. Accumulating evidence indicates that epigenetics plays a vital role in gastric carcinogenesis and progression, including histone modifications, DNA methylation and non-coding RNAs. Epigenetic biomarkers and drugs are currently under intensive evaluations to ensure efficient clinical utility in GC. In this review, key epigenetic alterations and related functions and mechanisms are summarized in GC. We focus on integration of existing epigenetic findings in GC for the bench-to-bedside translation of some pivotal epigenetic alterations into clinical practice and also describe the vacant field waiting for investigation.

Key Words: Gastric cancer; Epigenetics; Histone modifications; DNA methylation; Non-coding RNAs

Core Tip: Epigenetics plays a vital role in gastric carcinogenesis and progression. In this review, key epigenetic alterations and related functions and mechanisms are summarized in gastric cancer.

Citation: Tang SY, Zhou PJ, Meng Y, Zeng FR, Deng GT. Gastric cancer: An epigenetic view.

Received: March 21, 2021
Peer-review started: March 21, 2021
First decision: May 3, 2021
Revised: May 17, 2021
Accepted: December 21, 2021
Article in press: December 21, 2021
Published online: January 15, 2022

P-Reviewer: Burada F
S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

World J Gastrointest Oncol 2022; 14(1): 90-109
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/90.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.90

INTRODUCTION

Gastric cancer (GC) is one of the most common malignant tumors of the digestive tract and ranks as the fifth leading cause of morbidity and second leading cause of mortality worldwide, posing a serious threat to all human beings[1]. Residents in South and East of Asia including China, Japan and Korea are reported to have a higher risk of GC[2]. Due to the unconspicuous symptoms in the early stage of GC, many patients are first diagnosed as advanced GC accompanied by tumor infiltration and metastasis. Despite of combined treatment of surgery, chemotherapy, radiotherapy, and sometimes targeted therapy and immunotherapy, GC still shows a poor prognosis with the 5-year overall survival less than 30%[3,4]. Currently routine screening for GC is endoscopy and histological examination, which is costly, invasive and often painful to patients. Therefore, development of new or alternative methods for screening, diagnosis and treatment to GC is of great clinical significance.

Epigenetics has been illustrated to be associated with the diagnosis and treatment of GC patients. GC is highly complicated and heterogeneous in nature and often genetically divided into familial and sporadic disease. Familial GC, constituting about 10% of GC patients, has a close connection to genetic alterations[5]. Sporadic GC (90% of GC) is largely related to *Helicobacter pylori* (*H. pylori*) infection and evolves in a canonical model of chronic inflammation, atrophy, intestinal metaplasia, dysplasia and finally adenocarcinoma, which is characterized by typically epigenetic alterations but scarce genetic changes across over the stages[6]. With rapid progress in epigenomics, precise molecular classification towards GC seems admirable in research and clinical medicine. In 2014, The Cancer Genome Atlas identified GC into four molecular subtypes including Epstein–Barr virus (EBV) associated, microsatellite instable (MSI), chromosomal instability (CIN), and genomically stable (GS)[7]. Apparently, GS means the genome is stable in this type of GC[8]. Among the four classes, MSI patients have the best overall prognosis and the lowest frequency of recurrence with high incidence of gene mutations and DNA methylation. Patients in EBV-subtype are associated with Epstein-Barr virus infection and have extremely high DNA methylation status. In the patients with CIN subtype, the largest proportion of GC, is more prone to chromosomal diseases such as chromosome rearrangement and aberration. Radically distinct clinical outcomes are presented in different subtypes.

In this review, we mainly explore GC from an epigenetic view and summarize key epigenetic alterations and related functions and mechanisms, with special attention to histone modifications and the translational findings which guide us towards better clinical utility.

HISTONE MODIFICATIONS

Nucleosome, as a major unit of chromatin, consists of wrapped DNA and a histone octamer formed by two copies of H2A, H2B, H3 and H4 proteins[9]. Each histone contains an accessible amino terminal tail rich in lysine, arginine, serine and threonine residues, which is often modified post-translationally and the process is called posttranslational modifications (PTMs). Studies have shown that histone PTMs in GC mainly including acetylation, methylation, phosphorylation and ubiquitination are involved in various pathophysiological cellular functions such as carcinogenesis, inflammation and epithelial-mesenchymal transition (Figure 1)[10]. In recent years, some new modifications, such as succinylation, sumoylation, butyrylation and crotonylation, have been discovered in the occurrence and progression of other gastrointestinal tumors, such as esophageal, colorectal, and hepatocarcinoma liver cancer[11-14], which provide new insights in functions and mechanisms and even therapeutic potential for cancer diagnosis and treatments. Notably, those new types of histone modifications remain a vacant field in GC and thereby it may be an innovative and interesting field to explore in the near future.

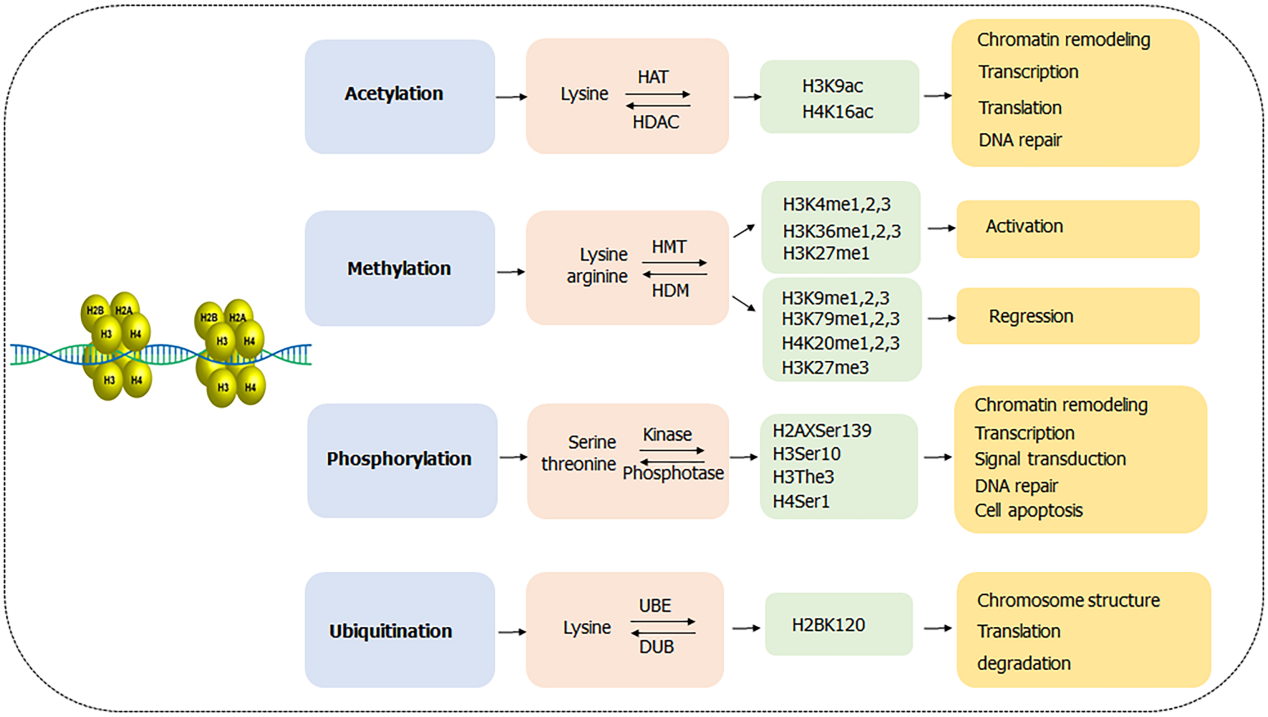


Figure 1 Histone modifications in gastric cancer. Layers show different histone modifications. Blue panel: Modification types; Pink panel: Modified residues and catalytic enzymes; Green panel: Epigenetic alterations sites; Yellow panel: Biological functions regulated by histone modifications. HAT; Histone acetylase; HDAC: Histone deacetylase; HMT: Histone methyltransferase; HDM: Histone demethylase; UBE: Ubiquitin enzyme; DUB: Deubiquitinase.

Histone acetylation

As the most common form of PTMs in GC, acetylation always occurs in N-terminal lysine residues of histone H3 and H4 and is associated with chromatin remodeling, regulation of transcription, translation and DNA repair. The acetylation of histones catalyzed by histone acetylase (HATs) transfers acetyl moieties from coenzyme A to lysine residues, opens the chromatin structure and makes it accessible to transcriptional factors, thus activating gene transcription. Instead, the histone deacetylase (HDACs) removes the acetyl groups from histone and results in repression of transcription. HATs consist of three families including GCN5, MYST and p300/CBP, while HDACs contain four classes including type I (HDAC 1,2,3,8), type II (HDAC 4,7,9,10), type III (SIRT 1-7) and type IV (HDAC 11)[15,16]. The reversible acetylation and deacetylation processes mainly facilitate GC progression by activating oncogene expression and silencing tumor suppressor gene expression.

Studies revealed that high H3K9Ac positive cells were associated with undifferentiated GC, suggesting poor prognosis of GC[17]. Further, BMP8B was highly expressed in GC tissues other than adjacent normal tissues, and reduced acetylation level of BMP8B loci on H3K9 and H4K16 influenced the development of poorly differentiated gastric tumors[18]. Many genes encoding HATs, such as KAT2B and EP300, are often genetically depleted or mutated in GC, and are significantly correlated with TNM staging[19,20]. IFN-γ-induced upregulation of histone H3 Lysine 9 acetylation (H3K9) level in gene promoter accelerates the expression of B7-H1, which contributes to tumor immune evasion in HGC-27 cells[21]. Wisnieski *et al*[22] demonstrated hypoacetylation of histone H3 in the initiator domain of CDKN1A decreased its mRNA level and reduced antitumor effect in GC. Besides, *H. pylori*-infection inhibited recruitment of HAT p300 to the p27 promoter which caused the hypoacetylation status in histone H4, then induced the downregulated p27 mRNA expression, and finally led to gastric carcinogenesis[23].

Histone methylation

Histone methylation usually takes place on H3 and H4 Lysine or arginine residues, catalyzed by histone methyltransferases (HMTs) and reversely controlled by histone demethylases (HDMs). The methylation could be single or multiple methylations to form mono-methylation (me1), di-methylation (me2) and tri-methylation (me3), participating in the formation and maintenance of chromatin structure, DNA repair, gene inactivation and transcription[24]. Methylations on different sites have different

functions in regulation of gene expression. In general, methylation of arginine residues, methylation of lysine H3K4 and H3K36, and monomethylation of H3K27 are associated with gene activation, while methylation of H3K9, H3K79 and H4K20, and dimethylation and trimethylation of H3K27 might cause gene silencing[25,26].

Specifically, repression of HDMs KDM5A and DPY300 subunits upregulated H3K4me level, inhibiting GC cell proliferation[27]. However, overexpression of HDMs LSD1 declined methylation of H3K4 in p21 promoter and repressed the transcription of p21, resulting in progression of GC[28]. An assay of familial GC patients identified INSR, FBXO24 and DOT1L as new susceptibility genes in diffuse gastric carcinoma, in which DOT1L was a histone methyltransferase involved in the mono, di and tri-methylation of H3K79, suggesting the contributing role of H3K79 in gastric carcinogenesis[29]. Methylation of H3K27 is well-investigated in GC. A paired-study of 117 GC patients showed that the level of H3K27me3 in GC and normal tissue was 56.4% and 7.25%, respectively, which negatively correlated with GC overall survival[30]. Besides, knockdown of demethylases SETDB2 was found to accelerate the expression of tumor suppressor genes WWOX and CADM1, and significantly reduced cell growth, migration and invasion in GC cells[31].

Histone phosphorylation

Histone phosphorylation is a dynamical process mediated by histone kinases and phosphatases, in which the phosphate group is transferred from ATP to the histone serine and threonine residues. There are several accessible sites in histone phosphorylation including H1.4 Ser27, H2AX Ser139 (also called γ-H2AX), H3 Ser10, H3 The3 and H4 Ser1[32,33]. Particularly, histone H3 is phosphorylated at Ser10 during mitosis in all eukaryotes and induction of phosphorylation in interphase has been shown to correlate with chromosome condensation prior to mitosis[34]. Histone phosphorylation functions as a switch on chromosomal folding, compression, segregation, transcriptional regulation, cell signal transduction, cell apoptosis, and DNA damage repair[35,36].

Histone phosphorylation frequently happens in H3 and H4 with a dual role in cancer progression[32,33]. For instance, phosphorylated histone H3 at position of serine10 (H3S10) by MSK1 promoted cell proliferation during gastric tumorigenesis *via* the activation of downstream transcriptional factor NFATc2-related inflammatory pathway[37]. H3S10 phosphorylation also played a vital prognostic role in defining negative resection margins in GC due to its lower expression in the surgical resection margins[38]. A cohort of 122 GC patients further indicated phosphorylated histone H3 overexpression could be an independent prognostic factor[39]. Moreover, repression of Aurora B-mediated H1.4 phosphorylation at Ser27, caused by Ras-ERK1/2 signaling, evidently participated in the progression of GC[40].

Histone ubiquitination

Unlike the three types of histone modifications described above, histone ubiquitination always works in the crosstalk with other modifications. Histone ubiquitination often acts subsequently after histone acetylation and methylation or modifies the stability and the activity of enzymes in these acetylation and methylation processes, which endures a synergic effect on cell division, cell cycle, DNA damage and cell apoptosis in GC[41]. When the histone, usually H2A and H2B, binds to one or several ubiquitins on lysine residues, it is called mono- or poly- ubiquitination and tends to work in the following three ways: Alterations of chromosome structure, recruitment and activation of downstream proteins, and degradation in proteasome pathway[42]. Ubiquitination is a reversible process in which ubiquitin is removed from polypeptides by deubiquitinases (DUBs), a superfamily of cysteine proteases and metalloproteases that cleave ubiquitin-protein bonds[43,44].

Hahn *et al*[45] identified that ring finger proteins RNF20 and RNF40 constituted a heterodimeric complex that functions as the E3 ubiquitin ligase for monoubiquitination of histone H2B at lysine 120 (H2B-K120) and the tumor suppressor CDC73 exerted antitumor effect in GC through the maintenance of H2B-K120 monoubiquitination. Besides, histone ubiquitination presents a therapeutic potential in GC as the expression of ubiquitinated-H2B was significantly lower in the malignant tissues and different differentiated tumors had variant levels of H2B ubiquitination[46].

DNA METHYLATION

In contrast to histone methylation, DNA methylation is a more frequent and compre-

hensive epigenetic modification (Figure 2), mediated by DNA methyltransferase (DNMTs) and demethylases. It refers to the transfer of the methyl group (CH3) from S.adenosylmethionine to C5 and forms 5-methylcytosine[47,48]. DNA methylation occurs in the dinucleotide CpG sequence, which may form CpG islands and dispersed sequences. CpG islands exist in around 60%-70% of gene promoters in human and consist of CpG core and shore area[49]. CpG core has a specific inhibitory effect on methylation, while the shore area, also known as transitional CpG region, is variable sites for dynamical alterations between hypomethylated and hypermethylated groups. In normal cells, CpG islands are non-methylated and other CpG sequence are methylated. Once stimulated by intrinsic or extrinsic factors, the methylation status changed and caused alterations in gene transcription, and consequently lead to tumorigenesis[48].

Aberrant DNA hypermethylation usually happens in the promoter of tumor suppressor genes in GC like p16, RASSF1A and hMLH1. Hypermethylation inhibits gene transcription by reducing binding to transcription factors, thereby impeding DNA readability and resulting in gene silencing[50]. Specifically, alteration of methylation in p16 promoter inhibited the cell cycle in G1 phase and induced 5-fluorurazil chemo-resistance in GC[51]. Abnormal methylation of RASSF1A gene promoter reduced RASSF1A expression, decreased cyclin D1 accumulation, and arrested cell cycle. Consistently, GC patients presented evidently higher frequency of aberrant methylation in RASSF1A promoter than control group, indicating the potential of methylated RASSF1A promoter as a molecular marker for the diagnosis of GC[52]. In addition to methylation alterations in promoter, hypomethylation at gene body regions has a distinct association with transcription and gene hypomethylation also exerts profound effects on cancer progression[53]. For instance, hypomethylation of SAT-α and L1 was associated with shortened survival in advanced GC patients[54]. And Lineage-specific RUNX3 hypomethylation constituted the immune component in GC and was associated with the early inflammatory, preneoplastic and tumor stages [55]. Genome-wide methylation sequencing studies in GC identified both hypo- and hyper-methylation events across the genome, suggesting a dual role of global genomic methylation in the stages of gastric carcinogenesis[56].

H. pylori-induced DNA Methylation is a hot research area in the development of GC. Numerous researches revealed that *H. pylori*, classified as Class I carcinogen by WHO, induced and accumulated aberrant DNA methylation through continuous chronic inflammation in gastric mucosae, and such high level of epigenetic field defects increased the risk of gastric carcinogenesis[57]. For example, *H. pylori* infection upregulated inflammatory response genes like IL-1β, Nos2, and Tnf, and promoted the infiltration of monocytes/macrophages with residual neutrophils in noncancerous mucosae, which induced a large number of aberrant DNA methylation in tumor suppressor genes and led to malignant transformation[58]. Eradication of *H. pylori* had subtle influence on the decrease of DNA methylation in gerbils, while application of immunosuppressive agent (*e.g.*, cyclosporin A) and demethylation agent (*e.g.*, 5-Aza-2-deoxycytidine) could evidently reduce level of DNA methylation and prevent development of GC[59,60]. Moreover, high levels of DNA methylation were found in gastric biopsies of inflammatory and precancerous lesions, comparing to adjacent normal tissue, and were also correlated with a greater risk of GC incidence[61]. *H. pylori*-induced DNA methylation takes place in various genes involved in cell adhesion, cell cycle, DNA damage repair, inflammation, and autophagy, which allows intensive interfered targets of such epigenetic defects in diagnostic biomarker and cancer prevention[58,62].

NON-CODING RNAS

Non-coding RNAs consist of microRNAs (miRNAs), long non coding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), *etc.*[63]. Since the first two non-coding RNA lineage defective 4 (lin-4)[64] and lethal 7 (let-7)[65] were identified in 1993 and 2000, researchers realized that in addition to protein, some RNAs lacking of protein-coding regions, which are called non-coding RNAs, were still conserved functional molecules and required for many biological processes. Among non-coding RNAs, miRNAs, lncRNAs and circRNAs were found to have plenty of functions in GC (Figure 3), including cell proliferation, cell cycle arrest, apoptosis, migration, invasion and chemo- or radio-sensitivity[66,67].

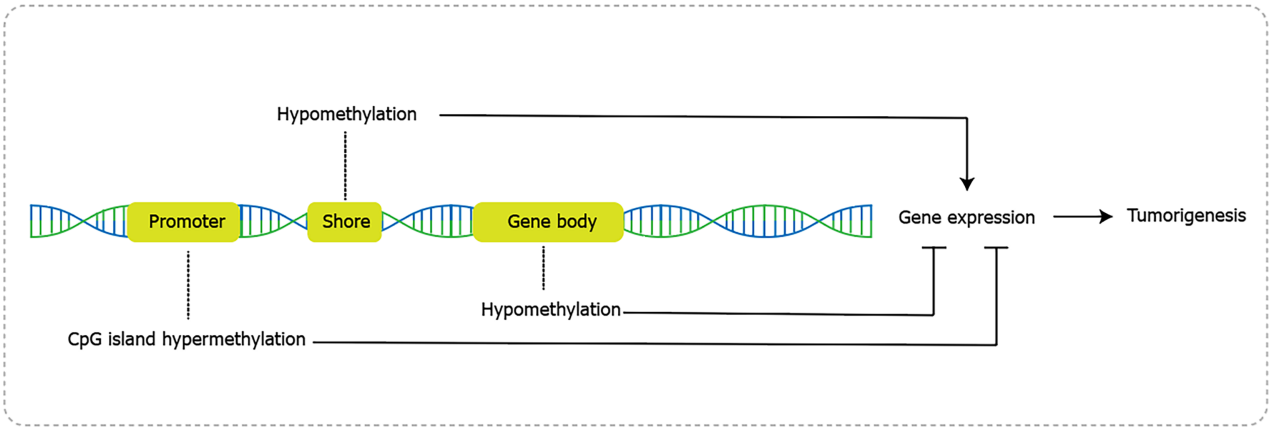


Figure 2 DNA methylation in gastric cancer. Aberrant methylation in promoter, shore area and gene body altered gene expression and involves in gastric carcinogenesis.

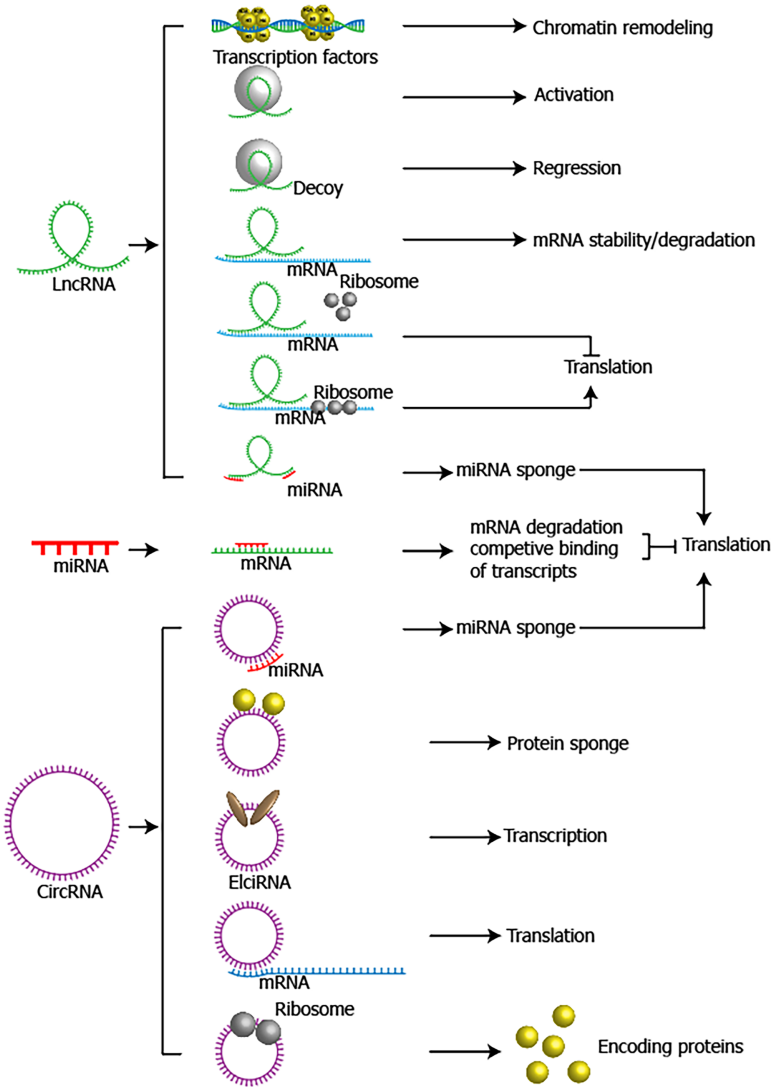


Figure 3 Non coding RNA in gastric cancer. The major mechanism and biological function of lncRNA, miRNA and circRNA in gastric cancer.

miRNAs

MicroRNAs are a class of small RNAs with 18-24 nucleotides and they repress translation process and silence target gene through complementary binding with 3′untranslated terminal region (UTR) of mRNA[68]. A shaped understanding towards miRNAs has been established in the past two decades due to numerous miRNAs

Table 1 Important miRNAs and their targets and biological functions in gastric cancer

miRNAs	Expression	Targets	Functions	Ref.
miR-21	Up	EMT	Tumor growth, metastasis	[89]
miR-183	Up	UVRAG	Cell proliferation, autophagy, apoptosis	[90]
miR-765	Up	BATF2	Chemosensitivity	[91]
miR-155	Up	TP53INP1	Cell cycle, proliferation, migration	[92]
miR-130b	Up	NFκB, p65	Cell proliferation, tumorigenesis	[93]
miR-92a-1-5p	Up	FOXD1	Metaplasia	[94]
miR-135b	Up	FOXN3/RECK	Cell invasion, CSC-like properties	[95]
miR-181a-5p	Up	AKT3	Cell proliferation, apoptosis, tumor growth	[96]
miR-224	Up	PAK4	Cell proliferation, migration	[97]
let-7i	Down	COL1A1	Cell invasion, metastasis	[98]
miR-146a	Down	-	Cell migration	[99]
MiR-12129	Down	SIRT1	Cell cycle, proliferation	[100]
miR-27b	Down	NR2F2	cell proliferation, tumor growth	[101]
miR-140-5p	Down	NOTCH1	Cell proliferation, migration, apoptosis	[102]
miR-34a	Down	Snail	Cell proliferation, invasion	[103]
miR-9	Down	TNFAIP8L3	Cell proliferation, migration	[104]
miR-195	Down	HMGB1	Chemosensitivity	[105]

arrays conducted in GC. Taking the largest scale of GC miRNAs array cohort for example, a general miRNAs signature profiling was developed, in which 22 oncogenic miRNAs and 13 tumor suppressor miRNAs were identified in 353 primary Japanese gastric tumor samples. In this study, authors also revealed that different histological subtypes had different miRNA signatures[69] as diffuse-type showed 2 folds of proportion in upregulated miRNAs to intestinal-type GC. Specifically, low expression of let-7g and miR-433 and high expression of miR-214 were associated with unfavorable outcomes in GC patients[69]. MiRNAs have an edge on GC diagnosis potential over other epigenetic factors because they alter quickly and are easy to be detected in the early stage of GC. Yu *et al*[70] performed a miRNAs microarray in early GC mouse model and the result showed that miR200-family promoted the initiation of GC and the integration of miR200-family's 15 target gene would provide superior predictive sensitivity and specificity for overall survival compared with each early GC indicator alone. Here we summarized the up- or down-regulated miRNAs in GC (Table 1).

LncRNAs

LncRNAs are longer than 200 nucleotides and exert profound influences on multiple biological functions through regulating transcription, chromatin remodeling and post-transcriptional process[71]. They work mainly in three ways: (1) Interact with mRNA, control transcription and regulate cellular signaling pathways; (2) Act as regulators of splicing and mRNA decay; (3) work as molecular decoys for miRNAs; and (4) interact with chromatin-modifying complexes or being a scaffold to maintain the structure of nuclear speckles[72-74]. Numerous lncRNAs have been uncovered the role and related mechanisms in GC. HOTAIR is a well-studied lncRNA and it is frequently overexpressed in GC, which may play a part in metastasis through following pathways: (1) Being a sponge of miR-330[75] and miR-331-3p[76] to upregulate the downstream targets; (2) Directly silencing HOXD[76] or miR34a expression[77]; (3) Regulating Wnt/β-catenin and PI3K/Akt pathways[77]; and (4) Inducing ubiquitination of Runx3 [78]. Therefore, HOTAIR was considered to be a potent diagnostic and prognostic biomarker in GC. Most of lncRNAs in GC were found to be oncogenic, like H19, MNX1-AS1, MALAT1, HULC, UCA1, *etc*. However, some lncRNAs like CRNDE were identified to inhibit GC progression. Here we summarized the up- or down-regulated lncRNAs and the related targets and functions in GC (Table 2).

Table 2 Important lncRNAs and their targets and biological functions in gastric cancer

LncRNAs	Expression	Targets	Functions	Ref.
MIAT	Up	miR-29a-3p/HDAC4	Cell proliferation, migration and invasion	[106]
PANDAR	Up	CDKN1A	Tumor growth	[107]
FOXD2-AS1	Up	EphB3	Tumorigenesis	[108]
SMARCC2	Up	miR-551b-3p/TMPRSS4	Cell proliferation, migration	[109]
H19	Up	miR-519d-p/LDHA	Aerobic glycolysis, proliferation, and immune escape	[110]
TINCR	Up	STAU1/CDKN2B	Cell proliferation, cell cycle	[111]
CCAT2	Up	E-cadherin, LATS2	Cell proliferation, invasion	[112]
AOC4P	Up	Vimentin, MMP9	Cell proliferation, migration, invasion	[113]
CTC-497E21.4	Up	miR-22-3p/NET1	Cell cycle, proliferation, invasion	[114]
BANCR	Up	ERK1/2, NF-κB1	Cell proliferation, apoptosis, chemosensitivity	[115, 116]
HOTTIP	Up	miR-216a-5p, miR-615-3p	Chemosensitivity, cell proliferation, apoptosis	[117, 118]
AC100830.4, CTC-501O10.1, RP11-210K20.5	Up	-	Differentially expressed in GC and normal tissue	[119]
INHBA-AS1, CEBPA-AS1, AK001058	Up	-	Differentially expressed in GC and normal tissue	[120]
CYTOP	Up	miR-103/RAB10	Cell proliferation, migration, apoptosis	[121]
NKX2-1-AS1	Up	SERPINE1/VEGFR-2	Cell proliferation, angiogenesis	[122]
NEAT1	Up	miR-17-5p/TGFβR2	Angiogenesis	[123]
ZFAS1	Up	EPAS1	Recurrence, metastasis	[124]
TSPEAR-AS2	Up	EZH2/GJA1, miR-1207-5p/CLDN4	Tumor progression	[125]
TMEM92-AS1	Up	YBX1/CCL5	Tumor progression	[126]
CRNDE	Down	NEDD4-1/PTEN	Chemosensitivity	[127]
MEG3	Down	miR-181a-5p/ ATP4B	Cell proliferation, migration, apoptosis	[128]
PCSK2-2:1	Down	-	Differentially expressed in GC and normal tissue	[129]
GNAQ-6:1	Down	-	Differentially expressed in GC and normal tissue	[130]
CTSLP4	Down	Hsp90α/HNRNPAB	Cell migration, invasion, EMT	[131]

CircRNAs

CircRNAs are a novel class of conserved single-stranded RNA molecules derived from exonic or intronic sequences by precursor mRNA back-splicing[79]. Compared to linear RNAs, the circular structure of circRNAs confers enhanced stability to exonuclease digestion[80]. Partially similar to lncRNAs, circRNAs could also act as miRNAs sponge, regulators of alternative splicing and tools of sequestering functional proteins in gene expression and posttranscriptional modification[81]. However, some circRNAs were identified to encode functional proteins[82]. CircRNAs were reported to exert influences on tumor growth, therapeutic resistance, recurrence and metastasis [83]. GC-related sequencing data revealed a variety of circRNAs with pro- or anti-tumor roles, including CircPVT1, CircRNA_001569, CircHIPK3, *etc.* CiRS-7, one of the mostly investigated circRNAs, is a sponge of miR-7. MiR-7 was known as a tumor suppressor miRNA, while ciRS-7 was found to act in an oncogenic role by antagonizing miR-7-mediated PTEN/PI3K/AKT pathway in GC. Overexpression of ciRS-7 accelerated the progression of GC[84]. Undoubtedly, circRNAs are of great value in research and are emerging as a rising star in the field of cancer biology and therapy. We listed some important circRNAs, as well as their targets and functions in Table 3.

Table 3 Important circRNAs and their targets and biological functions in gastric cancer

circRNAs	Expression	Targets	Functions	Ref.
circFAM73A	Up	miR-490-3p/ HMGA2	Cell proliferation, migration, CSC-like properties, chemosensitivity	[132]
circAFF2	Up	miR-6894-5p/ANTXR1	Cell proliferation, migration, invasion	[133]
circHIPK3	Up	miR-637 /AKT1	Tumorigenesis	[134]
circVAPA	Up	miR-125b-5p/STAT3	Chemosensitivity	[135]
circMAP7D1	Up	HER2	Cell proliferation, apoptosis	[136]
circ_0006282	Up	miR-144-5p/YWHAB	Cell proliferation, metastasis	[137]
circ_0081146	Up	miR-144/ HMGB1	Cell growth, migration, invasion	[138]
circ_SMAD4	Up	miR-1276/ CTNNB1	Tumorigenesis	[139]
circNEK9	Up	miR-409-3p/MAP7	Cell proliferation, migration, invasion	[140]
circ_0004104	Up	miR-539-3p/RNF2	Cell proliferation, metastasis, glutaminolysis	[141]
circPVT1	Up	miR-152-3p	Chemosensitivity	[142]
hsa_circ_0023409	Up	miR-542-3p/ IRS4	Cell proliferation, metastasis	[143]
circ_0044516	Up	miR-149-5p/HuR	Cell proliferation, migration, invasion, tumor growth	[144]
circLMO7	Up	miR-30a-3p/ WNT2	Cell growth, metastasis	[145]
hsa_circ_0001829	Up	miR-155-5p/SMAD2	Cell growth, metastasis	[146]
circCUL3	Up	miR-515-5p/STAT3/HK2	Cell proliferation, glucose consumption, lactate production, ATP quantity	[147]
circTMEM87A	Up	miR-142-5p/ULK1	Cell proliferation, metastasis	[148]
circPTPN22	Down	EMT	Cell proliferation, migration, EMT, invasion	[149]
hsa_circ_0004872	Down	miR-224/Smad4/ADAR1	Cell proliferation, migration, invasion, tumor growth, metastasis	[150]
hsa_circRNA_0009172	Down	miR-485-3p/NTRK3	Cell proliferation, migration, invasion, tumor growth	[151]
circ_002059	Down	miR-182/ MTSS1	Cell proliferation, migration	[152]
circ-ITCH	Down	miR-199a-5p/ Klotho	Metastasis	[153]
circCUL2	Down	miR-142-3p/ ROCK2	Cell transformation, chemosensitivity, tumorigenesis	[154]

TRANSLATIONAL APPLICATION OF EPIGENETICS

Researches on epigenetics not only revealed the underlying mechanism of cancer initiation and progression, but also provided novel diagnostic and prognostic candidate biomarkers and therapeutic targets. To the best of our knowledge, biomarkers in GC ranges from pivotal proteins, non-coding RNAs to plenty of modifications with various specificity and sensitivity, as well as epigenetic liquid biopsy, some of which have already shown favorable clinical utility (Table 4). Liquid biopsy is a simple, fast and non-invasive alternative to surgical biopsies, as blood or body fluid sample is always easy to collect. A sum of circulating tumor cells (CTCs) and cell-free nucleic acids (cfNAs) including DNA, mRNA and microRNAs could be detected in patient blood or body fluid[85]. Available information obtained from liquid biopsy could help doctors with cancer diagnosis and evaluation of clinical outcomes. Up to now, most of epigenetic liquid biopsies in GC were aberrant DNA methylations such as 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), CD40 and GHSR hypermethylation and they even could be used to identify specific cancer types[86-88]. Moreover, CTCs were often detected based on miRNA or mRNA PCR assay due to its low concentration in blood.

From the therapeutic perspective, targets involved in epigenetic modifications are potential drug targets and they are mainly divided into two groups including enzymes in histone acetylation (HAT or HDAC) and methylation (DNMT or DMT), and non-coding RNAs (miRNA or lncRNA). Some epigenetic drugs have been approved by FDA such as HDAC inhibitors (SAHA) in treatment of cutaneous T-cell lymphoma and DNMT inhibitors (vidaza, decitabine) in treatment of myelodysplatic syndromes [2]. However, most of epigenetic drugs are undergoing clinical or preclinical tests and none of them were currently ready for clinical utility in GC. As the rapid development

Table 4 Examples of biomarkers in gastric cancer

Genes	Purpose	Findings	Ref.
RUNX3	Diagnosis/prognosis	Methylation status correlates with liver metastasis	[155]
MLH1	Diagnosis/prognosis	Methylation status correlates with tumor stage	[156]
RASSF1A	Diagnosis/prognosis	Methylation status correlates with advanced stage, and lymph node positivity	[157]
MGMT	Diagnosis/prognosis	Methylation status correlates with distant metastasis	[156]
ANOS1	Diagnosis	Expression correlates with tumor progression	[158]
RPRML	Prognosis	Expression correlates with survival	[159]
CTD-2510F5.4	Diagnosis/prognosis	Expression correlates with clinicopathological classification and survival	[160]
lncRNA-GC1	Diagnosis	Circulating exosomal level correlates with early detection and disease progression	[161]
mesothelin	Diagnosis	Expression correlates with Peritoneal Recurrence	[162]
MiR-379-5pMiR-410-3p	Prognosis	Expression correlates with metastasis	[163]
S100A9	Diagnosis /Prognosis	Expression correlates with tumor aggressiveness	[164]
Notch1/2/3/4	Prognosis	Expression correlates with immune infiltration	[165]
KAT2A	Diagnosis	Expression correlates with depth of tumor invasion and tumor stage	[166]

Table 5 Examples of epigenetic drugs in gastric cancer

Drugs	Targets	Status	Ref.
Clinical			
Vorinostat + capecitabine + cisplatin	HDAC	Completed phase II test	[167]
Vorinostat + folinic acid+ 5□fluorouracil+ irinotecan	HDAC	Completed phase I test	[168]
Azacytidine + epirubicin/oxaliplatin/capecitabine	DNMT	Completed phase I test	[169]
Cholecalciferol + HDACi	HDAC	Induce apoptosis in GC cells; Prevent bone loss in preliminary trials;	[170, 171]
Preclinical			
SAHA	HDAC	Suppress proliferation, induce apoptosis, chemosensitivity in GC cells	[172, 173]
LBH589	HDAC	Suppress proliferation, induce chemosensitivity	[174, 175]
Resveratrol	HAT, HDAC	Suppress proliferation, invasion, tumorigenesis in GC cells	[176, 177]
Curcumin	HAT, HDAC	Suppress viability, proliferation, migration, induce autophagy, apoptosis in GC cells	[178, 179]
Quercetin	HAT, HDAC	Induce apoptosis, cell cycle arrest in GC cells	[180, 181]
Garcinol	HAT, HDAC, SIRTUIN	Suppress oxidation, inflammation, tumorigenesis in GC cells	[182, 183]
Sodium butyrate	HAT, HDAC	Induce apoptosis in GC cells	[184]
Tenovin 6	SIRTUIN	Induce apoptosis, autophagy in GC cells	[185]
DZNEP	HMT	Suppress proliferation, apoptosis, invasion, induce apoptosis in GC cells	[186, 187]
GSK126	HMT	Suppress proliferation, cell cycle angiogenesis EMT, tumorigenesis in GC cells	[188, 189]
Compound 26	Lysine demethylase	Suppress growth, migration, invasion in GC cells	[190]

of GC epigenetics research in recent decades, it is of great significance to integrate existing findings to ensure efficient translation applications (Table 5).

CONCLUSION

Accumulating evidence revealed the critical role of epigenetic alterations in cancer initiation and progression. Herein, we comprehensively discussed the functions and mechanisms of epigenetic factors in GC. Drugs targeted HAT, HDAC, DNMT are undergoing preclinical and clinical trials, which is promising for improving the efficacy and survival to GC. However, epigenetic studies in GC are still challenged by lack of innovative findings in new types of histone modifications. Succinylation and sumoylation, for instance, have already been reported to participate in tumorigenesis and progression in other gastrointestinal cancers including esophageal, colorectal and liver cancer. We believe combined technologies like single cell sequencing and multiple protein omics sequencing will further broaden epigenetic investigation in gastric malignancy and GC patients will benefit from numerous epigenetic drugs in the future.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Theuer CP**, Kurosaki T, Ziogas A, Butler J, Anton-Culver H. Asian patients with gastric carcinoma in the United States exhibit unique clinical features and superior overall and cancer specific survival rates. *Cancer* 2000; **89**: 1883-1892 [PMID: 11064344 DOI: 10.1002/1097-0142(20001101)89:9<1883::aid-cncr3>3.3.co;2-8]

3 **Jin H**, Pinheiro PS, Callahan KE, Altekruse SF. Examining the gastric cancer survival gap between Asians and whites in the United States. *Gastric Cancer* 2017; **20**: 573-582 [PMID: 27866287 DOI: 10.1007/s10120-016-0667-4]

4 **Cheong JH**, Yang HK, Kim H, Kim WH, Kim YW, Kook MC, Park YK, Kim HH, Lee HS, Lee KH, Gu MJ, Kim HY, Lee J, Choi SH, Hong S, Kim JW, Choi YY, Hyung WJ, Jang E, Huh YM, Noh SH. Predictive test for chemotherapy response in resectable gastric cancer: a multi-cohort, retrospective analysis. *Lancet Oncol* 2018; **19**: 629-638 [PMID: 29567071 DOI: 10.1016/S1470-2045(18)30108-6]

5 **Vogelaar IP**, van der Post RS, Bisseling TM, van Krieken JHJ, Ligtenberg MJ, Hoogerbrugge N. Familial gastric cancer: detection of a hereditary cause helps to understand its etiology. *Hered Cancer Clin Pract* 2012; **10**: 18 [PMID: 23231819 DOI: 10.1186/1897-4287-10-18]

6 **Tan P**, Yeoh KG. Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma. *Gastroenterology* 2015; **149**: 1153-1162.e3 [PMID: 26073375 DOI: 10.1053/j.gastro.2015.05.059]

7 **Cristescu R**, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 2015; **21**: 449-456 [PMID: 25894828 DOI: 10.1038/nm.3850]

8 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

9 **Kornberg RD**. Chromatin structure: a repeating unit of histones and DNA. *Science* 1974; **184**: 868-871 [PMID: 4825889 DOI: 10.1126/science.184.4139.868]

10 **Calcagno DQ**, Wisnieski F, Mota ERDS, Maia de Sousa SB, Costa da Silva JM, Leal MF, Gigek CO, Santos LC, Rasmussen LT, Assumpção PP, Burbano RR, Smith MA. Role of histone acetylation in gastric cancer: implications of dietetic compounds and clinical perspectives. *Epigenomics* 2019; **11**: 349-362 [PMID: 30672330 DOI: 10.2217/epi-2018-0081]

11 **Yang G**, Yuan Y, Yuan H, Wang J, Yun H, Geng Y, Zhao M, Li L, Weng Y, Liu Z, Feng J, Bu Y, Liu L, Wang B, Zhang X. Histone acetyltransferase 1 is a succinyltransferase for histones and non-histones and promotes tumorigenesis. *EMBO Rep* 2021; **22**: e50967 [PMID: 33372411 DOI: 10.15252/embr.202050967]

12 **Du L**, Fakih MG, Rosen ST, Chen Y. SUMOylation of E2F1 Regulates Expression of EZH2. *Cancer Res* 2020; **80**: 4212-4223 [PMID: 32816857 DOI: 10.1158/0008-5472.CAN-20-1259]

13 **Kishore C**. Epigenetic regulation and promising therapies in colorectal cancer. *Curr Mol Pharmacol* 2021 [PMID: 33573584 DOI: 10.2174/1874467214666210126105345]

14 **Wan J**, Liu H, Ming L. Lysine crotonylation is involved in hepatocellular carcinoma progression. *Biomed Pharmacother* 2019; **111**: 976-982 [PMID: 30841477 DOI: 10.1016/j.biopha.2018.12.148]

15 **Jenke R**, Reßing N, Hansen FK, Aigner A, Büch T. Anticancer Therapy with HDAC Inhibitors: Mechanism-Based Combination Strategies and Future Perspectives. *Cancers (Basel)* 2021; **13**

[PMID: 33562653 DOI: 10.3390/cancers13040634]
16 **Saha S**. Histone Modifications and Other Facets of Epigenetic Regulation in Trypanosomatids: Leaving Their Mark. *mBio* 2020; **11** [PMID: 32873754 DOI: 10.1128/mBio.01079-20]
17 **Park YS**, Jin MY, Kim YJ, Yook JH, Kim BS, Jang SJ. The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 2008; **15**: 1968-1976 [PMID: 18470569 DOI: 10.1245/s10434-008-9927-9]
18 **Wisnieski F**, Leal MF, Calcagno DQ, Santos LC, Gigek CO, Chen ES, Artigiani R, Demachki S, Assumpção PP, Lourenço LG, Burbano RR, Smith MC. BMP8B Is a Tumor Suppressor Gene Regulated by Histone Acetylation in Gastric Cancer. *J Cell Biochem* 2017; **118**: 869-877 [PMID: 27748538 DOI: 10.1002/jcb.25766]
19 **Wisnieski F**, Calcagno DQ, Leal MF, Chen ES, Gigek CO, Santos LC, Pontes TB, Rasmussen LT, Payão SL, Assumpção PP, Lourenço LG, Demachki S, Artigiani R, Burbano RR, Smith MC. Differential expression of histone deacetylase and acetyltransferase genes in gastric cancer and their modulation by trichostatin A. *Tumour Biol* 2014; **35**: 6373-6381 [PMID: 24668547 DOI: 10.1007/s13277-014-1841-0]
20 **Kim MS**, Lee SH, Yoo NJ. Frameshift mutations of tumor suppressor gene EP300 in gastric and colorectal cancers with high microsatellite instability. *Hum Pathol* 2013; **44**: 2064-2070 [PMID: 23759652 DOI: 10.1016/j.humpath.2012.11.027]
21 **Deng R**, Zhang P, Liu W, Zeng X, Ma X, Shi L, Wang T, Yin Y, Chang W, Wang G, Tao K. HDAC is indispensable for IFN-γ-induced B7-H1 expression in gastric cancer. *Clin Epigenetics* 2018; **10**: 153 [PMID: 30537988 DOI: 10.1186/s13148-018-0589-6]
22 **Wisnieski F**, Calcagno DQ, Leal MF, Santos LC, Gigek CO, Chen ES, Demachki S, Artigiani R, Assumpção PP, Lourenço LG, Burbano RR, Smith MC. CDKN1A histone acetylation and gene expression relationship in gastric adenocarcinomas. *Clin Exp Med* 2017; **17**: 121-129 [PMID: 26567008 DOI: 10.1007/s10238-015-0400-3]
23 **Byun SW**, Chang YJ, Chung IS, Moss SF, Kim SS. Helicobacter pylori decreases p27 expression through the delta opioid receptor-mediated inhibition of histone acetylation within the p27 promoter. *Cancer Lett* 2012; **326**: 96-104 [PMID: 22867947 DOI: 10.1016/j.canlet.2012.07.032]
24 **Michalak EM**, Burr ML, Bannister AJ, Dawson MA. The roles of DNA, RNA and histone methylation in ageing and cancer. *Nat Rev Mol Cell Biol* 2019; **20**: 573-589 [PMID: 31270442 DOI: 10.1038/s41580-019-0143-1]
25 **Song Y**, Wu F, Wu J. Targeting histone methylation for cancer therapy: enzymes, inhibitors, biological activity and perspectives. *J Hematol Oncol* 2016; **9**: 49 [PMID: 27316347 DOI: 10.1186/s13045-016-0279-9]
26 **Jarrold J**, Davies CC. PRMTs and Arginine Methylation: Cancer's Best-Kept Secret? *Trends Mol Med* 2019; **25**: 993-1009 [PMID: 31230909 DOI: 10.1016/j.molmed.2019.05.007]
27 **Torres IO**, Kuchenbecker KM, Nnadi CI, Fletterick RJ, Kelly MJ, Fujimori DG. Histone demethylase KDM5A is regulated by its reader domain through a positive-feedback mechanism. *Nat Commun* 2015; **6**: 6204 [PMID: 25686748 DOI: 10.1038/ncomms7204]
28 **Liu YW**, Xia R, Lu K, Xie M, Yang F, Sun M, De W, Wang C, Ji G. LincRNAFEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-Mediated H3K4me2 demethylation. *Mol Cancer* 2017; **16**: 39 [PMID: 28209170 DOI: 10.1186/s12943-017-0588-9]
29 **Donner I**, Kiviluoto T, Ristimäki A, Aaltonen LA, Vahteristo P. Exome sequencing reveals three novel candidate predisposition genes for diffuse gastric cancer. *Fam Cancer* 2015; **14**: 241-246 [PMID: 25576241 DOI: 10.1007/s10689-015-9778-z]
30 **He LJ**, Cai MY, Xu GL, Li JJ, Weng ZJ, Xu DZ, Luo GY, Zhu SL, Xie D. Prognostic significance of overexpression of EZH2 and H3k27me3 proteins in gastric cancer. *Asian Pac J Cancer Prev* 2012; **13**: 3173-3178 [PMID: 22994729 DOI: 10.7314/apjcp.2012.13.7.3173]
31 **Nishikawaji T**, Akiyama Y, Shimada S, Kojima K, Kawano T, Eishi Y, Yuasa Y, Tanaka S. Oncogenic roles of the SETDB2 histone methyltransferase in gastric cancer. *Oncotarget* 2016; **7**: 67251-67265 [PMID: 27572307 DOI: 10.18632/oncotarget.11625]
32 **Elmaci İ**, Altinoz MA, Sari R, Bolukbasi FH. Phosphorylated Histone H3 (PHH3) as a Novel Cell Proliferation Marker and Prognosticator for Meningeal Tumors: A Short Review. *Appl Immunohistochem Mol Morphol* 2018; **26**: 627-631 [PMID: 28777144 DOI: 10.1097/PAI.0000000000000499]
33 **Besant PG**, Attwood PV. Histone H4 histidine phosphorylation: kinases, phosphatases, liver regeneration and cancer. *Biochem Soc Trans* 2012; **40**: 290-293 [PMID: 22260708 DOI: 10.1042/BST20110605]
34 **Ajiro K**, Yoda K, Utsumi K, Nishikawa Y. Alteration of cell cycle-dependent histone phosphorylations by okadaic acid. Induction of mitosis-specific H3 phosphorylation and chromatin condensation in mammalian interphase cells. *J Biol Chem* 1996; **271**: 13197-13201 [PMID: 8662672 DOI: 10.1074/jbc.271.22.13197]
35 **Humphrey SJ**, James DE, Mann M. Protein Phosphorylation: A Major Switch Mechanism for Metabolic Regulation. *Trends Endocrinol Metab* 2015; **26**: 676-687 [PMID: 26498855 DOI: 10.1016/j.tem.2015.09.013]
36 **Murakami Y**. Phosphorylation of repressive histone code readers by casein kinase 2 plays diverse roles in heterochromatin regulation. *J Biochem* 2019; **166**: 3-6 [PMID: 31198932 DOI: 10.1093/jb/mvz045]
37 **Qi H**, Yang Z, Dai C, Wang R, Ke X, Zhang S, Xiang X, Chen K, Li C, Luo J, Shao J, Shen J.

STAT3 activates MSK1-mediated histone H3 phosphorylation to promote NFAT signaling in gastric carcinogenesis. *Oncogenesis* 2020; **9**: 15 [PMID: 32041943 DOI: 10.1038/s41389-020-0195-2]

38 **Khan SA**, Amnekar R, Khade B, Barreto SG, Ramadwar M, Shrikhande SV, Gupta S. p38-MAPK/MSK1-mediated overexpression of histone H3 serine 10 phosphorylation defines distance-dependent prognostic value of negative resection margin in gastric cancer. *Clin Epigenetics* 2016; **8**: 88 [PMID: 27588146 DOI: 10.1186/s13148-016-0255-9]

39 **Takahashi H**, Murai Y, Tsuneyama K, Nomoto K, Okada E, Fujita H, Takano Y. Overexpression of phosphorylated histone H3 is an indicator of poor prognosis in gastric adenocarcinoma patients. *Appl Immunohistochem Mol Morphol* 2006; **14**: 296-302 [PMID: 16932020 DOI: 10.1097/00129039-200609000-00007]

40 **Xu J**, Tian F, Chen X, Liu Z, Wu C, Zhao Z. Ras-ERK1/2 signaling participates in the progression of gastric cancer through repressing Aurora B-mediated H1.4 phosphorylation at Ser27. *J Cell Physiol* 2020 [PMID: 31907925 DOI: 10.1002/jcp.29432]

41 **Wang J**, Qiu Z, Wu Y. Ubiquitin Regulation: The Histone Modifying Enzyme's Story. *Cells* 2018; **7** [PMID: 30150556 DOI: 10.3390/cells7090118]

42 **Swatek KN**, Komander D. Ubiquitin modifications. *Cell Res* 2016; **26**: 399-422 [PMID: 27012465 DOI: 10.1038/cr.2016.39]

43 **Wilkinson KD**. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J* 1997; **11**: 1245-1256 [PMID: 9409543 DOI: 10.1096/fasebj.11.14.9409543]

44 **Eletr ZM**, Wilkinson KD. Regulation of proteolysis by human deubiquitinating enzymes. *Biochim Biophys Acta* 2014; **1843**: 114-128 [PMID: 23845989 DOI: 10.1016/j.bbamcr.2013.06.027]

45 **Hahn MA**, Dickson KA, Jackson S, Clarkson A, Gill AJ, Marsh DJ. The tumor suppressor CDC73 interacts with the ring finger proteins RNF20 and RNF40 and is required for the maintenance of histone 2B monoubiquitination. *Hum Mol Genet* 2012; **21**: 559-568 [PMID: 22021426 DOI: 10.1093/hmg/ddr490]

46 **Wang ZJ**, Yang JL, Wang YP, Lou JY, Chen J, Liu C, Guo LD. Decreased histone H2B monoubiquitination in malignant gastric carcinoma. *World J Gastroenterol* 2013; **19**: 8099-8107 [PMID: 24307806 DOI: 10.3748/wjg.v19.i44.8099]

47 **Zhang Q**, Wu Y, Xu Q, Ma F, Zhang CY. Recent advances in biosensors for in vitro detection and in vivo imaging of DNA methylation. *Biosens Bioelectron* 2021; **171**: 112712 [PMID: 33045657 DOI: 10.1016/j.bios.2020.112712]

48 **Ortiz-Barahona V**, Joshi RS, Esteller M. Use of DNA methylation profiling in translational oncology. *Semin Cancer Biol* 2020 [PMID: 33352265 DOI: 10.1016/j.semcancer.2020.12.011]

49 **Gardiner-Garden M**, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol* 1987; **196**: 261-282 [PMID: 3656447 DOI: 10.1016/0022-2836(87)90689-9]

50 **Héberlé É**, Bardet AF. Sensitivity of transcription factors to DNA methylation. *Essays Biochem* 2019; **63**: 727-741 [PMID: 31755929 DOI: 10.1042/EBC20190033]

51 **Wang M**, Li Y, Gao J, Zhou J, Gu L, Shen L, Deng D. p16 Methylation is associated with chemosensitivity to fluorouracil in patients with advanced gastric cancer. *Med Oncol* 2014; **31**: 988 [PMID: 24816738 DOI: 10.1007/s12032-014-0988-2]

52 **Balgkouranidou I**, Matthaios D, Karayiannakis A, Bolanaki H, Michailidis P, Xenidis N, Amarantidis K, Chelis L, Trypsianis G, Chatzaki E, Lianidou ES, Kakolyris S. Prognostic role of APC and RASSF1A promoter methylation status in cell free circulating DNA of operable gastric cancer patients. *Mutat Res* 2015; **778**: 46-51 [PMID: 26073472 DOI: 10.1016/j.mrfmmm.2015.05.002]

53 **Yang X**, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* 2014; **26**: 577-590 [PMID: 25263941 DOI: 10.1016/j.ccr.2014.07.028]

54 **Kim Y**, Wen X, Jeong S, Cho NY, Kim WH, Kang GH. Combinatory low methylation statuses of SAT-α and L1 are associated with shortened survival time in patients with advanced gastric cancer. *Gastric Cancer* 2019; **22**: 37-47 [PMID: 29926315 DOI: 10.1007/s10120-018-0852-8]

55 **Kurklu B**, Whitehead RH, Ong EK, Minamoto T, Fox JG, Mann JR, Judd LM, Giraud AS, Menheniott TR. Lineage-specific RUNX3 hypomethylation marks the preneoplastic immune component of gastric cancer. *Oncogene* 2015; **34**: 2856-2866 [PMID: 25088199 DOI: 10.1038/onc.2014.233]

56 **Leodolter A**, Alonso S, González B, Ebert MP, Vieth M, Röcken C, Wex T, Peitz U, Malfertheiner P, Perucho M. Somatic DNA Hypomethylation in H. pylori-Associated High-Risk Gastritis and Gastric Cancer: Enhanced Somatic Hypomethylation Associates with Advanced Stage Cancer. *Clin Transl Gastroenterol* 2015; **6**: e85 [PMID: 25928808 DOI: 10.1038/ctg.2015.14]

57 **Leung WK**, Man EP, Yu J, Go MY, To KF, Yamaoka Y, Cheng VY, Ng EK, Sung JJ. Effects of Helicobacter pylori eradication on methylation status of E-cadherin gene in noncancerous stomach. *Clin Cancer Res* 2006; **12**: 3216-3221 [PMID: 16707623 DOI: 10.1158/1078-0432.Ccr-05-2442]

58 **Perri F**, Cotugno R, Piepoli A, Merla A, Quitadamo M, Gentile A, Pilotto A, Annese V, Andriulli A. Aberrant DNA methylation in non-neoplastic gastric mucosa of H. Pylori infected patients and effect of eradication. *Am J Gastroenterol* 2007; **102**: 1361-1371 [PMID: 17509026 DOI: 10.1111/j.1572-0241.2007.01284.x]

59 **Niwa T**, Toyoda T, Tsukamoto T, Mori A, Tatematsu M, Ushijima T. Prevention of Helicobacter pylori-induced gastric cancers in gerbils by a DNA demethylating agent. *Cancer Prev Res (Phila)* 2013; **6**: 263-270 [PMID: 23559452 DOI: 10.1158/1940-6207.CAPR-12-0369]

60 **Nakajima T**, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, Oda I, Gotoda T, Ushijima T. Persistence of a component of DNA methylation in gastric mucosae after Helicobacter pylori eradication. *J Gastroenterol* 2010; **45**: 37-44 [PMID: 19821005 DOI: 10.1007/s00535-009-0142-7]

61 **Park JH**, Park J, Choi JK, Lyu J, Bae MG, Lee YG, Bae JB, Park DY, Yang HK, Kim TY, Kim YJ. Identification of DNA methylation changes associated with human gastric cancer. *BMC Med Genomics* 2011; **4**: 82 [PMID: 22133303 DOI: 10.1186/1755-8794-4-82]

62 **Muhammad JS**, Eladl MA, Khoder G. *Helicobacter pylori*-induced DNA Methylation as an Epigenetic Modulator of Gastric Cancer: Recent Outcomes and Future Direction. *Pathogens* 2019; **8** [PMID: 30781778 DOI: 10.3390/pathogens8010023]

63 **Tekcham DS**, Tiwari PK. Non-coding RNAs as emerging molecular targets of gallbladder cancer. *Gene* 2016; **588**: 79-85 [PMID: 27131889 DOI: 10.1016/j.gene.2016.04.047]

64 **Lee RC**, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**: 843-854 [PMID: 8252621 DOI: 10.1016/0092-8674(93)90529-y]

65 **Reinhart BJ**, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000; **403**: 901-906 [PMID: 10706289 DOI: 10.1038/35002607]

66 **Tsai MM**, Wang CS, Tsai CY, Huang HW, Chi HC, Lin YH, Lu PH, Lin KH. Potential Diagnostic, Prognostic and Therapeutic Targets of MicroRNAs in Human Gastric Cancer. *Int J Mol Sci* 2016; **17** [PMID: 27322246 DOI: 10.3390/ijms17060945]

67 **Wang J**, Sun J, Wang J, Song Y, Gao P, Shi J, Chen P, Wang Z. Long noncoding RNAs in gastric cancer: functions and clinical applications. *Onco Targets Ther* 2016; **9**: 681-697 [PMID: 26929639 DOI: 10.2147/OTT.S95412]

68 **Bartel DP**. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]

69 **Ueda T**, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol* 2010; **11**: 136-146 [PMID: 20022810 DOI: 10.1016/S1470-2045(09)70343-2]

70 **Yu L**, Wu D, Gao H, Balic JJ, Tsykin A, Han TS, Liu YD, Kennedy CL, Li JK, Mao JQ, Tan P, Oshima M, Goodall GJ, Jenkins BJ. Clinical Utility of a STAT3-Regulated miRNA-200 Family Signature with Prognostic Potential in Early Gastric Cancer. *Clin Cancer Res* 2018; **24**: 1459-1472 [PMID: 29330205 DOI: 10.1158/1078-0432.CCR-17-2485]

71 **Mercer TR**, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009; **10**: 155-159 [PMID: 19188922 DOI: 10.1038/nrg2521]

72 **Marchese FP**, Huarte M. Long non-coding RNAs and chromatin modifiers: their place in the epigenetic code. *Epigenetics* 2014; **9**: 21-26 [PMID: 24335342 DOI: 10.4161/epi.27472]

73 **Prasanth KV**, Prasanth SG, Xuan Z, Hearn S, Freier SM, Bennett CF, Zhang MQ, Spector DL. Regulating gene expression through RNA nuclear retention. *Cell* 2005; **123**: 249-263 [PMID: 16239143 DOI: 10.1016/j.cell.2005.08.033]

74 **Clemson CM**, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell* 2009; **33**: 717-726 [PMID: 19217333 DOI: 10.1016/j.molcel.2009.01.026]

75 **Bie L**, Luo S, Li D, Wei Y, Mu Y, Chen X, Wang S, Guo P, Lu X. HOTAIR Competitively Binds MiRNA330 as a Molecular Sponge to Increase the Resistance of Gastric Cancer to Trastuzumab. *Curr Cancer Drug Targets* 2020; **20**: 700-709 [PMID: 32364078 DOI: 10.2174/1568009620666200504114000]

76 **Endo H**, Shiroki T, Nakagawa T, Yokoyama M, Tamai K, Yamanami H, Fujiya T, Sato I, Yamaguchi K, Tanaka N, Iijima K, Shimosegawa T, Sugamura K, Satoh K. Enhanced expression of long non-coding RNA HOTAIR is associated with the development of gastric cancer. *PLoS One* 2013; **8**: e77070 [PMID: 24130837 DOI: 10.1371/journal.pone.0077070]

77 **Cheng C**, Qin Y, Zhi Q, Wang J, Qin C. Knockdown of long non-coding RNA HOTAIR inhibits cisplatin resistance of gastric cancer cells through inhibiting the PI3K/Akt and Wnt/β-catenin signaling pathways by up-regulating miR-34a. *Int J Biol Macromol* 2018; **107**: 2620-2629 [PMID: 29080815 DOI: 10.1016/j.ijbiomac.2017.10.154]

78 **Xue M**, Chen LY, Wang WJ, Su TT, Shi LH, Wang L, Zhang W, Si JM, Wang LJ, Chen SJ. HOTAIR induces the ubiquitination of Runx3 by interacting with Mex3b and enhances the invasion of gastric cancer cells. *Gastric Cancer* 2018; **21**: 756-764 [PMID: 29417297 DOI: 10.1007/s10120-018-0801-6]

79 **Tang X**, Ren H, Guo M, Qian J, Yang Y, Gu C. Review on circular RNAs and new insights into their roles in cancer. *Comput Struct Biotechnol J* 2021; **19**: 910-928 [PMID: 33598105 DOI: 10.1016/j.csbj.2021.01.018]

80 **Ruan Y**, Li Z, Shen Y, Li T, Zhang H, Guo J. Functions of circular RNAs and their potential applications in gastric cancer. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 85-92 [PMID: 31922886 DOI: 10.1080/17474124.2020.1715211]

81 **Zhang M**, Huang N, Yang X, Luo J, Yan S, Xiao F, Chen W, Gao X, Zhao K, Zhou H, Li Z, Ming L, Xie B, Zhang N. A novel protein encoded by the circular form of the SHPRH gene suppresses

glioma tumorigenesis. *Oncogene* 2018; **37**: 1805-1814 [PMID: 29343848 DOI: 10.1038/s41388-017-0019-9]

82 **Pamudurti NR**, Bartok O, Jens M, Ashwal-Fluss R, Stottmeister C, Ruhe L, Hanan M, Wyler E, Perez-Hernandez D, Ramberger E, Shenzis S, Samson M, Dittmar G, Landthaler M, Chekulaeva M, Rajewsky N, Kadener S. Translation of CircRNAs. *Mol Cell* 2017; **66**: 9-21.e7 [PMID: 28344080 DOI: 10.1016/j.molcel.2017.02.021]

83 **Guarnerio J**, Bezzi M, Jeong JC, Paffenholz SV, Berry K, Naldini MM, Lo-Coco F, Tay Y, Beck AH, Pandolfi PP. Oncogenic Role of Fusion-circRNAs Derived from Cancer-Associated Chromosomal Translocations. *Cell* 2016; **166**: 1055-1056 [PMID: 27518567 DOI: 10.1016/j.cell.2016.07.035]

84 **Pan H**, Li T, Jiang Y, Pan C, Ding Y, Huang Z, Yu H, Kong D. Overexpression of Circular RNA ciRS-7 Abrogates the Tumor Suppressive Effect of miR-7 on Gastric Cancer *via* PTEN/PI3K/AKT Signaling Pathway. *J Cell Biochem* 2018; **119**: 440-446 [PMID: 28608528 DOI: 10.1002/jcb.26201]

85 **Tsujiura M**, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Otsuji E. Liquid biopsy of gastric cancer patients: circulating tumor cells and cell-free nucleic acids. *World J Gastroenterol* 2014; **20**: 3265-3286 [PMID: 24696609 DOI: 10.3748/wjg.v20.i12.3265]

86 **Li W**, Zhang X, Lu X, You L, Song Y, Luo Z, Zhang J, Nie J, Zheng W, Xu D, Wang Y, Dong Y, Yu S, Hong J, Shi J, Hao H, Luo F, Hua L, Wang P, Qian X, Yuan F, Wei L, Cui M, Zhang T, Liao Q, Dai M, Liu Z, Chen G, Meckel K, Adhikari S, Jia G, Bissonnette MB, Zhao Y, Zhang W, He C, Liu J. 5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers. *Cell Res* 2017; **27**: 1243-1257 [PMID: 28925386 DOI: 10.1038/cr.2017.121]

87 **Amini M**, Ghorban K, Mokhtarzadeh A, Dadmanesh M, Baradaran B. CD40 DNA hypermethylation in primary gastric tumors; as a novel diagnostic biomarker. *Life Sci* 2020; **254**: 117774 [PMID: 32407843 DOI: 10.1016/j.lfs.2020.117774]

88 **Amini M**, Foroughi K, Talebi F, Aghagolzade Haji H, Kamali F, Jandaghi P, Hoheisel JD, Manoochehri M. GHSR DNA hypermethylation is a new epigenetic biomarker for gastric adenocarcinoma and beyond. *J Cell Physiol* 2019 [PMID: 30677130 DOI: 10.1002/jcp.28179]

89 **Xu G**, Meng L, Yuan D, Li K, Zhang Y, Dang C, Zhu K. MEG3/miR21 axis affects cell mobility by suppressing epithelialmesenchymal transition in gastric cancer. *Oncol Rep* 2018; **40**: 39-48 [PMID: 29749532 DOI: 10.3892/or.2018.6424]

90 **Yuan Y**, Zhang Y, Han L, Sun S, Shu Y. miR-183 inhibits autophagy and apoptosis in gastric cancer cells by targeting ultraviolet radiation resistance-associated gene. *Int J Mol Med* 2018; **42**: 3562-3570 [PMID: 30221685 DOI: 10.3892/ijmm.2018.3871]

91 **Lin W**, Miao Y, Meng X, Huang Y, Zhao W, Ruan J. miRNA-765 mediates multidrug resistance *via* targeting BATF2 in gastric cancer cells. *FEBS Open Bio* 2020; **10**: 1021-1030 [PMID: 32166887 DOI: 10.1002/2211-5463.12838]

92 **Shi SS**, Zhang HP, Yang CQ, Li LN, Shen Y, Zhang YQ. Exosomal miR-155-5p promotes proliferation and migration of gastric cancer cells by inhibiting TP53INP1 expression. *Pathol Res Pract* 2020; **216**: 152986 [PMID: 32527448 DOI: 10.1016/j.prp.2020.152986]

93 **Ding L**, Li Q, Chakrabarti J, Munoz A, Faure-Kumar E, Ocadiz-Ruiz R, Razumilava N, Zhang G, Hayes MH, Sontz RA, Mendoza ZE, Mahurkar S, Greenson JK, Perez-Perez G, Hanh NTH, Zavros Y, Samuelson LC, Iliopoulos D, Merchant JL. MiR130b from Schlafen4^{+} MDSCs stimulates epithelial proliferation and correlates with preneoplastic changes prior to gastric cancer. *Gut* 2020; **69**: 1750-1761 [PMID: 31980446 DOI: 10.1136/gutjnl-2019-318817]

94 **Li T**, Guo H, Li H, Jiang Y, Zhuang K, Lei C, Wu J, Zhou H, Zhu R, Zhao X, Lu Y, Shi C, Nie Y, Wu K, Yuan Z, Fan DM, Shi Y. MicroRNA-92a-1-5p increases CDX2 by targeting FOXD1 in bile acids-induced gastric intestinal metaplasia. *Gut* 2019; **68**: 1751-1763 [PMID: 30635407 DOI: 10.1136/gutjnl-2017-315318]

95 **Han TS**, Voon DC, Oshima H, Nakayama M, Echizen K, Sakai E, Yong ZWE, Murakami K, Yu L, Minamoto T, Ock CY, Jenkins BJ, Kim SJ, Yang HK, Oshima M. Interleukin 1 Up-regulates MicroRNA 135b to Promote Inflammation-Associated Gastric Carcinogenesis in Mice. *Gastroenterology* 2019; **156**: 1140-1155.e4 [PMID: 30508510 DOI: 10.1053/j.gastro.2018.11.059]

96 **Lu Z**, Luo T, Pang T, Du Z, Yin X, Cui H, Fang G, Xue X. MALAT1 promotes gastric adenocarcinoma through the MALAT1/miR-181a-5p/AKT3 axis. *Open Biol* 2019; **9**: 190095 [PMID: 31480991 DOI: 10.1098/rsob.190095]

97 **Xia M**, Wei J, Tong K. MiR-224 promotes proliferation and migration of gastric cancer cells through targeting PAK4. *Pharmazie* 2016; **71**: 460-464 [PMID: 29442033 DOI: 10.1691/ph.2016.6580]

98 **Shi Y**, Duan Z, Zhang X, Wang G, Li F. Down-regulation of the let-7i facilitates gastric cancer invasion and metastasis by targeting COL1A1. *Protein Cell* 2019; **10**: 143-148 [PMID: 29858755 DOI: 10.1007/s13238-018-0550-7]

99 **Shomali N**, Shirafkan N, Duijf PHG, Ghasabi M, Babaloo Z, Yousefi M, Mansoori B, Asadi M, Shanehbandi D, Baghbani E, Mohammadi A, Baradaran B. Downregulation of miR-146a promotes cell migration in Helicobacter pylori-negative gastric cancer. *J Cell Biochem* 2019; **120**: 9495-9505 [PMID: 30537266 DOI: 10.1002/jcb.28225]

100 **Zhang W**, Liao K, Liu D. MiRNA-12129 Suppresses Cell Proliferation and Block Cell Cycle Progression by Targeting SIRT1 in GASTRIC Cancer. *Technol Cancer Res Treat* 2020; **19**: 1533033820928144 [PMID: 32508267 DOI: 10.1177/1533033820928144]

101 **Feng Q**, Wu X, Li F, Ning B, Lu X, Zhang Y, Pan Y, Guan W. miR-27b inhibits gastric cancer

metastasis by targeting NR2F2. *Protein Cell* 2017; **8**: 114-122 [PMID: 27844448 DOI: 10.1007/s13238-016-0340-z]

102 **Wu K**, Zou J, Lin C, Jie ZG. MicroRNA-140-5p inhibits cell proliferation, migration and promotes cell apoptosis in gastric cancer through the negative regulation of THY1-mediated Notch signaling. *Biosci Rep* 2019; **39** [PMID: 31123165 DOI: 10.1042/bsr20181434]

103 **Zhang Y**, Yuan Y, Zhang Y, Cheng L, Zhou X, Chen K. SNHG7 accelerates cell migration and invasion through regulating miR-34a-Snail-EMT axis in gastric cancer. *Cell Cycle* 2020; **19**: 142-152 [PMID: 31814518 DOI: 10.1080/15384101.2019.1699753]

104 **Fan Y**, Shi Y, Lin Z, Huang X, Li J, Huang W, Shen D, Zhuang G, Liu W. miR-9-5p Suppresses Malignant Biological Behaviors of Human Gastric Cancer Cells by Negative Regulation of TNFAIP8L3. *Dig Dis Sci* 2019; **64**: 2823-2829 [PMID: 31140050 DOI: 10.1007/s10620-019-05626-2]

105 **Wang CQ**. MiR-195 reverses 5-FU resistance through targeting HMGA1 in gastric cancer cells. *Eur Rev Med Pharmacol Sci* 2019; **23**: 3771-3778 [PMID: 31115003 DOI: 10.26355/eurrev_201905_17803]

106 **Li Y**, Wang K, Wei Y, Yao Q, Zhang Q, Qu H, Zhu G. lncRNA-MIAT regulates cell biological behaviors in gastric cancer through a mechanism involving the miR-29a-3p/HDAC4 axis. *Oncol Rep* 2017; **38**: 3465-3472 [PMID: 29039602 DOI: 10.3892/or.2017.6020]

107 **Liu J**, Ben Q, Lu E, He X, Yang X, Ma J, Zhang W, Wang Z, Liu T, Zhang J, Wang H. Long noncoding RNA PANDAR blocks CDKN1A gene transcription by competitive interaction with p53 protein in gastric cancer. *Cell Death Dis* 2018; **9**: 168 [PMID: 29416011 DOI: 10.1038/s41419-017-0246-6]

108 **Xu TP**, Wang WY, Ma P, Shuai Y, Zhao K, Wang YF, Li W, Xia R, Chen WM, Zhang EB, Shu YQ. Upregulation of the long noncoding RNA FOXD2-AS1 promotes carcinogenesis by epigenetically silencing EphB3 through EZH2 and LSD1, and predicts poor prognosis in gastric cancer. *Oncogene* 2018; **37**: 5020-5036 [PMID: 29789713 DOI: 10.1038/s41388-018-0308-y]

109 **Yuan H**, Chen Z, Bai S, Wei H, Wang Y, Ji R, Guo Q, Li Q, Ye Y, Wu J, Zhou Y, Qiao L. Molecular mechanisms of lncRNA SMARCC2/miR-551b-3p/TMPRSS4 axis in gastric cancer. *Cancer Lett* 2018; **418**: 84-96 [PMID: 29337109 DOI: 10.1016/j.canlet.2018.01.032]

110 **Sun L**, Li J, Yan W, Yao Z, Wang R, Zhou X, Wu H, Zhang G, Shi T, Chen W. H19 promotes aerobic glycolysis, proliferation, and immune escape of gastric cancer cells through the microRNA-519d-3p/lactate dehydrogenase A axis. *Cancer Sci* 2021; **112**: 2245-2259 [PMID: 33756038 DOI: 10.1111/cas.14896]

111 **Xu TP**, Wang YF, Xiong WL, Ma P, Wang WY, Chen WM, Huang MD, Xia R, Wang R, Zhang EB, Liu YW, De W, Shu YQ. E2F1 induces TINCR transcriptional activity and accelerates gastric cancer progression *via* activation of TINCR/STAU1/CDKN2B signaling axis. *Cell Death Dis* 2017; **8**: e2837 [PMID: 28569791 DOI: 10.1038/cddis.2017.205]

112 **Wang YJ**, Liu JZ, Lv P, Dang Y, Gao JY, Wang Y. Long non-coding RNA CCAT2 promotes gastric cancer proliferation and invasion by regulating the E-cadherin and LATS2. *Am J Cancer Res* 2016; **6**: 2651-2660 [PMID: 27904778]

113 **Zhang K**, Lu C, Huang X, Cui J, Li J, Gao Y, Liang W, Liu Y, Sun Y, Liu H, Wei B, Chen L. Long noncoding RNA *AOC4P* regulates tumor cell proliferation and invasion by epithelial-mesenchymal transition in gastric cancer. *Therap Adv Gastroenterol* 2019; **12**: 1756284819827697 [PMID: 30815034 DOI: 10.1177/1756284819827697]

114 **Zong W**, Feng W, Jiang Y, Ju S, Cui M, Jing R. Evaluating the diagnostic and prognostic value of serum long non-coding RNA CTC-497E21.4 in gastric cancer. *Clin Chem Lab Med* 2019; **57**: 1063-1072 [PMID: 30763257 DOI: 10.1515/cclm-2018-0929]

115 **Miao X**, Liu Y, Fan Y, Wang G, Zhu H. LncRNA BANCR Attenuates the Killing Capacity of Cisplatin on Gastric Cancer Cell Through the ERK1/2 Pathway. *Cancer Manag Res* 2021; **13**: 287-296 [PMID: 33469371 DOI: 10.2147/CMAR.S269679]

116 **Zhang ZX**, Liu ZQ, Jiang B, Lu XY, Ning XF, Yuan CT, Wang AL. BRAF activated non-coding RNA (BANCR) promoting gastric cancer cells proliferation *via* regulation of NF-κB1. *Biochem Biophys Res Commun* 2015; **465**: 225-231 [PMID: 26248136 DOI: 10.1016/j.bbrc.2015.07.158]

117 **Xiao ZS**, Long H, Zhao L, Li HX, Zhang XN. LncRNA HOTTIP promotes proliferation and inhibits apoptosis of gastric carcinoma cells *via* adsorbing miR-615-3p. *Eur Rev Med Pharmacol Sci* 2020; **24**: 6692-6698 [PMID: 32633359 DOI: 10.26355/eurrev_202006_21656]

118 **Zhao R**, Zhang X, Zhang Y, Yang Y, Sun Y, Zheng X, Qu A, Umwali Y. HOTTIP Predicts Poor Survival in Gastric Cancer Patients and Contributes to Cisplatin Resistance by Sponging miR-216a-5p. *Front Cell Dev Biol* 2020; **8**: 348 [PMID: 32457911 DOI: 10.3389/fcell.2020.00348]

119 **Liu J**, Wang J, Song Y, Ma B, Luo J, Ni Z, Gao P, Sun J, Zhao J, Chen X, Wang Z. A panel consisting of three novel circulating lncRNAs, is it a predictive tool for gastric cancer? *J Cell Mol Med* 2018; **22**: 3605-3613 [PMID: 29700972 DOI: 10.1111/jcmm.13640]

120 **Ke D**, Li H, Zhang Y, An Y, Fu H, Fang X, Zheng X. The combination of circulating long noncoding RNAs AK001058, INHBA-AS1, MIR4435-2HG, and CEBPA-AS1 fragments in plasma serve as diagnostic markers for gastric cancer. *Oncotarget* 2017; **8**: 21516-21525 [PMID: 28423525 DOI: 10.18632/oncotarget.15628]

121 **Wei F**, Wang Y, Zhou Y, Li Y. Long noncoding RNA CYTOR triggers gastric cancer progression by targeting miR-103/RAB10. *Acta Biochim Biophys Sin (Shanghai)* 2021; **53**: 1044-1054 [PMID: 34110382 DOI: 10.1093/abbs/gmab071]

122 **Teng F**, Zhang JX, Chen Y, Shen XD, Su C, Guo YJ, Wang PH, Shi CC, Lei M, Cao YO, Liu SQ. LncRNA NKX2-1-AS1 promotes tumor progression and angiogenesis *via* upregulation of SERPINE1 expression and activation of the VEGFR-2 signaling pathway in gastric cancer. *Mol Oncol* 2021; **15**: 1234-1255 [PMID: 33512745 DOI: 10.1002/1878-0261.12911]

123 **Xu Y**, Li Y, Qiu Y, Sun F, Zhu G, Sun J, Cai G, Lin W, Fu Y, Wu H, Jiang S, Wen Z, Feng F, Luo J, Yang Y, Zhang Q. LncRNA NEAT1 Promotes Gastric Cancer Progression Through miR-17-5p/TGFβR2 Axis Up-Regulated Angiogenesis. *Front Cell Dev Biol* 2021; **9**: 705697 [PMID: 34552925 DOI: 10.3389/fcell.2021.705697]

124 **Zhu T**, Wang Z, Wang G, Hu Z, Ding H, Li R, Sun J. Long non-coding RNA ZFAS1 promotes the expression of EPAS1 in gastric cardia adenocarcinoma. *J Adv Res* 2021; **28**: 7-15 [PMID: 33364040 DOI: 10.1016/j.jare.2020.06.006]

125 **Ma ZH**, Shuai Y, Gao XY, Yan Y, Wang KM, Wen XZ, Ji JF. BTEB2-Activated lncRNA TSPEAR-AS2 Drives GC Progression through Suppressing GJA1 Expression and Upregulating CLDN4 Expression. *Mol Ther Nucleic Acids* 2020; **22**: 1129-1141 [PMID: 33294297 DOI: 10.1016/j.omtn.2020.10.022]

126 **Song S**, He X, Wang J, Song H, Wang Y, Liu Y, Zhou Z, Yu Z, Miao D, Xue Y. A novel long noncoding RNA, TMEM92-AS1, promotes gastric cancer progression by binding to YBX1 to mediate CCL5. *Mol Oncol* 2021; **15**: 1256-1273 [PMID: 33247987 DOI: 10.1002/1878-0261.12863]

127 **Xin L**, Zhou LQ, Liu C, Zeng F, Yuan YW, Zhou Q, Li SH, Wu Y, Wang JL, Wu DZ, Lu H. Transfer of LncRNA CRNDE in TAM-derived exosomes is linked with cisplatin resistance in gastric cancer. *EMBO Rep* 2021; **22**: e52124 [PMID: 34647680 DOI: 10.15252/embr.202052124]

128 **Ding L**, Tian Y, Wang L, Bi M, Teng D, Hong S. Hypermethylated long noncoding RNA MEG3 promotes the progression of gastric cancer. *Aging (Albany NY)* 2019; **11**: 8139-8155 [PMID: 31584879 DOI: 10.18632/aging.102309]

129 **Cai C**, Zhang H, Zhu Y, Zheng P, Xu Y, Sun J, Zhang M, Lan T, Gu B, Li S, Ma P. Serum Exosomal Long Noncoding RNA pcsk2-2:1 As A Potential Novel Diagnostic Biomarker For Gastric Cancer. *Onco Targets Ther* 2019; **12**: 10035-10041 [PMID: 31819499 DOI: 10.2147/OTT.S229033]

130 **Li S**, Zhang M, Zhang H, Hu K, Cai C, Wang J, Shi L, Ma P, Xu Y, Zheng P. Exosomal long noncoding RNA lnc-GNAQ-6:1 may serve as a diagnostic marker for gastric cancer. *Clin Chim Acta* 2020; **501**: 252-257 [PMID: 31730812 DOI: 10.1016/j.cca.2019.10.047]

131 **Pan T**, Yu Z, Jin Z, Wu X, Wu A, Hou J, Chang X, Fan Z, Li J, Yu B, Li F, Yan C, Yang Z, Zhu Z, Liu B, Su L. Tumor suppressor lnc-CTSLP4 inhibits EMT and metastasis of gastric cancer by attenuating HNRNPAB-dependent Snail transcription. *Mol Ther Nucleic Acids* 2021; **23**: 1288-1303 [PMID: 33717650 DOI: 10.1016/j.omtn.2021.02.003]

132 **Xia Y**, Lv J, Jiang T, Li B, Li Y, He Z, Xuan Z, Sun G, Wang S, Li Z, Wang W, Wang L, Xu Z. CircFAM73A promotes the cancer stem cell-like properties of gastric cancer through the miR-490-3p/HMGA2 positive feedback loop and HNRNPK-mediated β-catenin stabilization. *J Exp Clin Cancer Res* 2021; **40**: 103 [PMID: 33731207 DOI: 10.1186/s13046-021-01896-9]

133 **Bu X**, Chen Z, Zhang A, Zhou X, Zhang X, Yuan H, Zhang Y, Yin C, Yan Y. Circular RNA circAFF2 accelerates gastric cancer development by activating miR-6894-5p and regulating ANTXR 1 expression. *Clin Res Hepatol Gastroenterol* 2021; **45**: 101671 [PMID: 33722777 DOI: 10.1016/j.clinre.2021.101671]

134 **Yang D**, Hu Z, Zhang Y, Zhang X, Xu J, Fu H, Zhu Z, Feng D, Cai Q. CircHIPK3 Promotes the Tumorigenesis and Development of Gastric Cancer Through miR-637/AKT1 Pathway. *Front Oncol* 2021; **11**: 637761 [PMID: 33680975 DOI: 10.3389/fonc.2021.637761]

135 **Deng P**, Sun M, Zhao WY, Hou B, Li K, Zhang T, Gu F. Circular RNA circVAPA promotes chemotherapy drug resistance in gastric cancer progression by regulating miR-125b-5p/STAT3 axis. *World J Gastroenterol* 2021; **27**: 487-500 [PMID: 33642823 DOI: 10.3748/wjg.v27.i6.487]

136 **Yang H**, Wu Z, Liu X, Chen M, Zhang X, Jiang Y. NFIB promotes the progression of gastric cancer by upregulating circMAP7D1 to stabilize HER2 mRNA. *Mol Med Rep* 2021; **23** [PMID: 33576439 DOI: 10.3892/mmr.2021.11908]

137 **Hua Y**, Wang H, Wu X, Yang L, Wang C, Li X, Jin Y, Li M, Wang L, Dong C, Yin F. Circular RNA Circ_0006282 Promotes Cell Proliferation and Metastasis in Gastric Cancer by Regulating MicroRNA-144-5p/Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein β Axis. *Cancer Manag Res* 2021; **13**: 815-827 [PMID: 33536789 DOI: 10.2147/CMAR.S283952]

138 **Xu Q**, Liao B, Hu S, Zhou Y, Xia W. Circular RNA 0081146 facilitates the progression of gastric cancer by sponging miR-144 and up-regulating HMGB1. *Biotechnol Lett* 2021; **43**: 767-779 [PMID: 33496921 DOI: 10.1007/s10529-020-03058-x]

139 **Wang L**, Li B, Yi X, Xiao X, Zheng Q, Ma L. Circ_SMAD4 promotes gastric carcinogenesis by activating wnt/β-catenin pathway. *Cell Prolif* 2021; **54**: e12981 [PMID: 33458917 DOI: 10.1111/cpr.12981]

140 **Yu L**, Xie J, Liu X, Yu Y, Wang S. Plasma Exosomal CircNEK9 Accelerates the Progression of Gastric Cancer *via* miR-409-3p/MAP7 Axis. *Dig Dis Sci* 2021; **66**: 4274-4289 [PMID: 33449227 DOI: 10.1007/s10620-020-06816-z]

141 **Yue F**, Peng K, Zhang L, Zhang J. Circ_0004104 Accelerates the Progression of Gastric Cancer by Regulating the miR-539-3p/RNF2 Axis. *Dig Dis Sci* 2021; **66**: 4290-4301 [PMID: 33449226 DOI: 10.1007/s10620-020-06802-5]

142 **Wang X**, Zhang Y, Li W, Liu X. Knockdown of cir_RNA PVT1 Elevates Gastric Cancer Cisplatin Sensitivity *via* Sponging miR-152-3p. *J Surg Res* 2021; **261**: 185-195 [PMID: 33444948 DOI: 10.1016/j.jss.2020.12.013]

143 **Li J**, Yang Y, Xu D, Cao L. hsa_circ_0023409 Accelerates Gastric Cancer Cell Growth and Metastasis Through Regulating the IRS4/PI3K/AKT Pathway. *Cell Transplant* 2021; **30**: 963689720975390 [PMID: 33439739 DOI: 10.1177/0963689720975390]

144 **Yang Y**, Cai B, Shi X, Duan C, Tong T, Yu C. circ_0044516 functions in the progression of gastric cancer by modulating MicroRNA-149-5p/HuR axis. *Mol Cell Biochem* 2021 [PMID: 33417162 DOI: 10.1007/s11010-020-04026-9]

145 **Cao J**, Zhang X, Xu P, Wang H, Wang S, Zhang L, Li Z, Xie L, Sun G, Xia Y, Lv J, Yang J, Xu Z. Circular RNA circLMO7 acts as a microRNA-30a-3p sponge to promote gastric cancer progression *via* the WNT2/β-catenin pathway. *J Exp Clin Cancer Res* 2021; **40**: 6 [PMID: 33397440 DOI: 10.1186/s13046-020-01791-9]

146 **Niu Q**, Dong Z, Liang M, Luo Y, Lin H, Lin M, Zhong X, Yao W, Weng J, Zhou X. Circular RNA hsa_circ_0001829 promotes gastric cancer progression through miR-155-5p/SMAD2 axis. *J Exp Clin Cancer Res* 2020; **39**: 280 [PMID: 33308284 DOI: 10.1186/s13046-020-01790-w]

147 **Pu Z**, Xu M, Yuan X, Xie H, Zhao J. Circular RNA circCUL3 Accelerates the Warburg Effect Progression of Gastric Cancer through Regulating the STAT3/HK2 Axis. *Mol Ther Nucleic Acids* 2020; **22**: 310-318 [PMID: 33230436 DOI: 10.1016/j.omtn.2020.08.023]

148 **Wang H**, Sun G, Xu P, Lv J, Zhang X, Zhang L, Wang S, Cao J, Xia Y, Xuan Z, Li B, Huang X, Jiang T, Fang L, Xu Z. Circular RNA TMEM87A promotes cell proliferation and metastasis of gastric cancer by elevating ULK1 *via* sponging miR-142-5p. *J Gastroenterol* 2021; **56**: 125-138 [PMID: 33155080 DOI: 10.1007/s00535-020-01744-1]

149 **Ma S**, Kong S, Gu X, Xu Y, Tao M, Shen L, Shen X, Ju S. As a biomarker for gastric cancer, circPTPN22 regulates the progression of gastric cancer through the EMT pathway. *Cancer Cell Int* 2021; **21**: 44 [PMID: 33430866 DOI: 10.1186/s12935-020-01701-1]

150 **Ma C**, Wang X, Yang F, Zang Y, Liu J, Xu X, Li W, Jia J, Liu Z. Circular RNA hsa_circ_0004872 inhibits gastric cancer progression *via* the miR-224/Smad4/ADAR1 successive regulatory circuit. *Mol Cancer* 2020; **19**: 157 [PMID: 33172486 DOI: 10.1186/s12943-020-01268-5]

151 **Wang H**, Wang N, Zheng X, Wu L, Fan C, Li X, Ye K, Han S. Circular RNA hsa_circ_0009172 suppresses gastric cancer by regulation of microRNA-485-3p-mediated NTRK3. *Cancer Gene Ther* 2021; **28**: 1312-1324 [PMID: 33531648 DOI: 10.1038/s41417-020-00280-7]

152 **Li T**, Zuo X, Meng X. Circ_002059 suppresses cell proliferation and migration of gastric cancer *via* miR-182/MTSS1 axis. *Acta Biochim Biophys Sin (Shanghai)* 2021; **53**: 454-462 [PMID: 33686422 DOI: 10.1093/abbs/gmab015]

153 **Wang Y**, Wang H, Zheng R, Wu P, Sun Z, Chen J, Zhang L, Zhang C, Qian H, Jiang J, Xu W. Circular RNA ITCH suppresses metastasis of gastric cancer *via* regulating miR-199a-5p/Klotho axis. *Cell Cycle* 2021; **20**: 522-536 [PMID: 33499704 DOI: 10.1080/15384101.2021.1878327]

154 **Peng L**, Sang H, Wei S, Li Y, Jin D, Zhu X, Li X, Dang Y, Zhang G. circCUL2 regulates gastric cancer malignant transformation and cisplatin resistance by modulating autophagy activation *via* miR-142-3p/ROCK2. *Mol Cancer* 2020; **19**: 156 [PMID: 33153478 DOI: 10.1186/s12943-020-01270-x]

155 **Sakakura C**, Hamada T, Miyagawa K, Nishio M, Miyashita A, Nagata H, Ida H, Yazumi S, Otsuji E, Chiba T, Ito K, Ito Y. Quantitative analysis of tumor-derived methylated RUNX3 sequences in the serum of gastric cancer patients. *Anticancer Res* 2009; **29**: 2619-2625 [PMID: 19596937]

156 **Kolesnikova EV**, Tamkovich SN, Bryzgunova OE, Shelestyuk PI, Permyakova VI, Vlassov VV, Tuzikov AS, Laktionov PP, Rykova EY. Circulating DNA in the blood of gastric cancer patients. *Ann N Y Acad Sci* 2008; **1137**: 226-231 [PMID: 18837952 DOI: 10.1196/annals.1448.009]

157 **Pimson C**, Ekalaksananan T, Pientong C, Promthet S, Putthanachote N, Suwanrungruang K, Wiangnon S. Aberrant methylation of PCDH10 and RASSF1A genes in blood samples for non-invasive diagnosis and prognostic assessment of gastric cancer. *PeerJ* 2016; **4**: e2112 [PMID: 27330867 DOI: 10.7717/peerj.2112]

158 **Kanda M**, Shimizu D, Fujii T, Sueoka S, Tanaka Y, Ezaka K, Takami H, Tanaka H, Hashimoto R, Iwata N, Kobayashi D, Tanaka C, Yamada S, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Function and diagnostic value of Anosmin-1 in gastric cancer progression. *Int J Cancer* 2016; **138**: 721-730 [PMID: 26270236 DOI: 10.1002/ijc.29803]

159 **Alarcón MA**, Olivares W, Córdova-Delgado M, Muñoz-Medel M, de Mayo T, Carrasco-Aviño G, Wichmann I, Landeros N, Amigo J, Norero E, Villarroel-Espíndola F, Riquelme A, Garrido M, Owen GI, Corvalán AH. The Reprimo-Like Gene Is an Epigenetic-Mediated Tumor Suppressor and a Candidate Biomarker for the Non-Invasive Detection of Gastric Cancer. *Int J Mol Sci* 2020; **21** [PMID: 33322837 DOI: 10.3390/ijms21249472]

160 **Nanishi K**, Konishi H, Shoda K, Arita T, Kosuga T, Komatsu S, Shiozaki A, Kubota T, Fujiwara H, Okamoto K, Ichikawa D, Otsuji E. Circulating circERBB2 as a potential prognostic biomarker for gastric cancer: An investigative study. *Cancer Sci* 2020; **111**: 4177-4186 [PMID: 32896032 DOI: 10.1111/cas.14645]

161 **Guo X**, Lv X, Ru Y, Zhou F, Wang N, Xi H, Zhang K, Li J, Chang R, Xie T, Wang X, Li B, Chen Y, Yang Y, Chen L. Circulating Exosomal Gastric Cancer-Associated Long Noncoding RNA1 as a Biomarker for Early Detection and Monitoring Progression of Gastric Cancer: A Multiphase Study. *JAMA Surg* 2020; **155**: 572-579 [PMID: 32520332 DOI: 10.1001/jamasurg.2020.1133]

162 **Shin SJ**, Park S, Kim MH, Nam CM, Kim H, Choi YY, Jung MK, Choi HJ, Rha SY, Chung HC. Mesothelin Expression Is a Predictive Factor for Peritoneal Recurrence in Curatively Resected Stage III Gastric Cancer. *Oncologist* 2019; **24**: e1108-e1114 [PMID: 31015316 DOI: 10.1634/theoncologist.2018-0896]

163 **Liu X**, Chu KM. Exosomal miRNAs as circulating biomarkers for prediction of development of haematogenous metastasis after surgery for stage II/III gastric cancer. *J Cell Mol Med* 2020; **24**: 6220-6232 [PMID: 32383554 DOI: 10.1111/jcmm.15253]

164 **Zhao Z**, Zhang C, Zhao Q. S100A9 as a novel diagnostic and prognostic biomarker in human gastric cancer. *Scand J Gastroenterol* 2020; **55**: 338-346 [PMID: 32172630 DOI: 10.1080/00365521.2020.1737883]

165 **Hu J**, Yu J, Gan J, Song N, Shi L, Liu J, Zhang Z, Du J. Notch1/2/3/4 are prognostic biomarker and correlated with immune infiltrates in gastric cancer. *Aging (Albany NY)* 2020; **12**: 2595-2609 [PMID: 32028262 DOI: 10.18632/aging.102764]

166 **Meng X**, Zhao Y, Liu J, Wang L, Dong Z, Zhang T, Gu X, Zheng Z. Comprehensive analysis of histone modification-associated genes on differential gene expression and prognosis in gastric cancer. *Exp Ther Med* 2019; **18**: 2219-2230 [PMID: 31452712 DOI: 10.3892/etm.2019.7808]

167 **Yoo C**, Ryu MH, Na YS, Ryoo BY, Lee CW, Kang YK. Vorinostat in combination with capecitabine plus cisplatin as a first-line chemotherapy for patients with metastatic or unresectable gastric cancer: phase II study and biomarker analysis. *Br J Cancer* 2016; **114**: 1185-1190 [PMID: 27172248 DOI: 10.1038/bjc.2016.125]

168 **Yoo C**, Ryu MH, Na YS, Ryoo BY, Lee CW, Maeng J, Kim SY, Koo DH, Park I, Kang YK. Phase I and pharmacodynamic study of vorinostat combined with capecitabine and cisplatin as first-line chemotherapy in advanced gastric cancer. *Invest New Drugs* 2014; **32**: 271-278 [PMID: 23712440 DOI: 10.1007/s10637-013-9983-2]

169 **Schneider BJ**, Shah MA, Klute K, Ocean A, Popa E, Altorki N, Lieberman M, Schreiner A, Yantiss R, Christos PJ, Palmer R, You D, Viale A, Kermani P, Scandura JM. Phase I Study of Epigenetic Priming with Azacitidine Prior to Standard Neoadjuvant Chemotherapy for Patients with Resectable Gastric and Esophageal Adenocarcinoma: Evidence of Tumor Hypomethylation as an Indicator of Major Histopathologic Response. *Clin Cancer Res* 2017; **23**: 2673-2680 [PMID: 27836862 DOI: 10.1158/1078-0432.CCR-16-1896]

170 **Hu W**, Zhang L, Li MX, Shen J, Liu XD, Xiao ZG, Wu DL, Ho IHT, Wu JCY, Cheung CKY, Zhang YC, Lau AHY, Ashktorab H, Smoot DT, Fang EF, Chan MTV, Gin T, Gong W, Wu WKK, Cho CH. Vitamin D3 activates the autolysosomal degradation function against Helicobacter pylori through the PDIA3 receptor in gastric epithelial cells. *Autophagy* 2019; **15**: 707-725 [PMID: 30612517 DOI: 10.1080/15548627.2018.1557835]

171 **Ha J**, Lee JM, Lim Y, Kim MK, Kwon HS, Song KH, Jeon HM, Kang MI, Baek KH. Effect of bisphosphonate on the prevention of bone loss in patients with gastric cancer after gastrectomy: A randomized controlled trial. *Bone* 2020; **130**: 115138 [PMID: 31706052 DOI: 10.1016/j.bone.2019.115138]

172 **Seah KS**, Loh JY, Nguyen TTT, Tan HL, Hutchinson PE, Lim KK, Dymock BW, Long YC, Lee EJD, Shen HM, Chen ES. SAHA and cisplatin sensitize gastric cancer cells to doxorubicin by induction of DNA damage, apoptosis and perturbation of AMPK-mTOR signalling. *Exp Cell Res* 2018; **370**: 283-291 [PMID: 29959912 DOI: 10.1016/j.yexcr.2018.06.029]

173 **Xiong K**, Zhang H, Du Y, Tian J, Ding S. Identification of HDAC9 as a viable therapeutic target for the treatment of gastric cancer. *Exp Mol Med* 2019; **51**: 1-15 [PMID: 31451695 DOI: 10.1038/s12276-019-0301-8]

174 **Regel I**, Merkl L, Friedrich T, Burgermeister E, Zimmermann W, Einwächter H, Herrmann K, Langer R, Röcken C, Hofheinz R, Schmid R, Ebert MP. Pan-histone deacetylase inhibitor panobinostat sensitizes gastric cancer cells to anthracyclines *via* induction of CITED2. *Gastroenterology* 2012; **143**: 99-109.e10 [PMID: 22465428 DOI: 10.1053/j.gastro.2012.03.035]

175 **Singh A**, Patel P, Jageshwar, Patel VK, Jain DK, Kamal M, Rajak H. The Safety, Efficacy and Therapeutic Potential of Histone Deacetylase Inhibitors with Special Reference to Panobinostat in Gastrointestinal Tumors: A Review of Preclinical and Clinical Studies. *Curr Cancer Drug Targets* 2018; **18**: 720-736 [PMID: 28669336 DOI: 10.2174/1568009617666170630124643]

176 **Kim S**, Kim W, Kim DH, Jang JH, Kim SJ, Park SA, Hahn H, Han BW, Na HK, Chun KS, Choi BY, Surh YJ. Resveratrol suppresses gastric cancer cell proliferation and survival through inhibition of PIM-1 kinase activity. *Arch Biochem Biophys* 2020; **689**: 108413 [PMID: 32473133 DOI: 10.1016/j.abb.2020.108413]

177 **Yang T**, Zhang J, Zhou J, Zhu M, Wang L, Yan L. Resveratrol inhibits Interleukin-6 induced invasion of human gastric cancer cells. *Biomed Pharmacother* 2018; **99**: 766-773 [PMID: 29710474 DOI: 10.1016/j.biopha.2018.01.153]

178 **Barati N**, Momtazi-Borojeni AA, Majeed M, Sahebkar A. Potential therapeutic effects of curcumin in gastric cancer. *J Cell Physiol* 2019; **234**: 2317-2328 [PMID: 30191991 DOI: 10.1002/jcp.27229]

179 **Li W**, Zhou Y, Yang J, Li H, Zhang H, Zheng P. Curcumin induces apoptotic cell death and protective autophagy in human gastric cancer cells. *Oncol Rep* 2017; **37**: 3459-3466 [PMID: 28498433 DOI: 10.3892/or.2017.5637]

180 **Shang HS**, Lu HF, Lee CH, Chiang HS, Chu YL, Chen A, Lin YF, Chung JG. Quercetin induced cell apoptosis and altered gene expression in AGS human gastric cancer cells. *Environ Toxicol* 2018; **33**: 1168-1181 [PMID: 30152185 DOI: 10.1002/tox.22623]

181 **Hemati M**, Haghiralsadat F, Jafary F, Moosavizadeh S, Moradi A. Targeting cell cycle protein in gastric cancer with CDC20siRNA and anticancer drugs (doxorubicin and quercetin) co-loaded cationic PEGylated nanoniosomes. *Int J Nanomedicine* 2019; **14**: 6575-6585 [PMID: 31616144 DOI: 10.2147/IJN.S211844]

182 **Liu C**, Ho PC, Wong FC, Sethi G, Wang LZ, Goh BC. Garcinol: Current status of its anti-oxidative, anti-inflammatory and anti-cancer effects. *Cancer Lett* 2015; **362**: 8-14 [PMID: 25796441 DOI: 10.1016/j.canlet.2015.03.019]

183 **Zheng Y**, Guo C, Zhang X, Wang X, Ma A. Garcinol acts as an antineoplastic agent in human gastric cancer by inhibiting the PI3K/AKT signaling pathway. *Oncol Lett* 2020; **20**: 667-676 [PMID: 32565991 DOI: 10.3892/ol.2020.11585]

184 **Shin H**, Lee YS, Lee YC. Sodium butyrate-induced DAPK-mediated apoptosis in human gastric cancer cells. *Oncol Rep* 2012; **27**: 1111-1115 [PMID: 22160140 DOI: 10.3892/or.2011.1585]

185 **Ke X**, Qin Q, Deng T, Liao Y, Gao SJ. Heterogeneous Responses of Gastric Cancer Cell Lines to Tenovin-6 and Synergistic Effect with Chloroquine. *Cancers (Basel)* 2020; **12** [PMID: 32033497 DOI: 10.3390/cancers12020365]

186 **Huang R**, Jin X, Gao Y, Yuan H, Wang F, Cao X. DZNep inhibits Hif-1α and Wnt signalling molecules to attenuate the proliferation and invasion of BGC-823 gastric cancer cells. *Oncol Lett* 2019; **18**: 4308-4316 [PMID: 31579098 DOI: 10.3892/ol.2019.10769]

187 **Clermont PL**, Fornaro L, Crea F. Elevated expression of a pharmacologic Polycomb signature predicts poor prognosis in gastric and breast cancer. *Epigenomics* 2017; **9**: 1329-1335 [PMID: 28875726 DOI: 10.2217/epi-2017-0074]

188 **Liu S**, Rong G, Li X, Geng L, Zeng Z, Jiang D, Yang J, Wei Y. Diosgenin and GSK126 Produce Synergistic Effects on Epithelial-Mesenchymal Transition in Gastric Cancer Cells by Mediating EZH2 *via* the Rho/ROCK Signaling Pathway. *Onco Targets Ther* 2020; **13**: 5057-5067 [PMID: 32606728 DOI: 10.2147/OTT.S237474]

189 **Chen YT**, Zhu F, Lin WR, Ying RB, Yang YP, Zeng LH. The novel EZH2 inhibitor, GSK126, suppresses cell migration and angiogenesis *via* down-regulating VEGF-A. *Cancer Chemother Pharmacol* 2016; **77**: 757-765 [PMID: 26898301 DOI: 10.1007/s00280-016-2990-1]

190 **Zheng YC**, Duan YC, Ma JL, Xu RM, Zi X, Lv WL, Wang MM, Ye XW, Zhu S, Mobley D, Zhu YY, Wang JW, Li JF, Wang ZR, Zhao W, Liu HM. Triazole-dithiocarbamate based selective lysine specific demethylase 1 (LSD1) inactivators inhibit gastric cancer cell growth, invasion, and migration. *J Med Chem* 2013; **56**: 8543-8560 [PMID: 24131029 DOI: 10.1021/jm401002r]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 110-123

DOI: 10.4251/wjgo.v14.i1.110

ISSN 1948-5204 (online)

REVIEW

Statin as a therapeutic agent in gastroenterological cancer

Norio Uemura, Hiromitsu Hayashi, Hideo Baba

ORCID number: Norio Uemura 0000-0003-3192-4153; Hiromitsu Hayashi 0000-0002-1832-4287; Hideo Baba 0000-0002-6982-3457.

Author contributions: Uemura N and Hayashi H contributed equally to this work; Uemura N, Hayashi H and Baba H designed the research study; Uemura N and Hayashi H performed the research, analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

Conflict-of-interest statement: Authors have nothing to disclose.

Country/Territory of origin: Japan

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Norio Uemura, Hiromitsu Hayashi, Hideo Baba, Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan

Corresponding author: Hiromitsu Hayashi, FACS, MD, PhD, Surgeon, Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. hhayasi@kumamoto-u.ac.jp

Abstract

Statins inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme of the mevalonate pathway, and are widely used as an effective and safe approach handle hypercholesterolemia. The mevalonate pathway is a vital metabolic pathway that uses acetyl-CoA to generate isoprenoids and sterols that are crucial to tumor growth and progression. Multiple studies have indicated that statins improve patient prognosis in various carcinomas. Basic research on the mechanisms underlying the antitumor effects of statins is underway. The development of new anti-cancer drugs is progressing, but increasing medical costs from drug development have become a major obstacle. Readily available, inexpensive and well-tolerated drugs like statins have not yet been successfully repurposed for cancer treatment. Identifying the cancer patients that may benefit from statins is key to improved patient treatment. This review summarizes recent advances in statin research in cancer and suggests important considerations for the clinical use of statins to improve outcomes for cancer patients.

Key Words: Statin; HMG CoA reductase inhibitor; Mevalonate pathway; Cancer

Core Tip: Novel pharmacological therapies for cancer are in development, but the expense of new drug development has increased medical costs and placed a heavy financial burden on governments worldwide. Therefore, drug repositioning has become a major focus for new drug development because of reliability and cost effectiveness. Statins are one of the most studied drugs with potential drug repositioning for cancer treatment, but they have not reached clinical application. This review summarizes the results of recent research and clinical studies of statins in cancer, suggests strategies for clinical trial planning, and discusses the potential clinical application of statins for cancer treatment.

Received: April 29, 2021
Peer-review started: April 29, 2021
First decision: June 6, 2021
Revised: June 19, 2021
Accepted: November 24, 2021
Article in press: November 24, 2021
Published online: January 15, 2022

P-Reviewer: Meng FZ
S-Editor: Liu M
L-Editor: A
P-Editor: Liu M

Citation: Uemura N, Hayashi H, Baba H. Statin as a therapeutic agent in gastroenterological cancer. *World J Gastrointest Oncol* 2022; 14(1): 110-123
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/110.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.110

INTRODUCTION

Since the clinical application of statins in the late 1980s, statins have dramatically improved the clinical management of high cholesterol and ischemic heart disease, and their use has become widespread worldwide. Statins are specified inhibitors of the mevalonate (MVA) pathway, that is involved in the *de novo* synthesis of cholesterol and other nonsterol isoprenoids. The rate-limiting enzyme in MVA synthesis is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR)[1,2]. Statins function by inhibiting HMGCR and are effective in the management of hypercholesterolemia.

In addition to their functional role in normal physiology, the MVA pathway is noted to support tumorigenesis and be dysregulated in cancers[3-5]. The MVA pathway is a vital metabolic pathway that uses acetyl-CoA to generate isoprenoids and sterols, which are crucial to tumor growth and progression. Therefore, there is a great deal of interest in repurposing statins as anticancer drugs. Numerous cohort studies have announced that statin use is linked with lower risk of cancer development, lower cancer grade at diagnosis, and lower recurrence and cancer-related death[6]. Several randomized clinical trials have investigated the advantages of adding statins to anti-cancer agents. However, most of the trials did not show an improvement in prognosis and have not led to the clinical application of statins. The development of new anti-cancer drugs is progressing but increasing medical costs from drug development have become a major obstacle. Readily available, inexpensive and well-tolerated drugs like statins have not yet been successfully repurposed for cancer treatment. Planning clinical trials is difficult, and it is possible that the previous clinical trials were poorly designed[7]. In the age of precision medicine, defining the cancer patients that may benefit from statins is critical.

This review summarizes the results of recent basic research and clinical studies on statins in cancer and suggests strategies for future clinical trial planning. In addition, the potential for the clinical application of statins in cancer treatment is discussed.

MECHANISMS OF ACTION OF STATINS

The MVA pathway is a vital metabolic pathway that uses acetyl-CoA to generate isoprenoids and sterols, which are crucial to tumor growth and progression. In the first step of the MVA pathway, the rate-limiting enzyme HMGCR converts HMG-CoA to MVA (Figure 1). MVA is further metabolized to farnesyl pyrophosphate (FPP). FPP is the precursor in cholesterol and steroid biosynthesis as well as in the biosynthesis of dolichols. Intracellular cholesterol preserves sterol regulatory element-binding proteins (SREBPs) as an inactive form in their full-length. In a situation of cholesterol depletion, SREBP proteins are cleaved, releasing the active transcription factors involved in the MVA pathway and cholesterol transport.

Statins bind to the active site of HMGCR, compete with HMG-CoA, and reduce MVA synthesis. Hence, statins exhaust intracellular cholesterol, causing a homeostatic feedback machinery by the SREBP family of transcription factors. Activation of SREBPs increases the gene expression of low density lipoprotein (LDL) receptor (LDLR). Increased membrane expression of LDLR promotes the uptake of LDL cholesterol from the blood circulation and efficiently lowers serum cholesterol levels. Statins are generally prescribed to lower blood cholesterol, decrease the risk of cardiovascular disease, or enhance the survival rate of cases with cardiovascular disease.

MVA PATHWAY IN CANCER

The MVA pathway has been shown to play a multifaceted role in tumorigenesis[4,8]. The PI3K/AKT pathway is a critical regulator of cell proliferation and cell survival in response to growth factors. PI3K/AKT signaling activates the MVA pathway through

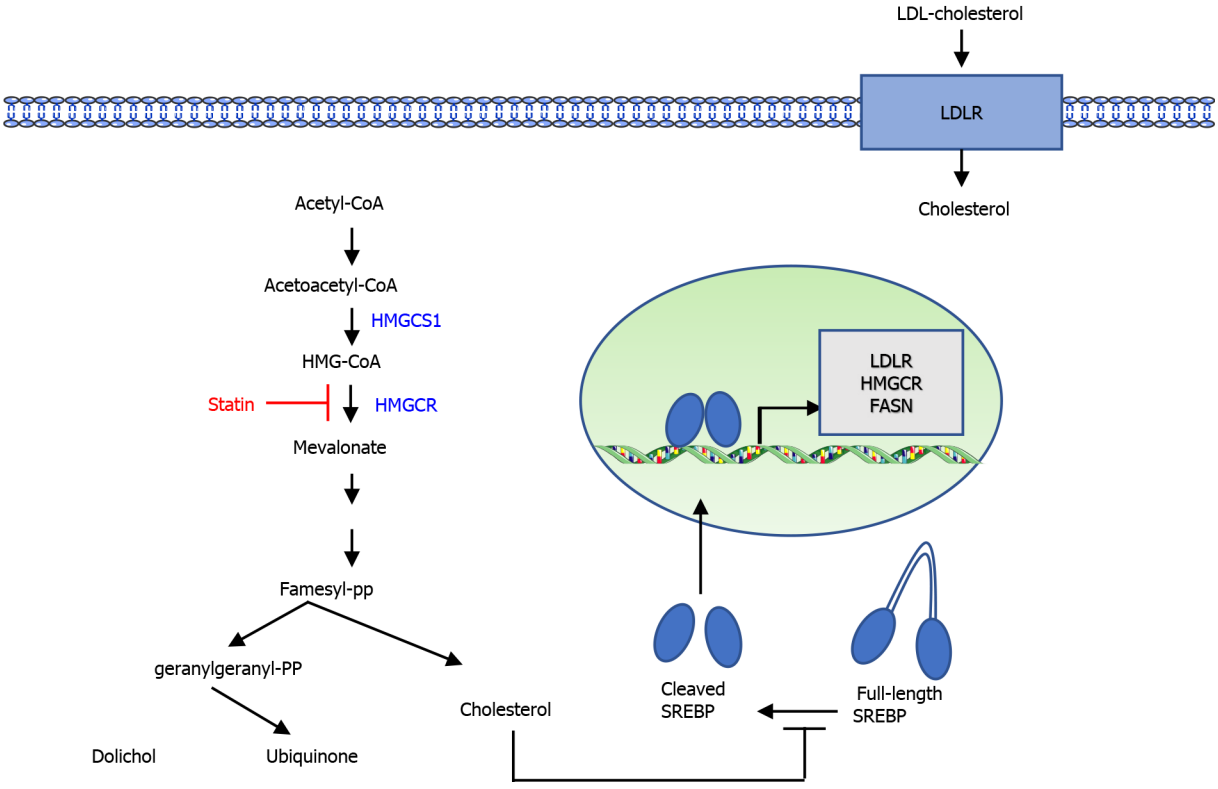


Figure 1 The mevalonate pathway and the SREBPs-mediated feedback response. SREBP: Sterol regulatory element-binding protein; LDL: Low density lipoprotein; LDLR: Low density lipoprotein receptor; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase.

increasing the expression of SREBPs. The increase in lipid and cholesterol generation regulated by the PI3K/AKT/SREBPs axis enhances the tumorigenesis and cancer growth[9,10]. Conversely, inhibition of the MVA pathway decreases PI3K activity through decreased RAS isoprenylation[11].

Two p53 mutants with gain-of-function mutations were shown to interact with nuclear SREBP2 and enhance the gene transcription of MVA pathway[12]. In contrast, wild-type p53 reduces lipid production by increasing LPIN1 expression under conditions of glucose starvation[13]. The tumor suppressor protein RB has also been involved as a MVA pathway regulator by interacting with SREBPs and reducing their binding to promoters of target genes[14,15]. The oncoproteins Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), both mediators of the Hippo pathway, are controlled by the SREBPs/MVA pathway[16]. The geranyl-geranyl pyrophosphate generated by the MVA cascade is essential for activation of Rho GTPases that, in turn, activate YAP/TAZ by inhibiting their phosphorylation and promoting their nuclear accumulation (Figure 2).

The Hedgehog (HH) signaling pathway, which has crucial roles in tumorigenesis, is controlled by cholesterol. Cholesterol and cholesterol-derived oxysterols activate HH signal transduction[17], whereas inhibition of the MVA pathway or downstream sterol biosynthesis decreases HH signaling and reduces cell proliferation.

Cholesterol is the precursor for steroid hormones such as estrogen and androgen. These hormones are implicated in hormone-driven breast cancers and prostate cancers *via* the activation of estrogen receptor-α (ERα) and androgen receptor, respectively[18,19]. Perhaps because of these functions, research into the antitumor effects of statins is the most advanced in the fields of breast cancer, ovarian cancer, and prostate cancer.

A recent report showed that the MVA pathway is involved in T lymphocyte metabolism and regulates T cell differentiation[20]. Improved understanding of MVA metabolism will enhance more efficient T cell manipulation for immunotherapy in cancer treatment.

STATINS AND ESOPHAGEAL CANCER

Three meta-analyses have been conducted on the effects of statins on esophageal cancer. In a meta-analysis of five cohort studies comprising 24576 patients, Zhou *et al*

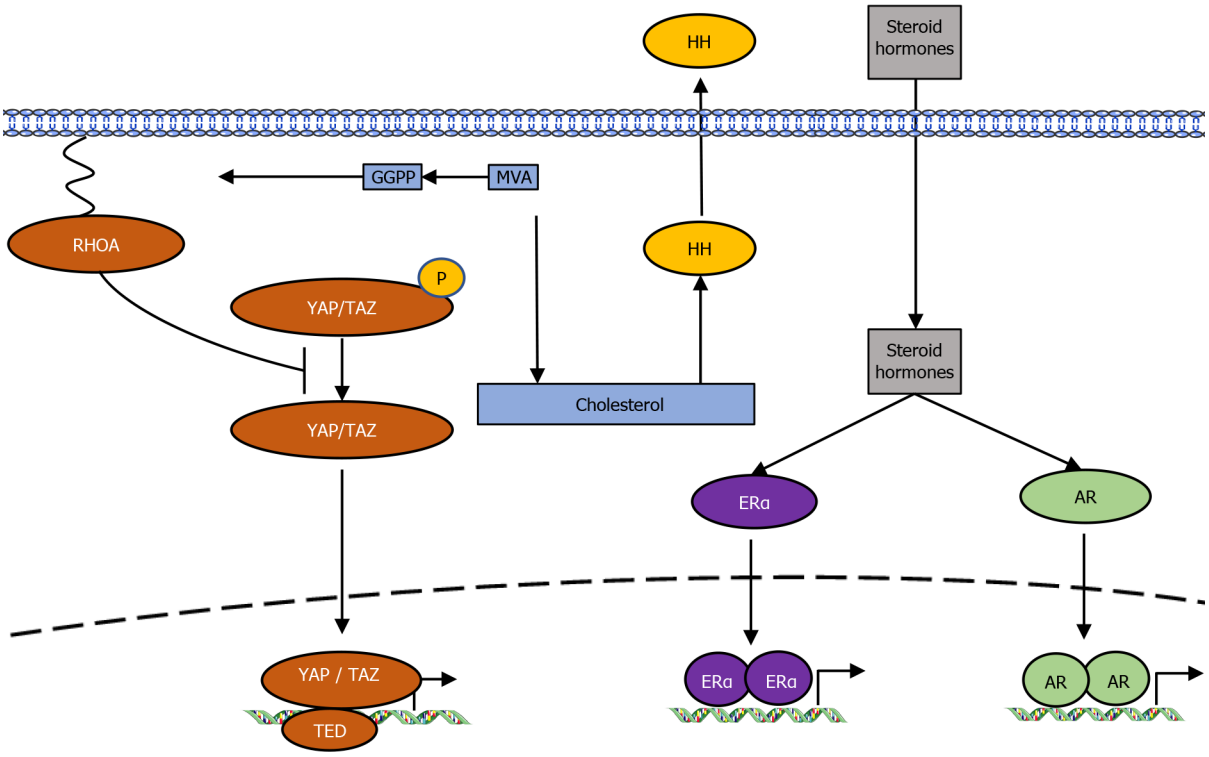


Figure 2 Activation of the mevalonate pathway drives oncogenic signaling pathways. MVA: Mevalonate; HH: Hedgehog; YAP: Yes-associated protein; TAZ: Transcriptional co-activator with PDZ-binding motif.

[21] reported that statin use in esophageal cancer patients was associated with a 26% improved overall survival [OS; 95% confidence interval (CI): 0.75–0.94] and disease-free survival (95%CI: 0.75–0.96)[22-26]. Deng *et al*[27] reported that statin use was considerably associated with decreased all-cause [random effects: Hazard ratio (HR) = 0.81, 95%CI: 0.75–0.89, $P < 0.001$] and cancer-specific mortality (fixed effects: HR = 0.84, 95%CI: 0.78–0.89, $P < 0.001$) in esophageal cancer from four cohort studies involving a total of 20435 patients[25]. In the subgroup analysis, both meta-analyses showed an effect of statins on improving prognosis regardless of the histological type of squamous cell carcinoma and adenocarcinoma. Thomas *et al*[28] insisted that statins might play a protective role against esophageal cancer development in cases with or without Barrett's esophagus.

STATINS AND GASTRIC CANCER

Two randomized controlled trials (RCTs) have examined the effects of statin combination therapy on gastric cancer. A phase III study that examined simvastatin (40 mg/d) plus capecitabine-cisplatin compared with capecitabin-cisplatin alone did not show increased progression-free survival[29]. A phase II study that examined pravastatin (40 mg/d) plus standard chemotherapy revealed no improvement of the progression-free survival rate at 6 mo compared with standard chemotherapy alone [30]. A matched case-control study reported that statin use in patients who underwent radical gastrectomy for stage II and III gastric cancer was associated with good prognosis. No significant differences were shown in relapse-free survival or OS between statin users and non-users. On the other hand, subgroup analysis revealed that patients who used statins for more than 6 mo showed better prognostic outcomes than non-users or those who used statins for less than 6 mo[31]. A population-based cohort study including 3833 patients with gastric cancer showed that statin use was linked with decreased cancer-specific mortality (adjusted HR = 0.83, 95%CI: 0.74–0.92) [32]. Several studies have shown that the use of statins reduces the risk of gastric cancer[33-35].

STATINS AND COLORECTAL CANCER

Many epidemiologic and clinical studies have been performed on statins and colorectal cancer (CRC). However, the results have been inconsistent. One notable observational study from Israel showed that 5 or more years of statin use was linked with a 45% decrease in CRC risk (95%CI: 0.40–0.74)[36]. An another study of United States veterans also revealed a 35% decrease in CRC risk with statin use (95%CI: 0.55–0.78)[37]. On the other hand, several meta-analyses of case-control and cohort studies have revealed smaller risk decreases[38,39], or no relationship[40,41]. The unconvincing results from observational studies could be due to healthier behaviors in statin users compared with nonusers, the different durations of statin intake[39], different hydrophilicity of specific statins[42], or different effects of statins on colon or rectal cancers[43,44].

STATINS AND HEPATOCELLULAR CARCINOMA

A nationwide population-based nested case-control study of patients with diabetes indicated a dose-dependent reduction of hepatocellular carcinoma (HCC) incidence with statin treatment[45]. In this study, statin users had a dose-dependent [cumulative defined daily dose (cDDD)] reduced risk of developing HCC [odds ratios (ORs) = 0.53, 0.36, 0.32, and 0.26 in ≤ 60, 60–180, 181–365, and > 365 cDDD, respectively; $P < 0.0001$]. The study also suggested that risk reduction was apparent in the presence of liver diseases such as chronic viral hepatitis, liver cirrhosis, alcoholic liver disease, and previous cancer (OR = 0.27, 95%CI: 0.14–0.50), but not significant in cases without liver disease (OR = 0.64, 95%CI: 0.32–1.29). Similar reports from Taiwan showed a dose-response relationship between statin use and the risk of HCV and HBV in an HCV cohort (HR = 0.66, 0.41, and 0.34 in 28–90, 91–365, and > 365 cDDD, respectively; $P < 0.0001$) and in an HBV cohort (HR = 0.66, 0.47, and 0.33 in 28–89, 90–180, and > 180 cDDD, respectively; $P < 0.0001$)[46,47]. In a cohort of 7248 HCV-infected patients in the United States ERCHIVES database, statin use was linked with a 44% decrease in the development of cirrhosis and a 49% decrease in incident HCC. Atorvastatin and fluvastatin were associated with more significant antifibrotic effects than other statins [48], and in 18080 patients with nonalcoholic fatty liver disease without cirrhosis, even higher HCC suppressive effects were suggested (HR = 0.29)[49]. Several reports have indicated that statins prevent liver fibrosis, and statins may delay the development of HCC by preventing fibrosis and inflammation of the liver[50]. A phase II trial to investigate the efficiency of a simvastatin *vs* placebo on the change in serum AFP-L3% from baseline to 6 mo following treatment initiation in cirrhotic patients with end-stage liver disease (NCT02968810) is currently underway. Atorvastatin is being investigated for tertiary prevention after curative resection or ablation for HCC (SHOT trial, NCT03024684).

STATINS AND PANCREATIC CANCER

A meta-analysis of 26 studies showed a considerable reduction in pancreatic cancer risk with statin use [relative risk (RR) = 0.84, 95%CI: 0.73–0.97; $P < 0.001$][51]. In subgroup analyses of the study, a non-significant relation was found between long-term statin use and the risk of pancreatic cancer (RR = 0.98, 95%CI: 0.86–1.11; $P = 0.718$). There was a non-significant relation between the use of lipophilic statins and the risk of pancreatic cancer (RR = 0.98, 95%CI: 0.84–1.15; $P = 0.853$). On the other hand, several studies revealed a reduced risk of pancreatic cancer among statin users [52-54], other reports showed no evidence of an association between statin use and pancreatic cancer[52,55]. A retrospective study of 2427 pancreatic cancer patients showed a 31% reduction in mortality in the group taking simvastatin and a 39% reduction in the group taking atorvastatin[56,57]. In another study of 1761 pancreatic cancer patients, the 5-year OS rate was 16.6% for statin users and 8.9% for nonusers ($P = 0.012$)[57]. Among 226 patients undergoing resection for pancreatic cancer, active use of moderate- to high-dose simvastatin was linked with favorable OS and disease-free survival[58].

Table 1 Randomized controlled trials of combination therapy with statins

Cancer type	Study type	Statin (dose)	Combination therapies	Outcome
Gastric cancer	Phase III	Simvastatin (40 mg/d)	Capecitabine andcisplatin	Simvastatin + capecitabine-cisplatin did not increase progression-free survival compared with capecitabine-cisplatin alone
	Phase II	Pravastatin (40 mg/d)	Epirubicin, cisplatinand capecitabine	Pravastatin + standard chemotherapy was well tolerated, but did not improve progression-free survival at 6 months compared with chemotherapy alone
Colorectal	Phase III	Simvastatin (40 mg/d)	FOLFIRI/XELIRI	Simvastatin + FOLFIRI/XELIRI did not increase progression-free survival compared with FOLFIRI/XELIRI alone
Hepatocellular	Phase III	Pravastatin (40 mg/d)	Sorafenib	Pravastatin + sorafenib did not improve overall or progression-free survival compared with sorafenib alone
	Phase II	Pravastatin (40 mg/d)	Transcatheter arterialembolization followedby fluorouracil	Pravastatin + standard therapy prolonged overall survival compared with standard therapy alone
Pancreatic	Phase II	Simvastatin (40 mg/d)	Gemcitabine	Simvastatin + gemcitabine was well tolerated, but did not decrease time to progression compared with gemcitabine alone

RCTS

Many retrospective cohort studies have identified a reduced risk of cancer mortality in patients taking statins to control cholesterol. However, prospective clinical studies have mostly not been successful (Table 1)[29,30,59-61]. Several causes might interpret these differences, including interpatient differences in the type of statins and the dose and duration of statin use. Besides, it is possible that not all cases benefit equally from statin treatment.

There are seven types of statins (simvastatin, atorvastatin, fluvastatin, lovastatin, pitavastatin, rosuvastatin, and pravastatin) that can be prescribed for hypercholesterolemia worldwide (Table 2). However, which statins are most effective against cancer remains unclear. In many *in vitro* studies, lipophilic statins are more effective in antiproliferation ability. Because lipophilic statins can cross biological membranes without requiring specific transporters, they have greater intracellular access and are thought to have more effective mechanisms than hydrophilic statins. One report examined differences in the effect of statins on pancreatic cancer using *in vivo* studies[62]. While simvastatin exerted the highest tumor suppressive effects *in vitro*, rosuvastatin and fluvastatin were the most potent compounds in an animal model. A retrospective cohort study examining the effects of different types of statins on advanced prostate cancer treated with androgen deprivation therapy found that atorvastatin, pravastatin, rosuvastatin, or pitavastatin showed a stronger effect on reduction in mortality compared with other statins[63]. It is necessary to determine the type of statin most effective against cancer to plan an optimal RCT.

Previous RCTs used simvastatin and pravastatin at 40 mg/d, which are moderate-intensity prescriptions (Table 1), and therefore higher doses or prescription of a higher-intensity statin might have provided enhanced responses in these studies. Drug combination strategies to reinforce the anti-cancer effect of statins should also be evaluated for future RCTs.

BIOMARKERS TO IDENTIFY CANCERS FOR WHICH STATINS ARE EFFECTIVE

SREBPs

The members of the SREBP family of transcription factors control the upregulation of *HMGCR* and other lipid metabolism genes and are activated to restore homeostasis in response to cholesterol depletion (Figure 1). A subset of cell lines and primary cells from multiple myeloma patients were unable to provoke the expression of SREBP target genes by statin treatment and readily undergo apoptosis[64]. On the contrary, cell lines with potent statin-induced activation of SREBPs were resistive to statin treatment. In prostate cancer, this sterol-regulated feedback loop may modulate statin sensitivity, and a combination therapy of statins and SREBP inhibitors has a synergistic effect in prostate cancer[65]. Although it is theoretically convincing that

Table 2 Properties of statins

Statin	Solubility[3]	Metabolism[3]	Human dose to lower cholesterol (mg)		
			Low	Moderate	High
Simvastatin	Lipophilic	CYP3A4	10	20-40	-
Atorvastatin	Lipophilic	CYP3A4/2C9	-	10-20	40-80
Fluvastatin	Lipophilic	CYP2C9	20-40	80	-
Pitavastatin	Lipophilic	Non-CYP450	-	1-4	-
Lovastatin	Lipophilic	CYP3A4/2C9	20	40-80	-
Rosuvastatin	Hydrophilic	Non-CYP450	-	5-10	20-40
Pravastatin	Hydrophilic	Non-CYP450	10-20	40-80	-

feedback dysregulation of the MVA pathway is involved in statin sensitivity, further research is required to verify whether SREBPs can be clinically useful biomarkers.

HMGCR

HMGCR is directly inhibited by statins, and SREBPs increase *HMGCR* expression through a feedback mechanism that is induced when intracellular cholesterol is depleted (Figure 1). High HMGCR protein expression is associated with poor prognosis in various cancers[65-67]. The efficacy of statins for cancer is inversely linked with high expression of cholesterol biosynthesis genes, including the *HMGCR* gene[64,68]. However, other reports suggested that HMGCR expression alone could not accurately predict the effect of statins[65,69]. Whether HMGCR expression alone can accurately predict statin susceptibility remains unclear. One possible interpretation for the conflicting data is the poor specificity of many commercially available HMGCR antibodies[70]. Further comprehensive studies using validated HMGCR reagents are required to properly investigate the utility of HMGCR expression as a predictive biomarker of the effects of statins.

A population-based case-control study of incident CRC in northern Israel showed that specific polymorphisms in the *HMGCR* gene modify the protective association between statins and CRC risk. Compared with non-statin users, the unadjusted OR of CRC among statin users with the A/A genotype of rs12654264 in *HMGCR* was 0.3 (95%CI: 0.18–0.51) and 0.66 among statin users with the T/T genotype (95%CI: 0.41–1.06; $P = 0.0012$)[71].

Mesenchymal cell markers

Several studies have demonstrated that tumor cells with higher vimentin expression (mesenchymal cell marker) and lower E-cadherin expression (epithelial cell marker) are highly sensitive to statin treatment[72-74]. Total vimentin and E-cadherin expression are not appropriate markers for the sensitivity of statins, but abundant cytosolic vimentin and absent cell surface E-cadherin expression indicate sensitivity to statins[73]. HRAS-induced epithelial-to-mesenchymal transition (EMT) through activation of zinc finger E-box binding homeobox 1 sensitized tumor cells to the antiproliferative activity of statins[74]. These studies also showed that statins preferentially kill cells induced to undergo EMT, suggesting that statins may be more effective against metastatic disease and prevent metastasis.

p53

Wild-type p53 represses the MVA pathway[12], while loss of *TP53* and two gain-of-function *TP53* mutants have been reported to enhance the expression of MVA pathway genes[11,75]. Tumors with loss of *TP53* or the two gain-of-function mutations are particularly vulnerable to statin treatment[76-78].

RAS mutations

The FPP and geranylgeranyl pyrophosphate produced by the MVA pathway serve as substrates for the post-translational prenylation of RAS. Therefore, RAS mutations have been hypothesized to be potential biomarkers of statin sensitivity. However, preclinical studies have shown that RAS mutation alone cannot predict statin susceptibility[74,79]. In a subgroup analysis of retrospective studies of CRC, statins were shown to have a higher prognostic effect in cancers with *KRAS* mutations[80].

However, other studies have reported no association between statin effects on CRC and *KRAS* status[39,81]. Further studies are needed to evaluate the utility of *KRAS* mutation status to predict the effect of statins on cancer.

ER

In breast cancer, the effect of statins has been linked with ER status, in which ER-negative breast cancer cells are notably sensitive to statin treatment[67]. These pre-clinical findings are further strengthened by clinical data demonstrating greater tumor cell apoptosis after fluvastatin treatment in women with ER-negative breast cancer[82].

COMBINATION OF STATINS WITH EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS

Several clinical trials have examined the introduction of simvastatin to epidermal growth factor receptor (EGFR) inhibitors therapy for *KRAS*-mutated CRC patients. The hypothesis behind these clinical trials is that the statin-induced depletion of MVA will inhibit *KRAS* prenylation, which will inhibit membrane localization and enhance the effectiveness of EGFR inhibitors[83,84]. Unfortunately, most trials have failed to show significant survival benefits from statins[85-87]. These results may suggest that *KRAS* mutation status is not a predictive biomarker of response to statin treatment. Another clinical trial showed that the addition of simvastatin to a cetuximab/irinotecan regimen overcame cetuximab resistance[88]. In this clinical trial, the therapeutic benefit of statin was only detectable in patients bearing tumors with mutant *KRAS* and a low Ras signature[88]. The Ras signature score is derived from the expression of Ras pathway-related genes across multiple databases and reflects other possible aberrations such as *BRAF* and *PI3KCA* mutations. Hence, factors other than *KRAS* mutation must be considered to predict the efficiency of statins in overcoming resistance to anti-EGFR therapy.

COMBINATION OF STATINS WITH RADIATION THERAPY

Statins may have synergistic effects with radiation therapy (RT) on cancer and may reduce inflammation and the gut and skin toxicities induced by RT. In retrospective cohort studies, patients taking statins during RT or chemo-RT for rectal, bladder, or prostate cancer treatment showed considerably higher rates of pathological complete response, local control and progression-free survival[89-93]. However, no study has shown an apparent benefit[94]. Furthermore, statins significantly reduced RT-induced bowel toxicity and skin injury[95-97]. However, a single-arm phase II trial of 53 prostate cancer patients taking lovastatin showed no reduced incidence of grade 2 or higher rectal toxicity compared with historical controls[98]. A RCT of simvastatin combined with standard chemotherapy and radiation in preoperative treatment for rectal cancer is underway.

COMBINATION OF STATINS WITH IMMUNOTHERAPY

Mevalonic acid metabolism is involved in controlling T cell activation[19,20,99,100]. Statins inhibit the geranylgeranylation of small GTPases, resulting in arrested endosomal maturation, prolonged antigen retention, enhanced antigen presentation, and T cell activation. It has been reported in multiple mouse cancer models that MVA pathway inhibitors are vigorous for cancer vaccinations and synergize with anti-PD-1 antibodies[101]. The tumor microenvironment is enriched with cholesterol. The high cholesterol in the tumor microenvironment induces CD8+ T cell exhaustion and upregulates the immune checkpoints PD-1, 2B4, TIM-3, and LAG-3[102]. Furthermore, lowering cholesterol levels in the tumor microenvironment by simvastatin restores the antitumor activity of CD8+ T cells. Many preclinical studies have demonstrated that the MVA pathway is involved in immune regulation. Future research into the immunomodulatory properties of statins has important clinical implications for cancer immunotherapy.

CONCLUSION

Clinical data that evaluated the utility of statins as anticancer agents have shown responses in some but not all cancers. Optimizing the type, dose, and duration of statins, as well as detecting biomarkers to recognize responders and developing combination therapies, will heighten the value of statins in cancer treatment.

REFERENCES

1 **Goldstein JL**, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990; **343**: 425-430 [PMID: 1967820 DOI: 10.1038/343425a0]

2 **Sinensky M**. Recent advances in the study of prenylated proteins. *Biochim Biophys Acta* 2000; **1484**: 93-106 [PMID: 10760460 DOI: 10.1016/s1388-1981(00)00009-3]

3 **Duncan RE**, El-Sohemy A, Archer MC. Mevalonate promotes the growth of tumors derived from human cancer cells *in vivo* and stimulates proliferation *in vitro* with enhanced cyclin-dependent kinase-2 activity. *J Biol Chem* 2004; **279**: 33079-33084 [PMID: 15155733 DOI: 10.1074/jbc.M400732200]

4 **Mullen PJ**, Yu R, Longo J, Archer MC, Penn LZ. The interplay between cell signalling and the mevalonate pathway in cancer. *Nat Rev Cancer* 2016; **16**: 718-731 [PMID: 27562463 DOI: 10.1038/nrc.2016.76]

5 **Carrer A**, Trefely S, Zhao S, Campbell SL, Norgard RJ, Schultz KC, Sidoli S, Parris JLD, Affronti HC, Sivanand S, Egolf S, Sela Y, Trizzino M, Gardini A, Garcia BA, Snyder NW, Stanger BZ, Wellen KE. Acetyl-CoA Metabolism Supports Multistep Pancreatic Tumorigenesis. *Cancer Discov* 2019; **9**: 416-435 [PMID: 30626590 DOI: 10.1158/2159-8290.CD-18-0567]

6 **Nielsen SF**, Nordestgaard BG, Bojesen SE. Statin use and reduced cancer-related mortality. *N Engl J Med* 2012; **367**: 1792-1802 [PMID: 23134381 DOI: 10.1056/NEJMoa1201735]

7 **Abdullah MI**, de Wolf E, Jawad MJ, Richardson A. The poor design of clinical trials of statins in oncology may explain their failure - Lessons for drug repurposing. *Cancer Treat Rev* 2018; **69**: 84-89 [PMID: 29936313 DOI: 10.1016/j.ctrv.2018.06.010]

8 **Yamauchi Y**, Furukawa K, Hamamura K. Positive feedback loop between PI3K-Akt-mTORC1 signaling and the lipogenic pathway boosts Akt signaling: induction of the lipogenic pathway by a melanoma antigen. *Cancer Res* 2011; **71**: 4989-4997 [PMID: 21632551 DOI: 10.1158/0008-5472.CAN-10-4108]

9 **Yi J**, Zhu J, Wu J, Thompson CB, Jiang X. Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis *via* SREBP-mediated lipogenesis. *Proc Natl Acad Sci U S A* 2020; **117**: 31189-31197 [PMID: 33229547 DOI: 10.1073/pnas.2017152117]

10 **Kusama T**, Mukai M, Iwasaki T, Tatsuta M, Matsumoto Y, Akedo H, Inoue M, Nakamura H. 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. *Gastroenterology* 2002; **122**: 308-317 [PMID: 11832446 DOI: 10.1053/gast.2002.31093]

11 **Freed-Pastor WA**, Mizuno H, Zhao X, Langerød A, Moon SH, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A, Bissell MJ, Osborne TF, Tian B, Lowe SW, Silva JM, Børresen-Dale AL, Levine AJ, Bargonetti J, Prives C. Mutant p53 disrupts mammary tissue architecture *via* the mevalonate pathway. *Cell* 2012; **148**: 244-258 [PMID: 22265415 DOI: 10.1016/j.cell.2011.12.017]

12 **Assaily W**, Rubinger DA, Wheaton K, Lin Y, Ma W, Xuan W, Brown-Endres L, Tsuchihara K, Mak TW, Benchimol S. ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. *Mol Cell* 2011; **44**: 491-501 [PMID: 22055193 DOI: 10.1016/j.molcel.2011.08.038]

13 **Shamma A**, Takegami Y, Miki T, Kitajima S, Noda M, Obara T, Okamoto T, Takahashi C. Rb Regulates DNA damage response and cellular senescence through E2F-dependent suppression of N-ras isoprenylation. *Cancer Cell* 2009; **15**: 255-269 [PMID: 19345325 DOI: 10.1016/j.ccr.2009.03.001]

14 **Sorrentino G**, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, Manfrin A, Ingallina E, Sommaggio R, Piazza S, Rosato A, Piccolo S, Del Sal G. Metabolic control of YAP and TAZ by the mevalonate pathway. *Nat Cell Biol* 2014; **16**: 357-366 [PMID: 24658687 DOI: 10.1038/ncb2936]

15 **Higashi T**, Hayashi H, Kitano Y, Yamamura K, Kaida T, Arima K, Taki K, Nakagawa S, Okabe H, Nitta H, Imai K, Hashimoto D, Chikamoto A, Beppu T, Baba H. Statin attenuates cell proliferative ability *via* TAZ (WWTR1) in hepatocellular carcinoma. *Med Oncol* 2016; **33**: 123 [PMID: 27734263 DOI: 10.1007/s12032-016-0845-6]

16 **Corcoran RB**, Scott MP. Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc Natl Acad Sci U S A* 2006; **103**: 8408-8413 [PMID: 16707575 DOI: 10.1073/pnas.0602852103]

17 **Nguyen VT**, Barozzi I, Faronato M, Lombardo Y, Steel JH, Patel N, Darbre P, Castellano L, Győrffy B, Woodley L, Meira A, Patten DK, Vircillo V, Periyasamy M, Ali S, Frige G, Minucci S, Coombes RC, Magnani L. Differential epigenetic reprogramming in response to specific endocrine therapies promotes cholesterol biosynthesis and cellular invasion. *Nat Commun* 2015; **6**: 10044 [PMID: 26610607 DOI: 10.1038/ncomms10044]

18 **Huang WC**, Li X, Liu J, Lin J, Chung LW. Activation of androgen receptor, lipogenesis, and oxidative stress converged by SREBP-1 is responsible for regulating growth and progression of prostate cancer cells. *Mol Cancer Res* 2012; **10**: 133-142 [PMID: 22064655 DOI: 10.1158/1541-7786.MCR-11-0206]

19 **Thurnher M**, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. *Sci Signal* 2015; **8**: re4 [PMID: 25829448 DOI: 10.1126/scisignal.2005970]

20 **MacIver NJ**, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* 2013; **31**: 259-283 [PMID: 23298210 DOI: 10.1146/annurev-immunol-032712-095956]

21 **Zhou C**, Zhong X, Gao P, Wu Z, Shi J, Guo Z, Wang Z, Song Y. Statin use and its potential therapeutic role in esophageal cancer: a systematic review and meta-analysis. *Cancer Manag Res* 2019; **11**: 5655-5663 [PMID: 31417309 DOI: 10.2147/CMAR.S193945]

22 **Nguyen T**, Khan A, Liu Y, El-Serag HB, Thrift AP. The Association Between Statin Use After Diagnosis and Mortality Risk in Patients With Esophageal Cancer: A Retrospective Cohort Study of United States Veterans. *Am J Gastroenterol* 2018; **113**: 1310 [PMID: 29946180 DOI: 10.1038/s41395-018-0169-6]

23 **Nimako GK**, Wintrob ZA, Sulik DA, Donato JL, Ceacareanu AC. Synergistic Benefit of Statin and Metformin in Gastrointestinal Malignancies. *J Pharm Pract* 2017; **30**: 185-194 [PMID: 26811340 DOI: 10.1177/0897190015627255]

24 **Cardwell CR**, Spence AD, Hughes CM, Murray LJ. Statin use after esophageal cancer diagnosis and survival: A population based cohort study. *Cancer Epidemiol* 2017; **48**: 124-130 [PMID: 28486205 DOI: 10.1016/j.canep.2017.04.015]

25 **Alexandre L**, Clark AB, Bhutta HY, Chan SS, Lewis MP, Hart AR. Association Between Statin Use After Diagnosis of Esophageal Cancer and Survival: A Population-Based Cohort Study. *Gastroenterology* 2016; **150**: 854-865.e1; quiz e16 [PMID: 26775632 DOI: 10.1053/j.gastro.2015.12.039]

26 **Lacroix O**, Couttenier A, Vaes E, Cardwell CR, De Schutter H, Robert A. Statin use after diagnosis is associated with an increased survival in esophageal cancer patients: a Belgian population-based study. *Cancer Causes Control* 2019; **30**: 385-393 [PMID: 30820714 DOI: 10.1007/s10552-019-01149-3]

27 **Deng HY**, Lan X, Zheng X, Zha P, Zhou J, Wang RL, Jiang R, Qiu XM. The association between statin use and survival of esophageal cancer patients: A systematic review and meta-analysis. *Medicine (Baltimore)* 2019; **98**: e16480 [PMID: 31335710 DOI: 10.1097/MD.0000000000016480]

28 **Thomas T**, Loke Y, Beales ILP. Systematic Review and Meta-analysis: Use of Statins Is Associated with a Reduced Incidence of Oesophageal Adenocarcinoma. *J Gastrointest Cancer* 2018; **49**: 442-454 [PMID: 28691139 DOI: 10.1007/s12029-017-9983-0]

29 **Kim ST**, Kang JH, Lee J, Park SH, Park JO, Park YS, Lim HY, Hwang IG, Lee SC, Park KW, Lee HR, Kang WK. Simvastatin plus capecitabine-cisplatin *vs* placebo plus capecitabine-cisplatin in patients with previously untreated advanced gastric cancer: a double-blind randomised phase 3 study. *Eur J Cancer* 2014; **50**: 2822-2830 [PMID: 25218337 DOI: 10.1016/j.ejca.2014.08.005]

30 **Konings IR**, van der Gaast A, van der Wijk LJ, de Jongh FE, Eskens FA, Sleijfer S. The addition of pravastatin to chemotherapy in advanced gastric carcinoma: a randomised phase II trial. *Eur J Cancer* 2010; **46**: 3200-3204 [PMID: 20727735 DOI: 10.1016/j.ejca.2010.07.036]

31 **Nam DH**, Lee H, Park JC, Shin SK, Lee SK, Hyung WJ, Lee YC, Kang MW, Noh SH. Long-term statin therapy improves oncological outcome after radical gastrectomy for stage II and III gastric cancer. *Anticancer Res* 2014; **34**: 355-361 [PMID: 24403487]

32 **Spence AD**, Busby J, Hughes CM, Johnston BT, Coleman HG, Cardwell CR. Statin use and survival in patients with gastric cancer in two independent population-based cohorts. *Pharmacoepidemiol Drug Saf* 2019; **28**: 460-470 [PMID: 30456916 DOI: 10.1002/pds.4688]

33 **Singh PP**, Singh S. Statins are associated with reduced risk of gastric cancer: a systematic review and meta-analysis. *Ann Oncol* 2013; **24**: 1721-1730 [PMID: 23599253 DOI: 10.1093/annonc/mdt150]

34 **Wu XD**, Zeng K, Xue FQ, Chen JH, Chen YQ. Statins are associated with reduced risk of gastric cancer: a meta-analysis. *Eur J Clin Pharmacol* 2013; **69**: 1855-1860 [PMID: 23748751 DOI: 10.1007/s00228-013-1547-z]

35 **Cheung KS**, Chan EW, Wong AYS, Chen L, Seto WK, Wong ICK, Leung WK. Statins Were Associated with a Reduced Gastric Cancer Risk in Patients with Eradicated *Helicobacter Pylori* Infection: A Territory-Wide Propensity Score Matched Study. *Cancer Epidemiol Biomarkers Prev* 2020; **29**: 493-499 [PMID: 31792089 DOI: 10.1158/1055-9965.EPI-19-1044]

36 **Poynter JN**, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, Low M, Greenson JK, Rennert G. Statins and the risk of colorectal cancer. *N Engl J Med* 2005; **352**: 2184-2192 [PMID: 15917383 DOI: 10.1056/NEJMoa043792]

37 **Farwell WR**, Scranton RE, Lawler EV, Lew RA, Brophy MT, Fiore LD, Gaziano JM. The association between statins and cancer incidence in a veterans population. *J Natl Cancer Inst* 2008; **100**: 134-139 [PMID: 18182618 DOI: 10.1093/jnci/djm286]

38 **Taylor ML**, Wells BJ, Smolak MJ. Statins and cancer: a meta-analysis of case-control studies. *Eur J Cancer Prev* 2008; **17**: 259-268 [PMID: 18414198 DOI: 10.1097/CEJ.0b013e3282b721fe]

39 **Li Y**, He X, Ding Y, Chen H, Sun L. Statin uses and mortality in colorectal cancer patients: An updated systematic review and meta-analysis. *Cancer Med* 2019; **8**: 3305-3313 [PMID: 31069997 DOI: 10.1002/cam4.2151]

40 **Graaf MR**, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. *J Clin Oncol* 2004; **22**: 2388-2394 [PMID: 15197200 DOI: 10.1200/JCO.2004.02.027]

41 **Dale KM**, Coleman CI, Henyan NN, Kluger J, White CM. Statins and cancer risk: a meta-analysis. *JAMA* 2006; **295**: 74-80 [PMID: 16391219 DOI: 10.1001/jama.295.1.74]

42 **Liu Y**, Tang W, Wang J, Xie L, Li T, He Y, Deng Y, Peng Q, Li S, Qin X. Association between statin use and colorectal cancer risk: a meta-analysis of 42 studies. *Cancer Causes Control* 2014; **25**: 237-249 [PMID: 24265089 DOI: 10.1007/s10552-013-0326-6]

43 **Lee JE**, Baba Y, Ng K, Giovannucci E, Fuchs CS, Ogino S, Chan AT. Statin use and colorectal cancer risk according to molecular subtypes in two large prospective cohort studies. *Cancer Prev Res (Phila)* 2011; **4**: 1808-1815 [PMID: 21680706 DOI: 10.1158/1940-6207.CAPR-11-0113]

44 **Ibáñez-Sanz G**, Guinó E, Pontes C, Quijada-Manuitt MÁ, de la Peña-Negro LC, Aragón M, Domínguez M, Rodríguez-Alonso L, Blasco A, García-Rodríguez A, Morros R, Moreno V. Statin use and the risk of colorectal cancer in a population-based electronic health records study. *Sci Rep* 2019; **9**: 13560 [PMID: 31537841 DOI: 10.1038/s41598-019-49877-5]

45 **Kim G**, Jang SY, Han E, Lee YH, Park SY, Nam CM, Kang ES. Effect of statin on hepatocellular carcinoma in patients with type 2 diabetes: A nationwide nested case-control study. *Int J Cancer* 2017; **140**: 798-806 [PMID: 27861855 DOI: 10.1002/ijc.30506]

46 **Tsan YT**, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. *J Clin Oncol* 2013; **31**: 1514-1521 [PMID: 23509319 DOI: 10.1200/JCO.2012.44.6831]

47 **Tsan YT**, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. *J Clin Oncol* 2012; **30**: 623-630 [PMID: 22271485 DOI: 10.1200/JCO.2011.36.0917]

48 **Simon TG**, Bonilla H, Yan P, Chung RT, Butt AA. Atorvastatin and fluvastatin are associated with dose-dependent reductions in cirrhosis and hepatocellular carcinoma, among patients with hepatitis C virus: Results from ERCHIVES. *Hepatology* 2016; **64**: 47-57 [PMID: 26891205 DOI: 10.1002/hep.28506]

49 **Simon TG**, King LY, Zheng H, Chung RT. Statin use is associated with a reduced risk of fibrosis progression in chronic hepatitis C. *J Hepatol* 2015; **62**: 18-23 [PMID: 25135867 DOI: 10.1016/j.jhep.2014.08.013]

50 **Chang FM**, Wang YP, Lang HC, Tsai CF, Hou MC, Lee FY, Lu CL. Statins decrease the risk of decompensation in hepatitis B virus- and hepatitis C virus-related cirrhosis: A population-based study. *Hepatology* 2017; **66**: 896-907 [PMID: 28318053 DOI: 10.1002/hep.29172]

51 **Zhang Y**, Liang M, Sun C, Qu G, Shi T, Min M, Wu Y, Sun Y. Statin Use and Risk of Pancreatic Cancer: An Updated Meta-analysis of 26 Studies. *Pancreas* 2019; **48**: 142-150 [PMID: 30640225 DOI: 10.1097/MPA.0000000000001226]

52 **Bonovas S**, Filioussi K, Sitaras NM. Statins are not associated with a reduced risk of pancreatic cancer at the population level, when taken at low doses for managing hypercholesterolemia: evidence from a meta-analysis of 12 studies. *Am J Gastroenterol* 2008; **103**: 2646-2651 [PMID: 18684187 DOI: 10.1111/j.1572-0241.2008.02051.x]

53 **Walker EJ**, Ko AH, Holly EA, Bracci PM. Statin use and risk of pancreatic cancer: results from a large, clinic-based case-control study. *Cancer* 2015; **121**: 1287-1294 [PMID: 25649483 DOI: 10.1002/cncr.29256]

54 **Archibugi L**, Arcidiacono PG, Capurso G. Statin use is associated to a reduced risk of pancreatic cancer: A meta-analysis. *Dig Liver Dis* 2019; **51**: 28-37 [PMID: 30314951 DOI: 10.1016/j.dld.2018.09.007]

55 **Kirkegård J**, Lund JL, Mortensen FV, Cronin-Fenton D. Statins and pancreatic cancer risk in patients with chronic pancreatitis: A Danish nationwide population-based cohort study. *Int J Cancer* 2020; **146**: 610-616 [PMID: 30861115 DOI: 10.1002/ijc.32264]

56 **Huang BZ**, Chang JI, Li E, Xiang AH, Wu BU. Influence of Statins and Cholesterol on Mortality Among Patients With Pancreatic Cancer. *J Natl Cancer Inst* 2017; **109** [PMID: 28040693 DOI: 10.1093/jnci/djw275]

57 **Lee HS**, Lee SH, Lee HJ, Chung MJ, Park JY, Park SW, Song SY, Bang S. Statin Use and Its Impact on Survival in Pancreatic Cancer Patients. *Medicine (Baltimore)* 2016; **95**: e3607 [PMID: 27175667 DOI: 10.1097/MD.0000000000003607]

58 **Wu BU**, Chang J, Jeon CY, Pandol SJ, Huang B, Ngor EW, Difronzo AL, Cooper RM. Impact of statin use on survival in patients undergoing resection for early-stage pancreatic cancer. *Am J Gastroenterol* 2015; **110**: 1233-1239 [PMID: 26195180 DOI: 10.1038/ajg.2015.217]

59 **Jouve JL**, Lecomte T, Bouché O, Barbier E, Khemissa Akouz F, Riachi G, Nguyen Khac E, Ollivier-Hourmand I, Debette-Gratien M, Faroux R, Villing AL, Vergniol J, Ramee JF, Bronowicki JP, Seitz JF, Legoux JL, Denis J, Manfredi S, Phelip JM; PRODIGE-11 investigators/collaborators. Pravastatin combination with sorafenib does not improve survival in advanced hepatocellular carcinoma. *J Hepatol* 2019; **71**: 516-522 [PMID: 31125576 DOI: 10.1016/j.jhep.2019.04.021]

60 **Kawata S**, Yamasaki E, Nagase T, Inui Y, Ito N, Matsuda Y, Inada M, Tamura S, Noda S, Imai Y, Matsuzawa Y. Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. *Br J Cancer* 2001; **84**: 886-891 [PMID: 11286466 DOI: 10.1054/bjoc.2000.1716]

61 **Hong JY**, Nam EM, Lee J, Park JO, Lee SC, Song SY, Choi SH, Heo JS, Park SH, Lim HY, Kang WK, Park YS. Randomized double-blinded, placebo-controlled phase II trial of simvastatin and

gemcitabine in advanced pancreatic cancer patients. *Cancer Chemother Pharmacol* 2014; **73**: 125-130 [PMID: 24162380 DOI: 10.1007/s00280-013-2328-1]

62 **Gbelcová H**, Lenícek M, Zelenka J, Knejzlík Z, Dvoráková G, Zadinová M, Poucková P, Kudla M, Balaz P, Ruml T, Vítek L. Differences in antitumor effects of various statins on human pancreatic cancer. *Int J Cancer* 2008; **122**: 1214-1221 [PMID: 18027870 DOI: 10.1002/ijc.23242]

63 **Wu SY**, Fang SC, Shih HJ, Wen YC, Shao YJ. Mortality associated with statins in men with advanced prostate cancer treated with androgen deprivation therapy. *Eur J Cancer* 2019; **112**: 109-117 [PMID: 30827745 DOI: 10.1016/j.ejca.2018.11.032]

64 **Clendening JW**, Pandyra A, Li Z, Boutros PC, Martirosyan A, Lehner R, Jurisica I, Trudel S, Penn LZ. Exploiting the mevalonate pathway to distinguish statin-sensitive multiple myeloma. *Blood* 2010; **115**: 4787-4797 [PMID: 20360469 DOI: 10.1182/blood-2009-07-230508]

65 **Longo J**, Mullen PJ, Yu R, van Leeuwen JE, Masoomian M, Woon DTS, Wang Y, Chen EX, Hamilton RJ, Sweet JM, van der Kwast TH, Fleshner NE, Penn LZ. An actionable sterol-regulated feedback loop modulates statin sensitivity in prostate cancer. *Mol Metab* 2019; **25**: 119-130 [PMID: 31023626 DOI: 10.1016/j.molmet.2019.04.003]

66 **Chang WC**, Cheng WC, Cheng BH, Chen L, Ju LJ, Ou YJ, Jeng LB, Yang MD, Hung YC, Ma WL. Mitochondrial Acetyl-CoA Synthetase 3 is Biosignature of Gastric Cancer Progression. *Cancer Med* 2018; **7**: 1240-1252 [PMID: 29493120 DOI: 10.1002/cam4.1295]

67 **Goard CA**, Chan-Seng-Yue M, Mullen PJ, Quiroga AD, Wasylishen AR, Clendening JW, Sendorek DH, Haider S, Lehner R, Boutros PC, Penn LZ. Identifying molecular features that distinguish fluvastatin-sensitive breast tumor cells. *Breast Cancer Res Treat* 2014; **143**: 301-312 [PMID: 24337703 DOI: 10.1007/s10549-013-2800-y]

68 **Kimbung S**, Lettiero B, Feldt M, Bosch A, Borgquist S. High expression of cholesterol biosynthesis genes is associated with resistance to statin treatment and inferior survival in breast cancer. *Oncotarget* 2016; **7**: 59640-59651 [PMID: 27458152 DOI: 10.18632/oncotarget.10746]

69 **Bjarnadottir O**, Romero Q, Bendahl PO, Jirström K, Rydén L, Loman N, Uhlén M, Johannesson H, Rose C, Grabau D, Borgquist S. Targeting HMG-CoA reductase with statins in a window-of-opportunity breast cancer trial. *Breast Cancer Res Treat* 2013; **138**: 499-508 [PMID: 23471651 DOI: 10.1007/s10549-013-2473-6]

70 **Clendening JW**, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, Martirosyan A, Hakem A, Hakem R, Jurisica I, Penn LZ. Dysregulation of the mevalonate pathway promotes transformation. *Proc Natl Acad Sci U S A* 2010; **107**: 15051-15056 [PMID: 20696928 DOI: 10.1073/pnas.0910258107]

71 **Lipkin SM**, Chao EC, Moreno V, Rozek LS, Rennert H, Pinchev M, Dizon D, Rennert G, Kopelovich L, Gruber SB. Genetic variation in 3-hydroxy-3-methylglutaryl CoA reductase modifies the chemopreventive activity of statins for colorectal cancer. *Cancer Prev Res (Phila)* 2010; **3**: 597-603 [PMID: 20403997 DOI: 10.1158/1940-6207.CAPR-10-0007]

72 **Viswanathan VS**, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, Kaffenberger SD, Eaton JK, Shimada K, Aguirre AJ, Viswanathan SR, Chattopadhyay S, Tamayo P, Yang WS, Rees MG, Chen S, Boskovic ZV, Javaid S, Huang C, Wu X, Tseng YY, Roider EM, Gao D, Cleary JM, Wolpin BM, Mesirov JP, Haber DA, Engelman JA, Boehm JS, Kotz JD, Hon CS, Chen Y, Hahn WC, Levesque MP, Doench JG, Berens ME, Shamji AF, Clemons PA, Stockwell BR, Schreiber SL. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* 2017; **547**: 453-457 [PMID: 28678785 DOI: 10.1038/nature23007]

73 **Warita K**, Warita T, Beckwitt CH, Schurdak ME, Vazquez A, Wells A, Oltvai ZN. Statin-induced mevalonate pathway inhibition attenuates the growth of mesenchymal-like cancer cells that lack functional E-cadherin mediated cell cohesion. *Sci Rep* 2014; **4**: 7593 [PMID: 25534349 DOI: 10.1038/srep07593]

74 **Yu R**, Longo J, van Leeuwen JE, Mullen PJ, Ba-Alawi W, Haibe-Kains B, Penn LZ. Statin-Induced Cancer Cell Death Can Be Mechanistically Uncoupled from Prenylation of RAS Family Proteins. *Cancer Res* 2018; **78**: 1347-1357 [PMID: 29229608 DOI: 10.1158/0008-5472.CAN-17-1231]

75 **Moon SH**, Huang CH, Houlihan SL, Regunath K, Freed-Pastor WA, Morris JP 4th, Tschaharganeh DF, Kastenhuber ER, Barsotti AM, Culp-Hill R, Xue W, Ho YJ, Baslan T, Li X, Mayle A, de Stanchina E, Zender L, Tong DR, D'Alessandro A, Lowe SW, Prives C. p53 Represses the Mevalonate Pathway to Mediate Tumor Suppression. *Cell* 2019; **176**: 564-580.e19 [PMID: 30580964 DOI: 10.1016/j.cell.2018.11.011]

76 **Turrell FK**, Kerr EM, Gao M, Thorpe H, Doherty GJ, Cridge J, Shorthouse D, Speed A, Samarajiwa S, Hall BA, Griffiths M, Martins CP. Lung tumors with distinct p53 mutations respond similarly to p53 targeted therapy but exhibit genotype-specific statin sensitivity. *Genes Dev* 2017; **31**: 1339-1353 [PMID: 28790158 DOI: 10.1101/gad.298463.117]

77 **Parrales A**, Ranjan A, Iyer SV, Padhye S, Weir SJ, Roy A, Iwakuma T. DNAJA1 controls the fate of misfolded mutant p53 through the mevalonate pathway. *Nat Cell Biol* 2016; **18**: 1233-1243 [PMID: 27775703 DOI: 10.1038/ncb3427]

78 **Tutuska K**, Parrilla-Monge L, Di Cesare E, Nemajerova A, Moll UM. Statin as anti-cancer therapy in autochthonous T-lymphomas expressing stabilized gain-of-function mutant p53 proteins. *Cell Death Dis* 2020; **11**: 274 [PMID: 32332697 DOI: 10.1038/s41419-020-2466-4]

79 **Wong WW**, Clendening JW, Martirosyan A, Boutros PC, Bros C, Khosravi F, Jurisica I, Stewart AK, Bergsagel PL, Penn LZ. Determinants of sensitivity to lovastatin-induced apoptosis in multiple myeloma. *Mol Cancer Ther* 2007; **6**: 1886-1897 [PMID: 17575117 DOI:

10.1158/1535-7163.MCT-06-0745]
80 **Ling Y**, Yang L, Huang H, Hu X, Zhao C, Ying Y. Prognostic Significance of Statin Use in Colorectal Cancer: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)* 2015; **94**: e908 [PMID: 26107680 DOI: 10.1097/MD.0000000000000908]
81 **Voorneveld PW**, Reimers MS, Bastiaannet E, Jacobs RJ, van Eijk R, Zanders MMJ, Herings RMC, van Herk-Sukel MPP, Kodach LL, van Wezel T, Kuppen PJK, Morreau H, van de Velde CJH, Hardwick JCH, Liefers GJ. Statin Use After Diagnosis of Colon Cancer and Patient Survival. *Gastroenterology* 2017; **153**: 470-479.e4 [PMID: 28512021 DOI: 10.1053/j.gastro.2017.05.011]
82 **Garwood ER**, Kumar AS, Baehner FL, Moore DH, Au A, Hylton N, Flowers CI, Garber J, Lesnikoski BA, Hwang ES, Olopade O, Port ER, Campbell M, Esserman LJ. Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. *Breast Cancer Res Treat* 2010; **119**: 137-144 [PMID: 19728082 DOI: 10.1007/s10549-009-0507-x]
83 **Shimoyama S**. Statins are logical candidates for overcoming limitations of targeting therapies on malignancy: their potential application to gastrointestinal cancers. *Cancer Chemother Pharmacol* 2011; **67**: 729-739 [PMID: 21327931 DOI: 10.1007/s00280-011-1583-2]
84 **Krens LL**, Baas JM, Gelderblom H, Guchelaar HJ. Therapeutic modulation of k-ras signaling in colorectal cancer. *Drug Discov Today* 2010; **15**: 502-516 [PMID: 20594936 DOI: 10.1016/j.drudis.2010.05.012]
85 **Baas JM**, Krens LL, ten Tije AJ, Erdkamp F, van Wezel T, Morreau H, Gelderblom H, Guchelaar HJ. Safety and efficacy of the addition of simvastatin to cetuximab in previously treated KRAS mutant metastatic colorectal cancer patients. *Invest New Drugs* 2015; **33**: 1242-1247 [PMID: 26386973 DOI: 10.1007/s10637-015-0285-8]
86 **Baas JM**, Krens LL, Bos MM, Portielje JE, Batman E, van Wezel T, Morreau H, Guchelaar HJ, Gelderblom H. Safety and efficacy of the addition of simvastatin to panitumumab in previously treated KRAS mutant metastatic colorectal cancer patients. *Anticancer Drugs* 2015; **26**: 872-877 [PMID: 26053280 DOI: 10.1097/CAD.0000000000000255]
87 **Krens LL**, Simkens LH, Baas JM, Koomen ER, Gelderblom H, Punt CJ, Guchelaar HJ. Statin use is not associated with improved progression free survival in cetuximab treated KRAS mutant metastatic colorectal cancer patients: results from the CAIRO2 study. *PLoS One* 2014; **9**: e112201 [PMID: 25375154 DOI: 10.1371/journal.pone.0112201]
88 **Lee J**, Hong YS, Hong JY, Han SW, Kim TW, Kang HJ, Kim TY, Kim KP, Kim SH, Do IG, Kim KM, Sohn I, Park SH, Park JO, Lim HY, Cho YB, Lee WY, Yun SH, Kim HC, Park YS, Kang WK. Effect of simvastatin plus cetuximab/irinotecan for KRAS mutant colorectal cancer and predictive value of the RAS signature for treatment response to cetuximab. *Invest New Drugs* 2014; **32**: 535-541 [PMID: 24468885 DOI: 10.1007/s10637-014-0065-x]
89 **Tsai HK**, Katz MS, Coen JJ, Zietman AL, Kaufman DS, Shipley WU. Association of statin use with improved local control in patients treated with selective bladder preservation for muscle-invasive bladder cancer. *Urology* 2006; **68**: 1188-1192 [PMID: 17141846 DOI: 10.1016/j.urology.2006.08.1078]
90 **Mace AG**, Gantt GA, Skacel M, Pai R, Hammel JP, Kalady MF. Statin therapy is associated with improved pathologic response to neoadjuvant chemoradiation in rectal cancer. *Dis Colon Rectum* 2013; **56**: 1217-1227 [PMID: 24104995 DOI: 10.1097/DCR.0b013e3182a4b236]
91 **Armstrong D**, Raissouni S, Price Hiller J, Mercer J, Powell E, MacLean A, Jiang M, Doll C, Goodwin R, Batuyong E, Zhou K, Monzon JG, Tang PA, Heng DY, Cheung WY, Vickers MM. Predictors of Pathologic Complete Response After Neoadjuvant Treatment for Rectal Cancer: A Multicenter Study. *Clin Colorectal Cancer* 2015; **14**: 291-295 [PMID: 26433487 DOI: 10.1016/j.clcc.2015.06.001]
92 **Katz MS**, Minsky BD, Saltz LB, Riedel E, Chessin DB, Guillem JG. Association of statin use with a pathologic complete response to neoadjuvant chemoradiation for rectal cancer. *Int J Radiat Oncol Biol Phys* 2005; **62**: 1363-1370 [PMID: 16029794 DOI: 10.1016/j.ijrobp.2004.12.033]
93 **Kollmeier MA**, Katz MS, Mak K, Yamada Y, Feder DJ, Zhang Z, Jia X, Shi W, Zelefsky MJ. Improved biochemical outcomes with statin use in patients with high-risk localized prostate cancer treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2011; **79**: 713-718 [PMID: 20452139 DOI: 10.1016/j.ijrobp.2009.12.006]
94 **Hardie C**, Jung Y, Jameson M. Effect of statin and aspirin use on toxicity and pathological complete response rate of neo-adjuvant chemoradiation for rectal cancer. *Asia Pac J Clin Oncol* 2016; **12**: 167-173 [PMID: 26947944 DOI: 10.1111/ajco.12468]
95 **Nübel T**, Damrot J, Roos WP, Kaina B, Fritz G. Lovastatin protects human endothelial cells from killing by ionizing radiation without impairing induction and repair of DNA double-strand breaks. *Clin Cancer Res* 2006; **12**: 933-939 [PMID: 16467108 DOI: 10.1158/1078-0432.CCR-05-1903]
96 **Holler V**, Buard V, Gaugler MH, Guipaud O, Baudelin C, Sache A, Perez Mdel R, Squiban C, Tamarat R, Milliat F, Benderitter M. Pravastatin limits radiation-induced vascular dysfunction in the skin. *J Invest Dermatol* 2009; **129**: 1280-1291 [PMID: 19212344 DOI: 10.1038/jid.2008.360]
97 **Teo MT**, Sebag-Montefiore D, Donnellan CF. Prevention and Management of Radiation-induced Late Gastrointestinal Toxicity. *Clin Oncol (R Coll Radiol)* 2015; **27**: 656-667 [PMID: 26129746 DOI: 10.1016/j.clon.2015.06.010]
98 **Jameson MB**, Gormly K, Espinoza D, Hague W, Asghari G, Jeffery GM, Price TJ, Karapetis CS, Arendse M, Armstrong J, Childs J, Frizelle FA, Ngan S, Stevenson A, Oostendorp M, Ackland SP. SPAR - a randomised, placebo-controlled phase II trial of simvastatin in addition to standard

chemotherapy and radiation in preoperative treatment for rectal cancer: an AGITG clinical trial. *BMC Cancer* 2019; **19**: 1229 [PMID: 31847830 DOI: 10.1186/s12885-019-6405-7]

99 **Wang R**, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* 2012; **13**: 907-915 [PMID: 22990888 DOI: 10.1038/ni.2386]

100 **Pearce EL**, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013; **38**: 633-643 [PMID: 23601682 DOI: 10.1016/j.immuni.2013.04.005]

101 **Xia Y**, Xie Y, Yu Z, Xiao H, Jiang G, Zhou X, Yang Y, Li X, Zhao M, Li L, Zheng M, Han S, Zong Z, Meng X, Deng H, Ye H, Fa Y, Wu H, Oldfield E, Hu X, Liu W, Shi Y, Zhang Y. The Mevalonate Pathway Is a Druggable Target for Vaccine Adjuvant Discovery. *Cell* 2018; **175**: 1059-1073.e21 [PMID: 30270039 DOI: 10.1016/j.cell.2018.08.070]

102 **Ma X**, Bi E, Lu Y, Su P, Huang C, Liu L, Wang Q, Yang M, Kalady MF, Qian J, Zhang A, Gupte AA, Hamilton DJ, Zheng C, Yi Q. Cholesterol Induces $CD8^+$ T Cell Exhaustion in the Tumor Microenvironment. *Cell Metab* 2019; **30**: 143-156.e5 [PMID: 31031094 DOI: 10.1016/j.cmet.2019.04.002]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 124-152

DOI: 10.4251/wjgo.v14.i1.124

ISSN 1948-5204 (online)

REVIEW

Development of artificial intelligence technology in diagnosis, treatment, and prognosis of colorectal cancer

Feng Liang, Shu Wang, Kai Zhang, Tong-Jun Liu, Jian-Nan Li

ORCID number: Feng Liang 0000-0002-8798-0229; Shu Wang 0000-0002-8758-0508; Kai Zhang 0000-0002-4499-7186; Tong-Jun Liu 0000-0001-5712-4838; Jian-Nan Li 0000-0001-9744-7666.

Author contributions: Liang F wrote the paper; Liang F, Wang S, and Li JN performed the revision and approval of the final version; Zhang K and Liu TJ performed literature research; Wang S corrected the writing of the paper; Li JN designed the review; Liang F and Wang S contributed equally to this work.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Supported by The Science and Technology Development Project of Jilin Province, No. 3D5197434429; and National Natural Science Foundation of China, No. 32000953.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review report's scientific quality classification

Grade A (Excellent): A

Feng Liang, Kai Zhang, Tong-Jun Liu, Jian-Nan Li, Department of General Surgery, The Second Hospital of Jilin University, Changchun 130041, Jilin Province, China

Shu Wang, Department of Radiotherapy, Jilin University Second Hospital, Changchun 130041, Jilin Province, China

Corresponding author: Jian-Nan Li, PhD, Professor, Department of General Surgery, The Second Hospital of Jilin University, No. 218 Ziqiang Street, Changchun 130041, Jilin Province, China. jnli@ciac.ac.cn

Abstract

Artificial intelligence (AI) technology has made leaps and bounds since its invention. AI technology can be subdivided into many technologies such as machine learning and deep learning. The application scope and prospect of different technologies are also totally different. Currently, AI technologies play a pivotal role in the highly complex and wide-ranging medical field, such as medical image recognition, biotechnology, auxiliary diagnosis, drug research and development, and nutrition. Colorectal cancer (CRC) is a common gastrointestinal cancer that has a high mortality, posing a serious threat to human health. Many CRCs are caused by the malignant transformation of colorectal polyps. Therefore, early diagnosis and treatment are crucial to CRC prognosis. The methods of diagnosing CRC are divided into imaging diagnosis, endoscopy, and pathology diagnosis. Treatment methods are divided into endoscopic treatment, surgical treatment, and drug treatment. AI technology is in the weak era and does not have communication capabilities. Therefore, the current AI technology is mainly used for image recognition and auxiliary analysis without in-depth communication with patients. This article reviews the application of AI in the diagnosis, treatment, and prognosis of CRC and provides the prospects for the broader application of AI in CRC.

Key Words: Artificial intelligence; Colorectal cancer; Diagnosis; Treatment; Prognosis

Core Tip: The current artificial intelligence (AI) technology is mainly used for image

Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: June 15, 2021
Peer-review started: June 15, 2021
First decision: August 8, 2021
Revised: August 19, 2021
Accepted: November 15, 2021
Article in press: November 15, 2021
Published online: January 15, 2022

P-Reviewer: Mitsala A, Morya AK
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Yan JP

recognition and auxiliary analysis without in-depth communication with patients. We here review the application of AI in the diagnosis, treatment, and prognosis of colorectal cancer (CRC) and look at the prospects for the broader application of AI in CRC.

Citation: Liang F, Wang S, Zhang K, Liu TJ, Li JN. Development of artificial intelligence technology in diagnosis, treatment, and prognosis of colorectal cancer. *World J Gastrointest Oncol* 2022; 14(1): 124-152
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/124.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.124

INTRODUCTION

With the invention of the computer, heavy scientific and engineering calculations have shifted from being done primarily by the human brain to being done more quickly and accurately by computers. Artificial intelligence (AI) has evolved rapidly with the continuous development of computer science and technology. AI is an umbrella term that helps humans perform tasks including computer simulation, decision-making, language understanding, problem-solving, voice and image recognition, and other "intelligent" tasks[1-3]. AI can be divided into machine learning (ML), deep learning (DL), anti-learning, quasi-supervised learning (QSL), and active learning (AL)[4-7]. ML is a subset of AI algorithm which uses statistical techniques to adjust and improve itself[1,3]. ML produces algorithms for analyzing data and learning to predict models, which means that ML is data-driven, with a little human intervention as possible in the decision-making process[4,8]. The model created by ML can be used as an independent executable system to predict the clinical phenotype[9]. The relevant technologies in ML include support vector machine (SVM), neural network (NN), random forest (RF), decision tree, and regression analysis[10]. Based on the association of class labels, ML is generally divided into supervised learning, unsupervised learning, and semi-supervised learning (SSL)[8,9]. Supervised learning is mainly used for solving classification and regression problems. Unsupervised learning is used for a cluster, density estimation, and dimensionality reduction[9]. SSL can significantly improve the learning accuracy when unlabeled data combined with a limited number of labeled data are used in SSL[11]. At present, supervised learning plays a leading role in AI and ML in the medical field[2]. Supervised learning provides more accurate results than other AI techniques because it considers the characteristics of the patients [10].

DL is a kind of developed ML based on an artificial NN (ANN)[2], which is inspired by the biological characteristics of the human brain, especially the connection of neurons[2,4]. DL can not only automatically find lesions, make recommendations for differential diagnosis, and write elementary medical reports, but can also be self-learning, *i.e.*, key characters and quantities can be extracted without a manual indication if the training data is provided[4]. Moreover, DL aims to copy the brain's learning process and process a large amount of high-dimensional data[12]. QSL is a statistical learning algorithm that avoids the manual marking of normal tissue and cancer tissue samples in traditional supervised learning and greatly reduces the intervention of experts[5]. ML usually needs a large number of annotated training sets, which are expensive to create. AI reduces the size of the required annotation set and generates a better classification model[7]. In some research, to predict the stage of colorectal cancer (CRC) from immune attributes, the anti-learning method has better performance than a series of ML algorithms[6].

CRC is the second reason for cancer death in males and the third reason for cancer death in females[13]. If colonic polyps, which may lead to at least 80%-95% of CRC [14], are detected by the screening procedure and resected in the precancerous stage, it can help prevent CRC development[15]. Although early and intensive screening can reduce cancer incidence and mortality, patients avoid CRC screening due to the complexity and cost of screening[15-17]. Generally, the methods of diagnosing CRC are divided into imaging diagnosis, endoscopy, and pathology diagnosis. Treatment methods are divided into endoscopic treatment, surgical treatment, and drug treatment. If lymph node metastasis is not confirmed preoperatively, lymph node

dissection is not required intraoperatively[18]. AI has great diagnostic potential because it can learn from a large data set. In the clinical image, AI is superior to medical experts and existing biomarkers[10]. This paper will describe the use of AI in the diagnosis, treatment, and prognosis of CRC. Web of Science and PubMed databases were searched using keywords "artificial intelligence" and "colorectal cancer".

USE OF AI IN DIAGNOSIS OF CRC

DL in imaging diagnosis

The DL intelligent assistant diagnosis system can help the clinical diagnosis and treatment of CRC[19]. The computer-aided diagnosis (CAD) system usually analyzes the nature of the selected area (cancerous or noncancerous) through the informative characteristics of the known potential (cancerous) structure[20]. The CAD system can help radiologists diagnose CRC by visual cues (CAD marks) associated with potential pathology. In addition, CAD can help determine the location of the disease (computer-aided detection, CADe) and determine whether the abnormality is benign or malignant. Regardless of the outcome, doctors must ultimately decide whether to "believe" the CAD mark[21]. The key for radiologists accepting the clinical use of CAD systems is to have a high detection sensitivity and a low false-positive rate (FP)[20]. Apart from polyps and cancer, other colorectal pathological morphologies are rare, which can explain why CAD solutions for computed tomography colonography (CTC) have developed so rapidly[22]. CAD of CTC has indeed improved sensitivity in finding polyps without disproportionately decreasing specificity, but the lesions mistaken for false-negative are significantly large and irregular[21-23]. Regge *et al*[21] believed that the difficulty of characterization (irregular and flat morphology) was the main determinant of radiologists' rejection of true positive CAD indications.

Although the consequence of CRC misdiagnosis is much more severe than that of polyp misdiagnosis, the research of CADe for CRC in CTC is still very limited[24]. The reason may be that the lack of literature on the detection characteristics of early CRC [25] and the fact that it remains a problem to effectively distinguish masses from normal colonic anatomy based on the design features of mathematical images[24]. Taylor *et al*[25] collected the morphological characteristics of flat tumors by locating tumors to distinguish tumors from normal tissue structure and found that the CAD system combined with CTC was relatively effective for detecting flat (non-polypoid) cancer. CAD can improve the speed of image interpretation, find out the polyps missed by experts, reduce the variability between observers, and improve the sensitivity of polyp detection[26,27]. However, the increase of FP generated by CAD may reduce the efficiency[22]. Deep transfer learning can greatly improve the accuracy of polyp detection in CTC[28]. Because the virtual intracavity images of polyp filtered by the CADe system can be used to modify the deep convolutional NN (DCNN) trained by millions of non-medical images, the DCNN can identify polyps[28]. It can significantly improve the detection of polyps for inexperienced doctors using a visualization scheme in CTC. Combined with the CAD system, the visualization scheme can reduce radiologists' interpretation time and improve the detection of colon tumors in CTC[29]. Van Wijk *et al*[30] presented a method by measuring the protrusion of candidate objects in a scale adaptive way to evaluate polyps larger than or equal to 6 mm, with a 95% sensitivity obtained. It was believed that identifying the size of polyps can reduce the risk of missed diagnosis of large polyps more than identifying the shape[30]. Kim *et al*[31] collected the CTC dataset interpreted by the CAD algorithm from polyp patients. The CTC dataset was designed to describe the lumpy structure extending into the lumen and could identify large polyps (> 6 mm) with a high sensitivity and acceptable FP. Based on the characteristics of volume and shape, Nappi *et al*[32] developed a CADe method to detect the location of colonic polyps and used this method to evaluate the serrated polyps confirmed by colonoscopy and biopsy. The results showed that the detection accuracy of the method was much higher than that of the traditional CADe system[32]. Therefore, the application of CAD diagnosis has a promising prospect. However, more data sets and effective annotations are still needed to enhance the accuracy of AI diagnosis[21].

The optimal portal venous phase timing recognition scan was selected for classifying the contrast enhancement time, which could help analyze the radiologic characteristics of the tumor and evaluate the efficacy of patients with advanced CRC [33]. Soomro *et al*[34] found that three-dimensional (3D) fully convolutional NNs combined with 3D level-set showed a higher sensitivity than 3D fully convolutional

NNs alone in the segmentation of CRC on magnetic resonance imaging (MRI), which helped for the diagnosis of CRC. In 3D-T2 weighted MRI, the 3D full collaborative network architecture based on DL could segment CRC more reasonably and effectively than other techniques[35]. In the high-resolution MRI image of rectal cancer, the use of a faster region-based convolution NN (Faster R-CNN) had a high accuracy in evaluating tumor boundaries[36,37]. Circumferential resection margin is one of the key factors affecting the treatment decision of CRC patients. Joshi *et al*[38] proposed an automatic calculation and visualization method of circumferential resection margin distance in MRI images of CRC to segment the middle rectal fascia, the corresponding tumor, and lymph node into different regions. The segmentation was used to analyze the shortest cut edge automatically, and the results obtained were almost identical to the experts' judgment[38].

DL in pathological diagnosis

If CRC is detected early, it is almost curable. However, in order to make a correct diagnosis, a double examination of biopsy and colonoscopy image is required, so the cost of diagnosis has increased[39]. Thus, the use of DL and automatic image analysis in pathology is increasing, which is called the third revolution of pathology[40]. Although the automatic coding in DL is considered helpful in extracting multi-layer image features and deep NNs can classify the features, it takes much time to train artificial neurons[41].

Convolutional NN (CNN) is a common method in pathological image analysis. Compared with other methods, CNN has the advantages of convenience for end-to-end learning (CNN learning parameters and representations are designed manually), flexibility, and high capacity[2]. The choice of color space is important for identifying cancer tissue because it deeply affects the performance of the classification model. CNN is used to analyze the tissue classification of different color spaces. Tiwari S proved that hue, saturation, value (HSV) color space was more suitable than any other color model for cancer tissue classification[42]. Because of the heterogeneity of the cells, texture, and cell contact complexity, it is challenging to detect and classify the nuclei in the pathological images of cancer tissues stained with hematoxylin and eosin (H&E)[43,44]. A space-constrained CNN based on DL was proposed for nuclear detection, which might provide a possibility for quantitative analysis of tissue components and clarify the tumor microenvironment. Moreover, the neighbor ensemble predictor combined with CNN could accurately predict the detected nuclear markers and classify the nuclei[43]. Although qualitative and quantitative analysis of histopathological images can clarify the tumor and explore various options for cancer treatment, it remains challenging due to cell heterogeneity. Zhang *et al*[45] proved that it had a good accuracy and lower cost of time when Faster R-CNN was used in feature extraction, providing a useful quantitative analysis group for pathological practice.

CNN, widely used to analyze histopathological images, only performs directly on the histopathological images, ignoring the histopathological images' stain decomposition. Xu *et al*[46] reported a new model based on DCNN to classify the H&E and immunohistochemistry images of epithelial and stromal cells in colon cancer. For distinguishing stromal from epithelial cells, the DCNN based model was always better than the traditional hand-made model. The morphology of glands and nuclei is used to evaluate the malignant degree of adenocarcinoma. As a necessity for quantitative diagnosis, the accurate detection and segmentation of the histological image are challenging due to its appearance variation, strong similarity, and tissue degradation. Chen *et al*[47] attempted to use a depth profile awareness network, which could output the accurate probability map of histological objects and draw clear contour lines, to improve the accuracy of detection and segmentation.

Digital pathology is a new field. The development of digital pathology may help pathologists to improve the quality of routine pathological operations[48]. The key to promoting the development of digital pathology is the CAD system, based on the principle of extracting histopathological features that pathologists consider important. Then, the existence of these features was explained quantitatively by computer calculation[49,50]. There are two important steps towards the CAD: Tumor segmentation of the whole section image in the histological section and the automatic segmentation of tumors in the H&E staining histological image[51]. Qaiser *et al*[51] found that tumor and non-tumor plaques had distinct homology, and proved the robustness and significance of persistent homology by exploring connectivity between nucleus. A method called persistent homology maps (PHPs) was proposed, which could distinguish tumor area from the normal area by simulating the atypical characteristics of tumor cell nucleus[51]. PHPs outperform other methods, including traditional CNN[51]. Two different tumor segmentation methods are proposed:

Targeting speed without affecting accuracy and targeting higher accuracy. The combination of PHPs and CNN features was shown to be better than competition algorithms[51].

DL in endoscopic diagnosis

Colonoscopy is a common method to screen polyps. The detection and removing of adenomatous polyps can reduce the incidence and mortality rates of CRC [13]. AI is necessary to improve machine performance and diagnosis accuracy, reducing the variability between operators and helping rapid treatment decision-making[3]. In addition, AI has a great potential to improve the detection rate of adenoma and reduce the cost of polypectomy[52]. The quality of intestinal preparation is an important factor influencing the effect of colonoscopy examination[53]. When the fecal residues are present in the colon, the rate of missed diagnosis of polyps will increase. Although the endoscopic image diagnostic program based on CNN has yielded good results, its diagnostic ability depends heavily on the quality and quantity of training data[4,54]. The use of CNN and colonoscopy procedure is expected to improve the detection rate and diagnosis accuracy of polyps[55]. Zhou *et al*[53] developed a CNN based system that was trained by collecting colonoscopy images. Through a human-machine competition, the system was found to be more reliable than endoscopic physicians in diagnosis of CRC. Taha *et al*[56] introduced a DL solution for polyps from colonoscopy, a pre-training architecture for feature extraction, used together with the classical SVM classifier. As the solution can avoid the high computational complexity and high resource requirements of CNN, it outperforms other models in the early screening of CRC[56]. Yao *et al*[57] proved that the features in red, green, blue (RGB) and HSV color space could well describe the frames in colonoscopy videos. It could improve the model's efficiency by integrating the prior knowledge based on vision into the data extracted by DL. Therefore, a feature extraction algorithm in HSV color space was designed to effectively improve the accuracy of diagnosis and reduce the cost[57]. McNeil *et al*[58] proposed an automatic quality control system based on DCNN, improving colonoscopy quality by cleaning the mucosal wall and reexamining the rushed segment. The system could increase the detection rate of polyps and have great significance for the early diagnosis and prevention of CRC.

The missed diagnosis rate of traditional colonoscopy approaches 25%[59,60], partly due to the lack of depth information, inter-observer variation, and contrast on the surface of the colon[60,61]. Computer-aided technology is important for polyp detection in endoscopic video. The method based on DL takes the lead in the evolution of algorithm performance[62]. It is a challenging task for CAD to minimize the FP of colonic polyps[63]. Mahmood *et al*[61] used a joint depth learning and graphics model-based framework to estimate depth from endoscopic images. At the same time, they used the texture-free colon model to generate training images and trained the model with those images[61]. The system could estimate the depth of virtual data with a relative error of 0.164, which was helpful to perfect the CAD system and identify lesions[61]. Komeda *et al*[64] believed that CNN had the advantage of learning from large data and led to high precision and fast processing time, and they designed a CNN-CAD system to study endoscopic images extracted from colonoscopy[64]. The analysis and cross-validation of 1200 cases of colonoscopy confirmed that the CNN-CAD system was helpful for the rapid diagnosis of colonic polyps and could simplify the decision-making process of colorectal polypectomy[64]. Compared with other algorithms, the CAD method (named RYCO) had the potential for rapid and accurate computer-aided polyp detection in colonoscopy. The fast target detection algorithm ResYOLO was pre-trained using a large non-medical image database, and the colonoscopy image was fine-tuned. At the same time, the time information was combined by a tracker named Efficient Convolution Operator to improve the detection results given by ResYOLO. RYCO could clarify the spatial characteristics of colorectal polyps directly and improve the detection efficiency of colorectal polyps[65]. In order to distinguish stage T1b and Tis/T1a CRC, the optical diagnostic system developed by CNN was proposed[66]. Zhu *et al*[66] selected the early CRC digital images without magnification and under a pure white light endoscope as the training dataset. At the end of the training process, 122 early CRC images were used to evaluate the diagnostic performance. The results showed that optical diagnoses by CNN had a high sensitivity but low specificity, which was different from humans[66]. Variations in polyp size and shape made the diagnosis of polyp in colonoscopy video challenging[67]. However, the Faster R-CNN could reduce the risk of polyp loss during colonoscopy[62]. Furthermore, Akbari *et al*[67] presented a fully convolutional network (FCN) method of polyp segmentation based on CNN. In the test phase, they did effective post-processing for the probability map generated by the network. The CVC-ColonDB

database was used to evaluate the method. The result showed that FCN could get more accurate segmentation results[67]. 3D-FCN could learn more representative spatiotemporal features from colonoscopy video and had stronger recognition ability than FCN[68].

The goal of the real-time endoscopic image diagnosis support system is to use AI during colonoscopy without interrupting the operation of any doctor[69]. Based on the DL method, the real-time optical detection and analysis of polyps can be carried out by white light endoscopy alone[70]. A real-time automatic polyp detection system can help endoscopists detect lesions that may correspond to adenomas quickly and reliably[13]. The accuracy of endoscopic differential diagnosis enables the "resection and discard" mode of small-scale colorectal polyps[71]. To relieve the high cost, long time consuming, and patients' discomfort, Lund Henriksen *et al*[71] explored a system for automatic polyp detection to assist and automate the examination procedures. By comparing root mean square propagation, stochastic gradient descent, and adaptive moment estimation, when stochastic gradient descent was used as the training optimizer, the detection rate increased while the number of FP was relatively stable [71].

Although optical biopsy is a promising field, tissue biopsy remains the gold standard. Whether the surface microstructure accurately reflects the histological characteristics of lesions will affect the results of optical biopsy[3,13,72,73]. The widespread clinical use of microscopic technology, especially the combination of virtual chromoendoscopy and microscopic imaging, has brought more attention to the field of optical biopsy[74]. Endoscopists can reliably diagnose and differentiate micro-adenomatous and hyperplastic polyps using established optical evaluation criteria [75]. The development of CAD and AI algorithms may overcome the main obstacles of optical biopsy and change the treatment of colorectal lesions[74,76]. Endocytoscopy is an effective method for deep diagnosis of CRC because of the high resolution[73]. Kudo *et al*[77] developed an AI-based system called EndoBRAIN which could identify the colon tumor by analyzing the nucleus, crypt structure, and microvasculature in the endoscopic image. The initial training of EndoBRAIN was carried out using endoscopic images. The diagnostic efficiency of endoscopists and the diagnostic performance of EndoBRAIN were analyzed retrospectively. The result showed that EndoBRAIN could increase the accuracy of the diagnosis[77]. Mahmood *et al*[78] proposed a new monocular endoscope depth estimation and terrain reconstruction system, which took advantage of the joint training framework based on CNN and conditional random field. The system used the synthetic endoscope data for training and the colon model data for fine-tuning. It could be integrated into the endoscope system, which provided a basis for improving the CAD algorithm to detect, segment, and classify lesions[78].

ML in imaging diagnosis

ML has to extract the most relevant or predictive features from many tested features and use these to determine the categories of new image samples[79,80]. The features will help diagnose CRC in imaging. It is very important to segment colorectal tumors accurately in MRI images, while the manual or semi-manual method is very tedious, time-consuming, and operator-dependent[81]. CAD plays an important role in many medical analyses, especially in computed tomography (CT) image analysis. Although many methods are designed, there are still some deficiencies in structure segmentation [82]. Onder *et al*[5] reported that ML methods including SVM and logistic regression could achieve better classification performance and improve the accuracy of the baseline CAD system. The ideal colon segmentation effect could be achieved in a CT image using the NN algorithm to remove the turbid liquid of the large intestine[83]. Jian *et al*[81] proposed a segmentation method based on the FCN framework. The normalization method was used to reduce the difference between images. The segmentation method could extract features from standardized images and generate corresponding predictions for reference using the idea of transfer learning. Finally, all predictions were fused to determine the final tumor boundary[81]. Compared with manual segmentation of T2 weighted MRI images of CRC, the FCN based segmentation method had a higher accuracy. The FCN based segmentation method might replace the time-consuming manual method[81]. In order to achieve accurate segmentation, a regression NN-augmented lagrangian genetic algorithm (RNN-ALGA) based on ML was proposed. Using RNN-ALGA, an accuracy of 97% could be achieved under the condition of small error. RNN-ALGA was suitable for abdominal CT image slices and could improve structural segmentation accuracy and time efficiency in diagnosing colonic diseases[82].

ML in pathological diagnosis

Computational pathology based on AI and ML methods is most promising. The computer model has better image recognition ability than human experts[2]. Large-scale and high-quality training datasets are necessary for an ML-based image classifier to achieve high performance[84]. ML-based tissue classification is a valuable method for manual histological analysis. However, high-resolution image classification is a complex and computationally expensive task. In addition, the goal of many tissue analysis tasks is to identify rare areas in the tissue. In colon cancer, tumor budding (TB) exists in the front of the tumor-infiltrating area, which is an important sign of tumor invasiveness[85]. When the image is examined at a low resolution, the small objects are difficult or impossible to detect. Sun *et al*[85] provided a two-tier CNN classification method that was explored to identify the small and important tissue areas in the whole slice tissue. The processing time of the method is reduced by 43%. The two-tier classifier provided an effective tissue classification by reducing the task area and increasing the chance of tumor bud recognition[85]. A variety of serum tumor markers can be used in the diagnosis of CRC. There is a wide range of variability in the types and quantities of routinely used markers. The traditional single cut-off point also hinders the effective use of tumor markers. In order to improve the diagnosis accuracy and reduce the cost, it is important to optimize the inspection combination and make full use of the inspection value. Shi *et al*[86] proposed an AI algorithm called diagnosis strategy of serum tumor maker, which proved that two markers were enough for diagnosis. Compared with SVM and decision tree, the multiple tumor markers with multiple cut-off values (MVMTM) algorithm could greatly improve the diagnosis efficiency of CRC using carcinoembryonic antigen, carbohydrate antigen 19-9 (CA19-9), and CA50[87]. The establishment of an image database for colorectal tumor biopsy is an important step to detect the tumor. The automatic classification of tumor cells can improve the rapidity and accuracy of tumor diagnosis. Image processing and ML can be used to distinguish different cell types in digital biopsy sections. In addition to using conventional RGB/grayscale images, multispectral images often provide extensive information to support classification tasks. Kunhoth *et al*[88] used a multispectral image acquisition system to develop a colorectal biopsy section database divided into training sets and test sets. In order to avoid the deviation, 50 iterations were run, and the results of a single operation were averaged, which finally proved that the database had a high classification accuracy. The colorectal biopsy section database could help diagnose CRC[88].

ML in endoscopic diagnosis

With good results in computer vision and other fields, ML still requires certain manual guidance[4]. Removal of precancerous polyps is important for colon cancer prevention. However, the detection rate of adenomatous polyps is quite different among endoscopists[89]. By calculating the risk and difference of detecting polyps, adenomas, and CRC, Barua *et al*[90] compared colonoscopy with AI and colonoscopy without AI. It was found that an AI-based polyp detection system in colonoscopy could increase the detection rate of nonprogressive small adenomas and polyps but could not increase the detection rate of progressive adenomas[90]. Wang *et al*[89] developed the ENDOANGEL system and compared AI colonoscopy with colonoscopy without AI through random-control experiments. The results showed that AI significantly improved the detection rate of adenoma in colonoscopy[91]. Lui *et al*[92] suggested that the DL AI model could detect adenomas missed in routine colonoscopy in the real-time examination. They believed that the combination of AI and auxiliary equipment could eliminate the risk of missing lesions in colonoscopy when the intestine was well prepared[92]. Elastic scattering spectroscopy (ESS) for optical guided biopsy had a high accuracy in tumor detection. Rodriguez-Diaz *et al*[93] proposed two spectral classification frameworks, called ensemble classification and misclassification rejection, for clinical problems of non-tumor and tumor colorectal lesion classification based on ESS measurement. When the two frameworks were used to develop the diagnosis algorithm together, the classification effect would be better, and the medical cost would be reduced[93]. Near-infrared spectroscopy could also be used to diagnose CRC and differentiate malignant tumors. Kondepati *et al*[94] collected the spectrum of cancer tissue and normal tissue from colonic tissue with an optical fiber probe. Major spectral differences could be observed. The spectrum was divided into cancer tissue and normal tissue with an accuracy of 89% using ANN, linear discriminant analysis, and other pattern recognition methods[94]. The method based on AL could perform real-time detection during colonoscopy and enhance detection performance at the same time. However, the possibility of increased FP

made the algorithm difficult to use in daily clinical practice[95]. Colon cancer might cause anemia as a common indication of colonoscopy. Hemoglobin concentration could be used as an indicator for the diagnosis of colon cancer, but it was not enough to diagnose colon cancer by hemoglobin concentration alone[96]. The AI-based ColonFlagTM might be an appropriate indicator, which used all indicators of whole blood count, age, and gender. At the same time, ColonFlagTM could provide appropriate treatment suggestions for patients who did not accept the fecal examination or colonoscopy[96]. Tian *et al*[97] believed that enhanced patient education (EPE) can be realized through visual aids, telephone, mobile and social media applications, multimedia education, and other software. EPE was used to guide the intestinal preparation of patients with colonoscopy and improve the detection rate of polyps, adenomas, and sessile serrated adenomas[97].

QSL and SSL in diagnosis

QSL eliminates the need for traditional supervised learning for manual labeling and reduces expert intervention. QSL texture labeling may be useful in the analysis and classification of pathological sections, but further research is needed[5]. The main purpose of analyzing millions of pixel histological images is to help pathologists predict cancer. At present, most methods are limited to the classification of tumors and stroma. Moreover, most of the existing methods are based on fully supervised learning and require many annotations that are difficult to obtain[98]. Javed *et al*[98] proposed a new group detection algorithm based on SSL, which could identify six different phenotypes in millions of pixels of image data. Two independent CRC datasets showed that the SSL algorithm was superior to the latest method[98]. ANNs are a class of models inspired by biological NNs, which are used to estimate functions that depend on a large number of general unknown inputs[99]. ANNs are usually shown as interconnected neuron systems, exchanging information with each other. Each connection has a digital weight, adjusted according to experience to make the input flexible and learn[4,9,99]. The establishment of diagnosis models based on ANN is helpful for clinicians to diagnose CRC, predicting postoperative outcomes, and screening high-risk prognosis subgroups[99]. ANNs have a good prospect in the general survey of CRC by establishing a clinical data model. This method is simple, low-cost, and non-invasive[100]. Other studies also described the application of AI in the diagnosis of CRC[101-107].

It is important to increase the sensitivity and specificity of early detection of CRC. First, massive endoscopic image datasets of early CRC should be set, with the early screening performed by colonoscopy and AI automatic recognition system. Second, early identification and timely warning for high-risk groups with a family history can be realized through new media and smartphone software. Third, with many pathological images and optical maps, we can identify whether the cutting edge is negative after endoscopic intervention in real time to adjust the treatment plan in time and avoid secondary surgery. Fourth, the government should establish a timely and effective national physical examination plan through AI to conduct early intervention and treatment for the high-risk population (Table 1).

USE OF AI IN TREATMENT OF CRC

AI in treatment decision

AI has become an irresistible trend in the medical field[108]. At present, oncologists are familiar with clinical practice guidelines (CPGs) and provide follow-up treatment for patients based on CPGs. On the contrary, physicians may not be familiar with the guidelines[109]. Passi *et al*[109] developed a decision support system (DSS) that used CRC follow-up data as a source of knowledge to generate appropriate follow-up recommendations for patients. Passi *et al*[109] designed and proposed the semantic framework of the web application, combining the current web technology and database storage with the designed ontology, and realized the unified development of DSS. Passi *et al*[109] also designed a web application interface to provide doctors with the functions of CPGs. DSS development could help physicians and nurses provide postoperative care for CRC patients[109]. Watson for Oncology provided oncologists with various cancer treatment suggestions, such as recommended, representing the preferred method; for consideration, not recommended. The absolute consistency of the treatment regimen with the recommendations of the multidisciplinary team of oncologists was studied. Lee *et al*[110] used Watson for Oncology to process cases and compared the results with the actual treatment received by patients. Key findings

Table 1 Artificial intelligence in diagnosis of colorectal cancer

Type of study	Ref.	No. of participants	Method	Control and interventions	Conclusion
Case control study	Yang *et al*[19], 2019	241	Depth-learning intelligent assistant diagnosis system	By comparing the accuracy of different algorithms on MRI images of patients with CRC, the algorithms that were conducive to the diagnosis of CRC were defined	T2-weighted imaging method had obvious advantages over other methods in differentiating CRC
Analytical research	Liu *et al*[20], 2011	429	SVM	Compared the performance of new and old classification methods in colorectal polyps CAD system	SVM could help CAD system get excellent classification performance
Review	Regge *et al*[21], 2013	NA	CAD system	NA	CAD system helped radiologists diagnose CRC with visual markers
Case control study	Summers *et al*[22], 2008	104	CAD system	The sensitivity of adenoma was measured by CAD system and compared with previous studies	CAD system had high accuracy in detecting and distinguishing adenoma
Descriptive research	Chowdhury *et al*[23], 2008	53	CAD-CTC system	The sensitivity of CAD-CTC system and manual CTC was compared through the image data of 53 patients	CAD-CTC system could effectively identify polyps and cancers with clinical significance in CT images
Case control study	Nappi *et al*[24], 2018	196	ResNets	Based on the clinical data of 196 patients, the classification performance of different models in distinguishing masses from normal colonic anatomy was compared	ResNets solved the practical problem of how to optimize the performance of DL
Case control study	Taylor *et al*[25], 2008	24	CAD system	The effectiveness of CAD system in detecting tumors was tested using the clinical data of 24 patients	CAD could effectively detect flat carcinoma by tumor morphology
Case control study	Summers *et al*[26], 2010	394	CAD-CTC system	The CTC data sets of 394 patients were trained in CAD system. It was confirmed that the experimental group could reduce the missed diagnosis rate of cancer	CAD-CTC system used advanced image processing and ML to reduce the occurrence of FP results
Case control study	Lee *et al*[27], 2011	65	CAD system	The CTC data sets of patient polyps were divided into a training data set and a test data set to compare the detection performance of CAD system	CAD system included colon wall segmentation, polyp specific volume filter, cluster size counting and thresholding, which had high detection performance of polyps and cancer tissue
Case control study	Nappi *et al*[28], 2015	154	DCNN	The clinical data were divided into a training data set and a test data set to compare the polyp detection performance of multiple classifiers	DCNN could greatly improve the accuracy of automatic detection of polyps in CTC
Case control study	Näppi *et al*[29], 2005	14	CAD system	The clinical data of 14 patients were used to test the effect of different staining methods on the effectiveness of polyp detection	CAD system helped to improve the ability to detect polyps in CTC
Case control study	van Wijk *et al*[30], 2010	84	CAD-CTC system	The polyp detection performance of different classification methods was tested through the clinical data of 84 patients	The sensitivity of the CAD-CTC system to distinguish polyps over 6 mm was very high
Case control study	Kim *et al*[31], 2007	35	CAD system	The sensitivity of CAD polyp detection was tested using colonoscopy data of 35 patients	CAD system helped to distinguish polyps and cancer tissue larger than or equal to 6 mm
Case control study	Nappi *et al*[32], 2017	101	CADe system	The polyp detection accuracy	CADe system could improve

				of novel and old CADe systems was compared by colonoscopy data of 101 patients	the accuracy of detecting serrated polyps or cancer tissues
Case control study	Ma *et al*[33], 2020	681	Portal venous phase timing algorithm	Training through 479 CT scan data sets; 202 CT scans were used for retrospective analysis and algorithm development and verification	It was helpful to quantitatively describe the characteristics of tumor enhancement
Case control study	Soomro *et al*[34], 2018	12	3D fully convolutional neural networks	The effects of polyp segmentation and recognition of different models were compared using MRI data of 12 patients	3D fully convolutional neural networks provided a more accurate segmentation result of colon MRI
Case control study	Soomro *et al*[35], 2019	43	DL	43 patients with CRC were evaluated by MRI. The data set was divided into 30 volumes for training and 13 volumes for testing	DL achieved better performance in colorectal tumor segmentation in volumetric MRI
Retrospective study	Wang *et al*[36], 2020	240	Faster R-CNN	The Faster R-CNN was trained using pelvic MRI images to establish an AI platform. The diagnosis results of AI platform were compared with those of senior radiologists	It was highly feasible to segment the circumcision positive margin with Faster R-CNN in MRI image of rectal cancer
Retrospective study	Wu *et al*[37], 2021	183	Faster R-CNN	The MRI data of 183 patients were collected as training objects. The platform was constructed using Faster R-CNN. The diagnostic accuracy was compared with that of radiologists	AI could effectively predict the T stage of rectal cancer
Case control study	Joshi *et al*[38], 2010	10	Non-parametric mixture model	Compared the accuracy of the algorithm and expert conclusions through the patient's MRI images	The algorithm could be used to distinguish T3 and T4 tumors accurately
Case control study	Shiraishi *et al*[40], 2020	314	CNN	The prognostic significance was evaluated by CNN based on the expression of tumor markers in 314 patients	CNN could help to evaluate the diagnosis and prognosis of tumor markers
Case control study	Pham[41], 2017	NA	DL	NA	DL could reduce training time and improve classification rate
Case control study	Tiwari[42], 2018	10	CNN	CNN was used to compare the accuracy of image classification methods for seven different tissue types	CNN determined the most suitable color for cancer tissue classification (HSV color space) by classifying tissues in different color spaces
Case control study	Sirinukunwattana *et al*[43], 2016	100	SC-CNN	Through the comparative evaluation on the image data set of 100 cases of CRC, SC-CNN was helpful to the quantitative analysis of tissue components	SC-CNN can help to predict the nuclear class tags more accurately
Case control study	Koohababni *et al*[44], 2018	NA	DL	NA	DL could combine the probability maps of a single nucleus to generate the final image, so as to improve the diagnostic performance of complex colorectal adenocarcinoma datasets
Case control study	Zhang *et al*[45], 2018	NA	Faster R-CNN	NA	Faster R-CNN provided quantitative analysis of tissue composition in pathological practice
Case control study	Xu *et al*[46], 2016	1376	DCNN	Compared the classification effects of AI and manual methods on the same pathological image dataset	DCNN can help to improve the accuracy of differentiation between epithelial and mesenchymal regions in digital tumor tissue microarray

Retrospective study	Chen *et al*[47], 2017	85	Deep contour-aware network	The classification performance of different segmentation methods on the same pathological image dataset was compared	Output accurate probability map of gland cells, draw clear outline to separate the originally gathered cells, and further improve the segmentation performance
Case control study	Yoshida *et al*[48], 2017	1328	An automated image analysis system	The classification results of the same dataset by human pathologists and electronic pathologists were compared	Compared with manual classification, the system had higher classification accuracy
Retrospective study	Saito *et al*[49], 2013	NA	CAD system	NA	CAD system could be used for quality control, double check diagnosis, and prevention of missed diagnosis of cancer
Descriptive research	Jin *et al*[50], 2019	NA	AI	NA	AI accelerated the transformation of pathology to quantitative direction, and provided annotation storage, sharing, and visualization services
Case control study	Qaiser *et al*[51], 2019	75	CNN	The segmentation and recognition effects of different methods on the same pathological dataset were compared	CNN and PHPs can more accurately and quickly distinguish tumor regions from normal regions by simulating the atypical characteristics of tumor nuclei
Retrospective study	Zhou *et al*[53], 2020	120	DCNN	In the man-machine competition of 120 images, the accuracy of AI and endoscopists was compared	DCNN helped to establish an objective and stable bowel preparation system
Case control study	de Almeida *et al*[54], 2019	NA	CNN	NA	CNN improved the accuracy of polyp segmentation. It can help to automatically increase the sample number of medical image analysis dataset
Case control study	Taha *et al*[56], 2017	15	DL	The effectiveness of the DL method for identifying polyps in colonoscopy images was verified on the public database	In the early screening of CRC, it was better than other single models
Case control study	Yao *et al*[57], 2019	NA	DL	NA	A DL algorithm in HSV color space was designed to effectively improve the accuracy of diagnosis and reduce the cost
Case control study	Bravo *et al*[59], 2018	NA	Supervised learning model	NA	Supervised learning model could help to detect polyps more than 5 mm automatically with high accuracy
Review	de Lange *et al*[60], 2018	NA	CAD system	NA	CAD system could eliminate the leakage rate of polyps, thus avoiding polyps from developing into CRC
Case control study	Mahmood *et al*[61], 2018	NA	CAD system	NA	CAD system combined with depth map could more accurately identify polyps or early cancer tissue
Retrospective study	Mo *et al*[62], 2018	16	DL	Compared the performance of multiple algorithms in the same dataset	DL was in the leading position in many aspects such as the performance of evolutionary algorithm, and was an effective clinical method
Case control study	Zhu *et al*[63], 2010	50	CAD system	Through the database of 50 patients, the performance differences of different segmentation strategies were compared	Initial polyp candidates could greatly facilitate the FP reduction process of CAD system
Case control study	Komeda *et al*[64], 2017	1200	CNN-CAD system	The efficiency of CNN-CAD system was evaluated by maintaining cross validation	CNN-CAD system can quickly diagnose colorectal polyp classification

				for 10 times	
Retrospective study	Zhang *et al*[65], 2018	18	CNN-CAD system	Through the video of 18 cases of colonoscopy, the efficiency of polyp detection between CNN-CAD system and existing methods was compared	CNN-CAD system can reduce the chance of missed diagnosis of polyps
Case control study	Zhu *et al*[66], 2019	357	CNN	The diagnostic performance of CNN was trained, fine-tuned, and evaluated using endoscopic data of 357 patients, and compared with that of manual diagnosis	The sensitivity of CNN optical diagnosis is higher than that of endoscopy, but the specificity is lower than that of endoscopy
Retrospective study	Akbari *et al*[67], 2018	300	FCN	The polyp segmentation method based on CNN was evaluated using CVC ColonDB database	FCN proposed a new method of image block selection and the probability map was processed effectively
Retrospective study	Yu *et al*[68], 2017	NA	3D-FCN	NA	3D-FCN could learn representative spatiotemporal features, and it had strong recognition ability
Case control study	Yamada *et al*[69], 2019	4395	AI	The AI system was trained through a large amount of data to make it sufficient to detect missed non polypoid lesions with high accuracy	AI could automatically detect the early features of CRC and improve the early detection rate of CRC
Retrospective study	Lund *et al*[71], 2019	20	DL	Polyp video dataset was used as training data. At the same time, a 5-fold cross validation method was used to evaluate the accuracy of the system	DL could improve the network training efficiency of polyp detection accuracy
Meta-analysis	Takamaru *et al*[73], 2020	NA	Endocytoscopy	NA	AI combined with endocytoscopy could greatly improve the efficiency of optical biopsy of CRC
Review	Djinbachian *et al*[76], 2019	NA	AI	NA	The sensitivity of optical diagnosis based on AI could be comparable to that of experienced endoscopists
Retrospective study	Kudo *et al*[77], 2019	69142	EndoBRAIN	A retrospective comparative analysis was performed between EndoBRAIN and 30 endoscopists on the diagnostic performance of endoscopic images in the same dataset	In the image of color cell endoscopy, EndoBRAIN could distinguish between tumor and non-tumor lesions accurately
Retrospective study	Mahmood *et al*[78], 2018	NA	CRF	NA	CRF estimated the depth of the colonoscopy image and reconstructed the surface structure of the colon
Case control study	Jian *et al*[81], 2018	2772	FCN	Quantitative comparison of manual and AI segmentation results of 2772 cases of CRC in MRI images	FCN was helpful for accurate segmentation of colorectal tumors
Case control study	Sivaganesan[82], 2016	20	RNN-ALGA	In the same database, milestone algorithms such as graph cut and level set were compared with RNN-ALGA algorithm	RNN-ALGA is suitable for abdominal slice of CT image, which can improve the accuracy and time efficiency of structure segmentation
Case control study	Gayathri *et al*[83], 2015	NA	NN	NA	NN can help to remove the colonic effusion and obtain the ideal colon segmentation effect
Retrospective study	Therrien *et al*[84], 2018	NA	SVM, CNN	NA	Using multiple datasets to train SVM and CNN could more accurately distinguish CRC staining tissue than single dataset
Case control study	Sun *et al*[85], 2019	NA	ML	NA	ML increased the chance of recognizing tumor bud by narrowing the region, thus

					providing effective tissue classification
Case control study	Shi *et al*[86], 2010	NA	DS-STM	NA	DS-STM could reduce the cost of diagnosis
Case control study	Su *et al*[87], 2012	212	MVMTM	The training set included 124 cases. The validation set included 88 cases. Comparedthe diagnostic efficiency of different methods for CRC	Compared with the traditional ML method, MVMTM has the advantages of low cost
Case control study	Kunhoth *et al*[88], 2017	80	Multispectral image acquisition system	A group of 20 samples were selected from 4 different types of colorectal cells. Compared the accuracy of different feature extraction methods	The database developed by this system had high classification accuracy
Case control study	Wang *et al*[89], 2018	1290	DL	Through the data of 1290 patients, an AI algorithm for real-time polyp detection was developed and verified	Compared with ML, DL could detect polyps in real time and reduce the cost
Meta-analysis	Barua *et al*[90], 2021	NA	AI	NA	AI based polyp detection system could increase the detection of small non-progressive adenomas and polyps
Randomized controlled study	Gong *et al*[91], 2020	704	ENDOANGEL system	704 patients were randomly assigned to use the ENDOANGEL system for colonoscopy or unaided (control) colonoscopy to compare the efficiency of ENDOANGEL system with conventional colonoscopy	The system significantly improved the detection rate of adenoma in colonoscopy
Meta-analysis	Lui *et al*[92], 2020	NA	AI	NA	AI system could improve the detection rate of adenoma and reduce the missed lesions in real-time colonoscopy
Case control study	Rodriguez-Diaz *et al*[93], 2011	134	A diagnostic algorithm with ESS	80 patients were randomly assigned to the training set, and the remaining 54 patients were assigned to the test set for prospective verification by the new algorithm	The algorithm with ESS reduced the risk and cost of biopsy, avoided the removal of non-neoplastic polyps, and reduced the operation time
Case control study	Kondepati *et al*[94], 2007	37	ANN	The tumor recognition accuracy of different algorithms was compared by collecting the spectra of cancer tissue and normal tissue	The spectrum was divided into cancer tissue group and normal tissue group by ANN, and the accuracy was 89%
Case control study	Angermann *et al*[95], 2016	NA	AL	NA	AL helped to realize real-time detection and distinguish between polyps and cancer tissues
Case control study	Ayling *et al*[96], 2019	619	ColonFlagTM	Through the clinical data of 619 patients, the performance of different systems in detecting CRC and high adenoma was compared	ColonFlagTM could help special patients establish an appropriate safety net
Meta-analysis	Tian *et al*[97], 2020	4560	EPE	Ten randomized controlled trials were included and 4560 participants were included for meta-analysis	EPE could guide the intestinal preparation of patients undergoing colonoscopy, and improve the detection rate of polyps, adenomas, and sessile serrated adenomas
Retrospective study	Javed *et al*[98], 2018	NA	QSL	NA	The prevalent communities found by QSL represented different tissue phenotypes with biological significance
Case control study	Wang *et al*[99], 2019	328	ANN	Different diagnostic models were established by back propagation and other	ANN combined with gene expression profile data could improve the diagnosis mode of

				methods, and the performance of each model was evaluated by cross validation test	CRC
Case control study	Battista *et al*[100], 2019	345	ANN	The diagnostic performance and FP of the new model were measured in the experimental group (patients with CRC) and the control group (patients with good health)	ANN could help to establish an easily available, low-cost mathematical tool for CRC screening
Review	Zhang *et al*[101], 2021	NA	ML	NA	ML based on cell-free DNA and microbiome data helped diagnose CRC
Case control study	Wang *et al*[102], 2021	9631	DCNN	The diagnostic accuracy of AI tools and experienced expert pathologists was compared through the same database	A novel strategy for clinic CRC diagnosis using weakly labeled pathological whole-slide image patches based on DCNN
Review	Jones *et al*[103], 2021	NA	AI	NA	Electronic health record type data combined with AI could help diagnose early cancer
Case control study	Lorenzovici *et al*[104], 2021	33	A computer aided diagnosis system	The accuracy of the system in diagnosing CRC was tested through a dataset of 33 patients	The system used ML to improve the accuracy of CRC diagnosis
Review and Meta-analysis	Xu *et al*[105], 2021	NA	CNN	NA	Through the comparative study of online database, CNN system had good diagnostic performance for CRC
Case control study	Öztürk *et al*[106], 2021	NA	CNN	NA	CNN was the most successful method that could effectively classify gastrointestinal image datasets with a small amount of labeled data
Review	Echle *et al*[107], 2021	NA	DL	NA	DL could directly extract the hidden information from the conventional histological images of cancer, so as to provide potential clinical information

NA: Not available; DL: Deep learning; ML: Machine learning; AL: Active learning; QSL: Quasi-supervised learning; CNN: Convolutional neural network; CRC: Colorectal cancer; SVM: Support vector machine; CAD: Computer-aided diagnosis; CTC: Computed tomography colonography; CT: Computed tomography; FP: False-positive rate; DCNN: Deep convolutional neural network; CADe: Computer-aided detection; 3D: Three-dimensional; MRI: Magnetic resonance imaging; AI: Artificial intelligence; R-CNN: Region-based convolutional neural network; SC-CNN: Space-constrained convolutional neural network; PHPs: Persistent homology maps; HSV: Hue, saturation, value; FCN: Fully convolutional network; CRF: Conditional random field; DS-STM: Diagnosis strategy of serum tumor maker; MVMTM: Multiple tumor markers with multiple cut-off values; ANN: Artificial neural network.

included an increased consistency rate after multiple disciplinary team implementation, a low consistency rate in elderly patients, and a high consistency rate in patients receiving chemotherapy. The results proved that Watson for Oncology might be helpful to simulate the effect of multiple disciplinary teams. Using evidence-based guidelines and simplifying treatment pathways, multidisciplinary care could provide best practices[110]. It is crucial to achieving personalized treatment since radiotherapy and chemotherapy are very painful. However, it is impossible to individualize patient treatment because the clinical situation of patients cannot easily link with DNA mutation[111]. Siddiqi *et al*[111] designed a MATCH system that provided a unique combination of clinical and genetic sequence data and constructed a database for all users. The MATCH system was currently providing hundreds of data samples, including clinical information, tumor markers, proteome sequences, gene inhibitors, *etc.* The importance of all data attributes and the corresponding processing information were modifiable[111]. Moreover, the system was developed with web services, which guaranteed interoperability among hospitals, pharmaceutical laboratories, and research centers, allowing them to access and exchange samples, information, and data models. The MATCH system helped identify the correlation between medical features so that oncologists could understand each patient's individual situation[111]. Nanorobots are expected to become intelligent drug delivery systems that respond to small molecular triggers[112]. Felfoul *et al*[113] developed a nanorobot that could deliver drugs to cancer cells. The robot sensed the concentration of hypoxia and

delivered drugs in the "anoxic area" generated by the active proliferation of cancer cells. The robot achieved an accurate effect of attacking cancer tumors[113]. Li *et al* [112] developed a nanorobot, which could kill cancer cells by releasing procoagulant substances in the cancer tissue, interrupting the blood supply to the cancer tissue. The greatest progress of robots is that it can significantly improve the targeting of chemotherapy drugs and reduce the killing effect of chemotherapy drugs on human normal tissues.

ML in immunotherapy pathway

Computational pathology can help obtain complete and repeatable datasets to promote individualized prediction of immunotherapy. ML can help evaluate the expression of immunohistochemical markers, tumor morphology, and the spatial distribution of tumor-infiltrating lymphocytes. The methylome group features queried by ML are proved to be suitable for predicting the response to immunosuppressive checkpoint inhibitors. Similar to image analysis, this method considers both tumor cells and reactive cells. The immune profiling is detected by spatial analysis and multiplexing of tumor immune cell interaction, and it is used as a predictor of patients' response to cancer treatment[114]. ML can be used to inhibit the Wnt/beta-catenin signaling, which is beneficial in cancer therapy[115], and it has the potential to provide new therapeutic strategies for patients by recognizing the interaction of tumor cells [114].

AI in endoscopic and surgical therapy

The estimation of the invasion depth is an important step in successfully implementing endoscopic submucosal dissection[116]. At present, narrow-band imaging with magnifying endoscopy is a practical method to estimate the invasion depth of CRC. Lee *et al*[116] used AI to interpret the cell endoscope images. Processing thousands of images, the algorithm could diagnose more than 90% of invasive CRC in hundreds of images detected[116]. Although the incidence of lymph node metastasis is relatively low, most T1 CRCs still need to undergo colectomy and lymphadenectomy [117]. Ichimasa *et al*[117] used the data of hundreds of patients in the AI model. The model analyzed 45 clinical and pathological factors and predicted positive or negative lymph node metastasis. The operation specimen is the gold standard of lymph node metastasis. Model validation results showed that patients received many unnecessary surgeries without lymph node metastasis[117]. AI can reduce unnecessary surgeries after endoscopic resection of T1 CRCs by predicting the presence of lymph node metastasis[117].

Compared with open surgery, a minimally invasive one is superior in short-term prognosis and long-term efficacy[118]. With the increasing popularity of laparoscopic surgery, the number of robotic surgeries is also growing. Surgeons can control the robot system 100% and perform more accurate operations at any time[119]. Kim[119] reported an animal experiment in which the effect of using smart tissue autonomous robots was comparable or even superior to open surgery, laparoscopic surgery, or robotic surgery[119]. The smart tissue autonomous robot integrates the sewing tool, robot arm, force sensor, and camera in hardware and software. The robot has the ability to stitch soft tissue. The efficiency of the robot sutured on the plane was 5 times faster than that of the surgeons, and 9 times faster than that of the surgeons using laparoscopic manual tools. Experiments also showed that the stitching robot was more accurate and consistent[120]. Compared with the Da Vinci Si robot system, the new Da Vinci Xi increased more flexibility of operation, and it was expected to promote the performance of multi quadrant surgery[121]. The clinicopathological characteristics and perioperative outcomes of patients with two kinds of robot systems were analyzed. The results showed that the ileostomy rate of Xi group was low, the operation time was short, the amount of bleeding was small, and the recovery was fast [121]. Surgeons can input operation instructions, order medical robots to perform complicated operations, and constantly monitor the operation on the monitor. During the operation, the surgeon can see the anatomical structure without opening the abdomen. Because the fluorescent dye is injected before the operation, the malignant cells and tissues can be visible. As a result, doctors can remove lesions more precisely [119]. Because of the precise recognition and detailed operation of robotic surgery, the learning curve of robotic colorectal surgery is shorter than that of laparoscopic surgery.

Robotic surgeries are beneficial in minimally invasive surgery of tumors, such as high-resolution and stable 3D views, optimal *in situ* free movement, and elimination of natural tremors[118]. However, in the face of a real surgical suture, the robot exposes its limitations. The complex structure of the human body requires the robot to spend

much time processing information about the anastomosis, which is obviously not beneficial in the time-consuming operation. Therefore, improving the image recognition and processing ability of the robot is the right direction to improve and develop the robot's autonomous stitching[120]. Compared with traditional laparoscopic surgery, robot surgery has some benefits, such as less urinary and sexual dysfunction and less intraoperative blood loss. However, more powerful evidence is needed[122]. Due to the high cost of robot, it will take a while to collect the data of robot surgery. However, as competition can decrease the price of robotic surgical systems, its promotion will be accelerated in the future. In robotic CRC surgery, many limitations have presented, such as the lack of unified technical standards and excessive dependence on surgical robot equipment. The problems will be solved by establishing training system and integrating medicine, research, and production. During clinical studies and large data analysis, robotic surgery will be the new development trend of colorectal surgery[122]. Other studies also described the application of AI in the therapy of CRC[123-127] (Table 2).

USE OF AI IN PROGNOSIS EVALUATION OF CRC

As one of the most common cancers globally, CRC is a result of multi-step and multi-factor action. The key to early diagnosis and improving the overall survival rate is determining the high-risk population[128]. Some related risk factors may increase the possibility of CRC, such as age, lifestyle, personal disease history, and genetic syndrome[129]. In order to establish a risk prediction model of CRC, appropriate feature selection is needed. It is important to identify features with predictive power for taking appropriate interventions to address risks[130]. Each AI technology generates different important attributes to evaluate tumor prognosis based on potential biases and assumptions. Based on the accuracy and the minimum deviation, it is clear that the most significant tumor characteristics are lymphocyte infiltration, Dukes stage, age, and mitotic count[131]. Tumor invasiveness score is a new prognostic factor for predicting tumor stage in colon cancer patients[132]. It helps use ML to increase patient ethnicity in cancer survivability prediction and support personalized general medicine[133]. Most medical studies concentrate on treatment and etiology rather than prediction because prediction tends to be uncertain and risky. The decision tree classifier can predict recurrence or death according to various factors. It is beneficial for doctors to make further treatment decisions and avoid unnecessary treatments[134]. An accurate prognosis is a basis of making an appropriate treatment plan for cancer patients. Because of the heterogeneity of the disease and the inherent limitations of the pathological reporting system, the outcomes are very different for patients in similar stages of pathology. ML used different types of features that could be easily collected from immunofluorescence images to predict phase II mortality, and ML had more accuracy than current clinical guidelines[135].

ML in prognosis evaluation

The molecular subtype of CRC can be used as a prognostic indicator of relapse-free survival rate. The determination of molecular subtype depends on the analysis of hundreds of genes[136]. Popovici *et al*[136] proposed a method to recognize CRC molecular subtypes from conventional histological images based on an SVM classifier. They used the DCNN to extract the local descriptors and then construct the dictionary representation of each tumor sample. A set of SVM classifiers were trained to solve different binary decision problems. The combined output was used to predict the molecular subtype. The overall accuracy of the results was very high[136]. It was beneficial to improve the accuracy of prognosis prediction. Zhang e*t al*[128] collected genetic variation and environmental information of CRC patients and cancer-free controls, trained the model with the large data, and established a multi-method integrated model. The model could effectively predict CRC risk[128]. The improved heterogeneous integrated learning model and generalized kernel recursive maximum correlation entropy algorithm had higher prediction ability than SVM[128]. ML is used to extract disease prediction models from electronic medical records[137]. ML can also solve many electronic medical record data, such as timeliness, imprecision, and integrity[129,138]. Hoogendoorn *et al*[137] could extract useful information from consulting notes, and the prediction performance of the ontology-based extraction method was significantly beyond the age and gender benchmark. It has been proved that the best way to predict CRC is by linking medical record texts with medical concepts[137].

Table 2 Artificial intelligence in treatment of colorectal cancer

Type of study	Ref.	Method	Conclusion
Retrospective study	Passi *et al*[109], 2015	DSS system	DSS system used follow-up data as a knowledge source to generate appropriate follow-up recommendations for patients receiving treatment
Retrospective study	Lee *et al*[110], 2018	Watson for Oncology	Watson for Oncology could provide evidence-based treatment advice for oncologists
Retrospective study	Siddiqi *et al*[111], 2008	MATCH system	MATCH system could provide hundreds of data samples to help doctors choose the most personalized treatment plan
Retrospective study	Li *et al*[112], 2018	Nanorobot	Nanorobots were relatively safe and immune inert. DNA nanorobots might represent a strategy for precise drug delivery in cancer treatment
Experimental study	Felfoul *et al*[113], 2016	Nanorobot	The robot achieved an accurate effect of attacking cancer tumors
Review	Koelzer *et al*[114], 2019	ML	The combination of ML and computational pathology could inform the clinical choice and prognosis stratification of CRC patients
Retrospective study	Lee *et al*[116], 2019	Narrow-band imaging	Narrow-band imaging helped doctors to predict the histology of colorectal polyps and estimate the depth of invasion
Meta-analysis, Case control study	Ichimasa *et al* [117], 2018	AI	AI could reduce unnecessary surgery after endoscopic resection of stage T1 CRC without loss of lymph node metastasis
Review	Kirchberg *et al* [118], 2019	Operation robot	Robotic surgery had great potential, but it still needed high-quality evidence-based medicine
Experimental study	Leonard *et al* [120], 2014	Smart tissue autonomous robot	Smart tissue autonomous robot was more accurate than surgeons using the most advanced robotic surgical system
Case control study	Huang *et al*[121], 2019	Operation robot	The operation robot had the advantages of short operation time, low estimated bleeding, and fast recovery after operation
Review	Zheng *et al*[122], 2020	Operation robot	There were some limitations, such as the disunity of technical standards and the excessive dependence on surgical robot equipment
Review	Mitsala *et al*[123], 2021	Computer-assisted drug delivery techniques	The technology could help to enhance the sensitivity and accuracy of targeted drugs
Case control study	Aikemu *et al* [124], 2020	AI	AI provided personalized and novel evidence-based clinical treatment strategies for CRC
Review	Hamamoto *et al* [125], 2020	AI	AI provided a variety of new technologies for the treatment of CRC, such as surgical robots, drug localization technology, and various medical devices
Review	Pritzker[126], 2020	AI	AI could screen individual biomarkers for comprehensive and individualized treatment of colon cancer with low toxicity
Experimental study	Ding *et al*[127], 2020	AI	The drug dose optimization technology based on AI could achieve more accurate individualized treatment than traditional methods

AI: Artificial intelligence; CRC: Colorectal cancer; DSS: Decision support system; ML: Machine learning.

The visual estimation of stroma ratio in microscopic images provides a strong predictor of survival rate in patients with CRC[139,140]. However, visual assessment is highly influenced by the observer and interstitial variation. Based on supervised learning, an objective quantitative method of tumor and stroma was established. Compared with the visual estimation of pathologists, the automatic tissue quantitative method was reliable and practical because it provided a new way to evaluate the prognosis and was crucial to predicting the tumor's survival ability[139]. Wang *et al* [141] developed a two-stage model to predict the survival of patients with advanced cancer. The first stage predicted whether patients could survive for more than 5 years. The second stage predicted the exact survival time of patients who could not survive for 5 years (in months). With low prediction error and good generalization performance, the two-stage model could help make treatment decisions, improve patient satisfaction, save medical resources, and reduce medical costs[141]. Based on the knowledge representation method of probability, Oliveira *et al*[142] designed a Clinical Decision Support System (CDSS) which, based on the cancer patients' records and the precise knowledge of experts, could propose an effective treatment scheme and solve the uncertainty of prognosis after surgery[142]. CDSS could complete four basic tasks: Data organization, data collection, the combination of various principles and specific data, and user-friendly display of analysis results. CDSS screened out appropriate

treatment methods from the aspects of curative effect, total survival rate, and side effect rate[143]. By comparing the treatment and prognosis of 250 cancer patients, Aikemu *et al*[124] found that Watson for Oncology could replace oncologists to provide patients with cutting-edge medical research and knowledge to a certain extent. It was also believed that the use of Watson for Oncology and other decision support tools could help achieve the promise of precision medicine[124].

Although resection of colon polyps can reduce the incidence rate and mortality of CRC by 75%, there is no individualized surveillance plan for polyp recurrence risk. Harrington *et al*[144] extracted polyp features from colonoscopy and pathological reports. The features extracted from these records and other demographic and anthropometric information were used to develop and compare ML models to predict polyp recurrence. The evaluation of the ML model further emphasized the important characteristics of predicting polyp recurrence from population and health records. RF model could detect patients with a high risk of recurrence and promote frequent follow-ups [144]. It is of great significance for individualized medical treatment. In order to improve the classification of polyps, Xie *et al*[145] proposed biometric modeling and ML methods to build polyp classifiers and screened the results of colonoscopy in a Chinese formation. The results showed that the RF model could improve the prediction performance compared with other methods[145]. Xie *et al*[145] also provided evidence that emotional state might be an influential factor in the early growth of CRC in China.

DL in prognosis evaluation

A deep network can directly predict the prognosis of CRC according to the morphological characteristics of tumor tissue samples[61]. Patients with CRC will benefit from the detection of TB, which is a reliable prognostic biomarker. DL can greatly reduce the number of FPs by detecting TB in H&E stained sections[146]. Zhao *et al*[147] proposed a DL model for automatic tumor-stroma ratio quantification using HE staining images of CRC. The model could eliminate the errors caused by traditional visual evaluation and reduce the work intensity of pathologists. Therefore, Zhao *et al* [147] believed that the model was suitable for clinical practice and might be helpful for clinical prognosis prediction and decision-making. Multimodal Deep Boltzmann Machine (DBM) is a DL structure used to predict patients' survival time. Syafiandini *et al*[148] integrated gene expression and clinical data into a new data form. The new data had few eigenvalues. In the multi-mode DBM architecture, these data were extracted from the joint hidden layer to identify gene subtypes, predict the response to a certain treatment, and find the most suitable treatment for patients[148]. Roadknight *et al*[149] described a dataset on the cellular and physical conditions of CRC patients who underwent surgical resection. These data provided unique immune status information for tumor resection, tumor classification, and postoperative survival[149]. Roadknight *et al*[149] studied the clustering and ML of these data to prove that the integrated method could predict the prognosis of patients. Compared with SVM, the better way to predict the tumor-node-metastasis stage from immunohistochemical markers is to use the anti-learning method[149]. Compared with other algorithms, the anti-learning method can more accurately predict cancer stage and survival rate from immune attributes[6].

SSL in prognosis evaluation

SSL methods use labeled or unlabeled data and graph regularization to predict patient survival and cancer recurrence[150,151]. The data of gene expression is transformed into the graph structure of SSL, and the data of protein interaction and gene expression are integrated to select gene pairs[151]. SSL methods can result in more accurate prediction than traditional SVM[11,150]. Recognition of cancer-related mutations is essential for understanding the cancer genomes that cause cancer gene activation or tumor suppressor gene inactivation[152]. Du *et al*[152] proposed a new feature selection method based on supervised learning that could identify gene mutations. The model was composed of the best features in candidate features' set with rotation forest. The method had a high accuracy and high prediction performance[152]. Chi *et al*[153] used the semi-supervised logistic regression method to establish the clinical prediction model of CRC survival risk. The performance of the model was strictly compared with that of other supervised learning models[153]. The model of CRC survival risk prediction established by the SSL method had good correction ability, popularization, interpretability, and clinical practicability. Other commonly used supervised learning methods, such as SVM, RF, and NN, showed poor calibration performance[153]. The SSL model might have more potential to develop a better risk prediction model in the actual clinical environment than the supervision model[153].

Other algorithms of AI in prognosis evaluation

The CRC recurrence support (CARES) system guided the prognosis by comparing the patients with new CRC and those with previous CRC to determine the high-risk group. As a result, only high-risk patients could receive more stringent examinations with reduced medical costs, while low-risk patients could be free from frequent and unnecessary examinations[154]. Immune cores could predict the prognosis of patients with colon cancer, and AI could detect additional prognostic markers on pathological sections. Digital tumor parameters (DGMate) were used to detect the digital parameters related to prognosis in tumor cells. The higher density of CD3+ tumor core, CD3+ invasive margin, and CD8+ tumor core was found, and the longer relapse-free survival was reported. CD3+ tumor core had a similar value to the classical CD3/CD8 immune core in prognosis. It was indicated that AI could help pathologists determine the prognosis of patients with colon cancer, which might improve patient treatments[155]. The existing methods describe the coordination among multiple genes by the additive representation of expression spectrum and use a fast heuristic method to identify the disjointed subnetworks. The methods may not be suitable for the potential combination of the disjointed genes[156]. Chowdhury *et al*[156] designed the Crane algorithm to solve this problem and proposed that the Crane algorithm was better than the addition algorithm in predicting CRC metastasis. In addition, AI could also be used to build CRC education software, whose menu contained an introduction, signs and symptoms, risk factors, preventive measures, and CRC screening procedures. The education software could achieve publicity and popularization of common sense through the communication between clinicians and patient representatives[157]. Other study also described the application of AI in the prognosis of CRC [158] (Table 3).

DISCUSSION

AI plays an important role in the fields of computer, internet, and vehicle engineering. The four main directions of future medical development are "personalization, precision, minimal invasion, and remoteness"[159]. In the field of medicine, first, AI gradually shows its advantages in disease diagnosis, treatment, and prognosis. CRC is one of the common human cancers, and its early diagnosis and standardized treatment have a profound impact on the prognosis. The development of AI for CRC has gone through the following stages: (1) Understanding cancer at the molecular and cellular levels through DL; (2) Assisting in the diagnosis of CRC according to images and pathological specimens; (3) Clinical drug designing and screening; and (4) Promoting the individualization of CRC diagnosis and treatment[159]. The diagnosis of CRC is mainly divided into imaging diagnosis and pathological diagnosis. Most of the imaging datasets are objective datasets with a high degree of information standardization. The CAD system based on DL realizes the automatic analysis and optimization of diversified images by extracting features from experts, extensive image training, making classification rules, and establishing mathematical models. Second, AI is beneficial to medical image analysis. Highly efficient image processing and analysis speed can quickly give auxiliary judgment results. Good sensitivity can reduce the missed diagnosis rate. Expert knowledge learning and quantitative data analysis can improve the quality of the basic inspection. Third, in clinical pathology, many digital sections of CRC have been accumulated, and some have been preliminarily developed with the technology of image recognition and DL. However, at present, AI cannot be separated from the auxiliary role. AI application at the functional level mainly includes disease diagnosis support and treatment decision support. The development of disease diagnosis support is active in treatment decision support. Advanced technologies are integrated with medicine and gradually play a necessary role in assisting diagnosis and early screening of major diseases.

Although AI is developing rapidly, it is still in the experimental stage and still faces many development bottlenecks. For example, first of all, the development of AI overemphasizes "probability association," but diseases always exist in unknown areas. How to combine data and medical knowledge is the key to the development of image AI. Second, AI-based DL requires much label data for training. Although labeled data has more influence on training results than algorithms, high-quality data acquisition for training is a big problem. Third, the image data standardization is low. The level of image system interaction operation in different hospitals is low. Moreover, the datasets of each imaging system are scattered all over the country with a low level of interaction. Forth, the difficulty of data annotation is great. The AI training requires a

Table 3 Artificial intelligence in prognosis evaluation of colorectal cancer

Type of study	Ref.	Method	Conclusion
Case control study	Zhang *et al*[128], 2017	Heterogeneous ensemble learning model	Heterogeneous ensemble learning model could use big data to identify high-risk groups of CRC patients
Retrospective study	Morgado *et al*[129], 2017	Decision support system	Decision support system could evaluate the risk of CRC by processing incomplete, unknown, or even contradictory data
Case control study	Anand *et al*[131], 1999	Intelligent hybrid system	Each AI technology produced a different set of important attributes. Intelligent hybrid system would be the trend of prognosis evaluation in the future
Case control study	Gupta *et al*[132], 2019	ML	ML could help to predict tumor stage and survival period
Case control study	Li *et al*[133], 2018	ML	Combining ML and database, clinicians might add race factor to evaluate prognosis
Case control study	Barsainya *et al*[134], 2018	Decision tree classifier	Decision tree classifier could predict recurrence and death according to various influencing factors
Cohort study	Dimitriou *et al*[135], 2018	ML	A framework for accurate prognosis prediction of CRC based on ML datasets
Case control study	Popovici *et al*[136], 2017	SVM	The accuracy of using SVM to distinguish CRC subtypes was very high
Experimental study	Hoogendoorn *et al* [137], 2016	AI	AI helped doctors to extract useful predictors from non-coding medical records
Experimental study	Kop *et al*[138], 2016	ML	The combination of ML and electronic medical records could help early detection and intervention
Case control study	Geessink *et al*[139], 2015	Supervised learning	Supervised learning helped to predict the survival ability of tumor, so as to accurately stratify the prognosis of tumor patients
Review	Wright *et al*[140], 2014	RF	RF could reduce the workload of pathologists by automatically calculating the area ratio of each slide
Meta-analysis	Wang *et al*[141], 2019	A two-stage ML model	Compared with the single-stage regression model, the two-stage model could obtain more accurate prediction results
Experimental study	Oliveira *et al*[142], 2013	CDSS	CDSS based on cancer patients records and knowledge could provide support for surgeons
Meta-analysis	Lo *et al*[143], 2000	CDSS	CDSS could select the appropriate treatment from the aspects of curative effect, overall survival rate, and side effect rate
Case control study	Harrington *et al* [144], 2018	ML	ML could be used to predict the risk of recurrence of colon polyps and cancer based on the pathological characteristics of medical records
Case control study	Xie *et al*[145], 2018	RF model	RF model helped to speculate the influencing factors of early CRC in China
Retrospective study	Bokhorst *et al*[146], 2018	DL	DL helped reduce FP by detecting tumor bud
Cohort study	Zhao *et al*[147], 2020	DL	The method allowed objective and standardized application while reducing the workload of pathologists
Retrospective study	Syafiandini *et al* [148], 2016	DBM	DBM helped to predict the survival time of cancer patients
Retrospective study	Roadknight *et al* [149], 2013	ML	ML helped predict the prognosis of patients according to the immune status and other information
Case control study	Cui *et al*[150], 2013	SSL	SSL improved the accuracy of predicting clinical results according to gene expression profile
Retrospective study	Park *et al*[151], 2014	SSL	SSL could improve the accuracy of predicting cancer recurrence
Retrospective study	Du *et al*[152], 2014	Supervised learning	Supervised learning could help to improve the accuracy of identifying cancer-related mutations
Case control study	Chi *et al*[153], 2019	Semi-supervised logistic regression method	Semi-supervised logistic regression method had better clinical prediction effect than supervised learning method
Review	Ong *et al*[154], 1997	CARES system	CARES system helped early detection of cancer recurrence in high-risk patients
Case control study	Reichling *et al*[155], 2020	DGMate	DGMate could judge the prognosis of tumor by detecting immunophenotype

Experimental study	Chowdhury *et al* [156], 2011	Crane algorithm	Crane algorithm helped to describe the coordination of multiple genes and effectively predicted the metastasis of CRC
Review	Mohamad *et al*[157], 2019	Nominal group technique	Nominal group technique was used in the content development of mobile app and the app used as a tool for CRC screening education
Retrospective study	Hacking *et al*[158], 2020	AI	AI could improve the prognosis of patients by increasing the diagnostic accuracy of slide images

CRC: Colorectal cancer; AI: Artificial intelligence; ML: Machine learning; SVM: Support vector machine; RF: Random forest; CDSS: Clinical Decision Support System; DBM: Deep Boltzmann Machine; SSL: Semi-supervised learning.

large amount of labeled image data, and the annotation needs to spend a lot of manual costs, which directly impacts the training results.

Meanwhile, the "black box" problem in ML raises several concerns clinically. ML can help read imaging and pathological pictures, recommend diagnosis and treatment options, and predict prognosis. However, due to the "black box" problem, the clinical application of AI tools progressed slowly. To further develop AI medicine, it is necessary to improve the interpretability of ML algorithms. The small steps of biological interpretation and clinical experience in ML algorithm can gradually solve the "black box" problem. In order to solve the above problems, data preprocessing is needed to complete the standardization, which requires the integration and fusion of heterogeneous data sets, such as images, physiological data, and information texts. At the same time, automatic software is used to analyze the medical image data quantitatively and extract a large number of features, including texture analysis, shape description, and other quantitative indicators.

The treatments for CRC are mainly surgery and chemotherapy. AI enables individual precision medicine by selecting appropriate treatment measures through big data analysis and comparison. At the same time, the development of robot technology provides a guarantee for the high accuracy of surgery and the high targeting of chemotherapy drugs. However, the quality of the data collection is still not enough to support AI to make treatment decisions independently. The complexity of the human body also reduces the speed of analysis and decision-making of AI in operations. In addition, robots cannot be widely used because of the high economic cost. Patients are often afraid of the unknown survival period after surgery, so giving a specific survival period can eliminate the psychological burden of patients. AI can predict the survival time and recurrence risk through patient information, surgery, and pathology and guide patients' prognosis and nursing. Therefore, high-quality, accurate data and standard operating specifications are required. In other words, the accuracy of prediction risk depends on the quality of the prognosis data, which in turn depends on the quality of data generated by diagnosis and treatment.

As diagnostic technology evolves, the information available to doctors is becoming more and more complex. In terms of treatment, new drugs are constantly developed, and new treatment schemes and methods are emerging. It is challenging for busy clinicians to have enough time and energy to obtain, screen, and use the information. With the continuous development of AI technology and image recognition, and the continued improvement of other aspects, AI will play an important role in CRC diagnosis and treatment. Therefore, the establishment of an AI standard system will be the top priority of future development. The standardization of images, features, medical record information, and other datasets will improve the accuracy of diagnosis and treatment. DL and ML will fully be combined to enable robots to complete surgery independently. Medical services include not only medical technology but also the guidance of patients' mental health. In the future, robots will provide nursing and adjust the psychological state of patients. However, moral and ethical issues must be well considered for the proper use of AI robots in today's medical environment.

Various countries have been trying to establish ethical, legal, and regulatory compliance standards for AI development. But there are many difficulties before fully accepting AI robots. First, patients' trust and acceptance will become an important factor in developing AI robotic surgery. The "black box" that has been used in many non-surgical applications has little theoretical transparency. In the medical field, lack of transparency impairs the doctors and patients' trust and acceptance of AI. Second, the safety of AI robot surgery is still an important issue to be concerned. The development of AI robot surgery involves a series of security problems, such as patient information protection, network security, robot autonomy, and machine failure. If the control of the AI robot is lost due to external factors such as network transmission delay and hacker attack, the immeasurable loss will happen. Third, the

responsibility attribution of medical malpractice remains a problem. Given the limitations of AI robots, the issues of medical malpractice responsibility will lead to a debate about the gray area of law. The solution of this problem will boost AI development[160].

CONCLUSION

Currently, AI is in the era of weak AI and does not have communication capabilities. Therefore, the current AI technology is mainly used for image recognition and auxiliary analysis without in-depth communication with patients. With the continuous development of AI technology, the role of AI in the diagnosis and treatment of CRC will continue to increase until the robot can complete surgery independently. At that time, AI will change the medical technologies and even the medical model.

ACKNOWLEDGEMENTS

We thank the anonymous referees for their useful suggestions.

REFERENCES

1 **Sivapalaratnam S**. Artificial intelligence and machine learning in haematology. *Br J Haematol* 2019; **185**: 207-208 [PMID: 30729496 DOI: 10.1111/bjh.15774]
2 **Acs B**, Rantalainen M, Hartman J. Artificial intelligence as the next step towards precision pathology. *J Intern Med* 2020; **288**: 62-81 [PMID: 32128929 DOI: 10.1111/joim.13030]
3 **El Hajjar A**, Rey JF. Artificial intelligence in gastrointestinal endoscopy: general overview. *Chin Med J (Engl)* 2020; **133**: 326-334 [PMID: 31929362 DOI: 10.1097/CM9.0000000000000623]
4 **Min JK**, Kwak MS, Cha JM. Overview of Deep Learning in Gastrointestinal Endoscopy. *Gut Liver* 2019; **13**: 388-393 [PMID: 30630221 DOI: 10.5009/gnl18384]
5 **Onder D**, Sarioglu S, Karacali B. Automated labelling of cancer textures in colorectal histopathology slides using quasi-supervised learning. *Micron* 2013; **47**: 33-42 [PMID: 23415158 DOI: 10.1016/j.micron.2013.01.003]
6 **Roadknight C**, Aickelin U, Qiu G, Scholefield J, Durrant L. Supervised learning and anti-learning of colorectal cancer classes and survival rates from cellular biology parameters. Proceedings 2012 IEEE international conference on systems, man, and cybernetics. 2012: 797-802 [DOI: 10.1109/icsmc.2012.6377825]
7 **Chen Y**, Carroll RJ, Hinz ER, Shah A, Eyler AE, Denny JC, Xu H. Applying active learning to high-throughput phenotyping algorithms for electronic health records data. *J Am Med Inform Assoc* 2013; **20**: e253-e259 [PMID: 23851443 DOI: 10.1136/amiajnl-2013-001945]
8 **Le Berre C**, Sandborn WJ, Aridhi S, Devignes MD, Fournier L, Smaïl-Tabbone M, Danese S, Peyrin-Biroulet L. Application of Artificial Intelligence to Gastroenterology and Hepatology. *Gastroenterology* 2020; **158**: 76-94.e2 [PMID: 31593701 DOI: 10.1053/j.gastro.2019.08.058]
9 **Jagga Z**, Gupta D. Machine learning for biomarker identification in cancer research - developments toward its clinical application. *Per Med* 2015; **12**: 371-387 [PMID: 29771660 DOI: 10.2217/pme.15.5]
10 **Low SK**, Nakamura Y. The road map of cancer precision medicine with the innovation of advanced cancer detection technology and personalized immunotherapy. *Jpn J Clin Oncol* 2019; **49**: 596-603 [PMID: 31135897 DOI: 10.1093/jjco/hyz073]
11 **Shi M**, Zhang B. Semi-supervised learning improves gene expression-based prediction of cancer recurrence. *Bioinformatics* 2011; **27**: 3017-3023 [PMID: 21893520 DOI: 10.1093/bioinformatics/btr502]
12 **Gulati S**, Patel M, Emmanuel A, Haji A, Hayee B, Neumann H. The future of endoscopy: Advances in endoscopic image innovations. *Dig Endosc* 2020; **32**: 512-522 [PMID: 31286574 DOI: 10.1111/den.13481]
13 **Wang P**, Berzin TM, Glissen Brown JR, Bharadwaj S, Becq A, Xiao X, Liu P, Li L, Song Y, Zhang D, Li Y, Xu G, Tu M, Liu X. Real-time automatic detection system increases colonoscopic polyp and adenoma detection rates: a prospective randomised controlled study. *Gut* 2019; **68**: 1813-1819 [PMID: 30814121 DOI: 10.1136/gutjnl-2018-317500]
14 **Kang J**, Gwak J. Ensemble of instance segmentation models for polyp segmentation in colonoscopy images. *IEEE Access* 2019; **7**: 26440-26447 [DOI: 10.1109/access.2019.2900672]
15 **Eisner R**, Greiner R, Tso V, Wang H, Fedorak RN. A machine-learned predictor of colonic polyps based on urinary metabolomics. *Biomed Res Int* 2013; **2013**: 303982 [PMID: 24307992 DOI: 10.1155/2013/303982]
16 **Köküer M**, Naguib RN, Jancovic P, Younghusband HB, Green RC. Cancer risk analysis in families

with hereditary nonpolyposis colorectal cancer. *IEEE Trans Inf Technol Biomed* 2006; **10**: 581-587 [PMID: 16871728 DOI: 10.1109/titb.2006.872054]

17 **Bell CS**, Puerto GA, Mariottini G-L, Valdastri P. Six DOF motion estimation for teleoperated flexible endoscopes using optical flow: A comparative study. 2014 IEEE international conference on robotics and automation. 2014: 5386-5392 [DOI: 10.1109/icra.2014.6907651]

18 **Liu Z**, Wang S, Dong D, Wei J, Fang C, Zhou X, Sun K, Li L, Li B, Wang M, Tian J. The Applications of Radiomics in Precision Diagnosis and Treatment of Oncology: Opportunities and Challenges. *Theranostics* 2019; **9**: 1303-1322 [PMID: 30867832 DOI: 10.7150/thno.30309]

19 **Yang T**, Liang N, Li J, Yang Y, Li Y, Huang Q, Li R, He X, Zhang H. Intelligent imaging technology in diagnosis of colorectal cancer using deep learning. *IEEE Access* 2019; **7**: 178839-178847 [DOI: 10.1109/access.2019.2958124]

20 **Dalca A**, Danagoulian G, Kikinis R, Schmidt E, Golland P. Sparse classification for computer aided diagnosis using learned dictionaries. Medical Image Computing and Computer-Assisted Intervention, 2011: 537-545

21 **Regge D**, Halligan S. CAD: how it works, how to use it, performance. *Eur J Radiol* 2013; **82**: 1171-1176 [PMID: 22595503 DOI: 10.1016/j.ejrad.2012.04.022]

22 **Summers RM**, Handwerker LR, Pickhardt PJ, Van Uitert RL, Deshpande KK, Yeshwant S, Yao J, Franaszek M. Performance of a previously validated CT colonography computer-aided detection system in a new patient population. *AJR Am J Roentgenol* 2008; **191**: 168-174 [PMID: 18562741 DOI: 10.2214/AJR.07.3354]

23 **Chowdhury TA**, Whelan PF, Ghita O. A fully automatic CAD-CTC system based on curvature analysis for standard and low-dose CT data. *IEEE Trans Biomed Eng* 2008; **55**: 888-901 [PMID: 18334380 DOI: 10.1109/TBME.2007.909506]

24 **Nappi JJ**, Hironaka T, Yoshida H. Detection of colorectal masses in CT colonography: Application of deep residual networks for differentiating masses from normal colon anatomy. Medical imaging 2018: Computer-aided diagnosis. Bellingham: Spie-Int Soc Optical Engineering, 2018 [DOI: 10.1117/12.2293848]

25 **Taylor SA**, Iinuma G, Saito Y, Zhang J, Halligan S. CT colonography: computer-aided detection of morphologically flat T1 colonic carcinoma. *Eur Radiol* 2008; **18**: 1666-1673 [PMID: 18389248 DOI: 10.1007/s00330-008-0936-7]

26 **Summers RM**. Current concepts in computer-aided detection for CT colonography. 2010 7th IEEE international symposium on biomedical imaging: From nano to macro. 2010: 269-272 [DOI: 10.1109/isbi.2010.5490363]

27 **Lee JG**, Hyo Kim J, Hyung Kim S, Sun Park H, Ihn Choi B. A straightforward approach to computer-aided polyp detection using a polyp-specific volumetric feature in CT colonography. *Comput Biol Med* 2011; **41**: 790-801 [PMID: 21762887 DOI: 10.1016/j.compbiomed.2011.06.015]

28 **Nappi JJ**, Hironaka T, Regge D, Yoshida H. Deep transfer learning of virtual endoluminal views for the detection of polyps in CT colonography. Medical imaging 2016: Computer-aided diagnosis. Bellingham: Spie-Int Soc Optical Engineering, 2015 [DOI: 10.1117/12.2217260]

29 **Näppi J**, Frimmel H, Yoshida H. Virtual endoscopic visualization of the colon by shape-scale signatures. *IEEE Trans Inf Technol Biomed* 2005; **9**: 120-131 [PMID: 15787014 DOI: 10.1109/titb.2004.837834]

30 **van Wijk C**, van Ravesteijn VF, Vos FM, van Vliet LJ. Detection and segmentation of colonic polyps on implicit isosurfaces by second principal curvature flow. *IEEE Trans Med Imaging* 2010; **29**: 688-698 [PMID: 20199908 DOI: 10.1109/TMI.2009.2031323]

31 **Kim SH**, Lee JM, Lee JG, Kim JH, Lefere PA, Han JK, Choi BI. Computer-aided detection of colonic polyps at CT colonography using a Hessian matrix-based algorithm: preliminary study. *AJR Am J Roentgenol* 2007; **189**: 41-51 [PMID: 17579150 DOI: 10.2214/AJR.07.2072]

32 **Nappi JJ**, Pickhardt P, Kim DH, Hironaka T, Yoshida H. Deep learning of contrast-coated serrated polyps for computer-aided detection in CT colonography. Medical imaging 2017: Computer-aided diagnosis, 2017 [DOI: 10.1117/12.2255634]

33 **Ma J**, Dercle L, Lichtenstein P, Wang D, Chen A, Zhu J, Piessevaux H, Zhao J, Schwartz LH, Lu L, Zhao B. Automated Identification of Optimal Portal Venous Phase Timing with Convolutional Neural Networks. *Acad Radiol* 2020; **27**: e10-e18 [PMID: 31151901 DOI: 10.1016/j.acra.2019.02.024]

34 **Soomro MH**, De Cola G, Conforto S, Schmid M, Giunta G, Guidi E, Neri E, Caruso D, Ciolina M, Laghi A. Automatic segmentation of colorectal cancer in 3D MRI by combining deep learning and 3D level-set algorithm-a preliminary study. 2018 IEEE 4th middle east conference on biomedical engineering. 2018: 198-203 [DOI: 10.1109/mecbme.2018.8402433]

35 **Soomro MH**, Coppotelli M, Conforto S, Schmid M, Giunta G, Del Secco L, Neri E, Caruso D, Rengo M, Laghi A. Automated Segmentation of Colorectal Tumor in 3D MRI Using 3D Multiscale Densely Connected Convolutional Neural Network. *J Healthc Eng* 2019; **2019**: 1075434 [PMID: 30838121 DOI: 10.1155/2019/1075434]

36 **Wang D**, Xu J, Zhang Z, Li S, Zhang X, Zhou Y, Lu Y. Evaluation of Rectal Cancer Circumferential Resection Margin Using Faster Region-Based Convolutional Neural Network in High-Resolution Magnetic Resonance Images. *Dis Colon Rectum* 2020; **63**: 143-151 [PMID: 31842158 DOI: 10.1097/DCR.0000000000001519]

37 **Wu QY**, Liu SL, Sun P, Li Y, Liu GW, Liu SS, Hu JL, Niu TY, Lu Y. Establishment and clinical application value of an automatic diagnosis platform for rectal cancer T-staging based on a deep

neural network. *Chin Med J (Engl)* 2021; **134**: 821-828 [PMID: 33797468 DOI: 10.1097/CM9.0000000000001401]

38 **Joshi N**, Bond S, Brady M. The segmentation of colorectal MRI images. *Med Image Anal* 2010; **14**: 494-509 [PMID: 20378393 DOI: 10.1016/j.media.2010.03.002]

39 **Dabass M**, Vashisth S, Vig R. Review of classification techniques using deep learning for colorectal cancer imaging modalities. 2019 6th International Conference on Signal Processing and Integrated Networks, 2019 [DOI: 10.1109/spin.2019.8711776]

40 **Shiraishi T**, Shinto E, Nearchou IP, Tsuda H, Kajiwara Y, Einama T, Caie PD, Kishi Y, Ueno H. Prognostic significance of mesothelin expression in colorectal cancer disclosed by area-specific four-point tissue microarrays. *Virchows Arch* 2020; **477**: 409-420 [PMID: 32107600 DOI: 10.1007/s00428-020-02775-y]

41 **Pham TD**. Scaling of texture in training autoencoders for classification of histological images of colorectal cancer. Advances in neural networks, 2017: 524-532 [DOI: 10.1007/978-3-319-59081-3_61]

42 **Tiwari S**. An analysis in tissue classification for colorectal cancer histology using convolution neural network and colour models. *IJISMD* 2018; **9**: 1-19 [DOI: 10.4018/ijismd.2018100101]

43 **Sirinukunwattana K**, Ahmed Raza SE, Yee-Wah Tsang, Snead DR, Cree IA, Rajpoot NM. Locality Sensitive Deep Learning for Detection and Classification of Nuclei in Routine Colon Cancer Histology Images. *IEEE Trans Med Imaging* 2016; **35**: 1196-1206 [PMID: 26863654 DOI: 10.1109/TMI.2016.2525803]

44 **Koohababni NA**, Jahanifar M, Gooya A, Rajpoot N. Nuclei detection using mixture density networks. Machine learning in medical imaging, 2018: 241-248 [DOI: 10.1007/978-3-030-00919-9_28]

45 **Zhang X**, Chen G, Saruta K, Terata Y. An end-to-end cells detection approach for colon cancer histology images. 10th international conference on digital image processing, 2018 [DOI: 10.1117/12.2503067]

46 **Xu J**, Luo X, Wang G, Gilmore H, Madabhushi A. A Deep Convolutional Neural Network for segmenting and classifying epithelial and stromal regions in histopathological images. *Neurocomputing* 2016; **191**: 214-223 [PMID: 28154470 DOI: 10.1016/j.neucom.2016.01.034]

47 **Chen H**, Qi X, Yu L, Dou Q, Qin J, Heng PA. DCAN: Deep contour-aware networks for object instance segmentation from histology images. *Med Image Anal* 2017; **36**: 135-146 [PMID: 27898306 DOI: 10.1016/j.media.2016.11.004]

48 **Yoshida H**, Yamashita Y, Shimazu T, Cosatto E, Kiyuna T, Taniguchi H, Sekine S, Ochiai A. Automated histological classification of whole slide images of colorectal biopsy specimens. *Oncotarget* 2017; **8**: 90719-90729 [PMID: 29207599 DOI: 10.18632/oncotarget.21819]

49 **Saito A**, Cosatto E, Kiyuna T, Sakamoto M. Dawn of the digital diagnosis assisting system, can it open a new age for pathology? Medical imaging 2013: Digital pathology, 2013 [DOI: 10.1117/12.2008967]

50 **Jin Y**, Zhou C, Teng X, Ji J, Wu H, Liao J. Pai-wsit: An AI service platform with support for storing and sharing whole-slide images with metadata and annotations. *IEEE Access* 2019; **7**: 54780-54786 [DOI: 10.1109/access.2019.2913255]

51 **Qaiser T**, Tsang YW, Taniyama D, Sakamoto N, Nakane K, Epstein D, Rajpoot N. Fast and accurate tumor segmentation of histology images using persistent homology and deep convolutional features. *Med Image Anal* 2019; **55**: 1-14 [PMID: 30991188 DOI: 10.1016/j.media.2019.03.014]

52 **Chao WL**, Manickavasagan H, Krishna SG. Application of Artificial Intelligence in the Detection and Differentiation of Colon Polyps: A Technical Review for Physicians. *Diagnostics (Basel)* 2019; **9** [PMID: 31434208 DOI: 10.3390/diagnostics9030099]

53 **Zhou J**, Wu L, Wan X, Shen L, Liu J, Zhang J, Jiang X, Wang Z, Yu S, Kang J, Li M, Hu S, Hu X, Gong D, Chen D, Yao L, Zhu Y, Yu H. A novel artificial intelligence system for the assessment of bowel preparation (with video). *Gastrointest Endosc* 2020; **91**: 428-435.e2 [PMID: 31783029 DOI: 10.1016/j.gie.2019.11.026]

54 **de Almeida Thomaz V**, Sierra-Franco CA, Raposo AB. Training data enhancements for robust polyp segmentation in colonoscopy images. 2019 IEEE 32nd International Symposium on Computer-Based Medical Systems (CBMS), 2019: 192-197 [DOI: 10.1109/cbms.2019.00047]

55 **Azer SA**. Challenges Facing the Detection of Colonic Polyps: What Can Deep Learning Do? *Medicina (Kaunas)* 2019; **55** [PMID: 31409050 DOI: 10.3390/medicina55080473]

56 **Taha B**, Dias J, Werghi N, Ieee. Convolutional neural network as a feature extractor for automatic polyp detection. 2017 24th IEEE international conference on image processing, 2017: 2060-2064 [DOI: 10.1109/icip.2017.8296644]

57 **Yao H**, Stidham RW, Soroushmehr R, Gryak J, Najarian K. Automated Detection of Non-Informative Frames for Colonoscopy Through a Combination of Deep Learning and Feature Extraction. *Annu Int Conf IEEE Eng Med Biol Soc* 2019; **2019**: 2402-2406 [PMID: 31946383 DOI: 10.1109/EMBC.2019.8856625]

58 **McNeil MB**, Gross SA. Siri here, cecum reached, but please wash that fold: Will artificial intelligence improve gastroenterology? *Gastrointest Endosc* 2020; **91**: 425-427 [PMID: 32036947 DOI: 10.1016/j.gie.2019.10.027]

59 **Bravo D**, Ruano J, Gomez M, Romero E. Automatic detection of colorectal polyps larger than 5 mm during colonoscopy procedures using visual descriptors. 14th international symposium on medical information processing and analysis, 2018 [DOI: 10.1117/12.2511577]

60 **de Lange T**, Halvorsen P, Riegler M. Methodology to develop machine learning algorithms to improve performance in gastrointestinal endoscopy. *World J Gastroenterol* 2018; **24**: 5057-5062 [PMID: 30568383 DOI: 10.3748/wjg.v24.i45.5057]

61 **Mahmood F**, Durr NJ. Deep learning-based depth estimation from a synthetic endoscopy image training set. Medical imaging 2018: Image processing. Bellingham: Spie-Int Soc Optical Engineering, 2018 [DOI: 10.1117/12.2293785]

62 **Mo X**, Tao K, Wang Q, Wang G. An efficient approach for polyps detection in endoscopic videos based on faster R-CNN. 2018 24th international conference on pattern recognition, 2018: 3929-3934 [DOI: 10.1109/icpr.2018.8545174]

63 **Zhu H**, Fan Y, Lu H, Liang Z. Improving initial polyp candidate extraction for CT colonography. *Phys Med Biol* 2010; **55**: 2087-2102 [PMID: 20299733 DOI: 10.1088/0031-9155/55/7/019]

64 **Komeda Y**, Handa H, Watanabe T, Nomura T, Kitahashi M, Sakurai T, Okamoto A, Minami T, Kono M, Arizumi T, Takenaka M, Hagiwara S, Matsui S, Nishida N, Kashida H, Kudo M. Computer-Aided Diagnosis Based on Convolutional Neural Network System for Colorectal Polyp Classification: Preliminary Experience. *Oncology* 2017; **93** Suppl 1: 30-34 [PMID: 29258081 DOI: 10.1159/000481227]

65 **Zhang R**, Zheng Y, Poon CCY, Shen D, Lau JYW. Polyp detection during colonoscopy using a regression-based convolutional neural network with a tracker. *Pattern Recognit* 2018; **83**: 209-219 [PMID: 31105338 DOI: 10.1016/j.patcog.2018.05.026]

66 **Zhu X**, Nemoto D, Mizuno T, Nakajima Y, Utano K, Aizawa M, Takezawa T, Sagara Y, Hayashi Y, Katsuki S, Yamamoto H, Hewett DG, Togashi K. Identification of deeply invasive colorectal cancer on non-magnified endoscopic images using artificial intelligence. *Gastrointest Endosc* 2019; **89**

67 **Akbari M**, Mohrekesh M, Nasr-Esfahani E, Soroushmehr SMR, Karimi N, Samavi S, Najarian K. Polyp Segmentation in Colonoscopy Images Using Fully Convolutional Network. *Annu Int Conf IEEE Eng Med Biol Soc* 2018; **2018**: 69-72 [PMID: 30440343 DOI: 10.1109/EMBC.2018.8512197]

68 **Lequan Yu**, Hao Chen, Qi Dou, Jing Qin, Pheng Ann Heng. Integrating Online and Offline Three-Dimensional Deep Learning for Automated Polyp Detection in Colonoscopy Videos. *IEEE J Biomed Health Inform* 2017; **21**: 65-75 [PMID: 28114049 DOI: 10.1109/JBHI.2016.2637004]

69 **Yamada M**, Saito Y, Imaoka H, Saiko M, Yamada S, Kondo H, Takamaru H, Sakamoto T, Sese J, Kuchiba A, Shibata T, Hamamoto R. Development of a real-time endoscopic image diagnosis support system using deep learning technology in colonoscopy. *Sci Rep* 2019; **9**: 14465 [PMID: 31594962 DOI: 10.1038/s41598-019-50567-5]

70 **Allescher HD**, Weingart V. Optimizing Screening Colonoscopy: Strategies and Alternatives. *Visc Med* 2019; **35**: 215-225 [PMID: 31602382 DOI: 10.1159/000501835]

71 **Lund Henriksen F**, Jensen R, Kvale Stensland H, Johansen D, Riegler MA, Halvorsen P. Performance of data enhancements and training optimization for neural network: A polyp detection case study. 2019 IEEE 32nd International Symposium on Computer-Based Medical Systems (CBMS), 2019: 287-293 [DOI: 10.1109/cbms.2019.00067]

72 **Ahmad OF**, Soares AS, Mazomenos E, Brandao P, Vega R, Seward E, Stoyanov D, Chand M, Lovat LB. Artificial intelligence and computer-aided diagnosis in colonoscopy: current evidence and future directions. *Lancet Gastroenterol Hepatol* 2019; **4**: 71-80 [PMID: 30527583 DOI: 10.1016/S2468-1253(18)30282-6]

73 **Takamaru H**, Wu SYS, Saito Y. Endocytoscopy: technology and clinical application in the lower GI tract. *Transl Gastroenterol Hepatol* 2020; **5**: 40 [PMID: 32632391 DOI: 10.21037/tgh.2019.12.04]

74 **Rath T**, Morgenstern N, Vitali F, Atreya R, Neurath MF. Advanced Endoscopic Imaging in Colonic Neoplasia. *Visc Med* 2020; **36**: 48-59 [PMID: 32110657 DOI: 10.1159/000505411]

75 **Shahidi N**, Rex DK, Kaltenbach T, Rastogi A, Ghalehjegh SH, Byrne MF. Use of Endoscopic Impression, Artificial Intelligence, and Pathologist Interpretation to Resolve Discrepancies Between Endoscopy and Pathology Analyses of Diminutive Colorectal Polyps. *Gastroenterology* 2020; **158**: 783-785.e1 [PMID: 31863741 DOI: 10.1053/j.gastro.2019.10.024]

76 **Djinbachian R**, Dubé AJ, von Renteln D. Optical Diagnosis of Colorectal Polyps: Recent Developments. *Curr Treat Options Gastroenterol* 2019; **17**: 99-114 [PMID: 30746593 DOI: 10.1007/s11938-019-00220-x]

77 **Kudo SE**, Misawa M, Mori Y, Hotta K, Ohtsuka K, Ikematsu H, Saito Y, Takeda K, Nakamura H, Ichimasa K, Ishigaki T, Toyoshima N, Kudo T, Hayashi T, Wakamura K, Baba T, Ishida F, Inoue H, Itoh H, Oda M, Mori K. Artificial Intelligence-assisted System Improves Endoscopic Identification of Colorectal Neoplasms. *Clin Gastroenterol Hepatol* 2020; **18**: 1874-1881.e2 [PMID: 31525512 DOI: 10.1016/j.cgh.2019.09.009]

78 **Mahmood F**, Durr NJ. Topographical reconstructions from monocular optical colonoscopy images via deep learning. 2018 IEEE 15th international symposium on biomedical imaging, 2018: 216-219 [DOI: 10.1109/isbi.2018.8363558]

79 **Vidyasagar M**. Machine learning methods in the computational biology of cancer. *Proc Math Phys Eng Sci* 2014; **470**: 20140081 [PMID: 25002826 DOI: 10.1016/j.arcontrol.2017.03.007]

80 **Ebigbo A**, Palm C, Probst A, Mendel R, Manzeneder J, Prinz F, de Souza LA, Papa JP, Siersema P, Messmann H. A technical review of artificial intelligence as applied to gastrointestinal endoscopy: clarifying the terminology. *Endosc Int Open* 2019; **7**: E1616-E1623 [PMID: 31788542 DOI: 10.1055/a-1010-5705]

81 **Jian J**, Xiong F, Xia W, Zhang R, Gu J, Wu X, Meng X, Gao X. Fully convolutional networks

(FCNs)-based segmentation method for colorectal tumors on T2-weighted magnetic resonance images. *Australas Phys Eng Sci Med* 2018; **41**: 393-401 [PMID: 29654521 DOI: 10.1007/s13246-018-0636-9]

82 **Sivaganesan D**. Wireless distributive personal communication for early detection of collateral cancer using optimized machine learning methodology. *Wireless Personal Communicat* 2016; **94**: 2291-2302 [DOI: 10.1007/s11277-016-3411-9]

83 **Gayathri Devi K**, Radhakrishnan R. Automatic segmentation of colon in 3D CT images and removal of opacified fluid using cascade feed forward neural network. *Comput Math Methods Med* 2015; **2015**: 670739 [PMID: 25838838 DOI: 10.1155/2015/670739]

84 **Therrien R**, Doyle S. Role of training data variability on classifier performance and generalizability. Medical imaging 2018: Digital pathology, 2018 [DOI: 10.1117/12.2293919]

85 **Sun CY**, Liu W, Doyle S. Two-tier classifier for identifying small objects in histological tissue classification: Experiments with colon cancer tissue mapping. Medical imaging 2019: Digital pathology, 2019 [DOI: 10.1117/12.2512973]

86 **Shi J**, Su Q, Zhang C, Huang G, Zhu Y. An intelligent decision support algorithm for diagnosis of colorectal cancer through serum tumor markers. *Comput Methods Programs Biomed* 2010; **100**: 97-107 [PMID: 20346535 DOI: 10.1016/j.cmpb.2010.03.001]

87 **Su Q**, Shi J, Gu P, Huang G, Zhu Y. An algorithm designed for improving diagnostic efficiency by setting multi-cutoff values of multiple tumor markers. *Expert Syst Appl* 2012; **39**: 5784-5791 [DOI: 10.1016/j.eswa.2011.11.089]

88 **Kunhoth S**, Al Maadeed S. Building a multispectral image dataset for colorectal tumor biopsy. 2017 13th international wireless communications and mobile computing conference, 2017: 1745-1750 [DOI: 10.1109/iwcmc.2017.7986548]

89 **Wang P**, Xiao X, Glissen Brown JR, Berzin TM, Tu M, Xiong F, Hu X, Liu P, Song Y, Zhang D, Yang X, Li L, He J, Yi X, Liu J, Liu X. Development and validation of a deep-learning algorithm for the detection of polyps during colonoscopy. *Nat Biomed Eng* 2018; **2**: 741-748 [PMID: 31015647 DOI: 10.1038/s41551-018-0301-3]

90 **Barua I**, Vinsard DG, Jodal HC, Løberg M, Kalager M, Holme Ø, Misawa M, Bretthauer M, Mori Y. Artificial intelligence for polyp detection during colonoscopy: a systematic review and meta-analysis. *Endoscopy* 2021; **53**: 277-284 [PMID: 32557490 DOI: 10.1055/a-1201-7165]

91 **Gong D**, Wu L, Zhang J, Mu G, Shen L, Liu J, Wang Z, Zhou W, An P, Huang X, Jiang X, Li Y, Wan X, Hu S, Chen Y, Hu X, Xu Y, Zhu X, Li S, Yao L, He X, Chen D, Huang L, Wei X, Wang X, Yu H. Detection of colorectal adenomas with a real-time computer-aided system (ENDOANGEL): a randomised controlled study. *Lancet Gastroenterol Hepatol* 2020; **5**: 352-361 [PMID: 31981518 DOI: 10.1016/S2468-1253(19)30413-3]

92 **Lui TKL**, Leung WK. Is artificial intelligence the final answer to missed polyps in colonoscopy? *World J Gastroenterol* 2020; **26**: 5248-5255 [PMID: 32994685 DOI: 10.3748/wjg.v26.i35.5248]

93 **Rodriguez-Diaz E**, Castanon DA, Singh SK, Bigio IJ. Spectral classifier design with ensemble classifiers and misclassification-rejection: application to elastic-scattering spectroscopy for detection of colonic neoplasia. *J Biomed Opt* 2011; **16**: 067009 [PMID: 21721830 DOI: 10.1117/1.3592488]

94 **Kondepati VR**, Oszinda T, Heise HM, Luig K, Mueller R, Schroeder O, Keese M, Backhaus J. CH-overtone regions as diagnostic markers for near-infrared spectroscopic diagnosis of primary cancers in human pancreas and colorectal tissue. *Anal Bioanal Chem* 2007; **387**: 1633-1641 [PMID: 17205263 DOI: 10.1007/s00216-006-0960-x]

95 **Angermann Q**, Histace A, Romain O. Active learning for real time detection of polyps in videocolonoscopy. 20th conference on medical image understanding and analysis, 2016: 182-187 [DOI: 10.1016/j.procs.2016.07.017]

96 **Ayling RM**, Lewis SJ, Cotter F. Potential roles of artificial intelligence learning and faecal immunochemical testing for prioritisation of colonoscopy in anaemia. *Br J Haematol* 2019; **185**: 311-316 [PMID: 30714125 DOI: 10.1111/bjh.15776]

97 **Tian X**, Xu LL, Liu XL, Chen WQ. Enhanced Patient Education for Colonic Polyp and Adenoma Detection: Meta-Analysis of Randomized Controlled Trials. *JMIR Mhealth Uhealth* 2020; **8**: e17372 [PMID: 32347798 DOI: 10.2196/17372]

98 **Javed S**, Fraz MM, Epstein D, Snead D, Rajpoot NM. Cellular community detection for tissue phenotyping in histology images. Computational pathology and ophthalmic medical image analysis, 2018: 120-129 [DOI: 10.1007/978-3-030-00949-6_15]

99 **Wang Q**, Wei J, Chen Z, Zhang T, Zhong J, Zhong B, Yang P, Li W, Cao J. Establishment of multiple diagnosis models for colorectal cancer with artificial neural networks. *Oncol Lett* 2019; **17**: 3314-3322 [PMID: 30867765 DOI: 10.3892/ol.2019.10010]

100 **Battista A**, Battista RA, Battista F, Cinquanta L, Iovane G, Corbisieri M, Suozzo A. Development of a new mathematical tool for early colorectal cancer diagnosis and its possible use in mass screening. *J Int Math* 2019; **22**: 811-835 [DOI: 10.1080/09720502.2019.1649834]

101 **Zhang W**, Chen X, Wong KC. Noninvasive early diagnosis of intestinal diseases based on artificial intelligence in genomics and microbiome. *J Gastroenterol Hepatol* 2021; **36**: 823-831 [PMID: 33880763 DOI: 10.1111/jgh.15500]

102 **Wang KS**, Yu G, Xu C, Meng XH, Zhou J, Zheng C, Deng Z, Shang L, Liu R, Su S, Zhou X, Li Q, Li J, Wang J, Ma K, Qi J, Hu Z, Tang P, Deng J, Qiu X, Li BY, Shen WD, Quan RP, Yang JT, Huang LY, Xiao Y, Yang ZC, Li Z, Wang SC, Ren H, Liang C, Guo W, Li Y, Xiao H, Gu Y, Yun JP, Huang D, Song Z, Fan X, Chen L, Yan X, Huang ZC, Huang J, Luttrell J, Zhang CY, Zhou W,

Zhang K, Yi C, Wu C, Shen H, Wang YP, Xiao HM, Deng HW. Accurate diagnosis of colorectal cancer based on histopathology images using artificial intelligence. *BMC Med* 2021; **19**: 76 [PMID: 33752648 DOI: 10.1186/s12916-021-01942-5]

103 **Jones OT**, Calanzani N, Saji S, Duffy SW, Emery J, Hamilton W, Singh H, de Wit NJ, Walter FM. Artificial Intelligence Techniques That May Be Applied to Primary Care Data to Facilitate Earlier Diagnosis of Cancer: Systematic Review. *J Med Internet Res* 2021; **23**: e23483 [PMID: 33656443 DOI: 10.2196/23483]

104 **Lorenzovici N**, Dulf EH, Mocan T, Mocan L. Artificial Intelligence in Colorectal Cancer Diagnosis Using Clinical Data: Non-Invasive Approach. *Diagnostics (Basel)* 2021; **11** [PMID: 33799452 DOI: 10.3390/diagnostics11030514]

105 **Xu Y**, Ding W, Wang Y, Tan Y, Xi C, Ye N, Wu D, Xu X. Comparison of diagnostic performance between convolutional neural networks and human endoscopists for diagnosis of colorectal polyp: A systematic review and meta-analysis. *PLoS One* 2021; **16**: e0246892 [PMID: 33592048 DOI: 10.1371/journal.pone.0246892]

106 **Öztürk Ş**, Özkaya U. Residual LSTM layered CNN for classification of gastrointestinal tract diseases. *J Biomed Inform* 2021; **113**: 103638 [PMID: 33271341 DOI: 10.1016/j.jbi.2020.103638]

107 **Echle A**, Rindtorff NT, Brinker TJ, Luedde T, Pearson AT, Kather JN. Deep learning in cancer pathology: a new generation of clinical biomarkers. *Br J Cancer* 2021; **124**: 686-696 [PMID: 33204028 DOI: 10.1038/s41416-020-01122-x]

108 **Araujo RL**, Linhares MM. Pushing the limits of liver surgery for colorectal liver metastases: Current state and future directions. *World J Gastrointest Surg* 2019; **11**: 34-40 [PMID: 30842810 DOI: 10.4240/wjgs.v11.i2.34]

109 **Passi K**, Zhao H. A decision support system (DSS) for colorectal cancer follow-up program *via* a semantic framework. *Int J Healthcare Informat Syst Informatics* 2015; **10**: 17-38 [DOI: 10.4018/ijhisi.2015010102]

110 **Lee WS**, Ahn SM, Chung JW, Kim KO, Kwon KA, Kim Y, Sym S, Shin D, Park I, Lee U, Baek JH. Assessing Concordance With Watson for Oncology, a Cognitive Computing Decision Support System for Colon Cancer Treatment in Korea. *JCO Clin Cancer Inform* 2018; **2**: 1-8 [PMID: 30652564 DOI: 10.1200/CCI.17.00109]

111 **Siddiqi J**, Akhgar B, Gruzdz A, Zaefarian G, Ihnatowicz A. Automated diagnosis system to support colon cancer treatment: Match. 15th International Conference on Information Technology: New Generations, 2008: 201-205 [DOI: 10.1109/itng.2008.62]

112 **Li S**, Jiang Q, Liu S, Zhang Y, Tian Y, Song C, Wang J, Zou Y, Anderson GJ, Han JY, Chang Y, Liu Y, Zhang C, Chen L, Zhou G, Nie G, Yan H, Ding B, Zhao Y. A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo. *Nat Biotechnol* 2018; **36**: 258-264 [PMID: 29431737 DOI: 10.1038/nbt.4071]

113 **Felfoul O**, Mohammadi M, Taherkhani S, de Lanauze D, Zhong Xu Y, Loghin D, Essa S, Jancik S, Houle D, Lafleur M, Gaboury L, Tabrizian M, Kaou N, Atkin M, Vuong T, Batist G, Beauchemin N, Radzioch D, Martel S. Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions. *Nat Nanotechnol* 2016; **11**: 941-947 [PMID: 27525475 DOI: 10.1038/nnano.2016.137]

114 **Koelzer VH**, Sirinukunwattana K, Rittscher J, Mertz KD. Precision immunoprofiling by image analysis and artificial intelligence. *Virchows Arch* 2019; **474**: 511-522 [PMID: 30470933 DOI: 10.1007/s00428-018-2485-z]

115 **Dzobo K**, Thomford NE, Senthebane DA. Targeting the Versatile Wnt/β-Catenin Pathway in Cancer Biology and Therapeutics: From Concept to Actionable Strategy. *OMICS* 2019; **23**: 517-538 [PMID: 31613700 DOI: 10.1089/omi.2019.0147]

116 **Lee BI**, Matsuda T. Estimation of Invasion Depth: The First Key to Successful Colorectal ESD. *Clin Endosc* 2019; **52**: 100-106 [PMID: 30914629 DOI: 10.5946/ce.2019.012]

117 **Ichimasa K**, Kudo SE, Mori Y, Misawa M, Matsudaira S, Kouyama Y, Baba T, Hidaka E, Wakamura K, Hayashi T, Kudo T, Ishigaki T, Yagawa Y, Nakamura H, Takeda K, Haji A, Hamatani S, Mori K, Ishida F, Miyachi H. Artificial intelligence may help in predicting the need for additional surgery after endoscopic resection of T1 colorectal cancer. *Endoscopy* 2018; **50**: 230-240 [PMID: 29272905 DOI: 10.1055/s-0043-122385]

118 **Kirchberg J**, Weitz J. Evidence for robotic surgery in oncological visceral surgery. *Chirurg* 2019; **90**: 379-386 [PMID: 30778607 DOI: 10.1007/s00104-019-0812-9]

119 **Kim YJ**. The Future Medical Science and Colorectal Surgeons. *Ann Coloproctol* 2017; **33**: 207-209 [PMID: 29354602 DOI: 10.3393/ac.2017.33.6.207]

120 **Leonard S**, Wu KL, Kim Y, Krieger A, Kim PC. Smart tissue anastomosis robot (STAR): a vision-guided robotics system for laparoscopic suturing. *IEEE Trans Biomed Eng* 2014; **61**: 1305-1317 [PMID: 24658254 DOI: 10.1109/TBME.2014.2302385]

121 **Huang YM**, Huang YJ, Wei PL. Colorectal Cancer Surgery Using the Da Vinci Xi and Si Systems: Comparison of Perioperative Outcomes. *Surg Innov* 2019; **26**: 192-200 [PMID: 30501567 DOI: 10.1177/1553350618816788]

122 **Zheng P**, Feng QY, Xu JM. Current status and consideration of robotic surgery for colorectal cancer in China. *Zhonghua Wei Chang Wai Ke Za Zhi* 2020; **23**: 336-340 [PMID: 32306599 DOI: 10.3760/cma.j.cn.441530-20200216-00056]

123 **Mitsala A**, Tsalikidis C, Pitiakoudis M, Simopoulos C, Tsaroucha AK. Artificial Intelligence in Colorectal Cancer Screening, Diagnosis and Treatment. A New Era. *Curr Oncol* 2021; **28**: 1581-

1607 [PMID: 33922402 DOI: 10.3390/curroncol28030149]

124 **Aikemu B**, Xue P, Hong H, Jia H, Wang C, Li S, Huang L, Ding X, Zhang H, Cai G, Lu A, Xie L, Li H, Zheng M, Sun J. Artificial Intelligence in Decision-Making for Colorectal Cancer Treatment Strategy: An Observational Study of Implementing Watson for Oncology in a 250-Case Cohort. *Front Oncol* 2020; **10**: 594182 [PMID: 33628729 DOI: 10.3389/fonc.2020.594182]

125 **Hamamoto R**, Suvarna K, Yamada M, Kobayashi K, Shinkai N, Miyake M, Takahashi M, Jinnai S, Shimoyama R, Sakai A, Takasawa K, Bolatkan A, Shozu K, Dozen A, Machino H, Takahashi S, Asada K, Komatsu M, Sese J, Kaneko S. Application of Artificial Intelligence Technology in Oncology: Towards the Establishment of Precision Medicine. *Cancers (Basel)* 2020; **12** [PMID: 33256107 DOI: 10.3390/cancers12123532]

126 **Pritzker KPH**. Colon Cancer Biomarkers: Implications for Personalized Medicine. *J Pers Med* 2020; **10** [PMID: 33066312 DOI: 10.3390/jpm10040167]

127 **Ding X**, Chang VHS, Li Y, Li X, Xu H, Ho C-M, Ho D, Yen Y. Harnessing an artificial intelligence platform to dynamically individualize combination therapy for treating colorectal carcinoma in a rat model. *Adv Ther* 2020; **3** [DOI: 10.1002/adtp.201900127]

128 **Zhang L**, Zheng C, Li T, Xing L, Zeng H, Yang H, Cao J, Chen B, Zhou Z. Building up a robust risk mathematical platform to predict colorectal cancer. *Complexity* 2017; **2017**: 1-14 [DOI: 10.1155/2017/8917258]

129 **Morgado P**, Vicente H, Abelha A, Machado J, Neves J. A case-based approach to colorectal cancer detection. Information science and applications, 2017: 433-442 [DOI: 10.1007/978-981-10-4154-9_50]

130 **Cueto-López N**, García-Ordás MT, Dávila-Batista V, Moreno V, Aragonés N, Alaiz-Rodríguez R. A comparative study on feature selection for a risk prediction model for colorectal cancer. *Comput Methods Programs Biomed* 2019; **177**: 219-229 [PMID: 31319951 DOI: 10.1016/j.cmpb.2019.06.001]

131 **Anand SS**, Smith AE, Hamilton PW, Anand JS, Hughes JG, Bartels PH. An evaluation of intelligent prognostic systems for colorectal cancer. *Artif Intell Med* 1999; **15**: 193-214 [PMID: 10082181 DOI: 10.1016/s0933-3657(98)00052-9]

132 **Gupta P**, Chiang SF, Sahoo PK, Mohapatra SK, You JF, Onthoni DD, Hung HY, Chiang JM, Huang Y, Tsai WS. Prediction of Colon Cancer Stages and Survival Period with Machine Learning Approach. *Cancers (Basel)* 2019; **11** [PMID: 31842486 DOI: 10.3390/cancers11122007]

133 **Li S**, Razzaghi T. Personalized colorectal cancer survivability prediction with machine learning methods. 2018 IEEE international conference on big data, 2018: 2554-2558 [DOI: 10.1109/bigdata.2018.8622121]

134 **Barsainya A**, Sairam A, Patil AP. Analysis and prediction of survival after colorectal chemotherapy using machine learning models. 2018 International Conference on Advances in Computing, Communications and Informatics, 2018 [DOI: 10.1109/icacci.2018.8554832]

135 **Dimitriou N**, Arandjelović O, Harrison DJ, Caie PD. A principled machine learning framework improves accuracy of stage II colorectal cancer prognosis. *NPJ Digit Med* 2018; **1**: 52 [PMID: 31304331 DOI: 10.1038/s41746-018-0057-x]

136 **Popovici V**, Budinská E, Dušek L, Kozubek M, Bosman F. Image-based surrogate biomarkers for molecular subtypes of colorectal cancer. *Bioinformatics* 2017; **33**: 2002-2009 [PMID: 28158480 DOI: 10.1093/bioinformatics/btx027]

137 **Hoogendoorn M**, Szolovits P, Moons LMG, Numans ME. Utilizing uncoded consultation notes from electronic medical records for predictive modeling of colorectal cancer. *Artif Intell Med* 2016; **69**: 53-61 [PMID: 27085847 DOI: 10.1016/j.artmed.2016.03.003]

138 **Kop R**, Hoogendoorn M, Teije AT, Büchner FL, Slottje P, Moons LM, Numans ME. Predictive modeling of colorectal cancer using a dedicated pre-processing pipeline on routine electronic medical records. *Comput Biol Med* 2016; **76**: 30-38 [PMID: 27392227 DOI: 10.1016/j.compbiomed.2016.06.019]

139 **Geessink OGF**, Baidoshvili A, Freling G, Klaase JM, Slump CH, van der Heijden F. Toward automatic segmentation and quantification of tumor and stroma in whole-slide images of H&E stained rectal carcinomas. Medical imaging 2015: Digital pathology. Bellingham: Spie-Int Soc Optical Engineering, 2015 [DOI: 10.1117/12.2081665]

140 **Wright A**, Magee D, Quirke P, Treanor D. Towards automatic patient selection for chemotherapy in colorectal cancer trials. Medical imaging 2014: Digital pathology, 2014 [DOI: 10.1117/12.2043220]

141 **Wang Y**, Wang D, Ye X, Wang Y, Yin Y, Jin Y. A tree ensemble-based two-stage model for advanced-stage colorectal cancer survival prediction. *Informat Sci* 2019; **474**: 106-124 [DOI: 10.1016/j.ins.2018.09.046]

142 **Oliveira T**, Barbosa E, Martins S, Goulart A, Neves J, Novais P. A prognosis system for colorectal cancer. Proceedings of the 26th IEEE International Symposium on Computer-Based Medical Systems, 2013 [DOI: 10.1109/cbms.2013.6627846]

143 **Lo Bello L**, Pistone G, Restuccia S, Vinci E, Mazzoleni G, Malaguarnera M. 5-fluorouracil alone versus 5-fluorouracil plus folinic acid in the treatment of colorectal carcinoma: meta-analysis. *Int J Clin Pharmacol Ther* 2000; **38**: 553-562 [PMID: 11125868 DOI: 10.5414/cpp38553]

144 **Harrington L**, Suriawinata A, MacKenzie T, Hassanpour S. Application of machine learning on colonoscopy screening records for predicting colorectal polyp recurrence. Proceedings 2018 IEEE international conference on bioinformatics and biomedicine, 2018: 993-998 [DOI:

10.1109/bibm.2018.8621455]

145 **Xie X**, Xing J, Kong N, Li C, Li J, Zhang S. Improving colorectal polyp classification based on physical examination data-an ensemble learning approach. *EEE Robot Automat Lett* 2018; **3**: 434-441 [DOI: 10.1109/lra.2017.2746918]

146 **Bokhorst JM**, Rijstenberg L, Goudkade D, Nagtegaal I, van der Laak J, Ciompi F. Automatic detection of tumor budding in colorectal carcinoma with deep learning. Computational pathology and ophthalmic medical image analysis, 2018: 130-138 [DOI: 10.1007/978-3-030-00949-6_16]

147 **Zhao K**, Li Z, Yao S, Wang Y, Wu X, Xu Z, Wu L, Huang Y, Liang C, Liu Z. Artificial intelligence quantified tumour-stroma ratio is an independent predictor for overall survival in resectable colorectal cancer. *EBioMedicine* 2020; **61**: 103054 [PMID: 33039706 DOI: 10.1016/j.ebiom.2020.103054]

148 **Syafiandini AF**, Wasito I, Yazid S, Fitriawan A, Amien M. Cancer subtype identification using deep learning approach. 2016 International Conference on Computer, Control, Informatics and its Applications, 2016 [DOI: 10.1109/ic3ina.2016.7863033]

149 **Roadknight C**, Aickelin U, Scholefield J, Durrant L. Ensemble learning of colorectal cancer survival rates. 2013 IEEE international conference on computational intelligence and virtual environments for measurement systems and applications, 2013: 82-86 [DOI: 10.1109/civemsa.2013.6617400]

150 **Cui Y**, Cai X, Jin Z. Semi-supervised classification using sparse representation for cancer recurrence prediction. 2013 IEEE international workshop on genomic signal processing and statistics, 2013: 102-105 [DOI: 10.1109/gensips.2013.6735949]

151 **Park C**, Ahn J, Kim H, Park S. Integrative gene network construction to analyze cancer recurrence using semi-supervised learning. *PLoS One* 2014; **9**: e86309 [PMID: 24497942 DOI: 10.1371/journal.pone.0086309]

152 **Du X**, Cheng J. Identification and analysis of driver missense mutations using rotation forest with feature selection. *Biomed Res Int* 2014; **2014**: 905951 [PMID: 25250338 DOI: 10.1155/2014/905951]

153 **Chi S**, Li X, Tian Y, Li J, Kong X, Ding K, Weng C. Semi-supervised learning to improve generalizability of risk prediction models. *J Biomed Inform* 2019; **92**: 103117 [PMID: 30738948 DOI: 10.1016/j.jbi.2019.103117]

154 **Ong LS**, Shepherd B, Tong LC, Seow-Choen F, Ho YH, Tang CL, Ho YS, Tan K. The Colorectal Cancer Recurrence Support (CARES) System. *Artif Intell Med* 1997; **11**: 175-188 [PMID: 9413605 DOI: 10.1016/s0933-3657(97)00029-8]

155 **Reichling C**, Taieb J, Derangere V, Klopfenstein Q, Le Malicot K, Gornet JM, Becheur H, Fein F, Cojocarasu O, Kaminsky MC, Lagasse JP, Luet D, Nguyen S, Etienne PL, Gasmi M, Vanoli A, Perrier H, Puig PL, Emile JF, Lepage C, Ghiringhelli F. Artificial intelligence-guided tissue analysis combined with immune infiltrate assessment predicts stage III colon cancer outcomes in PETACC08 study. *Gut* 2020; **69**: 681-690 [PMID: 31780575 DOI: 10.1136/gutjnl-2019-319292]

156 **Chowdhury SA**, Nibbe RK, Chance MR, Koyutürk M. Subnetwork state functions define dysregulated subnetworks in cancer. *J Comput Biol* 2011; **18**: 263-281 [PMID: 21385033 DOI: 10.1089/cmb.2010.0269]

157 **Mohamad Marzuki MF**, Yaacob NA, Bin Yaacob NM, Abu Hassan MR, Ahmad SB. Usable Mobile App for Community Education on Colorectal Cancer: Development Process and Usability Study. *JMIR Hum Factors* 2019; **6**: e12103 [PMID: 30990454 DOI: 10.2196/12103]

158 **Hacking S**, Nasim R, Lee L, Vitkovski T, Thomas R, Shaffer E, Nasim M. Whole slide imaging and colorectal carcinoma: A validation study for tumor budding and stromal differentiation. *Pathol Res Pract* 2020; **216**: 153233 [PMID: 33068916 DOI: 10.1016/j.prp.2020.153233]

159 **Wang Y**, He X, Nie H, Zhou J, Cao P, Ou C. Application of artificial intelligence to the diagnosis and therapy of colorectal cancer. *Am J Cancer Res* 2020; **10**: 3575-3598 [PMID: 33294256]

160 **O'Sullivan S**, Nevejans N, Allen C, Blyth A, Leonard S, Pagallo U, Holzinger K, Holzinger A, Sajid MI, Ashrafian H. Legal, regulatory, and ethical frameworks for development of standards in artificial intelligence (AI) and autonomous robotic surgery. *Int J Med Robot* 2019; **15**: e1968 [PMID: 30397993 DOI: 10.1002/rcs.1968]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 153-162

DOI: 10.4251/wjgo.v14.i1.153

ISSN 1948-5204 (online)

MINIREVIEWS

Impact of the microenvironment on the pathogenesis of mucosa-associated lymphoid tissue lymphomas

Barbara Uhl, Katharina T Prochazka, Karoline Fechter, Katrin Pansy, Hildegard T Greinix, Peter Neumeister, Alexander JA Deutsch

ORCID number: Barbara Uhl 0000-0002-8460-8771; Katharina T Prochazka 0000-0002-7201-5987; Karoline Fechter 0000-0002-6894-2438; Katrin Pansy 0000-0001-6972-4937; Hildegard T Greinix 0000-0002-2780-3300; Peter Neumeister 0000-0002-2761-5334; Alexander JA Deutsch 0000-0003-0914-2809.

Author contributions: Uhl B and Deutsch AJA drafted the work; all authors conceived the work and made substantial revisions to and critiqued the content; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Country/Territory of origin: Austria

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Barbara Uhl, Katharina T Prochazka, Karoline Fechter, Katrin Pansy, Hildegard T Greinix, Peter Neumeister, Alexander JA Deutsch, Division of Hematology, Medical University of Graz, Graz 8036, Austria

Corresponding author: Alexander JA Deutsch, PhD, Research Assistant Professor, Division of Hematology, Medical University of Graz, Auenbruggerplatz 38, Graz 8036, Austria. alexander.deutsch@medunigraz.at

Abstract

Approximately 8% of all non-Hodgkin lymphomas are extranodal marginal zone B cell lymphomas of mucosa-associated lymphoid tissue (MALT), also known as MALT lymphomas. These arise at a wide range of different extranodal sites, with most cases affecting the stomach, the lung, the ocular adnexa and the thyroid. The small intestine is involved in a lower percentage of cases. Lymphoma growth in the early stages is associated with long-lasting chronic inflammation provoked by bacterial infections (*e.g.*, *Helicobacter pylori* or *Chlamydia psittaci* infections) or autoimmune conditions (*e.g.*, Sjögren's syndrome or Hashimoto thyroiditis). While these inflammatory processes trigger lymphoma cell proliferation and/or survival, they also shape the microenvironment. Thus, activated immune cells are actively recruited to the lymphoma, resulting in either direct lymphoma cell stimulation *via* surface receptor interactions and/or indirect lymphoma cell stimulation *via* secretion of soluble factors like cytokines. In addition, chronic inflammatory conditions cause the acquisition of genetic alterations resulting in autonomous lymphoma cell growth. Recently, novel agents targeting the microenvironment have been developed and clinically tested in MALT lymphomas as well as other lymphoid malignancies. In this review, we aim to describe the composition of the microenvironment of MALT lymphoma, the interaction of activated immune cells with lymphoma cells and novel therapeutic approaches in MALT lymphomas using immunomodulatory and/or microenvironment-targeting agents.

Key Words: Mucosa-associated lymphoid tissue lymphoma; Tumor microenvironment; Microenvironment; *Helicobacter pylori*; Activated immune cells

Received: February 25, 2021
Peer-review started: February 25, 2021
First decision: May 3, 2021
Revised: May 16, 2021
Accepted: December 9, 2021
Article in press: December 9, 2021
Published online: January 15, 2022

P-Reviewer: Asano N
S-Editor: Gao CC
L-Editor: A
P-Editor: Gao CC

Core Tip: This review summarizes and discusses the major findings in extranodal mucosa-associated lymphoid tissue lymphomas with a focus on the microenvironment. It describes how long-lasting chronic inflammatory processes promote the growth of malignant cells, which can be directly mediated by bacteria and/or interaction with activated immune cells. In addition, major genetic alterations are summarized, and models of how these might be acquired are discussed. Finally, novel therapies targeting the microenvironment are described.

Citation: Uhl B, Prochazka KT, Fechter K, Pansy K, Greinix HT, Neumeister P, Deutsch AJ. Impact of the microenvironment on the pathogenesis of mucosa-associated lymphoid tissue lymphomas. *World J Gastrointest Oncol* 2022; 14(1): 153-162
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/153.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.153

INTRODUCTION

Extranodal mucosa-associated lymphoid tissue (MALT) lymphomas account for 5%-8% of all non-Hodgkin lymphomas (NHLs) and were first described in 1983 by Isaacson and Wright[1-3]. MALT lymphomas arise at a wide range of extranodal sites, most frequently occurring in the stomach, followed by the lung, ocular adnexa, thyroid and small intestine[4]. The cells of this type of lymphoma have the same cytological and immunophenotypical (CD20+, CD21+, CD35+, IgM+, and IgD-) features as marginal zone B cells, prompting the World Health Organization to designate this lymphoma "extranodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)"[5]. The cell of origin of MALT lymphomas is the marginal zone (MZ) B cell. These B cells are a first line of defense against infectious agents and build up an innate-like antibody response in a T cell-independent and T cell-dependent manner[6,7]. MALT lymphomagenesis is highly dependent on microenvironmental factors and therefore often associated with chronic inflammation induced either by *Helicobacter pylori* (*H. pylori*), the most common pathogen in gastric MALT lymphomas, or by chronic inflammation as a result of autoimmune disease. These are known risk factors for the development of MZ lymphomas[8]. In addition to the antigenic drive, oncogenic events are important in the process of malignant transformation[8]. MALT lymphoma cell proliferation is driven by T cell signaling, chronic (auto) antigen stimulation of MZ B cells, and activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB pathway)[9].

ACQUIRED GENETIC ABNORMALITIES

In MALT lymphomas, recurrent chromosomal aberrations, such as trisomies, amplifications and deletions, chromosomal translocations, somatic point mutations, and promotor hypermethylation, have been described.

The most common cytogenetic alterations are trisomies 3, 12, and/or 18, which are present in 20%-35% of cases, and they are often associated with one of the four main translocations[10-13]. Trisomies 3 and 18 and losses at 6q23 occur in MALT lymphomas primarily involving the stomach, orbital adnexa, thyroid, salivary glands, and lung[13]. Several promising candidate genes are located on chromosome 3, such as the proto-oncogene *BCL6* and the transcription factor *FOXP1*[14]. Additionally, the chemokine receptor *CCR4*, genomically located on chromosome 3 (3p24), is highly expressed in trisomy 3-positive MALT lymphomas[15]. Furthermore, genome-wide DNA profiling revealed deletions in 1p and 6q, as well as gains on chromosomes 3 and 18 and the short arm of chromosome 6[10].

The most common chromosomal translocations associated with the pathogenesis of MALT lymphomas are t(1;14)(p22,q32) (involving the *IGHV* and *BCL10* genes), t(11;18)(q21,q21) (involving BIRC3/MALT1), t(14;18)(q32,q21) (involving *IGH*/*BCL2*) and t(3;14)(p14.1,q32) (involving *IGHV-FOXP1*)[8,10,16]. The frequency of genetic aberrations is dependent on the primary site of disease[10,17]. At least three translo-

cations, t(11;18), t(14;18) and t(1;14), involve the *BCL10* and *MALT1* genes and lead to activation of the NF-κB pathway in lymphocytes, thus indicating that these aberrations are oncogenic events[18,19].

We observed somatic missense mutations in *PIM1* and *cMYC* in 46% of gastric and 30% of extragastric MALT lymphomas[20]. In addition, missense and frameshift mutations in *p53* were described in 20.8% of MALT lymphomas (mainly of gastric origin)[10]. Moreover, whole exome sequencing of extragastric MALT lymphomas identified recurrent novel somatic mutations in *PIK3CD*, *TET2*, and *TNFRSF14* and in two G protein-coupled receptors (*GPR34* and *CCR6*), which have not been reported to be somatically mutated in human tumors thus far. In addition, recurrent mutations were found in two genes (*TBL1XR1* and *NOTCH1*), for which somatic mutations were already reported in ocular adnexal MALT lymphomas. The mutation frequencies of these genes were remarkably variable among MALT lymphomas affecting different sites[21]. Sequencing of NF-κB signaling pathway-related genes — *A20*, *Card11*, *CD79B*, and *Myd88*, known to be frequently mutated in aggressive lymphomas[10,22] — demonstrated that 6% of MALT lymphoma cases exhibited missense or frameshift mutations in the *Myd88* locus. A total of 28.6% of the ocular adnexal MALT lymphomas had mutations in the *A20* locus[10,23]. *Card11* and *CD79B* were not affected in ocular adnexal MALT lymphomas[10].

Finally, promoter hypermethylation of the tumor suppressor genes *p16* and *p57* has been reported in low-grade MALT lymphoma cases[24]. CpG hypermethylation of *A20* has been detected in 26% of investigated MALT lymphomas, including ocular adnexal cases and lymphomas located in the salivary and thyroid glands[10].

Aberrant somatic hypermutation is associated with genetic lesions in malt lymphomas

Aberrant somatic hypermutation (ASHM) has been identified to be crucial for the development of lymphoid neoplasms. ASHM occurs commonly in diffuse large B cell lymphomas but is rare in indolent lymphomas[10,25,26]. The pathogenesis of most lymphomas is associated with distinct genetic lesions arising from mistakes during class switch recombination (CSR) and somatic hypermutation (SHM)[10,27]. Activation-induced cytidine deaminase (AID) is an enzyme required for CSR and SHM. Mistargeting of AID to known proto-oncogenes combined with a breakdown of protective high-fidelity repair mechanisms has been shown to be a principal contributor to the pathogenesis of B-NHL[10]. Our research group has demonstrated that the expression levels of AID are associated with the mutational load caused by ASHM in MALT lymphomas[25]. However, the mechanism causing the upregulation of AID has not been identified thus far. It has been demonstrated that *H. pylori* infection upregulates AID expression *via* NF-κB in gastric cells *in vitro* and *in vivo*, resulting in the accumulation of *p53* mutations[28]. Hence, it might be speculated that *H. pylori* infection is also participates in the upregulation of AID in B cells, leading to the accumulation of genetic alterations.

CHRONIC INFLAMMATION SHAPES THE MICROENVIRONMENT AND THEREBY PLAYS A KEY ROLE IN MALT LYMPHOMAGENESIS

It is well known that MALT lymphomas are commonly associated with long-lasting chronic inflammation caused by microbial pathogens and/or autoimmune diseases that trigger sustained lymphoid proliferation. The low activation threshold of MZ B cells may predispose them to neoplastic transformation[29].

Gastric MALT lymphomas show a strong association with chronic *H. pylori* infection [30]. Other infectious associations have been reported for *Borrelia burgdorferi* (skin)[31], *Campylobacter jejuni* (intestine)[32], *Achromobacter xylosoxidans* (lung)[33], *Chlamydia psittaci* (ocular, nongastrointestinal MALT lymphomas)[34-36] and hepatitis C virus (splenic marginal zone lymphoma)[37]. The strength of these associations shows vast geographical discrepancies[38-40]. In addition, an association of MALT lymphomas with chronic inflammation induced by autoimmune disease is found in primary Sjögren's syndrome (pSS)[41-43] and Hashimoto thyroiditis[44].

Long-lasting chronic inflammation, *e.g.*, induced by *H. pylori* infection or pSS, is the trigger for a multistage process in the evolution of MALT lymphomas due direct effects on B cell proliferation and/or survival and/or indirect effects on the activation of innate and adaptive immune cells[9,43,45] as shown in Figure 1.

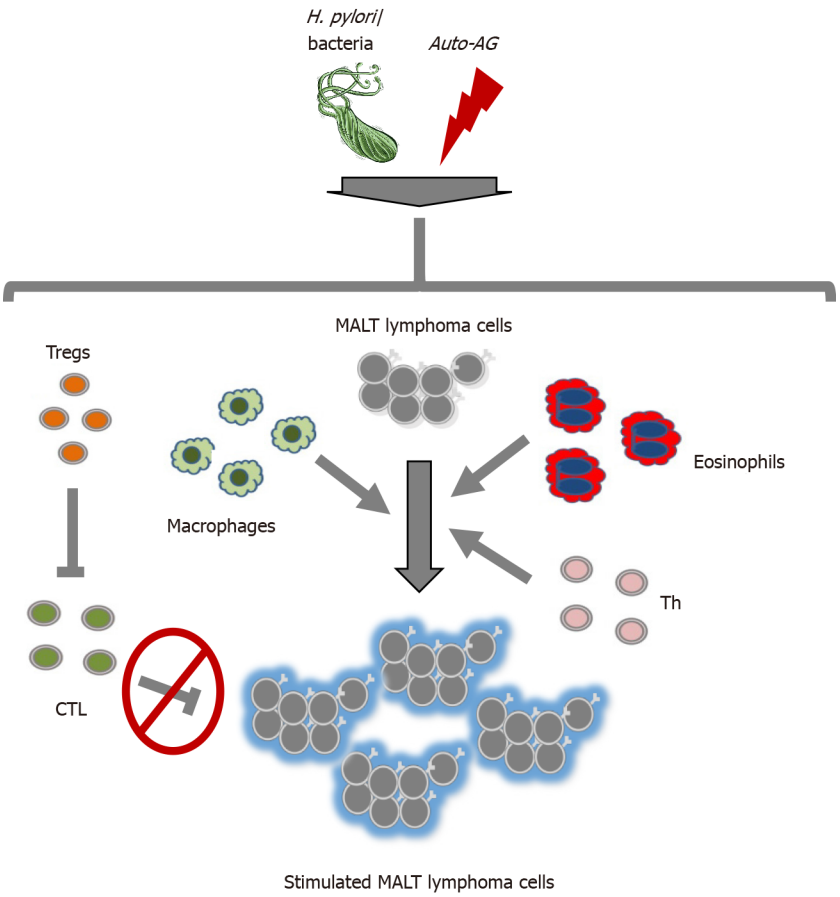


Figure 1 Graphical depiction of the interplay of mucosa-associated lymphoid tissue lymphoma cells with their microenvironment. *Helicobacter pylori* (*H. pylori*), other bacteria and/or autoantigens (auto-AGs) support an immune regulatory microenvironment promoting mucosa-associated lymphoid tissue (MALT) lymphomagenesis in different organs. First, regulatory T cells are activated and suppress the immune response by maintaining *H. pylori* colonialization and influencing cytotoxic T cells, which possess malfunctions and therefore cannot inhibit the expansion of MALT lymphoma cells. Second, eosinophils and macrophages express a proliferation-inducing ligand (APRIL) and B cell-activating factor, supporting lymphomagenesis. The production of APRIL is induced by *H. pylori* antigens and *H. pylori*-specific T cells. Third, T helper cells and their cytokines (IL-4, Il-5, and IL-10) promote the growth and differentiation of lymphoma cells and are stimulated by *H. pylori* and/or auto-AGs. MALT: Mucosa-associated lymphoid tissue; *H. pylori*: *Helicobacter pylori*; Tregs: Regulatory T cells; CTL: Cytotoxic T cell; Auto-AG: Autoantigen; Th: T helper.

H. pylori strains expressing cytotoxin-associated gene A (CagA) are associated with the lymphomagenesis of gastric MALT lymphoma[46,47]. CagA is involved in the promotion of proliferation and the inhibition of apoptosis of B lymphocytes through activation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) and upregulation of BCL-2 and BCL-xL[47]. Second, the CagA tyrosine phosphorylation-independent pathway impairs p53 *via* AKT serine/threonine kinase 1 (AKT1) and human homolog of double minute 2 (HDM2)[9,45]. In general, cell wall lipopolysaccharide has been shown to be responsible for triggering a pattern of mucosal inflammation *via* Toll-like receptor signaling, resulting in activation of MAPK, phosphoinositide 3-kinase (PI3K) and NF-κB pathways in *H. pylori* infection [48,49].

As already mentioned, long-lasting chronic inflammatory processes might also influence MALT lymphomagenesis through the direct (auto) antigen-mediated interaction of lymphoma cells with immune cells and/or the secretion of soluble factors like cytokines. In this case, a direct immune cell-lymphoma cell interaction and subsequent activation result. Activated T cells targeting *H. pylori* represent cells targeting autoantigens in the case of pSS, and these T cells are present in MALT lymphomas and are able to promote lymphoma cell growth *via* CD40-mediated signaling and T helper (Th) type-2 cytokine (IL-4, IL-5, and IL-10) effects[50,51]. Examples of two cytokines are a proliferation-inducing ligand (APRIL) and B cell-activating factor (BAFF), which are members of the tumor necrosis factor family and play a key role in B cells and autoimmunity. Both cytokines are secreted by eosinophils and/or macrophages and stimulate MALT lymphoma cells[52-54]. Both the CD40/CD40L interaction and APRIL and/or BAFF signaling cause the activation of important downstream signaling pathways, *e.g.*, NF-κB and/or MAPK[55,56], and

thereby have an important impact on MALT lymphomagenesis.

Chronic inflammatory processes in MALT lymphomas not only promote B cell growth/proliferation but also actively induce immunosuppressive conditions, which also play a major role in the development and progression of this B cell malignancy. These effects are partially mediated by recruited regulatory T cells (Tregs)[57,58], which suppress anticancer immunity by secreting anti-inflammatory cytokines and/or expressing immune inhibitory surface receptors[59,60]. Furthermore, activated tumor-infiltrating T cells have dysfunctional cytolytic capacity in MALT lymphomas[61,62].

It has been demonstrated that T cells, macrophages and neutrophils recruited during long-lasting chronic inflammation contribute to the formation of genetic aberrations, DNA damage and genetic instability in B cells, leading to antigen-independent lymphoma cell growth. These effects are mediated by activation of ASHM and class-switching recombination in MALT lymphomas[63] and are associated with epigenetic and genetic changes in $p57^{KIP}$[24], $p16^{INK4A}$[24,64] and p53 [10] as well as chromosomal translocation of *cMYC* and *BCL6*[10,65].

TUMOR MICROENVIRONMENT-TARGETING THERAPIES

As already described, MALT lymphomas with long-lasting chronic infections cause B cell proliferation and/or survival either directly and/or indirectly *via* activation of immune cells[9,43,45,66]. Therefore, these interactions provide multiple potential targets for new immunomodulatory treatments beyond the established treatment options for *H. pylori* eradication by antibiotics, radiation, chemotherapy and treatment with the anti-CD20 antibody therapy rituximab[67].

Immunomodulatory drugs (IMiDs) represent a novel therapeutic approach to target the tumor microenvironment of MALT lymphomas. IMiDs, consisting of thalidomide, lenalidomide and pomalidomide, are approved for the treatment of multiple myeloma, and lenalidomide is approved for the treatment of relapsed follicular lymphoma[53,68-70]. IMiDs exert anti-inflammatory effects, such as decreased production of cytokines and increased production of Th1 type cytokines; furthermore, they decrease vascular endothelial growth factor (VEGF) levels and show modulating effects on basic cellular mechanisms (T cell costimulation and alteration of FOXP3+ Tregs and natural killer cells)[68,69]. The efficacy of lenalidomide in MALT lymphomas has been reported in studies with induction of remission after treatment for up to 32 mo[71]. Raderer and Kiesewetter[53] conducted a phase II study with a combination therapy consisting of lenalidomide and rituximab, which achieved an overall response rate of 80% and a complete remission rate of 54%.

Further therapeutic targets are related to Tregs, which are recruited into the microenvironment of MALT lymphoma[58,72] and suppress antitumoral immune reactions[58,60,73,74]. It has been shown that the Bruton's kinase inhibitor ibrutinib reduces the number of Tregs in the early course of treatment in chronic lymphocytic leukemia (CLL), in addition to inhibiting the BCR pathway[75]. Ibrutinib has been tested in relapsed/refractory marginal zone B cell lymphoma (MZL) and possesses a remarkable response rate with tolerable toxicity[53]. However, no data are available thus far for the treatment of MALT lymphoma.

As reported in section 3, in MALT lymphoma cells, the NF-κB pathway is strongly activated by genetic alterations[18,76,77] or by interaction with activated immune cells *via* the CD40/CD40L[52-54,56] and/or APRIL axes[77-79].

Bortezomib, a proteasome inhibitor with inhibitory effects on the NF-κB signaling pathway[10], showed promising response rates in MALT lymphoma patients in phase II trials[80]. Furthermore, bortezomib was reported to reverses the tumor-induced dysfunction of CD8+ T cells by increasing the expression of Notch cascade genes[81]. Moreover, bortezomib enacts immunostimulatory effects by activating tumor-infiltrating CD8+ T cells[61,62]. Taken together, these findings suggest that the anti-lymphoma effects of bortezomib are mediated by NF-κB inhibition and by reversal of the observed T cell malfunction[52-54].

Another possibility to suppress NF-κB activation in MALT lymphoma cells is the disruption of the APRIL axis[82] with use of an anti-APRIL antibody; one such antibody was developed by Guadagnoli *et al*[82] and has shown promising results in CLL in a preclinical setting[83-89]. However, this strategy has not been tested in MALT lymphoma patients thus far.

It has also been demonstrated that macrolides, which are used for eradication of bacterial infection in MALT lymphomas, have certain immunomodulatory effects, *e.g.*, they decrease the number and inhibit the function of neutrophils as well as eosinophils

and inhibit Th2 cell functions[83-89]. Thus, it is likely that the immunomodulatory effects significantly impact the response rates of MALT lymphomas when these antimicrobial drugs are used.

CONCLUSION

MALT lymphomas represent a heterogeneous group of lymphoid neoplasms arising at different extranodal sites and are associated with a variety of long-lasting chronic infections. In the current pathogenic model, (auto) antigen stimuli trigger lymphoma cell growth, survival, and recruitment of immune cells to the microenvironment, which in turn stimulate lymphoma cells directly *via* surface receptor interactions and/or indirectly *via* cytokine secretion. Moreover, it has been shown that inflammatory processes may lead to the acquisition of further genetic alterations resulting in lymphoma cell growth independent of (auto) antigen stimuli. Many agents targeting/blocking the interaction of immune cells of the microenvironment with lymphoma cells, as well as eradicating the antigen stimuli, have been developed within recent years, indicating that the basis for novel therapeutic strategies is already available. Despite these advances, the number of comprehensive studies on the microenvironment composition and its interaction with lymphoma cells needs to be significantly increased to gain further knowledge on targets for innovative and efficient therapy.

ACKNOWLEDGEMENTS

We thank Waha JE for reviewing the manuscript for clarity in English as a native speaker.

REFERENCES

1 **Isaacson P**, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. *Cancer* 1983; **52**: 1410-1416 [PMID: 6193858 DOI: 10.1002/1097-0142(19831015)52:8<1410::AID-CNCR2820520813>3.0.CO;2-3]
2 A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; **89**: 3909-3918 [PMID: 9166827 DOI: 10.1182/blood.v89.11.3909]
3 **Olszewski AJ**, Castillo JJ. Survival of patients with marginal zone lymphoma: analysis of the Surveillance, Epidemiology, and End Results database. *Cancer* 2013; **119**: 629-638 [PMID: 22893605 DOI: 10.1002/cncr.27773]
4 **Isaacson PG**, Du MQ. MALT lymphoma: from morphology to molecules. *Nat Rev Cancer* 2004; **4**: 644-653 [PMID: 15286744 DOI: 10.1038/nrc1409]
5 **Swerdlow SH**, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H. Weltgesundheitsorganisation. WHO classification of tumours of haematopoietic and lymphoid tissues. [cited 12 January 2021]. In: International Agency for Research on Cancer [Internet]. Available from: https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Haematopoietic-And-Lymphoid-Tissues-2017
6 **Efremov DG**, Turkalj S, Laurenti L. Mechanisms of B Cell Receptor Activation and Responses to B Cell Receptor Inhibitors in B Cell Malignancies. *Cancers (Basel)* 2020; **12** [PMID: 32481736 DOI: 10.3390/cancers12061396]
7 **Cerutti A**, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol* 2013; **13**: 118-132 [PMID: 23348416 DOI: 10.1038/nri3383]
8 **Zucca E**, Bertoni F, Vannata B, Cavalli F. Emerging role of infectious etiologies in the pathogenesis of marginal zone B-cell lymphomas. *Clin Cancer Res* 2014; **20**: 5207-5216 [PMID: 25320370 DOI: 10.1158/1078-0432.CCR-14-0496]
9 **Kiesewetter B**, Raderer M. Immunomodulatory treatment for mucosa-associated lymphoid tissue lymphoma (MALT lymphoma). *Hematol Oncol* 2020; **38**: 417-424 [PMID: 32469432 DOI: 10.1002/hon.2754]
10 **Troppan K**, Wenzl K, Neumeister P, Deutsch A. Molecular Pathogenesis of MALT Lymphoma. *Gastroenterol Res Pract* 2015; **2015**: 102656 [PMID: 25922601 DOI: 10.1155/2015/102656]
11 **Krugmann J**, Tzankov A, Dirnhofer S, Fend F, Wolf D, Siebert R, Probst P, Erdel M. Complete or partial trisomy 3 in gastro-intestinal MALT lymphomas co-occurs with aberrations at 18q21 and correlates with advanced disease stage: a study on 25 cases. *World J Gastroenterol* 2005; **11**: 7384-7385 [PMID: 16437648 DOI: 10.3748/wjg.v11.i46.7384]
12 **Taji S**, Nomura K, Matsumoto Y, Sakabe H, Yoshida N, Mitsufuji S, Nishida K, Horiike S,

Nakamura S, Morita M, Taniwaki M. Trisomy 3 may predict a poor response of gastric MALT lymphoma to Helicobacter pylori eradication therapy. *World J Gastroenterol* 2005; **11**: 89-93 [PMID: 15609403 DOI: 10.3748/wjg.v11.i1.89]

13 **Kwee I**, Rancoita PM, Rinaldi A, Ferreri AJ, Bhagat G, Gascoyne RD, Canzonieri V, Gaidano G, Doglioni C, Zucca E, Ponzoni M, Bertoni F. Genomic profiles of MALT lymphomas: variability across anatomical sites. *Haematologica* 2011; **96**: 1064-1066 [PMID: 21459788 DOI: 10.3324/haematol.2011.040402]

14 **Dierlamm J**, Wlodarska I, Michaux L, Stefanova M, Hinz K, Van Den Berghe H, Hagemeijer A, Hossfeld DK. Genetic abnormalities in marginal zone B-cell lymphoma. *Hematol Oncol* 2000; **18**: 1-13 [PMID: 10797525 DOI: 10.1002/(SICI)1099-1069(200003)18:1<1::AID-HON647>3.0.CO;2-G]

15 **Deutsch AJ**, Aigelsreiter A, Steinbauer E, Frühwirth M, Kerl H, Beham-Schmid C, Schaider H, Neumeister P. Distinct signatures of B-cell homeostatic and activation-dependent chemokine receptors in the development and progression of extragastric MALT lymphomas. *J Pathol* 2008; **215**: 431-444 [PMID: 18561120 DOI: 10.1002/path.2372]

16 **Willis TG**, Jadayel DM, Du MQ, Peng H, Perry AR, Abdul-Rauf M, Price H, Karran L, Majekodunmi O, Wlodarska I, Pan L, Crook T, Hamoudi R, Isaacson PG, Dyer MJ. Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. *Cell* 1999; **96**: 35-45 [PMID: 9989495 DOI: 10.1016/S0092-8674(00)80957-5]

17 **Bertoni F**, Zucca E. Delving deeper into MALT lymphoma biology. *J Clin Invest* 2006; **116**: 22-26 [PMID: 16395399 DOI: 10.1172/JCI27476]

18 **Lucas PC**, Yonezumi M, Inohara N, McAllister-Lucas LM, Abazeed ME, Chen FF, Yamaoka S, Seto M, Nunez G. Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J Biol Chem* 2001; **276**: 19012-19019 [PMID: 11262391 DOI: 10.1074/jbc.M009984200]

19 **Uren AG**, O'Rourke K, Aravind LA, Pisabarro MT, Seshagiri S, Koonin EV, Dixit VM. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol Cell* 2000; **6**: 961-967 [PMID: 11090634 DOI: 10.1016/s1097-2765(00)00094-0]

20 **Deutsch AJ**, Frühwirth M, Aigelsreiter A, Cerroni L, Neumeister P. Primary cutaneous marginal zone B-cell lymphomas are targeted by aberrant somatic hypermutation. *J Invest Dermatol* 2009; **129**: 476-479 [PMID: 18704108 DOI: 10.1038/jid.2008.243]

21 **Moody S**, Thompson JS, Chuang SS, Liu H, Raderer M, Vassiliou G, Wlodarska I, Wu F, Cogliatti S, Robson A, Ashton-Key M, Bi Y, Goodlad J, Du MQ. Novel *GPR34* and *CCR6* mutation and distinct genetic profiles in MALT lymphomas of different sites. *Haematologica* 2018; **103**: 1329-1336 [PMID: 29674500 DOI: 10.3324/haematol.2018.191601]

22 **Lenz G**, Davis RE, Ngo VN, Lam L, George TC, Wright GW, Dave SS, Zhao H, Xu W, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Chan WC, Staudt LM. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* 2008; **319**: 1676-1679 [PMID: 18323416 DOI: 10.1126/science.1153629]

23 **Li ZM**, Rinaldi A, Cavalli A, Mensah AA, Ponzoni M, Gascoyne RD, Bhagat G, Zucca E, Bertoni F. MYD88 somatic mutations in MALT lymphomas. *Br J Haematol* 2012; **158**: 662-664 [PMID: 22640364 DOI: 10.1111/j.1365-2141.2012.09176.x]

24 **Min KO**, Seo EJ, Kwon HJ, Lee EJ, Kim WI, Kang CS, Kim KM. Methylation of p16(INK4A) and p57(KIP2) are involved in the development and progression of gastric MALT lymphomas. *Mod Pathol* 2006; **19**: 141-148 [PMID: 16357845 DOI: 10.1038/modpathol.3800505]

25 **Deutsch AJ**, Aigelsreiter A, Staber PB, Beham A, Linkesch W, Guelly C, Brezinschek RI, Fruhwirth M, Emberger W, Buettner M, Beham-Schmid C, Neumeister P. MALT lymphoma and extranodal diffuse large B-cell lymphoma are targeted by aberrant somatic hypermutation. *Blood* 2007; **109**: 3500-3504 [PMID: 17197434 DOI: 10.1182/blood-2006-06-030494]

26 **Bödör C**, Bognár A, Reiniger L, Szepesi A, Tóth E, Kopper L, Matolcsy A. Aberrant somatic hypermutation and expression of activation-induced cytidine deaminase mRNA in mediastinal large B-cell lymphoma. *Br J Haematol* 2005; **129**: 373-376 [PMID: 15842661 DOI: 10.1111/J.1365-2141.2005.05454.X]

27 **Küppers R**, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene* 2001; **20**: 5580-5594 [PMID: 11607811 DOI: 10.1038/sj.onc.1204640]

28 **Matsumoto Y**, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, Azuma T, Okazaki IM, Honjo T, Chiba T. Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med* 2007; **13**: 470-476 [PMID: 17401375 DOI: 10.1038/nm1566]

29 **Isaacson PG**, Spencer J. The biology of low grade MALT lymphoma. *J Clin Pathol* 1995; **48**: 395-397 [PMID: 7629280 DOI: 10.1136/jcp.48.5.395]

30 **Wotherspoon AC**, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; **338**: 1175-1176 [PMID: 1682595 DOI: 10.1016/0140-6736(91)92035-Z]

31 **Cerroni L**, Zöchling N, Pütz B, Kerl H. Infection by Borrelia burgdorferi and cutaneous B-cell lymphoma. *J Cutan Pathol* 1997; **24**: 457-461 [PMID: 9331890 DOI: 10.1111/j.1600-0560.1997.tb01318.x]

32 **Parsonnet J**, Isaacson PG. Bacterial infection and MALT lymphoma. *N Engl J Med* 2004; **350**: 213-

215 [PMID: 14724298 DOI: 10.1056/nejmp038200]
33 **Adam P**, Czapiewski P, Colak S, Kosmidis P, Tousseyn T, Sagaert X, Boudova L, Okoń K, Morresi-Hauf A, Agostinelli C, Pileri S, Pruneri G, Martinelli G, Du MQ, Fend F. Prevalence of Achromobacter xylosoxidans in pulmonary mucosa-associated lymphoid tissue lymphoma in different regions of Europe. *Br J Haematol* 2014; **164**: 804-810 [PMID: 24372375 DOI: 10.1111/bjh.12703]
34 **Ferreri AJ**, Guidoboni M, Ponzoni M, De Conciliis C, Dell'Oro S, Fleischhauer K, Caggiari L, Lettini AA, Dal Cin E, Ieri R, Freschi M, Villa E, Boiocchi M, Dolcetti R. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. *J Natl Cancer Inst* 2004; **96**: 586-594 [PMID: 15100336 DOI: 10.1093/jnci/djh102]
35 **Chanudet E**, Adam P, Nicholson AG, Wotherspoon AC, Ranaldi R, Goteri G, Pileri SA, Ye H, Müller-Hermelink HK, Du MQ. Chlamydiae and Mycoplasma infections in pulmonary MALT lymphoma. *Br J Cancer* 2007; **97**: 949-951 [PMID: 17876330 DOI: 10.1038/sj.bjc.6603981]
36 **Aigelsreiter A**, Gerlza T, Deutsch AJ, Leitner E, Beham-Schmid C, Beham A, Popper H, Borel N, Pospischil A, Raderer M, Kessler HH, Neumeister P. Chlamydia psittaci Infection in nongastrointestinal extranodal MALT lymphomas and their precursor lesions. *Am J Clin Pathol* 2011; **135**: 70-75 [PMID: 21173126 DOI: 10.1309/AJCPXMDRT1SY6KIV]
37 **Negri E**, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1-8 [PMID: 15185336 DOI: 10.1002/ijc.20205]
38 **Aoyama S**, Masaki A, Sakamoto Y, Takino H, Murase T, Ohshima K, Yoshino T, Kato S, Inagaki H. Achromobacter Infection Is Rare in Japanese Patients with Pulmonary B-cell Lymphoma. *Intern Med* 2018; **57**: 789-794 [PMID: 29151525 DOI: 10.2169/internalmedicine.9430-17]
39 **Carugi A**, Onnis A, Antonicelli G, Rossi B, Mannucci S, Luzzi A, Lazzi S, Bellan C, Tosi GM, Sayed S, De Falco G, Leoncini L. Geographic variation and environmental conditions as cofactors in Chlamydia psittaci association with ocular adnexal lymphomas: a comparison between Italian and African samples. *Hematol Oncol* 2010; **28**: 20-26 [PMID: 19728399 DOI: 10.1002/hon.921]
40 **Chanudet E**, Zhou Y, Bacon CM, Wotherspoon AC, Müller-Hermelink HK, Adam P, Dong HY, de Jong D, Li Y, Wei R, Gong X, Wu Q, Ranaldi R, Goteri G, Pileri SA, Ye H, Hamoudi RA, Liu H, Radford J, Du MQ. Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. *J Pathol* 2006; **209**: 344-351 [PMID: 16583361 DOI: 10.1002/path.1984]
41 **Wöhrer S**, Troch M, Streubel B, Zwerina J, Skrabs C, Formanek M, Hauff W, Hoffmann M, Müllauer L, Chott A, Raderer M. MALT lymphoma in patients with autoimmune diseases: a comparative analysis of characteristics and clinical course. *Leukemia* 2007; **21**: 1812-1818 [PMID: 17554381 DOI: 10.1038/sj.leu.2404782]
42 **Ferraccioli GF**, Sorrentino D, De Vita S, Casatta L, Labombarda A, Avellini C, Dolcetti R, Di Luca D, Beltrami CA, Boiocchi M, Bartoli E. B cell clonality in gastric lymphoid tissues of patients with Sjögren's syndrome. *Ann Rheum Dis* 1996; **55**: 311-316 [PMID: 8660105 DOI: 10.1136/ard.55.5.311]
43 **Nocturne G**, Mariette X. Sjögren Syndrome-associated lymphomas: an update on pathogenesis and management. *Br J Haematol* 2015; **168**: 317-327 [PMID: 25316606 DOI: 10.1111/bjh.13192]
44 **Troch M**, Woehrer S, Streubel B, Weissel M, Hoffmann M, Müllauer L, Chott A, Raderer M. Chronic autoimmune thyroiditis (Hashimoto's thyroiditis) in patients with MALT lymphoma. *Ann Oncol* 2008; **19**: 1336-1339 [PMID: 18334510 DOI: 10.1093/annonc/mdn049]
45 **Umehara S**, Higashi H, Ohnishi N, Asaka M, Hatakeyama M. Effects of Helicobacter pylori CagA protein on the growth and survival of B lymphocytes, the origin of MALT lymphoma. *Oncogene* 2003; **22**: 8337-8342 [PMID: 14614457 DOI: 10.1038/sj.onc.1207028]
46 **Kuo SH**, Wu MS, Yeh KH, Lin CW, Hsu PN, Chen LT, Cheng AL. Novel Insights of Lymphomagenesis of *Helicobacter pylori*-Dependent Gastric Mucosa-Associated Lymphoid Tissue Lymphoma. *Cancers (Basel)* 2019; **11** [PMID: 30999581 DOI: 10.3390/cancers11040547]
47 **Lin WC**, Tsai HF, Kuo SH, Wu MS, Lin CW, Hsu PI, Cheng AL, Hsu PN. Translocation of Helicobacter pylori CagA into Human B lymphocytes, the origin of mucosa-associated lymphoid tissue lymphoma. *Cancer Res* 2010; **70**: 5740-5748 [PMID: 20587516 DOI: 10.1158/0008-5472.CAN-09-4690]
48 **Slomiany BL**, Slomiany A. Syk: a new target for attenuation of Helicobacter pylori-induced gastric mucosal inflammatory responses. *Inflammopharmacology* 2019; **27**: 203-211 [PMID: 30820719 DOI: 10.1007/s10787-019-00577-6]
49 **Slomiany BL**, Slomiany A. Role of LPS-elicited signaling in triggering gastric mucosal inflammatory responses to H. pylori: modulatory effect of ghrelin. *Inflammopharmacology* 2017; **25**: 415-429 [PMID: 28516374 DOI: 10.1007/s10787-017-0360-1]
50 **Clark EA**, Ledbetter JA. How B and T cells talk to each other. *Nature* 1994; **367**: 425-428 [PMID: 8107800 DOI: 10.1038/367425a0]
51 **Nakamura H**, Kawakami A, Tominaga M, Migita K, Kawabe Y, Nakamura T, Eguchi K. Expression of CD40/CD40 Ligand and Bcl-2 family proteins in labial salivary glands of patients with Sjögren's syndrome. *Lab Invest* 1999; **79**: 261-269 [PMID: 10092062]
52 **Blosse A**, Peru S, Levy M, Marteyn B, Floch P, Sifré E, Giese A, Prochazkova-Carlotti M, Azzi Martin L, Dubus P, Mégraud F, Ruskone Fournestraux A, Fabiani B, Copie Bergman C, Robe C, Hahne M, Huard B, Lehours P. APRIL-producing eosinophils are involved in gastric MALT lymphomagenesis induced by Helicobacter sp infection. *Sci Rep* 2020; **10**: 14858 [PMID: 32908188 DOI: 10.1038/s41598-020-71792-3]

53 **Raderer M**, Kiesewetter B. What you always wanted to know about gastric MALT-lymphoma: a focus on recent developments. *Ther Adv Med Oncol* 2021; **13**: 17588359211033825 [PMID: 34621332 DOI: 10.1177/17588359211033825]

54 **Stergiou IE**, Poulaki A, Voulgarelis M. Pathogenetic Mechanisms Implicated in Sjögren's Syndrome Lymphomagenesis: A Review of the Literature. *J Clin Med* 2020; **9** [PMID: 33255258 DOI: 10.3390/jcm9123794]

55 **Elgueta R**, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* 2009; **229**: 152-172 [PMID: 19426221 DOI: 10.1111/j.1600-065X.2009.00782.x]

56 **Bossen C**, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. *Semin Immunol* 2006; **18**: 263-275 [PMID: 16914324 DOI: 10.1016/j.smim.2006.04.006]

57 **Craig VJ**, Cogliatti SB, Arnold I, Gerke C, Balandat JE, Wündisch T, Müller A. B-cell receptor signaling and CD40 Ligand-independent T cell help cooperate in Helicobacter-induced MALT lymphomagenesis. *Leukemia* 2010; **24**: 1186-1196 [PMID: 20428202 DOI: 10.1038/leu.2010.76]

58 **García M**, Bellosillo B, Sánchez-González B, García-Payarols F, Seoane A, Ferrer AM, Gimeno E, Barranco LE, Torner A, Solé F, Besses C, Serrano S, Salar A. Study of regulatory T-cells in patients with gastric malt lymphoma: influence on treatment response and outcome. *PLoS One* 2012; **7**: e51681 [PMID: 23284739 DOI: 10.1371/journal.pone.0051681]

59 **Curiel TJ**. Regulatory T cells and treatment of cancer. *Curr Opin Immunol* 2008; **20**: 241-246 [PMID: 18508251 DOI: 10.1016/j.coi.2008.04.008]

60 **Togashi Y**, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat Rev Clin Oncol* 2019; **16**: 356-371 [PMID: 30705439 DOI: 10.1038/s41571-019-0175-7]

61 **D'Elios MM**, Amedei A, Manghetti M, Costa F, Baldari CT, Quazi AS, Telford JL, Romagnani S, Del Prete G. Impaired T-cell regulation of B-cell growth in Helicobacter pylori--related gastric low-grade MALT lymphoma. *Gastroenterology* 1999; **117**: 1105-1112 [PMID: 10535873 DOI: 10.1016/S0016-5085(99)70395-1]

62 **Bergman MP**, D'Elios MM. Cytotoxic T cells in H. pylori-related gastric autoimmunity and gastric lymphoma. *J Biomed Biotechnol* 2010; **2010**: 104918 [PMID: 20617132 DOI: 10.1155/2010/104918]

63 **Thieblemont C**, Bertoni F, Copie-Bergman C, Ferreri AJ, Ponzoni M. Chronic inflammation and extra-nodal marginal-zone lymphomas of MALT-type. *Semin Cancer Biol* 2014; **24**: 33-42 [PMID: 24333758 DOI: 10.1016/j.semcancer.2013.11.005]

64 **Neumeister P**, Hoefler G, Beham-Schmid C, Schmidt H, Apfelbeck U, Schaider H, Linkesch W, Sill H. Deletion analysis of the p16 tumor suppressor gene in gastrointestinal mucosa-associated lymphoid tissue lymphomas. *Gastroenterology* 1997; **112**: 1871-1875 [PMID: 9178679 DOI: 10.1053/gast.1997.v112.pm9178679]

65 **Liang R**, Chan WP, Kwong YL, Xu WS, Srivastava G, Ho FC. High incidence of BCL-6 gene rearrangement in diffuse large B-cell lymphoma of primary gastric origin. *Cancer Genet Cytogenet* 1997; **97**: 114-118 [PMID: 9283593 DOI: 10.1016/S0165-4608(96)00388-3]

66 **Hussell T**, Isaacson PG, Crabtree JE, Spencer J. Helicobacter pylori-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *J Pathol* 1996; **178**: 122-127 [PMID: 8683376 DOI: 10.1002/(SICI)1096-9896(199602)178:2<122::AID-PATH486>3.0.CO;2-D]

67 **Zucca E**, Arcaini L, Buske C, Johnson PW, Ponzoni M, Raderer M, Ricardi U, Salar A, Stamatopoulos K, Thieblemont C, Wotherspoon A, Ladetto M; ESMO Guidelines Committee. Marginal zone lymphomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2020; **31**: 17-29 [PMID: 31912792 DOI: 10.1016/j.annonc.2019.10.010]

68 **Kotla V**, Goel S, Nischal S, Heuck C, Vivek K, Das B, Verma A. Mechanism of action of lenalidomide in hematological malignancies. *J Hematol Oncol* 2009; **2**: 36 [PMID: 19674465 DOI: 10.1186/1756-8722-2-36]

69 **Quach H**, Ritchie D, Stewart AK, Neeson P, Harrison S, Smyth MJ, Prince HM. Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma. *Leukemia* 2010; **24**: 22-32 [PMID: 19907437 DOI: 10.1038/leu.2009.236]

70 **Leonard JP**, Trneny M, Izutsu K, Fowler NH, Hong X, Zhu J, Zhang H, Offner F, Scheliga A, Nowakowski GS, Pinto A, Re F, Fogliatto LM, Scheinberg P, Flinn IW, Moreira C, Cabeçadas J, Liu D, Kalambakas S, Fustier P, Wu C, Gribben JG; AUGMENT Trial Investigators. AUGMENT: A Phase III Study of Lenalidomide Plus Rituximab Versus Placebo Plus Rituximab in Relapsed or Refractory Indolent Lymphoma. *J Clin Oncol* 2019; **37**: 1188-1199 [PMID: 30897038 DOI: 10.1200/JCO.19.00010]

71 **Kiesewetter B**, Troch M, Mayerhoefer ME, Dolak W, Simonitsch-Klupp I, Raderer M. Delayed Efficacy After Treatment With Lenalidomide or Thalidomide in Patients With Mucosa-Associated Lymphoid Tissue Lymphoma. *Oncologist* 2016; **21**: 72-75 [PMID: 26621040 DOI: 10.1634/theoncologist.2015-0176]

72 **Bagheri N**, Azadegan-Dehkordi F, Rahimian G, Rafieian-Kopaei M, Shirzad H. Role of Regulatory T-cells in Different Clinical Expressions of Helicobacter pylori Infection. *Arch Med Res* 2016; **47**: 245-254 [PMID: 27664483 DOI: 10.1016/j.arcmed.2016.07.013]

73 **Laur AM**, Floch P, Chambonnier L, Benejat L, Korolik V, Giese A, Dubus P, Mégraud F, Bandeira A, Lehours P. Regulatory T cells may participate in Helicobacter pylori persistence in gastric MALT lymphoma: lessons from an animal model. *Oncotarget* 2016; **7**: 3394-3402 [PMID: 26657504 DOI:

10.18632/oncotarget.6492]
74 **Iwaya Y**, Kobayashi M, Momose M, Hiraoka N, Sakai Y, Akamatsu T, Tanaka E, Ohtani H, Fukuda M, Nakayama J. High levels of FOXP3$^+$ regulatory T cells in gastric MALT lymphoma predict responsiveness to Helicobacter pylori eradication. *Helicobacter* 2013; **18**: 356-362 [PMID: 23551894 DOI: 10.1111/hel.12051]
75 **Podhorecka M**, Goracy A, Szymczyk A, Kowal M, Ibanez B, Jankowska-Lecka O, Macheta A, Nowaczynska A, Drab-Urbanek E, Chocholska S, Jawniak D, Hus M. Changes in T-cell subpopulations and cytokine network during early period of ibrutinib therapy in chronic lymphocytic leukemia patients: the significant decrease in T regulatory cells number. *Oncotarget* 2017; **8**: 34661-34669 [PMID: 28416773 DOI: 10.18632/oncotarget.16148]
76 **Liu F**, Karube K, Kato H, Arita K, Yoshida N, Yamamoto K, Tsuzuki S, Kim W, Ko YH, Seto M. Mutation analysis of NF-κB signal pathway-related genes in ocular MALT lymphoma. *Int J Clin Exp Pathol* 2012; **5**: 436-441 [PMID: 22808296]
77 **McAllister-Lucas LM**, Inohara N, Lucas PC, Ruland J, Benito A, Li Q, Chen S, Chen FF, Yamaoka S, Verma IM, Mak TW, Núñez G. Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. *J Biol Chem* 2001; **276**: 30589-30597 [PMID: 11387339 DOI: 10.1074/jbc.M103824200]
78 **Hozak RR**, Manji GA, Friesen PD. The BIR motifs mediate dominant interference and oligomerization of inhibitor of apoptosis Op-IAP. *Mol Cell Biol* 2000; **20**: 1877-1885 [PMID: 10669762 DOI: 10.1128/mcb.20.5.1877-1885.2000]
79 **Panwalkar A**, Verstovsek S, Giles F. Nuclear factor-kappaB modulation as a therapeutic approach in hematologic malignancies. *Cancer* 2004; **100**: 1578-1589 [PMID: 15073843 DOI: 10.1002/cncr.20182]
80 **Thounaojam MC**, Dudimah DF, Pellom ST Jr, Uzhachenko RV, Carbone DP, Dikov MM, Shanker A. Bortezomib enhances expression of effector molecules in anti-tumor CD8+ T lymphocytes by promoting Notch-nuclear factor-κB crosstalk. *Oncotarget* 2015; **6**: 32439-32455 [PMID: 26431276 DOI: 10.18632/oncotarget.5857]
81 **Pellom ST Jr**, Dudimah DF, Thounaojam MC, Uzhachenko RV, Singhal A, Richmond A, Shanker A. Bortezomib augments lymphocyte stimulatory cytokine signaling in the tumor microenvironment to sustain CD8+T cell antitumor function. *Oncotarget* 2017; **8**: 8604-8621 [PMID: 28052005 DOI: 10.18632/oncotarget.14365]
82 **Guadagnoli M**, Kimberley FC, Phan U, Cameron K, Vink PM, Rodermond H, Eldering E, Kater AP, van Eenennaam H, Medema JP. Development and characterization of APRIL antagonistic monoclonal antibodies for treatment of B-cell lymphomas. *Blood* 2011; **117**: 6856-6865 [PMID: 21543761 DOI: 10.1182/blood-2011-01-330852]
83 **Zimmermann P**, Ziesenitz VC, Curtis N, Ritz N. The Immunomodulatory Effects of Macrolides-A Systematic Review of the Underlying Mechanisms. *Front Immunol* 2018; **9**: 302 [PMID: 29593707 DOI: 10.3389/fimmu.2018.00302]
84 **Ghosh N**, Tucker N, Zahurak M, Wozney J, Borrello I, Huff CA. Clarithromycin overcomes resistance to lenalidomide and dexamethasone in multiple myeloma. *Am J Hematol* 2014; **89**: E116-E120 [PMID: 24723438 DOI: 10.1002/ajh.23733]
85 **Van Nuffel AM**, Sukhatme V, Pantziarka P, Meheus L, Sukhatme VP, Bouche G. Repurposing Drugs in Oncology (ReDO)-clarithromycin as an anti-cancer agent. *Ecancermedicalscience* 2015; **9**: 513 [PMID: 25729426 DOI: 10.3332/ecancer.2015.513]
86 **Li L**, Sun R, Miao Y, Tran T, Adams L, Roscoe N, Xu B, Manyam GC, Tan X, Zhang H, Xiao M, Tzankov A, Visco C, Dybkaer K, Bhagat G, Tam W, Hsi ED, van Krieken JH, You H, Huh J, Ponzoni M, Ferreri AJM, Møller MB, Piris MA, Zhang M, Winter JN, Medeiros LJ, Rassidakis GZ, Vaupel CA, Li Y, Dakappagari N, Xu-Monette ZY, Young KH. PD-1/PD-L1 expression and interaction by automated quantitative immunofluorescent analysis show adverse prognostic impact in patients with diffuse large B-cell lymphoma having T-cell infiltration: a study from the International DLBCL Consortium Program. *Mod Pathol* 2019; **32**: 741-754 [PMID: 30666052 DOI: 10.1038/s41379-018-0193-5]
87 **Ferreri AJ**, Sassone M, Kiesewetter B, Govi S, Scarfò L, Donadoni G, Raderer M. High-dose clarithromycin is an active monotherapy for patients with relapsed/refractory extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT): the HD-K phase II trial. *Ann Oncol* 2015; **26**: 1760-1765 [PMID: 25935794 DOI: 10.1093/annonc/mdv214]
88 **Ferreri AJM**, Cecchetti C, Kiesewetter B, Sassone M, Calimeri T, Perrone S, Ponzoni M, Raderer M. Clarithromycin as a "repurposing drug" against MALT lymphoma. *Br J Haematol* 2018; **182**: 913-915 [PMID: 28771670 DOI: 10.1111/bjh.14878]
89 **Govi S**, Dognini GP, Licata G, Crocchiolo R, Resti AG, Ponzoni M, Ferreri AJ. Six-month oral clarithromycin regimen is safe and active in extranodal marginal zone B-cell lymphomas: final results of a single-centre phase II trial. *Br J Haematol* 2010; **150**: 226-229 [PMID: 20433679 DOI: 10.1111/j.1365-2141.2010.08179.x]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 163-180

DOI: 10.4251/wjgo.v14.i1.163

ISSN 1948-5204 (online)

MINIREVIEWS

Immunotherapy in liver transplantation for hepatocellular carcinoma: Pros and cons

Yi Luo, Fei Teng, Hong Fu, Guo-Shan Ding

ORCID number: Yi Luo 0000-0003-1455-4162; Fei Teng 0000-0001-9076-8862; Hong Fu 0000-0003-0223-1905; Guo-Shan Ding 0000-0001-8917-969X.

Author contributions: Luo Y and Teng F contributed to the conception and design of the study; Luo Y performed the literature review, data analysis, and manuscript drafting; Teng F made critical revisions and was responsible for the final manuscript; Fu H participated in data analysis; Ding GS was the supervisor and participated in the manuscript editing.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest to report.

Supported by National Natural Science Foundation of China, No. 81702923 and No. 81871262.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Grade B (Very good): 0

Yi Luo, Fei Teng, Hong Fu, Guo-Shan Ding, Department of Liver Surgery and Organ Transplantation, Changzheng Hospital, Naval Medical University, Shanghai 200003, China

Corresponding author: Fei Teng, MD, PhD, Attending Doctor, Surgeon, Surgical Oncologist, Department of Liver Surgery and Organ Transplantation, Changzheng Hospital, Naval Medical University, No. 415 Fengyang Road, Huangpu District, Shanghai 200003, China.
tengfei@smmu.edu.cn

Abstract

Liver transplantation (LT) has emerged as a curative strategy for hepatocellular carcinoma (HCC), but contributes to a higher predisposition to HCC recurrence in the immunosuppression context, especially for tumors beyond the Milan criteria. Although immunotherapy has dramatically improved survival for immunocompetent patients and has become the standard of care for a variety of tumors, including HCC, it is mainly used outside the scope of organ transplantation owing to potentially fatal allograft rejection. Nevertheless, accumulative evidence has expanded the therapeutic paradigms of immunotherapy for HCC, from downstaging or bridging management in the pretransplant setting to the salvage or adjuvant strategy in the posttransplant setting. Generally, immunotherapy mainly includes immune checkpoint inhibitors (ICIs), adoptive cell transfer (ACT) and vaccine therapy. ICIs, followed by ACT, have been most investigated in LT, with some promising results. Because of the complex tumor microenvironment and immunoreactivity when immunosuppressants are combined with immunotherapy, it is difficult to reach formulations for immunosuppressant adjustment and the optimal selection of immunotherapy as well as patients. In addition, the absence of effective biomarkers for identifying rejection and tumor response is still an unresolved barrier to successful clinical immunotherapy applications for LT. In this review, we comprehensively summarize the available evidence of immunotherapy used in LT that is specific to HCC. Moreover, we discuss clinically concerning issues regarding the concurrent goals of graft protection and antitumor response.

Key Words: Hepatocellular carcinoma; Liver transplantation; Immunotherapy; Immune checkpoint inhibitors; Adoptive cell transfer; Immunosuppressant

Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 22, 2021
Peer-review started: March 22, 2021
First decision: June 16, 2021
Revised: June 30, 2021
Accepted: December 7, 2021
Article in press: December 7, 2021
Published online: January 15, 2022

P-Reviewer: Li L
S-Editor: Gao CC
L-Editor: A
P-Editor: Gao CC

Core Tip: This review addresses revolutionized immunotherapy for hepatocellular carcinoma (HCC) in liver transplantation (LT), from downstaging or bridging management in the pretransplant setting to adjuvant or salvage strategy in the posttransplant setting. Considering that the benefit of the antitumor response outweighs the incremental risk of rejection, it is worthwhile to take immunotherapy into account as the salvage option when HCC recurs after LT. More prospective studies are required to provide direct evidence regarding immunosuppressant adjustment, biomarkers for response and the optimal selection of immunotherapy as well as patients.

Citation: Luo Y, Teng F, Fu H, Ding GS. Immunotherapy in liver transplantation for hepatocellular carcinoma: Pros and cons. *World J Gastrointest Oncol* 2022; 14(1): 163-180
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/163.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.163

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers, is the fourth leading cause of cancer-related death and is the sixth most commonly diagnosed cancer worldwide[1]. Liver transplantation (LT) is a well-established and highly effective curative therapy for HCC patients with limited tumor burden who are not candidates for resection. However, even for those that meet the strictest Milan criteria based on the explant tumor burden (*i.e.*, a single nodule ≤ 5 cm in diameter or up to three nodules, with none larger than 3 cm in diameter and without tumor invasion into blood vessels or lymph nodes), the risk of HCC recurrence at 5 years after LT is estimated to be 10% to 15%[2]. Moreover, many countries outside the United States adopt expanded criteria rather than the Milan criteria, leading to an even higher incidence of HCC recurrence. When HCC recurs, the fate of the liver transplant recipient may be worse than that of the inoperable patient with advanced HCC, as immune checkpoint inhibitors (ICIs), the most significant breakthrough in recent years in cancer immunotherapy, are used outside the scope of transplantation. Immunotherapy has dramatically improved the survival of immunocompetent patients, with a long-term response and even complete cancer remission, and has become the standard of care for a variety of tumors, including HCC[3]. Immunotherapy, either by reactivating the suppressed intrinsic immune response or by transferring engineered immune cells, is aimed at immunopotentiation to eliminate tumors, which is contrary to immunosuppression for graft protection after transplantation. Therefore, rejection is an inherent risk for liver transplant recipients receiving immunotherapy and presents as a severe pattern that usually progresses rapidly to induce graft loss. In contrast, some patients receive immunotherapy without any sign of rejection, not only in LT but also in other solid organ transplantations. Generally, immunotherapy includes ICIs, adoptive cell transfer (ACT) and vaccine therapy[4]. Currently, most of the published studies on immunotherapy in the setting of LT are related to ICIs, followed by ACT, while vaccine therapy in LT has not been reported thus far. The different types of immunotherapies, as well as different immunosuppressants, have distinct mechanisms of action. When immunotherapy is combined with immunosuppressants in the setting of transplant recipients with malignancies, the interaction among the immune system, graft and cancer is mediated by a much more complex network of biological pathways than any of these entities alone. Many questions regarding the efficacy and safety of immunotherapy in this subgroup of patients remain unanswered. A recent review analyzed 91 patients treated with ICIs after kidney, liver or heart transplantation for different types of cancer and showed that 37 (41%) experienced rejection. Eight (10%) of 80 patients with an available survival status died due to rejection of the transplant, and 41 (51%) died of cancer progression[5]. As cancer progression is a greater threat and because immunotherapy appears to be the last therapeutic option for these patients, it is worth the risk of rejection. In this review, we focus on immunotherapy that is specific to HCC and used perioperatively in liver transplant recipients. We also discuss clinically concerning issues regarding the concurrent goals of graft protection and antitumor response that warrant further investigation.

IMMUNOTHERAPY AS A DOWNSTAGING OR BRIDGING APPROACH TO LT FOR HCC PATIENTS

The American Association for the Study of Liver Diseases suggests that patients beyond the Milan criteria be considered for LT after successful downstaging into the Milan criteria, which has been accepted by the United Network for Organ Sharing and provides a means for making formerly ineligible patients eligible for transplantation. For a long time, ablation and transarterial therapies have been used as two main downstaging approaches as well as bridging approaches, reducing the drop-out risk in the waiting list. Currently, immunotherapy is joining this oncological armamentarium, as an increasing number of clinical trials have shown encouraging objective response rates, even a complete response rate as high as 5.5%[3,4]. To date, 11 reported cases have used immunotherapy before LT (Table 1): 9 in a single-center series and 2 in two separate reports[6-8]. All 11 patients were treated with nivolumab, a programmed cell death protein-1 (PD-1) monoclonal antibody that belongs to ICIs, at a dose of 240 mg every 2 wk. The intra- and posttransplant immunosuppressant regimens were similar. One patient developed acute hepatic necrosis on postoperative day 5 that was likely related to the preoperative use of nivolumab and refractory to high-dose methylprednisolone and rabbit antithymocyte globulin and died on postoperative day 10. Another patient developed acute rejection, probably due to low tacrolimus levels, and responded rapidly to increasing dosages. The native liver explants of 4 patients showed > 90% tumor necrosis. After a follow-up of 16.0 ± 5.8 mo, none of the 10 surviving patients developed tumor recurrence.

Due to the small sample size and selective bias, it was difficult to determine risk factors associated with fatal rejection for those receiving immunotherapy as a downstaging or bridging approach to LT. However, the patient with fatal hepatic necrosis provided some clues. First, he received the longest immunotherapy of nivolumab (nearly 2 years) and underwent LT shortly after the last dose (8 d before transplantation). However, it is worth noting that 3 other patients who received the last dose less than 8 d before LT did not experience rejection, and one even received the last dose 1 d before transplantation with a total duration of 64 wk. However, a short interval between the last dose and LT should be avoided, as the half-life period of nivolumab is approximately 4 wk. Second, pathology of his explant revealed complete tumor necrosis and no evidence of residual HCC. Currently, there are no guidelines proposed for when and how to discontinue or taper ICIs. However, when a patient receiving immunotherapy achieves stable or regressive disease and is listed as a potential candidate for LT, a taper strategy should be considered. Third, the donor of the patient was positive for the HCV antibody, although without active HCV viremia, and there was no evidence of hepatitis or fibrosis on back-table biopsy of the donor liver. The relationship between an HCV-positive donor liver and severe rejection in the setting of immunotherapy needs further investigation.

IMMUNOTHERAPY AS ADJUVANT THERAPY FOR HCC AFTER LT

LT completely removes the primary tumors as well as potential lesions within the diseased liver. Circulating tumor cells or extrahepatic undetected lesions are origins of HCC recurrence. Theoretically, adjuvant therapy after LT can eliminate residual tumor cells, as the tumor burden, if still present, decreases to the lowest level. However, current evidence does not support adjuvant systematic therapies with chemotherapy or sorafenib to reduce the risk of HCC recurrence after LT[9]. A retrospective cohort study of 60 HCC patients within the University of California San Francisco criteria, published in 2018, assessed the posttransplant antirecurrence efficacy of Licardin in single and multiple administrations, a radioisotope iodine (^{131}I)-labeled antibody fragment targeting the HCC-associated antigen HAb18G/CD147, and showed that adjuvant therapy with Licardin significantly reduced HCC recurrence after LT and that multiple administrations had little additional antirecurrence efficacy[10]. However, subsequent studies with larger sample sizes are rare. Due to the unpredictable risk of rejection, which occurs mainly in transplant recipients taking ICIs, immunotherapy as adjuvant therapy after LT should be used cautiously. ACT using natural killer (NK) cells or cytokine-induced killer (CIK) cells seems to be safer than ICIs. Tanimine *et al*[11] reported adjuvant immunotherapy using liver allograft-derived NK cells in 24 HCC patients after living-donor LT at the 2015 American Transplant Congress and stated that the intravenous transfer of processed NK cells to recipients 4 d after LT with a median of 273.5 million cells/patient significantly

Table 1 Characteristics of hepatocellular carcinoma patients receiving immunotherapy as a downstaging or bridging approach to liver transplantation

No.	Ref.	Age	Sex	Underlying liver disease	MTD (cm)	Pathology milan in/out	Cycles/duration	Immunotherapy	Days before LT	Post-LT follow-up (mo)	Initial immunosuppression	Rejection
1	Tabrizian *et al*[6]	69	M	None	10	Milan out within UCSF	21 cycles	Nivolumab	18	23	Tapering steroids + tacrolimus + MMF	No
2	Tabrizian *et al*[6]	56	F	HCV	5.4	Milan out within UCSF	8 cycles	Nivolumab	22	22	Tapering steroids + tacrolimus + MMF	No
3	Tabrizian *et al*[6]	58	M	HBV	21	Milan in	32 cycles	Nivolumab	1	22	Tapering steroids + tacrolimus + MMF	No
4	Tabrizian *et al*[6]	63	M	HCV, HIV	4.4	Milan in	4 cycles	Nivolumab	2	21	Tapering steroids + tacrolimus + MMF	No
5	Tabrizian *et al*[6]	30	M	HBV	3.2	Milan in	25 cycles	Nivolumab	22	16	Tapering steroids + tacrolimus + MMF	Mild
6	Tabrizian *et al*[6]	63	M	HBV	2	Milan in	4 cycles	Nivolumab	13	14	Tapering steroids + tacrolimus + MMF	No
7	Tabrizian *et al*[6]	66	M	HBV	2.5	Milan in	9 cycles	Nivolumab	253	14	Tapering steroids + tacrolimus + MMF	No
8	Tabrizian *et al*[6]	55	F	HBV	2.8	Milan in	12 cycles	Nivolumab	7	8	Tapering steroids + tacrolimus + MMF	No
9	Tabrizian *et al*[6]	53	F	NASH	8.7	Milan out within UCSF	2 cycles	Nivolumab	30	8	Tapering steroids + tacrolimus + MMF	No
10	Schwacha-Eipper *et al*[7]	66	M	Alcohol-associated liver cirrhosis	6.4	Milan out	34 cycles	Nivolumab	105	12	NA	No
11	Nordness *et al*[8]	65	M	HCV	5.5	Milan in	2 yr	Nivolumab	8	Death at day 10	Tacrolimus + MMF + steroids	Yes

M: Male; F: Female; MTD: Max tumor diameter; HBV: Hepatitis B virus; HCV: Hepatitis B virus; UCSF: The University of California San Francisco criteria; LT: Liver transplantation; NASH: Nonalcoholic steatohepatitis; MMF: Mycophenolate mofetil; NA: Not available.

improved the 5-year recurrence-free survival and overall survival rates of patients pathologically exceeding the Milan criteria without any safety issues. Another case report on adjuvant immunotherapy using 5×10^9 CIK cells for 4 cycles one month after LT also showed no severe adverse effects, including rejection[12]. If we can distinguish patients with a low risk of rejection, immunotherapy, especially with ICIs, will be a very promising adjuvant therapy for those at a high risk of HCC recurrence after LT because of its superior performance on tumor response compared with other systemic therapies.

IMMUNOTHERAPY FOR HCC RECURRENCE AFTER LT

As described previously, HCC patients after LT are exposed to an inevitable risk of HCC recurrence, and unfortunately, there is a limited therapeutic arsenal available for the HCC recurrence subpopulation with progressive disease (PD) after routine treatment failure. However, in more recent years, growing research on immunotherapeutic applications in the transplant setting has yielded promising results that have revolutionized the therapeutic landscape of cancer recurrence after transplantation. Thus far, the cumulative literature on transplant immunotherapy is primarily focused on kidney transplantation[5]. A multicenter retrospective study covering 69 kidney transplant patients receiving ICIs reported improved overall survival (OS) despite a

concomitant increased risk of rejection[13]. Given the satisfactory clinical outcomes, mounting research has been conducted to explore the potential of immunotherapy in liver transplant recipients with recurrence or *de novo* malignancy. Various malignancies can occur after LT, and melanoma patients seem to exhibit a favorable tumor response to immunotherapy and acceptable rejection rate[14-16]. In a review of ICIs for 6 melanoma patients after LT, 2 achieved complete remission (CR), 2 achieved partial remission (PR), and the remaining 2 developed PD; of note, no patient experienced allograft rejection[14]. There are also emerging reports on HCC recurrence treated with immunotherapy after LT (Table 2). To our knowledge, 29 patients with HCC recurrence had received immunotherapy after LT: 19 received ICIs [PD-1 inhibitors in 15 patients and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) inhibitor in 4 patients], and 10 received cell-based immunotherapy [9 based on T cell receptor (TCR) T cells and one based on allogenic NK cells]. The median patient age was 56 (14-70) years, and 78% of patients were male. Among the patients with recurrence sites reported, patients who developed intrahepatic HCC recurrence alone after LT accounted for 11% (2/18), those who developed extrahepatic recurrence alone accounted for 56% (10/18), and those who developed both accounted for 33% (6/18). The estimation of the efficacy and safety of immunotherapy was performed based on the summarized data (Table 2).

Efficacy: The last chance for liver transplant recipients who develop HCC recurrence

Multiple treatments were used before the initiation of immunotherapy, including sorafenib (n = 14), regorafenib (n = 5), lenvatinib (n = 2), chemotherapy (n = 7), radiotherapy (n = 5), transarterial chemoembolization (n = 4), ablation (n = 3) and surgery (n = 1), and all failed to control the disease. Therefore, salvage immunotherapy has been increasingly utilized as the last option for such subpopulations. Excluding patients whose responses were not reported or could not be assessed because of rapid progression to death or immunotherapy discontinuance after rejection, a total of 16 (55.2%) patients were eligible for response evaluation. The overall response rate (ORR) (CR + PR) was 31.3% (5/16) [including 18.8% (3/16) with CR and 12.5% (2/16) with PR], although 68.8% (11/16) of patients failed to respond to immunotherapy. In the ICI subgroup, the ORR was 25% (3/12), which manifested a numerically improved antitumor response in transplant patients compared to that in nontransplant patients with advanced HCC, where the ORRs to nivolumab and pembrolizumab were 15% and 18.3%, respectively[17,18]. Such a difference was difficult to interpret in terms of clinical benefit due to the limited sample size and selection bias, so further studies are necessary to establish whether each individual immunotherapy agent plays a different role through a specific mechanism in the liver transplant setting. For a single immunotherapy agent, the tumor response rates of patients treated with nivolumab, pembrolizumab, and ipilimumab were 11% (1/9), 50% (1/2), and 100% (1/1), respectively. This is discordant with a previously reported review across multiple organ transplantations, where the tumor response rates were 31% (8/26), 48% (12/25), and 29% (4/14), respectively[5].

On the other hand, 9 patients, all from a consecutive cohort led by researchers in Singapore, were treated with HBV-specific TCR T cells[19-21]. Three patients were reported to have a response, one achieved PR with a follow-up of 1 year, and two had PD. Furthermore, HBV-specific TCR T cells were engineered by researchers using the electroporation technique to gain short-term immunosuppressant resistance, which would be very promising in the setting of LT[19]. Another patient who achieved PR was an isolated case with a follow-up of 18 mo; in this patient, allogenic NK cells combined with iodine-125 seed implantation were used[22]. Whether NK cell transfer plays a dominant role in this combined immune-radiotherapy should be investigated through further studies.

In general, considering that the promising antitumor response outweighs the incremental risk of rejection when immunotherapy is used as a non-first-line protocol for liver transplant recipients who develop HCC recurrence, it is worthwhile to take immunotherapy into account as the last salvage option.

Safety: Rejection can be fatal, while PD inevitably leads to death

Because of the advanced stage of HCC when immunotherapy was administered, PD, which rapidly led to death, was the most common response status (12/16). The median duration of immunotherapy was 8.6 wk (IQR 4, 23 wk) and was not long enough to fully expose other immunotherapy-related adverse effects (irAEs) apart from rejection, which might also be related to immunosuppressant usage. Four

Table 2 Characteristics and reported outcomes of published cases with hepatocellular carcinoma recurrence receiving immunotherapy after liver transplantation

No.	Ref.	Age	Sex	HCC recurrence	Immunosuppression protocol before immunotherapy	Compound	Duration of IMT (wk)	Interval from LT to IMT (yr)	Graft rejection	Tumor response	Follow-up (mo)	Cause of death
1	De Toni and Gerbes [27]	41	M	IR and ER	Low-dose tacrolimus	Nivolumab	30	1	No	PD	10	-
2	Friend *et al* [59]	20	M	ER	Sirolimus	Nivolumab	4	4	Yes, lethal (17 d)	NA	1	OF (4 wk after ICI initiation)
3	Friend *et al* [59]	14	M	ER	Tacrolimus	Nivolumab	2	3	Yes, lethal (7 d)	NA	1	OF (5 wk after ICI initiation)
4	Varkaris *et al*[25]	70	M	ER	Low-dose tacrolimus	Pembrolizumab	11.3	8	No	PD	3	PD
5	DeLeon *et al*[60]	57	M	HCC recurrence	Tacrolimus	Nivolumab	5.1	2.7	No	PD	1.2	Probably PD
6	DeLeon *et al*[60]	56	M	HCC recurrence	Sirolimus + MMF	Nivolumab	4.7	7.8	No	PD	1.1	Probably PD
7	DeLeon *et al*[60]	35	F	HCC recurrence	Tacrolimus	Nivolumab	5.6	3.7	No	PD	1.3	Probably PD
8	DeLeon *et al*[60]	64	M	HCC recurrence	Tacrolimus	Nivolumab	1.3	1.2	No	NA	0.3	MOF
9	DeLeon *et al*[60]	68	M	HCC recurrence	Sirolimus	Nivolumab	3.9	1.1	Yes (27 d)	NA	0.9	PD
10	Gassmann *et al*[58]	53	F	ER	Everolimus + MMF + steroids	Nivolumab	2	3	Yes, lethal (7 d)	NA	0.8	OF (2 wk after ICI initiation)
11	Rammohan *et al*[32]	57	M	ER	Tacrolimus + MMF + steroid + mTOR inhibitor	Pembrolizumab	42.9	4.3	No	CR	10	Alive
12	Zhuang *et al*[90]	54	M	ER	Tacrolimus	Nivolumab	62	2.7	No	PD	20	PD
13	Al Jarroudi *et al*[91]	70	M	IR	Tacrolimus	Nivolumab	8	> 3.0	Yes (45 d)	NA	4	PD
14	Al Jarroudi *et al*[91]	62	F	ER	Tacrolimus	Nivolumab	10	2.5	No	PD	2.5	Alive
15	Al Jarroudi *et al*[91]	66	M	IR and ER	Tacrolimus	Nivolumab	12	> 4.75	No	PD	3	Alive
16	Amjad *et al* [24]	62	F	IR and ER	-	Nivolumab	82.7	1.3	No	CR	20	Alive
17	Wang *et al* [92]	48	M	ER	Sirolimus + tacrolimus	Pembrolizumab	3	1	Yes (5 d)	NA	8	Alive
18	Qiu *et al* [93]	54	M	IR and ER	Sirolimus	Camrelizumab	39	4.3	No	PD	11	PD
19	Tan *et al* [21]	56	M	ER	Tacrolimus + MMF	HBV-TCR T cells	52	1.1	No	PR	12	Alive
20	Tan *et al* [21]	45	M	IR and ER	Sirolimus	HBV-TCR T cells	16	4.4	No	PD	3.7	Alive
21	Qasim *et al* [20]	70	M	ER	Tacrolimus	HBV-TCR T cells	8.6	11	No	PD	2	PD
22	Hafezi *et al* [19]	-	-	HCC recurrence	Tacrolimus + sirolimus	HBV-TCR T cells	10	1.5	-	-	-	-
23	Hafezi *et al*	-	-	HCC	Tacrolimus + sirolimus	HBV-TCR T	4	1	-	-	-	-

	[19]			recurrence	+ MMF	cells						
24	Hafezi *et al* [19]	-	-	HCC recurrence	Tacrolimus + sirolimus	HBV-TCR T cells	9	1.8	-	-	-	-
25	Hafezi *et al* [19]	-	-	HCC recurrence	Tacrolimus + MMF	HBV-TCR T cells	4	0.4	-	-	-	-
26	Hafezi *et al* [19]	-	-	HCC recurrence	Sirolimus	HBV-TCR T cells	4	0.5	-	-	-	-
27	Hafezi *et al* [19]	-	-	HCC recurrence	Tacrolimus + sirolimus	HBV-TCR T cells	8	0.7	-	-	-	-
28	Xie *et al*[22]	29	M	IR	-	NK cells	12.9	1.5	No	PR	18	Alive
29	Pandey and Cohen [49]	54	F	IR and ER	Tacrolimus	Ipilimumab	55.7	7.5	No	CR	27	Alive

M: Male; F: Female; IMT: Immunotherapy; IR: Intrahepatic recurrence; ER: Extrahepatic recurrence; MMF: Mycophenolate mofetil; CR: Complete response/remission; PR: Partial response/remission; PD: Disease progression/progressive disease; NA: Not available; OF: Organ failure; MOF: Multiple organ failure.

patients developed grade 1-2 transaminitis, two patients developed a biliary stricture that needed stent implantation, and one patient experienced chills, fatigue, and fever. In the ICI immunotherapy subgroups, survival status was determined for 19 patients, and 32% (6/19), including 5 receiving nivolumab and 1 receiving pembrolizumab, experienced rejection. Interestingly, patients who developed both intra- and extrahepatic recurrence appeared to have a lower predisposition to rejection than those who developed intra- or extrahepatic recurrence alone; the incidence of rejection was 0% (0/5), 100% (1/1), and 50% (4/8), respectively. Allograft rejection exhibited a tendency to occur shortly after immunotherapy initiation, at a median time of 12 d (range 5-45 d). No difference in the interval from LT to ICI initiation was detected between patients who did and did not experience rejection (P = 0.191). The mean interval was 2.5 ± 1.2 years for those who experienced rejection and 4.0 ± 2.5 years for those who did not. Although statistical significance was not achieved, perhaps partially due to limited data, patients with a short interval seemed to be at a higher risk of rejection than those with a long interval. After a median follow-up of 3 (0.3-27) months, 68% (13/19) of patients died, but only 23% (3/13) of deaths were attributed to immediate rejection. This result was consistent with the preexisting literature on immunotherapy across multiple solid organ transplantation, which demonstrated that rejection-specific mortality was far less frequent than cancer-specific mortality (23% *vs* 77% in our pooled analysis)[5]. In addition, the graft rejection rates of patients treated with nivolumab, pembrolizumab, and ipilimumab were 36% (5/14), 33% (1/3), and 0% (0/1), respectively. In a systematic review of ICIs for organ transplant patients with a variety of cancers published in 2020[23], among all transplant recipients, the graft rejection rates of patients treated with nivolumab, pembrolizumab, and ipilimumab were 54.2%, 44% and 23%, respectively, and among all liver transplant recipients, the graft rejection rates were 33%, 25% and 12.8%, respectively. This tendency is consistent with our pooled analysis, which indicates that PD-1 inhibitors contribute to a higher risk of graft rejection than CTLA-4 inhibitors. Of note, one patient who experienced two episodes of acute cellular rejection before immunotherapy did not experience rejection after immunotherapy, which revealed that a history of rejection might not be a contraindication for immunotherapy[24].

In the cell-based immunotherapy subgroups, 10 patients received immune cell infusion, and 4 had evaluable graft rejection information. Notably, all 4 patients were successfully infused without severe irAEs or allograft rejection at a median duration of 10.8 wk (range 8.6-52 wk), which suggests that ACT might be superior to ICIs in terms of safety profile. Additionally, intensified immunosuppressive regimens were not applied during the ACT infusion, and tacrolimus-based immunosuppressive regimens accounted for 80% (8/10). Minimal but therapeutic immunosuppressive protocols merit further exploration for ACT immunotherapy.

Taken together, these results suggest that although allograft rejection can be fatal, the relatively low risk of rejection-associated death warrants consideration of immunotherapy as an alternative strategy because disease progression inevitably leads to death.

HOW TO BALANCE GRAFT-PROTECTIVE IMMUNOSUPPRESSION AND ANTITUMOR IMMUNOPOTENTIATION

Lifelong immunosuppression is required for liver transplant recipients to maintain graft protection. However, immunosuppressants might exert adverse pressure on the antitumor efficacy of immunotherapy by dampening host immune capacity[25,26]. According to the currently available data, favorable immunological and oncological responses are still obtained, even noninferior to those in the nontransplant setting, which suggests an incompletely antagonistic relationship between immunosuppression and the antitumor efficacy of immunotherapy. Nevertheless, on the one hand, conventional immunosuppressant regimens for liver recipients receiving immunotherapy may lead to neither graft rejection nor significant antitumor efficacy[27]. On the other hand, the usage of immunotherapy recommended for nontransplant HCC patients might not be fully applicable for liver transplant recipients who develop HCC recurrence. Therefore, how to balance graft-protective immunosuppression and antitumor immunopotentiation remains a critical issue, and further comprehensive investigations are required to explore individual usage and mechanisms in the simultaneous utilization of immunosuppression and immunotherapy.

Adjustment of the immunosuppressant regimen

The immunosuppressive microenvironment plays an important role in immune tolerance and graft protection. Currently, the major immunosuppressants used for liver recipients include calcineurin inhibitors (CNIs), steroids, antimetabolites, and mammalian target of rapamycin (mTOR) inhibitors, which inhibit T cell activation by blocking signaling pathways (signal 1: Antigen presentation and recognition, HLA-TCR/CD3, signal 2: Costimulatory signaling, and signal 3 cytokine priming)[28]. The major clinical immunosuppressants target signals 1 and 3, while cancer immunotherapy targets signal 2[29]. CNIs, such as FK506, which targets signal 1, partially block IL-2 expression by disrupting the activation of nuclear factor of activated T cells [28,30]. Due to the unquestionable capacity of rejection reduction, CNIs are extensively used for the majority of liver transplant recipients[31]. In our pooled analysis, 70% (19/27) of patients were administered a tacrolimus-based immunosuppression protocol during immunotherapy, and 3 achieved a tumor response (2 CRs and 1 PR). Of concern is that low-dose tacrolimus, the minimal immunosuppression strategy, does not increase the burden of rejection and concomitantly avoids interference with the antitumor immune activity of immunotherapy[25,27,32]. Different from CNIs, mTOR inhibitors, including sirolimus and everolimus, block signal 3 of final T cell activation by inhibiting the cell cycle transition from G1 to S phase and thereby influence both the proliferation and activation of T lymphocytes[33,34]. Additionally, mTOR inhibitors have antitumor properties, and as a result, mTOR inhibitors are inclined to be used for liver transplant patients with HCC[35]. However, whether mTOR inhibitors play an essential biological role in graft protection and antitumor efficacy for liver transplant patients who develop HCC recurrence remains unclear. More recently, two studies tended to support the notion that mTOR inhibitors had the potential to uncouple the efficacy and rejection of ICIs in renal transplantation[13,36]. Compared to non-mTOR inhibitor subsets, the administration of mTOR inhibitors in renal transplant patients with malignancy presented a lower predisposition to rejection and simultaneously resulted in improved rejection-free graft survival and overall graft survival[13]. Apart from the aforementioned immunosuppressants, an increasing number of immunosuppressants appear to be associated with a low risk of rejection without affecting the ORR of the tumor to ICIs. Therefore, based on preliminary evidence, regimens combining mTOR inhibitors with low-dose tacrolimus may warrant consideration as an alternative strategy.

Moreover, whether additional steroids may antagonize the therapeutic profile of immunotherapy also remains controversial. Murakami *et al*[13] reported that steroids can diminish the effect of immunotherapy. Conversely, some studies of immunotherapy for organ transplant patients indicated that additional steroids may not exert a negative effect on the efficacy of immunotherapy and may even decrease the risk of irAEs[32,37]. A systematic review involving 39 allograft transplant patients treated with ICIs revealed that individual immunosuppressive regimens had different effects on allograft rejection and tumor response[38]. The allograft rejection rates with a single agent, including prednisone, mTOR inhibitors, or CNIs, and the combination regimen were 78% (7/9), 67% (2/3), 11% (1/9), and 29% (5/17), respectively. The tumor response rates to ICIs were 63% (5/8), 50% (1/2), 25% (2/8), and 50% (7/14)[38]. It is presumed that a single steroid regimen may be insufficient to prevent rejection,

despite a satisfactory tumor response. Thus, steroids combined with other low-dose immunosuppressants, such as CNIs and mTOR inhibitors, may yield promising outcomes in specifically stratified subgroups. Nevertheless, there is no definitive conclusion on the respective contributions of immunosuppressants in HCC patients after LT in our pooled analysis due to the absence of supporting information. Taken together, these findings indicate that given the limitation and heterogeneity of the experimental data, the optimal immunosuppressant regimen cannot be determined, and a combined strategy of mTOR inhibitors and low-dose tacrolimus, with or without steroids, warrants further validation.

Choice of immunotherapy and whether use it in a modified manner

The antitumor efficacy and rejection risk of each individual immunotherapy are distinctly different, and the identification of specific patients and selection of a reasonable management plan based on the respective biological properties of each immunotherapy are urgent matters. The most clinically relevant inhibitory costimulatory pathways (signal 2) are the PD-1:PD-L1/PD-L2 and CTLA-4/B7 axes, which are considered to function at different phases of the T cell response. Both of these inhibitory pathways contribute to immune tolerance; in addition, the PD-1 axis is thought to be the most essential for graft tolerance primarily during the maintenance phase across the posttransplant process, while the CTLA-4/B7 axis functions during the induction phase[39-41]. Therefore, PD-1 inhibitors (nivolumab, pembrolizumab) are more likely to give rise to graft rejection than CTLA-4 inhibitors (ipilimumab), as delineated in our analysis and a previous review[23]. Given that the CTLA-4 axis functions during the induction phase of immune tolerance, some studies have reported that CTLA-4 blockade at the late stage resulted in a lower risk of rejection than that at the early stage[23,42,43]. From the scant evidence, CTLA-4 inhibitors (ipilimumab) are likely more appropriate than PD-1 inhibitors for patients at a high risk of rejection or with a remote LT history.

PD-L2, unlike PD-L1 (the major ligand for PD-1 in peripheral tissues), is more commonly expressed on monocytes and dendritic cells than on tumor cells, and both PD-L1 and PD-L2 are considered to play crucial roles in allograft tolerance[44]. Therefore, from the clinical perspective, PD-L1-specific blockade (preventing the binding of PD-L1 to PD-1) may contribute to a lower predisposition to allograft rejection than PD-1 blockade (preventing the interactions of PD-1 with PD-L1 and PD-L2), partially owing to the preserved biological effects of the PD1/PD-L2 axis in immune tolerance. However, the therapeutic differences in activity and toxicity between PD-1 inhibitors and PD-L2 inhibitors remain to be further evaluated.

To date, no solid conclusion has been drawn regarding whether a modified method is required for immunotherapy. All patients with available information in our analysis were administered ICI immunotherapy in accordance with the instructions. From the perspective of the dose-effect relationship, low-dose exposure to nivolumab (≥ 0.3 mg/kg) could competitively saturate peripheral receptor occupancy and contribute to comparable antitumor efficacy[45,46]. In particular, low- but therapeutic-dose immunotherapy may not only relatively reduce adverse events and financial burden but also not compromise efficacy. Further prospective investigations are needed to explore the precise dose-effect relationship of each individual agent in HCC patients undergoing LT.

Notably, given that the efficacy of ICIs usually appears within 3 mo after initiation [47] and that PD-1 receptor occupancy lasts up to 85 d[48], a markedly prolonged duration is inadvisable because of the increased risk of rejection. Nordness *et al*[8] reported a case in which a recipient who received nivolumab for 2 years prior to LT developed fatal rejection, but pathology of his explants revealed complete tumor necrosis and no evidence of residual HCC. In another published case report, a partial tumor response occurred after three doses of ipilimumab (3 mg/kg), and CR was eventually achieved following the fourth dose of a 3-wk schedule conversion to a 12-wk schedule; notably, a durable response of 27 mo was obtained after a 13-mo ipilimumab regimen[49]. In view of the above results, a tumor response may develop at a relatively early stage, and a prolonged duration of immunotherapy would lead to immunotherapy resistance or severe adverse events. As a result, a prolonged cycle interval and even withdrawal need to be taken into consideration after a definitely complete tumor response based on periodic evaluations and timely identification.

Currently, the exploited cell subgroups of ACT mainly include tumor-infiltrating lymphocytes (TILs), CIK cells, lymphokine-activated killer cells, NK cells, T cells, and genetically redirected cells. In several accomplished studies, chimeric antigen receptor (CAR) T cell immunotherapy targeting tumor-associated antigens (TAAs) showed strong antitumor capacities but also nonnegligible adverse events, such as cytokine

release syndrome (CRS) and neurotoxicity, which limited its clinical applications in the liver transplant setting[50,51]. Unlike CAR-T cell therapy, CAR-NK cell therapy rarely elicits CRS or neurotoxicity; thus, CAR-NK cell therapy might be more suitable for translation into organ transplantation[52]. In our pooled data, CIK cells, NK cells, and HBV-TCR T cells were used in a liver transplant setting with promising clinical results. However, there are many unsolved problems regarding highly efficient production, dosing adjustment, and identification of tumor-specific antigens. Based on existing experiences, dose escalation and a relatively low-dose regimen might be favorable in the liver transplant setting. Considering the high heterogeneity of HCC, engineered cells with multiple targets and combined regimens represent new frontiers.

As mentioned above, there is still no study reporting vaccine therapy in the setting of LT. Even in a nontransplant setting, only a few trials of vaccine therapy targeting HCC-associated antigens have been performed, and none of them has provided clinically meaningful results. However, a strategy using neoantigens has emerged as a promising approach to develop cancer vaccines with intense tumor-specific nontoxic responses due to advancements in the field of high-throughput screening. The ability to predict highly immunogenic neoantigens with antitumor activity as vaccines using this approach has been shown in melanoma[53] and glioblastoma[54]. Although vaccines are traditionally considered a stand-alone therapy, there is a tendency to combine them with ICIs or ACT.

Surveillance and management of immunotherapy-related rejection

Immunotherapy-related rejection remains the major barrier to clinical immunotherapy promotion in HCC patients after LT. For liver transplant recipients receiving immunotherapy, the identification of rejection is easily confounded by immune-related hepatitis, a kind of irAE, which is characterized mainly by mild transaminitis (grades 1-2)[55]. Thus, caution is strongly warranted to distinguish immune-related hepatitis and rejection when apparent liver malfunction is detected. Compared with rejection, hepatitis occurs at a later stage following immunotherapy initiation (median time, 22 d *vs* 5-6 wk)[5,55] and rarely leads to fatal outcomes. Beyond this, immune-related hepatitis is more common in patients treated with CTLA-4 inhibitors[56], whereas allograft rejection is more frequently recorded in liver transplant patients treated with PD-1 inhibitors[23]. When a definite diagnosis cannot be made by virtue of the information above, graft biopsy should be performed and evaluated based on the Banff schema[57]. Generally, immune-related hepatitis is primarily characterized by acute lobular hepatitis, whereas allograft rejection is predominantly characterized by portal inflammation, bile duct damage, and endotheliitis[58]. Clinically, hepatitis and rejection do not seem to be completely distinct, and to some extent, they could be partially homologous. If a single liver biopsy presents both pathological features simultaneously, it is difficult to identify potential mutual interactions involved in disease progression; therefore, given the potential benefit for rejection control, further studies are required to explore the underlying relationship of hepatitis and rejection.

In particular, surveillance should focus on stratified populations who tend to be susceptible to rejection. Although no difference was detected in the interval from LT to ICI initiation between patients who did and did not experience rejection (2.5 ± 1.2 years *vs* 4.0 ± 2.5 years, $P = 0.191$), patients with a narrow interval from LT to immunotherapy initiation exhibited a tendency to have a higher risk of rejection. Moreover, in our analysis, rejection usually occurred shortly after immunotherapy initiation, at a median time of 12 d (range 5-45 d) or at a short cycle (range 1-4 cycles); therefore, more intensive surveillance is recommended during the early period after immunotherapy initiation. Of concern, PD-L1 expression on graft lymphocytes was reported to be strongly associated with rejection after ICI initiation[59,60]; however, Nordness *et al*[8] reported a case of rejection whose PD-L1 staining appeared to be negative before transplantation but positive after transplantation. It can be speculated that PD-L1 expression manifests as a secondary phenomenon following rejection, and therefore, liver biopsy should be performed routinely to validate its predictive efficacy.

Since allograft rejection largely appears to be life-threatening, effective preventive and therapeutic interventions are critically required in clinical practice. Evidence indicates that a cellular-mediated mechanism plays a key role in graft rejection, whereas an antibody-mediated mechanism is secondary only to the former[61,62]. In accordance with this evidence, all 3 evaluable patients enrolled in our analysis experienced cellular-mediated rejection, and 2 experienced both cellular- and antibody-mediated rejection. Typically, in liver transplant recipients who do not receive ICI treatment, approximately 75% of acute cell-mediated rejection can be mitigated with high-dose steroids[58,63]. Comparatively, in this population taking ICIs, only 29% of patients with allograft rejection were salvaged throughout the

treatment course; most patients experienced graft failure[23]. This is consistent with our analysis, where only 2 of 6 (33%) recipients showed a response to steroids. Furthermore, dialysis is often used as an alternative option for rejection in renal transplant recipients, but whether it is feasible in liver transplant recipients remains unclear[38]. Some scholars recommend plasmapheresis as a viable alternative solution for immunotherapy-induced rejection. Although plasmapheresis is mainly thought to alleviate acute antibody-mediated rejection rather than cell-mediated rejection, it can substantially accelerate clearance from the circulation and thus mitigate immunotherapy-induced rejection[58,64]. In addition, antithymocyte globulin and infliximab were reported to be successfully used for acute rejection in liver transplant recipients, but further investigation is needed[65,66]. In summary, an in-depth collaboration involving the patient, surgeon, and oncologist is urgently necessary to identify individualized risk-benefit profiles because of the absence of highly effective therapeutic means available.

Immunotherapy combined with other treatments

To achieve a higher response rate, combination strategies based on immunotherapy might be a promising direction toward optimal antitumor efficacy in liver transplant recipients who develop HCC recurrence. Combination with conventional HCC therapies is the first option. Locoregional liver-directed therapies, such as ablation and transarterial therapies, exhibit the dual effects of robust tumor destruction to liberate substantial TAAs and strongly activate the immune response by priming tumor-specific T cells[67]. Such therapy-induced immunogenic modulation of tumors might amplify the antitumor efficacy of CD8+ effector T cells activated by ICIs[67].

In addition, molecularly targeted therapies with immunotherapies have become the standard of care for advanced HCC. The FDA, EMA and other regulatory agencies worldwide have approved the PD-L1 inhibitor atezolizumab plus vascular endothelial growth factor (VEGF) inhibitor for first-line therapy in HCC. Atezolizumab plus bevacizumab is now listed as the preferred regimen in first-line systemic therapies by National Comprehensive Cancer Network guidelines for HCC, replacing sorafenib and lenvatinib[68,69]. The combination with lenvatinib was associated with double the response rate compared with that observed with single-agent pembrolizumab, but this came at the cost of increased toxicity[70]. In addition, tyrosine kinase inhibitors (TKIs), such as sorafenib, regorafenib and lenvatinib, have been shown to have immune-associated antitumor capacity independent of anti-VEGFR mechanisms[71]. Accumulative studies have demonstrated that sorafenib can stimulate antitumor efficacy by strengthening CD4+ and CD8+ T cell function and infiltration and inhibiting T-reg cells[72-74]. In the liver transplant setting, it has been reported that an HCC patient following LT developed metastatic lung lesions and subsequently received sorafenib but experienced disease progression after 1 year. Then, pembrolizumab was added to sorafenib treatment, and ultimately, the patient achieved CR without allograft rejection[32], which indicated the crucial synergistic antitumor efficacy of the combination of PD-1 inhibitors with TKIs even though TKIs failed as a first-line treatment. Currently, a number of phase III clinical trials using a combination of molecularly targeted therapies and immunotherapies are being conducted. If one or more of them also show positive results, the choice of preferred treatment will depend substantially on patient characteristics, tolerability and toxicity profile, and the preferred strategy would offer concrete experience to draw upon for HCC patients in the LT setting.

Growing evidence indicates that the gut microbiota affects the liver microenvironment in allograft rejection and HCC development[75-77]. Recently, several human studies have suggested that increased microbial diversity exerts a profound effect on the response to PD-1 inhibitors, which might be mediated by increased intratumor CD8+ T cell infiltration[78-80]. However, which specific bacterial taxa contribute to an improved tumor response to PD-1 inhibitors remains an unsolved issue. Hence, fecal microbiota transplantation (FMT), which shifts the entire gut microbiota to patients, may be an alternative. In the liver transplant setting, PD-1 inhibitors in combination with FMT might substantially improve the tumor response and allograft rejection, but more prospective studies are required.

Biomarkers for the response to immunotherapy

Effective biomarkers for identifying potential responders to ICIs would allow physicians to select optimal candidates for immunotherapy. PD-L1 expression on tumor cells was reported to be associated with the tumor response to PD-1 inhibitors [81]; however, in contrast, the CHECKMATE-040 trial suggested that the tumor response occurred regardless of PD-L1 staining[82]. Thus, PD-L1 expression in tumor

tissues does not seem sufficient as a single predictor to identify potential responders to PD-1 blockade. It is thought that immunotherapies, particularly ICIs, work in part by reactivating preexisting TILs. TILs are a class of lymphocytes in the tumor microenvironment that affect carcinogenesis and include CD8+ T cells, CD4+ T cells, tumor-associated macrophages (TAMs), tumor-associated neutrophils, myeloid-derived suppressor cells (MDSCs) and NK cells. An increased density of specific TIL phenotypes, particularly activated CD8+ TILs, is correlated with small tumor size, early TNM stage and better prognosis in HCC patients[83], and the CD8+ TIL density of responders was higher than that of nonresponders[84]. In addition, positive TILs in the tumor margin might be more associated with the tumor response than those in the tumor center[85,86]. In the tumor microenvironment, CD8+ TILs are exhausted or dysfunctional. The failure of CD8+ TILs to kill tumor cells involves signals from multiple cells, including MDSCs, Tregs, and TAMs. The interaction of PD-L1 with PD-1 on CD8+ TILs causes suppression and a decrease in their effector function, leading to decreased tumor cell death. Furthermore, the galectin-9 and T cell immunoglobulin and mucin-domain containing (TIM)-3 interaction on MDSCs and IL-10 secretion by Tregs have a similar effect[87]. Therefore, TILs and PD-L1 should be combined to guide the development of immunotherapies and predict their clinical responses in cancers. A recent study by DeLeon *et al*[60], covering 5 recipients with PD-L1 staining and 4 with TIL assessments, presumed that the combined expression of PD-L1 and TILs might be more reliable in liver transplant recipients. Additionally, the KEYNOTE-224 trial established a score involving both PD-L1-positive tumor cells and the immune cell ratio to the total number of viable tumor cells, with a positive score indicating a higher likelihood of tumor response[88]. In addition to the markers mentioned above, microsatellite instability, mismatch repair deficiency, and tumor mutational burden were thought to be potential biomarkers for predicting the response to ICIs; however, whether these biomarkers work well in the liver transplant setting requires further investigation. Some predictive biomarkers have been proposed to identify which patients are likely to benefit from CTLA-4 blockade; these include the absolute lymphocyte count and T cell activation marker-inducible costimulator [89]. However, to date, no biomarker has been validated in liver transplant recipients with CTLA-4 blockade. Herein, given the frustration with the inability to identify specific responder subsets, PD-1 inhibitors might be taken into consideration prior to CTLA-4 inhibitors to maximize tumor response. In addition, it is recommended that liver biopsy be conducted both pre- and postimmunotherapy together with a relevant biomarker quantitative assessment for a better stratification of HCC patients after LT.

CONCLUSION

Within the last decade, breakthroughs in immunotherapy have greatly expanded the treatment armamentarium for HCC. However, there is still an unlit corner for HCC patients awaiting LT or after LT due to the deep concern about lethal rejection induced by immunotherapy. On the one hand, there will be an increasing number of HCC patients after immunotherapy who are bridged or downstaged to be candidates for LT, as immunotherapy is now gradually becoming a part of routine or even preferred regimens for HCC systemic therapy. There are also many patients with HCC recurrence after LT who fail to respond to other therapies, and immunotherapy may be their last option. We must face the demand for immunotherapy in the setting of LT. On the other hand, the rejection rate, especially the lethal pattern, is higher than we can afford, and there are many unsolved problems when immunotherapy coexists with immunosuppressants in the setting of LT. Therefore, we need to explore immunotherapies in LT for HCC with caution regarding immunosuppressant adjustment, biomarkers for safety and efficacy, and selection strategies for different immunotherapies and patients.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Mehta N**, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, Lee D, Burns JM, Sanchez W, Greig PD, Grant DR, Roberts JP, Yao FY. Validation of a Risk Estimation of Tumor Recurrence After Transplant (RETREAT) Score for Hepatocellular Carcinoma Recurrence After Liver Transplant.

JAMA Oncol 2017; **3**: 493-500 [PMID: 27838698 DOI: 10.1001/jamaoncol.2016.5116]

3 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

4 **Kole C**, Charalampakis N, Tsakatikas S, Vailas M, Moris D, Gkotsis E, Kykalos S, Karamouzis MV, Schizas D. Immunotherapy for Hepatocellular Carcinoma: A 2021 Update. *Cancers (Basel)* 2020; **12** [PMID: 33020428 DOI: 10.3390/cancers12102859]

5 **Delyon J**, Zuber J, Dorent R, Poujol-Robert A, Peraldi MN, Anglicheau D, Lebbe C. Immune Checkpoint Inhibitors in Transplantation-A Case Series and Comprehensive Review of Current Knowledge. *Transplantation* 2021; **105**: 67-78 [PMID: 32355121 DOI: 10.1097/TP.0000000000003292]

6 **Tabrizian P**, Florman SS, Schwartz ME. PD-1 inhibitor as bridge therapy to liver transplantation? *Am J Transplant* 2021; **21**: 1979-1980 [PMID: 33316117 DOI: 10.1111/ajt.16448]

7 **Schwacha-Eipper B**, Minciuna I, Banz V, Dufour JF. Immunotherapy as a Downstaging Therapy for Liver Transplantation. *Hepatology* 2020; **72**: 1488-1490 [PMID: 32171041 DOI: 10.1002/hep.31234]

8 **Nordness MF**, Hamel S, Godfrey CM, Shi C, Johnson DB, Goff LW, O'Dell H, Perri RE, Alexopoulos SP. Fatal hepatic necrosis after nivolumab as a bridge to liver transplant for HCC: Are checkpoint inhibitors safe for the pretransplant patient? *Am J Transplant* 2020; **20**: 879-883 [PMID: 31550417 DOI: 10.1111/ajt.15617]

9 **Pelizzaro F**, Gambato M, Gringeri E, Vitale A, Cillo U, Farinati F, Burra P, Russo FP. Management of Hepatocellular Carcinoma Recurrence after Liver Transplantation. *Cancers (Basel)* 2021; **13** [PMID: 34638365 DOI: 10.3390/cancers13194882]

10 **Li J**, Qin L, Ding EC, Shen J, Li JP. [Efficacy and safety of licartin with repeated administration in treatment of HCC patients after liver transplantation]. *Zhonghua Yi Xue Za Zhi* 2018; **98**: 2645-2649 [PMID: 30220152 DOI: 10.3760/cma.j.issn.0376-2491.2018.33.007]

11 **Tanimine N**, Tanaka Y, Ishiyama K, Ohira M, Shimizu S, Yano T, Ohdan H. Adoptive Immunotherapy with Liver allograft-derived NK Cells Improves Recurrence-free Survival after Living-donor Liver Transplantation inPatients with Hepatocellular Carcinoma [abstract]. *Am J Transplant* 2015; **15** (suppl 3)

12 **Li R**, Yan F, Liu L, Li H, Ren B, Hui Z, Ren X. Cytokine-induced killer cell therapy for the treatment of primary hepatocellular carcinoma subsequent to liver transplantation: A case report. *Oncol Lett* 2016; **11**: 1885-1888 [PMID: 26998094 DOI: 10.3892/ol.2016.4109]

13 **Murakami N**, Mulvaney P, Danesh M, Abudayyeh A, Diab A, Abdel-Wahab N, Abdelrahim M, Khairallah P, Shirazian S, Kukla A, Owoyemi IO, Alhamad T, Husami S, Menon M, Santeusanio A, Blosser CD, Zuniga SC, Soler MJ, Moreso F, Mithani Z, Ortiz-Melo D, Jaimes EA, Gutgarts V, Lum E, Danovitch GM, Cardarelli F, Drews RE, Bassil C, Swank JL, Westphal S, Mannon RB, Shirai K, Kitchlu A, Ong S, Machado SM, Mothi SS, Ott PA, Rahma O, Hodi FS, Sise ME, Gupta S, Leaf DE, Devoe CE, Wanchoo R, Nair VV, Schmults CD, Hanna GJ, Sprangers B, Riella LV, Jhaveri KD; Immune Checkpoint Inhibitors in Solid Organ Transplant Consortium. A multi-center study on safety and efficacy of immune checkpoint inhibitors in cancer patients with kidney transplant. *Kidney Int* 2021; **100**: 196-205 [PMID: 33359528 DOI: 10.1016/j.kint.2020.12.015]

14 **De Bruyn P**, Van Gestel D, Ost P, Kruse V, Brochez L, Van Vlierberghe H, Devresse A, Del Marmol V, Le Moine A, Aspeslagh S. Immune checkpoint blockade for organ transplant patients with advanced cancer: how far can we go? *Curr Opin Oncol* 2019; **31**: 54-64 [PMID: 30694841 DOI: 10.1097/CCO.0000000000000505]

15 **Schvartsman G**, Perez K, Sood G, Katkhuda R, Tawbi H. Immune Checkpoint Inhibitor Therapy in a Liver Transplant Recipient With Melanoma. *Ann Intern Med* 2017; **167**: 361-362 [PMID: 28761949 DOI: 10.7326/L17-0187]

16 **Morales RE**, Shoushtari AN, Walsh MM, Grewal P, Lipson EJ, Carvajal RD. Safety and efficacy of ipilimumab to treat advanced melanoma in the setting of liver transplantation. *J Immunother Cancer* 2015; **3**: 22 [PMID: 26082835 DOI: 10.1186/s40425-015-0066-0]

17 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

18 **Yau T**, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Han KH, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Begic D, Chen G, Neely J, Anderson J, Sangro B. CheckMate 459: A randomized, multi-center phase III study of nivolumab (NIVO) *vs* sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Ann Oncol* 2019; **30**: 874

19 **Hafezi M**, Lin M, Chia A, Chua A, Ho ZZ, Fam R, Tan D, Aw J, Pavesi A, Krishnamoorthy TL, Chow WC, Chen W, Zhang Q, Wai LE, Koh S, Tan AT, Bertoletti A. Immunosuppressive Drug-Resistant Armored T-Cell Receptor T Cells for Immune Therapy of HCC in Liver Transplant Patients. *Hepatology* 2021; **74**: 200-213 [PMID: 33249625 DOI: 10.1002/hep.31662]

20 **Qasim W**, Brunetto M, Gehring AJ, Xue SA, Schurich A, Khakpoor A, Zhan H, Ciccorossi P, Gilmour K, Cavallone D, Moriconi F, Farzhenah F, Mazzoni A, Chan L, Morris E, Thrasher A, Maini

MK, Bonino F, Stauss H, Bertoletti A. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015; **62**: 486-491 [PMID: 25308176 DOI: 10.1016/j.jhep.2014.10.001]

21 **Tan AT**, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, Zhao X, Tan HS, Chia A, Le Bert N, Low D, Tan HK, Kumar R, Irani FG, Ho ZZ, Zhang Q, Guccione E, Wai LE, Koh S, Hwang W, Chow WC, Bertoletti A. Use of Expression Profiles of HBV-DNA Integrated Into Genomes of Hepatocellular Carcinoma Cells to Select T Cells for Immunotherapy. *Gastroenterology* 2019; **156**: 1862-1876.e9 [PMID: 30711630 DOI: 10.1053/j.gastro.2019.01.251]

22 **Xie S**, Wu Z, Zhou L, Liang Y, Wang X, Niu L, Xu K, Chen J, Zhang M. Iodine-125 seed implantation and allogenic natural killer cell immunotherapy for hepatocellular carcinoma after liver transplantation: a case report. *Onco Targets Ther* 2018; **11**: 7345-7352 [PMID: 30498359 DOI: 10.2147/OTT.S166962]

23 **Kumar V**, Shinagare AB, Rennke HG, Ghai S, Lorch JH, Ott PA, Rahma OE. The Safety and Efficacy of Checkpoint Inhibitors in Transplant Recipients: A Case Series and Systematic Review of Literature. *Oncologist* 2020; **25**: 505-514 [PMID: 32043699 DOI: 10.1634/theoncologist.2019-0659]

24 **Amjad W**, Kotiah S, Gupta A, Morris M, Liu L, Thuluvath PJ. Successful Treatment of Disseminated Hepatocellular Carcinoma After Liver Transplantation With Nivolumab. *J Clin Exp Hepatol* 2020; **10**: 185-187 [PMID: 32189935 DOI: 10.1016/j.jceh.2019.11.009]

25 **Varkaris A**, Lewis DW, Nugent FW. Preserved Liver Transplant After PD-1 Pathway Inhibitor for Hepatocellular Carcinoma. *Am J Gastroenterol* 2017; **112**: 1895-1896 [PMID: 29215617 DOI: 10.1038/ajg.2017.387]

26 **Munker S**, De Toni EN. Use of checkpoint inhibitors in liver transplant recipients. *United European Gastroenterol J* 2018; **6**: 970-973 [PMID: 30228883 DOI: 10.1177/2050640618774631]

27 **De Toni EN**, Gerbes AL. Tapering of Immunosuppression and Sustained Treatment With Nivolumab in a Liver Transplant Recipient. *Gastroenterology* 2017; **152**: 1631-1633 [PMID: 28384452 DOI: 10.1053/j.gastro.2017.01.063]

28 **Halloran PF**. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715-2729 [PMID: 15616206 DOI: 10.1056/NEJMra033540]

29 **Ho CM**, Chen HL, Hu RH, Lee PH. Harnessing immunotherapy for liver recipients with hepatocellular carcinoma: a review from a transplant oncology perspective. *Ther Adv Med Oncol* 2019; **11**: 1758835919843463 [PMID: 31065295 DOI: 10.1177/1758835919843463]

30 **Diehl R**, Ferrara F, Müller C, Dreyer AY, McLeod DD, Fricke S, Boltze J. Immunosuppression for *in vivo* research: state-of-the-art protocols and experimental approaches. *Cell Mol Immunol* 2017; **14**: 146-179 [PMID: 27721455 DOI: 10.1038/cmi.2016.39]

31 **Geissler EK**, Schlitt HJ. Immunosuppression for liver transplantation. *Gut* 2009; **58**: 452-463 [PMID: 19052024 DOI: 10.1136/gut.2008.163527]

32 **Rammohan A**, Reddy MS, Farouk M, Vargese J, Rela M. Pembrolizumab for metastatic hepatocellular carcinoma following live donor liver transplantation: The silver bullet? *Hepatology* 2018; **67**: 1166-1168 [PMID: 29023959 DOI: 10.1002/hep.29575]

33 **Klawitter J**, Nashan B, Christians U. Everolimus and sirolimus in transplantation-related but different. *Expert Opin Drug Saf* 2015; **14**: 1055-1070 [PMID: 25912929 DOI: 10.1517/14740338.2015.1040388]

34 **Wong M**. Mammalian target of rapamycin (mTOR) pathways in neurological diseases. *Biomed J* 2013; **36**: 40-50 [PMID: 23644232 DOI: 10.4103/2319-4170.110365]

35 **Schnitzbauer AA**, Filmann N, Adam R, Bachellier P, Bechstein WO, Becker T, Bhoori S, Bilbao I, Brockmann J, Burra P, Chazoullières O, Cillo U, Colledan M, Duvoux C, Ganten TM, Gugenheim J, Heise M, van Hoek B, Jamieson N, de Jong KP, Klein CG, Klempnauer J, Kneteman N, Lerut J, Mäkisalo H, Mazzaferro V, Mirza DF, Nadalin S, Neuhaus P, Pageaux GP, Pinna AD, Pirenne J, Pratschke J, Powel J, Rentsch M, Rizell M, Rossi G, Rostaing L, Roy A, Scholz T, Settmacher U, Soliman T, Strasser S, Söderdahl G, Troisi RI, Turrión VS, Schlitt HJ, Geissler EK. mTOR Inhibition Is Most Beneficial After Liver Transplantation for Hepatocellular Carcinoma in Patients With Active Tumors. *Ann Surg* 2020; **272**: 855-862 [PMID: 32889867 DOI: 10.1097/SLA.0000000000004280]

36 **Esfahani K**, Al-Aubodah TA, Thebault P, Lapointe R, Hudson M, Johnson NA, Baran D, Bhulaiga N, Takano T, Cailhier JF, Piccirillo CA, Miller WH. Targeting the mTOR pathway uncouples the efficacy and toxicity of PD-1 blockade in renal transplantation. *Nat Commun* 2019; **10**: 4712 [PMID: 31624262 DOI: 10.1038/s41467-019-12628-1]

37 **Biondani P**, De Martin E, Samuel D. Safety of an anti-PD-1 immune checkpoint inhibitor in a liver transplant recipient. *Ann Oncol* 2018; **29**: 286-287 [PMID: 29293878 DOI: 10.1093/annonc/mdx548]

38 **Abdel-Wahab N**, Safa H, Abudayyeh A, Johnson DH, Trinh VA, Zobniw CM, Lin H, Wong MK, Abdelrahim M, Gaber AO, Suarez-Almazor ME, Diab A. Checkpoint inhibitor therapy for cancer in solid organ transplantation recipients: an institutional experience and a systematic review of the literature. *J Immunother Cancer* 2019; **7**: 106 [PMID: 30992053 DOI: 10.1186/s40425-019-0585-1]

39 **Bour-Jordan H**, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol Rev* 2011; **241**: 180-205 [PMID: 21488898 DOI: 10.1111/j.1600-065X.2011.01011.x]

40 **Tanaka K**, Albin MJ, Yuan X, Yamaura K, Habicht A, Murayama T, Grimm M, Waaga AM, Ueno T, Padera RF, Yagita H, Azuma M, Shin T, Blazar BR, Rothstein DM, Sayegh MH, Najafian N. PDL1 is required for peripheral transplantation tolerance and protection from chronic allograft

rejection. *J Immunol* 2007; **179**: 5204-5210 [PMID: 17911605 DOI: 10.4049/jimmunol.179.8.5204]
41 **Yu S**, Su C, Luo X. Impact of infection on transplantation tolerance. *Immunol Rev* 2019; **292**: 243-263 [PMID: 31538351 DOI: 10.1111/imr.12803]
42 **Fisher J**, Zeitouni N, Fan W, Samie FH. Immune checkpoint inhibitor therapy in solid organ transplant recipients: A patient-centered systematic review. *J Am Acad Dermatol* 2020; **82**: 1490-1500 [PMID: 31302190 DOI: 10.1016/j.jaad.2019.07.005]
43 **Aguirre LE**, Guzman ME, Lopes G, Hurley J. Immune Checkpoint Inhibitors and the Risk of Allograft Rejection: A Comprehensive Analysis on an Emerging Issue. *Oncologist* 2019; **24**: 394-401 [PMID: 30413665 DOI: 10.1634/theoncologist.2018-0195]
44 **Fife BT**, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity *via* the CTLA-4 and PD-1 pathways. *Immunol Rev* 2008; **224**: 166-182 [PMID: 18759926 DOI: 10.1111/j.1600-065X.2008.00662.x]
45 **Yoo SH**, Keam B, Kim M, Kim SH, Kim YJ, Kim TM, Kim DW, Lee JS, Heo DS. Low-dose nivolumab can be effective in non-small cell lung cancer: alternative option for financial toxicity. *ESMO Open* 2018; **3**: e000332 [PMID: 30094065 DOI: 10.1136/esmoopen-2018-000332]
46 **Agrawal S**, Feng Y, Roy A, Kollia G, Lestini B. Nivolumab dose selection: challenges, opportunities, and lessons learned for cancer immunotherapy. *J Immunother Cancer* 2016; **4**: 72 [PMID: 27879974 DOI: 10.1186/s40425-016-0177-2]
47 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]
48 **Brahmer JR**, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 2010; **28**: 3167-3175 [PMID: 20516446 DOI: 10.1200/JCO.2009.26.7609]
49 **Pandey A**, Cohen DJ. Ipilumumab for hepatocellular cancer in a liver transplant recipient, with durable response, tolerance and without allograft rejection. *Immunotherapy* 2020; **12**: 287-292 [PMID: 32248723 DOI: 10.2217/imt-2020-0014]
50 **Sun B**, Yang D, Dai H, Liu X, Jia R, Cui X, Li W, Cai C, Xu J, Zhao X. Eradication of Hepatocellular Carcinoma by NKG2D-Based CAR-T Cells. *Cancer Immunol Res* 2019; **7**: 1813-1823 [PMID: 31484657 DOI: 10.1158/2326-6066.CIR-19-0026]
51 **Shi D**, Shi Y, Kaseb AO, Qi X, Zhang Y, Chi J, Lu Q, Gao H, Jiang H, Wang H, Yuan D, Ma H, Li Z, Zhai B. Chimeric Antigen Receptor-Glypican-3 T-Cell Therapy for Advanced Hepatocellular Carcinoma: Results of Phase I Trials. *Clin Cancer Res* 2020; **26**: 3979-3989 [PMID: 32371538 DOI: 10.1158/1078-0432.CCR-19-3259]
52 **Loh K**, Khan K, Kroemer A. CAR-NK Therapy: Translating a Novel Immunotherapy Treatment to Solid Organ Transplantation. *Transplantation* 2020; **104**: 1523-1524 [PMID: 32732821 DOI: 10.1097/TP.0000000000003267]
53 **Ott PA**, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, Li S, Lieb DJ, Eisenhaure T, Gjini E, Stevens J, Lane WJ, Javeri I, Nellaiappan K, Salazar AM, Daley H, Seaman M, Buchbinder EI, Yoon CH, Harden M, Lennon N, Gabriel S, Rodig SJ, Barouch DH, Aster JC, Getz G, Wucherpfennig K, Neuberg D, Ritz J, Lander ES, Fritsch EF, Hacohen N, Wu CJ. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 2017; **547**: 217-221 [PMID: 28678778 DOI: 10.1038/nature22991]
54 **Keskin DB**, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, Shukla SA, Hu Z, Li L, Le PM, Allesøe RL, Richman AR, Kowalczyk MS, Abdelrahman S, Geduldig JE, Charbonneau S, Pelton K, Iorgulescu JB, Elagina L, Zhang W, Olive O, McCluskey C, Olsen LR, Stevens J, Lane WJ, Salazar AM, Daley H, Wen PY, Chiocca EA, Harden M, Lennon NJ, Gabriel S, Getz G, Lander ES, Regev A, Ritz J, Neuberg D, Rodig SJ, Ligon KL, Suvà ML, Wucherpfennig KW, Hacohen N, Fritsch EF, Livak KJ, Ott PA, Wu CJ, Reardon DA. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 2019; **565**: 234-239 [PMID: 30568305 DOI: 10.1038/s41586-018-0792-9]
55 **Thompson JA**, Schneider BJ, Brahmer J, Andrews S, Armand P, Bhatia S, Budde LE, Costa L, Davies M, Dunnington D, Ernstoff MS, Frigault M, Hoffner B, Hoimes CJ, Lacouture M, Locke F, Lunning M, Mohindra NA, Naidoo J, Olszanski AJ, Oluwole O, Patel SP, Reddy S, Ryder M, Santomasso B, Shofer S, Sosman JA, Wahidi M, Wang Y, Johnson-Chilla A, Scavone JL. Management of Immunotherapy-Related Toxicities, Version 1.2019. *J Natl Compr Canc Netw* 2019; **17**: 255-289 [PMID: 30865922 DOI: 10.6004/jnccn.2019.0013]
56 **Remash D**, Prince DS, McKenzie C, Strasser SI, Kao S, Liu K. Immune checkpoint inhibitor-related hepatotoxicity: A review. *World J Gastroenterol* 2021; **27**: 5376-5391 [PMID: 34539139 DOI: 10.3748/wjg.v27.i32.5376]
57 **Demetris AJ**, Ruppert K, Dvorchik I, Jain A, Minervini M, Nalesnik MA, Randhawa P, Wu T, Zeevi A, Abu-Elmagd K, Eghtesad B, Fontes P, Cacciarelli T, Marsh W, Geller D, Fung JJ. Real-time monitoring of acute liver-allograft rejection using the Banff schema. *Transplantation* 2002; **74**: 1290-1296 [PMID: 12451268 DOI: 10.1097/00007890-200211150-00016]
58 **Gassmann D**, Weiler S, Mertens JC, Reiner CS, Vrugt B, Nägeli M, Mangana J, Müllhaupt B, Jenni

F, Misselwitz B. Liver Allograft Failure After Nivolumab Treatment-A Case Report With Systematic Literature Research. *Transplant Direct* 2018; **4**: e376 [PMID: 30255136 DOI: 10.1097/TXD.0000000000000814]

59 **Friend BD**, Venick RS, McDiarmid SV, Zhou X, Naini B, Wang H, Farmer DG, Busuttil RW, Federman N. Fatal orthotopic liver transplant organ rejection induced by a checkpoint inhibitor in two patients with refractory, metastatic hepatocellular carcinoma. *Pediatr Blood Cancer* 2017; **64** [PMID: 28643391 DOI: 10.1002/pbc.26682]

60 **DeLeon TT**, Salomao MA, Aqel BA, Sonbol MB, Yokoda RT, Ali AH, Moss AA, Mathur AK, Chascsa DM, Rakela J, Bryce AH, Borad MJ. Pilot evaluation of PD-1 inhibition in metastatic cancer patients with a history of liver transplantation: the Mayo Clinic experience. *J Gastrointest Oncol* 2018; **9**: 1054-1062 [PMID: 30603124 DOI: 10.21037/jgo.2018.07.05]

61 **Lefaucheur C**, Loupy A, Vernerey D, Duong-Van-Huyen JP, Suberbielle C, Anglicheau D, Vérine J, Beuscart T, Nochy D, Bruneval P, Charron D, Delahousse M, Empana JP, Hill GS, Glotz D, Legendre C, Jouven X. Antibody-mediated vascular rejection of kidney allografts: a population-based study. *Lancet* 2013; **381**: 313-319 [PMID: 23182298 DOI: 10.1016/S0140-6736(12)61265-3]

62 **Lipson EJ**, Bagnasco SM, Moore J Jr, Jang S, Patel MJ, Zachary AA, Pardoll DM, Taube JM, Drake CG. Tumor Regression and Allograft Rejection after Administration of Anti-PD-1. *N Engl J Med* 2016; **374**: 896-898 [PMID: 26962927 DOI: 10.1056/NEJMc1509268]

63 **Adams DH**, Neuberger JM. Treatment of acute rejection. *Semin Liver Dis* 1992; **12**: 80-88 [PMID: 1570553 DOI: 10.1055/s-2007-1007379]

64 **Kamar N**, Lavayssière L, Muscari F, Selves J, Guilbeau-Frugier C, Cardeau I, Esposito L, Cointault O, Nogier MB, Peron JM, Otal P, Fort M, Rostaing L. Early plasmapheresis and rituximab for acute humoral rejection after ABO-compatible liver transplantation. *World J Gastroenterol* 2009; **15**: 3426-3430 [PMID: 19610146 DOI: 10.3748/wjg.15.3426]

65 **Schmitt TM**, Phillips M, Sawyer RG, Northup P, Hagspiel KD, Pruett TL, Bonatti HJ. Anti-thymocyte globulin for the treatment of acute cellular rejection following liver transplantation. *Dig Dis Sci* 2010; **55**: 3224-3234 [PMID: 20238251 DOI: 10.1007/s10620-010-1149-x]

66 **Spain L**, Diem S, Larkin J. Management of toxicities of immune checkpoint inhibitors. *Cancer Treat Rev* 2016; **44**: 51-60 [PMID: 26874776 DOI: 10.1016/j.ctrv.2016.02.001]

67 **Dromi SA**, Walsh MP, Herby S, Traughber B, Xie J, Sharma KV, Sekhar KP, Luk A, Liewehr DJ, Dreher MR, Fry TJ, Wood BJ. Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. *Radiology* 2009; **251**: 58-66 [PMID: 19251937 DOI: 10.1148/radiol.2511072175]

68 **Benson AB**, D'Angelica MI, Abbott DE, Anaya DA, Anders R, Are C, Bachini M, Borad M, Brown D, Burgoyne A, Chahal P, Chang DT, Cloyd J, Covey AM, Glazer ES, Goyal L, Hawkins WG, Iyer R, Jacob R, Kelley RK, Kim R, Levine M, Palta M, Park JO, Raman S, Reddy S, Sahai V, Schefter T, Singh G, Stein S, Vauthey JN, Venook AP, Yopp A, McMillian NR, Hochstetler C, Darlow SD. Hepatobiliary Cancers, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2021; **19**: 541-565 [PMID: 34030131 DOI: 10.6004/jnccn.2021.0022]

69 **National Comprehensive Cancer Network**. (NCCN) Clinical Practice Guidelines in Oncology. Hepatobiliary Cancer, Version 3. 2021. [cited 20 June 2021]. In: National Comprehensive Cancer Network [Internet]. Available from: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1438

70 **Finn RS**, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, Okusaka T, Kobayashi M, Kumada H, Kaneko S, Pracht M, Mamontov K, Meyer T, Kubota T, Dutcus CE, Saito K, Siegel AB, Dubrovsky L, Mody K, Llovet JM. Phase Ib Study of Lenvatinib Plus Pembrolizumab in Patients With Unresectable Hepatocellular Carcinoma. *J Clin Oncol* 2020; **38**: 2960-2970 [PMID: 32716739 DOI: 10.1200/JCO.20.00808]

71 **Cheng AL**, Hsu C, Chan SL, Choo SP, Kudo M. Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J Hepatol* 2020; **72**: 307-319 [PMID: 31954494 DOI: 10.1016/j.jhep.2019.09.025]

72 **Farsaci B**, Donahue RN, Coplin MA, Grenga I, Lepone LM, Molinolo AA, Hodge JW. Immune consequences of decreasing tumor vasculature with antiangiogenic tyrosine kinase inhibitors in combination with therapeutic vaccines. *Cancer Immunol Res* 2014; **2**: 1090-1102 [PMID: 25092771 DOI: 10.1158/2326-6066.CIR-14-0076]

73 **Chen ML**, Yan BS, Lu WC, Chen MH, Yu SL, Yang PC, Cheng AL. Sorafenib relieves cell-intrinsic and cell-extrinsic inhibitions of effector T cells in tumor microenvironment to augment antitumor immunity. *Int J Cancer* 2014; **134**: 319-331 [PMID: 23818246 DOI: 10.1002/ijc.28362]

74 **Sunay MM**, Foote JB, Leatherman JM, Edwards JP, Armstrong TD, Nirschl CJ, Hicks J, Emens LA. Sorafenib combined with HER-2 targeted vaccination can promote effective T cell immunity in vivo. *Int Immunopharmacol* 2017; **46**: 112-123 [PMID: 28282575 DOI: 10.1016/j.intimp.2017.02.028]

75 **Ren Z**, Li A, Jiang J, Zhou L, Yu Z, Lu H, Xie H, Chen X, Shao L, Zhang R, Xu S, Zhang H, Cui G, Sun R, Wen H, Lerut JP, Kan Q, Li L, Zheng S. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 2019; **68**: 1014-1023 [PMID: 30045880 DOI: 10.1136/gutjnl-2017-315084]

76 **Kato K**, Nagao M, Miyamoto K, Oka K, Takahashi M, Yamamoto M, Matsumura Y, Kaido T, Uemoto S, Ichiyama S. Longitudinal Analysis of the Intestinal Microbiota in Liver Transplantation. *Transplant Direct* 2017; **3**: e144 [PMID: 28405600 DOI: 10.1097/TXD.0000000000000661]

77 **Sun LY**, Yang YS, Qu W, Zhu ZJ, Wei L, Ye ZS, Zhang JR, Sun XY, Zeng ZG. Gut microbiota of

liver transplantation recipients. *Sci Rep* 2017; **7**: 3762 [PMID: 28630433 DOI: 10.1038/s41598-017-03476-4]

78 **Gopalakrishnan V**, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, Cogdill AP, Zhao L, Hudgens CW, Hutchinson DS, Manzo T, Petaccia de Macedo M, Cotechini T, Kumar T, Chen WS, Reddy SM, Szczepaniak Sloane R, Galloway-Pena J, Jiang H, Chen PL, Shpall EJ, Rezvani K, Alousi AM, Chemaly RF, Shelburne S, Vence LM, Okhuysen PC, Jensen VB, Swennes AG, McAllister F, Marcelo Riquelme Sanchez E, Zhang Y, Le Chatelier E, Zitvogel L, Pons N, Austin-Breneman JL, Haydu LE, Burton EM, Gardner JM, Sirmans E, Hu J, Lazar AJ, Tsujikawa T, Diab A, Tawbi H, Glitza IC, Hwu WJ, Patel SP, Woodman SE, Amaria RN, Davies MA, Gershenwald JE, Hwu P, Lee JE, Zhang J, Coussens LM, Cooper ZA, Futreal PA, Daniel CR, Ajami NJ, Petrosino JF, Tetzlaff MT, Sharma P, Allison JP, Jenq RR, Wargo JA. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018; **359**: 97-103 [PMID: 29097493 DOI: 10.1126/science.aan4236]

79 **Routy B**, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, Fidelle M, Flament C, Poirier-Colame V, Opolon P, Klein C, Iribarren K, Mondragón L, Jacquelot N, Qu B, Ferrere G, Clémenson C, Mezquita L, Masip JR, Naltet C, Brosseau S, Kaderbhai C, Richard C, Rizvi H, Levenez F, Galleron N, Quinquis B, Pons N, Ryffel B, Minard-Colin V, Gonin P, Soria JC, Deutsch E, Loriot Y, Ghiringhelli F, Zalcman G, Goldwasser F, Escudier B, Hellmann MD, Eggermont A, Raoult D, Albiges L, Kroemer G, Zitvogel L. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; **359**: 91-97 [PMID: 29097494 DOI: 10.1126/science.aan3706]

80 **Baruch EN**, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, Adler K, Dick-Necula D, Raskin S, Bloch N, Rotin D, Anafi L, Avivi C, Melnichenko J, Steinberg-Silman Y, Mamtani R, Harati H, Asher N, Shapira-Frommer R, Brosh-Nissimov T, Eshet Y, Ben-Simon S, Ziv O, Khan MAW, Amit M, Ajami NJ, Barshack I, Schachter J, Wargo JA, Koren O, Markel G, Boursi B. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* 2021; **371**: 602-609 [PMID: 33303685 DOI: 10.1126/science.abb5920]

81 **Lu S**, Stein JE, Rimm DL, Wang DW, Bell JM, Johnson DB, Sosman JA, Schalper KA, Anders RA, Wang H, Hoyt C, Pardoll DM, Danilova L, Taube JM. Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade: A Systematic Review and Meta-analysis. *JAMA Oncol* 2019; **5**: 1195-1204 [PMID: 31318407 DOI: 10.1001/jamaoncol.2019.1549]

82 **Yau T**, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, Melero I, Kudo M, Hou MM, Matilla A, Tovoli F, Knox JJ, Ruth He A, El-Rayes BF, Acosta-Rivera M, Lim HY, Neely J, Shen Y, Wisniewski T, Anderson J, Hsu C. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: e204564 [PMID: 33001135 DOI: 10.1001/jamaoncol.2020.4564]

83 **Xu X**, Tan Y, Qian Y, Xue W, Wang Y, Du J, Jin L, Ding W. Clinicopathologic and prognostic significance of tumor-infiltrating CD8+ T cells in patients with hepatocellular carcinoma: A meta-analysis. *Medicine (Baltimore)* 2019; **98**: e13923 [PMID: 30633166 DOI: 10.1097/MD.0000000000013923]

84 **Duffy AG**, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, Davis JL, Hughes MS, Heller T, ElGindi M, Uppala A, Korangy F, Kleiner DE, Figg WD, Venzon D, Steinberg SM, Venkatesan AM, Krishnasamy V, Abi-Jaoudeh N, Levy E, Wood BJ, Greten TF. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017; **66**: 545-551 [PMID: 27816492 DOI: 10.1016/j.jhep.2016.10.029]

85 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505 DOI: 10.1038/nature13954]

86 **Riaz N**, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, Hodi FS, Martín-Algarra S, Mandal R, Sharfman WH, Bhatia S, Hwu WJ, Gajewski TF, Slingluff CL Jr, Chowell D, Kendall SM, Chang H, Shah R, Kuo F, Morris LGT, Sidhom JW, Schneck JP, Horak CE, Weinhold N, Chan TA. Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab. *Cell* 2017; **171**: 934-949.e16 [PMID: 29033130 DOI: 10.1016/j.cell.2017.09.028]

87 **Patel P**, Schutzer SE, Pyrsopoulos N. Immunobiology of hepatocarcinogenesis: Ways to go or almost there? *World J Gastrointest Pathophysiol* 2016; **7**: 242-255 [PMID: 27574562 DOI: 10.4291/wjgp.v7.i3.242]

88 **Herbst RS**, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab *vs* docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**: 1540-1550 [PMID: 26712084 DOI: 10.1016/S0140-6736(15)01281-7]

89 **Callahan MK**, Postow MA, Wolchok JD. Immunomodulatory therapy for melanoma: ipilimumab and beyond. *Clin Dermatol* 2013; **31**: 191-199 [PMID: 23438382 DOI: 10.1016/j.clindermatol.2012.08.006]

90 **Zhuang L**, Mou HB, Yu LF, Zhu HK, Yang Z, Liao Q, Zheng SS. Immune checkpoint inhibitor for hepatocellular carcinoma recurrence after liver transplantation. *Hepatobiliary Pancreat Dis Int* 2020;

19: 91-93 [PMID: 31706859 DOI: 10.1016/j.hbpd.2019.09.011]

91 **Al Jarroudi O**, Ulusakarya A, Almohamad W, Afqir S, Morere JF. Anti-Programmed Cell Death Protein 1 (PD-1) Immunotherapy for Metastatic Hepatocellular Carcinoma After Liver Transplantation: A Report of Three Cases. *Cureus* 2020; **12**: e11150 [PMID: 33133796 DOI: 10.7759/cureus.11150]

92 **Wang GY**, Tang H, Zhang YC, Li H, Yi SH, Jiang N, Wang GS, Zhang J, Zhang Q, Yang Y, Chen GH. Programmed death receptor (PD) -1 monoclonal antibody-induced acute immune hepatitis in the treatment of recurrent hepatocellular carcinoma after liver transplantation: a case report. *Organ Transplant* 2016; **1**: 44-47

93 **Qiu J**, Tang W, Du C. Immune Checkpoint Inhibitors in Patients with Recurrent Hepatocellular Carcinoma after Liver Transplantation: A Case Report and Literature Review. *Curr Cancer Drug Targets* 2020; **20**: 720-727 [PMID: 32433005 DOI: 10.2174/1568009620666200520084415]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 181-202

DOI: 10.4251/wjgo.v14.i1.181

ISSN 1948-5204 (online)

MINIREVIEWS

Trimodality treatment in gastric and gastroesophageal junction cancers: Current approach and future perspectives

Nikolaos Charalampakis, Sergios Tsakatikas, Dimitrios Schizas, Stylianos Kykalos, Maria Tolia, Rodanthi Fioretzaki, Georgios Papageorgiou, Ioannis Katsaros, Ahmed Adel Fouad Abdelhakeem, Matheus Sewastjanow-Silva, Jane E Rogers, Jaffer A Ajani

ORCID number: Nikolaos Charalampakis 0000-0002-8756-9659; Sergios Tsakatikas 0000-0001-9643-3262; Dimitrios Schizas 0000-0002-7046-0112; Stylianos Kykalos 0000-0002-8687-685X; Maria Tolia 0000-0002-3896-8426; Rodanthi Fioretzaki 0000-0003-1219-8715; Georgios Papageorgiou 0000-0002-8370-9802; Ioannis Katsaros 0000-0002-6743-8942; Ahmed Adel Fouad Abdelhakeem 0000-0001-7186-1332; Matheus Sewastjanow-Silva 0000-0003-4804-1891; Jane E Rogers 0000-0002-7275-7008; Jaffer A Ajani 0000-0001-9946-0629.

Author contributions: Charalampakis N, Tsakatikas S and Schizas D wrote the paper; Kykalos S, Tolia M, Fioretzaki R, Papageorgiou G and Katsaros I collected the data; Abdelhakeem Adel Fouad A, Sewastjanow-Silva M, Rogers EJ and Ajani AJ revised the manuscript.

Conflict-of-interest statement: The authors have no conflict of interest related to the manuscript.

Country/Territory of origin: Greece

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed

Nikolaos Charalampakis, Sergios Tsakatikas, Rodanthi Fioretzaki, Georgios Papageorgiou, Department of Medical Oncology, Metaxa Cancer Hospital of Piraeus, Piraeus 18537, Greece

Dimitrios Schizas, TheFirst Department of Surgery, National and Kapodistrian University of Athens, Laikon General Hospital, Athens 11527, Greece

Stylianos Kykalos, TheSecond Propedeutic Department of Surgery, National and Kapodistrian University of Athens, Laikon General Hospital, Athens 11527, Greece

Maria Tolia, Department of Radiation Oncology, University Hospital of Crete, Heraklion 71110, Greece

Ioannis Katsaros, Department of General Surgery, Metaxa Cancer Hospital of Piraeus, Piraeus 18537, Greece

Ahmed Adel Fouad Abdelhakeem, Matheus Sewastjanow-Silva, Jane E Rogers, Jaffer A Ajani, Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States

Corresponding author: Nikolaos Charalampakis, MD, PhD, Medical Oncologist, Department of Medical Oncology, Metaxa Cancer Hospital of Piraeus, Mpotasi 51, Piraeus 18537, Greece. nick301178@yahoo.com

Abstract

Gastric and gastroesophageal junction (GEJ) cancers represent an aggressive group of malignancies with poor prognosis even when diagnosed in relatively early stage, with an increasing incidence both in Asia and in Western countries. These cancers are characterized by heterogeneity as a result of different pathogenetic mechanisms as shown in recent molecular analyses. Accordingly, the understanding of phenotypic and genotypic correlations/classifications has been improved. Current therapeutic strategies have also advanced and moved beyond surgical extirpation alone, with the incorporation of other treatment modalities, such as radiation and chemotherapy (including biologics). Chemoradiotherapy has been used as postoperative treatment after suboptimal gastrectomy to ensure local disease control but also improvement in survival. Preoperative chemoradiotherapy/chemotherapy has been employed to increase the chance of a successful R0 resection and pathologic complete response rate,

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A, A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

Received: March 25, 2021
Peer-review started: March 25, 2021
First decision: June 16, 2021
Revised: June 28, 2021
Accepted: December 10, 2021
Article in press: December 10, 2021
Published online: January 15, 2022

P-Reviewer: Chen SY, Chisthi MM, Fu T, Song L
S-Editor: Zhang H
L-Editor: A
P-Editor: Zhang H

which is associated with improved long-term outcomes. Several studies have defined various chemotherapy regimens to accompany radiation (before and after surgery). Recently, addition of immunotherapy after trimodality of gastroesophageal cancer has produced an advantage in disease-free interval. Targeted agents used in the metastatic setting are being investigated in the early setting with mixed results. The aim of this review is to summarize the existing data on trimodality approaches for gastric and GEJ cancers, highlight the remaining questions and present the current research effort addressing them.

Key Words: Gastric cancer; Gastroesophageal junction cancer; Trimodality treatment; Chemoradiotherapy; Surgery; Immunotherapy

Core Tip: Trimodality treatment combining chemotherapy, radiation and surgery is an effective management of locally advanced gastric and gastroesophageal cancers, although the extent of benefit remains to be answered in future clinical trials. Addition of newer therapeutic agents, such as immune checkpoint inhibitors may further enhance the curative potential of this approach.

Citation: Charalampakis N, Tsakatikas S, Schizas D, Kykalos S, Tolia M, Fioretzaki R, Papageorgiou G, Katsaros I, Abdelhakeem AAF, Sewastjanow-Silva M, Rogers JE, Ajani JA. Trimodality treatment in gastric and gastroesophageal junction cancers: Current approach and future perspectives. *World J Gastrointest Oncol* 2022; 14(1): 181-202
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/181.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.181

INTRODUCTION

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer mortality with a varying incidence worldwide[1]. The highest incidence (> 20 per 100000 in men) is seen in China, Japan, Korea, Latin America and Eastern Europe, whereas the lowest incidence (< 10 per 100000 in men) is seen in North America, parts of Africa and Northern Europe[2]. In the West, cancers located at the gastroesophageal junction (GEJ) are less frequent than in the stomach, however, incidence of GEJ cancer has been steadily rising during the last decade[3]. Only 27% of newly diagnosed GCs are localized with a 5-year overall survival (OS) rate of 30.4%, which, unfortunately, remains stable over the last 30 to 40 years[4]. Surgery is still the only chance for cure and implementation of a multimodality treatment approach is utilized to further improve survival. Advanced disease carries a dismal prognosis and treatment remains challenging with a 5-year OS rate less than 5%. Thus, despite decreasing incidence, GC and GEJ cancers remain a serious health burden globally with high mortality rates.

Histology

The vast majority (95%) of gastric malignant neoplasms are adenocarcinomas, which are typically classified based on anatomic location and histologic subtype by Lauren as intestinal and diffuse[5]. Two different mechanisms of carcinogenesis have been implicated for GC correlating with the two different histologic subtypes. Intestinal-type gastric adenocarcinoma has been heavily associated with Helicobacter pylori infection as well as other environmental factors, such as alcohol, processed meat, smoking, and obesity. Diffuse-type gastric adenocarcinoma usually arises from defective intracellular adhesion molecules due to loss of E-cadherin protein expression that is encoded by *CDH1* gene[6]. Genomic mutation in *CDH1* gene is the cause of the Hereditary Diffuse Gastric Cancer Syndrome, which increases the chance for diffuse gastric cancer throughout a person's lifetime up to 70%[7].

Esophageal cancers are histologically diverse and can be either squamous cell carcinomas (SCC) or adenocarcinomas. Carcinomas of the GEJ are classified into the esophageal cancer group according to the 8th edition of American Joint Committee on Cancer/Union for International Cancer Control[8]. According to their histology and

molecular characteristics, GEJ tumors may be further grouped within either esophageal or gastric cancer groups. Specifically, adenocarcinomas of the GEJ share similar molecular traits with distal esophageal adenocarcinoma and upper gastric adenocarcinoma, as highlighted in the Cancer Genome Atlas analysis discussed further in the article[9]. Each histologic subtype differs in terms of primary tumor location and somewhat in prognosis[10]. Esophageal SCC arises from the squamous lining of the esophagus through progression of premalignant precursor lesions that occur in the presence of risk factors that cause chronic irritation and inflammation, is more likely to localize near the tracheal bifurcation, has a proclivity for earlier lymphatic spread, and is associated with a poorer prognosis[11]. Tobacco and alcohol consumption are major risk factors for SCC, whereas tobacco use is a moderate risk factor for adenocarcinoma. SCC usually arises in the middle third of the esophagus (or higher), followed by the lower third and then less commonly to the upper third[12,13]. Adenocarcinomas arise in the distal esophagus or the GEJ and have been positively associated with GERD, obesity with an increased risk in people with BMI over 30 kg/m^2[14]. This can be attributed partially to the increased risk of gastroesophageal reflux disease in obese individuals, which may lead to development of Barrett esophagus, a pre-malignant condition in which regular squamous epithelium of the esophagus is replaced by metaplastic columnar epithelium[15].

Molecular classifications

Apart from the traditional histologic subtypes, analysis from TCGA project has also classified GC into 4 different categories based on their genomic profile[4]: Tumors containing Epstein–Barr virus (EBV) account for approximately 10% of GCs and are characterized by high prevalence of DNA hypermethylation, amplification of *JAK2*, *PD-L1* and *PD-L2* genes. Moreover, nearly 80% have an amino acid-changing alteration in the *PIK3CA* gene. EBV-associated tumors are usually located in the proximal stomach and are associated with non-diffuse type. Large meta-analyses of multiple multicenter studies have concluded that EBV (+) GC has more favorable outcomes in comparison to EBV (-)[16]. Furthermore, Sohn *et al*[17] concluded that EBV (+) GC has the best prognosis among all other subtypes.

Microsatellite instability (MSI-H) is present in around 20% of GCs. Tumors showing mismatch repair deficiency contain a high rate of mutations, including mutations of genes encoding targetable oncogenic proteins and take place due to malfunctioning in the DNA repair mechanisms. These tumors are characterized by MLH1 hypermethylation and CIMP[18]. In terms of prognosis, MSI GC has worse prognosis than EBV (+) and better than genomically stable (GS) subtype, according to Sohn's prognostic model [17]. Finally, patients with MSI-H and EBV positive tumors have shown high rates of response to immunotherapy and durable survival outcomes[18].

Chromosomally instable (CIN) tumors are the most frequent, accounting for around 50% of GCs and they usually appear at the GEJ. These tumors display marked aneuploidy and have a considerable number of genomic amplifications of key receptor tyrosine kinases, cell cycle regulation genes and transcription factors. They are associated with intestinal histology and most tumors carry *TP53* mutations and RTK-RAS activation[19]. Prognosis is similar to MSI subtype, however, CIN subtype seems to receive the largest benefit from adjuvant chemotherapy[17].

GS subtype lacks the molecular characteristics of the other three subtypes and has tumors enriched for the diffuse histologic variant, with approximately 30% of them having mutations or fusions in the CDH1 and RHOA signaling pathway. This group accounts for 20% of GCs that are characterized by the lack of high levels of aneuploidy and high metastatic potential. It carries the worst prognosis of all the subtypes and receives little benefit from adjuvant chemotherapy, according to Sohn's model[17]. CDH1 germline mutations are usually associated with diffuse GC hereditary syndrome[7].

The TCGA project examined GEJ tumors separately in the molecular analysis of esophageal carcinoma[9]. Study results concluded that adenocarcinomas of the esophagus, including GEJ, more closely resemble GC, especially of the CIN subtype. GEJ tumors are characterized by DNA hypermethylation and while most tumors are classified to the CIN subtype, EBV or MSIH positivity is not uncommon[9].

TREATMENT

Curative treatment of GC can be achieved at the early stage through surgical or endoscopic resection. Other treatment modalities, such as radiation and chemothe-

rapy, are frequently employed to increase the chances of successful resection and prevent distant relapse. Several studies have explored this combination of modalities and the ideal sequencing is yet unclear.

Role of preoperative chemotherapy

Preoperative chemotherapy is being employed in many different solid tumors as it offers certain advantages over postoperative therapy. Preoperative chemotherapy has a chance of reducing tumor size and facilitates surgical excision with negative margins (R0 resection)[20]. Furthermore, patients have a higher chance of completing preoperative chemotherapy rather than postoperative, due to possible postoperative complications or decrease in performance status associated with gastrectomy-related comorbidities. Prevention of distant metastases until surgery is also an important goal of preoperative chemotherapy[21]. Finally, response to preoperative chemotherapy has been shown to have prognostic value in patients with breast and rectal cancer, with patients achieving pathologic complete response (pCR) enjoying longer disease-free survival (DFS)[22]. Newer retrospective data also suggested a similar pattern for gastric and GEJ cancer[23,24], while results from the prospective trials cited below showed a trend for improved outcomes in patients achieving complete response in preoperative therapy.

Principles of surgical excision

Adenocarcinomas of the GEJ can be classified according to the Siewert classification, into type I, which arise from the distal esophagus, type II which is true junctional carcinoma of the cardia/esophagus and type III, which is subcardial carcinoma may invade the GEJ from below[25]. The extent of gastrectomy as well as the reconstruction technique used depends on tumor location. Gastrectomy is accompanied by lymph node dissection, which can be classified as D1, D2 or D3 dissection. D1 Lymphadenectomy includes all N1 nodes (perigastric nodes) whereas D2 Lymphadenectomy consists of removal of both N1 and N2 nodal groups (distant perigastric nodes and nodes along main arteries supplying the stomach)[26]. D3 Lymphadenectomy is an extensive resection including N3 nodes (para-aortic lymph nodes). D2 resection has been proved to have an advantage in improving OS, however D3 dissection failed to show any benefit in comparison to D2, and is associated with increased postoperative morbidity[27].

Role of radiotherapy

Radiation therapy has been employed as a mean of local tumor control in most solid tumors, including gastric cancer. Ionizing radiation targets cells during their proliferative phase, and tumor cells are more susceptible to radiation damage than regular tissue cells[28]. Radiotherapy may be used with a curative or palliative intent, depending on the total dose received and the urgency for local tumor control. In metastatic gastric and gastroesophageal cancer, radiation has been used to alleviate symptoms such as bleeding, pain and obstruction with variable but generally satisfying results in different observation studies[29]. In the early setting, radiation is being used concomitantly with chemotherapy to achieve better tumor control before or after surgical excision. Whether the addition or the timing of radiation therapy to standard chemotherapy offers additional clinical benefits remains the question of ongoing clinical trials which will be further discussed in this paper.

Combination of chemotherapy and radiotherapy

Chemotherapy is being used concomitantly or sequentially with radiotherapy in the treatment of many localized solid tumors. Definitive chemoradiation is the modality of choice in locally advanced head and neck cancers, lung and rectal cancers[30]. Apart from the separate cytotoxic effects of each type of treatment, concurrent use of chemotherapy and radiotherapy has shown to produce a synergistic effect that enhances antitumor response, which is more apparent than when used sequentially [31]. Chemotherapy acts as a radiosensitizer, probably by allowing cells to inappropriately progress through the S phase of the cell cycle, thus not permitting to repair the DNA damage caused by radiation[32]. Furthermore, combination treatment modulates tumor microenvironment[33], and allows for simultaneous control of both systemic micrometastases and local disease. Radiation and chemotherapy combination has been thoroughly studied and has solidified its role in the treatment of gastric and esophageal cancer, particularly in locally advanced SCC of the esophagus, where disease control can be achieved with chemoradiation alone and surgery can be reserved for refractory or relapsed disease[34].

Addition of immunotherapy

Recently, with the increasing use of checkpoint inhibitors, significant interaction has been noted between immunotherapy and radiotherapy that may lead to increased antitumor response. One possible mechanism to explain this effect is the release of tumor neoantigens in the tumor microenvironment after cellular destruction due to radiation. Exposure of stromal immune cells to tumor neoantigens may induce an anti-tumor response, further enhanced by the presence of immune checkpoint inhibitors [35]. Several preclinical data support this notion. For example, a study by Deng *et al* [36] in mouse models of breast and colorectal cancer showed that combination of radiation and PD-L1 inhibitors, controlled tumor growth more effectively than radiation or immunotherapy alone (587.3 ± 169.1 mm with anti–PD-L1 alone *vs* 25.59 ± 10.26 mm with radiation plus anti–PD-L1, P = 0.0022, 402.8 ± 76.73 mm with radiation alone vs. 25.59 ± 10.26 mm with radiation plus anti–PD-L1, P = 0.0002).

Postoperative chemoradiotherapy: Addition of radiotherapy to chemotherapy after gastrectomy has been explored in several trials (Table 1). A classic study by Macdonald *et al*[37] examined the effectiveness of adding postoperative chemoradiotherapy to surgical resection in patients with adenocarcinoma of the stomach and GEJ. The trial included a total of 556 patients which were randomized to receive surgery alone or surgery with postoperative chemoradiotherapy. Most patients (67%) had T3 disease and positive lymph nodes (84%). Gastric antrum was the most common primary tumor location. However, more than half of the patients underwent suboptimal lymph node dissection (54% D0, 36% D1, 10% D2). Chemotherapy consisted of 5-fluorouracil (5-FU) and leucovorin and was administered concurrently with radiation. Addition of chemoradiotherapy increased OS to 36 mo, in comparison to 27 mo in the surgery-only arm, in a statistically significant manner. Grade 3 adverse events were more common in patients in the chemoradiotherapy arm[37].

Similarly, a phase II study by McNamara *et al*[38] tested the effectiveness of adjuvant chemoradiotherapy with cisplatin and 5-FU after neoadjuvant chemotherapy with epirubicin, oxaliplatin and 5-FU, in patients with resectable adenocarcinoma of the esophagus and GEJ. 60 patients were recruited and GEJ was the most common primary tumor location. An objective response was achieved in 41% of the patients and a pCR in 5% of them. The 3-year locoregional control rate reached 84% and distant metastasis control rate was 44%, while total relapse free survival was projected at 39%. 3-year OS rate was calculated at 42%. Clinical response to induction therapy was strongly associated with better outcomes[38].

Another small phase II study by Adelstein *et al*[39] examined the effectiveness of postoperative chemoradiotherapy in patients with locally advanced tumors of the esophagus and GEJ. The study enrolled 50 patients in total. Among them, 43 patients were diagnosed with adenocarcinoma, 36 had a tumor located in the GEJ and 86% of patients had node-positive disease. The study also included 3 patients with M1a disease. In the overall study population, the 4-year projected OS was 51%, freedom from recurrence was 50% and distant metastases control rate was 56%. No major difference was observed among different patient subgroups, apart from disease stage and a marginal benefit for patients with SCC in comparison to adenocarcinoma[39].

A retrospective pooled analysis by Dikken *et al*[40] evaluated the effectiveness of adjuvant chemoradiotherapy in comparison to surgical excision alone in 91 patients with GC from two phase I/II studies and 694 patients from the Dutch Gastric Cancer Group Trial (DGCT). Patients in the DGCT group underwent only surgery and were randomly assigned to D1 or D2 Lymph-node dissection. Patients in the phase I/II trials received chemotherapy with 5-FU and leucovorin, capecitabine alone or capecitabine with cisplatin. Local recurrence at 2 years was significantly higher in the surgery-only group (17% *vs* 5%) in the overall study population. Subgroup analysis according to the extent of lymph node dissection showed a statistically significantly lower recurrence rate in the chemoradiation arm in the D1 subgroup (2% *vs* 8%); however, no difference was observed in patients that had D2 surgery. Chemoradiation also improved outcomes of patients that underwent surgical excision with microscopically positive margins (R1 resection)[40].

A recent Turkish retrospective analysis of 354 patients with resectable GC associated postoperative chemoradiation with improved relapse free survival (RFS), albeit the percentage of patients that underwent D2 lymph node dissection is unclear. Median RFS in the whole study population reached 53.2 mo and median OS 136 mo. Interestingly, another factor associated with an increased risk for relapse was preoperative hypoalbuminemia[41].

Table 1 Postoperative chemoradiation clinical trials

Study name/phase	Size/stage/primary tumor location/histology	Intervention	Primary endpoint
INT-0116/phase III[37]	556 patients, IB-IVM0/stomach/adenocarcinoma	Gastrectomy D0-2 (both arms) AND E: 5-FU/LV + 45Gy radiation OR C: No post-surgical treatment	mOS: 27 m control vs 36 m experimental, HR: 1.35 (95% CI: 1.09-1.66, *P* = 0.005)
McNamara *et al* [87]/phase II	60 patients/T3-4/N1/M1a/esophagus/22% GEJ 78%/adenocarcinoma	Induction epirubicin, oxaliplatin, 5-FU → gastrectomy → E: adjuvant concurrent cisplatin, 5-FU + 50 to 55 Gy radiation	Surgical resection: 90% underwent surgical resection
Adelstein *et al*[39] /phase II	50 patients/T3/N1/M1a/esophagus/ 28% GEJ 72% adenocarcinoma 86% SCC 14%	Gastrectomy → E: cisplatin, 5-FU + 50.4-59.4 Gy radiation	OS rate: 51% 4-yr OS rate
Xie *et al*[42] /phase III	144 patients/T3-4/N1-3/stomach/adenocarcinoma	Gastrectomy D2 (both arms) AND E: capecitabine, oxaliplatin, 45 Gy radiation OR C: capecitabine, oxaliplatin	DFS rate: 72.8% experimental *vs* 76.3% control 3-yr DFS rate (*P* = 0.868)
ARTIST/phase III [43]	458 patients, IB-IV/stomach/adenocarcinoma (39% intestinal, 57% diffuse)	Gastrectomy D2 (both arms) AND E: capecitabine, cisplatin + 45 Gy radiation OR C: capecitabine, cisplatin	DFS rate: 78% experimental *vs* 74% control 3-yr DFS rate *P* = 0.0862
CRITICS/phase III[44]	788 patients/IB-IVA/stomach/ 83% GEJ 17% adenocarcinoma (32% intestinal 30% diffuse)	Preoperative epirubicin, cisplatin or oxaliplatin, capecitabine → Gastrectomy D1 (both arms) AND E: epirubicin, cisplatin or oxaliplatin, capecitabine or 5-FU + 45 Gy radiation OR C: epirubicin, cisplatin or oxaliplatin, capecitabine or 5-FU	mOS: 43 m control *vs* 37m experimental HR 1.01 (95%CI: 0.84-1.22; *P* = 0.90)
ARTIST 2/phase III[46]	538 patients/II/III N+/stomach/adenocarcinoma	Gastrectomy D2 (both arms) AND E1: S-1, oxaliplatin, + 45 Gy radiation OR E2: S-1, oxaliplatin OR C: S-1	DFS rate: 65% control, 78% experimental 2, 73% experimental 1 3-yr DFS rate experimental 2 *vs* experimental 1 HR 0.910, (*P* = 0.667)

mOS: Median overall survival; mDFS: Median disease-free survival; mPFS: Median progression-free survival; E: Experimental; C: Control; HR: Hazard ratio; 5-FU: 5-fluorouracil; LV: Leucovorin; Gy: Gray; S-1: Tegafur/gimeracil/oteracil; pCR: Pathologic complete response; GEJ: Gastroesophageal junction; CRT: Chemoradiation; AEG: Adenocarcinomas of the esophagogastric junction.

More recent trials, using optimized surgical techniques, have presented interesting data on the benefit of adjuvant chemoradiotherapy. A phase III trial by Xie *et al*[42] in 144 patients with locally advanced GC, staged as T3-4/N1-3 were randomized to receive either adjuvant capecitabine plus oxaliplatin or the same regimen with the addition of radiotherapy, after completion of D2 gastrectomy. 3-year disease-free survival did not differ significantly between arms, with 76.3% in the chemotherapy arm and 72.8% in the chemoradiation arm. A similar pattern was noted for OS. Rate of local RFS was also similar between arms, with no added benefit seen from the addition of radiotherapy. Similarly, no difference was noted in DFS in patients with lymph-node positive disease[42].

In a similar manner, the ARTIST study[43] compared the effectiveness of adding radiotherapy to adjuvant chemotherapy in prolonging DFS and OS in patients with GC. Investigators recruited 458 patients with GC that underwent gastrectomy with D2 Lymph node dissection. Over 80% of patients in each cohort had positive lymph nodes and the majority of patients had stage II and III disease. Most common location of the primary tumor was the body of the stomach. Patients were also stratified according to Lauren classification and around 60% in each arm belonged to the diffuse subtype. Patients were randomly assigned to either adjuvant chemotherapy with six cycles of capecitabine and cisplatin or to two cycles of capecitabine and cisplatin followed by chemoradiotherapy and two cycles of cisplatin and capecitabine after completion. DFS and OS showed no statistically significant difference among the two cohorts in the study's overall population. The only subgroups that seemed to derive a statistically significant benefit in DFS and OS from the addition of radiotherapy were patients with lymph node positive disease and intestinal subtype. Patients with stage III or IV disease showed a trend towards improvement, without reaching statistical significance [43].

Another study pointing to the same direction was CRITICS phase III study[44], in which 788 patients with gastric and GEJ adenocarcinomas were enrolled and randomized to receive either adjuvant chemotherapy with combination of epirubicin, cisplatin or oxaliplatin and capecitabine or adjuvant radiation therapy concurrently with cisplatin and capecitabine. All patients received preoperative chemotherapy and 741 patients underwent gastrectomy with at least D1+ lymph node dissection. Of

them, 478 patients received adjuvant therapy in their respective cohorts. Lauren histologic subtypes were equally represented within each cohort and only 17% of patients in each cohort had cancer of GEJ. Almost half of the patients in each cohort had node-negative disease after gastrectomy. Median OS was higher in the chemotherapy group *vs* the chemoradiotherapy group (43 mo *vs* 37 mo) although this difference did not reach statistical significance in any subgroup[44].

The recent ARTIST 2 trial[45] evaluated the addition of oxaliplatin or oxaliplatin and radiotherapy to adjuvant treatment of patients with stage II or III GC who underwent gastrectomy and D2 Lymph node dissection with positive lymph nodes. 538 patients were randomized into three cohorts according to the type of adjuvant treatment and were stratified according to the type of surgery, stage, and Lauren subtype. Patients in the first cohort received adjuvant S-1, in the second S-1 plus oxaliplatin and S-1 plus oxaliplatin and chemoradiotherapy in the third cohort. DFS was significantly lower in the S-1 only arm in comparison to S-1 plus oxaliplatin and S-1 plus oxaliplatin and chemoradiation, while there was no statistically significant benefit with the addition of radiotherapy. Interim results met the pre-specified endpoints sufficiently and the trial was terminated earlier than planned[46].

Postoperative chemoradiotherapy has improved local control rates and improved disease-free survival in earlier studies before adjuvant chemotherapy became standard of care in gastric and GEJ cancer. Although certain benefit in local control might exist for patients with less than D2 Lymphadenectomy, results from randomized phase III CRITICS and ARTIST 2 studies showed no additional benefit in clinical outcomes in comparison to adjuvant chemotherapy.

Preoperative chemoradiotherapy: The benefit of adding radiotherapy to preoperative chemotherapy in the management of GC has been a topic of debate, with many highly heterogeneous studies reporting conflicting results. Several small studies have been conducted, evaluating feasibility and effectiveness (Table 2).

A phase II study by Rivera *et al*[47] aimed to determine the benefit of adding chemoradiotherapy to preoperative chemotherapy with irinotecan and cisplatin in 23 patients with resectable, locally advanced, stage II-IV adenocarcinoma of the stomach and GEJ. Patients received two courses of irinotecan and cisplatin followed by irinotecan and cisplatin plus external beam radiation. In patients without progression, surgical resection was performed. Among the evaluable patients, 2 achieved pCR. Median OS was 14.5 mo and 2-year OS rate reached 35%[47].

The phase II RTOG 9904[48] study evaluated the effectiveness of neoadjuvant chemoradiotherapy in patients with resectable GC. 49 patients were enrolled and received preoperative chemotherapy with cisplatin, 5-FU and leucovorin concurrently with radiation, followed by surgery. The majority of patients had stage III disease. pCR and R0 rates reached 26% and 77% respectively and pCR was associated with favorable prognosis, in accordance with other previous studies. D2 resection was performed in only 50% of the patients[48].

A few other small studies have evaluated trimodality treatment in patients with esophageal and GEJ carcinomas. The phase II S0356 study[49] explored the impact of neoadjuvant chemoradiotherapy in patients with clinical stage II-III esophageal and GEJ adenocarcinomas. The study enrolled 93 patients, including 36 patients with adenocarcinomas of GEJ, who received a neoadjuvant combination of oxaliplatin and 5-FU and radiotherapy followed by surgical excision. After surgery, patients were planned to receive adjuvant chemotherapy with oxaliplatin and 5-FU. Genomic analysis of DNA and mRNA was also performed, seeking potential new prognostic and predictive biomarkers. The primary objective of this study was to achieve a pCR rate of 40%. 79 patients underwent surgery and 67.7% achieved R0 resections, 26 patients (28%) achieved pCR, thus not reaching the pre-specified endpoint of 40% and estimated median OS and 3-year OS were 28.3 mo and 45.1%, respectively. In terms of genomic analysis, ERCC-1 gene expression was associated with worse PFS and OS[49].

A similar phase II study by Ilson *et al*[50] evaluated the effectiveness of preoperative chemoradiation in patients with esophageal and GEJ carcinoma. The study included 55 patients in total, with both squamous (22%) and adenocarcinoma (75%) histologies. Primary tumor location was the GEJ in 33% of the patients. Patients received induction chemotherapy with combination of cisplatin and irinotecan, followed by concurrent chemoradiation. Out of them, 16% achieved pCR and median OS reached 31.7 mo. Patients were also evaluated for correlation between positron emission tomography (PET) response to induction chemotherapy and pCR rate, R0 resection rate, PFS and OS. PET response was significantly associated with higher pCR rate, PFS and chance of R0 resection. OS was increased in PET-responders, although not in a statistically significant manner[50]. Due to positive results from the addition of radiotherapy to

Table 2 Preoperative chemoradiation clinical trials

Study name/phase	Size/stage/primary tumor location/histology	Intervention	Primary endpoint
Rivera *et al*[47] /phase II	23 patients/II-IV, M0/stomach/57% GEJ 43% adenocarcinoma	Irinotecan, cisplatin + 45 Gy radiation → surgery	pCR: 2/23 (9%) achieved pCR after CRT
RTOG 9904/phase II [48]	43 patients/IB-III/stomach/adenocarcinoma	Cisplatin, 5-FU/LV + 45 Gy radiation → gastrectomy	pCR: 26% achieved pCR
S0356/phase II [49]	93 patients/II-III/esophagus/60% GEJ 40% adenocarcinoma	Oxaliplatin, 5-FU + 45 Gy radiation → surgery	pCR: 28% achieved pCR
Ilson *et al*[50] /phase II	55 patients/uT1N1M0-uT2-4NanyM0/esophagus 67% GEJ 33%/adenocarcinoma 75% SCC 22%	Cisplatin, irinotecan + 50.4 Gy radiation → surgery	pCR: 16% achieved pCR
Ajani *et al*[51] /phase II	126 patients/II-III/esophagus 3.2% GEJ 96.8% (AEG1 64.3%, AEG2 32.5%)/adenocarcinoma 96.8% SCC 3.2%	E: induction oxaliplatin, 5-FU OR C: no induction chemotherapy AND oxaliplatin, 5-FU + 50.4 Gy radiation → surgery (both arms)	pCR: 13% control *vs* 26% experimental (P = 0.094)
NeoRes/phase II [54]	181 patients/T1-3, Nany (except T1N0)/esophagus 82% GEJ 18%/adenocarcinoma 72% SCC 28%	E: cisplatin, 5-FU + 40 Gy radiation OR C: cisplatin, 5-FUAND surgery (both arms)	pCR: 28% experimental *vs* 9% control
CALGB 9781/phase III [56]	56 patients/T1-3Nany/esophagus/ GEJ adenocarcinoma 75% SCC 25%	E: cisplatin, 5-FU + 50.4 Gy radiation OR C: no preoperative treatment AND surgery (both arms)	mOS: 4.48 y experimental vs 1.79 y control (P = 0.002)
POET/phase III [52]	119 patients/T3-4/GEJ/adenocarcinoma	E: induction cisplatin, 5-FU/LV → cisplatin, etoposide + 30 Gy radiation OR C: cisplatin, 5-FU/LV AND surgery	OS rate: 46.7% experimental *vs* 26.1% control 3-yr OS HR 0.65, (95%CI: 0.42-1.01, P = 0.055)
CROSS/phase III [57]	366 patients/T1N1, T2-3N0-1/esophagus 73.2% GEJ 24%/adenocarcinoma 75% SCC 23%	E: carboplatin, paclitaxel + 41.4 Gy radiation OR C: no chemoradiation AND surgery (both arms)	mOS: 49.4 m experimental *vs* 24 m control HR 0.657, (95%CI: 0.495-0.871, P = 0.003)
Neo-AEGIS/phase III [60]	377/cT2-3N0-3M0/esophagus GEJ/adenocarcinoma	E: carboplatin, paclitaxel + 41.4 Gy radiation OR C: epirubicin, cisplatin/oxaliplatin, 5-FU/capecitabine or docetaxel, oxaliplatin, leucovorin, 5-FU ANDsurgery (all arms)	OS rate: 56% experimental *vs* 57% 3-yr OS HR 1.02, (95%CI: 0.74-1.42)

mOS: Median overall survival; mDFS: Median disease-free survival; mPFS: Median progression-free survival; E: Experimental; C: Control; HR: Hazard ratio; 5-FU: 5-fluorouracil; LV: Leucovorin; Gy: Gray; S-1: Tegafur/gimeracil/oteracil; pCR: Pathologic complete response; GEJ: Gastroesophageal junction; CRT: Chemoradiation; AEG: Adenocarcinomas of the esophagogastric junction.

neoadjuvant chemotherapy from single arm trials, direct comparison with a chemotherapy or surgery only approach has been employed in smaller phase II studies and paved the way for larger phase III trials.

A phase II randomized trial on the effectiveness of trimodality treatment by Ajani *et al*[51] randomized 126 patients with esophageal and GEJ carcinoma to neoadjuvant chemoradiotherapy with or without induction chemotherapy with oxaliplatin and 5-FU, followed by surgical resection. 122 patients (96.8%) were diagnosed with adenocarcinoma and 122 patients (96.8%) had tumors located in the GEJ. Median OS was 45.6 mo for all patients, with median OS being 45.6 mo in the no-induction arm and 43.6 mo in the induction arm, with this difference not reaching statistical significance. The pCR rate was numerically higher in the induction chemotherapy arm (26% *vs* 13%)[51].

Similarly, the POET trial[52] recruited 119 patients with locally advanced GEJ adenocarcinoma (Siewert types I-III) and randomized them to receive either chemotherapy with cisplatin and 5-FU alone or chemotherapy and chemoradiation. Patients in both cohorts were treated with surgical resection afterwards. Local PFS after tumor resection was significantly improved in the chemoradiation arm and there was a trend towards improvement of OS, without reaching the pre-specified endpoint for statistical significance[52].

Accordingly, the NeoRes study[53] conducted in Norway and Sweden recruited 181 patients with malignant tumors of the esophagus and GEJ. The most prevalent histologic type was adenocarcinoma (72% of patients) and 17% of tumors were located in the GEJ. Patients were equally randomized to receive neoadjuvant chemotherapy alone or with the addition of radiotherapy. The pCR rate was higher in the chemoradiation arm and lymph node positivity was lower at the time of surgery. OS did not

differ between the two arms. Later results confirm that the addition of radiation to neoadjuvant chemotherapy did not significantly affect 5-year PFS and OS[53,54].

Despite the large number of trials addressing this topic, conclusions cannot be clearly drawn, due to a large number of confounding factors, low adherence to treatment protocols and high group heterogeneity. A meta-analysis by Zhao *et al*[55] of six clinical trials, including the ones by Stahl and Klevebro, that included 866 patients with adenocarcinoma and SCC of the esophagus and GEJ, concluded that 3-year and 5-year OS rates were improved with the addition of radiotherapy to neoadjuvant chemotherapy in a statistically significant manner. Furthermore, neoadjuvant chemoradiotherapy increased the chance of R0 resection and pCR. This benefit seems to apply to patients with both adenocarcinomas and SCCs[55].

Larger phase III trials have attempted to produce more robust evidence and provide definitive answers on the benefit of trimodality treatment. A small phase III trial CALGB 9781[56] evaluated the use of trimodality treatment with chemotherapy including cisplatin, 5-FU and radiotherapy before surgical resection of esophageal or GEJ carcinomas. The trial was terminated early due to poor accrual and only 56 patients were evaluable for response. 23 patients in the chemoradiotherapy cohort and 19 in the surgery-alone cohort had adenocarcinomas (77% and 73%, respectively). In the overall study population, median OS reached 4.48 years in comparison to 1.79 years with surgery-alone arm. However, the final population study was small, and no further data on different subgroups were evaluable[56].

The largest dataset from a randomized clinical trial currently available is from the CROSS phase III trial[57], which evaluated the effectiveness of neoadjuvant chemoradiotherapy in patients with resectable esophageal and GEJ carcinomas. 366 patients were randomly assigned to receive either combination of weekly carboplatin and paclitaxel with concurrent radiotherapy over a 5-wk period and then proceed to surgical resection, or to surgery-alone. The study included patients with different histologies, with the majority being adenocarcinomas (75%). In 88 patients, the primary tumor location was GEJ. Around 65% of patients in each cohort had lymph-node positive disease. Patients in the chemoradiotherapy group achieved a statistically significant higher degree of R0 resections in comparison to surgery alone (92% *vs* 69%), while 29% in the chemoradiotherapy group achieved pCR at the end of neoadjuvant treatment. Postoperative complications were similar between the two subgroups, and OS was significantly improved in the chemoradiotherapy group (49.4 mo *vs* 24.0 mo). In subgroup analysis, patients with node-negative disease at diagnosis and patients with squamous histology received clear benefit, while patients with adenocarcinoma showed a clear trend towards improvement[57].

Although CROSS study[44] proved that neoadjuvant chemoradiotherapy leads to improved outcomes in comparison to surgery alone, data from MAGIC trial[58] show also improved OS with preoperative chemotherapy, while FLOT4 trial[59] identified FLOT as a superior regimen. Thus, the ideal neoadjuvant approach is still unclear and the benefit of trimodality therapy is still under discussion.

Recent results from the phase III Neo-AEGIS are the first comparative data available answering this question. Neo-AEGIS is a phase III randomized European study comparing the efficacy and safety of preoperative chemoradiation, per CROSS study protocol, to chemotherapy alone, per MAGIC or FLOT4 protocol in patients with resectable esophageal and GEJ adenocarcinomas[60]. The study initially attempted to prove superiority of the CROSS regimen over chemotherapy, however after the first futility analysis a non-inferiority approach was adopted. Chemotherapy alone reached the primary endpoint of non-inferiority in terms of 3-year OS [57% for chemotherapy *vs* 56% for chemoradiation hazard ratio (HR) 1.02 (95%CI: 0.74-1.42)]. Of note, more patients in the CROSS arm achieved pCR and significant tumor shrinkage, in accordance with results from earlier clinical trials showing improved tumor local control with preoperative chemoradiation[60].

Results from the TOPGEAR study[61] are also awaited. TOPGEAR is an ongoing international phase III trial in patients with adenocarcinoma of the stomach and GEJ receiving induction perioperative chemotherapy with epirubicin/cisplatin/5-FU (ECF) alone or in combination with preoperative chemoradiation. The ECF group receives three preoperative cycles of ECF, while the chemoradiation group receives two cycles of ECF followed by chemoradiation. After surgical excision, patients in both groups are receiving three cycles of ECF. An interim analysis of 120 recruited patients indicated that 90% in the ECF group and 85% in the chemoradiotherapy group underwent gastrectomy. Results on effectiveness and comparison among groups are pending[61].

Analysis of recent real-world data can also add to the existing knowledge on the management of operable gastroesophageal cancer. A study of 1916 patients from the nationwide Netherlands Cancer Registry (NCR)[62], with esophageal or GEJ cancer undergoing curative treatment, with surgery or definitive chemoradiation, reported on real-world treatment outcomes. The majority of patients underwent surgery and only 21% received definitive chemoradiation. Out of patients with resected disease, 83% underwent neoadjuvant chemoradiation and 10% neoadjuvant chemotherapy, with or without adjuvant chemoradiation. Only 7% received surgery alone. Patients that received definitive chemoradiation had shorter median DFS (14.2 mo *vs* 26.4 mo) and median OS (20.9 mo *vs* 40.5 mo) than patients that underwent surgical resection. However, median age was higher and performance status was worse in the definitive chemoradiation group. Patients that received neoadjuvant chemoradiation had a median DFS of 25.2 mo and a median OS of 38 mo. Interestingly, this study included a separate subgroup analysis of patients that received adjuvant nivolumab after trimodality treatment with chemoradiation and surgery, as part of CheckMate-577 trial. Among these patients the median DFS and median OS were 19.2 and 29.4 mo, respectively[62].

Another retrospective study by Spencer *et al*[63] evaluated the role of neoadjuvant chemoradiation in patients with stage III or IVA locally advanced (T3/T4) adenocarcinoma of the esophagus and GEJ, followed by surgical resection. Patients with threatened circumferential resection margin by imaging received neoadjuvant chemoradiation, while those without a threatened margin received neoadjuvant chemotherapy. Patients that received neoadjuvant radiation also received a combination of carboplatin and paclitaxel. Most patients in the chemotherapy group received a platinum-fluoropyrimidine doublet. In total, results from 81 patients were reported. 18 patients received chemoradiation and 63 patients chemotherapy alone. Both groups included 5 patients with stage IVA disease. Rates of R0 resection were higher in the chemoradiation group and rate of local relapse was lower in comparison with the chemotherapy group. However, no difference was noted in OS and RFS. R1 resection in the chemotherapy group was a negative prognostic factor[63].

Jurkowski *et al*[64] reported on the outcomes of patients with locally advanced esophageal and GEJ adenocarcinomas treated with total neoadjuvant therapy, including induction chemotherapy followed by chemoradiation. Induction chemotherapy included doublet or triplet regimens of 5-FU, cisplatin or oxaliplatin and docetaxel, while carboplatin and paclitaxel, or oxaliplatin and 5-FU were used concurrently with radiation. 37 out of 59 evaluable patients underwent surgical resection. 9 patients opted out of surgery since they achieved clinical complete response. Among the patients who received surgery, R0 rate was 89% and pCR rate was 19%. For the entire population of the study, median DFS was 2.4 years and median OS 4.7 years. Patients who underwent surgery had a higher DFS and median OS (3.5 years *vs* 1.5 years and 5.8 years *vs* 4.2 years). The subgroup that achieved clinical complete response had worse 3-year DFS in comparison to operated patients with pCR (42% *vs* 83%), however 3-year OS was improved (89% *vs* 83%)[64].

Preoperative radiation is frequently employed in patients with gastroesophageal cancer and represents the standard of care in some many high-volume centres. Prospective and retrospective date suggest a role for preoperative chemoradiation in improving local tumor control and achieving higher pCR rates. Whether this translates to long-standing improvement in overall survival remains the subject of ongoing clinical trials, such as TOPGEAR and Neo-AEGIS. While Neo-AEGIS showed non-inferiority of preoperative chemotherapy to the CROSS regimen, it is unclear whether addition of chemoradiation to induction chemotherapy would maximize clinical benefit until results from the TOPGEAR study are announced. Moreover, only 27 of 184 patients in the chemotherapy-alone arm received FLOT, which has proved to be superior to the MAGIC regimen[59].

Combination with newer therapeutic agents

Newer treatment modalities have been constantly added to the therapeutic arsenal in metastatic gastroesophageal cancer. Targeting agents, such as anti-HER2 antibodies, and immune checkpoint inhibitors, as well as new predictive and prognostic biomarkers have all prolonged survival and improved quality of life in patients with unresectable disease[65]. Several modalities are being investigated in the early setting and some have already produced encouraging results (Table 3).

Chemoradiation and immunotherapy: The first trial of immunotherapy to produce positive results in early gastroesophageal cancer is CheckMate 577[66]. It is a global, multi-center, randomized, double-blind phase III study that explores the addition of

Table 3 Chemoradiation and targeted therapies clinical trials

Study name/phase	Size/stage/primary tumor location/histology	Intervention	Primary endpoint
Ku *et al*[72]/phase II	33 patients/uT2-3N0-1/esophagus 33% GEJ 66%/adenocarcinoma	E: cisplatin, irinotecan + bevacizumab + 50.4 Gy radiation → surgery	Tolerability: 59% grade 3/4 hematologic toxicity 42% grade 3/4 non-hematologic toxicity (including deep vein thrombosis)
TOXAG/phase II [70]	34 patients/76% > IIIA/stomach GEJ/adenocarcinoma HER2 positive	E: gastrectomy D2 → oxaliplatin, capecitabine + 45 Gy radiation + trastuzumab	Tolerability: 90.3% completed 3 cycles of treatment
SAKK 75/08/phase III [71]	300 patients/T2N1-3, T3Nany, T4aNany/esophagus 50% GEJ 50%/adenocarcinoma 64% SCC 36%	E: docetaxel, cisplatin + 45 Gy radiation + cetuximab → surgery → cetuximab OR C: docetaxel, cisplatin + 45 Gy radiation AND surgery (both arms)	mPFS: 2.9 y experimental *vs* 2 y control HR 0.79; (95%CI: 0.58 to 1.07, $P = 0.13$)
RTOG 1010/phase III[69]	203 patients/T1N1-2, T2-3N0-2/esophagus GEJ/adenocarcinoma HER2 positive	E: carboplatin, paclitaxel + 50.4 Gy radiation + trastuzumab → surgery → trastuzumab ORC: carboplatin, paclitaxel + 50.4Gy radiation → Surgery	mDFS: 19.6 m experimental *vs* 14.2 control HR 0.97 (95%CI: 0.69-1.36)
CheckMate 577/phase III[66]	794 patients/II-III, ≥ ypT1 or ≥ ypN1/esophagus 60% GEJ 40%/adenocarcinoma 71% SCC 29%	Neoadjuvant chemoradiation → surgery (both arms) AND E: nivolumab OR C: placebo	mDFS: 22.4 m experimental *vs* 11 m control HR 0.69 (95%CI: 0.56-0.86, $P = 0.0003$)

mOS: Median overall survival; mDFS: Median disease-free survival; mPFS: Median progression-free survival; E: Experimental; C: Control; HR: Hazard ratio; 5-FU: 5-fluorouracil; LV: Leucovorin; Gy: Gray; S-1: Tegafur/gimeracil/oteracil; pCR: Pathologic complete response; GEJ: Gastroesophageal junction; CRT: Chemoradiation; AEG: Adenocarcinomas of the esophagogastric junction.

anti-PD1 checkpoint inhibitor nivolumab to trimodality treatment for esophageal and GEJ carcinoma. 794 patients with resected, stage II/III, esophageal/GEJ carcinoma who received neoadjuvant chemoradiation and did not achieve a pCR were randomized 2:1 to receive nivolumab 240 mg every 2 wk for 16 wk, followed by 480 mg every 4 wk up to 1 year or placebo. Most dominant histology was adenocarcinoma (71%) and most patients had positive lymph nodes (60%). The primary endpoint of the study was median DFS, which was doubled with the addition of nivolumab in comparison to placebo (22.4 mo *vs* 11.0 mo). Severe treatment-related adverse events (TRAEs) occurred in 8% of nivolumab patients and 3% of placebo patients. The most common TRAEs were fatigue, pruritus, diarrhea and rash. Grade 3 immune related adverse events occurred in less than 1% of patients in the nivolumab arm[66].

Apart from the proven benefit of nivolumab, a newer phase II[67] study is examining the role of adding durvalumab to neoadjuvant chemoradiotherapy in patients with esophageal and GEJ adenocarcinoma. Study design was based on the results of CALGB 80803[68], a study that evaluated response to induction chemotherapy by PET/computed tomography (CT). After 2 cycles of mFOLFOX6, PET responders received chemoradiation with capecitabine and oxaliplatin and radiation to 50.4 Gy, while PET non-responders switched to different chemotherapy regimen of carboplatin/paclitaxel concurrent with radiation. Durvalumab was added to all patients, 2 wk before chemoradiation and was continued during chemoradiation, and was continued after R0 resection. According to preliminary data, 36 patients have been recruited, 25 with adenocarcinoma of GEJ and 11 with adenocarcinoma of the esophagus. Out of them, 72% showed disease response to induction chemotherapy at PET/CT. 25 patients underwent surgical resection and 6 achieved pCR, while 5 patients were downstaged to ypT1N0 and 2 patients to ypT0N1, showing 99% response. Another 20 patients had more than 90% response. Grade 3/4 neutropenia was observed in 8 patients and grade 3 hepatitis in 1 patient. More data are still pending[67].

Immunotherapy has gained importance in gastric and gastroesophageal cancer due to recent results from first-line phase III studies (Checkmate 649, Keynote 570) showing efficacy in the metastatic setting. As a result, combination of chemotherapy and immunotherapy is being used earlier in the therapeutic algorithm. CheckMate 577 is the first study to prove the benefit of adding postoperative immunotherapy in patients with gastroesophageal cancer and residual disease following preoperative chemoradiation. Ongoing and future studies will address the question of incorporating immunotherapy in trimodality treatment of gastric cancer, either concurrently or sequentially with chemotherapy and/or radiation therapy.

Combinations with targeted therapies: Given the effectiveness of anti-HER2 antibody trastuzumab in the management of advanced HER2 overexpressing GC, several trials have evaluated the benefit of HER2 inhibition in the early setting in combination with trimodality treatment.

The phase III RTOG 1010 trial[69] evaluated the effectiveness of adding the anti-HER2 agent trastuzumab concurrently with chemoradiation in patients with resectable, HER2 overexpressing (determined by immunohistochemistry and fluorescence in situ hybridization) adenocarcinoma of the esophagus and GEJ. Chemoradiotherapy consisted of carboplatin and paclitaxel and radiation of 50.4 Gy of 3D-chemoradiation therapy or intensity modulated radiotherapy. Trastuzumab was administered throughout the chemoradiation period and for 13 more cycles after surgery. In total, 203 patients were randomized to receive neoadjuvant chemoradiotherapy with or without trastuzumab. The study did not reach its primary objective, which was statistically significant improvement in DFS. Median DFS was 19.6 mo in the experimental arm and 14.2 mo in the control arm (HR 0.97, 95%CI: 0.69-1.36). Median OS also did not differ between the two arms[69].

The phase II TOXAG study[70] also evaluates the safety and efficacy of adding trastuzumab to neoadjuvant chemoradiation with oxaliplatin, capecitabine and radiation in patients with HER2+ adenocarcinoma of the stomach and GEJ who will undergo curative surgical resection. 212 patients have been screened and 34 underwent surgical resection. The combination regimen of oxaliplatin, capecitabine, trastuzumab and radiation has achieved a high rate of tolerability of 90.3% and 97% of patients achieved D2 Lymph node dissection. At 25 mo follow-up, 59.8% of patients were still alive, and 12 patients have died because of disease progression. More data on effectiveness of treatment are still pending[70].

Smaller studies have investigated anti-EGFR antibody cetuximab and anti-VEGF antibody bevacizumab in combination with chemoradiotherapy and surgery. In the phase III SAKK 75/08 trial[71], 300 patients with esophageal cancer were randomly assigned to receive neoadjuvant chemoradiation with or without the anti-EGFR antibody cetuximab, followed by surgical resection. More than half of patients (63%) were diagnosed with adenocarcinoma. Chemoradiotherapy consisted of cisplatin and docetaxel and radiation of 45 Gy. Cetuximab was given throughout chemoradiation and as adjuvant monotherapy after surgery. The study did not meet its primary endpoint, which was a statistically significant improvement in PFS [2.9 years in cetuximab arm *vs* 2 years in control arm (HR 0.79, 95%CI: 0.58-1.07; $P = 0.13$)]. Median OS was numerically improved (5.1 years *vs* 3 years) but this difference did not reach statistical significance. Similarly, subgroup analysis did not show any advantage with the addition of cetuximab, regardless of histologic type. However, locoregional control was significantly better in the cetuximab arm[71].

A phase II study by Ku *et al*[72] examined the effectiveness of adding the anti-VEGF antibody bevacizumab to preoperative induction chemotherapy and chemoradiation with cisplatin and irinotecan in patients with resectable locally advanced esophageal and GEJ adenocarcinomas. The final evaluable population of the study consisted of 33 patients, all with cancer of the GEJ. 25 patients achieved R0 resections after neoadjuvant treatment with a pCR rate of 15%. Median PFS and OS were 15.1 mo and 30.5 mo, respectively[72].

Anti-HER2 and antiangiogenic agents have improved clinical outcomes in patients with metastatic gastric and gastroesophageal cancer. However, in the early setting, targeted agents combined with standard of care have not yet produced satisfactory results. The phase III RTOG 1010 of trastuzumab plus chemoradiation has failed to prove additional clinical benefit with the addition of trastuzumab. The anti-EGFR agent cetuximab did not improve PFS in the phase III SAKK 75/08 in comparison to standard chemoradiotherapy alone. Comparative data are not yet available on the use of anti-VEGF agents.

Choice of chemotherapy regimen

As mentioned, there is an adequate amount of evidence on the addition of radiation therapy to chemotherapy in resectable gastric and GEJ cancer. However, as mentioned in the studies above, it is clear that there is heterogeneity concerning the chemotherapy regimen used. Several efforts have been made to identify the ideal chemotherapy regimen to partner with preoperative or postoperative radiation (Table 4).

A retrospective study by Jiang DM *et al*[73] compared carboplatin plus paclitaxel with cisplatin plus fluorouracil as chemotherapy regimens in patients with resectable tumors of the esophagus and GEJ that received neoadjuvant chemoradiotherapy. The study also included patients who did not proceed to surgical resection and received

Table 4 Clinical trials comparing chemotherapy regimens

Study name/phase	Size/stage/primary tumor location/histology	Intervention	Primary endpoint
ECOG E7296/phase II[74]	38 patients/T2N1-2, T3-4Nany/stomach 45% GEJ 55%/adenocarcinoma 95% SCC 5%	E: neoadjuvant cisplatin, paclitaxel → surgery → 5-FU/LV + 45 Gy radiation	Tolerability: 66% grade 3/4 toxicities during neoadjuvant treatment 7.9% completed per protocol treatment
CALGB 80803/ Phase II [76]	241 patients/T1N1-3M0, T2-4NanyM0 (resectable)/esophagus GEJ adenocarcinoma	E1: induction oxaliplatin, 5-FU/LV OR E2: induction carboplatin, paclitaxelAND PET scan → non-responders change chemotherapy arm, responders continue → chemotherapy + 50,4 Gy radiation → surgery (both arms)	pCR: 18% [95%CI (7.5-33.5)] experimental 1; and 20% [95%CI (10, 33.7)] experimental 2 of PET non-responders who switched chemotherapy arm achieved pCR
E1201/phase II[77]	81 patients/II-Iva/esophagus GEJ/adenocarcinoma	E1: preoperative cisplatin, irinotecan + 45 Gy radiation → surgery → cisplatin, irinotecan OR E2: preoperative cisplatin, paclitaxel + 45 Gy radiation → surgery → cisplatin, paclitaxel	pCR: 15.4%, exact, unadjusted 90% binomial CI: 6.9%-28.1% experimental 1 and 16.7%, exact, unadjusted 90% binomial CI: 8.1%-29.0% experimental 2 achieved pCR
RTOG-0114/phase II[78]	73 patients/IB-IIIB/stomach/adenocarcinoma	Gastrectomy (both arms) AND E1: cisplatin, paclitaxel, 5-FU + 45 Gy radiation OR E2: cisplatin, paclitaxel + 45 Gy radiation	DFS rate: E1 closed early due to toxicity (14.6 m DFS) 52% (95%CI: 36%-68%) experimental 2 2-yr DFS
NCCTG N0849/phase II [79]	42 patients/T3-4N0, TanyN+/III-IVA/esophagus/55% GEJ 40% cardia 3.6% adenocarcinoma	E: induction docetaxel, oxaliplatin, capecitabine OR C: no induction AND oxaliplatin, 5-FU + 50.4 Gy radiation → surgery (both arms)	pCR: 33% experimental and 48% control achieved pCR
CALGB 80101/phase III [80]	546 patients/IB-IV (M0)/stomach 78% (4% proximal gastric, 41% distal gastric, 15% stomach NOS, 17% multicentric) GEJ 22%/adenocarcinoma	Surgery (both arms) AND E: epirubicin, cisplatin, 5-FU → 5-FU + 45 Gy radiation → chemotherapy OR C: 5-FU/LV → 5-FU + 45 Gy radiation → chemotherapy	OS rate: 44% control *vs* 44% experimental multivariate HR 0.98 (95%CI: 0.78-1.24, P = 0.69) 5-yr OS
PRODIGE5/ACCORD17 phase II/III[81]	259 patients/I-IVA/esophagus/adenocarcinoma 14% SCC 86%	E: oxaliplatin, 5-FU/LV + 50 Gy radiation OR C: cisplatin, 5-FU + 50 Gy radiation	mPFS: 9.7 m experimental *vs* 9.4 m control HR 0.93 (95%CI: 0.70-1.24, P = 0.64)

mOS: Median overall survival; mDFS: Median disease-free survival; mPFS: Median progression-free survival; E: Experimental; C: Control; HR: Hazard ratio; 5-FU: 5-fluorouracil; LV: Leucovorin; Gy: Gray; S-1: Tegafur/gimeracil/oteracil; pCR: Pathologic complete response; PET: Positron emission tomography; GEJ: Gastroesophageal junction; CRT: Chemoradiation; AEG: Adenocarcinomas of the esophagogastric junction.

definitive chemoradiation. 93 patients with esophageal (49%) and GEJ (51%) tumors that received neoadjuvant (72%) or definitive (28%) chemoradiation were identified. 53 patients had received cisplatin-5-FU and 40 carboplatin-paclitaxel. 59 patients were diagnosed with adenocarcinoma and 34 with SCC. In patients who received surgery, no difference was observed between the two treatment groups. However, in the definitive chemoradiation subgroup, carboplatin-paclitaxel was associated with significantly worse 3-year OS (36% *vs* 63% P = 0.001 HR 3.1, 95%CI: 1.2-7.7) and DFS (0% *vs* 41%; P = 0.004; HR 3.6, 95%CI: 1.4-8.9)[73].

In the phase II, ECOG E7296 study[74], patients with carcinomas of the stomach and GEJ received adjuvant chemoradiation after induction chemotherapy and surgical resection. 38 patients were enrolled and among them, 36 were diagnosed with adenocarcinoma and 21 with tumor of the GEJ. Induction chemotherapy consisted of cisplatin and paclitaxel, while adjuvant chemotherapy of leucovorin and 5-FU. No pCR was noted after induction chemotherapy and only 3 out of 38 patients completed treatment per protocol design. Median OS in the overall population was 1.6 years. The regimen used in this study was evaluated to be highly toxic and, thus, further development was discouraged by the investigators[74].

Another study, CALGB 80803, a phase II study[68] attempted to individualize chemotherapy in patients with resectable esophageal and GEJ adenocarcinomas receiving preoperative chemoradiation, by using early PET/CT scan. 241 patients were enrolled and were randomized to receive induction chemotherapy with either FOLFOX6 or carboplatin/paclitaxel and were subsequently evaluated with PET scan. PET non-responders switched to the opposite arm during chemoradiation. Median OS was 48.8 mo for PET responders and 27.4 mo for PET non-responders. Among patients who did not respond to induction chemotherapy, 18% in the induction FOLFOX arm

and 20% in the carboplatin/paclitaxel arm achieved pCR by switching to the alternative regimen during chemoradiation[68,75,76] . Remarkably, pCR rates in responders that received induction with FOLFOX reached 40.3%[76].

E1201 is a phase II randomized clinical trial[77] comparing neoadjuvant chemoradiotherapy regimens of cisplatin-paclitaxel and cisplatin-irinotecan in patients with resectable adenocarcinomas of the esophagus and GEJ. At the end of the study, 81 patients had received trimodality treatment and were evaluable for response rate, DFS and OS. The GEJ was the primary tumor location in 60 patients. Patients received preoperative chemotherapy and radiation and postoperative chemotherapy. Median OS reached 35 mo in cisplatin-irinotecan arm and 5-year OS was 46%, whereas in cisplatin-paclitaxel arm median OS was 21 mo and 5-year OS was 27%. Median PFS was 39.8 mo in cisplatin-irinotecan arm and 12.4 mo in cisplatin-paclitaxel (P = 0.046). Investigators decided that there was no significant advantage with the use of any of these two regimens, in comparison to other chemoradiotherapy combinations from other studies[77].

In the randomized, multi-center phase II RTOG 0114 study[78], 78 patients with resected GC were randomized to receive adjuvant chemotherapy with cisplatin-paclitaxel and fluorouracil, or cisplatin-paclitaxel alone. Both arms would receive subsequent chemoradiation with either infusional 5-FU or infusional paclitaxel and 45 Gy of radiation. Rate of grade 3 or higher adverse events was very high in the triplet chemotherapy arm, thus this cohort was terminated early. Patients who received cisplatin-paclitaxel achieved a 2-year DFS of 52%. This study failed to surpass the 67% 2-year DFS from INT0116 study, which was the study's primary endpoint[78].

In the Alliance N0849 randomized phase II trial[79], extended neoadjuvant chemoradiotherapy was compared to standard chemoradiotherapy in patients with locally advanced adenocarcinomas of the esophagus and GEJ. Patients in the extended arm received a combination of docetaxel, oxaliplatin and capecitabine, followed by 5-FU and oxaliplatin concurrently with 50.4 Gy radiation, while patients in the control arm received only chemoradiation. The study's interim analysis included 42 randomized patients. Among them, 71% had stage III disease and 55% and 40% adenocarcinoma of the esophagus and GEJ, respectively. The primary endpoint was pCR rates, which did not differ significantly between the two arms (33% with extended neoadjuvant therapy and 48% with chemoradiotherapy alone). Rate of grade 4 adverse events was numerically higher in the experimental arm (38% *vs* 24%), although this difference did not reach statistical significance[79].

The phase III CALGB 80101 trial[80] compared two different chemotherapy regimens to be used as part of postoperative chemoradiation treatment plan in patients with gastric or gastroesophageal adenocarcinomas. 546 patients who underwent curative resection were randomized to receive either combination of 5-FU and leucovorin, or postoperative combination of epirubicin, cisplatin and 5-FU, before and after concurrent radiotherapy and 5-FU. 5-year OS was 44% in both arms, with no difference in any subgroups[80].

In the phase II/III PRODIGE5/ACCORD17 trial[81], FOLFOX regimen was compared to combination of cisplatin-5-FU in terms of safety and effectiveness in patients with esophageal carcinoma of various histologies, receiving definitive chemoradiation. 134 patients were randomized to FOLFOX group and 133 to cisplatin-5-FU group. In each group, 85% of patients had squamous histologic type. Median PFS was 9.7 mo in the FOLFOX arm and 9.4 mo in the cisplatin-5-FU arm. One death attributed to toxicity took place in the FOLFOX arm and 6 in the cisplatin-5-FU. Rates of grade 3/4 toxicities were similar between the two cohorts. In general, paresthesia and sensory neuropathy was significantly more common in the FOLFOX arm, while serum creatinine increase and mucositis were significantly more frequent in the cisplatin-5-FU arm[81].

A single arm pilot study by Wo *et al*[82] examined the use of induction FOLFIRINOX before chemoradiation with carboplatin and paclitaxel in patients with locally advanced gastric and gastroesophageal cancer undergoing surgical resection. 25 patients were enrolled, and 20 patients underwent surgical excision and were evaluable for response. At an interim analysis, 37% of the evaluable patients had achieved pCR. Grade 3+ toxicities occurred in 28% of patients. This regimen will be evaluated in further trials[82].

Several chemotherapy regimens have been used adjunct to radiation therapy in the pre- or postoperative setting of gastric and gastroesophageal cancer. The most frequently used is the regimen of carboplatin and paclitaxel from the CROSS study, or the combination of cisplatin and 5-FU. Recent data from the PRODIGE5/ACCORD17 study suggest a clear role for the use of FOLFOX, since it achieved similar results with cisplatin-5-FU with no added toxicity. Triplet regimen of cisplatin, paclitaxel and 5-FU

followed by radiation was deemed too toxic in the RTOG 0114 study. Finally, the CALGB 80803 study used a more creative approach, by incorporating intermittent restage with PET-CT and shifting non-responders from FOLFOX or carboplatin, paclitaxel to the other regimen, achieving pCR rates in patients with initially resistant tumors.

Ongoing clinical trials

Several ongoing clinical trials attempt to answer clinical questions posed from previous studies or explore novel treatment modalities (Table 5). The multi-center, randomized, phase II CRITICS II trial[83] explores the ideal treatment preoperative modality in patients with resectable GC. Enrolled patients will be randomized in 3 arms, to receive either 4 cycles of neoadjuvant chemotherapy with docetaxel, oxaliplatin and capecitabine, or 2 cycles of DOC followed by chemoradiotherapy with a combination of carboplatin and paclitaxel with 45 Gy radiation, or chemoradiation alone. Primary endpoint is event-free survival at 1 year after randomization, which includes local, regional or distant disease progression and death from any cause[83]. The phase III multicenter, randomized NEO-CRAG trial (NCT01815853) evaluates the safety and efficacy of adding 45 Gy radiation to perioperative chemotherapy with 6 cycles of capecitabine and oxaliplatin (CAPOX) in patients with resectable, locally advanced GC. Similarly, the phase III randomized PREACT study[84] compares preoperative chemotherapy to chemoradiotherapy in patients with locally advanced gastric or GEJ adenocarcinomas, planned to undergo surgical resection. Chemotherapy consists of 3 preoperative cycles of S-1 and oxaliplatin (SOX), while chemoradiation consists of two cycles of SOX and concurrent radiotherapy. Patients in both arms will receive postoperative chemotherapy with 3 cycles of SOX. Primary endpoint is an increase in 3-year DFS with chemoradiotherapy regimen. The study also aims to identify microRNAs as a potential predictive biomarker of response to chemoradiotherapy[84].

A study that attempts to answer different questions with a complex design is ITACA-S2 (NCT01989858). ITACA-S2 is a randomized, multi-center, phase III trial in which patients with operable GC are randomized into 4 arms of different perioperative treatment modalities. Patients in arm A will receive perioperative chemotherapy with three cycles of combination of epirubicin, cisplatin and capecitabine or 5-FU, and will continue the same regimen after surgery. In arm B, patients will receive the same regimen only after gastrectomy. Arms C and D include the same chemotherapy regimens, with the addition of adjuvant chemoradiotherapy with 45 Gy radiation concurrently with 5-FU or capecitabine. The aim of this study is to evaluate the effect of preoperative *vs* postoperative chemotherapy on OS, and also to assess the benefit of postoperative chemoradiotherapy. Finally, the ENRICHED phase III multicenter randomized clinical trial (NCT03680261) compares adjuvant chemotherapy with 6 cycles CAPOX or SOX to adjuvant chemoradiotherapy with 45 Gy radiation concurrently with capecitabine or S-1, followed by 3 cycles of CAPOX or SOX, in patients with resected lymph node positive GC. Primary endpoint of the study is 3-year OS.

Several trials are currently exploring the role of adding concurrent anti-PD1 checkpoint inhibitor pembrolizumab to neoadjuvant chemoradiotherapy in patients with resectable gastric, GEJ and esophageal carcinoma of different histologic types[85] (NCT02730546, NCT03064490, NCT03257163). Furthermore, in a small pilot phase I/II study, addition of combination immunotherapy of nivolumab and ipilimumab to chemoradiation after induction chemotherapy, is being explored in patients with resectable GC (NCT03776487). Newer studies are also focusing on a combination of trimodality treatment and targeting agents, such as the anti-EGFR antibody cetuximab and the VEGFR, EGFR and RET inhibitor vandetanib in the treatment of early GC (NCT00857246, NCT01183559).

Optimization of the radiation component of trimodality treatment is an important part of ongoing trials. NCT04162665 is evaluating magnetic resonance imaging-guided radiotherapy and NCT04523818 the safety and effectiveness of a shorter radiation course in patients with resectable adenocarcinoma (NCT04523818). In the Danish CURE study[86], ctDNA is being used as a predictive biomarker to response and disease progression after different treatment modalities, including chemoradiation in patients with gastroesophageal cancer (NCT04576858). A phase II trial still in recruitment is exploring the role of neoadjuvant laparoscopic hyperthermic intraperitoneal chemotherapy during diagnostic laparoscopy, followed by neoadjuvant chemoradiation, for locally advanced GC (NCT04308837).

Combination of different treatment modalities is an ongoing research field in early gastric cancer, due to sub-optimal results of the current standard of care. Many phase III studies such as TOPGEAR, CRITICS II, NEO-CRAG and PREACT will offer

Table 5 Ongoing clinical trials

Study name/phase	Size/stage/primary tumor location/histology	Intervention	Primary endpoint
NCT02730546/Phase Ib/II suspended	68 patients/IB-IIIB/GEJ cardia/adenocarcinoma	E: carboplatin, paclitaxel + radiation or 5-FU, oxaliplatin, leucovorin + pembrolizumab → surgery	pCR and 1-yr PFS rate
PROCEED/phase II/NCT03064490recruiting	38 patients/locally advanced stomach GEJ esophagus/adenocarcinoma	E: carboplatin, paclitaxel + 45 Gy radiation + pembrolizumab → surgery → pembrolizumab	pCR
NCT03257163/phase II recruiting	40 patients/IB-IIIC/gastric adenocarcinoma MSI or EBV positive	E: pembrolizumab → surgery → pembrolizumab + capecitabine + radiation	3-yr RFS rate
NCT03776487/phase I/II recruiting	30 patients/0-IVA/stomach GEJ/adenocarcinoma	E: oxaliplatin, 5-FU + nivolumab, ipilimumab + radiation → surgery → nivolumab	Tolerability
NCT00857246/phase II/completed	30 patients/T3N0, T4, TanyN1-3, M0/stomach GEJ	E: induction cisplatin, irinotecan + cetuximab → surgery → cetuximab + 5-FU/LV + 37.5 Gy radiation	RR
NCT01183559/phase I/completed	9 patients/potentially resectable/esophagus GEJ stomach	E: vandetanib + carboplatin, paclitaxel + 5-FU + 45 Gy radiation → surgery	Maximum tolerated dose: 200 mg vandetanib
NCT04162665/recruiting	36 patients/T1-T2N1, T3Nany/stomach AEG3/adenocarcinoma	E: MRI guided 25 Gy radiation + oxaliplatin, capecitabine	pCR
NCT04308837/phase II recruiting	29 patients/uT3-4NanyM0/stomach	E: laparoscopic hyperthermic intraperitoneal chemotherapy → carboplatin, paclitaxel + IM radiation T>6M → gastrectomy D2 → oxaliplatin, 5-FU/LV	pCR
CRITICS-II/phase II/NCT02931890/recruiting[83]	207 patients/IB-IIIC/resectable/stomach GEJ/adenocarcinoma	E1: docetaxel, oxaliplatin, capecitabine OR E2: induction docetaxel, oxaliplatin, capecitabine → carboplatin, paclitaxel + 45 Gy radiation OR E3: carboplatin, paclitaxel + 45 Gy radiation AND gastrectomy (all arms)	1-yr EFS rate
Neo-CRAG/phase III	620 patients/cT3N2/N3M0, cT4aN + M0, cT4bNanyM0/gastric adenocarcinoma	E: oxaliplatin, capecitabine + 45 Gy radiation OR C: oxaliplatin, capecitabine AND gastrectomy D2 → oxaliplatin, capecitabine (both arms)	3-yr DFS rate
PREACT/phase III	682 patients/IIB (T3N1 only)-IIIC (excluding T2N3)/stomach GEJ (excluding AEG1)/adenocarcinoma	E: S-1, oxaliplatin + 45 Gy radiation OR C: S-1, oxaliplatin AND gastrectomy D2 → S-1, oxaliplatin (both arms)	3-yr DFS rate
ITACA S-2/phase III/NCT01989858/ terminated	1180 patients/T3-4N0M0, TanyN + M0/stomach/adenocarcinoma	E1: epirubicin, oxaliplatin, capecitabine (EOX) or epirubicin, cisplatin, 5-FU (ECF) → gastrectomy → EOX or ECF OR C1: gastrectomy → EOX or ECF E2: EOX or ECF → gastrectomy → EOX or ECF → capecitabine or 5-FU + 45 Gy radiation OR C2: gastrectomy → EOX or ECF → capecitabine or 5-FU + 45 Gy	5-yr OS rate
Enriched-CRT 2017/phase III/NCT03680261/not yet recruiting	556 patients/pT2-4aN1-3M0 LVI+/stomach GEJ/adenocarcinoma	Gastrectomy D1/D2 (both arms) AND E: oxaliplatin, capecitabine or S-1 + 45 Gy radiation OR C: oxaliplatin, capecitabine or S-1	3-yr OS rate
TOPGEAR/phase III[61]	620 patients/IB (T1N1 only)-IIIC/resectable stomach GEJ/adenocarcinoma	E: induction EOX or ECF or epirubicin, cisplatin, capecitabine (ECX) or docetaxel, oxaliplatin, 5-FU/LV (FLOT) → 5-FU or capecitabine + 45 Gy radiation OR C: induction EOX or ECF or ECX or FLOT AND gastrectomy D1+ → EOX or ECF or ECX or FLOT (all arms)	5-yr OS rate

mOS: Median overall survival; mDFS: Median disease-free survival; mPFS: Median progression-free survival; E: Experimental; C: Control; HR: Hazard ratio; 5-FU: 5-fluorouracil; LV: Leucovorin; Gy: Gray; S-1: Tegafur/gimeracil/oteracil; pCR: Pathologic complete response; GEJ: Gastroesophageal junction; CRT: Chemoradiation; AEG: Adenocarcinomas of the esophagogastric junction; EOX: Epirubicin, oxaliplatin, capecitabine; ECF: Epirubicin, cisplatin, 5-fluorouracil; ECX: Epirubicin, cisplatin, capecitabine; FLOT: Docetaxel, oxaliplatin, 5-fluorouracil/leucovorin.

concrete data on whether addition of radiation therapy to the newest preoperative chemotherapy regimens, such as FLOT, impacts clinical outcomes. ITACA-S2 is a multi-arm comprehensive study evaluating both the ideal regimen and timing of perioperative treatment, while the ENRICHED trial repeats the adjuvant chemoradiation design in a modern setting. Finally, a significant number of trials incorporated different checkpoint inhibitors in the preoperative setting, after the success of Checkmate 577.

CONCLUSION

Complete surgical resection of the tumor provides the best chance for cure; however, a significant proportion of patients presents with unresectable disease. Adjunctive therapy besides gastrectomy is recommended in a multidisciplinary approach and preoperative therapy is the cornerstone of management in the West. The evidence-based approach should include perioperative chemotherapy or postoperative chemoradiotherapy for selected patients. Similarly, for resectable GEJ cancers, preoperative chemoradiotherapy will enhance surgical outcomes and improve the pCR rate. Evidence from the ARTIST 2 and CRITICS study do not support the adjuvant use of radiation therapy in adequately resected tumors. In the preoperative setting, although prospective data comparing neoadjuvant chemotherapy to chemoradiotherapy are still immature, there is a trend to improved local control and pCR rates, which may translate into significant clinical outcomes in the future. Long term results from the Neo-AEGIS and TOPGEAR trial are eagerly awaited. Moreover, future research should also focus is on optimizing the chemotherapy regimen, defining the role of radiotherapy and immunotherapy and exploring the effect of treatment timing (preoperative, postoperative or both).

REFERENCES

1 **Jemal A**, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1893-1907 [PMID: 20647400 DOI: 10.1158/1055-9965.EPI-10-0437]

2 **Karimi P**, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 700-713 [PMID: 24618998 DOI: 10.1158/1055-9965.EPI-13-1057]

3 **Then EO**, Lopez M, Saleem S, Gayam V, Sunkara T, Culliford A, Gaduputi V. Esophageal Cancer: An Updated Surveillance Epidemiology and End Results Database Analysis. World J Oncol 2020; 11(2): 55-64 [PMID: 32284773 DOI: 10.14740/wjon1254]

4 **Charalampakis N**, Economopoulou P, Kotsantis I, Tolia M, Schizas D, Liakakos T, Elimova E, Ajani JA, Psyrri A. Medical management of gastric cancer: a 2017 update. *Cancer Med* 2018; **7**: 123-133 [PMID: 29239137 DOI: 10.1002/cam4.1274]

5 **Dicken BJ**, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: review and considerations for future directions. *Ann Surg* 2005; **241**: 27-39 [PMID: 15621988 DOI: 10.1097/01.sla.0000149300.28588.23]

6 **Ma J**, Shen H, Kapesa L, Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. *Oncol Lett* 2016; **11**: 2959-2964 [PMID: 27123046 DOI: 10.3892/ol.2016.4337]

7 **Hansford S**, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H, Schrader KA, Schaeffer DF, Shumansky K, Zogopoulos G, Santos TA, Claro I, Carvalho J, Nielsen C, Padilla S, Lum A, Talhouk A, Baker-Lange K, Richardson S, Lewis I, Lindor NM, Pennell E, MacMillan A, Fernandez B, Keller G, Lynch H, Shah SP, Guilford P, Gallinger S, Corso G, Roviello F, Caldas C, Oliveira C, Pharoah PD, Huntsman DG. Hereditary Diffuse Gastric Cancer Syndrome: CDH1 Mutations and Beyond. *JAMA Oncol* 2015; **1**: 23-32 [PMID: 26182300 DOI: 10.1001/jamaoncol.2014.168]

8 **Rice TW**, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. *Ann Cardiothorac Surg* 2017; **6**: 119-130 [PMID: 28447000 DOI: 10.21037/acs.2017.03.14]

9 **Cancer Genome Atlas Research Network**; Analysis Working Group: Asan University; BC Cancer Agency; Brigham and Women's Hospital; Broad Institute; Brown University; Case Western Reserve University; Dana-Farber Cancer Institute; Duke University; Greater Poland Cancer Centre; Harvard Medical School; Institute for Systems Biology; KU Leuven; Mayo Clinic; Memorial Sloan Kettering Cancer Center; National Cancer Institute; Nationwide Children's Hospital; Stanford University; University of Alabama; University of Michigan; University of North Carolina; University of Pittsburgh; University of Rochester; University of Southern California; University of Texas MD Anderson Cancer Center; University of Washington; Van Andel Research Institute; Vanderbilt University; Washington University; Genome Sequencing Center: Broad Institute; Washington University in St. Louis; Genome Characterization Centers: BC Cancer Agency; Broad Institute; Harvard Medical School; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; University of North Carolina; University of Southern California Epigenome Center; University of Texas MD Anderson Cancer Center; Van Andel Research Institute; Genome Data Analysis Centers: Broad Institute; Brown University:; Harvard Medical School; Institute for Systems Biology; Memorial Sloan Kettering Cancer Center; University of California Santa Cruz; University of Texas MD Anderson Cancer Center; Biospecimen Core Resource: International Genomics Consortium; Research Institute at Nationwide Children's Hospital; Tissue Source Sites: Analytic Biologic Services; Asan Medical Center; Asterand Bioscience; Barretos Cancer Hospital; BioreclamationIVT; Botkin Municipal Clinic; Chonnam National University Medical School;

Christiana Care Health System; Cureline; Duke University; Emory University; Erasmus University; Indiana University School of Medicine; Institute of Oncology of Moldova; International Genomics Consortium; Invidumed; Israelitisches Krankenhaus Hamburg; Keimyung University School of Medicine; Memorial Sloan Kettering Cancer Center; National Cancer Center Goyang; Ontario Tumour Bank; Peter MacCallum Cancer Centre; Pusan National University Medical School; Ribeirão Preto Medical School; St. Joseph's Hospital &Medical Center; St. Petersburg Academic University; Tayside Tissue Bank; University of Dundee; University of Kansas Medical Center; University of Michigan; University of North Carolina at Chapel Hill; University of Pittsburgh School of Medicine; University of Texas MD Anderson Cancer Center; Disease Working Group: Duke University; Memorial Sloan Kettering Cancer Center; National Cancer Institute; University of Texas MD Anderson Cancer Center; Yonsei University College of Medicine; Data Coordination Center: CSRA Inc; Project Team: National Institutes of Health. Integrated genomic characterization of oesophageal carcinoma. *Nature* 2017; **541**: 169-175 [PMID: 28052061 DOI: 10.1038/nature20805]

10 **Mariette C**, Finzi L, Piessen G, Van Seuningen I, Triboulet JP. Esophageal carcinoma: prognostic differences between squamous cell carcinoma and adenocarcinoma. *World J Surg* 2005; **29**: 39-45 [PMID: 15599738 DOI: 10.1007/s00268-004-7542-x]

11 **Siewert JR**, Ott K. Are squamous and adenocarcinomas of the esophagus the same disease? *Semin Radiat Oncol* 2007; **17**: 38-44 [PMID: 17185196 DOI: 10.1016/j.semradonc.2006.09.007]

12 **Domper Arnal MJ**, Ferrández Arenas Á, Lanas Arbeloa Á. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. *World J Gastroenterol* 2015; **21**: 7933-7943 [PMID: 26185366 DOI: 10.3748/wjg.v21.i26.7933]

13 **Berry MF**. Esophageal cancer: staging system and guidelines for staging and treatment. *J Thorac Dis* 2014; **6** Suppl 3: S289-S297 [PMID: 24876933 DOI: 10.3978/j.issn.2072-1439.2014.03.11]

14 **Coleman HG**, Xie SH, Lagergren J. The Epidemiology of Esophageal Adenocarcinoma. *Gastroenterology* 2018; **154**: 390-405 [PMID: 28780073 DOI: 10.1053/j.gastro.2017.07.046]

15 **Jain S**, Dhingra S. Pathology of esophageal cancer and Barrett's esophagus. *Ann Cardiothorac Surg* 2017; **6**: 99-109 [PMID: 28446998 DOI: 10.21037/acs.2017.03.06]

16 **Ignatova E**, Seriak D, Fedyanin M, Tryakin A, Pokataev I, Menshikova S, Vakhabova Y, Smirnova K, Tjulandin S, Ajani JA. Epstein-Barr virus-associated gastric cancer: disease that requires special approach. *Gastric Cancer* 2020; **23**: 951-960 [PMID: 32514646 DOI: 10.1007/s10120-020-01095-z]

17 **Sohn BH**, Hwang JE, Jang HJ, Lee HS, Oh SC, Shim JJ, Lee KW, Kim EH, Yim SY, Lee SH, Cheong JH, Jeong W, Cho JY, Kim J, Chae J, Lee J, Kang WK, Kim S, Noh SH, Ajani JA, Lee JS. Clinical Significance of Four Molecular Subtypes of Gastric Cancer Identified by The Cancer Genome Atlas Project. *Clin Cancer Res* 2017; **23**: 4441-4449 [PMID: 28747339 DOI: 10.1158/1078-0432.CCR-16-2211]

18 **Kim ST**, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, Park YS, Lim HY, Lee H, Choi M, Talasaz A, Kang PS, Cheng J, Loboda A, Lee J, Kang WK. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018; **24**: 1449-1458 [PMID: 30013197 DOI: 10.1038/s41591-018-0101-z]

19 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

20 **Tokunaga M**, Sato Y, Nakagawa M, Aburatani T, Matsuyama T, Nakajima Y, Kinugasa Y. Perioperative chemotherapy for locally advanced gastric cancer in Japan: current and future perspectives. *Surg Today* 2020; **50**: 30-37 [PMID: 31612329 DOI: 10.1007/s00595-019-01896-5]

21 **Choi AH**, Kim J, Chao J. Perioperative chemotherapy for resectable gastric cancer: MAGIC and beyond. *World J Gastroenterol* 2015; **21**: 7343-7348 [PMID: 26139980 DOI: 10.3748/wjg.v21.i24.7343]

22 **Li Y**, Wang J, Ma X, Tan L, Yan Y, Xue C, Hui B, Liu R, Ma H, Ren J. A Review of Neoadjuvant Chemoradiotherapy for Locally Advanced Rectal Cancer. *Int J Biol Sci* 2016; **12**: 1022-1031 [PMID: 27489505 DOI: 10.7150/ijbs.15438]

23 **Kaltenmeier C**, Althans A, Mascara M, Nassour I, Khan S, Hoehn R, Zureikat A, Tohme S. Pathologic Complete Response Following Neoadjuvant Therapy for Gastric Adenocarcinoma: A National Cancer Database Analysis on Incidence, Predictors, and Outcomes. *Am Surg* 2021; **87**: 1145-1154 [PMID: 33342268 DOI: 10.1177/0003134820972083]

24 **Xiang M**, Chang DT, Heestand GM, Pollom EL. Survival after neoadjuvant approaches to gastroesophageal junction cancer. *Gastric Cancer* 2020; **23**: 175-183 [PMID: 31230228 DOI: 10.1007/s10120-019-00980-6]

25 **Siewert JR**, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. *Br J Surg* 1998; **85**: 1457-1459 [PMID: 9823902 DOI: 10.1046/j.1365-2168.1998.00940.x]

26 **Johnston FM**, Beckman M. Updates on Management of Gastric Cancer. *Curr Oncol Rep* 2019; **21**: 67 [PMID: 31236716 DOI: 10.1007/s11912-019-0820-4]

27 **Weledji EP**. The principles of the surgical management of gastric cancer. *Int J Surg Oncol (N Y)* 2017; **2**: e11 [PMID: 29177225 DOI: 10.1097/IJ9.0000000000000011]

28 **Baskar R**, Lee KA, Yeo R, Yeoh KW. Cancer and radiation therapy: current advances and future directions. *Int J Med Sci* 2012; **9**: 193-199 [PMID: 22408567 DOI: 10.7150/ijms.3635]

29 **Tey J**, Soon YY, Koh WY, Leong CN, Choo BA, Ho F, Vellayappan B, Lim K, Tham IW. Palliative radiotherapy for gastric cancer: a systematic review and meta-analysis. *Oncotarget* 2017; **8**: 25797-25805 [PMID: 28445941 DOI: 10.18632/oncotarget.15554]

30 **Hennequin C**, Guillerm S, Quero L. Combination of chemotherapy and radiotherapy: A thirty years evolution. *Cancer Radiother* 2019; **23**: 662-665 [PMID: 31473087 DOI: 10.1016/j.canrad.2019.07.157]

31 **Curran WJ Jr**, Paulus R, Langer CJ, Komaki R, Lee JS, Hauser S, Movsas B, Wasserman T, Rosenthal SA, Gore E, Machtay M, Sause W, Cox JD. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. *J Natl Cancer Inst* 2011; **103**: 1452-1460 [PMID: 21903745 DOI: 10.1093/jnci/djr325]

32 **Lawrence TS**, Blackstock AW, McGinn C. The mechanism of action of radiosensitization of conventional chemotherapeutic agents. *Semin Radiat Oncol* 2003; **13**: 13-21 [PMID: 12520460 DOI: 10.1053/srao.2003.50002]

33 **Hirata E**, Sahai E. Tumor Microenvironment and Differential Responses to Therapy. *Cold Spring Harb Perspect Med* 2017; **7** [PMID: 28213438 DOI: 10.1101/cshperspect.a026781]

34 **Vellayappan BA**, Soon YY, Ku GY, Leong CN, Lu JJ, Tey JC. Chemoradiotherapy versus chemoradiotherapy plus surgery for esophageal cancer. *Cochrane Database Syst Rev* 2017; **8**: CD010511 [PMID: 28829911 DOI: 10.1002/14651858.CD010511.pub2]

35 **Sharabi AB**, Lim M, DeWeese TL, Drake CG. Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy. *Lancet Oncol* 2015; **16**: e498-e509 [PMID: 26433823 DOI: 10.1016/S1470-2045(15)00007-8]

36 **Deng L**, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, Fu YX. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* 2014; **124**: 687-695 [PMID: 24382348 DOI: 10.1172/JCI67313]

37 **Macdonald JS**, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, Haller DG, Ajani JA, Gunderson LL, Jessup JM, Martenson JA. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med* 2001; **345**: 725-730 [PMID: 11547741 DOI: 10.1056/NEJMoa010187]

38 **McNamara MJ**, Adelstein DJ, Bodmann JW, Greskovich JF Jr, Ives DI, Mason DP, Murthy SC, Rice TW, Saxton JP, Sohal D, Stephans K, Rodriguez CP, Videtic GM, Rybicki LA. A phase II trial of induction epirubicin, oxaliplatin, and fluorouracil, followed by surgery and postoperative concurrent cisplatin and fluorouracil chemoradiotherapy in patients with locoregionally advanced adenocarcinoma of the esophagus and gastroesophageal junction. *J Thorac Oncol* 2014; **9**: 1561-1567 [PMID: 25170643 DOI: 10.1097/JTO.0000000000000312]

39 **Adelstein DJ**, Rice TW, Rybicki LA, Saxton JP, Videtic GM, Murthy SC, Mason DP, Rodriguez CP, Ives DI. Mature results from a phase II trial of postoperative concurrent chemoradiotherapy for poor prognosis cancer of the esophagus and gastroesophageal junction. *J Thorac Oncol* 2009; **4**: 1264-1269 [PMID: 19668013 DOI: 10.1097/JTO.0b013e3181b26f8e]

40 **Mansouri H**, Zemni I, Achouri L, Mahjoub N, Ayedi MA, Ben Safta I, Ben Dhiab T, Chargui R, Rahal K. Chemoradiotherapy or chemotherapy as adjuvant treatment for resected gastric cancer: should we use selection criteria? *Rep Pract Oncol Radiother* 2021; **26** (2): 266-280 [PMID: 34211778 DOI: 10.5603/RPOR.a2021.0040]

41 **Yekeduz E**, Dogan I, Akbulut H, Karabulut S, Utkan G, Urun Y. A retrospective multicenter study: Clinicopathological factors affecting recurrence-free survival in operable gastric cancer patients. *J Clin Oncol* 2021; **39**: 185 [DOI: 10.1200/JCO.2021.39.3_suppl.185]

42 **Xie C**, Jin X, Chen D. Capecitabine plus oxaliplatin vs capecitabine plus oxaliplatin with concurrent radiotherapy in the treatment of gastric cancer after D2 gastrectomy. *J Clin Oncol* 2018; **36**: 4059 [DOI: 10.1200/JCO.2018.36.15_suppl.4059]

43 **Wang MJ**, Li C, Sun Y, Shen FJ, Wang CB. Prognostic effect of adjuvant chemoradiotherapy for patients with gastric cancer: an updated evidence of randomized controlled trials. Oncotarget 2017; 8(61): 102880-102887 [PMID: 29262530 DOI: 10.18632/oncotarget.21983]

44 **Cats A**, Jansen EPM, van Grieken NCT, Sikorska K, Lind P, Nordsmark M, Meershoek-Klein Kranenbarg E, Boot H, Trip AK, Swellengrebel HAM, van Laarhoven HWM, Putter H, van Sandick JW, van Berge Henegouwen MI, Hartgrink HH, van Tinteren H, van de Velde CJH, Verheij M; CRITICS investigators. Chemotherapy versus chemoradiotherapy after surgery and preoperative chemotherapy for resectable gastric cancer (CRITICS): an international, open-label, randomised phase 3 trial. *Lancet Oncol* 2018; **19**: 616-628 [PMID: 29650363 DOI: 10.1016/S1470-2045(18)30132-3]

45 **Park SH**, Lee J, Sohn TS, Lim DH, Kim K-M, An JY, Choi MG, Lee JH, Bae JM, Kim S, Lee SJ, Kim ST, Park JO, Park YS, Lim HY, Kang WK. Results from the safety interim analysis of the adjuvant chemoradiotherapy in stomach tumors 2 trial: a multicenter, randomized phase III clinical trial. Precis Future Med 2019; 3(1): 24-29 [DOI: 10.23838/pfm.2018.00177]

46 **Park SH**, Zang DY, Han B, Ji JH, Kim TG, Oh SY, Hwang IG, Kim JH, Shin D, Lim DH, Kim KM, An JY, Choi M-G, Lee J-H, Sohn TS, Bae J-M, Kim S, Lee J, Kang WK. ARTIST 2: Interim results of a phase III trial involving adjuvant chemotherapy and/or chemoradiotherapy after D2-gastrectomy in stage II/III gastric cancer (GC). *J Clin Oncol* 2019; **37**: 4001 [DOI: 10.1200/JCO.2019.37.15_suppl.4001]

47 **Rivera F**, Galán M, Tabernero J, Cervantes A, Vega-Villegas ME, Gallego J, Laquente B, Rodríguez E, Carrato A, Escudero P, Massutí B, Alonso-Orduña V, Cardenal A, Sáenz A, Giralt J, Yuste AL, Antón A, Aranda E; Spanish Cooperative Group for Digestive Tumor Therapy. Phase II trial of preoperative irinotecan-cisplatin followed by concurrent irinotecan-cisplatin and radiotherapy for resectable locally advanced gastric and esophagogastric junction adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2009; **75**: 1430-1436 [PMID: 19540072 DOI: 10.1016/j.ijrobp.2008.12.087]

48 **Ludmir EB**, Das P. Shifting sands: the role of radiotherapy for patients with gastric and gastroesophageal adenocarcinoma. Transl Gastroenterol Hepatol 2021; 6: 50 [PMID: 34423171 DOI: 10.21037/tgh.2020.03.01]

49 **Turgeman I**, Ben-Aharon I. Evolving treatment paradigms in esophageal cancer. Ann Transl Med 2021; 9(10): 903 [PMID: 34164537 DOI: 10.21037/atm.2020.03.110]

50 **Ilson DH**, Minsky BD, Ku GY, Rusch V, Rizk N, Shah M, Kelsen DP, Capanu M, Tang L, Campbell J, Bains M. Phase 2 trial of induction and concurrent chemoradiotherapy with weekly irinotecan and cisplatin followed by surgery for esophageal cancer. *Cancer* 2012; **118**: 2820-2827 [PMID: 21990000 DOI: 10.1002/cncr.26591]

51 **van der Wilk BJ**, Eyck BM, Lagarde SM, van der Gaast A, Nuyttens J, Wijnhoven BPL, van Lanschot JJB. The optimal neoadjuvant treatment of locally advanced esophageal cancer. J Thorac Dis 2019; 11(Suppl 5): S621-S631 [PMID: 31080638 DOI: 10.21037/jtd.2018.11.143]

52 **Stahl M**, Walz MK, Riera-Knorrenschild J, Stuschke M, Sandermann A, Bitzer M, Wilke H, Budach W. Preoperative chemotherapy versus chemoradiotherapy in locally advanced adenocarcinomas of the oesophagogastric junction (POET): Long-term results of a controlled randomised trial. *Eur J Cancer* 2017; **81**: 183-190 [PMID: 28628843 DOI: 10.1016/j.ejca.2017.04.027]

53 **Ma Z**, Yuan M, Bao Y, Wang Y, Men Y, Hui Z. Survival of Neoadjuvant and Adjuvant Therapy Compared With Surgery Alone for Resectable Esophageal Squamous Cell Carcinoma: A Systemic Review and Network Meta-Analysis. Front Oncol 2021; 11: 728185 [PMID: 34745950 DOI: 10.3389/fonc.2021.728185]

54 **von Döbeln GA**, Klevebro F, Jacobsen AB, Johannessen HO, Nielsen NH, Johnsen G, Hatlevoll I, Glenjen NI, Friesland S, Lundell L, Yu J, Nilsson M. Neoadjuvant chemotherapy versus neoadjuvant chemoradiotherapy for cancer of the esophagus or gastroesophageal junction: long-term results of a randomized clinical trial. *Dis Esophagus* 2019; **32** [PMID: 30137281 DOI: 10.1093/dote/doy078]

55 **Zhao X**, Ren Y, Hu Y, Cui N, Wang X, Cui Y. Neoadjuvant chemotherapy versus neoadjuvant chemoradiotherapy for cancer of the esophagus or the gastroesophageal junction: A meta-analysis based on clinical trials. *PLoS One* 2018; **13**: e0202185 [PMID: 30138325 DOI: 10.1371/journal.pone.0202185]

56 **Hou S**, Pan Z, Hao X, Hang Q, Ding Y. Recent Progress in the Neoadjuvant Treatment Strategy for Locally Advanced Esophageal Cancer. *Cancers (Basel)* 2021; **13** (20) [PMID: 34680311 DOI: 10.3390/cancers13205162]

57 **van Hagen P**, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hospers GA, Bonenkamp JJ, Cuesta MA, Blaisse RJ, Busch OR, ten Kate FJ, Creemers GJ, Punt CJ, Plukker JT, Verheul HM, Spillenaar Bilgen EJ, van Dekken H, van der Sangen MJ, Rozema T, Biermann K, Beukema JC, Piet AH, van Rij CM, Reinders JG, Tilanus HW, van der Gaast A; CROSS Group. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012; **366**: 2074-2084 [PMID: 22646630 DOI: 10.1056/NEJMoa1112088]

58 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992 DOI: 10.1056/NEJMoa055531]

59 **Al-Batran SE**, Homann N, Pauligk C, Goetze TO, Meiler J, Kasper S, Kopp HG, Mayer F, Haag GM, Luley K, Lindig U, Schmiegel W, Pohl M, Stoehlmacher J, Folprecht G, Probst S, Prasnikar N, Fischbach W, Mahlberg R, Trojan J, Koenigsmann M, Martens UM, Thuss-Patience P, Egger M, Block A, Heinemann V, Illerhaus G, Moehler M, Schenk M, Kullmann F, Behringer DM, Heike M, Pink D, Teschendorf C, Löhr C, Bernhard H, Schuch G, Rethwisch V, von Weikersthal LF, Hartmann JT, Kneba M, Daum S, Schulmann K, Weniger J, Belle S, Gaiser T, Oduncu FS, Güntner M, Hozaeel W, Reichart A, Jäger E, Kraus T, Mönig S, Bechstein WO, Schuler M, Schmalenberg H, Hofheinz RD; FLOT4-AIO Investigators. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. *Lancet* 2019; **393**: 1948-1957 [PMID: 30982686 DOI: 10.1016/S0140-6736(18)32557-1]

60 **Reynolds JV**, Preston SR, O'Neill B, Lowery MA, Baeksgaard L, Crosby T, Cunningham M, Cuffe S, Griffiths GO, Roy R, Falk S, Hanna G, Bartlett FR, Parker I, Alvarez-Iglesias A, Nilsson M, Piessen G, Risum S, Ravi N, McDermott RS. Neo-AEGIS (Neoadjuvant trial in Adenocarcinoma of the Esophagus and Esophago-Gastric Junction International Study): Preliminary results of phase III RCT of CROSS versus perioperative chemotherapy (Modified MAGIC or FLOT protocol). (NCT01726452). *J Clin Oncol* 2021; **39**: 4004 [DOI: 10.1200/JCO.2021.39.15_suppl.4004]

61 **Leong T**, Smithers BM, Haustermans K, Michael M, Gebski V, Miller D, Zalcberg J, Boussioutas A, Findlay M, O'Connell RL, Verghis J, Willis D, Kron T, Crain M, Murray WK, Lordick F, Swallow C, Darling G, Simes J, Wong R. TOPGEAR: A Randomized, Phase III Trial of Perioperative ECF Chemotherapy with or Without Preoperative Chemoradiation for Resectable Gastric Cancer: Interim Results from an International, Intergroup Trial of the AGITG, TROG, EORTC and CCTG. *Ann Surg Oncol* 2017; **24**: 2252-2258 [PMID: 28337660 DOI: 10.1245/s10434-017-5830-6]

62 **Pape M**, Vissers PAJ, Beerepoot L, Van Berge Henegouwen MI, Lagarde S, Mook S, Bertwistle D, McDonald L, Van Laarhoven HWM, Verhoeven R. Disease-free and overall survival in nonmetastatic esophageal or gastroesophageal junctional cancer after treatment with curative intent: A nationwide

population-based study. *J Clin Oncol* 2021; **39**: 246 [DOI: 10.1200/JCO.2021.39.3_suppl.246]

63 **Tim Spencer**, Jonathan Helbrow, Eleanor Pilsworth, Stephen Falk, Helen Winter, Thomas Bird. Selecting the optimal neoadjuvant treatment approach based on the risk of an involved surgical margin in locally advanced adenocarcinoma of the esophagus and esophagogastric junction. *J Clin Oncol* 2021; **39**: 198 [DOI: 10.1200/JCO.2021.39.3_suppl.198]

64 **Jurkowski L**, Shreenivas AV, Chakrabarti S, Kamgar M, Thomas JP, Puckett L, Shukla M, Gore E, Evans J, Johnstone CA, Gasparri M, Linsky P, Johnstone D, Dua K, Khan AH, Madhavan S, Szabo A, George B. Association of total neoadjuvant therapy with favorable clinical outcomes in patients with locally advanced esophageal and gastroesophageal junction adenocarcinomas (LA-GEJ CA). *J Clin Oncol* 2021; **39**: 231 [DOI: 10.1200/JCO.2021.39.3_suppl.231]

65 **Schizas D**, Charalampakis N, Kole C, Mylonas KS, Katsaros I, Zhao M, Ajani JA, Psyrri A, Karamouzis MV, Liakakos T. Immunotherapy for esophageal cancer: a 2019 update. *Immunotherapy* 2020; **12**: 203-218 [PMID: 32208794 DOI: 10.2217/imt-2019-0153]

66 **Kelly RJ**, Ajani JA, Kuzdzal J, Zander T, Van Cutsem E, Piessen G, Mendez G, Feliciano JL, Motoyama S, Lièvre A, Uronis H, Elimova E, Grootscholten C, Geboes K, Zhang J, Zhu L, Lei M, Kondo K, Cleary JM, Moehler M. LBA9_PR Adjuvant nivolumab in resected esophageal or gastroesophageal junction cancer (EC/GEJC) following neoadjuvant chemoradiation therapy (CRT): First results of the CheckMate 577 study. *Ann Oncol* 2020; **31**: S1193-S1194 [DOI: 10.1016/j.annonc.2020.08.2299]

67 **Ku GY**, Abraham Jing-Ching Wu, Smita Sihag, Bernard J. Park, David Randolph Jones, Ping Gu, Steven Brad Maron, Ryan Sugarman, Sree Bhavani Chalasani, Marina Shcherba, Lara Feder, Kendall Cowie, Jaclyn Norris, Valerie Johnson, Laura H. Tang, David H. Ilson, Yelena Y. Janjigian, Daniela Molena. Durvalumab (D) and PET-directed chemoradiation (CRT) after induction FOLFOX for esophageal adenocarcinoma. *J Clin Oncol* 2021; **39**: 226 [DOI: 10.1200/JCO.2021.39.3_suppl.226]

68 **Goodman KA**, Hall N, Bekaii-Saab TS, Ou F-S, Twohy E, Meyers MO, Boffa DJ, Mitchell K, Perry K, Frankel WL, Venook AP, O'Reilly EM, Ilson DH. Survival outcomes from CALGB 80803 (Alliance): A randomized phase II trial of PET scan-directed combined modality therapy for esophageal cancer. *J Clin Oncol* 2018; **36**: 4012 [DOI: 10.1200/JCO.2018.36.15_suppl.4012]

69 **Safran H**, Winter KA, Wigle DA, DiPetrillo TA, Haddock MG, Hong TS, Leichman LP, Rajdev L, Resnick MB, Kachnic LA, Seaward SA, Mamon HJ, Pardo DAD, Anderson CM, Shen X, Sharma AK, Katz AW, Salo JC, Leonard KL, Crane CH. Trastuzumab with trimodality treatment for esophageal adenocarcinoma with HER2 overexpression: NRG Oncology/RTOG 1010. *J Clin Oncol* 2020; **38**: 4500 [DOI: 10.1200/JCO.2020.38.15_suppl.4500]

70 **Abali H**, Yalcin S, Onal C, Dane F, Oksuzoglu B, Ozdemir N, Mertsoylu H, Artac M, Camci C, Karabulut B, Basal FB, Budakoglu B, Sendur MAN, Goktas B, Ozdener F, Baygul A. A study of the combination of oxaliplatin, capecitabine, and trastuzumab and chemo-radiotherapy in the adjuvant setting in operated patients with HER2+ gastric or gastroesophageal junction cancer (TOXAG study). *J Clin Oncol* 2018; **36**: 26 [DOI: 10.1200/JCO.2018.36.4_suppl.26]

71 **Ruhstaller T**, Thuss-Patience P, Hayoz S, Schacher S, Knorrenschild JR, Schnider A, Plasswilm L, Budach W, Eisterer W, Hawle H, Mariette C, Hess V, Mingrone W, Montemurro M, Girschikofsky M, Schmidt SC, Bitzer M, Bedenne L, Brauchli P, Stahl M. Neoadjuvant chemotherapy followed by chemoradiation and surgery with and without cetuximab in patients with resectable esophageal cancer: a randomized, open-label, phase III trial (SAKK 75/08). *Ann Oncol* 2018; **29**: 1386-1393 [DOI: 10.1093/annonc/mdy105]

72 **Ku GY**, Bains MS, Park DJ, Janjigian YY, Rusch VW, Rizk NP, Yoon SS, Millang B, Capanu M, Goodman KA, Ilson DH. Phase II study of bevacizumab and preoperative chemoradiation for esophageal adenocarcinoma. *J Gastrointest Oncol* 2016; **7**: 828-837 [PMID: 28078107 DOI: 10.21037/jgo.2016.08.09]

73 **Jiang DM**, Sim HW, Espin-Garcia O, Chan BA, Natori A, Lim CH, Moignard S, Chen EX, Liu G, Darling G, Swallow CJ, Brar S, Brierley J, Ringash J, Wong R, Kim J, Rogalla P, Hafezi-Bakhtiari S, Knox JJ, Jang RW, Elimova E. Chemoradiotherapy Using Carboplatin plus Paclitaxel versus Cisplatin plus Fluorouracil for Esophageal or Gastroesophageal Junction Cancer. *Oncology* 2021; **99**: 49-56 [PMID: 33053548 DOI: 10.1159/000510446]

74 **Chakravarthy AB**, Catalano PJ, Mondschein JK, Rosenthal DI, Haller DG, Whittington R, Spitz FR, Wagner H, Sigurdson ER, Tschetter LK, Bayer GK, Mulcahy MF, Benson AB. Phase II Trial of Paclitaxel/Cisplatin Followed by Surgery and Adjuvant Radiation Therapy and 5-Fluorouracil/Leucovorin for Gastric Cancer (ECOG E7296). *Gastrointest Cancer Res* 2012; **5**: 191-197 [PMID: 23293700]

75 **Goodman KA**, Niedzwiecki D, Hall N, Bekaii-Saab TS, Ye X, Meyers MO, Mitchell-Richards K, Boffa DJ, Frankel WL, Venook AP, Hochster HS, Crane CH, O'Reilly EM, Ilson DH. Initial results of CALGB 80803 (Alliance): A randomized phase II trial of PET scan-directed combined modality therapy for esophageal cancer. *J Clin Oncol* 2017; **35**: 1 [DOI: 10.1200/JCO.2017.35.4_suppl.1]

76 **Zhao Z**, Yang S, Zhou A, Li X, Fang R, Zhang S, Zhao G, Li P. Small Extracellular Vesicles in the Development, Diagnosis, and Possible Therapeutic Application of Esophageal Squamous Cell Carcinoma. Front Oncol 2021; 11: 732702 [PMID: 34527593 DOI: 10.3389/fonc.2021.732702]

77 **Kleinberg LR**, Catalano PJ, Forastiere AA, Keller SM, Mitchel EP, Anne PR, Benson AB 3rd. Eastern Cooperative Oncology Group and American College of Radiology Imaging Network Randomized Phase 2 Trial of Neoadjuvant Preoperative Paclitaxel/Cisplatin/Radiation Therapy (RT) or Irinotecan/Cisplatin/RT in Esophageal Adenocarcinoma: Long-Term Outcome and Implications for

Trial Design. *Int J Radiat Oncol Biol Phys* 2016; **94**: 738-746 [PMID: 26972646 DOI: 10.1016/j.ijrobp.2015.12.009]

78 **Schernberg A**, Rivin Del Campo E, Rousseau B, Matzinger O, Loi M, Maingon P, Huguet F. Adjuvant chemoradiation for gastric carcinoma: State of the art and perspectives. Clin Transl Radiat Oncol 2018; 10: 13-22 [PMID: 29928701 DOI: 10.1016/j.ctro.2018.02.005]

79 **Alberts SR**, Soori GS, Shi Q, Wigle DA, Sticca RP, Miller RC, Leenstra JL, Peller PJ, Wu T-T, Yoon HH, Drevyanko TF, Ko S, Mattar BI, Nikcevich DA, Behrens RJ, Khalil MF, Kim GP. Randomized phase II trial of extended vs standard neoadjuvant therapy for esophageal cancer, NCCTG (Alliance) trial N0849. *J Clin Oncol* 2013; **31**: 4026 [DOI: 10.1200/JCO.2013.31.15_suppl.4026]

80 **Wu L**, Feng Y, Wu Z, Xu H, Zhang C, Ning J, Wang R, Chen J, Xie M, Zhang Y, Bu L, Hao J, Ma T. Survival outcomes of adjuvant taxanes, platinum plus fluoropyrimidines versus platinum and fluoropyrimidines for gastric cancer patients after D2 gastrectomy: a retrospective propensity score-matched analysis. *World J Surg Oncol* 2021; **19** (1): 272 [PMID: 34507562 DOI: 10.1186/s12957-021-02390-4]

81 **Conroy T**, Galais MP, Raoul JL, Bouché O, Gourgou-Bourgade S, Douillard JY, Etienne PL, Boige V, Martel-Lafay I, Michel P, Llacer-Moscardo C, François E, Créhange G, Abdelghani MB, Juzyna B, Bedenne L, Adenis A; Fédération Francophone de Cancérologie Digestive and UNICANCER-GI Group. Definitive chemoradiotherapy with FOLFOX versus fluorouracil and cisplatin in patients with oesophageal cancer (PRODIGE5/ACCORD17): final results of a randomised, phase 2/3 trial. *Lancet Oncol* 2014; **15**: 305-314 [PMID: 24556041 DOI: 10.1016/S1470-2045(14)70028-2]

82 **Wo JY-L**, Clark JW, Allen JN, Blaszkowsky LS, Keane F, Drapek LC, Ryan DP, Corcoran RB, Roeland E, Parikh AR, Khandekar MJ, Heist RS, Morse C, Yeap BY, Ulysse CA, Christopher B, Lanuti M, Berger DL, Mullen JT, Hong TS. A pilot study of neoadjuvant FOLFIRINOX followed by chemoradiation for gastric and gastroesophageal cancer: Preliminary results. *J Clin Oncol* 2019; **37**: 4057 [DOI: 10.1200/JCO.2019.37.15_suppl.4057]

83 **Slagter AE**, Jansen EPM, van Laarhoven HWM, van Sandick JW, van Grieken NCT, Sikorska K, Cats A, Muller-Timmermans P, Hulshof MCCM, Boot H, Los M, Beerepoot LV, Peters FPJ, Hospers GAP, van Etten B, Hartgrink HH, van Berge Henegouwen MI, Nieuwenhuijzen GAP, van Hillegersberg R, van der Peet DL, Grabsch HI, Verheij M. CRITICS-II: a multicentre randomised phase II trial of neo-adjuvant chemotherapy followed by surgery versus neo-adjuvant chemotherapy and subsequent chemoradiotherapy followed by surgery versus neo-adjuvant chemoradiotherapy followed by surgery in resectable gastric cancer. *BMC Cancer* 2018; **18**: 877 [PMID: 30200910 DOI: 10.1186/s12885-018-4770-2]

84 **Liu X**, Cai H, Sheng W, Huang H, Long Z, Wang Y. microRNAs expression profile related with response to preoperative radiochemotherapy in patients with locally advanced gastric cancer. *BMC Cancer* 2018; **18**: 1048 [PMID: 30373600 DOI: 10.1186/s12885-018-4967-4]

85 **Hsu CH**, Guo JC, Huang TC, Kuo HY, Lin CC, Hsu FM, Cheng JC, Huang PM, Lee JM. Phase II study of pembrolizumab after chemoradiotherapy (CRT) as adjuvant therapy for locally advanced esophageal squamous cell carcinoma (LA-ESCC) patients at high risk of recurrence following preoperative CRT plus surgery. *J Clin Oncol* 2021; **39** [DOI: 10.1200/JCO.2021.39.3_suppl.TPS259]

86 **Lumish MA**, Tarazona N, Janjigian YY. Postoperative ctDNA monitoring: a canary in a coalmine. *Ann Oncol* 2021; **32**: 431-433 [PMID: 33484835 DOI: 10.1016/j.annonc.2021.01.005]

87 **McNamara MJ**, Rice TW, Rybicki LA, Rodriguez CP, Videtic GMM, Saxton JP, Stephans KL, Greskovich J, Sohal D, Mason DP, Murthy SC, Ives DI, Bodmann J, Adelstein DJ. A phase II trial of induction epirubicin, oxaliplatin, and flourouracil, surgery and post-operative concurrent cisplatin and fluorouracil chemoradiotherapy (CRT) in patients (pts) with loco-regionally advanced (LRA) adenocarcinoma (ACA) of the esophagus (E) and gastroesophageal junction (GEJ). *J Clin Oncol* 2013; **31**: 4087 [DOI: 10.1200/JCO.2013.31.15_suppl.4087]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 203-215

DOI: 10.4251/wjgo.v14.i1.203

ISSN 1948-5204 (online)

MINIREVIEWS

Radiofrequency ablation in the management of primary hepatic and biliary tumors

Richard Hendriquez, Tara Keihanian, Jatinder Goyal, Rtika R Abraham, Rajnish Mishra, Mohit Girotra

ORCID number: Richard Hendriquez 0000-0001-6958-9931; Tara Keihanian 0000-0002-1753-985X; Jatinder Goyal 0000-0002-8699-6149; Rtika R Abraham 0000-0002-4608-7899; Rajnish Mishra 0000-0003-1295-1260; Mohit Girotra 0000-0002-7086-7211.

Author contributions: Henriquez R, Keihanian T and Goyal G performed data review, prepared and edited the manuscript; Abraham RR and Mishra R performed edited manuscript; Girotra M conceptualized and prepared, edited the manuscript and provided final approval; all authors wrote, read and approved the final manuscript.

Conflict-of-interest statement: The authors have no disclosures or conflicts of interest.

Country/Territory of origin: United States

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Grade B (Very good): 0

Richard Hendriquez, Department of Internal Medicine, University of Central Florida, Orlando, FL 32816, United States

Tara Keihanian, Department of Gastroenterology and Hepatology, University of Miami, Miami, FL 33136, United States

Jatinder Goyal, Department of Gastroenterology and Hepatology, Wellspan Digestive Health - York Hospital, York, PA 17403, United States

Rtika R Abraham, Department of Endocrinology, Swedish Medical Center, Seattle, WA 98104, United States

Rajnish Mishra, Mohit Girotra, Digestive Health Institute, Section of Gastroenterology and Interventional Endoscopy, Swedish Medical Center, Seattle, WA 98104, United States

Corresponding author: Mohit Girotra, FACG, FACP, MD, Digestive Health Institute, Section of Gastroenterology and Interventional Endoscopy, Swedish Medical Center, 1221 Madison Street, Ste 1220, Seattle, WA 98104, United States. girotra.mohit@gmail.com

Abstract

In the United States, 80%-90% of primary hepatic tumors are hepatocellular carcinomas and 10%-15% are cholangiocarcinomas (CCA), both with high mortality rate, particularly CCA, which portends a worse prognosis. Traditional management with surgery has good outcomes in appropriately selected patients; however, novel ablative treatment options have emerged, such as radiofrequency ablation (RFA), which can improve the prognosis of both hepatic and biliary tumors. RFA is aimed to generate an area of necrosis within the targeted tissue by applying thermal therapy *via* an electrode, with a goal to completely eradicate the tumor while preserving surrounding healthy tissue. Role of RFA in management of hepatic and biliary tumors forms the focus of our current mini-review article.

Key Words: Radiofrequency ablation; Radiofrequency ablation; Hepatic tumor; Biliary tumor; Cholangiocarcinoma; Hepatocellular carcinoma; Cholangiocarcinomas; Hepatocellular carcinomas

Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 28, 2021
Peer-review started: March 28, 2021
First decision: June 7, 2021
Revised: June 15, 2021
Accepted: December 7, 2021
Article in press: December 7, 2021
Published online: January 15, 2022

P-Reviewer: Lang SA
S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

Core Tip: Radiofrequency ablation (RFA) generates an area of necrosis within the targeted tissue by applying thermal therapy *via* an electrode, with a goal to completely eradicate the tumor while preserving surrounding healthy tissue. RFA can maintain biliary drainage by tumor ablation within the biliary ducts or occluded metallic stents, which improves survival and quality of life in unresectable cholangiocarcinomas patients. In hepatocellular carcinoma, RFA is used alone or in combination (with hepatectomy/transcatheter arterial chemoembolization) for ablation of tumors < 2 cm, and improves local tumor progression and recurrence-free survival, and considered by some to be comparative to hepatectomy.

Citation: Hendriquez R, Keihanian T, Goyal J, Abraham RR, Mishra R, Girotra M. Radiofrequency ablation in the management of primary hepatic and biliary tumors. *World J Gastrointest Oncol* 2022; 14(1): 203-215
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/203.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.203

INTRODUCTION

Most primary hepatic tumors are found to be either Hepatocellular Carcinoma (HCC) or Cholangiocarcinoma (CCA). Specifically, within the United States, 80%-90% of these tumors are found to be HCCs, and the remaining 10%-15% being CCAs[1-9]. These hepatic tumors have a high mortality rate, particularly CCA, which portends a worse prognosis[7,10-14]. Traditionally, surgical resection has been shown to have good outcomes in appropriately selected patients. However, with the advent of novel ablative treatment options such as radiofrequency ablation (RFA), the prognosis of both hepatic and biliary tumors can be improved[12,15,16].

Radiofrequency ablation (RFA) is aimed to generate an area of necrosis within the targeted tissue by applying thermal therapy *via* an electrode[17-20], with a goal to eradicate the tumor while preserving surrounding healthy tissue[12,21,22]. Thermal ablation has been used for management of a wide range of lesions, from renal tumors to uterine fibroids. However, more data is emerging in its role as a curative or palliative option in those with primary and secondary hepatobiliary malignancies[11,18-20]. In this mini-review article, we discuss the role of RFA in patients with primary-hepatic and biliary tumors.

RFA TECHNIQUE AND PROCEDURE

RFA utilizes electrodes to provide an alternating current, causing the ions to reverberate rapidly, thereby increasing tissue temperature[8,16,23-26]. This thermal energy induces coagulative necrosis and subsequent death of the malignant cells. RFA can be accomplished through multiple approaches, including surgical, percutaneous, and more recently, endoscopic modality[19,27,28]. Several studies have explored the safety, efficacy and feasibility of RFA for loco-regional control of tumor growth. To facilitate this, a specialized catheter named Endo Luminal RadiofrequencyAblation (ELRA) was developed (STARmed, Goyang, Korea), which is a 7-Fr bipolar catheter with a 1750 mm length, with an automatic temperature probe, allowing the user to avoid excessive heating and collateral damage to surrounding healthy tissue, thus decreasing the rates of procedure-related adverse events[29-32]. Four different exposure lengths are available (11, 18, 22 and 33 mm) to allow RFA of strictures of varying lengths, with recommended power setting of 7-10 W and target temperature of 80 °C for up to 2 min. A similar Habib EndoHPB catheter (Boston Scientific, Marlborough, MA, United States) is an 1800 mm long 8-Fr device with two distal tip electrodes placed 8 mm apart, to achieve biliary RFA. Novel devices have been developed to achieve the same *via* endoscopic ultrasound (EUS) approach. For example, the Habib endoscopic ultrasound (EUS) RFA device is a 1-Fr wire monopolar electrode inserted inside a standard EUS fine-needle aspiration (FNA) needle that can achieve coagulation of specific target tissue[12,21,33,34]. During endoscopic retrograde cholangiography (ERCP), after biliary cannulation a guidewire is passed through the

strictured segment of bile duct, over which the RFA catheter is advanced and electrodes are positioned under fluoroscopic guidance to achieve ablation over bursts of 60 s. For longer strictures, stepwise ablation is performed to cover the entire length, or alternately catheter with varying exposure length can be utilized, if available. This modality can also be used to treat tumor/tissue ingrowth within metallic stents placed for cholangiocarcinoma (Figure 1). For strictures involving the hilum, ablation of both right and left hepatic ducts is performed after placement of two bilateral guidewires. After improvement of stricture, upstream debris removal is performed, followed by cholangiogram to assess for complications including bile leak, prior to stent placement.

RFA IN CCC

CCA represents approximately 2%-3% of malignancies arising from the gastrointestinal (GI) system, but is second most common primary liver tumor[7,34,35]. Specifically, these malignancies arise from the cells that line the biliary tree, and categorized as extra-hepatic or intra-hepatic, depending on their extent of ductal infiltration and location in relation to the cystic duct insertion; as most famously reported using the Bismuth-Corlette system[13,34]. Supplementary classifications of CCAs have been proposed, which in addition to tumor extent within the biliary system also take into account the size of the tumor, vascular (hepatic artery/portal vein) and lymph node involvement, distant metastases, and estimated post-resection hepatic volume [36], which have advantage over Bismuth-Corlette system which does not provide information on vascular encasement or metastatic disease, includes only peri-hilar CCA but not intrahepatic CCA, and does not necessarily determine local resectability, and hence of limited prognostic value. In fact, there is emerging evidence that although resection of type IV CCA is technically demanding with high morbidity, it can be performed with low mortality and offers better survival probability in selected patients[37].

When it comes to the treatment of CCAs, anatomical location and resectability play a crucial role. For those lesions that are considered resectable, surgical resection can be curable. Chemotherapy and radiation are typically utilized for unresectable lesions or can be used as neoadjuvant therapy for resectable tumors. For those tumors that cause obstruction, biliary drainage is usually the mainstay therapy with stent placement[29,32,38]. At present, extra-hepatic CCA is considered the condition most effectively treated with biliary RFA. Performance of RFA for intrahepatic CCA is challenging, and can be achieved *via* ERCP or EUS or percutaneous approach. RFA has also been employed to prolong the patency of stents in malignant obstructive tumors[27,38,39]. Typically the deployment of a self-expandable metallic stent (SEMS) is the mainstay palliative therapy in these patients. By prolonging the patency of stents, it improves survival and quality of life in patients with unresectable CCA.

Multiple studies have appraised the efficacy of RFA in the treatment of CCA and stent patency[33,40,41]. Cui and colleagues evaluated the effect of RFA on stent patency in malignant biliary obstruction, and while there was no significant difference in the overall survival, patency time was significantly increased in the RFA group at 7.6 mo when compared to 4.3 mo in the stent without RFA group. Another retrospective study by Li *et al*[29] determined hat stent patency was prolonged in those patients who underwent RFA plus stenting compared to stenting alone (81% *vs* 35%) with a $P < 0.05$. Furthermore, a meta-analysis by Sofi and colleagues, which included eight observational studies and one randomized controlled trial of RFA in malignant biliary obstruction showed not only a significantly prolonged stent patency in the RFA group when compared to the control group, but also a significant increase in overall survival in the RFA group ($n = 504$; 95%CI: 1.145-1.7; $P < 0.01$)[18]. Yang *et al*[20] performed a randomized control trial on patients with unresectable distal CCA and perihilar CCA; one group received RFA plus stenting ($n = 32$) and the other group received stenting alone ($n = 33$). Compared to stenting alone, the RFA plus stent group had a statistically significant increase in both patency (6.8 mo; 95%CI: 3.6-8.2 *vs* 3.4 mo; 95%CI: 2.4-6.5) and overall survival (13.2 mo *vs* 8.3 mo)[20]. These results are in contrast to previous reports, like by Wu *et al*[32], which has shown efficacy of RFA for stent patency, but no survival benefit. A detailed summary is provided in Table 1. Few studies have also compared Photodynamic therapy (PDT) with RFA, mostly without any statistically significant difference in overall survival between the two treatment approaches[42]. However, one of the retrospective studies did show that RFA conferred a short-term advantage in decline in bilirubin[43,44].

Table 1 Utilization of radiofrequency ablation for cholangiocarcinoma

Technique	Ref.	Number of Patients	Location	Stent type	Mean number of sessions	Patency of stent (d, median)	Stent occlusion	Survival	Adverse events
	Mizandari *et al*[78], 2013	39	CCA (17); Bismuth I (5); II (1); IIIa (4); IV (7)-Panc CA (11), GB CA (4), HCC (1), Ampullary CA (1), Metastatic CA (5)	SEMS (all)	1	84.5	1	3 mo (median)	Abdominal pain (15)
	Wu *et al* [32], 2017	71[RFA and stenting = 35, stenting alone = 36]	Extra-hepatic distal CCA	Covered SEMS (7); uncovered SEMS (28)	1	Uncovered SEMS (241); covered SEMS (212)	-	Uncovered SEMS (245 d, median); covered SEMS (278 d, median)	Abdominal pain (27)
Percutaneous	Li *et al* [29], 2015	26[RFA and stenting = 12, stenting alone = 14]	Hilar (2), middle and distal CBD(7), Panc CA (2), ampullary CA (1)	SEMS (all)	1	RFA group (0), control group (3)	RFA group 100%; control group 85% at 90 d	-	Cholangitis (3)
	Wu *et al* [31], 2015	47	Hilar (7), distal CBD (16);ampullary CA (8); Panc CA (6); GB CA (4); HCC(2); Metastatic disease(4)	SEMS	1.38	149	11	6 mo	Abdominal pain (21), intra-abdominal hemorrhage (1)
	Wang *et al* [28], 2016	9	Bismuth IIIa (1); IIIb (1); IV (7)	SEMS	1 (only 1 patient had 2 sessions)	100	-	5.3 mo	Abdominal pain (3); Cholangitis (4)
	Wang *et al* [39], 2016	12	Bismuth I (5); IIIa (1); IV (3); Gastric CA (1); HCC(1); Congenital Choledochal cyst (1)	Plastic (7); SEMs (4)	1	125	-	7.7 mo (median)	Fever (2), pancreatitis (1)
	Laquière *et al*[81], 2016	12	Bismuth I (4); II (3); III (2); IV (3)	Plastic and Metallic (does not quantify)	1.63	-	4	12.3 mo	Sepsis (1), early stent migration (1), late stent migration(1), cholangitis (1)
Endoscopic	Sharaiha *et al*[86], 2015	69	Hilar (23); proximal CBD (7); distal CBD (7); Bismuth I (4); Bismuth III (2); Bismuth IV (5); Panc CA (19); GB CA (2); Gastric CA (1), Metastasis disease (3)	Metallic (49); Plastic (20)	1.3	95% at 30 d	3	17.7 ± 15.4 mo	Pancreatitis (1); Cholecystitis(2); Haemobilia (1); abdominal pain (3)
	Strand *et al*[87], 2014	16	Intrahepatic/proximal (1); Hilar (13); Extrahepatic/distal (2)	Plastic (3); fully covered SEMS (3); uncovered SEMS (11)	1.19	-	0.06	9.6 mo	Stent migration (0.02); cholangitis (0.13); hepatic abscess (0.02); need for percutaneous drainage (0.01); severe abdominal pain (0.02) (occurrence per month)
	Sharaiha *et al*[30], 2014	64	CCA (18); Panc CA (8)	Covered SEMS (8); uncovered SEMS (7); Plastic (11)	1	100% at 90 d	0	5.9 mo	Abdominal pain(3); Pancreatitis (1); Cholecystitis (1)
	Alis *et al* [88], 2013	10	Bismuth I (4); Distal CBD (6)	SEMS (all)	1	270	0	-	Pancreatitis (2)
	Figueroa	20	CCA (11); Panc CA (7);	Plastic (6);	1.25	100% at 30	0	-	Abdominal pain

	Barojas *et al*[49], 2013		Gastric Ca (1), IPMN with high grade dysplasia (1)	covered SEMS (13); uncovered SEMS (1)		d			(5); Pancreatitis (1); Cholecystitis (1)
	Steel *et al* [19], 2011	21	CCA (6); Panc CA (16)	Uncovered SEMS (all)	2	114 (median stent patency at 9- d)	4	-	Pancreatitis (1); cholecystitis (2), obstructive jaundice/death (1)
Percutaneous and endoscopic	Dolak *et al* [27], 2014	58	Bismuth I (5); II (1); III (6); IV (33); distal CBD (5);Panc CA (4), central HCC,mCRC(3)	Plastic (19); SEMS (35); no stent (4)	1.44	170 (Metallic stent = 218, Plastic stent = 115)	-	10.9 mo (median)	Cholangitis (5); hemobilia (2); sepsis (2); hepatic coma (1); hepatic infarction (1)

CBD: Common bile duct; CCA: Cholangiocarcinoma; GA Ca: Gallbladder cancer; Panc CA: Pancreatic cancer; mCRC: Metastatic colorectal cancer; SEMS: Self-expanding metallic stent.

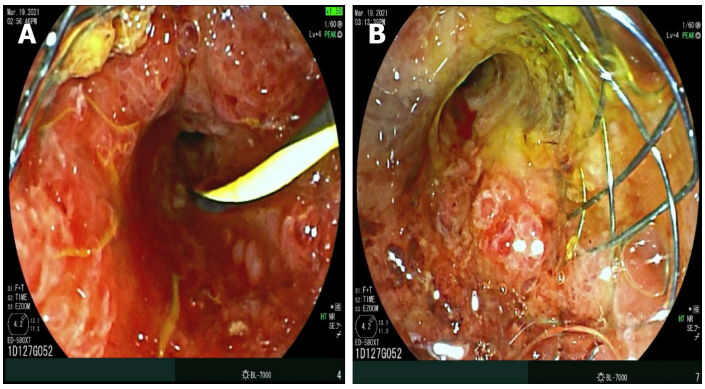


Figure 1 Cholangiocarcinoma stricture and radiofrequency ablation. A: Tumor ingrowth into uncovered metallic stent (placed for distal cholangiocarcinoma), allowing passage of guidewire but no other equipment; B: Treated with Habib radiofrequency ablation probe, to achieve patency of stent, which allowed successful biliary drainage.

To summarize, RFA is a successful strategy for loco-regional management of extra-hepatic CCA, management of malignant biliary obstruction, as well as blocked metallic stents. The performance of RFA is operator dependent, and not protocol based, and hence timing and interval of RFA remains unclear, as well as choice of stents (plastic *vs* metallic). For intra-hepatic CCA, the access using ERCP-RFA catheters can be challenging, and alternative approaches may include EUS-RFA or percutaneous RFA by Interventional Radiology. Alternatives to RFA include Photodynamic Therapy (PDT), Microwave Ablation (MWA) and Irreversible Electroporation Ablation (IRE), all of which are complex and expensive procedures, which require highly specialized equipment, have side effects (photosensitivity with PDT) and complication profile, and hence not commonly performed worldwide. IRE is a non-thermal ablation modality, the basic principle of which is to create irreversible pores in cellular bi-lipid membranes by subjecting them to series of high intensity electrical pulses for short duration of time, resulting in cell death due to apoptosis, especially used for tumors located close to porta-hepatis. On the other hand, in MWA, tumor tissue is destroyed by direct hyperthermic injury produced by electromagnetic waves emitted from non-insulated portions of antenna, resulting in larger volume of active heating resulting in shorter procedure times, higher tissue temperatures beyond the threshold of water vaporization and less susceptibility to the heat sink effect of blood flow. Detailed discussion regarding these modalities is beyond the scope of this mini-review manuscript.

RFA IN HCC

HCC is the most common primary liver cancer and has the third-highest cancer-related mortality worldwide, exceeding 700000 deaths per year[2,3,5,6,45-47]. There

are different causes of HCC, which vary worldwide; in Africa, aflatoxin B1 and chronic Hepatitis B infection seem to account for the most incidence of HCC, whereas cases in North America, Japan, and Europe are related to alcoholism and Hepatitis C infection [2,3,5,6,45-47]. Currently, the curative management options for HCC include liver transplantation, hepatectomy, or ablative therapies. Most patients diagnosed with HCC are not surgical candidates due to the advanced tumor size, invasion, or presence of metastasis[1-4,48]. Most management algorithms worldwide employ a specific scoring system named Barcelona Clinic Liver Cancer guidelines[1-6,48-50], to aids clinicians in determining the most appropriate management modality. Patients with early-stage HCC without any vascular invasion are classified as BCLC-A, which are suitable candidates for resection, ablation, or transplantation. On the other end of the treatment spectrum, patients with extra-hepatic tumor spread or vascular invasion are classified as BCLC-C, and are best managed with systemic therapies such as Sorafenib [1-6,48-50].

With the introduction of the Milan criteria, an increase in liver transplantation has been witnessed, ushering in a new era of curative treatment for HCC[51,52]. However, transplantation is dependent on donor availability, and since there are a limited number of donors, only a finite number of patients can undergo successful treatment. More importantly, patients may spend long periods of time awaiting transplant, allowing cancer to progress, which may disqualify formerly eligible patients from transplantation. To avoid this clinical dilemma, ablative techniques such as RFA become important for the crucial role they can play in delaying the malignancy progression[24,53-56]. A distinct advantage of these ablation techniques is that they can be performed safely on suboptimal surgical candidates.

RFA is the most widely used thermal ablative procedure used in patients with HCC [49,54,57,58], the success of which is inversely related to tumor size. Complete remission is achieved in approximately 90%-92% in those with tumor size < 2 cm whereas remission rates decrease to 20%-40% in those ≥ 2 cm in size[59]. While theoretically, multipolar electrodes may expand the ablation zone of RFA, this has not panned out in clinical studies. Cartier *et al*[60] compared traditional monopolar electrode RFA with multipolar electrodes in patients with tumors > 2.5 cm, and found no difference in residual tumor or recurrence. RFA seems to be a safe treatment option with procedure-related mortality of approximately 0.2% and an overall complication rate of 2.2%[61,62]. A novel RFA technique being studied is the "no-touch RFA protocol," which involves inserting multiple electrodes within the tissue that surrounds the tumor[62], which avoids direct contact with the tumor, allowing thermal ablation to be conducted with decreased risk of tumor seeding by the probe.

Several studies have investigated the effectiveness of RFA in HCC (Table 2). Liao *et al*[63] randomized 96 patients into those undergoing wide margin (WM ≥ 10 mm) ablation (n = 48) and normal margin (NM: ≥ 5 but < 10 mm) ablation (n = 48), and followed for mean period of 38.3 ± 4.8 mos. When analyzed based on intention-to-treat strategy, the 3-year incidences of local tumor progression (LTP) (14.9% *vs* 30.2%), intrahepatic recurrence (IHR) (15.0% *vs* 32.7%), and recurrence-free survival (RFS) (31.7 ± 12.1 *vs* 24.0 ± 11.7 mo) for WM group were significantly improved compared to NM group[63]. Getting recurrence-free survival advantage with RFA is a major success, for which RFA is adopted widely worldwide for smaller HCC, especially in non-resectable candidates. In regards to the "no-touch RFA protocol," a multicenter retrospective study of HCC < 5 cm in diameter (n = 362) showed effectiveness of this approach over monopolar RFA in terms of recurrence rates[62,63], but no statistical difference in 5-year survival rates (monopolar 37.2% *vs* no-touch multipolar 46.4% P = 0.378). Some investigators have proposed that stereotactic body radiotherapy (SBRT) was more effective than RFA, which has been challenged in recent studies[64,65]. In 2018, Rajyaguru *et al*[64] compared the effectiveness of RFA (n = 3684) against SBRT (n = 296), and their analysis support superior survival with RFA for non-surgically managed patients with stage I or II HCC. Various studies have investigated predictive factors to achieve improved outcomes in HCC when utilizing RFA. In a recent meta-analysis by Giardini *et al*[61,65-68] (34 studies; n = 11,216), alpha-fetoprotein (AFP) < 20 ng/mL, Child-Pugh class A and albumin-bilirubin index of 1 were noted to confer increased survival benefit. In addition, survival also increased in patients with single tumor < 2 cm in diameter and preserved hepatic function[61,69-71].

Several studies have also explored the comparative success rates of RFA *vs* hepatectomy in HCC. A meta-analysis by Xu *et al*[72] indicated that RFA and surgical hepatectomy had similar overall survival at 1 year (relative risk [RR], 1.39; 95% confidence interval [CI]: 0.36, 5.33; P = 0.63) and 3 years (RR, 1.40; 95%CI: 0.75, 2.62; P = 0.29), whereas RFA resulted in decreased overall survival compared with HR at 5 years (RR: 1.91; 95%CI: 1.32, 2.79; P = 0.001)[72]. However, closer analysis of subgroup

Table 2 Utilization of Radiofrequency ablation for hepatocellular carcinoma

Ref.	Type	N	Technique	Survival	Recurrence	Adverse Events	Outcome
Zhang *et al* [89], 2013	Retrospective	155	RFA (78- 93 sessions) and MWA (77-91 sessions)	1-, 3-, and 5-year overall survival rates: RFA: 91.0%, 64.1% and 41.3%; MWA: 92.2%, 51.7%, and 38.5%	RFA: 11/93 (11.8%) and MWA: 11/105 (10.5%)	RFA group: persistent jaundice (n = 1) and biliary fistula (n = 1). MWA group: hemothorax and intrahepatic hematoma (n = 1) and peritoneal hemorrhage (n = 1)	No significant differences LTP, DR, and overall survival
Karla *et al* [90], 2017	Prospective	50	RFA alone (25) and RFA + alcohol ablation (25)	RFA alone 84%; RFA + alcohol (80%) (at 6 month)	Local recurrence (11); Distant intrahepatic tumor recurrence (4)	Hemoperitoneum (1)	Combined use of RFA and alcohol did not improve the local tumor control and survival
Abdelaziz *et al* [91], 2017	Retrospective	67	TACE-RFA (22) and TACE-MWA (45)	Survival at 1, 2 and 3 years: TACE-MWA: 83.3%, 64.7%, 64.7%; TACE-RFA: 73.1%, 40.6% and 16.2% (P = 0.08)	TACE-RFA: 4 (18.2%); TACE-MWA: 8 (17.8%)	TACE-RFA: bone metastases 1 (4.5%), Ascites 3 (13.6%), variceal bleeding 5 (22.7%); TACE-MWA: portal vein thrombosis: 1 (2.2%), ascites 6 (13.3%), variceal bleeding: 4 (8.9%)	No significant difference in overall survival was observed
Gyori *et al* [92], 2017	Retrospective	150	54% (n = 81) received TACE-based LRT, 26% (n = 39) PEI/RFA regimen, and 17% (n = 26) had no treatment while on the waiting list	No difference in overall survival after liver transplantation when comparing TACE- and RFA-based regimens.			TACE- and RFA-based regimens showed equal outcomes in terms of transplantation rate, tumor response, and post-transplant survival. Lower survival in recipients of Multimodality LRT.
Hao *et al*[93], 2017	Retrospective	237	50 pathologically early HCCs, 187 typical HCCs		LTP observed in 1 Early HCC (2%); 46 Typical HCC (24.6%)	Fever, abdominal pain and elevated liver enzyme levels.	Rate of LTP for early HCCs after RFA was significantly lower than typical HCCs.
Liao *et al*[63], 2017	Prospective randomized	96	48 patients wide margin (WM) ablation (≥ 10 mm) and 48 normal margin (NM) ablation (≥ 5 mm but < 10 mm)	The 1-, 2-, and 3-year survival rates: WM: 95.8%, 91.6%, and 74.6%; NM: 95.8%, 78.4%, and 60.2%	3-year LTP: WM: 14.9%; NM: 30.2% Intrahepatic recurrence (IHR): WM: 15.0% NM: 32.7%	Perihepatic bile collection (1); intrahepatic hemorrhage(1); fever(1); liver infarction (1); thermal skin injury (1); pleural effusion (1)	WM-RFA may reduce the incidence of tumor recurrence among cirrhotic patients with small HCCs
Rajyaguru *et al*[64], 2018	Observational	3980	RFA (3,684) and SBRT (296)	5 yr overall survival: RFA: 29.8% (95%CI: 24.5-35.3%); SBRT: 19.3% (95%CI: 13.5-25.9%)			Treatment with RFA yields superior survival compared with SBRT for nonsurgically managed patients with stage I or II HCC
Parick *et al* [65], 2018	Retrospective cohort	440	RFA (408) and SBRT (32)	RFA patients had better overall survival (P < 0.001)			SBRT (HR 1.80; 95%CI: 1.15-2.82) associated with worse survival
Santambrogio *et al*[94], 2018	Prospective controlled	264	Laparoscopic hepatic resection (LHR = 59) *vs* laparoscopic ablation therapy (LAT = 205)	Survival rates LHR at 1, 3, and 5 years were 93, 82, and 56%. In LAT = 91%, 62%, and 40%	LHR = 24/59 (41%); LAT = 135/205 (66%)		LAT found to be adequate alternative

OLT: Orthotopic liver transplantation; LRT: Locoregional treatment; LTP: Local Tumor Progression; TACE: Transarterial chemoembolization; PEI: Percutaneous ethanol injection; SBRT: Stereotactic body radiotherapy; MWA: Microwave ablation; DR: Distant recurrence.

data, results showed no difference in survival between the groups in tumors less than 2.0 cm in size[72]. The Surveillance, Epidemiology and End Results (SEER) database explored the same question further stratified by age[65], and noted that patients older than 65 years with tumors less than 2 cm had similar survival to their propensity-matched group age less than 65 years. Interestingly, those < 65 years and tumors >3.0 cm had an increased overall survival with hepatectomy compared to RFA. However,

large-scale studies have not been able to incorporate the novel RFA techniques previously discussed compared to hepatectomy[59,73,74]. Further studies will need to be conducted to answer this question.

Several studies have explored the role of combination therapy with RFA. In a recent meta-analysis, the pooled results showed that the 1-, 3-, 5-year overall survival rate in the combined RFA+hepatectomy group were comparable with those in the hepatectomy alone group (OR = 0.77, 0.96, 0.88; P = 0.33, 0.88, 0.70, respectively). Similarly, there was no significant difference in 1-, 3-, 5-year disease free survival rate between the combined group and the surgical alone group (OR = 0.57, 0.83, 0.72; P = 0.17, 0.37, 0.32, respectively). These results indicated that the hepatectomy combined with RFA could reach a long-term survival outcome similar to curative surgical resection for multifocal HCC patients, and this approach may be a promising alternative for patients with marginal liver function or complicated tumor distribution [75]. But, RFA+hepatectomy is limited due to its increased rate of post-op complications such as liver failure and death. Transcatheter arterial chemoembolization (TACE) is another commonly used percutaneous non-ablative treatment for HCC[76], which when combined with RFA yields a feasible treatment strategy with promising outcomes. In study by Kim *et al*[77], 1 mo, 6 mo, and 1-year tumor responses of TACE-RFA were similar to those of RFA and better than those of TACE. A distinct advantage of this combination therapy may be in patients with tumors located close to major vessels, wherein TACE occludes the hepatic artery flow, allowing a larger area for RFA ablation. This strategy minimizes the "heat-sink" effect associated with RFA. Regardless, the TACE-RFA group showed longer hospital stay and more frequent patient discomfort requiring medication than TACE or RFA monotherapy groups (P < 0.001), as well as the frequency of overall complications after TACE-RFA was higher than TACE (P = 0.006) or RFA (P = 0.009)[52,74,76-78]. Finally, RFA is also being utilized in combination with Sorafenib for management of HCC. A recent meta-analysis (15 studies, 2227 patients) showed that compared to RFA-alone, the patients in RFA+Sorafenib had longer 1-, 2- and 3-year overall survival (P < 0.05), better overall efficacy (P < 0.0001), longer RFA interval (P < 0.001) and lower 2-year recurrence rate (P = 0.02). However, this came at the cost of higher adverse reactions compared to RFA-alone group, including hand-foot skin reactions (P < 0.001), diarrhea and constipation (P < 0.0001), hypertension (P = 0.009) and alopecia (P < 0.001)[79]. Therefore, cognizance of overall adverse events is necessary while choosing the most optimal strategy. Despite these limitations, overall improvements in technology under development show promising prospects in the treatment of HCC.

ADVERSE EVENTS AND LIMITATIONS OF RFA

Several adverse events have been associated with RFA, the most common being post-procedure mild abdominal pain following either endoscopic or percutaneous RFA approaches. There seems to be a higher incidence of bleeding with percutaneous RFA, whereas a higher association of pancreatitis with the endoscopic approach[59,80]. Other post-procedure complications, such as hemobilia and hepatic artery pseudoaneurysms, have been postulated to be due to thermal injury[38,81]. This can be avoided with the newer ELRA RF catheter, which has a temperature probe. Further complications have been listed in Tables 1 and 2.

RFA does have its limitations. The therapeutic efficacy of RFA is inversely associated with tumor size and location[59]. RFA needs direct contact with the tissue, which can pose a challenge to treat tumors in inaccessible sites. Furthermore, tumors in close proximity to large vessels pose interesting therapeutic challenges[10,18,22,41,82,83]. Tumors located near large portal and hepatic vein branches can result in a "heat-sink" effect, which results in the inability to reach maximal ablation temperatures, thereby causing incomplete cell death[84]. It is important to keep in mind that RFA cannot be used in pregnancy or patients with cardiac devices or coagulopathy[24,73,82,83,85].

CONCLUSION

RFA has been established as novel and safe minimally invasive management tool for HCC. While multiple studies optimizing these techniques have shown promising results in patients with CCA, the low incidence of these biliary tumors makes it challenging to coordinate high-powered RCTs comparing various techniques and

treatment strategies. It is paramount that future studies are coordinated through collaboration between various institutions of excellence for the progress of this still novel technique.

REFERENCES

1 **Avolio AW**, Cillo U, Salizzoni M, De Carlis L, Colledan M, Gerunda GE, Mazzaferro V, Tisone G, Romagnoli R, Caccamo L, Rossi M, Vitale A, Cucchetti A, Lupo L, Gruttadauria S, Nicolotti N, Burra P, Gasbarrini A, Agnes S; Donor-to-Recipient Italian Liver Transplant (D2R-ILTx) Study Group. Balancing donor and recipient risk factors in liver transplantation: the value of D-MELD with particular reference to HCV recipients. *Am J Transplant* 2011; **11**: 2724-2736 [PMID: 21920017 DOI: 10.1111/j.1600-6143.2011.03732.x]

2 **Bosetti C**, Levi F, Boffetta P, Lucchini F, Negri E, La Vecchia C. Trends in mortality from hepatocellular carcinoma in Europe, 1980-2004. *Hepatology* 2008; **48**: 137-145 [PMID: 18537177 DOI: 10.1002/hep.22312]

3 **Bruix J**, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]

4 **Bruix J**, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

5 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

6 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/s0140-6736(11)61347-0]

7 **Green BL**, House MG. Nonsurgical Approaches to Treat Biliary Tract and Liver Tumors. *Surg Oncol Clin N Am* 2019; **28**: 573-586 [PMID: 31472906 DOI: 10.1016/j.soc.2019.06.013]

8 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226 DOI: 10.1148/radiol.2343040350]

9 **Loriaud A**, Denys A, Seror O, Vietti Violi N, Digklia A, Duran R, Trillaud H, Hocquelet A. Hepatocellular carcinoma abutting large vessels: comparison of four percutaneous ablation systems. *Int J Hyperthermia* 2018; **34**: 1171-1178 [PMID: 29457510 DOI: 10.1080/02656736.2018.1440017]

10 **Buerlein RCD**, Wang AY. Endoscopic Retrograde Cholangiopancreatography-Guided Ablation for Cholangiocarcinoma. *Gastrointest Endosc Clin N Am* 2019; **29**: 351-367 [PMID: 30846158 DOI: 10.1016/j.giec.2018.11.006]

11 **Chahal P**, Baron TH. Endoscopic palliation of cholangiocarcinoma. *Curr Opin Gastroenterol* 2006; **22**: 551-560 [PMID: 16891889 DOI: 10.1097/01.mog.0000239872.12081.a4]

12 **Labib PL**, Davidson BR, Sharma RA, Pereira SP. Locoregional therapies in cholangiocarcinoma. *Hepat Oncol* 2017; **4**: 99-109 [PMID: 29367874 DOI: 10.2217/hep-2017-0014]

13 **Qureshi K**, Jesudoss R, Al-Osaimi AM. The treatment of cholangiocarcinoma: a hepatologist's perspective. *Curr Gastroenterol Rep* 2014; **16**: 412 [PMID: 25183579 DOI: 10.1007/s11894-014-0412-2]

14 **Razumilava N**, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 13-21.e1; quiz e3 [PMID: 22982100 DOI: 10.1016/j.cgh.2012.09.009]

15 **Nault JC**, Sutter O, Nahon P, Ganne-Carrié N, Séror O. Percutaneous treatment of hepatocellular carcinoma: State of the art and innovations. *J Hepatol* 2018; **68**: 783-797 [PMID: 29031662 DOI: 10.1016/j.jhep.2017.10.004]

16 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-77; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]

17 **Lee EW**, Chen C, Prieto VE, Dry SM, Loh CT, Kee ST. Advanced hepatic ablation technique for creating complete cell death: irreversible electroporation. *Radiology* 2010; **255**: 426-433 [PMID: 20413755 DOI: 10.1148/radiol.10090337]

18 **Sofi AA**, Khan MA, Das A, Sachdev M, Khuder S, Nawras A, Lee W. Radiofrequency ablation combined with biliary stent placement *vs* stent placement alone for malignant biliary strictures: a systematic review and meta-analysis. *Gastrointest Endosc* 2018; **87**: 944-951.e1 [PMID: 29108980 DOI: 10.1016/j.gie.2017.10.029]

19 **Steel AW**, Postgate AJ, Khorsandi S, Nicholls J, Jiao L, Vlavianos P, Habib N, Westaby D. Endoscopically applied radiofrequency ablation appears to be safe in the treatment of malignant biliary obstruction. *Gastrointest Endosc* 2011; **73**: 149-153 [PMID: 21184881 DOI: 10.1016/j.gie.2010.09.031]

20 **Yang J**, Wang J, Zhou H, Zhou Y, Wang Y, Jin H, Lou Q, Zhang X. Efficacy and safety of endoscopic radiofrequency ablation for unresectable extrahepatic cholangiocarcinoma: a randomized

trial. *Endoscopy* 2018; **50**: 751-760 [PMID: 29342492 DOI: 10.1055/s-0043-124870]
21 **Larghi A**, Rimbaș M, Tringali A, Boškoski I, Rizzatti G, Costamagna G. Endoscopic radiofrequency biliary ablation treatment: A comprehensive review. *Dig Endosc* 2019; **31**: 245-255 [PMID: 30444547 DOI: 10.1111/den.13298]
22 **Lee DH**, Lee JM. Recent Advances in the Image-Guided Tumor Ablation of Liver Malignancies: Radiofrequency Ablation with Multiple Electrodes, Real-Time Multimodality Fusion Imaging, and New Energy Sources. *Korean J Radiol* 2018; **19**: 545-559 [PMID: 29962861 DOI: 10.3348/kjr.2018.19.4.545]
23 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]
24 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]
25 **Tateishi R**, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201-1209 [PMID: 15690326 DOI: 10.1002/cncr.20892]
26 **Yan K**, Chen MH, Yang W, Wang YB, Gao W, Hao CY, Xing BC, Huang XF. Radiofrequency ablation of hepatocellular carcinoma: long-term outcome and prognostic factors. *Eur J Radiol* 2008; **67**: 336-347 [PMID: 17765421 DOI: 10.1016/j.ejrad.2007.07.007]
27 **Dolak W**, Schreiber F, Schwaighofer H, Gschwantler M, Plieschnegger W, Ziachehabi A, Mayer A, Kramer L, Kopecky A, Schrutka-Kölbl C, Wolkersdörfer G, Madl C, Berr F, Trauner M, Püspök A; Austrian Biliary RFA Study Group. Endoscopic radiofrequency ablation for malignant biliary obstruction: a nationwide retrospective study of 84 consecutive applications. *Surg Endosc* 2014; **28**: 854-860 [PMID: 24196547 DOI: 10.1007/s00464-013-3232-9]
28 **Wang F**, Li Q, Zhang X, Jiang G, Ge X, Yu H, Nie J, Ji G, Miao L. Endoscopic radiofrequency ablation for malignant biliary strictures. *Exp Ther Med* 2016; **11**: 2484-2488 [PMID: 27284336 DOI: 10.3892/etm.2016.3235]
29 **Li TF**, Huang GH, Li Z, Hao CF, Ren JZ, Duan XH, Zhang K, Chen C, Han XW, Jiao DC, Zhang MF, Wang YL. Percutaneous transhepatic cholangiography and intraductal radiofrequency ablation combined with biliary stent placement for malignant biliary obstruction. *J Vasc Interv Radiol* 2015; **26**: 715-721 [PMID: 25817458 DOI: 10.1016/j.jvir.2015.01.037]
30 **Sharaiha RZ**, Natov N, Glockenberg KS, Widmer J, Gaidhane M, Kahaleh M. Comparison of metal stenting with radiofrequency ablation *vs* stenting alone for treating malignant biliary strictures: is there an added benefit? *Dig Dis Sci* 2014; **59**: 3099-3102 [PMID: 25033929 DOI: 10.1007/s10620-014-3264-6]
31 **Wu TT**, Li HC, Li WM, Ao GK, Lin H, Zheng F, Song JY. Percutaneous Intraluminal Radiofrequency Ablation for Malignant Extrahepatic Biliary Obstruction: A Safe and Feasible Method. *Dig Dis Sci* 2015; **60**: 2158-2163 [PMID: 25648642 DOI: 10.1007/s10620-015-3547-6]
32 **Wu TT**, Li WM, Li HC, Ao GK, Zheng F, Lin H. Percutaneous Intraductal Radiofrequency Ablation for Extrahepatic Distal Cholangiocarcinoma: A Method for Prolonging Stent Patency and Achieving Better Functional Status and Quality of Life. *Cardiovasc Intervent Radiol* 2017; **40**: 260-269 [PMID: 27743089 DOI: 10.1007/s00270-016-1483-2]
33 **Cui W**, Fan W, Lu M, Zhang Y, Yao W, Li J, Wang Y. The safety and efficacy of percutaneous intraductal radiofrequency ablation in unresectable malignant biliary obstruction: A single-institution experience. *BMC Cancer* 2017; **17**: 288 [PMID: 28438130 DOI: 10.1186/s12885-017-3278-5]
34 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125 [PMID: 15192785 DOI: 10.1055/s-2004-828889]
35 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
36 **Deoliveira ML**, Schulick RD, Nimura Y, Rosen C, Gores G, Neuhaus P, Clavien PA. New staging system and a registry for perihilar cholangiocarcinoma. *Hepatology* 2011; **53**: 1363-1371 [PMID: 21480336 DOI: 10.1002/hep.24227]
37 **Ebata T**, Mizuno T, Yokoyama Y, Igami T, Sugawara G, Nagino M. Surgical resection for Bismuth type IV perihilar cholangiocarcinoma. *Br J Surg* 2018; **105**: 829-838 [PMID: 28488733 DOI: 10.1002/bjs.10556]
38 **Tal AO**, Vermehren J, Friedrich-Rust M, Bojunga J, Sarrazin C, Zeuzem S, Trojan J, Albert JG. Intraductal endoscopic radiofrequency ablation for the treatment of hilar non-resectable malignant bile duct obstruction. *World J Gastrointest Endosc* 2014; **6**: 13-19 [PMID: 24527176 DOI: 10.4253/wjge.v6.i1.13]
39 **Wang Y**, Cui W, Fan W, Zhang Y, Yao W, Huang K, Li J. Percutaneous intraductal radiofrequency ablation in the management of unresectable Bismuth types III and IV hilar cholangiocarcinoma. *Oncotarget* 2016; **7**: 53911-53920 [PMID: 27322076 DOI: 10.18632/oncotarget.10116]
40 **Cui W**, Wang Y, Fan W, Lu M, Zhang Y, Yao W, Li J. Comparison of intraluminal radiofrequency ablation and stents *vs* stents alone in the management of malignant biliary obstruction. *Int J Hyperthermia* 2017; **33**: 853-861 [PMID: 28540797 DOI: 10.1080/02656736.2017.1309580]

41 **Lee YN**, Jeong S, Choi HJ, Cho JH, Cheon YK, Park SW, Kim YS, Lee DH, Moon JH. The safety of newly developed automatic temperature-controlled endobiliary radiofrequency ablation system for malignant biliary strictures: A prospective multicenter study. *J Gastroenterol Hepatol* 2019; **34**: 1454-1459 [PMID: 30861593 DOI: 10.1111/jgh.14657]

42 **Yang J**, Shen H, Jin H, Lou Q, Zhang X. Treatment of unresectable extrahepatic cholangiocarcinoma using hematoporphyrin photodynamic therapy: A prospective study. *Photodiagnosis Photodyn Ther* 2016; **16**: 110-118 [PMID: 27720942 DOI: 10.1016/j.pdpdt.2016.10.001]

43 **Patel J**, Rizk N, Kahaleh M. Role of photodynamic therapy and intraductal radiofrequency ablation in cholangiocarcinoma. *Best Pract Res Clin Gastroenterol* 2015; **29**: 309-318 [PMID: 25966430 DOI: 10.1016/j.bpg.2015.02.008]

44 **Schmidt A**, Bloechinger M, Weber A, Siveke J, von Delius S, Prinz C, Schmitt W, Schmid RM, Neu B. Short-term effects and adverse events of endoscopically applied radiofrequency ablation appear to be comparable with photodynamic therapy in hilar cholangiocarcinoma. *United European Gastroenterol J* 2016; **4**: 570-579 [PMID: 27536367 DOI: 10.1177/2050640615621235]

45 **Ikenaga N**, Chijiiwa K, Otani K, Ohuchida J, Uchiyama S, Kondo K. Clinicopathologic characteristics of hepatocellular carcinoma with bile duct invasion. *J Gastrointest Surg* 2009; **13**: 492-497 [PMID: 19011945 DOI: 10.1007/s11605-008-0751-0]

46 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/nejm199603143341104]

47 **Murray KF**, Carithers RL Jr; AASLD. AASLD practice guidelines: Evaluation of the patient for liver transplantation. *Hepatology* 2005; **41**: 1407-1432 [PMID: 15880505 DOI: 10.1002/hep.20704]

48 **Arii S**, Teramoto K, Kawamura T, Okamoto H, Kaido T, Mori A, Imamura M. Characteristics of recurrent hepatocellular carcinoma in Japan and our surgical experience. *J Hepatobiliary Pancreat Surg* 2001; **8**: 397-403 [PMID: 11702247 DOI: 10.1007/s005340100000]

49 **Figueroa-Barojas P**, Bakhru MR, Habib NA, Ellen K, Millman J, Jamal-Kabani A, Gaidhane M, Kahaleh M. Safety and efficacy of radiofrequency ablation in the management of unresectable bile duct and pancreatic cancer: a novel palliation technique. *J Oncol* 2013; **2013**: 910897 [PMID: 23690775 DOI: 10.1155/2013/910897]

50 **Freeman RB**, Edwards EB, Harper AM. Waiting list removal rates among patients with chronic and malignant liver diseases. *Am J Transplant* 2006; **6**: 1416-1421 [PMID: 16686765 DOI: 10.1111/j.1600-6143.2006.01321.x]

51 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

52 **Liu W**, Zheng Y, He W, Zou R, Qiu J, Shen J, Yang Z, Zhang Y, Wang C, Wang Y, Zuo D, Li B, Yuan Y. Microwave *vs* radiofrequency ablation for hepatocellular carcinoma within the Milan criteria: a propensity score analysis. *Aliment Pharmacol Ther* 2018; **48**: 671-681 [PMID: 30063081 DOI: 10.1111/apt.14929]

53 **DuBay DA**, Sandroussi C, Kachura JR, Ho CS, Beecroft JR, Vollmer CM, Ghanekar A, Guba M, Cattral MS, McGilvray ID, Grant DR, Greig PD. Radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *HPB (Oxford)* 2011; **13**: 24-32 [PMID: 21159100 DOI: 10.1111/j.1477-2574.2010.00228.x]

54 **Fisher RA**, Maluf D, Cotterell AH, Stravitz T, Wolfe L, Luketic V, Sterling R, Shiffman M, Posner M. Non-resective ablation therapy for hepatocellular carcinoma: effectiveness measured by intention-to-treat and dropout from liver transplant waiting list. *Clin Transplant* 2004; **18**: 502-512 [PMID: 15344951 DOI: 10.1111/j.1399-0012.2004.00196.x]

55 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137 [PMID: 15841454 DOI: 10.1002/hep.20688]

56 **Toso C**, Dupuis-Lozeron E, Majno P, Berney T, Kneteman NM, Perneger T, Morel P, Mentha G, Combescure C. A model for dropout assessment of candidates with or without hepatocellular carcinoma on a common liver transplant waiting list. *Hepatology* 2012; **56**: 149-156 [PMID: 22271250 DOI: 10.1002/hep.25603]

57 **Fontana RJ**, Hamidullah H, Nghiem H, Greenson JK, Hussain H, Marrero J, Rudich S, McClure LA, Arenas J. Percutaneous radiofrequency thermal ablation of hepatocellular carcinoma: a safe and effective bridge to liver transplantation. *Liver Transpl* 2002; **8**: 1165-1174 [PMID: 12474157 DOI: 10.1053/jlts.2002.36394]

58 **Hansen PD**, Cassera MA, Wolf RF. Ablative technologies for hepatocellular, cholangiocarcinoma, and metastatic colorectal cancer of the liver. *Surg Oncol Clin N Am* 2015; **24**: 97-119 [PMID: 25444471 DOI: 10.1016/j.soc.2014.09.003]

59 **Jiang YQ**, Wang ZX, Deng YN, Yang Y, Wang GY, Chen GH. Efficacy of Hepatic Resection *vs* Radiofrequency Ablation for Patients With Very-Early-Stage or Early-Stage Hepatocellular Carcinoma: A Population-Based Study With Stratification by Age and Tumor Size. *Front Oncol* 2019; **9**: 113 [PMID: 30863723 DOI: 10.3389/fonc.2019.00113]

60 **Cartier V**, Boursier J, Lebigot J, Oberti F, Fouchard-Hubert I, Aubé C. Radiofrequency ablation of hepatocellular carcinoma: Mono or multipolar? *J Gastroenterol Hepatol* 2016; **31**: 654-660 [PMID: 26414644 DOI: 10.1111/jgh.13179]

61 **Casadei Gardini A**, Marisi G, Canale M, Foschi FG, Donati G, Ercolani G, Valgiusti M, Passardi A, Frassineti GL, Scarpi E. Radiofrequency ablation of hepatocellular carcinoma: a meta-analysis of overall survival and recurrence-free survival. *Onco Targets Ther* 2018; **11**: 6555-6567 [PMID: 30323628 DOI: 10.2147/ott.S170836]

62 **Hocquelet A**, Aubé C, Rode A, Cartier V, Sutter O, Manichon AF, Boursier J, N'kontchou G, Merle P, Blanc JF, Trillaud H, Seror O. Comparison of no-touch multi-bipolar *vs* monopolar radiofrequency ablation for small HCC. *J Hepatol* 2017; **66**: 67-74 [PMID: 27422750 DOI: 10.1016/j.jhep.2016.07.010]

63 **Liao M**, Zhong X, Zhang J, Liu Y, Zhu Z, Wu H, Zeng Y, Huang J. Radiofrequency ablation using a 10-mm target margin for small hepatocellular carcinoma in patients with liver cirrhosis: A prospective randomized trial. *J Surg Oncol* 2017; **115**: 971-979 [PMID: 28334430 DOI: 10.1002/jso.24607]

64 **Rajyaguru DJ**, Borgert AJ, Smith AL, Thomes RM, Conway PD, Halfdanarson TR, Truty MJ, Kurup AN, Go RS. Radiofrequency Ablation Versus Stereotactic Body Radiotherapy for Localized Hepatocellular Carcinoma in Nonsurgically Managed Patients: Analysis of the National Cancer Database. *J Clin Oncol* 2018; **36**: 600-608 [PMID: 29328861 DOI: 10.1200/jco.2017.75.3228]

65 **Parikh ND**, Marshall VD, Green M, Lawrence TS, Razumilava N, Owen D, Singal AG, Feng M. Effectiveness and cost of radiofrequency ablation and stereotactic body radiotherapy for treatment of early-stage hepatocellular carcinoma: An analysis of SEER-medicare. *J Med Imaging Radiat Oncol* 2018; **62**: 673-681 [PMID: 29877615 DOI: 10.1111/1754-9485.12754]

66 **Nicoli N**, Casaril A, Marchiori L, Mangiante G, Hasheminia AR. Treatment of recurrent hepatocellular carcinoma by radiofrequency thermal ablation. *J Hepatobiliary Pancreat Surg* 2001; **8**: 417-421 [PMID: 11702250 DOI: 10.1007/s005340100003]

67 **Pai M**, Spalding D, Jiao L, Habib N. Use of bipolar radiofrequency in parenchymal transection of the liver, pancreas and kidney. *Dig Surg* 2012; **29**: 43-47 [PMID: 22441619 DOI: 10.1159/000335732]

68 **Pulvirenti A**, Garbagnati F, Regalia E, Coppa J, Marchiano A, Romito R, Schiavo M, Fabbri A, Burgoa L, Mazzaferro V. Experience with radiofrequency ablation of small hepatocellular carcinomas before liver transplantation. *Transplant Proc* 2001; **33**: 1516-1517 [PMID: 11267402 DOI: 10.1016/s0041-1345(00)02577-x]

69 **Brillet PY**, Paradis V, Brancatelli G, Rangheard AS, Consigny Y, Plessier A, Durand F, Belghiti J, Sommacale D, Vilgrain V. Percutaneous radiofrequency ablation for hepatocellular carcinoma before liver transplantation: a prospective study with histopathologic comparison. *AJR Am J Roentgenol* 2006; **186**: S296-S305 [PMID: 16632691 DOI: 10.2214/ajr.04.1927]

70 **Chan AC**, Poon RT, Cheung TT, Chok KS, Chan SC, Fan ST, Lo CM. Survival analysis of re-resection *vs* radiofrequency ablation for intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. *World J Surg* 2012; **36**: 151-156 [PMID: 22030561 DOI: 10.1007/s00268-011-1323-0]

71 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]

72 **Xu XL**, Liu XD, Liang M, Luo BM. Radiofrequency Ablation *vs* Hepatic Resection for Small Hepatocellular Carcinoma: Systematic Review of Randomized Controlled Trials with Meta-Analysis and Trial Sequential Analysis. *Radiology* 2018; **287**: 461-472 [PMID: 29135366 DOI: 10.1148/radiol.2017162756]

73 **Majumdar A**, Roccarina D, Thorburn D, Davidson BR, Tsochatzis E, Gurusamy KS. Management of people with early- or very early-stage hepatocellular carcinoma: an attempted network meta-analysis. *Cochrane Database Syst Rev* 2017; **3**: CD011650 [PMID: 28351116 DOI: 10.1002/14651858.CD011650.pub2]

74 **Mohkam K**, Dumont PN, Manichon AF, Jouvet JC, Boussel L, Merle P, Ducerf C, Lesurtel M, Rode A, Mabrut JY. No-touch multibipolar radiofrequency ablation *vs* surgical resection for solitary hepatocellular carcinoma ranging from 2 to 5 cm. *J Hepatol* 2018; **68**: 1172-1180 [PMID: 29410287 DOI: 10.1016/j.jhep.2018.01.014]

75 **Xu LL**, Zhang M, Yi PS, Zheng XB, Feng L, Lan C, Tang JW, Ren SS, Xu MQ. Hepatic resection combined with radiofrequency ablation *vs* hepatic resection alone for multifocal hepatocellular carcinomas: A meta-analysis. *J Huazhong Univ Sci Technolog Med Sci* 2017; **37**: 974-980 [PMID: 29270762 DOI: 10.1007/s11596-017-1836-3]

76 **Martin AN**, Wilkins LR, Das D, Johnston LE, Bauer TW, Adams RB, Zaydfudim VM. Efficacy of Radiofrequency Ablation *versus* Transarterial Chemoembolization for Patients with Solitary Hepatocellular Carcinoma ≤ 3 cm. *Am Surg* 2019; **85**: 150-155 [PMID: 30819290]

77 **Kim W**, Cho SK, Shin SW, Hyun D, Lee MW, Rhim H. Combination therapy of transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) for small hepatocellular carcinoma: comparison with TACE or RFA monotherapy. *Abdom Radiol (NY)* 2019; **44**: 2283-2292 [PMID: 30806742 DOI: 10.1007/s00261-019-01952-1]

78 **Mizandari M**, Pai M, Xi F, Valek V, Tomas A, Quaretti P, Golfieri R, Mosconi C, Guokun A, Kyriakides C, Dickinson R, Nicholls J, Habib N. Percutaneous intraductal radiofrequency ablation is a safe treatment for malignant biliary obstruction: feasibility and early results. *Cardiovasc Intervent Radiol* 2013; **36**: 814-819 [PMID: 23232859 DOI: 10.1007/s00270-012-0529-3]

79 **Jin M**, Yu Q, Liu Y, Xu W, Fu X, Ji B. Safety and Efficacy of Physical Thermal Ablation Combined Sorafenib for Hepatocellular Carcinoma: A Meta-analysis. *J Clin Transl Hepatol* 2021; **9**: 149-159 [PMID: 34007796 DOI: 10.14218/jcth.2020.00125]

80 **Germani G**, Pleguezuelo M, Gurusamy K, Meyer T, Isgrò G, Burroughs AK. Clinical outcomes of

radiofrequency ablation, percutaneous alcohol and acetic acid injection for hepatocelullar carcinoma: a meta-analysis. *J Hepatol* 2010; **52**: 380-388 [PMID: 20149473 DOI: 10.1016/j.jhep.2009.12.004]

81 **Laquière A**, Boustière C, Leblanc S, Penaranda G, Désilets E, Prat F. Safety and feasibility of endoscopic biliary radiofrequency ablation treatment of extrahepatic cholangiocarcinoma. *Surg Endosc* 2016; **30**: 1242-1248 [PMID: 26162420 DOI: 10.1007/s00464-015-4322-7]

82 **Kang TW**, Lim HK, Cha DI. Percutaneous ablation for perivascular hepatocellular carcinoma: Refining the current status based on emerging evidence and future perspectives. *World J Gastroenterol* 2018; **24**: 5331-5337 [PMID: 30598578 DOI: 10.3748/wjg.v24.i47.5331]

83 **Lurje I**, Czigany Z, Bednarsch J, Roderburg C, Isfort P, Neumann UP, Lurje G. Treatment Strategies for Hepatocellular Carcinoma - a Multidisciplinary Approach. *Int J Mol Sci* 2019; **20** [PMID: 30909504 DOI: 10.3390/ijms20061465]

84 **Weber JC**, Navarra G, Jiao LR, Nicholls JP, Jensen SL, Habib NA. New technique for liver resection using heat coagulative necrosis. *Ann Surg* 2002; **236**: 560-563 [PMID: 12409660 DOI: 10.1097/00000658-200211000-00004]

85 **Koda M**, Ueki M, Maeda Y, Mimura KI, Okamoto K, Matsunaga Y, Kawakami M, Hosho K, Murawaki Y. The influence on liver parenchymal function and complications of radiofrequency ablation or the combination with transcatheter arterial embolization for hepatocellular carcinoma. *Hepatol Res* 2004; **29**: 18-23 [PMID: 15135342 DOI: 10.1016/j.hepres.2004.02.001]

86 **Sharaiha RZ**, Sethi A, Weaver KR, Gonda TA, Shah RJ, Fukami N, Kedia P, Kumta NA, Clavo CM, Saunders MD, Cerecedo-Rodriguez J, Barojas PF, Widmer JL, Gaidhane M, Brugge WR, Kahaleh M. Impact of Radiofrequency Ablation on Malignant Biliary Strictures: Results of a Collaborative Registry. *Dig Dis Sci* 2015; **60**: 2164-2169 [PMID: 25701319 DOI: 10.1007/s10620-015-3558-3]

87 **Strand DS**, Cosgrove ND, Patrie JT, Cox DG, Bauer TW, Adams RB, Mann JA, Sauer BG, Shami VM, Wang AY. ERCP-directed radiofrequency ablation and photodynamic therapy are associated with comparable survival in the treatment of unresectable cholangiocarcinoma. *Gastrointest Endosc* 2014; **80**: 794-804 [PMID: 24836747 DOI: 10.1016/j.gie.2014.02.1030]

88 **Alis H**, Sengoz C, Gonenc M, Kalayci MU, Kocatas A. Endobiliary radiofrequency ablation for malignant biliary obstruction. *Hepatobiliary Pancreat Dis Int* 2013; **12**: 423-427 [PMID: 23924501 DOI: 10.1016/s1499-3872(13)60066-1]

89 **Zhang L**, Wang N, Shen Q, Cheng W, Qian GJ. Therapeutic efficacy of percutaneous radiofrequency ablation *vs* microwave ablation for hepatocellular carcinoma. *PLoS One* 2013; **8**: e76119 [PMID: 24146824 DOI: 10.1371/journal.pone.0076119]

90 **Kalra N**, Kang M, Duseja AK, Bhatia A, Singh V, Dhiman RK, Rajwanshi A, Chawla YK, Khandelwal N. Comparison of radiofrequency ablation alone and in combination with percutaneous ethanol injection for management of hepatocellular carcinoma. *Indian J Med Res* 2017; **146**: S30-S37 [PMID: 29578192 DOI: 10.4103/ijmr.IJMR_1812_15]

91 **Abdelaziz AO**, Abdelmaksoud AH, Nabeel MM, Shousha HI, Cordie AA, Mahmoud ShH, Medhat E, Omran D, Elbaz TM. Transarterial Chemoembolization Combined with Either Radiofrequency or Microwave Ablation in Management of Hepatocellular Carcinoma Asian Pac J Cancer Prev 2017; 18: 189-194 [PMID: 28240516 DOI: 10.22034/apjcp.2017.18.1.189]

92 **Györi GP**, Felsenreich DM, Silberhumer GR, Soliman T, Berlakovich GA. Multimodality locoregional treatment strategies for bridging HCC patients before liver transplantation. *Eur Surg* 2017; **49**: 236-243 [PMID: 29104589 DOI: 10.1007/s10353-017-0487-8]

93 **Hao Y**, Numata K, Ishii T, Fukuda H, Maeda S, Nakano M, Tanaka K. Rate of local tumor progression following radiofrequency ablation of pathologically early hepatocellular carcinoma. *World J Gastroenterol* 2017; **23**: 3111-3121 [PMID: 28533668 DOI: 10.3748/wjg.v23.i17.3111]

94 **Santambrogio R**, Barabino M, Bruno S, Mariani N, Maroni N, Bertolini E, Franceschelli G, Opocher E. Surgical Resection *vs* Ablative Therapies Through a Laparoscopic Approach for Hepatocellular Carcinoma: a Comparative Study. *J Gastrointest Surg* 2018; **22**: 650-660 [PMID: 29235004 DOI: 10.1007/s11605-017-3648-y]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 216-229

DOI: 10.4251/wjgo.v14.i1.216

ISSN 1948-5204 (online)

MINIREVIEWS

Immune evasion mechanisms and therapeutic strategies in gastric cancer

En-Si Ma, Zheng-Xin Wang, Meng-Qi Zhu, Jing Zhao

ORCID number: En-Si Ma 0000-0002-8702-9839; Zheng-Xin Wang 0000-0002-3354-339X; Meng-Qi Zhu 0000-0002-0092-3900; Jing Zhao 0000-0003-2446-7732.

Author contributions: Ma ES and Zhao J drafted the manuscript; Ma ES, Zhu MQ, and Zhao J prepared the figures and tables; Zhu MQ provided input to the writing of this paper; Wang ZX and Zhao J provided critical comments; Ma ES, Wang ZX, and Zhao J designed the outline and coordinated the writing of the paper.

Conflict-of-interest statement: No potential conflicts of interest are disclosed.

Supported by The Natural Science Foundation of China, No. 81672378, No. 81201521, No. 81873874 and No. 81773089; and the Clinical Research Plan of SHDC, No. SHDC2020CR2021B.

Country/Territory of origin: China

Specialty type: Geriatrics and Gerontology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

En-Si Ma, Zheng-Xin Wang, Jing Zhao, Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China

En-Si Ma, Zheng-Xin Wang, Institute of Organ Transplantation, Fudan University, Shanghai 200040, China

Meng-Qi Zhu, Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai 200040, China

Jing Zhao, Cancer Metastasis Institute, Fudan University, Shanghai 200040, China

Corresponding author: Jing Zhao, PhD, Associate Professor, Department of General Surgery, Huashan Hospital, Fudan University, No. 12 Urumqi Middle Road, Shanghai 200040, China. jingzhao@fudan.edu.cn

Abstract

Gastric cancer (GC) is a malignancy with a high incidence and mortality. The tumor immune microenvironment plays an important role in promoting cancer development and supports GC progression. Accumulating evidence shows that GC cells can exert versatile mechanisms to remodel the tumor immune microenvironment and induce immune evasion. In this review, we systematically summarize the intricate crosstalk between GC cells and immune cells, including tumor-associated macrophages, neutrophils, myeloid-derived suppressor cells, natural killer cells, effector T cells, regulatory T cells, and B cells. We focus on how GC cells alter these immune cells to create an immunosuppressive microenvironment that protects GC cells from immune attack. We conclude by compiling the latest progression of immune checkpoint inhibitor-based immunotherapies, both alone and in combination with conventional therapies. Anti-cytotoxic T-lymphocyte-associated protein 4 and anti-programmed cell death protein 1/programmed death-ligand 1 therapy alone does not provide substantial clinical benefit for GC treatment. However, the combination of immune checkpoint inhibitors with chemotherapy or targeted therapy has promising survival advantages in refractory and advanced GC patients. This review provides a comprehensive understanding of the immune evasion mechanisms of GC, and highlights promising immunotherapeutic strategies.

Key Words: Gastric cancer; Immune evasion; Immune checkpoint; Immunotherapy; Microenvironment

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: April 22, 2021
Peer-review started: April 22, 2021
First decision: June 16, 2021
Revised: June 22, 2021
Accepted: December 10, 2021
Article in press: December 10, 2021
Published online: January 15, 2022

P-Reviewer: Pinheiro RN
S-Editor: Wang JL
L-Editor: Filipodia
P-Editor: Wang JL

Core Tip: Gastric cancer (GC) is one of the most common malignancies with high incidence and mortality. GC can exert versatile mechanisms to induce immune evasion. Here, we systematically summarized the intricate crosstalk among GC cells and various immune cells and mainly focused on how GC cells educate immune cells to create an immunosuppressive microenvironment and facilitate GC cells from attack of the immune system. In addition, we retrieved the latest progression of immune checkpoint inhibitor-based immunotherapies and their combination with conventional therapies. This review provides a comprehensive understanding of the immune evasion mechanism and immunotherapeutic strategies in GC.

Citation: Ma ES, Wang ZX, Zhu MQ, Zhao J. Immune evasion mechanisms and therapeutic strategies in gastric cancer. *World J Gastrointest Oncol* 2022; 14(1): 216-229
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/216.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.216

INTRODUCTION

Gastric cancer (GC) is the fifth most common malignancy worldwide and causes the third most cancer-related deaths. Traditional treatment strategies, including gastrectomy, neoadjuvant chemotherapy, and targeted therapy, have made great progress in recent decades, all of which has markedly improved the prognosis of GC [1]. Recently, immune checkpoint inhibitors, such as programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) antibodies, have been approved for the treatment of refractory and metastatic GC patients[2,3]. However, only a small fraction of GC patients may benefit from immune checkpoint inhibitor treatment[4]. The overall and progression-free survival of GC remain disappointing. The median survival of refractory and advanced patients is usually less than 2 years[5]. Therefore, it is necessary to further explore the underlying mechanisms of GC development and to understand how GC cells escape from the antitumor immune response in order to identify novel biomarkers and develop effective therapeutic strategies for treating GC.

Accumulating evidence shows that the tumor microenvironment (TME) plays an important role in cancer development[6]. The TME is highly heterogeneous and includes a mix of stromal cells, macrophages, neutrophils, natural killer (NK) cells, T and B cells, and some negative regulatory cells. Intricate crosstalk between the cancer cells and immune cells can promote cancer progression, including in GC[7]. Theoretically, tumor cells have potential immunogenicity and should be recognized and eliminated by the host immune system. However, antitumor immunity is usually subverted by cancer cells[7]. It has been widely reported that GC cells can exert numerous mechanisms to evade immune attacks[8]. For instance, GC cells can release cytokines, chemokines, and growth factors to create an immunosuppressive microenvironment, recruit negative regulatory immune cells, or inhibit the antitumor activity of effector lymphocytes[8]. After exposure to GC cells, immune cells may lose their antitumor function and instead facilitate GC cell proliferation, metastasis, angiogenesis, and immune evasion. Successful cancer treatment should therefore both restore antitumor activity and block immunosuppression.

In the present review, we summarize the multiple mechanisms of immune evasion mediated by different immune cells and highlight the latest achievements in immunotherapy for treating GC. This systematic summary will provide a comprehensive understanding of cancer immunity and current immunotherapeutic strategies in GC.

MECHANISMS OF IMMUNE EVASION IN GC

The TME plays an important role in cancer development and progression. There is intricate crosstalk between cancer cells and the TME. Cancer cells can exert mechanisms that remodel the TME, thus triggering immune evasion and promoting the malignant progression of cancer. Several studies have delineated the interplay

between GC cells and specific immune cell types, which will be discussed in detail below.

Tumor-associated macrophages induce immunosuppression in GC

Macrophages are a major component of tumor-infiltrating lymphocytes. Tumor-associated macrophages (TAMs) play a crucial role in angiogenesis, metastasis, and immunosuppression. TAMs can be classified into two subtypes: M1 or classically activated macrophages, and M2 or alternatively activated macrophages. M1s have antitumor activity, whereas M2s support cancer development. M2-TAMs are remarkably enriched in GC samples and are closely associated with invasion, metastasis, peritoneal dissemination, and unfavorable prognosis[9,10]. TAMs can induce GC invasion through activating epidermal growth factor receptor, c-Src, Erk1/2, Akt, and small GTPase activity in GC cells[11]. Wang *et al*[9] showed that TAM-derived CCL5 bound to its receptor CCR5, resulting in signal transducer and activator of transcription 3 (STAT3) activation and increased DNMT1 expression, which epigenetically silenced the tumor suppressor GSN in GC. TAMs can also secrete CXCL1 and CXCL5 to trigger the CXCR2/STAT3 signaling cascade and increase tumor necrosis factor (TNF)-α release from GC cells[12]. Reciprocally, TNF-α can enhance CXCL1 and CXCL5 release from TAMs. The positive feedback loop between GC cells and TAMs promotes cancer metastasis[12].

Exosomes play a crucial role in mediating the crosstalk between GC and TAMs. GC-derived exosomes induced PD1$^+$TAM generation, which inhibited the function of CD8+ T cells and aggravated GC progression[13]. TAM-derived exosomes could transmit functional ApoE into GC cells, thereby activating the PI3K/Akt pathway to remodel the cytoskeleton and promote migration of GC cells[14]. In addition to inducing malignant features of GC cells, TAMs can also affect the antitumor function of immune cells. Peng *et al*[15] reported that TAMs impaired NK cell function through transforming growth factor (TGF)-β1 in GC, which decreased the expression of effectors including interferon (IFN)-γ, TNF-α, and Ki-67. TAM-derived CXCL8 abolished proliferation and infiltration of CD8+ T cells *via* autonomous PD-L1 expression in GC[16].

Although the immunosuppressive role of TAMs is widely substantiated, there remains a lack of effective approaches to target TAMs for cancer therapy. The underlying mechanisms and therapeutic implications of targeting TAMs still need to be explored.

Neutrophils mediate immune evasion in GC

Neutrophils are the predominant leukocytes in humans. The role of neutrophils in different cancer types is controversial because they can exert either tumor-promoting or tumor-suppressing effects depending on the cancer type. Neutrophils are abundant in peripheral blood and solid tumors, including in GC. Enriched neutrophils were significantly associated with larger tumor size, advanced TNM stage, and poor survival for patients with GC[17,18]. GC cells and the TME can exert multiple regulatory roles to remodel neutrophils and promote cancer development. GC cell-derived GM-CSF could activate and increase PD-L1 expression in neutrophils *via* activating the JAK/STAT3 signaling pathway. As a result, the PD-L1+ neutrophils inhibited proliferation and decreased IFN-γ expression in T cells, thereby inducing immunosuppression in GC[19]. Another study showed that the interleukin 17 (IL-17)+ neutrophil subpopulation was more abundant in the invasive margins of GC samples. This type of neutrophil can release IL-17 to recruit more neutrophils to the invasive frontier by CXC chemokines, which can in turn secrete matrix metallopeptidase 9 into the reprogrammed extracellular matrix and promote angiogenesis in GC[20].

Reciprocally, neutrophils can facilitate the acquisition of malignant phenotypes by cancer cells. The GC environment has high levels of CXCL5, which can stimulate the ERK pathway in GC cells to induce epithelial-mesenchymal transition. Conversely, CXCL5 also influences neutrophils to activate the ERK/P38 cascade and increase IL-6 and IL-23 expression, which in turn stimulates the invasion and metastasis of GC cells [21]. Recent studies have discovered that neutrophil extracellular traps (NETs) play an important role in cancer progression and may trap and protect cancer cells to facilitate distant metastasis. The DNA component of NETs can function as a chemotactic factor to attract cancer cells through its receptor CCDC25 on cancer cells and activate the ILK-β-parvin pathway to enhance cell migration[22]. NET levels were increased and linked to advanced tumor stage in GC. However, the mechanisms of NET formation and regulation remain unclear in GC and should be further investigated.

Myeloid-derived suppressor cells regulate immunosuppression in GC

Myeloid-derived suppressor cells (MDSCs) are rapidly becoming a hotspot in the field of cancer immunity. MDSCs act as immunosuppressive cells to stimulate tumor growth and metastasis and modulate immune evasion. MDSCs are attracted to the tumor parenchyma by the interaction between CCL5 and CCR5 in GC[23]. Anti-CCR5 could effectively block the recruitment of MDSCs, and enhance the efficacy of anti-PD-L1 treatment in mice[23]. Some other chemokines, including CXCL12, CXCL5, and CCL2, are also responsible for the recruitment of MDSCs in GC[24]. Tumor-derived exosomes can affect MDSCs and thus exert a tumorigenic role. For example, GC-derived exosome miR-107 is taken up by MDSCs where it silences the expression of its targets PTEN and DICER and activates the PI3K/AKT pathway, leading to MDSC expansion[25].

There are various MDSC subsets in GC. A subset of MDSCs with CD45+CD33^{low} CD11b^{dim} was specifically enriched in GC, which could effectively suppress CD8+ T cell proliferation and IFN-γ and granzyme B expression. Mechanistically, GC patient serum-derived IL-6 and IL-8 activated the PI3K/AKT signaling pathway in this MDSC subtype to increase AGRI expression and mediate T cell suppression. The presence of CD45+CD33^{low}CD11b^{dim} cells, as well as IL-6, IL-8, and ARG I serum levels were positively correlated with GC progression and were negatively linked to overall patient survival[26].

In a mouse model of GC, Hsu *et al*[27] found that silencing STK24 expression could accelerate orthotopic tumor growth and induce MDSC infiltration into the tumor. Chemotherapeutic treatment could reduce MDSC enrichment in spontaneous gastric tumors in mice and improve the effects of anti-PD-1 therapy. Combining PD-1/PD-L1 blockade with MDSC targeting may be a promising strategy to prevent the progression and development of GC[28].

NK cells and GC development

NK cells, as important effectors of host immunity, induce cancer cell apoptosis by secreting IFN-γ[29], TNF-α[30], or by forming the complexes Fas/FasL and TRAIL/TRAILR[31]. NKG2D is an essential receptor for NK activation, and MICA and MICB are the well-known suppressive ligands of NKG2D that inhibit NK function[32,33]. Several studies have found that GC cells could reduce NKG2D expression and inhibit NK cell function through the release of soluble MICA and MICB. For example, Midkine could increase CHOP expression and form complexes with transcriptional factor AP-1, thereby increasing MICA/B expression and inhibiting NK cytotoxicity [34]. STA21 increased MICB expression and secretion by inhibiting the STAT3 pathway, which in turn repressed NKG2D expression and impaired NK function[35]. Matrix metallopeptidase inhibition could upregulate NKG2D ligand expression and increase NK activity in GC[36].

Cytokines, including IL-10, TGF-βl, and PGE2, could abolish the antitumor function of NK cells in GC[37]. There is mounting evidence that NK cells preferentially target cancer stem cells[38]. Recent research has found that the vital cancer stem cell marker CD133 can effectively activate NK cells in an NKG2D-dependent manner[39]. However, DKK3 inhibits CD133-induced NK cell activation by suppressing the ERK pathway and immune synapse formation[39]. Another recent study found that vinculin could induce epithelial-mesenchymal transition in GC cells and affect NK cell infiltration, which potentially predicts inferior prognosis and distant metastasis of GC [40]. It is critical to restore NK cell function and cytotoxicity to effectively treat GC, which should be carefully considered in combinatory treatment strategies.

T cell immunity in GC

T cell immunity is the most important component in the immune response to cancer. There are many subtypes of T cells, such as CD4, CD8, helper T (Th) 17, Th22, memory T, and regulatory T cells (Tregs). Each of the T cell subsets has its specific function, which include antitumor activity and immune evasion. CD8+ T cells and Tregs are two currently well-established lymphocytes that are involved in cancer immunity.

Metabolic reprogramming plays an important role in T cell-mediated immunity against cancer. CD155 on the surface of GC cells can bind to the immune checkpoint molecule TIGIT on the surface of T cells, which prevents T cells from metabolizing glucose, decreases IFN-γ production, and abolishes the cytotoxicity activity of CD8+ T cells[41]. Recently, it was determined that cancer cells can compete with T cells for nutrients, rendering T cells inactive. Lin *et al*[42] found that the infiltration of tissue-resident memory T cells (Trm) was markedly associated with a favorable prognosis in GC. Trm cells mainly rely on fatty acid oxidation, rather than glucose, for their energy

supply. However, GC cells make use of a more efficient pathway to metabolize fatty acids than Trm cells, which results in Trm starvation and death[42].

Tregs are well-known immunoregulatory cells that can suppress the proliferation and cytokine secretion of T effector cells. However, the correlation between Treg infiltration and GC prognosis remains unclear, and there have been many contradictory results because of different Treg markers, location distances, and intracellular interactions. GC cells can release cytokines to recruit Tregs, including CCL17, CCL22, and CCL28. GC cells can also induce CD4+ naïve T cells to differentiate into Tregs *via* TGF-β and induced immunosuppression[43-45]. Tregs can exert mechanisms to abolish the cytotoxicity of T cells. Recently, Shi *et al*[46] reported that Tregs and the A2aR+/CD8+ T cell subpopulations were enriched in GC samples. Tregs can hydrolyze ATP into adenosine, which was taken up by CD8+ T cells through the adenosine A2aR pathway, inhibiting CD8+ T cell proliferation and inducing apoptosis [46]. An investigation on GC resistance to checkpoint blockade found that these patients frequently had an RHOA mutation. RHOA mutations activate the PI3K-AKT-mTOR signaling cascade, producing free fatty acids, which Tregs could consume more efficiently than effector T cells. This metabolic advantage of Tregs enabled them to accumulate within GC tissue and generate an immunosuppressive TME, thus limiting the efficacy of immune checkpoint blockade[47].

Understanding the regulatory mechanisms of T cell immunity is of critical importance to the goal of eliminating cancer cells, and there are still many unknown factors in this complex biological network.

B cells induce immune evasion in GC

B cells have a dual role in the immune system and can participate in antibody production and antigen presentation. CD20+ B cell infiltration is associated with better tumor prognosis[48]. A recent study found that the protective effect of CD20+ B cells may be related to the production of antibodies by sulfated glycosaminoglycan-induced functional B cells, which strongly inhibit the growth of GC[49]. In addition to the elimination of GC cells, a subpopulation of B cells with an inhibitory phenotype, known as Bregs, have recently come to the attention of researchers. Bregs can produce several inhibitory cytokines, including IL-10, TGF-β, and IL-35[50]. Furthermore, Bregs can express inhibitory molecules, such as FasL and PD-L1[51,52]. Bregs in GC can enhance IL-10 production by CD4+ and CD8+ T cells to accelerate tumor growth[53]. A different study showed that Bregs have no impact on the proliferation of CD3+ T cells or CD4+ Th cells but instead inhibit the secretion of IFN-γ and TNF-α by CD4+ Th cells and convert CD4+CD25 effector T cells to CD4+FoxP3+ Tregs *via* TGF-β1[54]. These findings demonstrate that Bregs can exert immunosuppressive effects in GC development, the detailed mechanisms of which require urgent clarification.

With the progression of high-throughput sequencing technology and multi-omics platforms, widespread cellular remodeling events have been identified in GC and the TME, including genomic alteration, transcriptional states, epigenetic reprogramming, intercellular interactions, and metabolic reprogramming. These new insights provide valuable knowledge that will explain cancer immune evasion and facilitate the development of novel immunotherapies.

Collectively, there exists complex interplay between GC cells and various immune cells as described in Figure 1.

PROGRESS OF CHECKPOINT INHIBITOR-BASED IMMUNOTHERAPY IN GC

Immunotherapy has achieved impressive success in cancer treatment to date. A series of clinical trials of immunotherapeutic agents have been carried out for the treatment of GC. In this section, we will introduce the effects of checkpoint inhibitor-based immunotherapy in GC and focus on methods for selecting patients who will benefit from such therapy.

The immune checkpoint is a class of signaling molecule that regulates antigen recognition of T cell receptors during the immune response. Immune checkpoints can be categorized into co-stimulatory and co-inhibitory subtypes. The most common immune checkpoint blockers, which target co-inhibitory receptors of T cells, are antibodies against CTLA4, PD-1, and PD-L1 that can reinvigorate the anti-tumor immune activity of T cells.

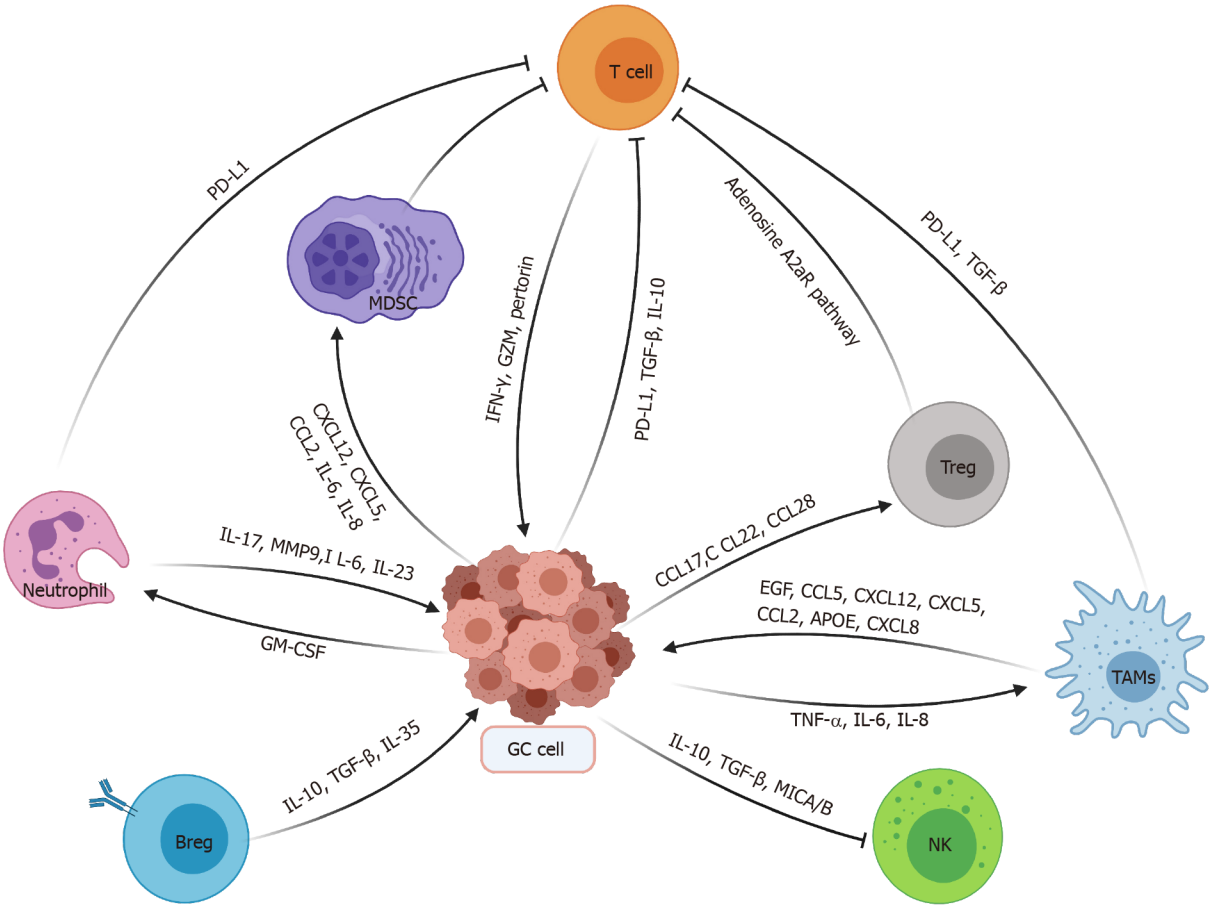


Figure 1 Overview of interactions between gastric cancer cells and various immune cells. PD-L1: Programmed death-ligand 1; TGF: Transforming growth factor; IL: Interleukin; TNF: Tumor necrosis factor; MDSC: Myeloid-derived suppressor cell; GC: Gastric cancer; NK: Natural killer; TAMs: Tumor-associated macrophages; IFN: Interferon; GZM: Granzyme; Treg: Regulatory T cell; Breg: Regulatory B cell; MMP: Matrix metalloproteinase; EGF: Epidermal growth factor.

Anti-CTLA4 in GC treatment

The initial study of anti-CTLA-4 in GC was a small phase II trial with the antibody tremelimumab, which enrolled 18 patients with metastatic gastric and esophageal adenocarcinomas. Patients were treated with an intravenous infusion of tremelimumab. Although some patients showed stabilization or even remission, the objective response rate (ORR) for tremelimumab alone was unsatisfactory, as only in 1 of 18 patients (5.5%) reached the primary endpoint[55]. Another study using anti-CTLA-4 antibodies was a phase II trial of ipilimumab, which enrolled 57 patients with advanced/metastatic gastric or gastroesophageal junction cancer (GEJC). The clinical endpoints of the study were immune-related progression-free survival (PFS), PFS, overall survival (OS), and immune-related best overall response rate. The best supportive care group had an immune-related best overall response rate of 7.0% (n = 4/57), the median immune-related PFS was 4.90 mo, and the 12-mo immune-related PFS was about 10% without any improvement. Based on these findings, targeting CTLA-4 alone is not considered to be an effective remedy for GC[56].

PD-1 and PD-L1 inhibitors in GC

Blockade of PD-1 and its ligand PD-L1 confers an encouraging survival advantage in several malignancies, including GC. KEYNOTE-012 was the first clinical trial of pembrolizumab, an antibody against PD-1, in 39 advanced GC patients. A sustained antitumor response was observed, and the median OS was 11.4 mo, which was better than the OS of 4-8 mo in the conventional chemotherapy group[57]. KEYNOTE-028 was another pembrolizumab trial that included 23 eligible patients with squamous cell carcinoma or adenocarcinoma of the esophagus or gastroesophageal junction who had failed standard therapy and had PD-L1-positive tumors. The ORR was 30%, and the median OS was 7.0 mo. In addition, this study developed a novel scoring system containing six immunomodulation-related IFN-γ-related genes, which significantly correlated with both PFS and ORR. Using this novel system, GC patients with higher scores frequently had a better response to pembrolizumab treatment[58].

Subsequently, a large-scale, randomized phase III trial, KEYNOTE-061, was carried out to compare the efficacy of pembrolizumab and paclitaxel in patients with advanced gastric or GEJC that progressed on first-line chemotherapy with platinum and fluoropyrimidine. In total, 196 patients were enrolled in the pembrolizumab cohort and 199 in the paclitaxel cohort. PD-L1 combined positive score (CPS) and microsatellite instability were two main criteria for subgroup analysis. As expected, the safety of pembrolizumab was superior to that of paclitaxel, and the OS in the CPS ≥ 1 group was better than in the low CPS group. Pembrolizumab had survival benefits in the long-term, with 12-mo and 18-mo survival rates of approximately 40% and 26%, respectively. Subgroup analysis suggested that the pembrolizumab response was more pronounced in patients with higher PD-L1 expression and high microsatellite instability levels[59].

Unlike pembrolizumab, which is an engineered humanized IgG4 antibody, nivolumab is a fully human IgG4 anti-PD-1 antibody. ATTRACTION-02, a phase III clinical trial, was conducted to evaluate the efficacy and safety of nivolumab in advanced and refractory GC or GEJC patients. Nivolumab showed a beneficial efficacy in GC and GEJC patients regardless of PD-L1 expression[2].

The efficacy of the PD-L1 monoclonal antibody avelumab has been compared with that of chemotherapy in chemotherapy-refractory GC or GEJC patients in the JAVELIN Gastric 300 trial. Unfortunately, avelumab failed to improve OS and PFS in this trial[60]. Subsequently, a global phase III clinical trial, named JAVELIN Gastric 100, was conducted to investigate the efficacy of avelumab after first-line induction chemotherapy for GC and GEJC. In line with previous results, avelumab alone seems to be slightly inferior in ORR, median PFS, and OS compared with chemotherapy[61]. A phase Ib clinical trial, named JAVELIN Solid Tumor trial, was conducted to investigate the antitumor activity and safety of avelumab as first-line switch-maintenance (1 L-mn) or second-line (2 L) treatment in patients with advanced GC/GEJC previously treated with chemotherapy. In this clinical trial avelumab showed clinical activity and an acceptable safety profile in patients with GC/GEJC [62]. However, avelumab was better tolerated, even in advanced-stage patients, than chemotherapy, suggesting that avelumab could be used as part of a combinatory therapy.

Combination of different immune checkpoint inhibitors in GC treatment

To explore whether combinations of different immune checkpoint inhibitors could synergistically resist cancer development, the CheckMate-032 trial was conducted to evaluate the safety and efficacy of nivolumab and nivolumab plus ipilimumab in chemotherapy-refractory GEJC patients. Nivolumab alone or combined with ipilimumab displayed a durable antitumor response and long-term OS benefits. Although increased toxicity was observed in the combination subgroup, the safety profile was manageable[63]. Recently, a phase Ib/II randomized controlled trial was performed to assess durvalumab and tremelimumab in combination or as monotherapy for chemotherapy-refractory GEJC patients. The response rates were low for both monotherapy or combination therapies. However, the combination therapy could significantly prolong the median OS and 12-mo OS. The tolerance of combination therapy was at an acceptable level. Therefore, this combination strategy may be an alternative option to improve the prognosis of these difficult-to-treat GC patients[64].

Immune checkpoint inhibitors combined with chemotherapy in GC

The clinical trial KEYNOTE-059 was initiated to evaluate the efficacy and safety of pembrolizumab alone or pembrolizumab combined with chemotherapy in patients with recurrent or metastatic GC and GEJC. The PD-L1 CPS score was found to be an effective tool to select patients who benefit from anti-PD-L1 treatment. Pembrolizumab monotherapy and in combination with chemotherapy displayed favorable antitumor activity and manageable tolerance as a first-line treatment[65]. Recently, the DURIGAST trial was designed to explore the efficacy of chemotherapy plus durvalumab (anti-PD-L1) *vs* chemotherapy plus durvalumab and tremelimumab (anti-CTLA-4) as second-line treatment of advanced GC and GEJC. At present, the safety profile is manageable, and further follow-up is ongoing. The trial results are eagerly anticipated[66].

Immune checkpoint inhibitors combined with anti-angiogenesis agents in GC

Ramucirumab is an antibody targeting angiogenesis factor VEGFR2 that has shown promising efficacy in GC treatment. Recently, ramucirumab was combined with

pembrolizumab in a Phase 1a/b JVDF Trial to treat naïve advanced GC and GEJC patients. PD-L1-positive patients acquired a better prognosis than PD-L1 negative patients; the median PFS was 8.6 mo *vs* 4.3 mo, and the median OS was 17.3 mo *vs* 11.3 mo, respectively. The adverse effects of ramucirumab plus pembrolizumab did not accumulate, suggesting a good safety profile[67]. Immunotherapy combined with targeted medicine may therefore be a novel option for treatment of advanced GC patients.

Overall, cancer immunotherapy has opened an exciting new avenue for cancer treatment. A series of immunotherapy and combination strategies have been conducted for the treatment of GC over the past few years as summarized in Table 1. Some of the clinical trials have achieved promising efficacy, and some have failed to improve prognosis. At present, there is still a lack of effective biomarkers to identify the patients that could potentially benefit from specific therapies. Novel strategies are needed to enhance the overall response rates and improve the prognosis of GC.

As shown in Table 1, some of the immunotherapeutic effects are not statistically significant. To figure out which factor is critical for immunotherapeutic outcome, we performed an extensive analysis and found that PD-L1 CPS is an essential determinant because the prognosis of patients with PD-L1 CPS ≥ 1 was significantly better than patients with PD-L1 CPS < 1 and the ORR value in PD-L1 CPS ≥ 1 subgroup nearly reached to twice that compared with the PD-L1 CPS < 1 patients[3,65,67]. These findings suggest that it is necessary to carry out precise PD-L1 CPS and identify the potential GC patients who may benefit from immunotherapy.

Although immunotherapy may achieve a marvelous effect in some specific patients, the expensive cost has become an unneglectable financial burden for patient families and the whole society[68]. The term "financial toxicity" is referred to this particular side effect of drug therapy, which directly affects the prognosis of patients[69]. Financial toxicity may limit drug availability, result in poor qualities of life and care, account for lower obedience to treatment, and further lead to disease deterioration and poverty. The vicious circle formed by financial toxicity and malignant cancer ultimately aggravates the poorer prognostic outcomes and even higher mortality[69]. To objectively evaluate the effects of financial toxicity, de Souza *et al*[70] created the Comprehensive Score for Financial Toxicity, a quantitative measure of financial distress in cancer patients. The Comprehensive Score for Financial Toxicity associates with income, psychosocial stress, and health-related life quality[71]. Meeker *et al*[72] demonstrated that economic burden could cause grievous emotions such as worry, tension, and anxiety at the psychological level, which led to dismal life quality and poor prognosis in cancer patients.

Taken together, both the biological context of the immune criterion and the sociological context of the Comprehensive Score for Financial Toxicity should be fully considered to acquire better therapeutic effects for gastric cancer patients.

CONCLUSION

Our knowledge of cancer immunology has made great progress in recent years. Numerous novel and rational immunotherapeutic approaches have been designed and have achieved favorable clinical benefits in GC treatment. However, there are still some challenges that need to be conquered, such as identifying patients that could benefit from a specific therapy, improving the response rates, and decreasing adverse effects. These intractable challenges highlight the importance of systematically investigating the intricate and dynamic crosstalk between immune cells and tumor cells. Consistent effort is required to overcome the gaps in our knowledge in the fields of cancer biology and immunology. In the near future, more precise personalized immunotherapeutic strategies will be developed, which will provide survival advantages for refractory and advanced GC patients.

Table 1 The summary of major clinical trials in gastric cancer involving immune checkpoint inhibitors

Therapeutic strategy	Trial identifier	Drug name	Stage	Number	Type of cancer	Immune criterion	ORR (%)	Median PFS (mo)	Median OS (mo)	6-mo PFS (%)	1-year PFS (%)	6-mo OS (%)	1-year OS (%)
Anti-CTLA-4	A Phase II trial of Tremelimumab [55]	Tremelimumab	II	18	Metastatic gastric and esophageal adenocarcinomas		5.5	2.83	4.83	-	-	-	33
	NCT01585987 [56]	Ipilimumab	II	57	Advanced/metastatic gastric or gastroesophageal junction cancer		1.8 (irBORR)	2.72; 2.92 (irPFS)	12.7	18.3; 22.3 (irPFS)	8.4; 10.6 (irPFS)	-	-
Anti-PD-1 or Anti-PD-L1 alone	KEYNOTE-012 (NCT01848834) [57]	Pembrolizumab	Ib	39	PD-L1-positive adenocarcinoma of the stomach or gastroesophageal junction		22	1.9	11.4	26	-	66	42
	KEYNOTE-028 (NCT02054806) [58]	Pembrolizumab	Ib	23	Squamous cell carcinoma or adenocarcinoma of the esophagus or gastroesophageal junction in whom standard therapy failed and who had PD-L1–positive		30	1.8	7	30	22	60	40
	KEYNOTE-061 (NCT02370498) [59]	Pembrolizumab	III	296	Advanced gastric or gastroesophageal junction cancer	PD-L1 CPS ≥ 1	16	1.5	9.1	-	-	-	40
	JAVELIN solid tumor trial (NCT01772004) first-line switch-maintenance [62]	Avelumab	Ib	90	Advanced gastric or gastroesophageal cancer		6.7	2.8	11.1	23	13	-	46.2
	ATTRACTION-2 (NCT02267343) [2]	Nivolumab	III	330	Advanced gastric or gastroesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens		-	-	5.26	-	-	46.1	26.2
	JAVELIN Gastric 100 (NCT02625610) [61]	Avelumab	III	249	Locally advanced or metastatic gastric or gastroesophageal junction cancer		-	3.2	10.4	-	-	-	-
	JAVELIN Gastric 300 (NCT02625623) [60]	Avelumab	III	185	Advanced gastric or gastroesophageal junction cancer		2.2	1.4	4.6	-	-	41	-
Immune checkpoints combination (Anti-PD-L1 and anti-CTLA4)	CheckMate-032 (NCT01928394) [63]	Nivolumab	I/II	59	Locally advanced or metastatic chemotherapy-refractory gastric, esophageal, or gastroesophageal junction cancer		12	1.4	-	-	8	-	39
		Nivolumab 1 mg/kg + ipilimumab 3 mg/kg		49			24	1.4	-	-	17	-	35
		Nivolumab 3 mg/kg + ipilimumab 1 mg/kg		52			8	1.6	-	-	10	-	24
	NCT02340975 [64]	Durvalumab + Tremelimumab (second-line)	Ib/II	44	Chemotherapy-refractory gastric cancer or gastroesophageal junction cancer		7.4	-	9.2	6.1	-	-	37
		Durvalumab		44			0	-	3.4	0	-	-	4.6

		(second-line)											
		Tremelimumab (second-line)		22			8.3	-	7.7	20	-	-	22.9
		Durvalumab + Tremelimumab (third-line)		25			4	-	9.2	15	-	-	38.8
Immune checkpoint combined with chemotherapy	KEYNOTE-059 (NCT02335411) [3,65]	Pembrolizumab	II	259	Previously treated gastric and gastroesophageal junction cancer		11.6	2	5.6	14.1	-	46.5	23.4
						PD-L1 CPS ≥ 1	15.5	-	-	-	-	-	-
						PD-L1 CPS < 1	6.4	-	-	-	-	-	-
		Pembrolizumab + chemotherapy		25	Advanced gastric or gastroesophageal junction cancer		60	6.6	13.8	68	-	-	52
						PD-L1 CPS ≥ 1	68.8	-	11.1	-	-	-	-
						PD-L1 CPS < 1	37.5	-	19.8	-	-	-	-
		Pembrolizumab		31			25.8	3.3	20.7	34.9	-	-	63
Immune checkpoint combined with target angiogenesis	NCT02443324 [67]	Ramucirumab + pembrolizumab	Ia/b	28	Advanced/metastatic gastric or gastroesophageal junction cancer		25	5.6	14.6	-	-	-	-
						PD-L1 CPS ≥ 1	32	8.6	17.3	-	-	-	66.7
						PD-L1 CPS < 1	17	4.3	11.3	-	-	-	41.7

OS: Overall survival; PFS: Progression-free survival; ORR: Objective response rate; irPFS: Immune-related progression free survival; irBORR: Immune-related best overall response rate; PD-L1: Programmed death-ligand 1: PD-L1 CPS: Programmed death-ligand 1 combined positive score; PD-1: Programed cell death protein 1.

REFERENCES

1 **Wang SM**, Zheng RS, Zhang SW, Zeng HM, Chen R, Sun KX, Gu XY, Wei WW, He J. [Epidemiological characteristics of gastric cancer in China, 2015]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2019; **40**: 1517-1521 [PMID: 32062908 DOI: 10.3760/cma.j.issn.0254-6450.2019.12.003]

2 **Kang YK**, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, Yeh KH, Yoshikawa T, Oh SC, Bai LY, Tamura T, Lee KW, Hamamoto Y, Kim JG, Chin K, Oh DY, Minashi K, Cho JY, Tsuda M, Chen LT. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **390**: 2461-2471 [PMID: 28993052 DOI: 10.1016/S0140-6736(17)31827-5]

3 **Fuchs CS**, Doi T, Jang RW, Muro K, Satoh T, Machado M, Sun W, Jalal SI, Shah MA, Metges JP, Garrido M, Golan T, Mandala M, Wainberg ZA, Catenacci DV, Ohtsu A, Shitara K, Geva R, Bleeker J, Ko AH, Ku G, Philip P, Enzinger PC, Bang YJ, Levitan D, Wang J, Rosales M, Dalal RP, Yoon HH. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial. *JAMA Oncol* 2018; **4**: e180013 [PMID: 29543932 DOI: 10.1001/jamaoncol.2018.0013]

4 **Li X**, Shao C, Shi Y, Han W. Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. *J Hematol Oncol* 2018; **11**: 31 [PMID: 29482595 DOI: 10.1186/s13045-018-0578-4]

5 **Ferro A**, Peleteiro B, Malvezzi M, Bosetti C, Bertuccio P, Levi F, Negri E, La Vecchia C, Lunet N. Worldwide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. *Eur J Cancer* 2014; **50**: 1330-1344 [PMID: 24650579 DOI: 10.1016/j.ejca.2014.01.029]

6 **Binnewies M**, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, Coussens LM, Gabrilovich DI, Ostrand-Rosenberg S, Hedrick CC, Vonderheide RH, Pittet MJ, Jain RK, Zou W, Howcroft TK, Woodhouse EC, Weinberg RA, Krummel MF. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* 2018; **24**: 541-550 [PMID: 29686425 DOI: 10.1038/s41591-018-0014-x]

7 **Galon J**, Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. *Immunity* 2020; **52**: 55-81 [PMID: 31940273 DOI: 10.1016/j.immuni.2019.12.018]

8 **Oya Y**, Hayakawa Y, Koike K. Tumor microenvironment in gastric cancers. *Cancer Sci* 2020; **111**: 2696-2707 [PMID: 32519436 DOI: 10.1111/cas.14521]

9 **Wang HC**, Chen CW, Yang CL, Tsai IM, Hou YC, Chen CJ, Shan YS. Tumor-Associated Macrophages Promote Epigenetic Silencing of Gelsolin through DNA Methyltransferase 1 in Gastric Cancer Cells. *Cancer Immunol Res* 2017; **5**: 885-897 [PMID: 28835422 DOI: 10.1158/2326-6066.CIR-16-0295]

10 **Yin S**, Huang J, Li Z, Zhang J, Luo J, Lu C, Xu H. The Prognostic and Clinicopathological

Significance of Tumor-Associated Macrophages in Patients with Gastric Cancer: A Meta-Analysis. *PLoS One* 2017; **12**: e0170042 [PMID: 28081243 DOI: 10.1371/journal.pone.0170042]

11 **Cardoso AP**, Pinto ML, Pinto AT, Oliveira MI, Pinto MT, Gonçalves R, Relvas JB, Figueiredo C, Seruca R, Mantovani A, Mareel M, Barbosa MA, Oliveira MJ. Macrophages stimulate gastric and colorectal cancer invasion through EGFR Y(1086), c-Src, Erk1/2 and Akt phosphorylation and smallGTPase activity. *Oncogene* 2014; **33**: 2123-2133 [PMID: 23644655 DOI: 10.1038/onc.2013.154]

12 **Zhou Z**, Xia G, Xiang Z, Liu M, Wei Z, Yan J, Chen W, Zhu J, Awasthi N, Sun X, Fung KM, He Y, Li M, Zhang C. A C-X-C Chemokine Receptor Type 2-Dominated Cross-talk between Tumor Cells and Macrophages Drives Gastric Cancer Metastasis. *Clin Cancer Res* 2019; **25**: 3317-3328 [PMID: 30796034 DOI: 10.1158/1078-0432.CCR-18-3567]

13 **Wang F**, Li B, Wei Y, Zhao Y, Wang L, Zhang P, Yang J, He W, Chen H, Jiao Z, Li Y. Tumor-derived exosomes induce PD1^{+} macrophage population in human gastric cancer that promotes disease progression. *Oncogenesis* 2018; **7**: 41 [PMID: 29799520 DOI: 10.1038/s41389-018-0049-3]

14 **Zheng P**, Luo Q, Wang W, Li J, Wang T, Wang P, Chen L, Zhang P, Chen H, Liu Y, Dong P, Xie G, Ma Y, Jiang L, Yuan X, Shen L. Tumor-associated macrophages-derived exosomes promote the migration of gastric cancer cells by transfer of functional Apolipoprotein E. *Cell Death Dis* 2018; **9**: 434 [PMID: 29567987 DOI: 10.1038/s41419-018-0465-5]

15 **Peng LS**, Zhang JY, Teng YS, Zhao YL, Wang TT, Mao FY, Lv YP, Cheng P, Li WH, Chen N, Duan M, Chen W, Guo G, Zou QM, Zhuang Y. Tumor-Associated Monocytes/Macrophages Impair NK-Cell Function *via* TGFβ1 in Human Gastric Cancer. *Cancer Immunol Res* 2017; **5**: 248-256 [PMID: 28148545 DOI: 10.1158/2326-6066.CIR-16-0152]

16 **Lv Y**, Zuo L, Zhu X, Zhao J, Xue H, Jiang Z, Zhuge Y, Zhang C, Sun J, Ding P, Ren W, Li Y, Zhang K, Zhang W, He C, Zhong J, Peng Q, Ma F, Luo J, Zhang M, Wang G, Sun M, Dong J, Bai W, Guo W, Wang Q, Yuan X, Wang Z, Yu T, Luo B, Li X, Yuan J, Han N, Zhu Y, Niu J, Li K, Yin Z, Nie Y, Fan D, Han G. Identifying optimal candidates for early TIPS among patients with cirrhosis and acute variceal bleeding: a multicentre observational study. *Gut* 2019; **68**: 1297-1310 [PMID: 30415233 DOI: 10.1136/gutjnl-2018-317057]

17 **Li ZY**, Wang JT, Chen G, Shan ZG, Wang TT, Shen Y, Chen J, Yan ZB, Peng LS, Mao FY, Teng YS, Liu JS, Zhou YY, Zhao YL, Zhuang Y. Expression, regulation and clinical significance of B7-H3 on neutrophils in human gastric cancer. *Clin Immunol* 2021; **227**: 108753 [PMID: 33945871 DOI: 10.1016/j.clim.2021.108753]

18 **Shan ZG**, Yan ZB, Peng LS, Cheng P, Teng YS, Mao FY, Fan K, Zhuang Y, Zhao YL. Granulocyte-Macrophage Colony-Stimulating Factor-Activated Neutrophils Express B7-H4 That Correlates with Gastric Cancer Progression and Poor Patient Survival. *J Immunol Res* 2021; **2021**: 6613247 [PMID: 33763491 DOI: 10.1155/2021/6613247]

19 **Wang TT**, Zhao YL, Peng LS, Chen N, Chen W, Lv YP, Mao FY, Zhang JY, Cheng P, Teng YS, Fu XL, Yu PW, Guo G, Luo P, Zhuang Y, Zou QM. Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. *Gut* 2017; **66**: 1900-1911 [PMID: 28274999 DOI: 10.1136/gutjnl-2016-313075]

20 **Li TJ**, Jiang YM, Hu YF, Huang L, Yu J, Zhao LY, Deng HJ, Mou TY, Liu H, Yang Y, Zhang Q, Li GX. Interleukin-17-Producing Neutrophils Link Inflammatory Stimuli to Disease Progression by Promoting Angiogenesis in Gastric Cancer. *Clin Cancer Res* 2017; **23**: 1575-1585 [PMID: 27620275 DOI: 10.1158/1078-0432.CCR-16-0617]

21 **Mao Z**, Zhang J, Shi Y, Li W, Shi H, Ji R, Mao F, Qian H, Xu W, Zhang X. CXCL5 promotes gastric cancer metastasis by inducing epithelial-mesenchymal transition and activating neutrophils. *Oncogenesis* 2020; **9**: 63 [PMID: 32632106 DOI: 10.1038/s41389-020-00249-z]

22 **Yang L**, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, Liu J, Xing Y, Chen X, Su S, Song E. DNA of neutrophil extracellular traps promotes cancer metastasis *via* CCDC25. *Nature* 2020; **583**: 133-138 [PMID: 32528174 DOI: 10.1038/s41586-020-2394-6]

23 **Yang L**, Wang B, Qin J, Zhou H, Majumdar APN, Peng F. Blockade of CCR5-mediated myeloid derived suppressor cell accumulation enhances anti-PD1 efficacy in gastric cancer. *Immunopharmacol Immunotoxicol* 2018; **40**: 91-97 [PMID: 29303012 DOI: 10.1080/08923973.2017.1417997]

24 **Zhou Y**, Guo F. A selective sphingosine-1-phosphate receptor 1 agonist SEW-2871 aggravates gastric cancer by recruiting myeloid-derived suppressor cells. *J Biochem* 2018; **163**: 77-83 [PMID: 29036438 DOI: 10.1093/jb/mvx064]

25 **Ren W**, Zhang X, Li W, Feng Q, Feng H, Tong Y, Rong H, Wang W, Zhang D, Zhang Z, Tu S, Zhu X, Zhang Q. Exosomal miRNA-107 induces myeloid-derived suppressor cell expansion in gastric cancer. *Cancer Manag Res* 2019; **11**: 4023-4040 [PMID: 31190980 DOI: 10.2147/CMAR.S198886]

26 **Mao FY**, Zhao YL, Lv YP, Teng YS, Kong H, Liu YG, Wu XL, Hao CJ, Chen W, Duan MB, Han B, Ma Q, Wang TT, Peng LS, Zhang JY, Cheng P, Su CY, Fu XL, Zou QM, Guo G, Guo XL, Zhuang Y. CD45^{+}CD33^{low}CD11b^{dim} myeloid-derived suppressor cells suppress CD8^{+} T cell activity *via* the IL-6/IL-8-arginase I axis in human gastric cancer. *Cell Death Dis* 2018; **9**: 763 [PMID: 29988030 DOI: 10.1038/s41419-018-0803-7]

27 **Hsu HP**, Wang CY, Hsieh PY, Fang JH, Chen YL. Knockdown of serine/threonine-protein kinase 24 promotes tumorigenesis and myeloid-derived suppressor cell expansion in an orthotopic immunocompetent gastric cancer animal model. *J Cancer* 2020; **11**: 213-228 [PMID: 31892988 DOI: 10.7150/jca.35821]

28 **Fabian KP**, Padget MR, Donahue RN, Solocinski K, Robbins Y, Allen CT, Lee JH, Rabizadeh S,

Soon-Shiong P, Schlom J, Hodge JW. PD-L1 targeting high-affinity NK (t-haNK) cells induce direct antitumor effects and target suppressive MDSC populations. *J Immunother Cancer* 2020; **8** [PMID: 32439799 DOI: 10.1136/jitc-2019-000450]

29 **Stetson DB**, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, Kronenberg M, Locksley RM. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J Exp Med* 2003; **198**: 1069-1076 [PMID: 14530376 DOI: 10.1084/jem.20030630]

30 **Wajant H**, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ* 2003; **10**: 45-65 [PMID: 12655295 DOI: 10.1038/sj.cdd.4401189]

31 **Martín-Antonio B**, Suñe G, Perez-Amill L, Castella M, Urbano-Ispizua A. Natural Killer Cells: Angels and Devils for Immunotherapy. *Int J Mol Sci* 2017; **18** [PMID: 28850071 DOI: 10.3390/ijms18091868]

32 **González S**, López-Soto A, Suarez-Alvarez B, López-Vázquez A, López-Larrea C. NKG2D ligands: key targets of the immune response. *Trends Immunol* 2008; **29**: 397-403 [PMID: 18602338 DOI: 10.1016/j.it.2008.04.007]

33 **Saito H**, Osaki T, Ikeguchi M. Decreased NKG2D expression on NK cells correlates with impaired NK cell function in patients with gastric cancer. *Gastric Cancer* 2012; **15**: 27-33 [PMID: 21626292 DOI: 10.1007/s10120-011-0059-8]

34 **Zhao S**, Wang H, Nie Y, Mi Q, Chen X, Hou Y. Midkine upregulates MICA/B expression in human gastric cancer cells and decreases natural killer cell cytotoxicity. *Cancer Immunol Immunother* 2012; **61**: 1745-1753 [PMID: 22415659 DOI: 10.1007/s00262-012-1235-3]

35 **Garrido-Tapia M**, Hernández CJ, Ascui G, Kramm K, Morales M, Ga Rate V, Zúñiga R, Bustamante M, Aguillón JC, Catala N D, Ribeiro CH, Molina MAC. STAT3 inhibition by STA21 increases cell surface expression of MICB and the release of soluble MICB by gastric adenocarcinoma cells. *Immunobiology* 2017; **222**: 1043-1051 [PMID: 28578917 DOI: 10.1016/j.imbio.2017.05.009]

36 **Shiraishi K**, Mimura K, Kua LF, Koh V, Siang LK, Nakajima S, Fujii H, Shabbir A, Yong WP, So J, Takenoshita S, Kono K. Inhibition of MMP activity can restore NKG2D ligand expression in gastric cancer, leading to improved NK cell susceptibility. *J Gastroenterol* 2016; **51**: 1101-1111 [PMID: 27002316 DOI: 10.1007/s00535-016-1197-x]

37 **Szkaradkiewicz A**, Karpiński TM, Drews M, Borejsza-Wysocki M, Majewski P, Andrzejewska E. Natural killer cell cytotoxicity and immunosuppressive cytokines (IL-10, TGF-beta1) in patients with gastric cancer. *J Biomed Biotechnol* 2010; **2010**: 901564 [PMID: 20445748 DOI: 10.1155/2010/901564]

38 **Ames E**, Canter RJ, Grossenbacher SK, Mac S, Chen M, Smith RC, Hagino T, Perez-Cunningham J, Sckisel GD, Urayama S, Monjazeb AM, Fragoso RC, Sayers TJ, Murphy WJ. NK Cells Preferentially Target Tumor Cells with a Cancer Stem Cell Phenotype. *J Immunol* 2015; **195**: 4010-4019 [PMID: 26363055 DOI: 10.4049/jimmunol.1500447]

39 **Xia P**, Xu XY. DKK3 attenuates the cytotoxic effect of natural killer cells on $CD133^+$ gastric cancer cells. *Mol Carcinog* 2017; **56**: 1712-1721 [PMID: 28218426 DOI: 10.1002/mc.22628]

40 **Li H**, Wang C, Lan L, Behrens A, Tomaschko M, Ruiz J, Su Q, Zhao G, Yuan C, Xiao X, Li B, Yan L, Wu W, Li W, Chen J, He Y, Zhang C. High expression of vinculin predicts poor prognosis and distant metastasis and associates with influencing tumor-associated NK cell infiltration and epithelial-mesenchymal transition in gastric cancer. *Aging (Albany NY)* 2021; **13**: 5197-5225 [PMID: 33535187 DOI: 10.18632/aging.202440]

41 **He W**, Zhang H, Han F, Chen X, Lin R, Wang W, Qiu H, Zhuang Z, Liao Q, Zhang W, Cai Q, Cui Y, Jiang W, Wang H, Ke Z. CD155T/TIGIT Signaling Regulates $CD8^+$ T-cell Metabolism and Promotes Tumor Progression in Human Gastric Cancer. *Cancer Res* 2017; **77**: 6375-6388 [PMID: 28883004 DOI: 10.1158/0008-5472.CAN-17-0381]

42 **Lin R**, Zhang H, Yuan Y, He Q, Zhou J, Li S, Sun Y, Li DY, Qiu HB, Wang W, Zhuang Z, Chen B, Huang Y, Liu C, Wang Y, Cai S, Ke Z, He W. Fatty Acid Oxidation Controls $CD8^+$ Tissue-Resident Memory T-cell Survival in Gastric Adenocarcinoma. *Cancer Immunol Res* 2020; **8**: 479-492 [PMID: 32075801 DOI: 10.1158/2326-6066.CIR-19-0702]

43 **Lu X**, Liu J, Li H, Li W, Wang X, Ma J, Tong Q, Wu K, Wang G. Conversion of intratumoral regulatory T cells by human gastric cancer cells is dependent on transforming growth factor-β1. *J Surg Oncol* 2011; **104**: 571-577 [PMID: 21695703 DOI: 10.1002/jso.22005]

44 **Yuan XL**, Chen L, Zhang TT, Ma YH, Zhou YL, Zhao Y, Wang WW, Dong P, Yu L, Zhang YY, Shen LS. Gastric cancer cells induce human CD4+Foxp3+ regulatory T cells through the production of TGF-β1. *World J Gastroenterol* 2011; **17**: 2019-2027 [PMID: 21528082 DOI: 10.3748/wjg.v17.i15.2019]

45 **Hu JL**, Yang Z, Tang JR, Fu XQ, Yao LJ. Effects of gastric cancer cells on the differentiation of Treg cells. *Asian Pac J Cancer Prev* 2013; **14**: 4607-4610 [PMID: 24083711 DOI: 10.7314/apjcp.2013.14.8.4607]

46 **Shi L**, Feng M, Du S, Wei X, Song H, Yixin X, Song J, Wenxian G. Adenosine Generated by Regulatory T Cells Induces $CD8^+$ T Cell Exhaustion in Gastric Cancer through A2aR Pathway. *Biomed Res Int* 2019; **2019**: 4093214 [PMID: 31930120 DOI: 10.1155/2019/4093214]

47 **Kumagai S**, Togashi Y, Sakai C, Kawazoe A, Kawazu M, Ueno T, Sato E, Kuwata T, Kinoshita T, Yamamoto M, Nomura S, Tsukamoto T, Mano H, Shitara K, Nishikawa H. An Oncogenic Alteration Creates a Microenvironment that Promotes Tumor Progression by Conferring a Metabolic Advantage to Regulatory T Cells. *Immunity* 2020; **53**: 187-203.e8 [PMID: 32640259 DOI:

10.1016/j.immuni.2020.06.016]

48 **Nielsen JS**, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, Nelson BH. CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. *Clin Cancer Res* 2012; **18**: 3281-3292 [PMID: 22553348 DOI: 10.1158/1078-0432.CCR-12-0234]

49 **Katoh H**, Komura D, Konishi H, Suzuki R, Yamamoto A, Kakiuchi M, Sato R, Ushiku T, Yamamoto S, Tatsuno K, Oshima T, Nomura S, Seto Y, Fukayama M, Aburatani H, Ishikawa S. Immunogenetic Profiling for Gastric Cancers Identifies Sulfated Glycosaminoglycans as Major and Functional B Cell Antigens in Human Malignancies. *Cell Rep* 2017; **20**: 1073-1087 [PMID: 28768193 DOI: 10.1016/j.celrep.2017.07.016]

50 **Mauri C**, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol* 2012; **30**: 221-241 [PMID: 22224776 DOI: 10.1146/annurev-immunol-020711-074934]

51 **Gotot J**, Gottschalk C, Leopold S, Knolle PA, Yagita H, Kurts C, Ludwig-Portugall I. Regulatory T cells use programmed death 1 ligands to directly suppress autoreactive B cells in vivo. *Proc Natl Acad Sci U S A* 2012; **109**: 10468-10473 [PMID: 22689978 DOI: 10.1073/pnas.1201131109]

52 **Dasgupta S**, Dasgupta S, Bandyopadhyay M. Regulatory B cells in infection, inflammation, and autoimmunity. *Cell Immunol* 2020; **352**: 104076 [PMID: 32143836 DOI: 10.1016/j.cellimm.2020.104076]

53 **Hu HT**, Ai X, Lu M, Song Z, Li H. Characterization of intratumoral and circulating IL-10-producing B cells in gastric cancer. *Exp Cell Res* 2019; **384**: 111652 [PMID: 31574287 DOI: 10.1016/j.yexcr.2019.111652]

54 **Wang WW**, Yuan XL, Chen H, Xie GH, Ma YH, Zheng YX, Zhou YL, Shen LS. CD19+CD24hiCD38hiBregs involved in downregulate helper T cells and upregulate regulatory T cells in gastric cancer. *Oncotarget* 2015; **6**: 33486-33499 [PMID: 26378021 DOI: 10.18632/oncotarget.5588]

55 **Ralph C**, Elkord E, Burt DJ, O'Dwyer JF, Austin EB, Stern PL, Hawkins RE, Thistlethwaite FC. Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. *Clin Cancer Res* 2010; **16**: 1662-1672 [PMID: 20179239 DOI: 10.1158/1078-0432.CCR-09-2870]

56 **Bang YJ**, Cho JY, Kim YH, Kim JW, Di Bartolomeo M, Ajani JA, Yamaguchi K, Balogh A, Sanchez T, Moehler M. Efficacy of Sequential Ipilimumab Monotherapy versus Best Supportive Care for Unresectable Locally Advanced/Metastatic Gastric or Gastroesophageal Junction Cancer. *Clin Cancer Res* 2017; **23**: 5671-5678 [PMID: 28655793 DOI: 10.1158/1078-0432.CCR-17-0025]

57 **Muro K**, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang YJ. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; **17**: 717-726 [PMID: 27157491 DOI: 10.1016/S1470-2045(16)00175-3]

58 **Doi T**, Piha-Paul SA, Jalal SI, Saraf S, Lunceford J, Koshiji M, Bennouna J. Safety and Antitumor Activity of the Anti-Programmed Death-1 Antibody Pembrolizumab in Patients With Advanced Esophageal Carcinoma. *J Clin Oncol* 2018; **36**: 61-67 [PMID: 29116900 DOI: 10.1200/JCO.2017.74.9846]

59 **Shitara K**, Özgüroğlu M, Bang YJ, Di Bartolomeo M, Mandalà M, Ryu MH, Fornaro L, Olesiński T, Caglevic C, Chung HC, Muro K, Goekkurt E, Mansoor W, McDermott RS, Shacham-Shmueli E, Chen X, Mayo C, Kang SP, Ohtsu A, Fuchs CS; KEYNOTE-061 investigators. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2018; **392**: 123-133 [PMID: 29880231 DOI: 10.1016/S0140-6736(18)31257-1]

60 **Bang YJ**, Ruiz EY, Van Cutsem E, Lee KW, Wyrwicz L, Schenker M, Alsina M, Ryu MH, Chung HC, Evesque L, Al-Batran SE, Park SH, Lichinitser M, Boku N, Moehler MH, Hong J, Xiong H, Hallwachs R, Conti I, Taieb J. Phase III, randomised trial of avelumab versus physician's choice of chemotherapy as third-line treatment of patients with advanced gastric or gastro-oesophageal junction cancer: primary analysis of JAVELIN Gastric 300. *Ann Oncol* 2018; **29**: 2052-2060 [PMID: 30052729 DOI: 10.1093/annonc/mdy264]

61 **Moehler M**, Dvorkin M, Boku N, Özgüroğlu M, Ryu MH, Muntean AS, Lonardi S, Nechaeva M, Bragagnoli AC, Coşkun HS, Cubillo Gracian A, Takano T, Wong R, Safran H, Vaccaro GM, Wainberg ZA, Silver MR, Xiong H, Hong J, Taieb J, Bang YJ. Phase III Trial of Avelumab Maintenance After First-Line Induction Chemotherapy Versus Continuation of Chemotherapy in Patients With Gastric Cancers: Results From JAVELIN Gastric 100. *J Clin Oncol* 2021; **39**: 966-977 [PMID: 33197226 DOI: 10.1200/JCO.20.00892]

62 **Chung HC**, Arkenau HT, Lee J, Rha SY, Oh DY, Wyrwicz L, Kang YK, Lee KW, Infante JR, Lee SS, Kemeny M, Keilholz U, Melichar B, Mita A, Plummer R, Smith D, Gelb AB, Xiong H, Hong J, Chand V, Safran H. Avelumab (anti-PD-L1) as first-line switch-maintenance or second-line therapy in patients with advanced gastric or gastroesophageal junction cancer: phase 1b results from the JAVELIN Solid Tumor trial. *J Immunother Cancer* 2019; **7**: 30 [PMID: 30717797 DOI: 10.1186/s40425-019-0508-1]

63 **Janjigian YY**, Bendell J, Calvo E, Kim JW, Ascierto PA, Sharma P, Ott PA, Peltola K, Jaeger D, Evans J, de Braud F, Chau I, Harbison CT, Dorange C, Tschaika M, Le DT. CheckMate-032 Study: Efficacy and Safety of Nivolumab and Nivolumab Plus Ipilimumab in Patients With Metastatic

Esophagogastric Cancer. *J Clin Oncol* 2018; **36**: 2836-2844 [PMID: 30110194 DOI: 10.1200/JCO.2017.76.6212]

64 **Kelly RJ**, Lee J, Bang YJ, Almhanna K, Blum-Murphy M, Catenacci DVT, Chung HC, Wainberg ZA, Gibson MK, Lee KW, Bendell JC, Denlinger CS, Chee CE, Omori T, Leidner R, Lenz HJ, Chao Y, Rebelatto MC, Brohawn PZ, He P, McDevitt J, Sheth S, Englert JM, Ku GY. Safety and Efficacy of Durvalumab and Tremelimumab Alone or in Combination in Patients with Advanced Gastric and Gastroesophageal Junction Adenocarcinoma. *Clin Cancer Res* 2020; **26**: 846-854 [PMID: 31676670 DOI: 10.1158/1078-0432.CCR-19-2443]

65 **Bang YJ**, Kang YK, Catenacci DV, Muro K, Fuchs CS, Geva R, Hara H, Golan T, Garrido M, Jalal SI, Borg C, Doi T, Yoon HH, Savage MJ, Wang J, Dalal RP, Shah S, Wainberg ZA, Chung HC. Pembrolizumab alone or in combination with chemotherapy as first-line therapy for patients with advanced gastric or gastroesophageal junction adenocarcinoma: results from the phase II nonrandomized KEYNOTE-059 study. *Gastric Cancer* 2019; **22**: 828-837 [PMID: 30911859 DOI: 10.1007/s10120-018-00909-5]

66 **Evrard C**, Louvet C, Hajbi FE, Fiore FD, Malicot KL, Aparicio T, Bouché O, Laurent-Puig P, Bibeau F, Lecomte T, Lièvre A, Guimbaud R, Kim S, Zaanan A, Sokol H, Chibaudel B, Desrame J, Pierre S, Gonzalez D, Lepage C, Tougeron D. PRODIGE 59-DURIGAST trial: A randomised phase II study evaluating FOLFIRI + Durvalumab ± Tremelimumab in second-line of patients with advanced gastric cancer. *Dig Liver Dis* 2021; **53**: 420-426 [PMID: 33358124 DOI: 10.1016/j.dld.2020.11.036]

67 **Chau I**, Penel N, Soriano AO, Arkenau HT, Cultrera J, Santana-Davila R, Calvo E, Le Tourneau C, Zender L, Bendell JC, Mi G, Gao L, McNeely SC, Oliveira JM, Ferry D, Herbst RS, Fuchs CS. Ramucirumab in Combination with Pembrolizumab in Treatment-Naïve Advanced Gastric or GEJ Adenocarcinoma: Safety and Antitumor Activity from the Phase 1a/b JVDF Trial. *Cancers (Basel)* 2020; **12** [PMID: 33076423 DOI: 10.3390/cancers12102985]

68 **Zafar SY**, Peppercorn JM, Schrag D, Taylor DH, Goetzinger AM, Zhong X, Abernethy AP. The financial toxicity of cancer treatment: a pilot study assessing out-of-pocket expenses and the insured cancer patient's experience. *Oncologist* 2013; **18**: 381-390 [PMID: 23442307 DOI: 10.1634/theoncologist.2012-0279]

69 **Zafar SY**. Financial Toxicity of Cancer Care: It's Time to Intervene. *J Natl Cancer Inst* 2016; **108** [PMID: 26657334 DOI: 10.1093/jnci/djv370]

70 **de Souza JA**, Yap BJ, Hlubocky FJ, Wroblewski K, Ratain MJ, Cella D, Daugherty CK. The development of a financial toxicity patient-reported outcome in cancer: The COST measure. *Cancer* 2014; **120**: 3245-3253 [PMID: 24954526 DOI: 10.1002/cncr.28814]

71 **de Souza JA**, Yap BJ, Wroblewski K, Blinder V, Araújo FS, Hlubocky FJ, Nicholas LH, O'Connor JM, Brockstein B, Ratain MJ, Daugherty CK, Cella D. Measuring financial toxicity as a clinically relevant patient-reported outcome: The validation of the COmprehensive Score for financial Toxicity (COST). *Cancer* 2017; **123**: 476-484 [PMID: 27716900 DOI: 10.1002/cncr.30369]

72 **Meeker CR**, Geynisman DM, Egleston BL, Hall MJ, Mechanic KY, Bilusic M, Plimack ER, Martin LP, von Mehren M, Lewis B, Wong YN. Relationships Among Financial Distress, Emotional Distress, and Overall Distress in Insured Patients With Cancer. *J Oncol Pract* 2016; **12**: e755-e764 [PMID: 27328795 DOI: 10.1200/JOP.2016.011049]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 230-241

DOI: 10.4251/wjgo.v14.i1.230

ISSN 1948-5204 (online)

MINIREVIEWS

Early-onset colorectal cancer: Current insights and future directions

Claudia Wing-Kwan Wu, Rashid N Lui

ORCID number: Claudia Wing-Kwan Wu 0000-0003-1401-0173; Rashid N Lui 0000-0003-1277-9595.

Author contributions: Wu CWK and Lui RN contributed equally to this work; all authors have read and approved the final manuscript.

Conflict-of-interest statement: All authors have no conflicts of interest to disclose.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Claudia Wing-Kwan Wu, Rashid N Lui, Division of Gastroenterology and Hepatology, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China

Rashid N Lui, Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong, China

Corresponding author: Rashid N Lui, MBChB, FRCP, Clinical Assistant Professor (Honorary), Division of Gastroenterology and Hepatology, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China.
rashidlui@cuhk.edu.hk

Abstract

Early-onset colorectal cancer (EOCRC) has seen an alarming rise worldwide over the past two decades. The reason for this global trend is poorly understood. EOCRC appears to have its own unique clinical and molecular features when compared with late-onset colorectal cancer. Younger patients appear to have more distal or rectal disease, a more advanced stage of disease at presentation, and more unfavorable histological features. Identifying risk factors for EOCRC is the first step in mitigating the rising burden of this disease. Here we summarize several noteworthy biological factors and environmental exposures that are postulated to be responsible culprits. This can hopefully translate in clinical practice to the development of better risk stratification tool for identifying high-risk individuals for early colorectal cancer screening, and identifying areas needed for further research to curb this rising trend.

Key Words: Early-onset colorectal cancer; Young-onset colorectal cancer; Risk factors; Environmental exposures; Microbiome; Genetics

Core Tip: The incidence of early onset colorectal cancer is on the rise. Herein, we discuss on various risk factors that have been implicated for these recent trends and point to where future research needs to be directed for better utilization of healthcare resources. Early recognition and diagnosis are essential for better outcomes of this preventable cancer.

Received: June 16, 2021
Peer-review started: June 16, 2021
First decision: July 29, 2021
Revised: August 2, 2021
Accepted: November 24, 2021
Article in press: November 24, 2021
Published online: January 15, 2022

P-Reviewer: Sacdalan DL
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Yan JP

Citation: Wu CWK, Lui RN. Early-onset colorectal cancer: Current insights and future directions. *World J Gastrointest Oncol* 2022; 14(1): 230-241
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/230.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.230

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer deaths worldwide. The International Agency for Research on Cancer estimated that there were 1.93 million new cases of CRC and 935000 deaths from CRC in 2020[1]. Early-onset CRC (EOCRC), largely defined as CRC occurring in adults younger than 50 years old, has seen an alarming rising trend in recent years[2-5].

A recent systemic review of 40 studies spanning 12 countries across five continents has found a nearly 30% increase in incidence of EOCRC around the world over the past 20 years, largely driven by increasing incidence in the United States, Australia, and Canada[6]. Since 1994, the incidence of EOCRC has been increasing by around 2% per year. This is alarming given that the overall incidence of and death from CRC has been on the decline[2]. An observational study done on CRC incidence in the United States population according to the Surveillance, Epidemiology, and End Results (SEER) registries found a steep increase in EOCRC incidence from age 49-50 years, with 92.9% of cases being invasive lesions picked up on screening[7]. This likely reflects that a significant proportion of the populations were screened too late, given that the goal of screening was to remove premalignant lesions to prevent malignant transformation. In 2018, the American Cancer Society (ACS) lowered their recommended age for average-risk adults to start screening at 45 years old[8]. Although this method allows early detection of advanced adenomas or CRC to reduce disease burden and mortality, this mass screening approach will likely lead to a substantial increase in cost and burden to the healthcare system.

EOCRC tends to have a predominantly left colonic and rectal distribution, a higher proportion of mucinous and signet ring histologic subtype, poorer cell differentiation, a higher pathologic grade, and a more advanced stage at presentation[9-11]. Although hereditary cancer syndromes and family history account for approximately 30% of EOCRC cases, the majority appear to arise sporadically[12]. To date, the underlying etiologies of this rising trend have not yet been fully elucidated. Identifying specific risk factors or causes to this trend can allow for the establishment of better risk-stratification models and more targeted screening to tackle this global phenomenon.

Multiple postulated risk factors have been identified that may be driving factors to the development of EOCRC. Exposure to many potential elements from an early age from conception to adulthood may predispose to a higher risk of EOCRC. This includes external factors such as socioeconomic background, lifestyle, diet, and antibiotic exposure; and intrinsic factors, such as genetics, gut microbiota, and oxidative stress[13].

Apart from the well-established risk factors for CRC such as male gender, smoking, alcoholism, family history of CRC, type 2 diabetes, and inflammatory bowel disease, many studies have attempted to study additional demographic and environmental factors that may be specific risk factors for EOCRC[10,14,15]. A meta-analysis examining 20 studies through MEDLINE and Embase database search found that Caucasian ethnicity, obesity, and hyperlipidemia, as well as male gender, alcohol, and history of CRC in a first-degree relative, were all significantly associated with the development of EOCRC[16]. A more sedentary lifestyle or occupation, ulcerative colitis, hypertension, and diet-related factors were also found to have an association with increased risk in some studies[14]. Here we discuss in more detail some of the key suspects implicated in the development of EOCRC (Figure 1).

RACIAL DISPARITIES

African Americans have been known to be at higher risk for the development of CRC compared with Caucasians, and this is usually associated with an earlier-onset and worse outcome[17]. Potential reasons for this disparity include lower socioeconomic status, limited access to healthcare, and lack of awareness of screening. Steps have

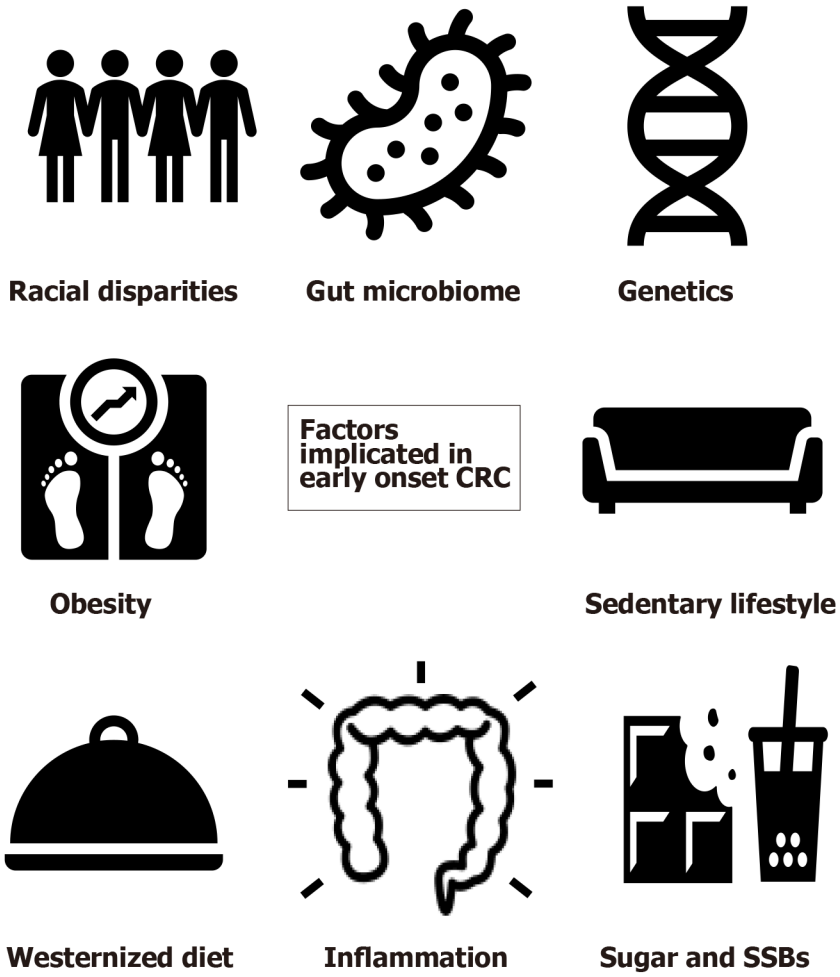


Figure 1 Factors implicated in early-onset colorectal cancer. Image source for colon: https://img.icons8.com. CRC: Colorectal cancer; SSB: Sugar sweetened beverages.

been taken over the years to close this gap in CRC risk with the American College of Gastroenterology and American Society of Gastrointestinal Endoscopists guidelines recommending an earlier age to start CRC screening for African Americans[18].

These efforts have led to tangible results with the gap closing between Whites and Blacks[19]. In fact, the incidence of rectal cancer in Whites has now surmounted that of the Blacks and Hispanics in recent years, and the overall incidence of EOCRC is now similar in the two groups since 2015[2]. Results of a SEER analysis examining the difference in incidence of CRC amongst White and Black EOCRC patients from 1992-1996 to 2010-2014 showed that there was a 47% relative increase in CRC incidence in Whites, compared to a 1% relative increase in Blacks[20]. The rise in EOCRC is mainly due to an increase in rectal cancer, which was seen most strikingly in the White population. This suggests that rectal cancer may have its own distinct characteristics and etiological differences from colon cancer. Nevertheless, the incidence of EOCRC is still climbing steadily regardless of ethnicity, highlighting the need for further research into meaningful interventions to curb this rise.

OBESITY AND SEDENTARY LIFESTYLE

Obesity has long been associated with an increased risk of CRC[21]. According to a recent propensity-weighted analysis which included 133008 adults diagnosed with EOCRC in the United States between 1999 and 2018, there was a strong association between EOCRC and a raised body mass index (BMI) of ≥ 30 kg/m^2, along with an earlier age of diabetes diagnosis[22]. A meta-analysis in 2017 found a 30% increased risk of CRC in men and a 12% increased risk of CRC in women for every 5 kg/m^2 increment increase in BMI[23]. There is also an increased risk of early-onset advanced adenoma amongst obese patients[24]. The underlying mechanism behind the

association between obesity and EOCRC is unclear, although it is postulated that there is an interplay between the risk of obesity, estrogen levels, and the risk of CRC, with obesity being a driver of chronic inflammation[25,26].

Of course, there are multiple confounding variables that may affect the relationship between obesity and EOCRC. This includes a reverse causality effect where CRC may induce weight loss. Obesity itself could also be a surrogate for other known risk factors for CRC. Metabolic syndrome, increased insulin resistance, raised insulin-like growth factor 1 (IGF-1), and raised low-density lipoprotein are all positively correlated with an increased risk for EOCRC[21,24].

Leading a sedentary lifestyle has also been recognized as an emerging global health problem due to increased desk work, the rising trend of e-commerce, and inactive media consumption since a young age[27]. A prospective study examining television viewing time (as a surrogate for sedentary time) in almost 90000 women aged 25 to 42 years in the United States found that more than 1 hour of daily TV viewing was associated with a 12% increased risk of CRC, particularly rectal cancer. More than 2 hours of TV viewing was associated with a 70% increase in risk. The risk appeared even higher in subgroups of patients with a high BMI, physical inactivity, and smokers [28].

Physical inactivity may result in lower energy use, higher caloric intake, and unhealthy dietary intake. It may also correlate with impaired glucose regulation or gut dysbiosis. Some studies have examined the role of increased physical activity to improve gut health by promoting certain bacterial species in the gut microbiome[29-31]. All in all, this highlights the importance of physical activity and controlling the obesity pandemic to prevent EOCRC.

WESTERN DIET

A growing adoption of a non-Mediterranean, Western diet worldwide has been consistently shown in the literature to be an important risk factor[32,33]. A diet high in red, processed meat, and low in fibre from a young age has been shown to affect the gut microbiota and drive inflammation processes[34-36]. Westernized cooking methods, such as deep-frying, grilling, or roasting, generate more advanced glycation end-products (AGEs), which are complex compounds produced from food that is rich in fat and protein[37,38]. They are involved in promoting oxidative stress and chronic inflammation, which in turn promote a microenvironment favorable for colorectal carcinogenesis. Many studies have shown that AGEs are responsible for signal pathways involved in colitis-associated colorectal carcinogenesis seen in inflammatory bowel disease[39]. Mediterranean food, on the other hand, has low AGE levels and has been found to be protective against the development of CRC[40-42].

A recent prospective cohort study, which examined dietary patterns in 29474 women who underwent colonoscopy at < 50 years of age, found that a Westernized diet was positively associated with high-risk distal or rectal adenomas, whereas healthier diets such as a prudent diet, Dietary Approaches to Stop Hypertension, Alternative Mediterranean Diet, and Alternative Healthy Eating Index were inversely associated with early onset adenomas[43]. Interestingly, some studies have found that the genetic composition of tumors associated with a Western diet tends to be KRAS wild-type, and BRAF-wild type[44]. These genetic compositions are consistent with the typical features of EOCRC.

A meta-analysis recently published suggested a strong association of higher intake of dietary fibre, calcium, and yoghurt with a reduced risk of CRC, with convincing evidence that intake of a Western diet and processed meat is associated with a higher risk of EOCRC[45]. Interestingly, the impact of yoghurt and calcium may be related with the modulation of the gut microbiome, such as the presence of lactic acid-producing bacteria, which may reduce the level of carcinogens in the gut. Yoghurt also creates a lower pH in the colon, which may be more accommodating for probiotics [46]. This supports the idea that modulating the gut microbiome with prebiotics and/or probiotics may have a potential role in preventing the development of CRC, which will be further discussed in a later section.

SUGAR

One of the other culprits in the plethora of Western food that may be a culprit for EOCRC is sugar. Refined sugars (including glucose, fructose, sucrose, and maltose) are

cheap and widely available worldwide. Sugar consumption in the form of snacks, desserts, sweets, or sugar-sweetened beverages has steeply increased especially during childhood and adolescence. Over the last decade, sugar consumption globally has grown from 154 to 171 million metric tons from 2009/2010 to 2019/2020[47]. This climb was found to be most significant in developing or low-income countries[48]. In a large United States cohort study that analyzed 95464 female registered nurses' dietary habits from the Nurses' Health Study II, it was found that high sugar (especially fructose) intake during adolescence was significantly associated with an increased risk of colorectal adenomas. Consuming two or more, rather than one, sugar-sweetened beverages a day in adolescence further increased the risk of EOCRC by two-fold[49].

Several mechanisms that tie sugar intake to the development of CRC have been postulated. High intake of sugar can promote obesity, insulin resistance, and type 2 diabetes[50,51]. Sugar, specifically fructose, may have a direct effect on the gut microbiome, leading to chronic inflammation and a heightened susceptibility of the colorectal epithelium to cellular damage[52]. Fructose also produces AGEs, which as previously discussed, has a potentially significant role in carcinogenesis[53]. Hyperinsulinemia and elevated IGF-1 levels can stimulate cell proliferation and differentiation, inhibit apoptosis, and in turn enhance tumor development. As adolescence is a period of pronounced physiological changes that include decreased insulin sensitivity and hyperinsulinemia, this stage of development may be particularly susceptible to the effects of a high sugar intake[49].

The link between diet, nutrients, and the pathogenesis of EOCRC is complex, with a myriad of processes involving immune signaling, genetic predisposition, and alterations in the gut microbiome. Other significant food exposures that may play a role in CRC include dietary additives, nitrate-containing foods, synthetic food colorings, monosodium glutamate, *etc*.[13,54]. Further studies on dietary causation links will bring to light any potential preventative measures for EOCRC.

GUT MICROBIOME

It is estimated that 100 billion bacteria reside in the gastrointestinal tract (with a large proportion present in the colon), maintaining a symbiotic relationship with the human host[55]. The gut microbiota maintains gut homeostasis and functions and is often considered the first line of defense against pathogens. The composition of the gut microbiome is dynamic and subject to change by multiple factors throughout our lives. The first 1-2 years of life are pivotal for the development of the gut microbiota[56]. From birth, the microbiota composition is believed to differ significantly depending on the mode of delivery. Vaginally delivered babies tend to have more *Lactobacilli*, whereas Caesarean-delivered babies tend to have delayed colonization of facultative anaerobes such as *Clostridium*[57]. Breast-fed and bottle-fed babies also have markedly different gut microbiota composition, with breastfed babies having a much higher abundance of bacteria that are thought to be beneficial, such as *Bifidobacterium* and *Lactobacillus* species[58]. The composition of the gut microbiota stabilizes in early adulthood, but is still influenced by exposures such as diet, antibiotics, stress, and inflammation. The gut microbiome is responsible for the synthesis of many important vitamins or molecules for our human body, such as butyrate, folate, biotin, and cobalamin[59]. Some of these molecules are important in reducing bacterial translocation and promoting anti-inflammatory properties, and are essential in maintaining gut barrier integrity[60].

Alterations of gut microbiome composition (or gut dysbiosis) can lead to dysregulation of multiple pathways in the body. Extensive or prolonged antibiotics use can destroy normal gut flora and lead to colonization of unwelcome pathogens. Several microorganisms, such as *Streptococcus bovis*, *Bacterioides fragilis*, *Salmonella enterica*, *Fusobacterium*, and *Escherichia coli*, have been discovered to have a role in colon carcinogenesis. These pathogens can promote gut inflammation, produce cancer-associated metabolites, and activate oncogenic signaling pathways[61]. Chronic inflammation from bacterial infection or inflammatory bowel disease can cause epithelial barrier dysfunction and weaken host defenses. Different dietary exposures can lead to significant shifts in the gut microbiome, favoring organisms capable of utilizing those specific nutrients. High-fat diets can lead to accumulation of lipopolysaccharides that can promote inflammation and increase VEGF-C expression, which is a key regulator for lymphangiogenesis and lymph node metastasis in CRC[62]. One study found that a drastic increase in fibre intake over 2 wk led to a change in microbiome composition to fibre-degrading bacteria, such as *Bifidobacterium* and

Lactobacillus, which has been associated with anti-oncogenic properties[63-65].

Probiotics have long been marketed to the general public as a dietary supplement for their potential beneficial effects on the gut[66]. The replenishment of beneficial intestinal microbial communities may help stimulate epithelial cell proliferation, reduce pathogenic overgrowth, ameliorate gut inflammation, and potentially reduce the risk of CRC[67-69]. Studies have also shown that certain strains of probiotics may be effective as an adjuvant agent to CRC treatment[70]. Yet, its effects specifically on CRC treatment are not well studied and further investigation is required.

Our diet from birth has a role in shaping our gut microbiome. Understanding the relationship between diet and gut dysbiosis teaches us that how we shape our diets at an early age could impact the development of CRC. Thus, it is important to encourage healthy eating habits from childhood to maintain a healthy microbiota. Nevertheless, it remains difficult to prove the causative link between dysbiosis in early human development and its association with EOCRC, and further research in this area is needed.

GENETIC FEATURES

Recognizing genetic alterations that can predispose to early onset of high-risk adenoma or CRC is crucial for deciding on early screening regimes and therapeutic strategies. Around 28% of EOCRC patients have a positive family history[71]. Patients with a first-degree relative of CRC have up to a four-fold increased lifetime risk of CRC[72]. Those with a known family history of a high-penetrance hereditary cancer syndrome, such as Lynch syndrome or adenomatous polyposis coli (APC), are at a particularly high risk and require an onset of colonoscopy screening at a much earlier age than the general population[73,74]. For non-hereditary cases, according to the ACS guidelines, those with a first-degree relative of CRC diagnosed before age 60 should also start colonoscopy screening from age 40, or 10 years younger than the earliest diagnosed relative[72]. However, low adherence to early screening guidelines is one of the major obstacles in EOCRC prevention. A study of 2473 patients with EOCRC found that family history-based early screening criteria were only adhered to in 25% of cases, and nearly all these patients could have had CRC diagnosed earlier or even prevented had they followed these guidelines[75]. This highlights the importance of public education on cancer screening programs.

Several studies have found that a significant proportion of EOCRC patients carrying a genetic mutation have no family history of CRC[10,71]. Apart from the well-recognized hereditary cancer syndromes accounting for around 13% of EOCRC cases, a wide spectrum of low to moderate penetrance sporadic mutations have recently been found in these patients, including some genes not traditionally associated with CRC [71,76]. A genome-wide association study found up to 140 single nucleotide polymorphisms associated with CRC[77]. Genetic mutations are much more common in EOCRC compared with those diagnosed at a later age[78] and may have a cumulative effect. However, in the absence of a positive family history, a proportion of these patients will not be enrolled into early screening programs with strategies to identify such patients being an unmet need[79].

The pathogenesis of CRC involves a complex sequence of multistep genetic alterations. There are three main genetic pathways of CRC carcinogenesis: Chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) pathways[80]. Each pathway is associated with specific genetic and epigenetic alterations. The CIN pathway is characterized by an accumulation of mutations in the tumor-suppressor and oncogenes, including *APC*, *KRAS*, and *TP53* amongst others, accounting for 85% of sporadic CRC cases. The MSI pathway, on the other hand, is a state of genetic hypermutability due to impaired DNA mismatch repair (MMR). MSI is the hallmark of Lynch syndrome-associated tumors, an autosomal dominant disorder characterized by the presence of DNA *MMR* genes (*e.g.*, *MLH1, MSH2, MSH6,* and *PMS2*), accounting for around 8% of EOCRC cases[71,81]. Lynch syndrome increases the lifetime risk of CRC to 52%-82% depending on the pathogenic variant involved[82]. The CIMP pathway and BRAF V600E mutation are thought to be the molecular hallmark of the serrated pathway and are usually associated with proximal lesions[83].

EOCRC has distinct genetic features compared with late-onset CRC. A retrospective review of around 36000 CRC patients comparing genetic characteristics in different age groups showed that EOCRC patients are more likely to be MSI and have CTNNB1, ATM mutations, and CIMP hypermethylation. The consensus molecular subtype 1

was the most common CRC subtype in patients younger than 40 years old. There were fewer BRAF V600 mutations (< 4%) in patients less than 30 years old. KRAS, NRAS, and BRAF mutations in the mitogen-activated protein kinase pathway were lowest in the 18-29-year-old group (48%), and highest in the 70-year-old or older group (65%-70%)[84]. Hypermethylation of *ESR1*, *GATA5*, and *WT1* genes were also found to be suggestive of earlier diagnosis of CRC[85].

Certain genetic mutations may infer a higher rate of progression or be predictive factors for treatment resistance. KRAS mutation confers resistance to anti-EGFR therapy. Several studies have demonstrated that MSI tumors have a lack of response to 5FU-based chemotherapy[86]. Given that around 1 in 5 patients with EOCRC have a germline mutation, broad germline testing should be considered for all EOCRC patients to guide treatment modalities, prognostication, counselling to family members, and chemoprevention strategies[76,87].

Establishing a good predictive model for risk stratification of many genetic variants predisposing to CRC is important for more targeted screening of high-risk patients. A study using a polygenic risk score (PRS) derived from 95 common genetic variants was able to predict the risk of EOCRC when testing 12197 early-onset CRC and 95865 late-onset CRC patients of European descent. A higher PRS is more strongly associated with EOCRC than late-onset patients. Those in the highest PRS quartile had a 3.7-fold increased risk of EOCRC compared with those in the lowest quartile. Interestingly, high PRS cases also had a tendency towards distal and rectal tumors[78]. PRS may therefore be a useful tool to stratify risk when used alongside the identification of other lifestyle and environmental risk factors, and may pick up some high risk patients within the average-risk screening group who would otherwise have not been identified based on conventional criteria. This may provide a more targeted and personalized approach for CRC screening than our current standard of care.

CONCLUSION

CRC is a genetic and molecularly heterogeneous disease. EOCRC represents a subgroup of CRC with unique characteristics. Genetic predisposition and multiple risk factors are being explored as potential contributors to this rising trend. Given the long process of transition from non-neoplastic cells to malignancy, exploring early-life exposures as potential culprits is important[13]. Increasing evidence has shown that obesity, sedentary lifestyle, Westernized diet, and high sugar intake are significant risk factors for EOCRC. Exposures as early as in the prenatal or perinatal stages of life, such as maternal diet or delivery methods, have been postulated to affect the composition of the gut microbiota. However, studies that prove causality remain elusive. Large epidemiological studies are still needed to further discover or verify potential causative factors.

The relationship between diet, lifestyle, and gut dysbiosis and their respective roles in colorectal carcinogenesis are complex. The composition of gut microbiome is dynamic and dependent on multiple factors including race, age, lifestyle, diet, medication use, stress, *etc.* There is currently no clear consensus for the definition of gut dysbiosis due to the high microbial heterogeneity in CRC[88]. Further investigations on the gut microenvironment from stool samples in CRC patients may help characterize the gut microbiome that predisposes to CRC, with emerging evidence that shows promise for its use in CRC screening and risk stratification. Future research on manipulating the gut microbiome through diet or drugs like probiotics may even play a role in cancer prevention.

Apart from the need for further research on exploring the unanswered questions of the underlying cause and mechanisms behind EOCRC, numerous barriers to the reduction of the incidence of EOCRC still exist. Poor compliance with early screening programs may be due to inadequate public awareness[89]. Information on family history may not be known to patients. Young patients and physicians alike tend to attribute early symptoms to non-sinister pathologies that may result in a delay of diagnosis. A study of young patients has shown that they present to a medical practitioner on average 294 d after the onset of rectal bleeding, which likely resulted in a more advanced stage of disease[90]. With regard to healthcare systems, there may be access, cost, or policy barriers to screening and treatment.

Steps to fight EOCRC include raising awareness of this growing threat through education and public promotion. This includes public awareness campaigns, educating the public on the dietary or lifestyle risks of CRC, and enhancing physician awareness of EOCRC. Advising young patients to stay vigilant of early symptoms,

such as per-rectal bleeding, abdominal pain, weight loss, and change in bowel habits and to seek timely medical attention is also important. Promoting awareness of early colonoscopy screening for high-risk groups, and referring patients who are eligible for genetic counselling and testing are essential for early identification of at-risk individuals. Further research on predisposing genetic and epigenetic signatures is needed. In the future, we should strive for specific genetic profiling through whole-genome sequencing for better risk stratification[91]. It may be useful to see how well specific risk stratification tools including lifestyle risks or PRS perform in the real world to identify high-risk patients for a more personalized screening strategy, which in turn may allow for better allocation of resources to those most in need to combat this global rise in EOCRC.

REFERENCES

1 **Cancer Today**. Global Cancer Observatory. International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today/fact-sheets-cancers

2 **Surveillance**, Epidemiology, and End Results. Cancer of the Colon and Rectum - Cancer Stat Facts. Available from: https://seer.cancer.gov/statfacts/html/colorect.html

3 **Siegel RL**, Torre LA, Soerjomataram I, Hayes RB, Bray F, Weber TK, Jemal A. Global patterns and trends in colorectal cancer incidence in young adults. *Gut* 2019; **68**: 2179-2185 [PMID: 31488504 DOI: 10.1136/gutjnl-2019-319511]

4 **Lui RN**, Tsoi KKF, Ho JMW, Lo CM, Chan FCH, Kyaw MH, Sung JJY. Global Increasing Incidence of Young-Onset Colorectal Cancer Across 5 Continents: A Joinpoint Regression Analysis of 1,922,167 Cases. *Cancer Epidemiol Biomarkers Prev* 2019; **28**: 1275-1282 [PMID: 31113868 DOI: 10.1158/1055-9965.EPI-18-1111]

5 **Sung JJY**, Chiu HM, Jung KW, Jun JK, Sekiguchi M, Matsuda T, Kyaw MH. Increasing Trend in Young-Onset Colorectal Cancer in Asia: More Cancers in Men and More Rectal Cancers. *Am J Gastroenterol* 2019; **114**: 322-329 [PMID: 30694865 DOI: 10.14309/ajg.0000000000000133]

6 **Saad El Din K**, Loree JM, Sayre EC, Gill S, Brown CJ, Dau H, De Vera MA. Trends in the epidemiology of young-onset colorectal cancer: a worldwide systematic review. *BMC Cancer* 2020; **20**: 288 [PMID: 32252672 DOI: 10.1186/s12885-020-06766-9]

7 **Abualkhair WH**, Zhou M, Ahnen D, Yu Q, Wu XC, Karlitz JJ. Trends in Incidence of Early-Onset Colorectal Cancer in the United States Among Those Approaching Screening Age. *JAMA Netw Open* 2020; **3**: e1920407 [PMID: 32003823 DOI: 10.1001/jamanetworkopen.2019.20407]

8 **Peterse EFP**, Meester RGS, Siegel RL, Chen JC, Dwyer A, Ahnen DJ, Smith RA, Zauber AG, Lansdorp-Vogelaar I. The impact of the rising colorectal cancer incidence in young adults on the optimal age to start screening: Microsimulation analysis I to inform the American Cancer Society colorectal cancer screening guideline. *Cancer* 2018; **124**: 2964-2973 [PMID: 29846933 DOI: 10.1002/cncr.31543]

9 **O'Connell JB**, Maggard MA, Liu JH, Etzioni DA, Livingston EH, Ko CY. Rates of colon and rectal cancers are increasing in young adults. *Am Surg* 2003; **69**: 866-872 [PMID: 14570365 DOI: 10.1046/j.1600-6143.2003.00209.x]

10 **Mauri G**, Sartore-Bianchi A, Russo AG, Marsoni S, Bardelli A, Siena S. Early-onset colorectal cancer in young individuals. *Mol Oncol* 2019; **13**: 109-131 [PMID: 30520562 DOI: 10.1002/1878-0261.12417]

11 **Khan M**, Korphaisarn K, Saif A, Foo WC, Kopetz S. Early-Onset Signet-Ring Cell Adenocarcinoma of the Colon: A Case Report and Review of the Literature. *Case Rep Oncol Med* 2017; **2017**: 2832180 [PMID: 28326211 DOI: 10.1155/2017/2832180]

12 **Wells K**, Wise PE. Hereditary Colorectal Cancer Syndromes. *Surg Clin North Am* 2017; **97**: 605-625 [PMID: 28501250 DOI: 10.1016/j.suc.2017.01.009]

13 **Hofseth LJ**, Hebert JR, Chanda A, Chen H, Love BL, Pena MM, Murphy EA, Sajish M, Sheth A, Buckhaults PJ, Berger FG. Early-onset colorectal cancer: initial clues and current views. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 352-364 [PMID: 32086499 DOI: 10.1038/s41575-019-0253-4]

14 **Rawla P**, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019; **14**: 89-103 [PMID: 31616522 DOI: 10.5114/pg.2018.81072]

15 **Jiang Y**, Ben Q, Shen H, Lu W, Zhang Y, Zhu J. Diabetes mellitus and incidence and mortality of colorectal cancer: a systematic review and meta-analysis of cohort studies. *Eur J Epidemiol* 2011; **26**: 863-876 [PMID: 21938478 DOI: 10.1007/s10654-011-9617-y]

16 **O'Sullivan DE**, Sutherland RL, Town S, Chow K, Fan J, Forbes N, Heitman SJ, Hilsden RJ, Brenner DR. Risk Factors for Early-Onset Colorectal Cancer: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2021 [PMID: 33524598 DOI: 10.1016/j.cgh.2021.01.037]

17 **Carethers JM**. Screening for colorectal cancer in African Americans: determinants and rationale for an earlier age to commence screening. *Dig Dis Sci* 2015; **60**: 711-721 [PMID: 25540085 DOI: 10.1007/s10620-014-3443-5]

18 **Shaukat A**, Kahi CJ, Burke CA, Rabeneck L, Sauer BG, Rex DK. ACG Clinical Guidelines: Colorectal Cancer Screening 2021. *Am J Gastroenterol* 2021; **116**: 458-479 [PMID: 33657038 DOI:

10.14309/ajg.0000000000001122]
19 **Muller C**, Ihionkhan E, Stoffel EM, Kupfer SS. Disparities in Early-Onset Colorectal Cancer. *Cells* 2021; **10** [PMID: 33925893 DOI: 10.3390/cells10051018]
20 **Murphy CC**, Wallace K, Sandler RS, Baron JA. Racial Disparities in Incidence of Young-Onset Colorectal Cancer and Patient Survival. *Gastroenterology* 2019; **156**: 958-965 [PMID: 30521807 DOI: 10.1053/j.gastro.2018.11.060]
21 **Ma Y**, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. *PLoS One* 2013; **8**: e53916 [PMID: 23349764 DOI: 10.1371/journal.pone.0053916]
22 **Hussan H**, Patel A, Hinton A, Ma Q, Tabung, Fred KT, Clinton S. The Associations Between Obesity and Early Onset Colorectal Cancer: A Propensity-Weighted Analysis of the National Health and Nutrition Examination Survey (NHANES). *Am J Gastroenterol* 2020; 115 [DOI: 10.14309/01.ajg.0000706892.59075.79]
23 **Kyrgiou M**, Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, Martin-Hirsch P, Tsilidis KK. Adiposity and cancer at major anatomical sites: umbrella review of the literature. *BMJ* 2017; **356**: j477 [PMID: 28246088 DOI: 10.1136/bmj.j477]
24 **Kim JY**, Jung YS, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Choi KY, Park DI. Different risk factors for advanced colorectal neoplasm in young adults. *World J Gastroenterol* 2016; **22**: 3611-3620 [PMID: 27053853 DOI: 10.3748/wjg.v22.i13.3611]
25 **Liu PH**, Wu K, Ng K, Zauber AG, Nguyen LH, Song M, He X, Fuchs CS, Ogino S, Willett WC, Chan AT, Giovannucci EL, Cao Y. Association of Obesity With Risk of Early-Onset Colorectal Cancer Among Women. *JAMA Oncol* 2019; **5**: 37-44 [PMID: 30326010 DOI: 10.1001/jamaoncol.2018.4280]
26 **Ye P**, Xi Y, Huang Z, Xu P. Linking Obesity with Colorectal Cancer: Epidemiology and Mechanistic Insights. *Cancers (Basel)* 2020; **12** [PMID: 32486076 DOI: 10.3390/cancers12061408]
27 **Park JH**, Moon JH, Kim HJ, Kong MH, Oh YH. Sedentary Lifestyle: Overview of Updated Evidence of Potential Health Risks. *Korean J Fam Med* 2020; **41**: 365-373 [PMID: 33242381 DOI: 10.4082/kjfm.20.0165]
28 **Nguyen LH**, Liu PH, Zheng X, Keum N, Zong X, Li X, Wu K, Fuchs CS, Ogino S, Ng K, Willett WC, Chan AT, Giovannucci EL, Cao Y. Sedentary Behaviors, TV Viewing Time, and Risk of Young-Onset Colorectal Cancer. *JNCI Cancer Spectr* 2018; **2**: pky073 [PMID: 30740587 DOI: 10.1093/jncics/pky073]
29 **Brennan CA**, Garrett WS. Gut Microbiota, Inflammation, and Colorectal Cancer. *Annu Rev Microbiol* 2016; **70**: 395-411 [PMID: 27607555 DOI: 10.1146/annurev-micro-102215-095513]
30 **Bressa C**, Bailén-Andrino M, Pérez-Santiago J, González-Soltero R, Pérez M, Montalvo-Lominchar MG, Maté-Muñoz JL, Domínguez R, Moreno D, Larrosa M. Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS One* 2017; **12**: e0171352 [PMID: 28187199 DOI: 10.1371/journal.pone.0171352]
31 **Mach N**, Fuster-Botella D. Endurance exercise and gut microbiota: A review. *J Sport Health Sci* 2017; **6**: 179-197 [PMID: 30356594 DOI: 10.1016/j.jshs.2016.05.001]
32 **Castelló A**, Amiano P, Fernández de Larrea N, Martín V, Alonso MH, Castaño-Vinyals G, Pérez-Gómez B, Olmedo-Requena R, Guevara M, Fernandez-Tardon G, Dierssen-Sotos T, Llorens-Ivorra C, Huerta JM, Capelo R, Fernández-Villa T, Díez-Villanueva A, Urtiaga C, Castilla J, Jiménez-Moleón JJ, Moreno V, Dávila-Batista V, Kogevinas M, Aragonés N, Pollán M; MCC-Spain researchers. Low adherence to the western and high adherence to the mediterranean dietary patterns could prevent colorectal cancer. *Eur J Nutr* 2019; **58**: 1495-1505 [PMID: 29582162 DOI: 10.1007/s00394-018-1674-5]
33 **Feng YL**, Shu L, Zheng PF, Zhang XY, Si CJ, Yu XL, Gao W, Zhang L. Dietary patterns and colorectal cancer risk: a meta-analysis. *Eur J Cancer Prev* 2017; **26**: 201-211 [PMID: 26945285 DOI: 10.1097/CEJ.0000000000000245]
34 **Albracht-Schulte K**, Islam T, Johnson P, Moustaid-Moussa N. Systematic Review of Beef Protein Effects on Gut Microbiota: Implications for Health. *Adv Nutr* 2021; **12**: 102-114 [PMID: 32761179 DOI: 10.1093/advances/nmaa085]
35 **Larsson SC**, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *Int J Cancer* 2006; **119**: 2657-2664 [PMID: 16991129 DOI: 10.1002/ijc.22170]
36 **Chan DS**, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, Norat T. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS One* 2011; **6**: e20456 [PMID: 21674008 DOI: 10.1371/journal.pone.0020456]
37 **Zhang Q**, Wang Y, Fu L. Dietary advanced glycation end-products: Perspectives linking food processing with health implications. *Compr Rev Food Sci Food Saf* 2020; **19**: 2559-2587 [PMID: 33336972 DOI: 10.1111/1541-4337.12593]
38 **Omofuma OO**, Turner DP, Peterson LL, Merchant AT, Zhang J, Steck SE. Dietary Advanced Glycation End-products (AGE) and Risk of Breast Cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). *Cancer Prev Res (Phila)* 2020; **13**: 601-610 [PMID: 32169887 DOI: 10.1158/1940-6207.CAPR-19-0457]
39 **Azizian-Farsani F**, Abedpoor N, Hasan Sheikhha M, Gure AO, Nasr-Esfahani MH, Ghaedi K. Receptor for Advanced Glycation End Products Acts as a Fuel to Colorectal Cancer Development. *Front Oncol* 2020; **10**: 552283 [PMID: 33117687 DOI: 10.3389/fonc.2020.552283]
40 **Lopez-Moreno J**, Quintana-Navarro GM, Delgado-Lista J, Garcia-Rios A, Delgado-Casado N,

Camargo A, Perez-Martinez P, Striker GE, Tinahones FJ, Perez-Jimenez F, Lopez-Miranda J, Yubero-Serrano EM. Mediterranean Diet Reduces Serum Advanced Glycation End Products and Increases Antioxidant Defenses in Elderly Adults: A Randomized Controlled Trial. *J Am Geriatr Soc* 2016; **64**: 901-904 [PMID: 27100598 DOI: 10.1111/jgs.14062]

41 **Farinetti A**, Zurlo V, Manenti A, Coppi F, Mattioli AV. Mediterranean diet and colorectal cancer: A systematic review. *Nutrition* 2017; **43-44**: 83-88 [PMID: 28935150 DOI: 10.1016/j.nut.2017.06.008]

42 **Grosso G**, Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S, Drago F, Basile F. Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. *Nutr Cancer* 2014; **66**: 558-565 [PMID: 24754383 DOI: 10.1080/01635581.2014.902975]

43 **Zheng X**, Hur J, Nguyen LH, Liu J, Song M, Wu K, Smith-Warner SA, Ogino S, Willett WC, Chan AT, Giovannucci E, Cao Y. Comprehensive Assessment of Diet Quality and Risk of Precursors of Early-Onset Colorectal Cancer. *J Natl Cancer Inst* 2021; **113**: 543-552 [PMID: 33136160 DOI: 10.1093/jnci/djaa164]

44 **Mehta RS**, Song M, Nishihara R, Drew DA, Wu K, Qian ZR, Fung TT, Hamada T, Masugi Y, da Silva A, Shi Y, Li W, Gu M, Willett WC, Fuchs CS, Giovannucci EL, Ogino S, Chan AT. Dietary Patterns and Risk of Colorectal Cancer: Analysis by Tumor Location and Molecular Subtypes. *Gastroenterology* 2017; **152**: 1944-1953.e1 [PMID: 28249812 DOI: 10.1053/j.gastro.2017.02.015]

45 **Veettil SK**, Wong TY, Loo YS, Playdon MC, Lai NM, Giovannucci EL, Chaiyakunapruk N. Role of Diet in Colorectal Cancer Incidence: Umbrella Review of Meta-analyses of Prospective Observational Studies. *JAMA Netw Open* 2021; **4**: e2037341 [PMID: 33591366 DOI: 10.1001/jamanetworkopen.2020.37341]

46 **Zheng X**, Wu K, Song M, Ogino S, Fuchs CS, Chan AT, Giovannucci EL, Cao Y, Zhang X. Yogurt consumption and risk of conventional and serrated precursors of colorectal cancer. *Gut* 2020; **69**: 970-972 [PMID: 31209182 DOI: 10.1136/gutjnl-2019-318374]

47 **Shahbandeh M**. Global Sugar Consumption, 2020/21. Statista. 2021 May 27. Available from: https://www.statista.com/statistics/249681/total-consumption-of-sugar-worldwide/

48 **OECD iLibrary**. OECD-FAO Agricultural Outlook 2019-2028. OECD Publishing. 2019. Available from: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2019-2028_bdef14fa-en

49 **Hur J**, Otegbeye E, Joh HK, Nimptsch K, Ng K, Ogino S, Meyerhardt JA, Chan AT, Willett WC, Wu K, Giovannucci E, Cao Y. Sugar-sweetened beverage intake in adulthood and adolescence and risk of early-onset colorectal cancer among women. *Gut* 2021 [PMID: 33958435 DOI: 10.1136/gutjnl-2020-323450]

50 **Slattery ML**, Benson J, Berry TD, Duncan D, Edwards SL, Caan BJ, Potter JD. Dietary sugar and colon cancer. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 677-685 [PMID: 9298574 DOI: 10.1016/S0278-6915(97)85474-9]

51 **Malik VS**, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* 2010; **121**: 1356-1364 [PMID: 20308626 DOI: 10.1161/CIRCULATIONAHA.109.876185]

52 **Zhao S**, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, Zeng X, Trefely S, Fernandez S, Carrer A, Miller KD, Schug ZT, Snyder NW, Gade TP, Titchenell PM, Rabinowitz JD, Wellen KE. Dietary fructose feeds hepatic lipogenesis *via* microbiota-derived acetate. *Nature* 2020; **579**: 586-591 [PMID: 32214246 DOI: 10.1038/s41586-020-2101-7]

53 **Sotokawauchi A**, Matsui T, Higashimoto Y, Yamagishi SI. Fructose causes endothelial cell damage *via* activation of advanced glycation end products-receptor system. *Diab Vasc Dis Res* 2019; **16**: 556-561 [PMID: 31375034 DOI: 10.1177/1479164119866390]

54 **Crowe W**, Elliott CT, Green BD. A Review of the In Vivo Evidence Investigating the Role of Nitrite Exposure from Processed Meat Consumption in the Development of Colorectal Cancer. *Nutrients* 2019; **11** [PMID: 31694233 DOI: 10.3390/nu11112673]

55 **Dahmus JD**, Kotler DL, Kastenberg DM, Kistler CA. The gut microbiome and colorectal cancer: a review of bacterial pathogenesis. *J Gastrointest Oncol* 2018; **9**: 769-777 [PMID: 30151274 DOI: 10.21037/jgo.2018.04.07]

56 **Bäckhed F**, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 2015; **17**: 690-703 [PMID: 25974306 DOI: 10.1016/j.chom.2015.04.004]

57 **Thursby E**, Juge N. Introduction to the human gut microbiota. *Biochem J* 2017; **474**: 1823-1836 [PMID: 28512250 DOI: 10.1042/BCJ20160510]

58 **Moore RE**, Townsend SD. Temporal development of the infant gut microbiome. *Open Biol* 2019; **9**: 190128 [PMID: 31506017 DOI: 10.1098/rsob.190128]

59 **O'Keefe SJ**, Ou J, Aufreiter S, O'Connor D, Sharma S, Sepulveda J, Fukuwatari T, Shibata K, Mawhinney T. Products of the colonic microbiota mediate the effects of diet on colon cancer risk. *J Nutr* 2009; **139**: 2044-2048 [PMID: 19741203 DOI: 10.3945/jn.109.104380]

60 **Chen J**, Vitetta L. The Role of Butyrate in Attenuating Pathobiont-Induced Hyperinflammation. *Immune Netw* 2020; **20**: e15 [PMID: 32395367 DOI: 10.4110/in.2020.20.e15]

61 **Tilg H**, Adolph TE, Gerner RR, Moschen AR. The Intestinal Microbiota in Colorectal Cancer. *Cancer Cell* 2018; **33**: 954-964 [PMID: 29657127 DOI: 10.1016/j.ccell.2018.03.004]

62 **Zhu G**, Huang Q, Huang Y, Zheng W, Hua J, Yang S, Zhuang J, Wang J, Ye J. Lipopolysaccharide

increases the release of VEGF-C that enhances cell motility and promotes lymphangiogenesis and lymphatic metastasis through the TLR4- NF-κB/JNK pathways in colorectal cancer. *Oncotarget* 2016; **7**: 73711-73724 [PMID: 27713159 DOI: 10.18632/oncotarget.12449]

63 **Oliver A**, Chase AB, Weihe C, Orchanian SB, Riedel SF, Hendrickson CL, Lay M, Sewall JM, Martiny JBH, Whiteson K. High-Fiber, Whole-Food Dietary Intervention Alters the Human Gut Microbiome but Not Fecal Short-Chain Fatty Acids. *mSystems* 2021; **6** [PMID: 33727392 DOI: 10.1128/mSystems.00115-21]

64 **Bahmani S**, Azarpira N, Moazamian E. Anti-colon cancer activity of Bifidobacterium metabolites on colon cancer cell line SW742. *Turk J Gastroenterol* 2019; **30**: 835-842 [PMID: 31530527 DOI: 10.5152/tjg.2019.18451]

65 **Wei H**, Chen L, Lian G, Yang J, Li F, Zou Y, Lu F, Yin Y. Antitumor mechanisms of bifidobacteria. *Oncol Lett* 2018; **16**: 3-8 [PMID: 29963126 DOI: 10.3892/ol.2018.8692]

66 **Swanson KS**, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, Scott KP, Holscher HD, Azad MB, Delzenne NM, Sanders ME. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 687-701 [PMID: 32826966 DOI: 10.1038/s41575-020-0344-2]

67 **Dikeocha IJ**, Al-Kabsi AM, Hussin S, Alshawsh MA. Role of probiotics in patients with colorectal cancer: a systematic review protocol of randomised controlled trial studies. *BMJ Open* 2020; **10**: e038128 [PMID: 32771989 DOI: 10.1136/bmjopen-2020-038128]

68 **Dos Reis SA**, da Conceição LL, Siqueira NP, Rosa DD, da Silva LL, Peluzio MD. Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutr Res* 2017; **37**: 1-19 [PMID: 28215310 DOI: 10.1016/j.nutres.2016.11.009]

69 **Cruz BCS**, Sarandy MM, Messias AC, Gonçalves RV, Ferreira CLLF, Peluzio MCG. Preclinical and clinical relevance of probiotics and synbiotics in colorectal carcinogenesis: a systematic review. *Nutr Rev* 2020; **78**: 667-687 [PMID: 31917829 DOI: 10.1093/nutrit/nuz087]

70 **Sivamaruthi BS**, Kesika P, Chaiyasut C. The Role of Probiotics in Colorectal Cancer Management. *Evid Based Complement Alternat Med* 2020; **2020**: 3535982 [PMID: 32148539 DOI: 10.1155/2020/3535982]

71 **Daca Alvarez M**, Quintana I, Terradas M, Mur P, Balaguer F, Valle L. The Inherited and Familial Component of Early-Onset Colorectal Cancer. *Cells* 2021; **10** [PMID: 33806975 DOI: 10.3390/cells10030710]

72 **Wilkins T**, McMechan D, Talukder A, Herline A. Colorectal Cancer Screening and Surveillance in Individuals at Increased Risk. *Am Fam Physician* 2018; **97**: 111-116 [PMID: 29365221]

73 **Perrod G**, Rahmi G, Cellier C. Colorectal cancer screening in Lynch syndrome: Indication, techniques and future perspectives. *Dig Endosc* 2021; **33**: 520-528 [PMID: 32314431 DOI: 10.1111/den.13702]

74 **van Leerdam ME**, Roos VH, van Hooft JE, Dekker E, Jover R, Kaminski MF, Latchford A, Neumann H, Pellisé M, Saurin JC, Tanis PJ, Wagner A, Balaguer F, Ricciardiello L. Endoscopic management of polyposis syndromes: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2019; **51**: 877-895 [PMID: 31342472 DOI: 10.1055/a-0965-0605]

75 **Gupta S**, Bharti B, Ahnen DJ, Buchanan DD, Cheng IC, Cotterchio M, Figueiredo JC, Gallinger SJ, Haile RW, Jenkins MA, Lindor NM, Macrae FA, Le Marchand L, Newcomb PA, Thibodeau SN, Win AK, Martinez ME. Potential impact of family history-based screening guidelines on the detection of early-onset colorectal cancer. *Cancer* 2020; **126**: 3013-3020 [PMID: 32307706 DOI: 10.1002/cncr.32851]

76 **Pearlman R**, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, Goldberg RM, Paskett E, Shields PG, Freudenheim JL, Stanich PP, Lattimer I, Arnold M, Liyanarachchi S, Kalady M, Heald B, Greenwood C, Paquette I, Prues M, Draper DJ, Lindeman C, Kuebler JP, Reynolds K, Brell JM, Shaper AA, Mahesh S, Buie N, Weeman K, Shine K, Haut M, Edwards J, Bastola S, Wickham K, Khanduja KS, Zacks R, Pritchard CC, Shirts BH, Jacobson A, Allen B, de la Chapelle A, Hampel H; Ohio Colorectal Cancer Prevention Initiative Study Group. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. *JAMA Oncol* 2017; **3**: 464-471 [PMID: 27978560 DOI: 10.1001/jamaoncol.2016.5194]

77 **Thomas M**, Sakoda LC, Hoffmeister M, Rosenthal EA, Lee JK, van Duijnhoven FJB, Platz EA, Wu AH, Dampier CH, de la Chapelle A, Wolk A, Joshi AD, Burnett-Hartman A, Gsur A, Lindblom A, Castells A, Win AK, Namjou B, Van Guelpen B, Tangen CM, He Q, Li CI, Schafmayer C, Joshu CE, Ulrich CM, Bishop DT, Buchanan DD, Schaid D, Drew DA, Muller DC, Duggan D, Crosslin DR, Albanes D, Giovannucci EL, Larson E, Qu F, Mentch F, Giles GG, Hakonarson H, Hampel H, Stanaway IB, Figueiredo JC, Huyghe JR, Minnier J, Chang-Claude J, Hampe J, Harley JB, Visvanathan K, Curtis KR, Offit K, Li L, Le Marchand L, Vodickova L, Gunter MJ, Jenkins MA, Slattery ML, Lemire M, Woods MO, Song M, Murphy N, Lindor NM, Dikilitas O, Pharoah PDP, Campbell PT, Newcomb PA, Milne RL, MacInnis RJ, Castellví-Bel S, Ogino S, Berndt SI, Bézieau S, Thibodeau SN, Gallinger SJ, Zaidi SH, Harrison TA, Keku TO, Hudson TJ, Vymetalkova V, Moreno V, Martín V, Arndt V, Wei WQ, Chung W, Su YR, Hayes RB, White E, Vodicka P, Casey G, Gruber SB, Schoen RE, Chan AT, Potter JD, Brenner H, Jarvik GP, Corley DA, Peters U, Hsu L. Genome-wide Modeling of Polygenic Risk Score in Colorectal Cancer Risk. *Am J Hum Genet* 2020; **107**: 432-444 [PMID: 32758450 DOI: 10.1016/j.ajhg.2020.07.006]

78 **Archambault AN**, Su YR, Jeon J, Thomas M, Lin Y, Conti DV, Win AK, Sakoda LC, Lansdorp-

Vogelaar I, Peterse EFP, Zauber AG, Duggan D, Holowatyj AN, Huyghe JR, Brenner H, Cotterchio M, Bézieau S, Schmit SL, Edlund CK, Southey MC, MacInnis RJ, Campbell PT, Chang-Claude J, Slattery ML, Chan AT, Joshi AD, Song M, Cao Y, Woods MO, White E, Weinstein SJ, Ulrich CM, Hoffmeister M, Bien SA, Harrison TA, Hampe J, Li CI, Schafmayer C, Offit K, Pharoah PD, Moreno V, Lindblom A, Wolk A, Wu AH, Li L, Gunter MJ, Gsur A, Keku TO, Pearlman R, Bishop DT, Castellví-Bel S, Moreira L, Vodicka P, Kampman E, Giles GG, Albanes D, Baron JA, Berndt SI, Brezina S, Buch S, Buchanan DD, Trichopoulou A, Severi G, Chirlaque MD, Sánchez MJ, Palli D, Kühn T, Murphy N, Cross AJ, Burnett-Hartman AN, Chanock SJ, de la Chapelle A, Easton DF, Elliott F, English DR, Feskens EJM, FitzGerald LM, Goodman PJ, Hopper JL, Hudson TJ, Hunter DJ, Jacobs EJ, Joshu CE, Küry S, Markowitz SD, Milne RL, Platz EA, Rennert G, Rennert HS, Schumacher FR, Sandler RS, Seminara D, Tangen CM, Thibodeau SN, Toland AE, van Duijnhoven FJB, Visvanathan K, Vodickova L, Potter JD, Männistö S, Weigl K, Figueiredo J, Martín V, Larsson SC, Parfrey PS, Huang WY, Lenz HJ, Castelao JE, Gago-Dominguez M, Muñoz-Garzón V, Mancao C, Haiman CA, Wilkens LR, Siegel E, Barry E, Younghusband B, Van Guelpen B, Harlid S, Zeleniuch-Jacquotte A, Liang PS, Du M, Casey G, Lindor NM, Le Marchand L, Gallinger SJ, Jenkins MA, Newcomb PA, Gruber SB, Schoen RE, Hampel H, Corley DA, Hsu L, Peters U, Hayes RB. Cumulative Burden of Colorectal Cancer-Associated Genetic Variants Is More Strongly Associated With Early-Onset vs Late-Onset Cancer. *Gastroenterology* 2020; **158**: 1274-1286.e12 [PMID: 31866242 DOI: 10.1053/j.gastro.2019.12.012]

79 **Mork ME**, You YN, Ying J, Bannon SA, Lynch PM, Rodriguez-Bigas MA, Vilar E. High Prevalence of Hereditary Cancer Syndromes in Adolescents and Young Adults With Colorectal Cancer. *J Clin Oncol* 2015; **33**: 3544-3549 [PMID: 26195711 DOI: 10.1200/JCO.2015.61.4503]

80 **Nguyen HT**, Duong HQ. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. *Oncol Lett* 2018; **16**: 9-18 [PMID: 29928381 DOI: 10.3892/ol.2018.8679]

81 **Yurgelun MB**, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, Ukaegbu CI, Brais LK, McNamara PG, Mayer RJ, Schrag D, Meyerhardt JA, Ng K, Kidd J, Singh N, Hartman AR, Wenstrup RJ, Syngal S. Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer. *J Clin Oncol* 2017; **35**: 1086-1095 [PMID: 28135145 DOI: 10.1200/JCO.2016.71.0012]

82 **Jang E**, Chung DC. Hereditary colon cancer: lynch syndrome. *Gut Liver* 2010; **4**: 151-160 [PMID: 20559516 DOI: 10.5009/gnl.2010.4.2.151]

83 **Tapial S**, Olmedillas-López S, Rueda D, Arriba M, García JL, Vivas A, Pérez J, Pena-Couso L, Olivera R, Rodríguez Y, García-Arranz M, García-Olmo D, González-Sarmiento R, Urioste M, Goel A, Perea J. Cimp-Positive Status is More Representative in Multiple Colorectal Cancers than in Unique Primary Colorectal Cancers. *Sci Rep* 2019; **9**: 10516 [PMID: 31324877 DOI: 10.1038/s41598-019-47014-w]

84 **Willauer AN**, Liu Y, Pereira AAL, Lam M, Morris JS, Raghav KPS, Morris VK, Menter D, Broaddus R, Meric-Bernstam F, Hayes-Jordan A, Huh W, Overman MJ, Kopetz S, Loree JM. Clinical and molecular characterization of early-onset colorectal cancer. *Cancer* 2019; **125**: 2002-2010 [PMID: 30854646 DOI: 10.1002/cncr.31994]

85 **Magnani G**, Furlan D, Sahnane N, Reggiani Bonetti L, Domati F, Pedroni M. Molecular Features and Methylation Status in Early Onset (≤40 Years) Colorectal Cancer: A Population Based, Case-Control Study. *Gastroenterol Res Pract* 2015; **2015**: 132190 [PMID: 26557847 DOI: 10.1155/2015/132190]

86 **Ribic CM**, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247-257 [PMID: 12867608 DOI: 10.1056/NEJMoa022289]

87 **Slomski A**. Aspirin Protects Against Colorectal Cancer in Lynch Syndrome. *JAMA* 2020; **324**: 733 [PMID: 32840595 DOI: 10.1001/jama.2020.14804]

88 **Kosumi K**, Mima K, Baba H, Ogino S. Dysbiosis of the gut microbiota and colorectal cancer: the key target of molecular pathological epidemiology. *J Lab Precis Med* 2018; **3** [PMID: 30345420 DOI: 10.21037/jlpm.2018.09.05]

89 **Hogan NM**, Hanley M, Hogan AM, Sheehan M, McAnena OJ, Regan MP, Kerin MJ, Joyce MR. Awareness and uptake of family screening in patients diagnosed with colorectal cancer at a young age. *Gastroenterol Res Pract* 2015; **2015**: 194931 [PMID: 25688262 DOI: 10.1155/2015/194931]

90 **Sandhu GS**, Anders R, Walde A, Leal AD, King GT, Leong S, Davis SL, Purcell WT, Goodman KA, Herter W, Meguid CL, Birnbaum EH, Ahrendt SA, Gleisner A, Schulick RD, Delchiaro M, McCarter M, Patel S, Messersmith WA, Lieu CH. High incidence of advanced stage cancer and prolonged rectal bleeding history before diagnosis in young-onset patients with colorectal cancer. *J Clin Oncol*2019 37: 3576-3576 [DOI: 10.1200/JCO.2019.37.15_suppl.3576]

91 **Valle L**, de Voer RM, Goldberg Y, Sjursen W, Försti A, Ruiz-Ponte C, Caldés T, Garré P, Olsen MF, Nordling M, Castellvi-Bel S, Hemminki K. Update on genetic predisposition to colorectal cancer and polyposis. *Mol Aspects Med* 2019; **69**: 10-26 [PMID: 30862463 DOI: 10.1016/j.mam.2019.03.001]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 242-252

DOI: 10.4251/wjgo.v14.i1.242

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Hydrogen-rich water exerts anti-tumor effects comparable to 5-fluorouracil in a colorectal cancer xenograft model

Fereshteh Asgharzadeh, Alex Tarnava, Asma Mostafapour, Majid Khazaei, Tyler W LeBaron

ORCID number: Fereshteh Asgharzadeh 0000-0002-8349-3722; Alex Tarnava 0000-0002-0796-5345; Asma Mostafapour 0000-0001-8337-2827; Majid Khazaei 0000-0002-8257-2699; Tyler W LeBaron 0000-0001-9164-6728.

Author contributions: All authors were involved in design of experiment; Asgharzadeh F, Mostafapour A, and Khazaei M carried out the experiments and wrote the methods and results sections; Tarnava A and LeBaron TW wrote the background, discussion and conclusion; and all authors agree to final manuscript.

Institutional review board statement: The Mashhad University of Medical Sciences Committee on Animal Ethics has approved all animal protocols used in this research.

Institutional animal care and use committee statement: The Mashhad University of Medical Sciences Committee on Animal Ethics has approved all animal protocols used in this research. Reference Number: 991229; Date: July 10, 2020.

Conflict-of-interest statement: Tarnava A is involved in commercial entities with interest in the marketing of hydrogen-rich

Fereshteh Asgharzadeh, Majid Khazaei, Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad 9177899191, Iran

Alex Tarnava, Drink HRW, New Westminster, BC V3j0b6 Canada

Asma Mostafapour, Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad 9177899191, Iran

Tyler W LeBaron, Centre of Experimental Medicine, Institute for Heart Research Slovak Academy of Sciences, Bratislava 984104, Slovakia

Tyler W LeBaron, Department of Kinesiology and Outdoor Recreation, Southern Utah University, UT 84720, United States

Tyler W LeBaron, Biological Research, Molecular Hydrogen Institute, UT 84721, United States

Tyler W LeBaron, Department of Physical Science, Southern Utah University, UT 84720, United States

Corresponding author: Tyler W LeBaron, BSc, MSc, Director, Instructor, Research Scientist, Centre of Experimental Medicine, Institute for Heart Research Slovak Academy of Sciences, D úbravská cesta 9, 841 04 Bratislava, Slovak Republic, Bratislava 984104, Slovakia. lebaront@molecularhydrogeninstitute.com

Abstract

BACKGROUND

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the world. Tumor removal remains the preferred frontline treatment; however, effective non-surgical interventions remain a high priority. 5-fluorouracil (5-FU) is a widely used chemotherapy agent, and molecular hydrogen (H_2) has been recognized for its antioxidant and anti-inflammatory effects, with research also suggesting its potential anti-tumor effects. Therefore, H_2 dissolved in water [hydrogen-rich water (HRW)], with or without 5-FU, may present itself as a novel therapeutic for CRC.

AIM

To investigate the effects of HRW, with or without 5-FU, as a novel therapeutic for CRC.

METHODS
CRC was induced in the left flank of inbred Balb/c mice. A total of 24 mice bearing tumors were randomly divided into four groups (n = 6 per group) and treated as follows: (1) Control group; (2) 5-FU group that received intraperitoneal injection of 5-FU (5 mg/kg) every other day; (3) H_2 group that received HRW, created and delivered *via* dissolving the H_2-generating tablet in the animals' drinking water, with 200 μL also delivered by oral gavage; and (4) The combination group, H_2 (administered in same way as for group three) combined with 5-FU administered same way as group two.

RESULTS
Administration of HRW + 5-FU significantly improved tumor weight, tumor size, collagen content and fibrosis as compared to the CRC control group. Specifically, HRW attenuated oxidative stress (OS) and potentiated antioxidant activity (AA), whereas 5-FU treatment exacerbated OS and blunted AA. The combination of HRW + 5-FU significantly reduced tumor weight and size, as well as reduced collagen deposition and the degree of fibrosis, while further increasing OS and decreasing AA compared to administration of 5-FU alone.

CONCLUSION
Administration of HRW, with or without 5-FU, may serve as a therapeutic for treating CRC.

Key Words: Colorectal cancer; Molecular hydrogen; 5-fluorouracil; Oxidative stress; Antioxidants; Inflammation

Core Tip: Colorectal cancer is a leading cause of death and is often treated with the chemotherapy drug, 5-fluorouracil (5-FU), which has some unwanted side effects. Molecular hydrogen (H_2 gas) has antioxidant, anti-inflammatory, and anti-cancer effects. H_2 gas can be dissolved in water to make hydrogen-rich water (HRW). The effects of HRW, 5-FU and the combination of HRW and 5-FU in a colorectal-cancer mouse model were examined. HRW and 5-FU decreased tumor size and weight with the combination being the most effective. In contrast to 5-FU, HRW attenuated oxidative stress and improved antioxidant activity.

Citation: Asgharzadeh F, Tarnava A, Mostafapour A, Khazaei M, LeBaron TW. Hydrogen-rich water exerts anti-tumor effects comparable to 5-fluorouracil in a colorectal cancer xenograft model. *World J Gastrointest Oncol* 2022; 14(1): 242-252
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/242.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.242

INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide, in which statistically 3.2% of men and 2.6% of women will die from the disease[1,2]. CRC has a survival rate of 91% if detected in stage 1. However, its overall 5-year survival rate is only 65%, according to 2020 data from the American Cancer Society published on the SEER database[3]. Surgical removal of rectal cancer remains the first-line treatment of CRC. However, non-surgical treatment options serve as important treatment tools, as rates of screening and surgery approvals between various nations can lead to differences in rate of survival[4].

Molecular hydrogen (H_2) has been studied extensively as a therapeutic gas, with an estimated 1500 publications to date exploring its potential therapeutic use in 170 disease models across every organ in the mammalian body. H_2 can be administered through several methods, such as H_2 inhalation, dissolving H_2 gas in water to make hydrogen-rich water (HRW) for oral consumption or topical application, or hydrogen-rich saline.

water; LeBaron TW has received travel reimbursement, honoraria, and speaking and consultancy fees from various academic and commercial entities regarding molecular hydrogen. All other authors report no conflict of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Supported by the HRW Natural Health Products Inc. (dba Drink HRW), as well as by a grant from Mashhad University of Medical Sciences.

Country/Territory of origin: United States

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: May 19, 2021

Peer-review started: May 19, 2021
First decision: June 16, 2021
Revised: June 30, 2021
Accepted: December 7, 2021
Article in press: December 7, 2021
Published online: January 15, 2022

P-Reviewer: Wu HL, Yao K
S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

5-fluorouracil (5-FU) is a widely used chemotherapeutic employed during cancer treatment[5]. H_2 has shown positive effects in terms of quality of life in human clinical research. For example, studies report that H_2 therapy was associated with improved liver function in patients who were administered chemotherapy, as well as reduced side effects for those receiving radiation therapy, and protective effects against radiation-induced bone marrow damage in cancer patients[6-8].

H_2 has been previously demonstrated to display anti-cancer properties when administered on its own. Hyperbaric H_2 therapy has been examined as a potential cancer therapy, revealing potent anti-tumor effects in mice with squamous cell carcinoma[9]. In a study involving mice with colon cancer, it was shown that drinking HRW dose-dependently potentiated the tumor-inhibitory activity of 5-FU by enhancing cellular apoptosis of the cancer cells[10]. In the present study, we aimed to explore the potential effects of a higher concentration of HRW than previously utilized, to further explore the effects of high-concentration HRW compared to control, 5-FU administration on its own, as well as HRW in combination with 5-FU, for the mitigation of CRC progression and accompanying outcomes.

MATERIALS AND METHODS

Chemicals and reagents

HRW was created using H_2-producing tablets (HRW Natural Health Products Inc., New Westminster BC, Canada) by dissolving it in a 500-mL beaker. HRW was made two times each day every 12 h. The concentration of HRW was > 1.5 mmol/L and remained > 0.1 mmol/L after 12 h as determined by redox titration (H2Blue; H2Sciences, Las Vegas, Nevada). 5-FU was obtained from EBEWE Pharma, Unterach, Austria. F12/Dulbecco's Modified Eagle Medium (DMEM/F12), fetal bovine serum (FBS), penicillin (Pen) and streptomycin (Strep) were obtained from Gibco BRL, Life Technologies Inc. (Gaithersburg, MD, United States).

Cell culture

The mouse colorectal adenocarcinoma cell line CT-26 was obtained from Pasteur Institute (Tehran, Iran). CT-26 cells were cultured in DMEM/F12 medium containing 10% FBS, Pen (50 U/mL) and Strep (50 μg/mL) in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C.

Xenografts in mice: Treatment and evaluation

Tumor xenograft experiments were conducted as previously described by Golovko *et al*[11]. In brief, 6- to 8-wk-old female inbred Balb/c mice were injected with 5×10^5 CT-26 cells (100 μL) into the left rear flank (day 0). When tumor volumes reached 80-100 mm^3 (-10 d), 24 mice bearing tumors were divided randomly into four groups (n = 6 per group) and treated as follows: (1) The control group; (2) The 5-FU group received intraperitoneal injections of 5-FU (5 mg/kg) every other day; (3) The H_2 group received HRW both from drinking water and by delivering 200 μL of the solution *via* oral gavage; and (4) The combination group, H_2 (administered in same way as group three) combined with 5-FU (administered in the same way as group two). The tumor volume was calculated every other day according to the following formula: V = (length × width^2)/2[12]. The animals were sacrificed on day 14 and the tumors were removed for further analysis.

Histological assay

Fixed tumor tissue samples were embedded in paraffin wax and then sectioned at 5 μm thickness with a microtome. The tumor tissue sections were deparaffinized and stained with Hematoxylin-Eosin for evaluation of tumor necrosis. Masson trichrome staining was also performed for evaluation of collagen content and fibrosis.

Tissue preparation for measurement of oxidative stress markers

The colon tissues samples were homogenized in ice with PBS and centrifuged. The supernatant was stored at -70 °C for the determination of the oxidative and antioxidative proteins.

Malondialdehyde measurement

Malondialdehyde (MDA) was measured by methods as previously described[13]. Briefly, 1 mL of homogenate was mixed with 2 mL of a solution containing thiobar-

bituric acid, trichloroacetic acid, and HCl in hot water (100 °C) for 45 min and centrifuged for ten minutes. The MDA levels were determined by measuring the absorbance of the solution.

Total thiol group measurement

We used di-thio nitrobenzoic acid (DTNB) reagent for measurement of total thiol group as previously described[13]. Briefly, 1 mL of Tris-EDTA buffer (pH = 8.6) was added to the colon homogenate and absorbance was measured. Similarly, 20 μL of DTNB reagents was added to the sample absorbance and the absorbance was measured again; subsequently, the total molar concentration of thiol was determined as previously described[14].

Evaluation of superoxide dismutase and catalase

Superoxide dismutase (SOD) was determined with a colorimetric assay described by Madesh *et al*[15]. The method is centered on the synthesis of SOD by pyrogallol autoxidation and inhibition of superoxide-dependent reduction of 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) to its formazan. Catalase was measured by evaluating the kinetics of H_2O_2 hydrolysis at 240 nm in a buffer of sodium phosphate. The velocity of the enzyme activity can be determined by converting H_2O_2 to H_2O and O_2 within 60 s of normal conditions[15].

Ethics statement

The Mashhad University of Medical Sciences Committee on Animal Ethics has approved all animal protocols used in this research. Reference Number: 991229; Date: July 10, 2020.

Data analysis

The statistical methods of this study were reviewed by Dr. Mohammad Taghi Shakeri, a member of the Biostatistic Department of Mashad University of Medical Sciences. All the results are presented as means and standard error of the mean (mean ± SEM). The differences in the mean values among different groups were determined by a one-way analysis of variance (ANOVA) using the SPSS 22.0 program. Significance was set at values of $P < 0.05$.

RESULTS

H_2 suppresses tumor growth and enhances the antitumor efficacy of 5-FU in a colon cancer xenograft model

We studied the influence of H_2 on tumor growth in a CRC xenograft model. Administration of HRW significantly decreased tumor growth in mice (Figure 1). The suppressive effect of HRW on tumor growth was slightly, but not statistically, more potent than 5-FU, and not as effective as the combination therapy. Specifically, the average control tumor size was 2698.85 mm^3, whereas the average tumor size in the HRW group was 2047.23 mm^3 (24.1% suppression compared to control), while the average tumor size in the 5-FU group was 2097.32 mm^3 (22.3% suppression compared to control). The combination group of HRW + 5-FU resulted in the greatest tumor size suppression, with the average size of 1177.5 mm^3 (56.4% suppression compared to control) (Figure 1A).

Similarly, a comparison of tumor weight between the groups showed that both 5-FU and HRW significantly reduced tumor weight ($P < 0.05$), and this decrease was potentiated in the combination group of HRW + 5-FU ($P < 0.001$) (Figure 1B). Treatment with 5-FU reached statistical significance at day 12 through 14 ($P < 0.05$) as compared to control, whereas the combination treatment of HRW + 5-FU reached significance by day 6 ($P < 0.05$) and continued its suppressive effect at day 8 ($P < 0.01$) and days 10-14 ($P < 0.001$) compared to control. Moreover, combination treatment was more effective compared to 5-FU treatment alone, reaching significance by day 8 ($P < 0.05$) with days 10 through 14 being even more significant ($P < 0.01$).

The effects of H_2 and 5-FU on redox status

We investigated the effects of H_2 administered *via* drinking HRW on levels of markers of OS in tissue homogenates. As shown in Figure 2, HRW treatment decreased MDA levels in tumor tissues compared to control ($P < 0.05$) and 5-FU treatment ($P < 0.001$). However, 5-FU treatment increased MDA levels compared to control (Figure 2A, $P <$

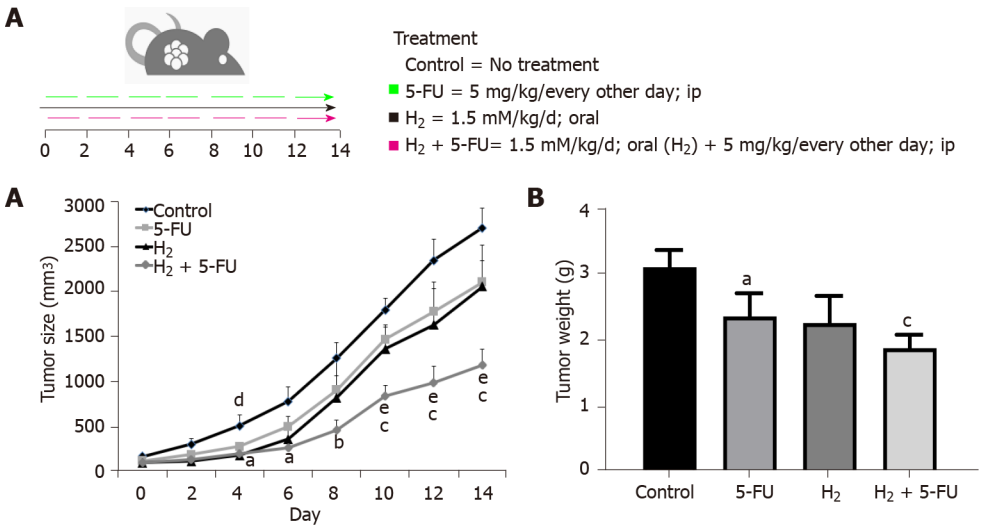


Figure 1 Hydrogen, 5-fluorouracil and their combination reduced tumor growth and tumor weight in a murine model of colorectal cancer. A: Tumor size; B: Tumor weight change in mice treated with hydrogen-rich water (HRW), 5-fluorouracil (5-FU) and their combination. $P < 0.05$ and $P < 0.001$ compared to control, $P < 0.01$ compared to 5-FU, $P < 0.05$ compared to HRW and $P < 0.05$, $P < 0.01$ and $P < 0.001$ compared to combination groups; $n = 6$ per group. a: $P < 0.05$ compare to control; b: $P < 0.01$ compare to control; c: $P < 0.001$ compare to control; d: $P < 0.05$ compare to H_2; e: $P < 0.01$ compare to 5-FU. H_2: Hydrogen; 5-FU: 5-fluorouracil.

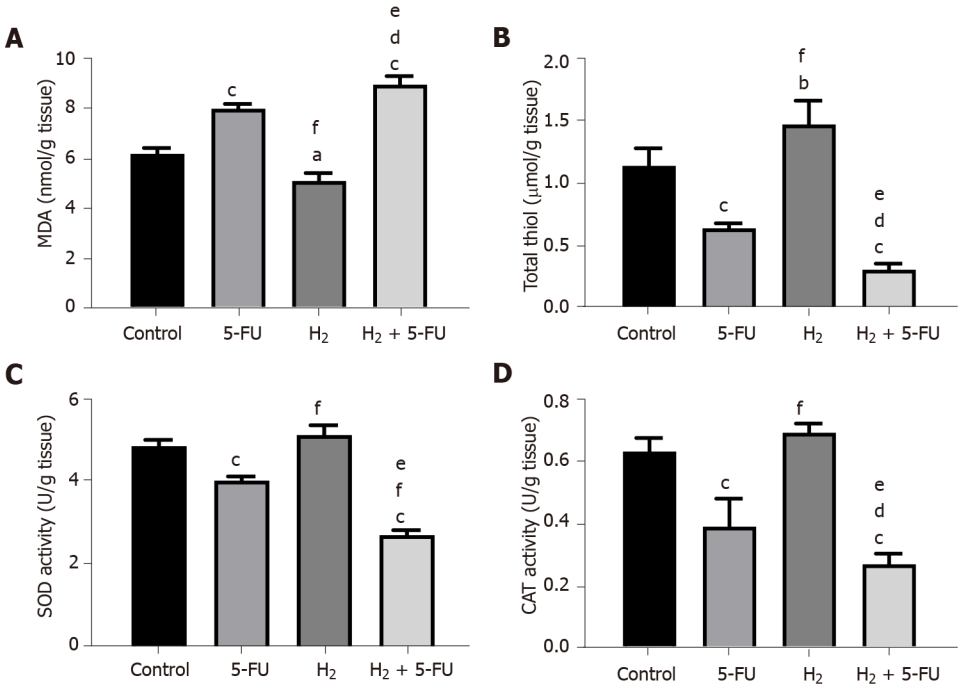


Figure 2 Effects of hydrogen and 5-fluorouracil on the oxidative stress index in colorectal cancer. A: Malondialdehyde; B: Total thiol; C: Superoxide dismutase; D: Catalase activity. $P < 0.05$, $P < 0.01$ and $P < 0.001$ compared to control, $P < 0.05$ and $P < 0.001$ compared to 5-fluorouracil, $P < 0.001$ compared to hydrogen groups; $n = 6$ per group. a: $P < 0.05$ compare to control; b: $P < 0.01$ compare to control; c: $P < 0.001$ compare to control; d: $P < 0.05$ compare to H_2; e: $P < 0.001$ compare to H_2; f: $P < 0.001$ compare to 5-FU. H_2: Hydrogen; 5-FU: 5-fluorouracil; SOD: Superoxide dismutase; MDA: Malondialdehyde; CAT: Catalase.

0.001). HRW tended to improve activity of all three antioxidant markers measured. For example, HRW increased thiol concentrations (Figure 2B) compared to control ($P <$ 0.01) and 5-FU treatment ($P < 0.001$). Additionally, we observed a trend towards an increase in SOD and catalase activity following H_2 treatment, although significance was only reached when compared to 5-FU treatment. Compared to the control group, there was a significant decrease in levels of all antioxidant markers following 5-FU treatment. In the combination group (HRW and 5-FU), a more prevalent rise in OS and suppression of AA was observed compared to 5-FU alone. MDA levels significantly increased in the combination group compared to control (Figures 2B, 2C and 2D; $P <$

0.001) and the 5-FU treatment ($P < 0.05$). Similarly, activity of all three antioxidants measured were suppressed by the combination compared to control and also when compared to 5-FU alone.

H_2 and 5-FU increased necrotic areas

Tumor necrosis was observed under a light microscope. As illustrated in Figures 3A and 3B, treatment of H_2 or 5-FU displayed interspersed tissue necrosis compared to the untreated group. In the H_2 + 5-FU group, we observed larger necrotic areas than the necrotic areas in either group alone.

H_2 and 5-FU decreased tumor fibrosis in the colon cancer xenograft model

We used Masson's trichrome staining to compare the collagen deposition in tumor tissues across the treatment and control groups. Our results demonstrate that administration of either H_2 or 5-FU suppressed collagen deposition and degree of fibrosis compared to the control group (Figure 4A). The increment in percentage of collagen deposition in all treated groups was significantly decreased when compared to the control group (Figure 4B; $P < 0.001$). Specifically, collagen deposition percentage in the control group was 24.6%. In contrast, with both H_2 and 5-FU alone, the percentage of collagen deposition in the tumor tissue was significantly reduced to about 13% ($P < 0.001$). However, administration of both H_2 + 5-FU in combination further reduced the percentage of collagen deposition in tumor tissue (= 3%) compared to both the 5-FU group and H_2 group ($P < 0.001$).

DISCUSSION

Non-surgical treatment options to improve outcomes in CRC remains a high priority. Ideal adjuvant treatment options should aim to improve quality of life, reduce symptoms, and work synergistically with standard care. Although 5-FU remains the front-line treatment option for a variety of cancers due to its effectiveness, it also has limitations. For instance, cardiotoxicity has shown to be a serious side effect of 5-FU administration, largely due to increases in OS and suppression of endogenous antioxidant mobilization[16]. Accordingly, molecular H_2 has been proposed as a novel approach for the treatment of cardiovascular disorders due to its ability to significantly reduce the effects of OS[17].

Our results demonstrate that the combination of HRW and 5-FU treatment potentiated the beneficial anti-tumor effects of both treatments on their own, such as tumor weight, size, the degree of fibrosis and collagen content in the tumor. Enigmatically, while treatment with HRW on its own significantly improved all three measured antioxidant markers while decreasing levels of MDA, the combination therapy of HRW and 5-FU significantly blunted AA and elevated MDA levels significantly above those measured with 5-FU alone. Acute temporal increases in OS after H_2 administration have been noted in other studies, and it has been previously suggested that molecular H_2 may act as a therapeutic hormetic agent similar to exercise[18-21]. Nogueira *et al*[19] (2018) examined the effects of molecular H_2 administration on exercise performance and noted an acute rise in OS in the H_2 treated group, followed by a greater antioxidant mobilization, leading to improved redox homeostasis shortly after the exercise period ended. Further, previously published human clinical research has demonstrated significant improvements in redox homeostasis following medium-term administration of HRW for 24 wk[22]. Since our short-term study was unable to determine the effects of H_2 + 5-FU administration on OS and AA over a longer treatment course, such as has been reported in previous research on HRW[23], future research is warranted to investigate this area.

When administered on its own, HRW demonstrated similar benefits in reducing tumor size compared to 5-FU. These results corroborate earlier reports that HRW can suppress early tumor formation in rats[24]. Additionally, molecular H_2 was shown to prevent tumor progression in a cell line model of lung cancer[25]. In our study, we demonstrated that HRW administration was associated with a significant decrease in pathological collagen content equivalent to that of 5-FU. In contrast to previous reports demonstrating that molecular H_2 upregulates collagen biosynthesis and expression, and corresponding to the results of another study reporting that molecular H_2 significantly reduced type III collagen depositions as observed *via* staining[26-28]. Molecular H_2 has shown to both be able to promote and suppress outcomes, model dependent, for many biological processes, which may indicate that contradictory reports do not undermine our understanding of the mechanisms by which H_2

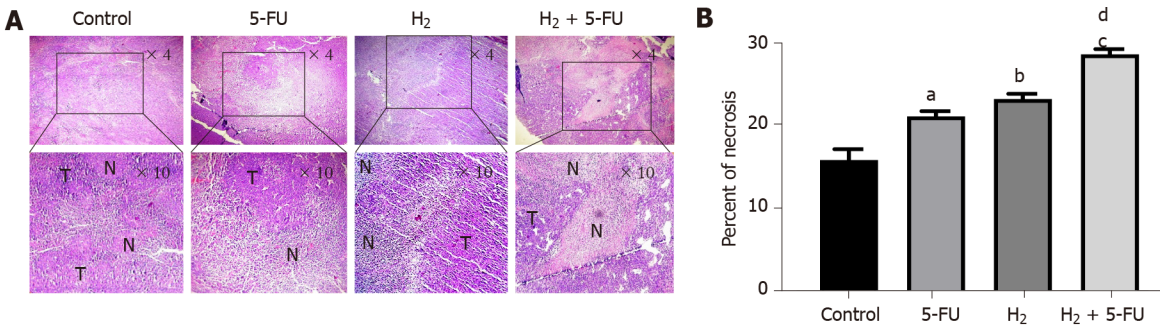


Figure 3 Hydrogen and 5-fluorouracil induce necrosis in tumor tissue of colorectal cancer. A: Hematoxylin-Eosin staining of tumor sections revealed that hydrogen (H_2) and 5-fluorouracil (5-FU) induce necrosis; B: Percent of tumor necrosis. $P < 0.05$, $P < 0.01$ and $P < 0.001$ compared to control, $P < 0.001$ compared to 5-FU, $P < 0.01$ compared to H_2 groups; $n = 6$ per group. a: $P < 0.05$ compare to control; b: $P < 0.01$ compare to control; c: $P < 0.001$ compare to control; d: $P < 0.001$ compare to 5-FU. H_2: Hydrogen; 5-FU: 5-fluorouracil; T: Tumor cells; N: Necrotic area.

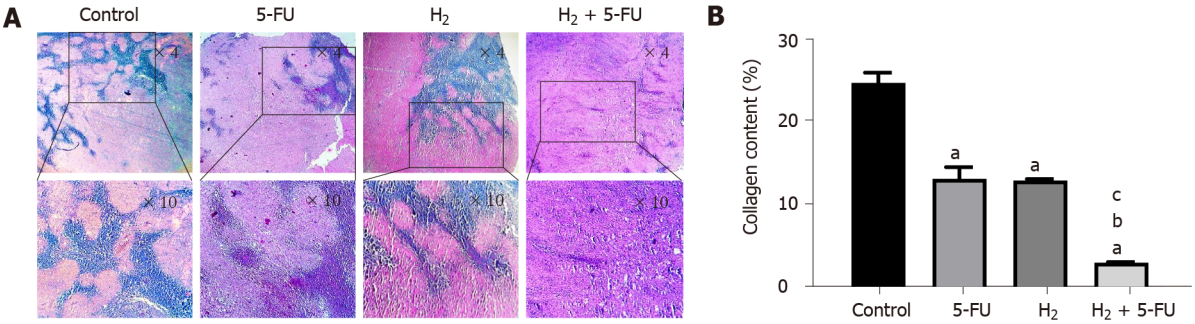


Figure 4 Hydrogen and 5-fluorouracil attenuate fibrosis in tumor tissue of colorectal cancer. A: Trichrome staining of tumor samples revealed that hydrogen (H_2) suppresses fibrosis in the murine model of colorectal cancer (collagen fiber accumulation appears in blue); B: Tumor fibrosis expressed as collagen content (%) in different groups. $P < 0.001$ compared to control, $P < 0.001$ compared to 5-fluorouracil, $P < 0.01$ compared to H_2 groups; $n = 6$ per group. a: $P < 0.001$ compare to control; b: $P < 0.001$ compare to 5-FU; c: $P < 0.001$ compare to H2. H_2: Hydrogen; 5-FU: 5-fluorouracil.

operates. HRW demonstrated similar outcomes to 5-FU for visual results of fibrosis from mass trichrome staining. Further, molecular H_2 has been previously demonstrated to reduce fibrosis in the lungs and abdomen[29,30].

Interestingly, the combination of 5-FU and HRW demonstrated significant reductions of tumor weight, size, collagen deposition and degree of fibrosis, while increasing markers of OS and blunting AA significantly beyond 5-FU alone. Previous studies have demonstrated that HRW generally reduces OS in most animal and human disease models when administered as a stand-alone intervention. Molecular H_2 has been observed to work in an additive or synergistic capacity with several other interventions in various models, as demonstrated by a recent study, in which administration of high-concentration HRW alongside minocycline improved outcomes following ischemic stroke in rats[31]. Additionally, molecular H_2 has shown to enhance the effects of photothermal therapy by inhibiting tumor progression in cell cultures and was also shown to act equivalently to sulfasalazine in a dextran sodium sulfate-induced mouse model of colitis, with the combination therapy of HRW + sulfasalazine demonstrating effects of significantly greater magnitude than either treatment on its own[32,33].

Treatment with 5-FU has been shown to result in DNA damage[34]. Alterations in various processes, such as nucleotide and amino acid metabolism, may lead to 5-FU resistance[35]. Autophagy plays an important role in nucleotide and amino acid metabolism, and H_2 has been shown to both stimulate and mitigate autophagy for beneficial outcomes[36-40]. It has also been suggested that therapies which mediate DNA repair alongside 5-FU and other cancer treatments should be further explored as a therapeutic target[41]. For instance, it has been shown that H_2 exerted significant protective effects against DNA damage in calf thymus tissue following exposure to radiation[42]. Future research is also needed in order to address both potential protective effects and long-term benefits of molecular H_2 delivered in conjunction with 5-FU and other conventional cancer therapies, exploring outcomes related to DNA damage, cell signaling, and survival rates.

So far, little is known regarding the effects of various dosages and different administration methods of HRW, H_2 inhalation, and hydrogen-rich saline on cancer cells. To date, several routes have shown potential benefits of HRW administration for cancer treatment, including the previously cited reports using inhalation studies in humans, and HRW use in murine models. Additionally, a recent report demonstrated that the use of H_2-producing reactive magnesium implants was associated with significant suppression of tumor growth in a mouse model of ovarian cancer[43]. However, since H_2 has demonstrated protective effects on healthy cells, it could also protect and stimulate cancer cell growth. For example, H_2 administration has been demonstrated to induce the mitochondrial unfolded protein response, which is also a proliferative signal in various cancer cells[44,45]. Therefore, the effects of different dosages and administration methods of molecular H_2 should be carefully analyzed to determine its effects.

CONCLUSION

Safe and well-tolerated adjuvant therapeutics with the potential to ameliorate the deleterious consequences of various cancer treatments, while simultaneously improving outcomes, are of high interest to cancer patients and the medical community. Molecular H_2 therapy demonstrates potential anti-cancer properties, as well as the ability to reduce the secondary effects of various treatments. In this study, we have shown that administered on its own, HRW demonstrates anti-cancer properties and improves markers of OS and AA compared to conventional treatment (5-FU). The combination of HRW and 5-FU suppressed tumor progression in a synergistic manner; however, the addition of HRW to 5-FU treatment increased OS levels and reduced AA. Limitations of this study include that the observation period during the study was only 14 d, with rates of survival and remission not examined. As such, interpretation of these results should be evaluated cautiously. Larger, longer-term studies are highly warranted to explore HRW as an adjuvant therapy for various cancers, alongside conventional therapy, with longer observational periods needed to address unanswered questions regarding potential positive and negative effects of molecular H_2 on redox homeostasis during cancer treatment.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide. Surgical removal remains the first-line treatment for CRC; however, nonsurgical options remain important tools for treatment. Currently, treatments such as 5-fluorouracil (5-FU), a widely administered chemotherapeutic agent utilized in the treatment of CRC, presents known beneficial effects, but also significant side effects. Hydrogen-rich water (HRW) has demonstrated beneficial effects in numerous species, including humans, in many disease models, including various cancers. One attractive aspect of HRW is the high safety profile and low rates of side effects combined with its promising therapeutic effects.

Research motivation

New treatments with potential positive effects in CRC are desperately needed, particularly treatments with high safety profiles and low side effects. HRW may fit the criteria as a safe potential treatment for CRC, either as a stand-alone treatment or in combination with conventional treatments.

Research objectives

We aimed to evaluate the efficacy of HRW on a CRC model compared to 5-FU and control, as well as the combination treatment of HRW and 5-FU compared to 5-FU alone, HRW alone, or control. We measured tumor size, tumor weight, fibrosis, and collagen content, as well as oxidative stress (OS) and antioxidant activity (AA) in mice with induced CRC. These objectives allow us to determine the therapeutic efficacy and mechanistic insight of HRW with or without 5-FU, as well as determine if there are additive benefits in a combinational treatment to guide future clinical studies.

Research methods

Six- to eight-week-old female inbred Balb/c mice were injected with 5×10^5 CT-26 cells (100 μL) into the left rear flank (day 0). When tumor volumes reached 80-100 mm^3, 24 mice bearing tumors were randomly divided into four groups. Mice were either left untreated (control) or treated with 5-FU (intraperitoneal injection, 5 mg/kg every other day), high-concentration HRW produced by magnesium tablets (ad libitum in drinking water, as well as by oral gavage 200 μL daily), or both HRW and 5-FU.

Research results

We report that molecular hydrogen dissolved in water (HRW) was as effective as 5-FU, with more preferential outcomes relating to higher AA and lower OS. Importantly, the combination of HRW and 5-FU was superior to either therapy on its own, presenting the possibility that HRW may be explored as an adjuvant therapy alongside conventional chemotherapeutics.

Research conclusions

HRW may be a novel safe adjuvant therapy for treating CRC, either as a stand-alone therapy, or preferably, alongside conventional chemotherapeutics.

Research perspectives

Clinical research to evaluate the effects of HRW as a treatment for CRC, both alone and in combination with 5-FU and other chemotherapeutics, is highly warranted.

ACKNOWLEDGEMENTS

We thank Mr. Alex Tarnava, CEO of HRW Natural Health Products Inc. for kindly donating Drink HRW tablets for this study and Mashhad University of Medical Sciences for their support.

REFERENCES

1 **Rawla P**, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019; **14**: 89-103 [PMID: 31616522 DOI: 10.5114/pg.2018.81072]
2 Colorectal cancer: Overview. [cited 5 May 2021] Available from: https://www.cedars-sinai.org/health-library/diseases-and-conditions/c/colorectal-cancer-overview.html
3 **Surveillance**, Epidemiology, and End Results (SEER) Program. (2021). Cancer Stat Facts: Colorectal Cancer. [cited 5 May 2021]. Available from: https://seer.cancer.gov/statfacts/html/colorect.html
4 **Benitez Majano S**, Di Girolamo C, Rachet B, Maringe C, Guren MG, Glimelius B, Iversen LH, Schnell EA, Lundqvist K, Christensen J, Morris M, Coleman MP, Walters S. Surgical treatment and survival from colorectal cancer in Denmark, England, Norway, and Sweden: a population-based study. *Lancet Oncol* 2019; **20**: 74-87 [PMID: 30545752 DOI: 10.1016/S1470-2045(18)30646-6]
5 **Longley DB**, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; **3**: 330-338 [PMID: 12724731 DOI: 10.1038/nrc1074]
6 **Yang Q**, Ji G, Pan R, Zhao Y, Yan P. Protective effect of hydrogen-rich water on liver function of colorectal cancer patients treated with mFOLFOX6 chemotherapy. *Mol Clin Oncol* 2017; **7**: 891-896 [PMID: 29142752 DOI: 10.3892/mco.2017.1409]
7 **Kang KM**, Kang YN, Choi IB, Gu Y, Kawamura T, Toyoda Y, Nakao A. Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors. *Med Gas Res* 2011; **1**: 11 [PMID: 22146004 DOI: 10.1186/2045-9912-1-11]
8 **Hirano SI**, Aoki Y, Li XK, Ichimaru N, Takahara S, Takefuji Y. Protective effects of hydrogen gas inhalation on radiation-induced bone marrow damage in cancer patients: a retrospective observational study. *Med Gas Res* 2021; **11**: 104-109 [PMID: 33942780 DOI: 10.4103/2045-9912.314329]
9 **Dole M**, Wilson FR, Fife WP. Hyperbaric hydrogen therapy: a possible treatment for cancer. *Science* 1975; **190**: 152-154 [PMID: 1166304 DOI: 10.1126/science.1166304]
10 **Runtuwene J**, Amitani H, Amitani M, Asakawa A, Cheng KC, Inui A. Hydrogen-water enhances 5-fluorouracil-induced inhibition of colon cancer. *PeerJ* 2015; **3**: e859 [PMID: 25870767 DOI: 10.7717/peerj.859]
11 **Golovko D**, Kedrin D, Yilmaz ÖH, Roper J. Colorectal cancer models for novel drug discovery. *Expert Opin Drug Discov* 2015; **10**: 1217-1229 [PMID: 26295972 DOI: 10.1517/17460441.2015.1079618]
12 **Moradi-Marjaneh R**, Hassanian SM, Rahmani F, Aghaee-Bakhtiari SH, Avan A, Khazaei M.

Phytosomal Curcumin Elicits Anti-tumor Properties Through Suppression of Angiogenesis, Cell Proliferation and Induction of Oxidative Stress in Colorectal Cancer. *Curr Pharm Des* 2018; **24**: 4626-4638 [PMID: 30636578 DOI: 10.2174/1381612825666190110145151]

13 **Amerizadeh F**, Rezaei N, Rahmani F, Hassanian SM, Moradi-Marjaneh R, Fiuji H, Boroumand N, Nosrati-Tirkani A, Ghayour-Mobarhan M, Ferns GA, Khazaei M, Avan A. Crocin synergistically enhances the antiproliferative activity of 5-flurouracil through Wnt/PI3K pathway in a mouse model of colitis-associated colorectal cancer. *J Cell Biochem* 2018; **119**: 10250-10261 [PMID: 30129057 DOI: 10.1002/jcb.27367]

14 **Binabaj MM**, Asgharzadeh F, Avan A, Rahmani F, Soleimani A, Parizadeh MR, Ferns GA, Ryzhikov M, Khazaei M, Hassanian SM. EW-7197 prevents ulcerative colitis-associated fibrosis and inflammation. *J Cell Physiol* 2019; **234**: 11654-11661 [PMID: 30478959 DOI: 10.1002/jcp.27823]

15 **Madesh M**, Balasubramanian KA. A microtiter plate assay for superoxide using MTT reduction method. *Indian J Biochem Biophys* 1997; **34**: 535-539 [PMID: 9594436]

16 **Polk A**, Vistisen K, Vaage-Nilsen M, Nielsen DL. A systematic review of the pathophysiology of 5-fluorouracil-induced cardiotoxicity. *BMC Pharmacol Toxicol* 2014; **15**: 47 [PMID: 25186061 DOI: 10.1186/2050-6511-15-47]

17 **LeBaron TW**, Kura B, Kalocayova B, Tribulova N, Slezak J. A New Approach for the Prevention and Treatment of Cardiovascular Disorders. Molecular Hydrogen Significantly Reduces the Effects of Oxidative Stress. *Molecules* 2019; **24** [PMID: 31159153 DOI: 10.3390/molecules24112076]

18 **Ichihara M**, Sobue S, Ito M, Hirayama M, Ohno K. Beneficial biological effects and the underlying mechanisms of molecular hydrogen - comprehensive review of 321 original articles. *Med Gas Res* 2015; **5**: 12 [PMID: 26483953 DOI: 10.1186/s13618-015-0035-1]

19 **Nogueira JE**, Passaglia P, Mota CMD, Santos BM, Batalhão ME, Carnio EC, Branco LGS. Molecular hydrogen reduces acute exercise-induced inflammatory and oxidative stress status. *Free Radic Biol Med* 2018; **129**: 186-193 [PMID: 30243702 DOI: 10.1016/j.freeradbiomed.2018.09.028]

20 **Murakami Y**, Ito M, Ohsawa I. Molecular hydrogen protects against oxidative stress-induced SH-SY5Y neuroblastoma cell death through the process of mitohormesis. *PLoS One* 2017; **12**: e0176992 [PMID: 28467497 DOI: 10.1371/journal.pone.0176992]

21 **LeBaron TW**, Laher I, Kura B, Slezak J. Hydrogen gas: from clinical medicine to an emerging ergogenic molecule for sports athletes [1]. *Can J Physiol Pharmacol* 2019; **97**: 797-807 [PMID: 30970215 DOI: 10.1139/cjpp-2019-0067]

22 **LeBaron TW**, Singh RB, Fatima G, Kartikey K, Sharma JP, Ostojic SM, Gvozdjakova A, Kura B, Noda M, Mojto V, Niaz MA, Slezak J. The Effects of 24-Week, High-Concentration Hydrogen-Rich Water on Body Composition, Blood Lipid Profiles and Inflammation Biomarkers in Men and Women with Metabolic Syndrome: A Randomized Controlled Trial. *Diabetes Metab Syndr Obes* 2020; **13**: 889-896 [PMID: 32273740 DOI: 10.2147/DMSO.S240122]

23 **Sha JB**, Zhang SS, Lu YM, Gong WJ, Jiang XP, Wang JJ, Qiao TL, Zhang HH, Zhao MQ, Wang DP, Xia H, Li ZW, Chen JL, Zhang L, Zhang CG. Effects of the long-term consumption of hydrogen-rich water on the antioxidant activity and the gut flora in female juvenile soccer players from Suzhou, China. *Med Gas Res* 2018; **8**: 135-143 [PMID: 30713665 DOI: 10.4103/2045-9912.248263]

24 **Li FY**, Zhu SX, Wang ZP, Wang H, Zhao Y, Chen GP. Consumption of hydrogen-rich water protects against ferric nitrilotriacetate-induced nephrotoxicity and early tumor promotional events in rats. *Food Chem Toxicol* 2013; **61**: 248-254 [PMID: 24140467 DOI: 10.1016/j.fct.2013.10.004]

25 **Wang D**, Wang L, Zhang Y, Zhao Y, Chen G. Hydrogen gas inhibits lung cancer progression through targeting SMC3. *Biomed Pharmacother* 2018; **104**: 788-797 [PMID: 29852353 DOI: 10.1016/j.biopha.2018.05.055]

26 **Nishiwaki H**, Ito M, Negishi S, Sobue S, Ichihara M, Ohno K. Molecular hydrogen upregulates heat shock response and collagen biosynthesis, and downregulates cell cycles: meta-analyses of gene expression profiles. *Free Radic Res* 2018; **52**: 434-445 [PMID: 29424253 DOI: 10.1080/10715762.2018.1439166]

27 **Safonov M**, You J, Lee, J, Safonov V L, Berman D, Zhu DH. Hydrogen generating patch improves skin cell viability, migration activity, and collagen expression. *ER* 2020; **1**: 1-5 [DOI: 10.1016/j.engreg.2020.05.001]

28 **Terasaki Y**, Ohsawa I, Terasaki M, Takahashi M, Kunugi S, Dedong K, Urushiyama H, Amenomori S, Kaneko-Togashi M, Kuwahara N, Ishikawa A, Kamimura N, Ohta S, Fukuda Y. Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. *Am J Physiol Lung Cell Mol Physiol* 2011; **301**: L415-L426 [PMID: 21764987 DOI: 10.1152/ajplung.00008.2011]

29 **Dong WW**, Zhang YQ, Zhu XY, Mao YF, Sun XJ, Liu YJ, Jiang L. Protective Effects of Hydrogen-Rich Saline Against Lipopolysaccharide-Induced Alveolar Epithelial-to-Mesenchymal Transition and Pulmonary Fibrosis. *Med Sci Monit* 2017; **23**: 2357-2364 [PMID: 28522797 DOI: 10.12659/msm.900452]

30 **Lu H**, Chen W, Liu W, Si Y, Zhao T, Lai X, Kang Z, Sun X, Guo Z. Molecular hydrogen regulates PTEN-AKT-mTOR signaling *via* ROS to alleviate peritoneal dialysis-related peritoneal fibrosis. *FASEB J* 2020; **34**: 4134-4146 [PMID: 31930571 DOI: 10.1096/fj.201901981R]

31 **Jiang Z**, Alamuri TT, Muir ER, Choi DW, Duong TQ. Longitudinal multiparametric MRI study of hydrogen-enriched water with minocycline combination therapy in experimental ischemic stroke in rats. *Brain Res* 2020; **1748**: 147122 [PMID: 32919984 DOI: 10.1016/j.brainres.2020.147122]

32 **Zhao P**, Jin Z, Chen Q, Yang T, Chen D, Meng J, Lu X, Gu Z, He Q. Local generation of hydrogen for enhanced photothermal therapy. *Nat Commun* 2018; **9**: 4241 [PMID: 30315173 DOI:

10.1038/s41467-018-06630-2]

33 **Barnier C**, Clerissi C, Lami R, Intertaglia L, Lebaron P, Grimaud R, Urios L. Description of Palleronia rufa sp. nov., a biofilm-forming and AHL-producing Rhodobacteraceae, reclassification of Hwanghaeicola aestuarii as Palleronia aestuarii comb. nov., Maribius pontilimi as Palleronia pontilimi comb. nov., Maribius salinus as Palleronia salina comb. nov., Maribius pelagius as Palleronia pelagia comb. nov. and emended description of the genus Palleronia. *Syst Appl Microbiol* 2020; **43**: 126018 [PMID: 31733924 DOI: 10.1016/j.syapm.2019.126018]

34 **De Angelis PM**, Svendsrud DH, Kravik KL, Stokke T. Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery. *Mol Cancer* 2006; **5**: 20 [PMID: 16709241 DOI: 10.1186/1476-4598-5-20]

35 **Zhang N**, Yin Y, Xu SJ, Chen WS. 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules* 2008; **13**: 1551-1569 [PMID: 18794772 DOI: 10.3390/molecules13081551]

36 **Guo JY**, Teng X, Laddha SV, Ma S, Van Nostrand SC, Yang Y, Khor S, Chan CS, Rabinowitz JD, White E. Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells. *Genes Dev* 2016; **30**: 1704-1717 [PMID: 27516533 DOI: 10.1101/gad.283416.116]

37 **Chen H**, Mao X, Meng X, Li Y, Feng J, Zhang L, Zhang Y, Wang Y, Yu Y, Xie K. Hydrogen alleviates mitochondrial dysfunction and organ damage *via* autophagymediated NLRP3 inflammasome inactivation in sepsis. *Int J Mol Med* 2019; **44**: 1309-1324 [PMID: 31432098]

38 **Jiang X**, Niu X, Guo Q, Dong Y, Xu J, Yin N, Qianqian Q, Jia Y, Gao L, He Q, Lv P. Corrigendum to "FoxO1-mediated autophagy plays an important role in the neuroprotective effects of hydrogen in a rat model of vascular dementia" [Behav. Brain Res. 356 (2019) 98-106]. *Behav Brain Res* 2019; **374**: 111839 [PMID: 31005352 DOI: 10.1016/j.bbr.2019.03.008]

39 **Wang H**, Huo X, Chen H, Li B, Liu J, Ma W, Wang X, Xie K, Yu Y, Shi K. Hydrogen-Rich Saline Activated Autophagy *via* HIF-1α Pathways in Neuropathic Pain Model. *Biomed Res Int* 2018; **2018**: 4670834 [PMID: 29888265 DOI: 10.1155/2018/4670834]

40 **Gao Y**, Yang H, Chi J, Xu Q, Zhao L, Yang W, Liu W. Hydrogen Gas Attenuates Myocardial Ischemia Reperfusion Injury Independent of Postconditioning in Rats by Attenuating Endoplasmic Reticulum Stress-Induced Autophagy. *Cell Physiol Biochem* 2017; **43**: 1503-1514 [PMID: 29035876 DOI: 10.1159/000481974]

41 **Wyatt MD**, Wilson DM 3rd. Participation of DNA repair in the response to 5-fluorouracil. *Cell Mol Life Sci* 2009; **66**: 788-799 [PMID: 18979208 DOI: 10.1007/s00018-008-8557-5]

42 **Abou-Hamdan M**, Gardette B, Cadet J, Gharib B, De Reggi M, Douki T, Triantaphylides C. Molecular hydrogen attenuates radiation-induced nucleobase damage to DNA in aerated aqueous solutions. *Int J Radiat Biol* 2016; **92**: 536-541 [PMID: 27438130 DOI: 10.1080/09553002.2016.1206234]

43 **Qiao S**, Wang Y, Zan R, Wu H, Sun Y, Peng H, Zhang R, Song Y, Ni J, Zhang S, Zhang X. Biodegradable Mg Implants Suppress the Growth of Ovarian Tumor. *ACS Biomater Sci Eng* 2020; **6**: 1755-1763 [PMID: 33455395 DOI: 10.1021/acsbiomaterials.9b01703]

44 **Sobue S**, Inoue C, Hori F, Qiao S, Murate T, Ichihara M. Molecular hydrogen modulates gene expression *via* histone modification and induces the mitochondrial unfolded protein response. *Biochem Biophys Res Commun* 2017; **493**: 318-324 [PMID: 28890349 DOI: 10.1016/j.bbrc.2017.09.024]

45 **Deng P**, Haynes CM. Mitochondrial dysfunction in cancer: Potential roles of ATF5 and the mitochondrial UPR. *Semin Cancer Biol* 2017; **47**: 43-49 [PMID: 28499833 DOI: 10.1016/j.semcancer.2017.05.002]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 253-264

DOI: 10.4251/wjgo.v14.i1.253

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Lnc524369 promotes hepatocellular carcinoma progression and predicts poor survival by activating YWHAZ-RAF1 signaling

Wei Zheng, Guo-Liang Shen, Ke-Yang Xu, Qiao-Qiao Yin, Tian-Chen Hui, Zhe-Wen Zhou, Cheng-An Xu, Shou-Hao Wang, Wen-Hao Wu, Ling-Fei Shi, Hong-Ying Pan

ORCID number: Wei Zheng 0000-0001-6639-2869; Guo-Liang Shen 0000-0003-4665-3501; Ke-Yang Xu 0000-0002-7049-2528; Qiao-Qiao Yin 0000-0003-2812-0496; Tian-Chen Hui 0000-0001-8128-7696; Zhe-Wen Zhou 0000-0002-4638-915X; Cheng-An Xu 0000-0003-4761-6518; Shou-Hao Wang 0000-0001-9947-9629; Wen-Hao Wu 0000-0002-0736-6561; Ling-Fei Shi 0000-0002-7170-2232; Hong-Ying Pan 0000-0002-8433-3851.

Author contributions: Zheng W designed the report and wrote the paper; Hui TC, Yin QQ, Zhou ZW, Xu CA, and Shi LF performed the majority of experiments and analyzed the data; Wu WH and Wang SH performed the molecular investigations; Shen GL designed and coordinated the research; Xu KY and Pan HY revised the paper.

Institutional review board statement: This study was reviewed and approved by the Zhejiang Provincial People's Hospital Ethics Committee.

Informed consent statement: All patients in our study provided informed consent.

Conflict-of-interest statement: The authors declare no conflict of interest.

Wei Zheng, Department of Clinical Medicine, Medical College of Soochow University, Suzhou 215006, Jiangsu Province, China

Wei Zheng, Qiao-Qiao Yin, Hong-Ying Pan, Department of Infectious Diseases, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou 310014, Zhejiang Province, China

Guo-Liang Shen, Department of Hepatobiliary & Pancreatic Surgery and Minimally Invasive Surgery, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou 310014, Zhejiang Province, China

Ke-Yang Xu, School of Chinese Medicine, Hongkong Baptist university, Hong Kong 999777, China

Tian-Chen Hui, Zhe-Wen Zhou, Department of Graduate School, Bengbu Medical College, Bengbu 233030, Anhui Province, China

Cheng-An Xu, Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang Province, China

Shou-Hao Wang, Wen-Hao Wu, Medical Department of Qingdao University, Qingdao University, Qingdao 266071, Shandong, China

Ling-Fei Shi, Diagnosis and Treatment Center of Osteoporosis, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hang Zhou 310014, Zhejiang Province, China

Corresponding author: Hong-Ying Pan, MD, Chief Doctor, Department of Infectious Diseases, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, No. 158 Shangtang Road, Hangzhou 310014, Zhejiang Province, China. hypanhz@139.com

Abstract

BACKGROUND

Liver cancer is one of the most highly malignant cancers, characterized by easy metastasis and chemoradiotherapy resistance. Emerging evidence indicates that long noncoding RNAs (LncRNAs), including Lnc524369, are highly involved in the initiation, progression, radioresistance, and chemoresistance of hepatocellular

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Supported by the Medical and Health Science and Technology Project of Zhejiang Province (No. 2021KY043) and Chinese Foundation for Hepatitis Prevention and Control-TianQing Liver Disease Research Fund Subject (No. TQGB2020168).

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: June 22, 2021
Peer-review started: June 22, 2021
First decision: July 4, 2021
Revised: July 28, 2021
Accepted: September 16, 2021
Article in press: September 16, 2021
Published online: January 15, 2022

carcinoma (HCC). However, the function of Lnc524369 remains unclear.

AIM

To explore the function of Lnc524369 in HCC.

METHODS

To investigate the effect of Lnc524369, tissue from 41 HCC patients were analyzed using CCK8, migration, and invasion assays. Lnc524369 and YWHAZ (also named 14-3-3ζ) mRNA were detected by qPCR, and YWHAZ and RAF1 proteins were detected by western blot in liver cancer cell lines and human HCC tissues. The Cancer Cell Line Encyclopedia (CCLE) databases, STRING database, Human Protein Atlas database, and the TCGA database were used for bioinformatic analysis.

RESULTS

Lnc524369 was significantly upregulated in the nucleus of liver cancer cells and human HCC tissues. Overexpression of Lnc524369 was associated with the proliferation, migration, and invasion of liver cancer cells. YWHAZ and RAF1 proteins and YWHAZ mRNA were overexpressed in liver cancer, which could be attenuated by overexpression of Lnc524369. Lnc524369 and its downstream target YWHAZ and RAF1 proteins were negatively associated with overall survival time.

CONCLUSION

Lnc524369 might be a promising target of HCC as it can enhance liver cancer progression and decrease the overall survival time of HCC by activating the YWHAZ/RAF1 pathway.

Key Words: Long noncoding RNAs; Lnc524369; Hepatocellular carcinoma; YWHAZ; RAF1

Core Tip: Lnc524369 is expressed at low levels in the cytoplasm but enriched in the nucleus of hepatocellular carcinoma (HCC) cells and might be strongly coexpressed with YWHAZ. Overexpression of Lnc524369 promoted the proliferation, migration, and invasion of liver cancer cells. The Lnc524369-mediated YWHAZ/RAF1 pathway was negatively associated with the overall survival time of HCC patients.

Citation: Zheng W, Shen GL, Xu KY, Yin QQ, Hui TC, Zhou ZW, Xu CA, Wang SH, Wu WH, Shi LF, Pan HY. Lnc524369 promotes hepatocellular carcinoma progression and predicts poor survival by activating YWHAZ-RAF1 signaling. *World J Gastrointest Oncol* 2022; 14(1): 253-264
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/253.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.253

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common pathological type of primary liver cancer[1]. Among all cancers, the incidence and mortality rates of HCC rank sixth and second in the world, respectively[2]. Common risk factors leading to HCC are hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholism, obesity, environmental toxins, and metabolic diseases[3]. At present, approximately 93 million hepatitis B carriers are at least partially responsible for the high incidences of liver fibrosis, cirrhosis, and HCC in China[4]. Due to HCC's high malignancy and insensitivity to chemoradiotherapy, the potential mechanisms of HCC need to be further clarified.

Long noncoding RNAs (lncRNAs) are a class of RNA transcripts with a length of more than 200 nucleotides that lack protein coding potential[5]. Increasing evidence shows that lncRNAs can regulate many important pathophysiological processes,

P-Reviewer: Youness RA
S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Yu HG

especially in the occurrence and development of malignant tumors[6]. For example, lncRNAs such as HOTAIR, HULC, and MALAT-1, are closely related to the proliferation, apoptosis, angiogenesis, invasion, metastasis, and prognosis of HCC[6]. Therefore, lncRNAs have therapeutic potential and diagnostic value in HCC. A previous study had suggested that Lnc524369 (ap003469.2) is expressed at low levels in the cytoplasm but enriched in the nucleus of HCC and might be strongly coexpressed with 14-3-3 protein zeta/delta (YWHAZ) using a fractionation-then-sequencing approach[7]. YWHAZ is clearly upregulated in HCC, promotes cell proliferation and metastasis, and its expression is a predictor of poor survival[8-11]. Analysis of the protein-to-protein interaction network indicated that YWHAZ is strongly coexpressed with RAF1. Furthermore, analysis of the Cancer Cell Line Encyclopedia (CCLE) revealed that overexpression of the YWHAZ and RAF1 proteins occurs frequently in liver cancer cell lines. In this study, we aimed to determine the function of Lnc524369 in the development of HCC.

MATERIALS AND METHODS

The human liver cancer cell lines Huh7 and HepG2, as well as 41 human HCC samples from Zhejiang Provincial People's Hospital, were used to determine Lnc524369 expression levels. However, due to limited human HCC sample tissue, only 5 of 41 HCC samples (as well as the liver cell lines) were used to examine YWHAZ and RAF1 protein or mRNA levels. The whole study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital.

Bioinformatic analysis

We analyzed the protein expression of YWHAZ and RAF1 in liver cancer cell lines by using data obtained from the CCLE (http://www.broadinstitute.org/ccle). In addition, we conducted survival analysis of YWHAZ and RAF1 by using the Human Protein Atlas database (http://www.proteinatlas.org/) and the Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga).

RNA isolation (cytoplasmic and nuclear)

RNA was isolated from 1 × 10^6 Huh7 cells or 15 mg human HCC tissue using a cytoplasmic and nuclear RNA purification kit from Norgen Biotek Corp. (Ontario, Canada). Cells or tissues washed with phosphate-buffered saline (PBS) were lysed with ice-cold lysis solution, and then the lysate was transferred to a microcentrifuge tube and spun at maximum speed for three minutes. The supernatant contained cytoplasmic RNA, while the pellet contained nuclear RNA. Binding solution was then added to the supernatant and pellet separately, and each fraction was mixed and resuspended well. The RNA samples were then bound to separate spin columns using centrifugation. Next, the columns were washed twice with 400 μL of the provided wash solution, and the fractionated RNA was eluted from the columns using 50 μL of the provided elution buffer.

Real-time PCR

Real-time PCR was performed to determine Lnc524369 expression in HepG2 and Huh7 cell lines, as well as human HCC and paracancerous tissue. Cells treated with the pcDNA3 control plasmid and the Lnc524369-pcdna3.1 overexpression plasmid, as well as human HCC and paracancerous tissue, were collected for analysis. A cytoplasmic and nuclear RNA purification kit (NGB21000; Norgen Biotek Corp.) was used to extract RNA from cells and tissues. Total RNA was extracted by TRIzol reagent, and cDNA was synthesized according to the instructions of the Quantitect reverse transcription kit (Qiagen, Hilden, German). Using GAPDH as an internal reference, real-time PCR was performed with a Powerup SYBRTM Green master mix kit (Thermo Fisher Scientific, Waltham, MA, United States). The specific primer sequences are shown in Table 1. Each experiment was repeated three times, and three complex holes were set in each sample. The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blot

Western blotting was performed to determine YWHAZ and RAF1 expression in the Huh7 cell line, as well as human HCC and paracancerous tissue. Anti-rabbit YWHAZ (1:2000; Proteintech, Rosemont, IL, United States), anti-mouse RAF1 (1:1000;

Table 1 Primer sequences used in study

Gene name	Gene accession No.	Primer (5'→3')	Length (bp)
Lnc524369	ENST00000524369.1	F: CAGCAGAACTGGGTGTTGGA	90
		R: GCGCTGCAGTTTCCTCCTT	
GAPDH	NM_002046.5	F: CCATGACAACTTTGGTATCGTGGAA	107
		R: GGCCATCACGCCACAGTTTC	

Proteintech), and anti-rabbit GAPDH (internal reference, 1:10000; Abcam, Cambridge, United Kingdom) were added to the cell or tissue samples and incubated overnight at 4 °C. Goat anti-rabbit IgG HRP secondary antibody (1:5000; Thermo Fisher Scientific) was added to the membrane for film exposure. After the film was exposed in a dark room, the optical density of the strips was analyzed by Image-Pro Plus 6.0 software, and the results were analyzed with GAPDH as an internal reference. Each experiment was repeated 3 times, and the results are expressed as the mean ± standard deviation (SD).

Culture of human liver cancer cell lines Huh7 and HepG2

The human liver cell lines Huh7 and HepG2 were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. They were resuspended in DMEM high glucose (containing 10% FBS and double antibodies). The cells were cultured at 37 °C and 5% CO_2 until the confluence was approximately 90%.

Construction of the Lnc524369-pcdna3.1 overexpression vector

According to the sequence of the Lnc524369 gene, the whole gene was synthesized by Shenggong Bioengineering (Shanghai) Co., Ltd. (Shanghai, China) with the addition of *BamH*I and *EcoR*I restriction sites the ends of the gene. The synthetic products of the Lnc524369 and pcDNA3.1 plasmids (Invitrogen, Waltham, MA, United States) were digested, purified, and linked with *BamH*I and *EcoR*I, respectively, and then transformed into E. coli DH5α competent cells and cultured overnight at 37 °C. The recombinant plasmid Lnc524369-pcDNA3.1 was extracted and sequenced. After identification, an endofree plasma maxi kit (Qiagen) was used to extract the Lnc524369-pcDNA3.1 overexpression plasmid and pcDNA3.1 control plasmid for subsequent transfection experiments.

Transfection of Lnc524369-pcdna3.1 overexpression vector

Huh7 cells were inoculated into 6 well plates at a density of 5 × 10^5 cells/mL and incubated overnight in a 5% CO_2 incubator at 37 °C. When the confluence of the Huh7 cells reached 70%-80%, the Lnc524369-pcDNA3.1 overexpression plasmid and pcDNA3.1 control plasmid were transfected according to the instructions of Lipofectamine 3000 (Thermo Fisher Scientific). After 8 h, the media was replaced with fresh complete culture medium. After 48 h, the total RNA was extracted for subsequent qPCR analysis and the protein was extracted for subsequent western blot detection.

CCK8 assay for cell proliferation

Huh7 cells transfected for 24 h with the pcDNA3.1 control plasmid and the Lnc524369-pcDNA3.1 overexpression plasmid were digested and collected, and the cell density was adjusted. According to the cell density of 2 × 10^3/100 μL in each well, the cells were recorded as 0 h after adherence. After 0 h, 24 h, 48 h and 72 h, 10 μL CCK8 was added to each well and incubated at 37 °C and 5% CO_2 for 2 h. Next, the absorbance value at 450 nm was detected using an enzyme-labeled measurement instrument.

Migration transwell assay

After counting, the density of Huh7 cells was 2 × 10^5/mL, and 200 μL transwell cell suspension in serum free mediums was added to each transwell chamber (8.0 μm), and 600 μL containing 15% FBS medium was added to the incubator, incubated 48 h in the 5% CO_2 incubator at 37 °C. The cells were then fixed with 4% paraformaldehyde for 30 min, and stained with 0.1% crystal violet for 30 min. After washing with PBS, an inverted microscope was used to take pictures and count the cells. Each group was provided with 3 multiple holes. Five visual fields were randomly selected from each well, and the average number of migrating cells was counted.

Table 2 Hepatocellular carcinoma patients based on Lnc524369 levels

		Higher expression (*n* = 22)	Lower expression (*n* = 19)	t/χ^2	*P*
Age		57.68 ± 10.77	54.21 ± 11.44	1.000	0.688
Male		13 (59.1%)	12 (63.16%)	0.004	0.952
Pathological grading	Low	9 (40.91%)	2 (10.53%)		
	Moderate	11 (50.00%)	10 (52.63%)	-	0.009
	High	2 (9.09%)	7 (36.84%)		

Fisher's exact test was used.

Invasion transwell assay

The 24 well Matrigel invasion chamber was taken out and allowed to recover to room temperature. Then, 500 μL serum-free medium was added and incubated at 37 °C for 2 h. The basement membrane was hydrated and the excess liquid was absorbed for standby. The cells were resuspended in serum-free medium for 24 h, and the cell concentration was adjusted to 2×10^5/mL. Two hundred microliters of cell suspension was added to the upper chamber, and 600 μL of cell culture medium containing 15% FBS was added to the lower chamber. After 48 h incubation in a 5% CO_2 incubator at 37 °C, the cells were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 30 min. After washing with PBS, an inverted microscope was used for counting the cells. Each group included 3 wells, and 5 visual fields were randomly selected under 200 magnification for each well, and the average number of invasive cells was counted.

Statistical analysis

SPSS 19.0 software (IBM Corp., Armonk, NY, United States) was used for statistical analysis. The results were expressed as the mean ± SD. Independent sample *t* tests or paired sample *t* tests were used for mean comparisons between the two groups, and one-way ANOVA was used for multigroup mean comparisons. The Spearman method was used for correlation analysis. Kaplan–Meier method was used for survival analysis. When $P < 0.05$ (*), the difference was significant; when $P < 0.01$ (**), the difference was very significant; and when $P < 0.001$ (***), the difference was extremely significant.

RESULTS

The transcriptional level of Lnc524369 level in HepG2 and Huh7 cells

The Lnc524369 level was relatively higher in Huh7 cells than in HepG2 cells and L02 cells (Figure 1A) and enriched in the nucleus of Huh7 cells (Figure 1B). Considering the weaker invasion and lower Lnc524369 level of HepG2, Huh7 cells were used to investigate overexpression of Lnc524369.

Lnc524369 upregulated the YWHAZ and RAF1 expression in Huh7 cells

Real-time PCR results showed that compared with the blank and pcDNA3.1 transfection groups, lnc52436-pcDNA3.1 was upregulated 443-fold in Huh7 cells, as shown in Figure 1C ($P < 0.01$). Compared with the blank and pcDNA3.1 transfection groups, the YWHAZ protein and mRNA levels, as well as the RAF1 protein levels in the Lnc52436-pcDNA3.1 transfection group were significantly increased, as shown in Figures 1D-F and 2B ($P < 0.01$).

Lnc524369 promoted the liver cancer cell proliferation, migration, and invasion

The CCK-8 assay was used to detect the viability of Huh7 cells after overexpression of Lnc524369. Compared with the blank and pcDNA3.1 groups, the viability of Huh7 cells increased significantly from 48 h (Figure 3A, $P < 0.01$). This result indicated that the proliferation of liver cancer cells was enhanced by overexpression of Lnc524369. Transwell assay results showed that compared with the blank and pcDNA3.1 transfection groups, the number of migrated Lnc52436-pcDNA3.1 cells was significantly increased, as shown in Figure 3B and 3D ($P < 0.01$). This result indicated

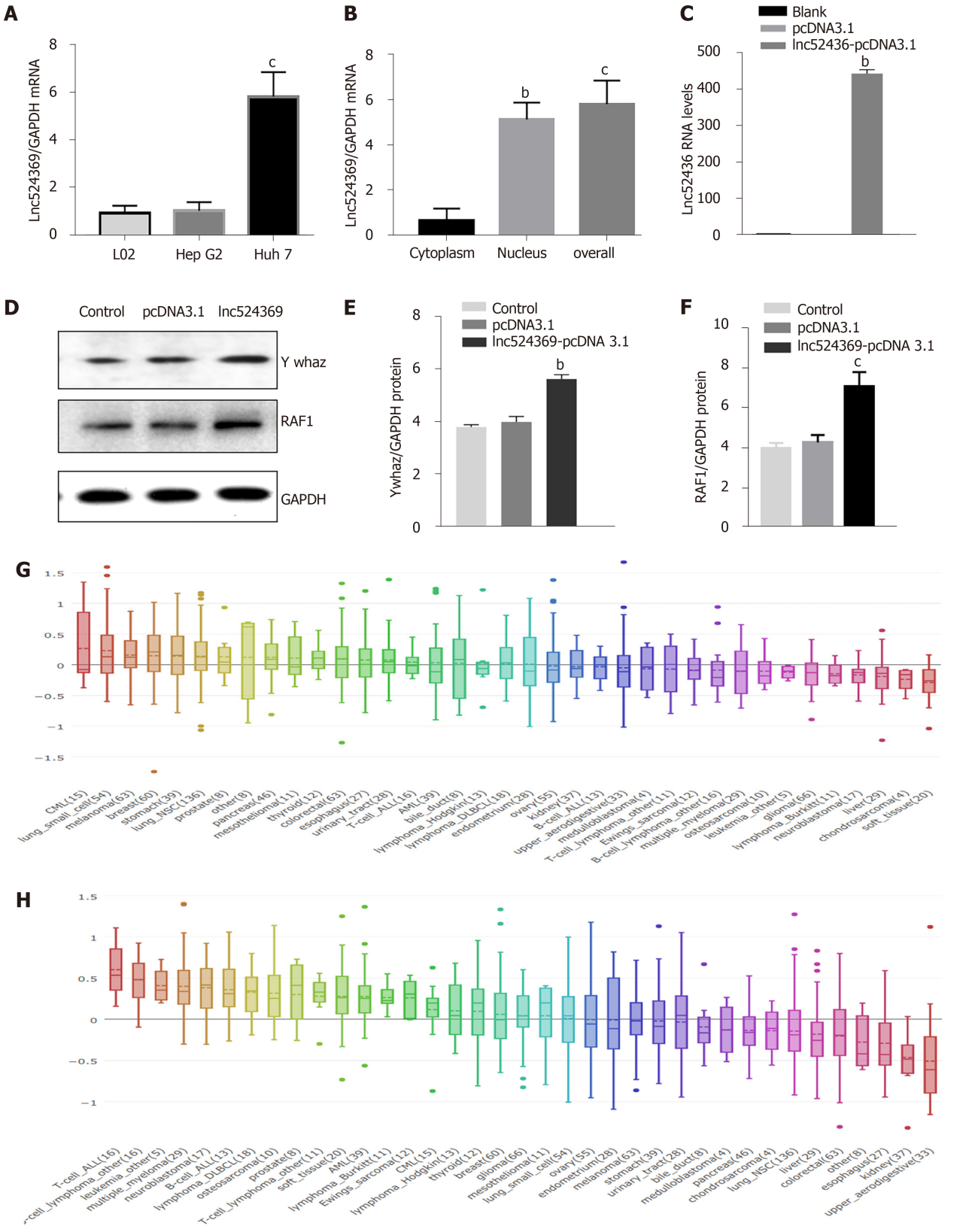


Figure 1 Expression of Lnc524369/YWHAZ/RAF1 *in vitro*. A: Expression of Lnc524369 in human cancer cell lines (HepG2 and Huh 7) and normal liver cells (L02); B: Expression of Lnc524369 in the cytoplasm and nucleus of Huh 7 cells; C: Knock-in of Lnc524369 in Huh 7 cells; D: Western blot bands of YWHAZ and RAF1 protein; E: YWHAZ expression was increased by overexpression of Lnc524369; F: RAF1 expression was increased by overexpression of Lnc524369; G: YWHAZ protein was expressed in liver cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) database; H: RAF1 protein was expressed in liver cancer cell lines from the CCLE database.

that the migration of liver cancer cells could be enhanced by overexpression of Lnc524369. Transwell assay results showed that compared with the blank and pcDNA3.1 transfection groups, the number of cells passing through the basement

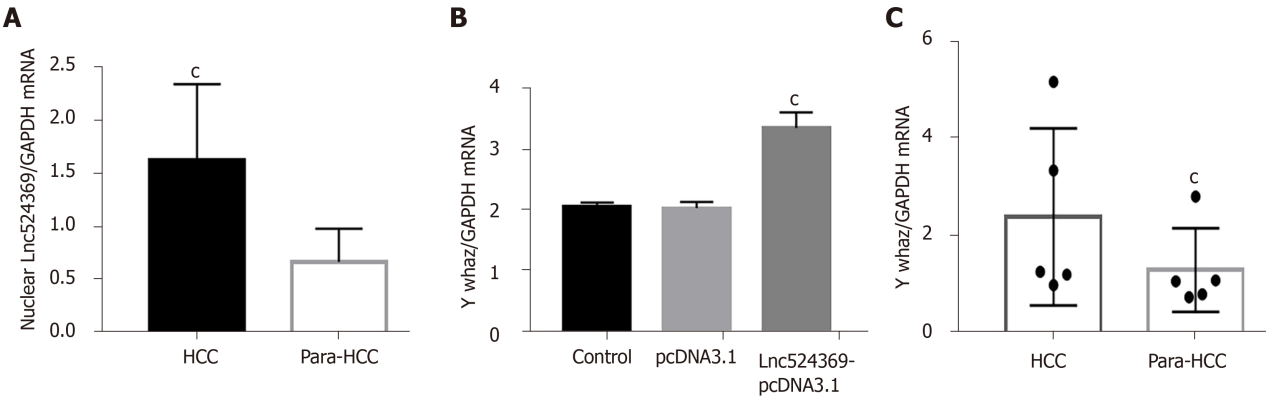


Figure 2 The expression of Lnc542369 and YWHAZ mRNA *in vitro* and in hepatocellular carcinoma patients. A: Nuclear Lnc542369 was significantly upregulated in hepatocellular carcinoma (HCC) tissues compared with para-HCC tissues; B: YWHAZ mRNA was significantly upregulated by overexpression of Lnc542369; C: YWHAZ mRNA was significantly upregulated in HCC tissues compared with para-HCC tissues.

membrane in the Lnc52436-pcDNA3.1 transfection group was significantly increased (Figure 3C and 3D) ($P < 0.01$). This result indicated that the invasion of liver cancer cells could be enhanced by overexpression of Lnc524369.

Lnc524369 was overexpressed in human HCC samples and predicted poor prognosis

Forty-one confirmed HCC patients were included in this study according to the HCC guidelines[12], and their baseline characteristics are shown in Table 2. Human HCC and para-HCC tissues were acquired by surgical resection, which was further confirmed by two histopathological doctors. The relative nuclear Lnc524369 expression level was significantly higher in HCC tissues than in para-HCC tissues ($P < 0.001$) (Figure 2A). There was a positive correlation between Lnc524369 and the pathological grade of HCC (correlation coefficient RS: 0.604, $P < 0.001$) (Figure 4D). The survival rate of patients with high expression of Lnc524369 was significantly lower than that of patients with low expression ($P = 0.013$) (Figure 4G).

YWHAZ and RAF1 levels in human HCC samples and survival analysis of the TCGA database

YWHAZ mRNA and protein levels were determined in our five included HCC samples by real-time PCR and western blot. Our results showed that the YWHAZ mRNA level and protein were significantly higher in HCC tissues than in para-HCC tissues ($P < 0.05$) (Figures 2C, 4E and 4I); RAF1 protein was also significantly higher in HCC tissues than in para-HCC tissues ($P < 0.05$) (Figure 4F and 4I). In addition, the YWHAZ mRNA data of 235 live and 130 deceased HCC patients (264 male; 119 female) in the TCGA database were included for survival analysis. The survival probability of patients with high YWHAZ and RAF1 mRNA expression was significantly decreased compared with those patients with low YWHAZ expression ($P < 0.001$) (Figure 4H and 4J).

DISCUSSION

Recently, lncRNAs have emerged as critical molecules in multiple biological processes involved in virus infection, metabolic diseases, vascular diseases, stem cell biology, fibrosis, and cancer[13-16]. Currently, the roles of lncRNAs have been widely reported in the progression of HCC and liver cancer stem cells[17]. A large number of lncRNAs localize in the nucleus and are highly involved in several cellular components, biological processes, and molecular functions, such as chromatin organization, structural scaffolds of nuclear domains, and transcriptional and posttranscriptional gene expression[18]. Previous fractionation-then-sequencing data from human HCC tissues have shown that Lnc524369 is enriched in the nucleus of liver cancer but not the cytoplasm[7]. In our study, we also confirmed that Lnc524369 expression was enriched in the nucleus of Huh 7 cells.

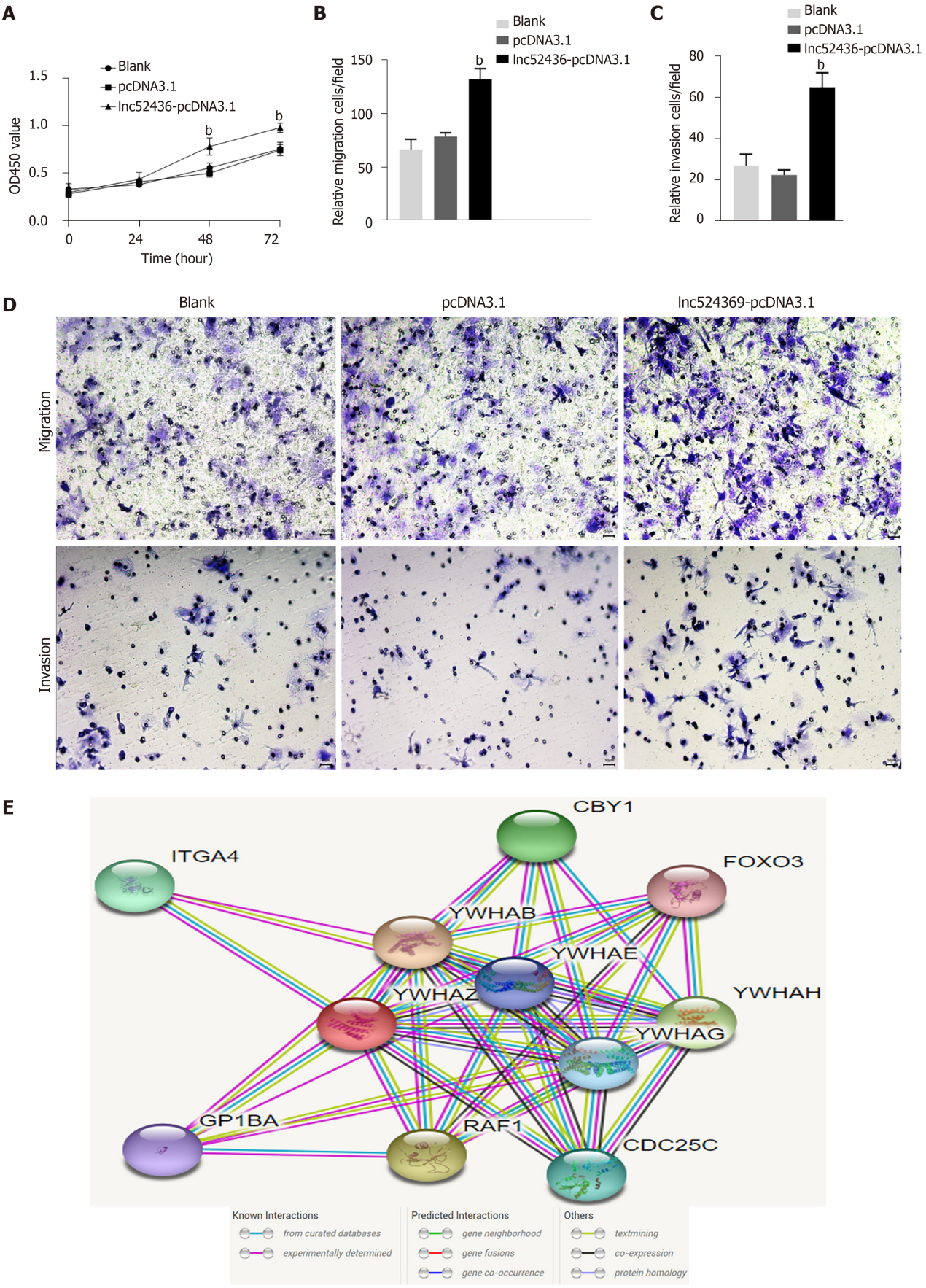


Figure 3 Overexpression of Lnc524369 *in vitro* and the protein-protein-interaction network of YWHAZ. A: Overexpression of Lnc524369 promoted the proliferation of Huh7 cells; B: Overexpression of Lnc524369 promoted the migration of Huh7 cells; C: Overexpression of Lnc524369 promoted the invasion of Huh7 cells; D: Images of migration and invasion; E: The protein-protein-interaction network of YWHAZ-RAF1 in the STRING database.

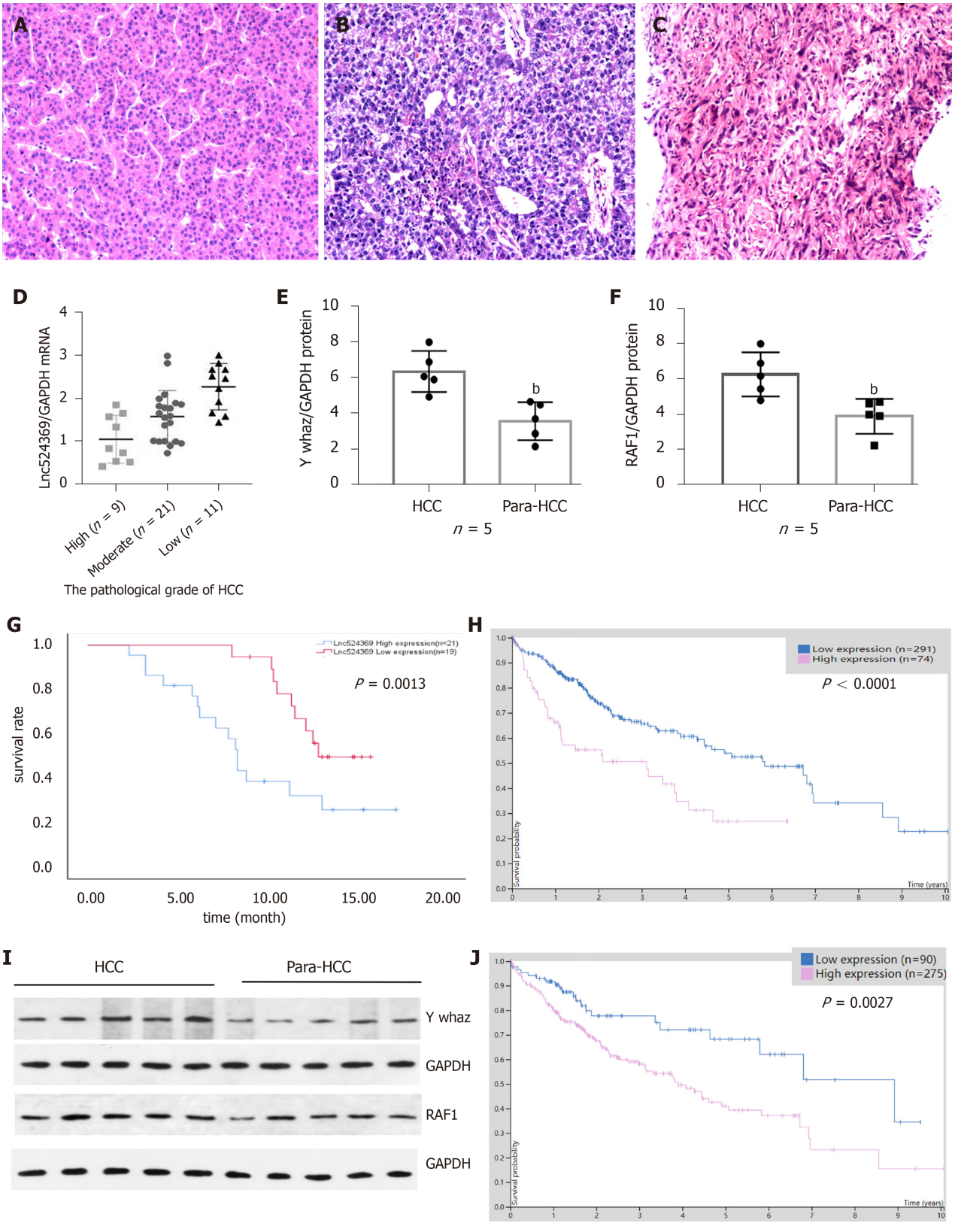


Figure 4 The expression of Lnc524369/YWHAZ/RAF1 protein in hepatocellular carcinoma patients and its survival analysis. A: High differentiation of human hepatocellular carcinoma (HCC) pathology; B: Moderate differentiation of human HCC pathology; C: Low differentiation of human HCC pathology; D: Lnc524369 relative level was positively corelated with pathological grade of HCC (RS: 0.604, $P < 0.01$); E: YWHAZ protein level was higher in HCC tissue than para-HCC tissue ($P < 0.01$); F: RAF1 protein level was higher in HCC tissue than para-HCC tissue ($P < 0.001$); G: The western blot band of YWHAZ protein; H: Survival analysis for YWHAZ mRNA of HCC patients at TCGA database; I: The RAF1 protein of the western blot in HCC tissue and para-HCC tissue; J: Survival analysis for RAF1 mRNA of HCC patients at TCGA database. HCC: Hepatocellular carcinoma.

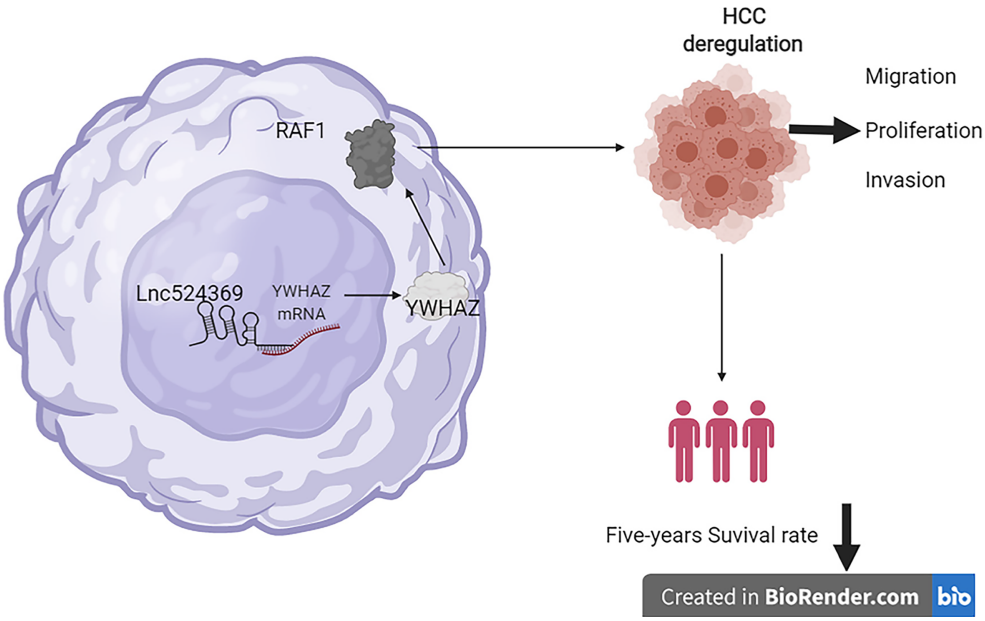


Figure 5 Possible mechanism of Lnc524369 effect on hepatocellular carcinoma. Lnc529439 might increase the expression of YWHAZ mRNA and then upregulate the YWHAZ protein level, which triggers RAF1 activation to promote hepatocellular carcinoma (HCC) progression.

Furthermore, we illustrated that the expression of nuclear Lnc524369 was significantly increased in human HCC tissues compared to HCC adjacent tissues (P < 0.01). Additionally, we found that the overexpression of Lnc524369 could clearly promote the proliferation, migration, and invasion of liver cancer cells and simultaneously enhance YWHAZ and RAF1 expression. This result suggested that Lnc524369 might positively correlate with the expression of YWHAZ and RAF1 in the development of HCC. Lnc524369 was significantly correlated with a poor survival rate of HCC patients.

YWHAZ has been shown to be commonly upregulated in multiple cancers, especially HCC, as it can promote tumorigenesis, metastasis, and chemoresistance in the progression of cancer[19-21]. Our bioinformatic analysis showed that RAF1 was strongly coexpressed with YWHAZ (Figure 3E). RAF1 is also highly involved in the development of HCC[22]. Again, our analysis of the CCLE databases revealed that YWHAZ and RAF1 were frequently expressed in liver cancer cell lines. Our study indicated that YWHAZ transcriptional and posttranscriptional levels were both overexpressed in HCC tissues compared to HCC adjacent tissues (P < 0.01). In addition, we found that YWHAZ and RAF1 mRNA levels were negatively related to overall survival time in the TCGA database. Previous studies have suggested that multiple noncoding RNAs, such as miR-451a, miR-22, and the long noncoding RNA MIR4435-2HG, are involved in HCC proliferation, invasion, and metastasis by targeting YWHAZ[8,9,11]. These results indicate that the overexpression of YWHAZ plays a critical role in the initiation and progression of HCC; therefore, the YWHAZ/RAF1 protein might have significant clinical potential for targeted therapy and early diagnosis.

There are some limitations in this study that will be improved in further studies. First, the expression of Lnc524369, YWHAZ, and RAF1 should be further knocked down for malignant function confirmation; second, the effect of Lnc524369 and YWHAZ in rodent models should be further studied; third, the included number of HCC patients for this study should be further enlarged. In summary, Lnc524369 and its downstream targets YWHAZ and RAF1 play a crucial role in the development of HCC and are negatively associated with the overall survival times of HCC patients, which provides new insight into the early diagnosis and targeted treatment of HCC (Figure 5).

CONCLUSION

Because Lnc524369 can enhance liver cancer progression and decrease the overall survival time of HCC by activating the YWHAZ/RAF1 pathway, might be a

promising target of HCC.

ARTICLE HIGHLIGHTS

Research background

Long noncoding RNAs, including Lnc524369, have the potential to regulate unknown cellular and molecular mechanisms in the initiation, progression, diagnosis, and prognosis of hepatocellular carcinoma (HCC). However, a critical gap in our knowledge is understanding how nucleus-enriched Lnc524369 promotes liver cancer growth.

Research motivation

To discover specific targets for the diagnosis and treatment of HCC.

Research objectives

To investigate the underlying mechanisms of Lnc524369 in HCC.

Research methods

The expression of Lnc524369, YWHAZ, and RAF1 was determined by qPCR or western blot. CCK-8, migration, and invasion assays were used to investigate Lnc524369 function. Forty-one HCC patients, the Cancer Cell Line Encyclopedia databases, STRING database, Human Protein Atlas database and the TCGA database were used for survival analysis.

Research results

Lnc524369 was significantly upregulated in the nucleus of HCC, which promoted the proliferation, migration, and invasion of liver cancer cells by upregulating YWHAZ and RAF1 expression. Lnc524369 and its downstream target YWHAZ/RAF1 protein could predict the poor overall survival time of HCC patients.

Research conclusions

The Lnc524369-mediated YWHAZ/RAF1 pathway is highly involved in the progression and prognosis of HCC.

Research perspectives

In the future, we will reveal the critical role of Lnc524369, which might enhance the early diagnosis of HCC and facilitate the further development of targeted therapy.

REFERENCES

1 **Massarweh NN**, El-Serag HB. Epidemiology of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. *Cancer Control* 2017; **24**: 1073274817729245 [PMID: 28975830 DOI: 10.1177/1073274817729245]

2 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

3 **Fujiwara N**, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J Hepatol* 2018; **68**: 526-549 [PMID: 28989095 DOI: 10.1016/j.jhep.2017.09.016]

4 **Trépo C**, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/S0140-6736(14)60220-8]

5 **Quinn JJ**, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 2016; **17**: 47-62 [PMID: 26666209 DOI: 10.1038/nrg.2015.10]

6 **Cai Z**, Xu K, Li Y, Lv Y, Bao J, Qiao L. Long noncoding RNA in liver cancer stem cells. *Discov Med* 2017; **24**: 87-93 [PMID: 28972877]

7 **Chow EY**, Zhang J, Qin H, Chan TF. Characterization of Hepatocellular Carcinoma Cell Lines Using a Fractionation-Then-Sequencing Approach Reveals Nuclear-Enriched HCC-Associated lncRNAs. *Front Genet* 2019; **10**: 1081 [PMID: 31781161 DOI: 10.3389/fgene.2019.01081]

8 **Wei GY**, Hu M, Zhao L, Guo WS. MiR-451a suppresses cell proliferation, metastasis and EMT *via* targeting YWHAZ in hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2019; **23**: 5158-5167 [PMID: 31298411 DOI: 10.26355/eurrev_201906_18180]

9 **Chen M**, Hu W, Xiong CL, Qu Z, Yin CQ, Wang YH, Luo CL, Guan Q, Yuan CH, Wang FB. miR-

22 targets YWHAZ to inhibit metastasis of hepatocellular carcinoma and its down-regulation predicts a poor survival. *Oncotarget* 2016; **7**: 80751-80764 [PMID: 27811373 DOI: 10.18632/oncotarget.13037]

10 **Zhao JF**, Zhao Q, Hu H, Liao JZ, Lin JS, Xia C, Chang Y, Liu J, Guo AY, He XX. The ASH1-miR-375-YWHAZ Signaling Axis Regulates Tumor Properties in Hepatocellular Carcinoma. *Mol Ther Nucleic Acids* 2018; **11**: 538-553 [PMID: 29858089 DOI: 10.1016/j.omtn.2018.04.007]

11 **Shen X**, Ding Y, Lu F, Yuan H, Luan W. Long noncoding RNA MIR4435-2HG promotes hepatocellular carcinoma proliferation and metastasis through the miR-22-3p/YWHAZ axis. *Am J Transl Res* 2020; **12**: 6381-6394 [PMID: 33194037]

12 **Zhou J**, Sun HC, Wang Z, Cong WM, Wang JH, Zeng MS, Yang JM, Bie P, Liu LX, Wen TF, Han GH, Wang MQ, Liu RB, Lu LG, Ren ZG, Chen MS, Zeng ZC, Liang P, Liang CH, Chen M, Yan FH, Wang WP, Ji Y, Cheng WW, Dai CL, Jia WD, Li YM, Li YX, Liang J, Liu TS, Lv GY, Mao YL, Ren WX, Shi HC, Wang WT, Wang XY, Xing BC, Xu JM, Yang JY, Yang YF, Ye SL, Yin ZY, Zhang BH, Zhang SJ, Zhou WP, Zhu JY, Liu R, Shi YH, Xiao YS, Dai Z, Teng GJ, Cai JQ, Wang WL, Dong JH, Li Q, Shen F, Qin SK, Fan J. Guidelines for Diagnosis and Treatment of Primary Liver Cancer in China (2017 Edition). *Liver Cancer* 2018; **7**: 235-260 [PMID: 30319983 DOI: 10.1159/000488035]

13 **Liu Y**, Feng J, Sun M, Yang G, Yuan H, Wang Y, Bu Y, Zhao M, Zhang S, Zhang X. Long non-coding RNA HULC activates HBV by modulating HBx/STAT3/miR-539/APOBEC3B signaling in HBV-related hepatocellular carcinoma. *Cancer Lett* 2019; **454**: 158-170 [PMID: 30981758 DOI: 10.1016/j.canlet.2019.04.008]

14 **Li Y**, Xu K, Chen S, Cao Y, Zhan H. Roles of Identified Long Noncoding RNA in Diabetic Nephropathy. *J Diabetes Res* 2019; **2019**: 5383010 [PMID: 30891461 DOI: 10.1155/2019/5383010]

15 **Chen S**, Sun X, Wu S, Jiang J, Zhu C, Xu K. Role of identified noncoding RNA in erectile dysfunction. *Andrologia* 2020; **52**: e13596 [PMID: 32441367 DOI: 10.1111/and.13596]

16 **Prabhakar B**, Lee S, Bochanis A, He W, Manautou JE, Rasmussen TP. lnc-RHL, a novel long non-coding RNA required for the differentiation of hepatocytes from human bipotent progenitor cells. *Cell Prolif* 2021; **54**: e12978 [PMID: 33393114 DOI: 10.1111/cpr.12978]

17 **Lv H**, Lv G, Han Q, Yang W, Wang H. Noncoding RNAs in liver cancer stem cells: The big impact of little things. *Cancer Lett* 2018; **418**: 51-63 [PMID: 29307614 DOI: 10.1016/j.canlet.2018.01.001]

18 **Sun Q**, Hao Q, Prasanth KV. Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression. *Trends Genet* 2018; **34**: 142-157 [PMID: 29249332 DOI: 10.1016/j.tig.2017.11.005]

19 **Liu Y**, Guo J, Shen K, Wang R, Chen C, Liao Z, Zhou J. Paclitaxel Suppresses Hepatocellular Carcinoma Tumorigenesis Through Regulating Circ-BIRC6/miR-877-5p/*YWHAZ* Axis. *Onco Targets Ther* 2020; **13**: 9377-9388 [PMID: 33061425 DOI: 10.2147/OTT.S261700]

20 **Zhao J**, Fu X, Chen H, Min L, Sun J, Yin J, Guo J, Li H, Tang Z, Ruan Y, Wang X, Sun Y, Huang L. G3BP1 interacts with YWHAZ to regulate chemoresistance and predict adjuvant chemotherapy benefit in gastric cancer. *Br J Cancer* 2021; **124**: 425-436 [PMID: 32989225 DOI: 10.1038/s41416-020-01067-1]

21 **Hui C**, Tian L, He X. Circular RNA circNHSL1 Contributes to Gastric Cancer Progression Through the miR-149-5p/YWHAZ Axis. *Cancer Manag Res* 2020; **12**: 7117-7130 [PMID: 32848466 DOI: 10.2147/CMAR.S253152]

22 **Ghousein A**, Mosca N, Cartier F, Charpentier J, Dupuy JW, Raymond AA, Bioulac-Sage P, Grosset CF. miR-4510 blocks hepatocellular carcinoma development through RAF1 targeting and RAS/RAF/MEK/ERK signalling inactivation. *Liver Int* 2020; **40**: 240-251 [PMID: 31612616 DOI: 10.1111/liv.14276]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 265-277

DOI: 10.4251/wjgo.v14.i1.265

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Characterization of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of gastric cardia and its influence on prognosis

Hai-Ling Wang, Xue-Ke Zhao, Fu-You Zhou, Xin Song, Liu-Yu Li, Gai-Rong Huang, Qi-De Bao, Ling-Ling Lei, Hai-Jun Yang, Li Li, Rui-Hua Xu, Ai-Li Li, Xian-Zeng Wang, Wen-Li Han, Jing-Li Ren, Li-Dong Wang

ORCID number: Hai-Ling Wang 0000-0003-4437-2052; Xue-Ke Zhao 0000-0002-9036-6342; Fu-You Zhou 0000-0002-6548-4099; Xin Song 0000-0002-7680-4908; Liu-Yu Li 0000-0001-6748-3295; Gai-Rong Huang 0000-0002-7034-0444; Qi-De Bao 0000-0002-5746-0273; Ling-Ling Lei 0000-0001-7540-7786; Hai-Jun Yang 0000-0002-8663-7779; Li Li 0000-0001-9567-5403; Rui-Hua Xu 0000-0002-6914-2168; Ai-Li Li 0000-0002-1143-7393; Xian-Zeng Wang 0000-0001-5691-8898; Wen-Li Han 0000-0002-3516-2195; Jing-Li Ren 0000-0001-9891-0579; Li-Dong Wang 0000-0002-7933-0410.

Author contributions: Wang LD and Wang HL designed the study and wrote the paper; Zhao XK, Song X and Huang GR performed data collection; Bao QD, Lei LL and Yang HJ conducted follow-up of the patients; Li LY, Li L and Xu RH performed the tissue microarray; Zhou FY and Li AL performed the immunohistochemical analysis; Wang XZ, Han WL and Ren JL contributed to data analysis; Wang LD revised the manuscript.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Zhengzhou

Hai-Ling Wang, Xue-Ke Zhao, Xin Song, Liu-Yu Li, Ling-Ling Lei, Rui-Hua Xu, Wen-Li Han, Li-Dong Wang, State Key Laboratory of Esophageal Cancer Prevention & Treatment and Henan Key Laboratory for Esophageal Cancer Research, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Fu-You Zhou, Department of Thoracic Surgery and Tumor Prevention Treatment, Anyang Tumor Hospital, Anyang 455000, Henan Province, China

Gai-Rong Huang, Li Li, Department of Geriatrics, Henan Provincial People's Hospital, Zhengzhou 450003, Henan Province, China

Qi-De Bao, Department of Oncology, Anyang District Hospital, Anyang 455000, Henan Province, China

Hai-Jun Yang, Department of Pathology, Anyang Tumor Hospital, Anyang 455000, Henan Province, China

Ai-Li Li, Department of Pathology, Linzhou Tumor Hospital, Linzhou 456500, Henan Province, China

Xian-Zeng Wang, Department of Thoracic Surgery, Linzhou People's Hospital, Linzhou 456500, Henan Province, China

Jing-Li Ren, Department of Pathology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, Henan Province, China

Corresponding author: Li-Dong Wang, MD, PhD, Professor, State Key Laboratory of Esophageal Cancer Prevention & Treatment and Henan Key Laboratory for Esophageal Cancer Research, The First Affiliated Hospital of Zhengzhou University, No. 40 Daxue Road, Zhengzhou 450052, Henan Province, China. ldwang2007@126.com

Abstract

BACKGROUND

Gastric cardia adenocarcinoma (GCA), which has been classified as type II adenocarcinoma of the esophagogastric junction in western countries, is of similar

University.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: We have no potential conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Supported by National Natural Science Foundation of China, No. 81872032, and No. U1804262; and National Key R&D Program of China, No. 2016YFC0901403.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: September 6, 2021
Peer-review started: September 6, 2021
First decision: November 8, 2021

geographic distribution with esophageal squamous cell carcinoma in China, and even referred as "sister cancer" by Chinese oncologists. The molecular mechanism for GCA is largely unknown. Recent studies have shown that decreased expression of E-cadherin is associated with the invasion and metastasis of multiple cancers. However, the E-cadherin expression has not been well characterized in gastric cardia carcinogenesis and its effect on GCA prognosis.

AIM
To characterize E-cadherin expression in normal gastric cardia mucosa, dysplasia and GCA tissues, and its influence on prognosis for GCA.

METHODS
A total of 4561 patients with GCA were enrolled from our previously established GCA and esophageal cancer databases. The enrollment criteria included radical surgery for GCA, but without any radio- or chemo-therapy before operation. The GCA tissue from 4561 patients and matched adjacent normal epithelial tissue (n = 208) and dysplasia lesions (n = 156) were collected, and processed as tissue microarray for immunohistochemistry. The clinicopathological characteristics were retrieved from the medical records in hospital and follow-up was carried out through letter, telephone or home interview. E-cadherin protein expression was determined by two step immunohistochemistry. Kaplan–Meier and Cox regression analyses were used to correlate E-cadherin protein expression with survival of GCA patients.

RESULTS
Of the 4561 GCA patients, there were 3607 males with a mean age of 61.6 ± 8.8 and 954 females with a mean age of 61.9 ± 8.6 years, respectively. With the lesions progressed from normal gastric cardia mucosa to dysplasia and GCA, the positive immunostaining rates for E-cadherin decreased significantly from 100% to 93.0% and 84.1%, respectively (R^2 = 0.9948). Furthermore, E-cadherin positive immunostaining rate was significantly higher in patients at early stage (0 and I) than in those at late stage (II and III) (92.7% *vs* 83.7%, P = 0.001). E-cadherin positive expression rate was significantly associated with degree of differentiation (P = 0.001) and invasion depth (P < 0.001). Multivariate analysis showed that the GCA patients with positive E-cadherin immunostaining had better survival than those with negative (P = 0.026). It was noteworthy that E-cadherin positive expression rate was similar in patients with positive and negative lymph node metastasis. However, in patients with negative lymph node metastasis, those with positive expression of E-cadherin had better survival than those with negative expression (P = 0.036). Similarly, in patients with late stage GCA, those with positive expression of E-cadherin had better survival than those with negative expression (P = 0.011).

CONCLUSION
E-cadherin expression may be involved in gastric cardia carcinogenesis and low expression of E-cadherin may be a promising early biomarker and overall survival predictor for GCA.

Key Words: E-cadherin expression; Immunohistochemistry; Gastric cardia adenocarcinoma; Dysplasia; Clinicopathological feature; Prognosis

Core Tip: In previous reports, there is no consistent conclusion on the association between E-cadherin expression and gastric cardia carcinogenesis and its effect on prognosis with gastric cardia adenocarcinoma (GCA) patients. It was notable that the positive immunostaining rates of E-cadherin decreased significantly from normal mucosa to dysplasia and GCA, as well as higher in early stage than those in advanced stage of GCA. Moreover, we found high expression of E–cadherin represented a better survival, especially for patients with negative lymph node metastasis. In conclusion, E-

Revised: November 18, 2021
Accepted: December 8, 2021
Article in press: December 8, 2021
Published online: January 15, 2022

P-Reviewer: Andrejic-Visnjic B, Micsik T
S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

cadherin may be involved in carcinogenesis and may be a predictor on prognosis for GCA.

Citation: Wang HL, Zhao XK, Zhou FY, Song X, Li LY, Huang GR, Bao QD, Lei LL, Yang HJ, Li L, Xu RH, Li AL, Wang XZ, Han WL, Ren JL, Wang LD. Characterization of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of gastric cardia and its influence on prognosis. *World J Gastrointest Oncol* 2022; 14(1): 265-277
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/265.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.265

INTRODUCTION

Gastric cardia adenocarcinoma (GCA), which has been classified as type II adenocarcinoma of the esophagogastric junction in western countries[1], is of similar geographic distribution with esophageal squamous cell carcinoma in China[2], and even referred as "sister cancer" by Chinese oncologists. In contrast to esophageal squamous cell carcinoma, the incidence for GCA is increasing worldwide[3,4]. Most GCA patients lack early warning symptoms, and > 90% of patients are diagnosed at an advanced stage, resulting in poor prognosis, with < 20% 5-year survival[5,6]. Obviously, early detection for GCA is crucial in decreasing the high mortality. Identification of unique molecular biomarkers at the early stage of GCA is crucial for screening high-risk individuals and early detection of GCA. Unfortunately, the molecular mechanism of human gastric cardia carcinogenesis is largely unknown.

Accumulated evidence indicates that E-cadherin protein, a member of the cadherin family encoded by the *CDH1* gene, may play an important role in intercellular adhesion, maintaining the stability of epithelial structure and function, cell polarity, and regulating intracellular signaling pathways[7]. Reduced expression of E-cadherin has been reported as a molecular biomarker of a cellular process called epithelial-mesenchymal transition, which is often associated with cancer progression[8]. The latest studies have indicated that decreased expression of E-cadherin is involved in many different types of cancer[9-11]. However, E-cadherin expression in GCA has not been well characterized.

In the present study, we detected the expression of E-cadherin in GCA, precancerous lesions and normal mucosa. We also evaluated the relationship of E-cadherin expression and survival of GCA.

MATERIALS AND METHODS

Patients

All the patients were enrolled from the 500000 esophageal and gastric cardia carcinoma databases (1973–2020) established by the State Key Laboratory for Esophageal Cancer Prevention & Treatment and Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). GCA patients were enrolled in the present study according to the following criteria: (1) Patients were diagnosed with GCA by postoperative histopathology; (2) Patients had tumors located in the esophagogastric junction; (3) Patients had no other malignant tumors except for GCA; (4) Patients received no chemotherapy or/and radiotherapy before surgery; and (5) The tissue samples of the patients were available. The exclusion criteria were: (1) Pathological type was not adenocarcinoma; (2) Clinicopathological information was incomplete; and (3) patients had received preoperative radiation or chemotherapy. A total of 4561 patients with GCA were enrolled in the study (Table 1). In addition, 208 matched adjacent normal epithelial tissue and 156 dysplasia lesions were selected.

The staged of patients with GCA were based on the 8th edition of the American Joint Committee. Positive smoking history was defined as having smoked continuously or accumulatively for 6 mo or more in one's lifetime and negative drinking history was defined less than 20 g of alcohol *per* day. Family history positive was defined as more than two patients with GCA in two consecutive generations.

Table 1 Clinical characteristics of patients with gastric cardia adenocarcinoma

Variables	Cases, *n* (%)	
Gender		
Female	954	20.9
Male	3607	79.1
Age at diagnosis (yr)		
< 60	1717	37.6
≥ 60	2844	62.4
Family history		
Negative	3366	73.8
Positive	1195	26.2
Cigarette smoking		
No	2166	47.5
Yes	2395	52.5
Alcohol consumption		
No	3206	70.3
Yes	1355	29.7
Differentiation		
Well	133	2.9
Moderate	2039	44.7
Poor	2389	52.4
T status		
T1	71	1.6
T2	308	6.8
T3	3044	66.7
T4	1138	25.0
Lymph node metastasis		
Negative	1637	35.9
Positive	2924	64.1
Staging		
Early stage	191	4.2
Advanced stage	4370	95.8

Histopathological diagnosis

Histopathological diagnoses for normal mucosa, dysplasia and adenocarcinoma of the gastric cardia were made according to established criteria[12]. The normal gastric cardia mucosa, composing of a single columnar epithelium and mucous glands composed only of mucous cells; dysplasia, neoplastic feature including nuclear atypia and/or architectural abnormalities confined to the gastric cardia epithelium, without invasion; GCA, invasion of neoplastic gastric cardia cells through the basement membrane.

Immunohistochemical staining and evaluation

E-cadherin protein expression was detected by immunohistochemical staining on normal mucosa, dysplasia and GCA with tissue microarray. The focal area of the cancer tissue was marked on the paraffin-embedded specimens, and a 7 × 16 microarray was designed. Punch holes with a diameter of 1.5 mm were made in the samples. The tissue chip model was then made and fixed. Immunohistochemistry was

carried out by a two-step protocol using the Roche Benchmark XT. In brief, the paraffin-embedded tissue sections were deparaffinized with xylene and anhydrous ethanol for rehydration and heated in citrate buffer (G1202, pH 6.0) for 25 min at 95 ℃ for antigen repair. The sections were then cooled for 60 min at room temperature, and immersed in 3% hydrogen peroxide solution (G0115) to neutralize endogenous peroxidase. A mouse monoclonal anti-E-cadherin antibody was used (cat. no. GB13083-1; dilution 1:500; Wuhan Servicebio Technology Co., Ltd, Wuhan, China). The anti-E-cadherin antibody was added and incubated overnight at 4 ℃. The secondary antibody was then added (cat. no. G1210-2). Between each incubation step, the slides were washed with phosphate buffered saline (PBS, pH 7.4, G0002) three times. Immunostaining was performed using the Roche Benchmark XT with diaminobenzidine (DAB, G1212-200) according to the manufacturer's instructions and the sections were subsequently counterstained with hematoxylin (G1004). The known positive sections were used as the positive control, and PBS was used as the negative control instead of the primary antibody. Observation was performed using a microscope at a magnification of 400 ×. The positive cells for E-cadherin protein expression showed yellow or brown staining in the cell membrane.

According to the staining intensity, the results were categorized as: 0 points, no staining; 1 point, light yellow; 2 points, brown yellow; and 3 points, tan. According to the ratio of the positive cell number, they were scored as 0 (< 10%), 1 (11%-25%), 2 (26%-50%) or 3 (> 50%). The two scores were multiplied, and the results were classified as negative (< 3) or positive (≥ 3). Immunohistochemical results were independently assessed by two pathologists. If the results were inconsistent, they were evaluated by the two pathologists together until a consensus was reached.

Follow-up

All the patients were followed up by letter, telephone or home interview every 3–6 mo after initial diagnosis and treatment. Before the 1990s, patients were usually followed up through letters. The data were saved in medical records. The patients who survived for > 5 years were followed up once a year until the end event (death) occurred. The last follow-up was on June 30, 2020. The median follow-up time was 5.4 [interquartile range (IQR) 3.4–7.6] years.

Statistical analysis

SPSS statistical software (version 25.0, IBM, Chicago, IL) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, California United States) were used to analyze the data. Variables with abnormal distribution were represented by a median (IQR). The χ^2 test and Fisher tests were used for the differences in clinicopathological characteristics and the protein expression of E-cadherin between the groups. The correlation of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of the gastric cardia was evaluated by linear regression analysis (R^2-value). The effect of a single factor on survival was analyzed by Kaplan–Meier method and log-rank test. Independent risk factors affecting survival were analyzed by Cox regression model. All the test levels were $\alpha = 0.05$. The statistical review of the study was performed by the biomedical statistician from Zhengzhou University.

RESULTS

Clinical characteristics of patients

From the clinical records, we retrieved the baseline clinical parameters for this group of GCA patients (Table 1). It shows the distribution of all GCA patients by gender, age, family history, cigarette smoking, alcohol consumption and histopathology. Among the 4561 patients with GCA, there were 954 women and 3607 men with a mean age of 61.6 ± 8.8 and 61.9 ± 8.6, respectively. The number of male patients was 3.8 times that of female patients. Family aggregation for GCA patients was evident with a positive family history in 26.2% of the patients. In addition, 2395 (52.5%) patients had a history of cigarette smoking and 1355 (29.7%) patients had a history of alcohol consumption. Among male patients, 65.7% (2370/3607) had a history of smoking and 36.8% (1327/3607) had alcohol consumption. The depth of invasion and lymph node metastasis were also classified. There were 2924 (64.1%) patients with positive lymph node metastasis in postoperative pathology. There were 191 (4.2%) patients at early stage (0 and I) and 4370 (95.8%) patients at advanced stage (II and III).

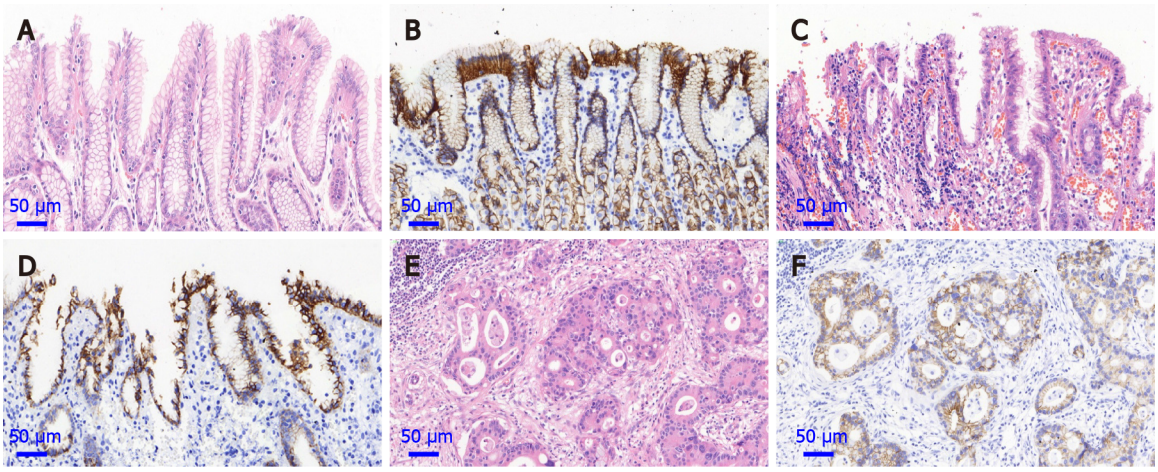


Figure 1 Hematoxylin and eosin staining sections and expression of E-cadherin of normal gastric cardia mucosa, dysplasia and gastric cardia adenocarcinoma (magnification, 400 ×). A: Hematoxylin and eosin staining (HE) section of normal gastric cardia mucosa; B: Positive protein expression of E-cadherin in normal gastric cardia mucosa; C: HE section of dysplasia (DYS); D: Positive protein expression of E-cadherin in DYS tissue; E: HE section of gastric cardia adenocarcinoma (GCA) tissue; F: Positive protein expression of E-cadherin in GCA.

Expression of E-cadherin protein in normal mucosa, dysplasia and GCA

The positive immunostaining reaction of E-cadherin protein expression was mainly located in the cell membrane (Figure 1). With the lesions progressed from normal gastric cardia mucosa to dysplasia and GCA, the positive immunostaining rates for E-cadherin decreased significantly from 100.0% (208/208), to 93.0% (145/156) and 84.1% (3836/4561), respectively (χ^2 = 47.439, P < 0.001; Table 2). In the linear analysis of E-cadherin protein expression in normal mucosa, dysplasia and GCA, the decreasing tendency was observed (y = -0.08x + 1.0833, R^2 = 0.9948, Figure 2).

Association of E-cadherin expression with clinicopathological features in patients with GCA

By comparing the relationship between E-cadherin expression and clinicopathological characteristics, the expression rate of E-cadherin in male patients was lower than that in female patients (83.5 *vs* 86.4%, χ^2 = 4.645, P = 0.031, Table 3). It was found that the positive rate of E-cadherin expression gradually decreased with the degree of differentiation (92.5% *vs* 85.4% *vs* 82.5%, χ^2 = 14.259, P = 0.001, Table 3). E-cadherin expression differed according to degree of tumor invasion (χ^2 = 22.490, P < 0.001, Table 3). The E-cadherin positive immunostaining rate was significantly higher in the patients at early stage (0 and I) than advanced stage (II and III) (92.7% *vs* 83.7%, χ^2 = 10.941, P = 0.001, Table 3). There was no significant difference in the expression of E-cadherin protein according to age at diagnosis, family history, cigarette smoking, alcohol consumption and lymph node metastasis (P > 0.05, Table 3).

E-cadherin expression is an independent risk factor for GCA prognosis

To evaluate the potential association of clinical factors with overall survival, we performed univariate Cox regression analysis. In univariate analysis, age at diagnosis (P < 0.001), degree of differentiation (P < 0.001), invasion depth (P < 0.001), lymph node metastasis (P < 0.001) and E-cadherin expression (P = 0.003) were survival factors (Table 4). There was no significant difference in overall survival among patients with different gender, family history, cigarette smoking and alcohol consumption (Table 4). Kaplan–Meier analysis showed that positive E-cadherin expression predicted better overall survival (P = 0.003; Figure 3A). Similarly, it showed that age < 60 years at diagnosis, well differentiation, T1 and negative lymph node metastasis predicted better overall survival (P < 0.001; Supplementary Figure 1). In multivariate analysis, E-cadherin expression was an independent factor of GCA survival (P = 0.026; Table 4).

Stratification analysis of the effect of E-cadherin expression on patient survival

According to the clinicopathological features, the patients were divided into different groups. In the group with negative lymph node metastasis, survival was better in patients with positive E-cadherin expression than negative expression (P = 0.036; Figure 3B). A similar result was found in the group with positive lymph node

Table 2 The difference of E-cadherin protein expression in normal tissue, dysplasia and gastric cardia adenocarcinoma

Lesion type	Total	E-cadherin protein expression		χ^2	*P* value
	n	Positive, *n* (%)	Negative, *n* (%)		
Normal	208	208 (100.0)	0 (0)	47.439	< 0.001
DYS	156	145 (93.0)	11 (7.0)		
GCA	4561	3836 (84.1)	725 (15.9)		

DYS: Dysplasia; GCA: Gastric cardia adenocarcinoma.

metastases (P = 0.048; Figure 3C). With regard to the patients at advanced stage (II and III), patients with positive E-cadherin expression survived better than those with negative expression (P = 0.011; Figure 3D).

DISCUSSION

As we know, the present study is the first report about the E-cadherin protein expression in the lesions progressed from normal gastric cardia mucosa to dysplasia and GCA, and the largest sample study of the expression of E-cadherin protein and its influence on survival with GCA[13,14].

It is well known that loss of E-cadherin expression resulting from CDH1 gene alterations is the primary carcinogenetic event in hereditary diffuse gastric cancer[15]. However, there was few report concerning the expression of E-cadherin in the gastric cardia carcinogenesis, progressed from normal gastric cardia to dysplasia and GCA. It is showed that, in our study, the significantly decreased immunostaining rate of E-cadherin protein presented from normal gastric cardia to dysplasia and GCA, which indicated that E-cadherin protein may be involved in the gastric cardia carcinogenesis and low expression of E-cadherin protein may accelerate the process. The result in our study was consistent with those reported on gastric cancer[16].

It was found that the positive rate of E-cadherin expression gradually decreased with the decline of the degree of differentiation (92.5% *vs* 85.4% *vs* 82.5%, χ^2 = 14.259, P = 0.001). The worse the differentiation, the lower the positive expression rate of E-cadherin. This is consistent with previous studies[17,18]. We think that E-cadherin may be a differentiation marker.

The present study demonstrated that patients with positive expression of E-cadherin protein had better survival than those with negative expression. Cox regression analysis indicated that positive expression of E-cadherin protein was an independent factor for better prognosis of patients with GCA, considered together with age at diagnosis, degree of differentiation, invasion depth and lymph node metastasis. The mechanism for the E-cadherin expression and cancer prognosis is largely unknown. E-cadherin gene, also known as CDH1, has been recognized as a tumor suppressor gene. Decreased expression of E-cadherin is reported to be related to prognosis in breast, colorectal and hepatocellular cancers[19,20]. However, less research has been conducted on GCA and controversial results have been observed in gastric cancer[21,22]. A meta-analysis of E-cadherin expression in 4383 patients with gastric cancer showed that the down-regulation of E-cadherin expression was significantly correlated with TNM stage, tumor invasion depth, lymph node metastasis, tumor differentiation, vascular invasion, tissue type and distant metastasis [23]. This study showed that negative E-cadherin was associated with poor differentiation and deep invasion of tumors, which suggested that tumor differentiation was related to cell adhesion, while tumors lacking adhesion were prone to regional lymph node or distant metastasis and had a relatively poor prognosis. The results of our study did not indicate that E-cadherin was associated with lymph node metastasis of GCA, which still needs to be confirmed by further studies.

Disruption of the cell adhesion molecule E-cadherin causes dysregulation of cell–cell adhesion properties. E-cadherin expression may be associated with epithelial–mesenchymal transition through activating the Akt and mitogen-activated protein kinase signaling pathways[24], and negative expression of E-cadherin could lead to a decline of proliferation and metastasis. Medicines for CDH1 mutations are being developed and it has been suggested that non-steroidal anti-inflammatory drugs

Table 3 Association of E-cadherin expression with clinicopathological features in patients with gastric cardia adenocarcinoma

Variables	Total	E-cadherin protein expression		χ^2	*P* value
	n	Positive, *n* (%)	Negative, *n* (%)		
Gender				4.645	0.031
Female	954	824 (86.4)	130 (13.6)		
Male	3607	3012 (83.5)	595 (16.5)		
Age at diagnosis (yr)				0.709	> 0.05
< 60	1717	1434 (83.5)	283 (16.5)		
≥ 60	2844	2402 (84.5)	442 (15.5)		
Family history				1.018	> 0.05
Negative	3366	2820 (83.8)	546 (16.2)		
Positive	1195	1016 (85.0)	179 (15.0)		
Cigarette smoking				1.408	> 0.05
No	2166	1841 (85.0)	325 (15.0)		
Yes	2395	2005 (83.7)	390 (16.3)		
Alcohol consumption				0.011	> 0.05
No	3206	2706 (84.4)	500 (15.6)		
Yes	1355	1142 (84.3)	213 (15.7)		
Differentiation				14.259	0.001
Well	133	123 (92.5)	10 (7.5)		
Moderate	2039	1742 (85.4)	297 (14.6)		
Poor	2389	1971 (82.5)	418 (17.5)		
T status				22.490	< 0.001
pT1	71	63 (88.7)	8 (11.3)		
pT2	308	278 (90.3)	30 (9.7)		
pT3	3044	2580 (84.8)	464 (15.2)		
pT4	1138	915 (80.4)	223 (19.6)		
Lymph node metastasis				0.481	> 0.05
Negative	1637	1385 (84.6)	252 (15.4)		
Positive	2924	2451 (83.8)	473 (16.2)		
Staging				10.941	0.001
Early stage	191	177 (92.7)	14 (7.3)		
Advanced stage	4370	3659 (83.7)	711 (16.3)		

can inhibit CDH1 methylation in human gastric mucosa[25].

Another interesting finding in the present study was that positive expression of E-cadherin protein in GCA patients at dysplasia lesion was higher than in GCA stage (93% *vs* 84.1%, $P = 0.003$), which indicates that E-cadherin protein may be an early potential biomarker for gastric cardia carcinogenesis. Accumulated evidence demonstrates that the germline mutations of E-cadherin gene are highly correlated with hereditary diffuse gastric cancer and lobular breast cancer, and are considered to be promising biomarkers, combined with endoscopy, for early detection of hereditary diffuse gastric cancer and breast cancer[26-29].

Lastly, we found that in the GCA patients with negative lymph node metastasis, positive expression of E-cadherin protein indicated better survival than negative expression. The difference in E-cadherin expression can further stratify the prognosis of patients with negative lymph node metastasis, indicating that E-cadherin protein expression may be a promising prognostic biomarker for non-surgical GCA patients. It

Table 4 Univariate and multivariate analyses of survival of patients with gastric cardia adenocarcinoma

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Gender			> 0.05			
Female	1					
Male	1.063	0.979-1.153				
Age at diagnosis (yr)			< 0.001			< 0.001
< 60	1			1		
≥ 60	1.335	1.246-1.431		1.352	1.262-1.449	
Family history			> 0.05			
Negative	1					
Positive	1.060	0.983-1.143				
Cigarette smoking			> 0.05			
No	1					
Yes	1.004	0.929-1.084				
Alcohol consumption			> 0.05			
No	1					
Yes	0.947	0.869-1.031				
Differentiation			< 0.001			< 0.001
Well	1			1		
Moderate	1.316	1.067-1.623		1.234	1.000-1.522	
Poor	1.791	1.454-2.206		1.480	1.199-1.827	
T status			< 0.001			< 0.001
pT1	1			1		
pT2	1.916	1.289-2.849		1.604	1.078-2.387	
pT3	2.829	1.949-4.107		2.074	1.426-3.018	
pT4	3.390	2.328-4.936		2.272	1.555-3.320	
Lymph node metastasis			< 0.001			< 0.001
Negative	1			1		
Positive	1.952	1.815-2.099		1.805	1.676-1.944	
E-cadherin			0.003			0.026
Positive	1			1		
Negative	1.144	1.048-1.248		1.104	1.012-1.206	

CI: Confidence interval; GCA: Gastric cardia adenocarcinoma; HR: Hazard ratio.

is well known that lymph node metastasis is a useful indicator for poor survival in almost all cancer patients, including GCA. However, clinically, GCA patients with negative lymph node metastasis also showed different survival. E-cadherin protein expression may shed a light on these phenomena.

This study has some limitations. Firstly, there might be some missing data about clinical information. Secondly, the patients' time span was long and the fact that they came from different hospitals also might have caused some bias. Further studies are needed to confirm the new findings.

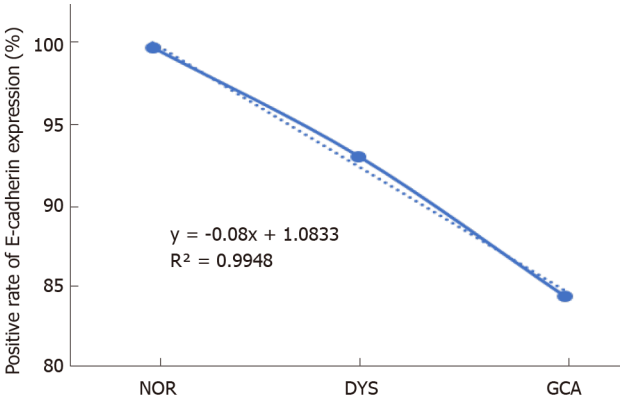


Figure 2 The linear analysis of E-cadherin protein expression in normal gastric cardia mucosa, dysplasia and gastric cardia adenocarcinoma. With the lesions progressed from normal gastric cardia mucosa to dysplasia and gastric cardia adenocarcinoma, the positive immunostaining rates for E-cadherin decreased significantly from 100% to 93.0% and 84.1%, respectively (y = - 0.08x + 1.0833, R^2 = 0.9948).

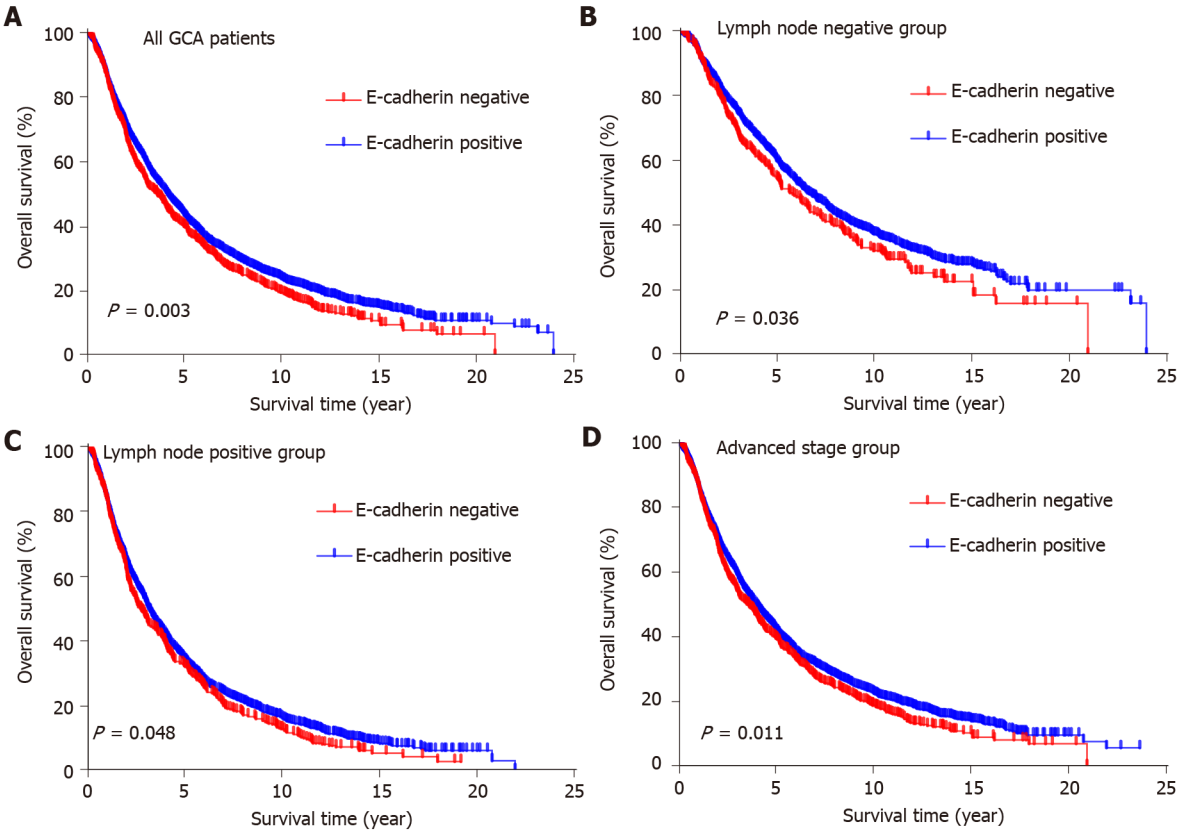


Figure 3 Kaplan–Meier analysis of the effect of E-cadherin expression on survival of gastric cardia adenocarcinoma patients. A: Kaplan-Meier curves of overall survival according to the E-cadherin expression in gastric cardia adenocarcinoma (GCA) patients (n = 4651; P = 0.003); B: Kaplan–Meier curves of overall survival in GCA patients with negative lymph node metastasis (n = 1637; P = 0.036); C: Kaplan–Meier curves of overall survival in GCA patients with positive lymph node metastasis (n = 2924; P = 0.048); D: Kaplan–Meier curves of overall survival in GCA patients with advanced stage (n = 4370; P = 0.011). GCA: Gastric cardia adenocarcinoma.

CONCLUSION

E-cadherin plays an important role in carcinogenesis of GCA. E-cadherin may be a promising biomarker for early warning and overall survival predictor for GCA patients. E-cadherin protein expression may also shed light on the clinical phenomena for the GCA patients with negative lymph node metastasis with different survival.

ARTICLE HIGHLIGHTS

Research background

Gastric cardia adenocarcinoma (GCA), which has been classified as type II adenocarcinoma of the esophagogastric junction in western countries, is of similar geographic distribution with esophageal squamous cell carcinoma in China, and even referred as "sister cancer" by Chinese oncologists. The molecular mechanism for GCA is largely unknown. Recent studies have shown that decreased expression of E-cadherin is associated with the invasion and metastasis of multiple cancers. However, the E-cadherin expression has not been well characterized in gastric cardia carcinogenesis and its effect on GCA prognosis.

Research motivation

In previous reports, there is no consistent conclusion on the association between E-cadherin expression and gastric cardia carcinogenesis and its effect on prognosis with GCA.

Research objectives

This study aimed to characterize E-cadherin expression in normal gastric cardia epithelium, dysplasia lesions and GCA tissues, and its influence on prognosis for GCA.

Research methods

Immunochemistry stating of E-cadherin was performed on GCA and matched adjacent normal epithelial tissue and dysplasia. The correlation on E-cadherin protein expression and prognosis of patients with GCA were analyzed using Kaplan–Meier and Cox regression test.

Research results

With the lesions progressed from normal gastric cardia mucosa to dysplasia and GCA, the positive immunostaining rates for E-cadherin decreased significantly from 100% to 93.0% and 84.1%, respectively ($R^2 = 0.9948$). E-cadherin had better survival than those with negative expression ($P = 0.026$). In the group with negative lymph node metastasis, survival was better in patients with positive E-cadherin expression than negative expression ($P = 0.036$). Similarly, in patients with late stage GCA, those with positive expression of E-cadherin had better survival than those with negative expression ($P = 0.011$).

Research conclusions

E-cadherin expression may be involved in gastric cardia carcinogenesis and low expression of E-cadherin may be a promising early biomarker and overall survival predictor for GCA.

Research perspectives

E-cadherin protein expression is expected to be a molecular marker for early detection and prognosis prediction for GCA.

ACKNOWLEDGEMENTS

We thank Professor Xue-Zhong Shi (Department of Epidemiology and Biostatistics, College of Public Health, Zhengzhou University) for help in statistical analysis.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Chen H**, Wang LD, Guo M, Gao SG, Guo HQ, Fan ZM, Li JL. Alterations of p53 and PCNA in cancer and adjacent tissues from concurrent carcinomas of the esophagus and gastric cardia in the same patient in Linzhou, a high incidence area for esophageal cancer in northern China. *World J Gastroenterol* 2003; **9**: 16-21 [PMID: 12508343 DOI: 10.3748/wjg.v9.i1.16]

3 **Arnold M**, Laversanne M, Brown LM, Devesa SS, Bray F. Predicting the Future Burden of Esophageal Cancer by Histological Subtype: International Trends in Incidence up to 2030. *Am J Gastroenterol* 2017; **112**: 1247-1255 [PMID: 28585555 DOI: 10.1038/ajg.2017.155]

4 **Dubecz A**, Solymosi N, Stadlhuber RJ, Schweigert M, Stein HJ, Peters JH. Does the Incidence of Adenocarcinoma of the Esophagus and Gastric Cardia Continue to Rise in the Twenty-First Century? *J Gastrointest Surg* 2013 [PMID: 24234242 DOI: 10.1007/s11605-013-2345-8]

5 **Liu K**, Yang K, Zhang W, Chen X, Zhang B, Chen Z, Chen J, Zhao Y, Zhou Z, Chen L, Hu J. Changes of Esophagogastric Junctional Adenocarcinoma and Gastroesophageal Reflux Disease Among Surgical Patients During 1988-2012: A Single-institution, High-volume Experience in China. *Ann Surg* 2016; **263**: 88-95 [PMID: 25647058 DOI: 10.1097/SLA.0000000000001148]

6 **Buas MF**, Vaughan TL. Epidemiology and risk factors for gastroesophageal junction tumors: understanding the rising incidence of this disease. *Semin Radiat Oncol* 2013; **23**: 3-9 [PMID: 23207041 DOI: 10.1016/j.semradonc.2012.09.008]

7 **Wheelock MJ**, Johnson KR. Cadherins as modulators of cellular phenotype. *Annu Rev Cell Dev Biol* 2003; **19**: 207-235 [PMID: 14570569 DOI: 10.1146/annurev.cellbio.19.011102.111135]

8 **Bure IV**, Nemtsova MV, Zaletaev DV. Roles of E-cadherin and Noncoding RNAs in the Epithelial-mesenchymal Transition and Progression in Gastric Cancer. *Int J Mol Sci* 2019; **20**: 2870 [PMID: 31212809 DOI: 10.3390/ijms20122870]

9 **Ishiguro H**, Wakasugi T, Terashita Y, Sakamoto N, Tanaka T, Mizoguchi K, Sagawa H, Okubo T, Takeyama H. Decreased expression of CDH1 or CTNNB1 affects poor prognosis of patients with esophageal cancer. *World J Surg Oncol* 2016; **14**: 240 [PMID: 27600761 DOI: 10.1186/s12957-016-0956-8]

10 **Lin Y**, Shen LY, Fu H, Dong B, Yang HL, Yan WP, Kang XZ, Dai L, Zhou HT, Yang YB, Liang Z, Chen KN. P21, COX-2, and E-cadherin are potential prognostic factors for esophageal squamous cell carcinoma. *Dis Esophagus* 2017; **30**: 1-10 [PMID: 27868288 DOI: 10.1111/dote.12522]

11 **Bruun J**, Kolberg M, Nesland JM, Svindland A, Nesbakken A, Lothe RA. Prognostic Significance of β-Catenin, E-Cadherin, and SOX9 in Colorectal Cancer: Results from a Large Population-Representative Series. *Front Oncol* 2014; **4**: 118 [PMID: 24904831 DOI: 10.3389/fonc.2014.00118]

12 **Giroux V**, Rustgi AK. Metaplasia: tissue injury adaptation and a precursor to the dysplasia-cancer sequence. *Nat Rev Cancer* 2017; **17**: 594-604 [PMID: 28860646 DOI: 10.1038/nrc.2017.68]

13 **Polkowski WP**, Skomra DG, Mielko J, Wallner GT, Szumiło J, Zinkiewicz K, Korobowicz EM, van Lanschot JJ. E-cadherin expression as predictive marker of proximal resection line involvement for advanced carcinoma of the gastric cardia. *Eur J Surg Oncol* 2004; **30**: 1084-1092 [PMID: 15522555 DOI: 10.1016/j.ejso.2004.07.022]

14 **Wijnhoven BP**, Tucker ET, Dinjens WN, Tilanus HW, Pignatelli M. Biochemical analysis and subcellular distribution of E-cadherin-catenin in adenocarcinomas of the gastro-oesophageal junction. *Anticancer Res* 2004; **24**: 1369-1375 [PMID: 15274297]

15 **Yakirevich E**, Resnick MB. Pathology of gastric cancer and its precursor lesions. *Gastroenterol Clin North Am* 2013; **42**: 261-284 [PMID: 23639640 DOI: 10.1016/j.gtc.2013.01.004]

16 **Zhou YN**, Xu CP, Han B, Li M, Qiao L, Fang DC, Yang JM. Expression of E-cadherin and beta-catenin in gastric carcinoma and its correlation with the clinicopathological features and patient survival. *World J Gastroenterol* 2002; **8**: 987-993 [PMID: 12439911 DOI: 10.3748/wjg.v8.i6.987]

17 **Corso G**, Figueiredo J, De Angelis SP, Corso F, Girardi A, Pereira J, Seruca R, Bonanni B, Carneiro P, Pravettoni G, Guerini Rocco E, Veronesi P, Montagna G, Sacchini V, Gandini S. E-cadherin deregulation in breast cancer. *J Cell Mol Med* 2020; **24**: 5930-5936 [PMID: 32301282 DOI: 10.1111/jcmm.15140]

18 **Graziano F**, Humar B, Guilford P. The role of the E-cadherin gene (CDH1) in diffuse gastric cancer susceptibility: from the laboratory to clinical practice. *Ann Oncol* 2003; **14**: 1705-1713 [PMID: 14630673 DOI: 10.1093/annonc/mdg486]

19 **Padmanaban V**, Krol I, Suhail Y, Szczerba BM, Aceto N, Bader JS, Ewald AJ. E-cadherin is required for metastasis in multiple models of breast cancer. *Nature* 2019; **573**: 439-444 [PMID: 31485072 DOI: 10.1038/s41586-019-1526-3]

20 **Wong SHM**, Fang CM, Chuah LH, Leong CO, Ngai SC. E-cadherin: Its dysregulation in carcinogenesis and clinical implications. *Crit Rev Oncol Hematol* 2018; **121**: 11-22 [PMID: 29279096 DOI: 10.1016/j.critrevonc.2017.11.010]

21 **Sun S**, Gong Q. The expressions and prognostic implications of Twist and E-cadherin in adenocarcinomas of the gastroesophageal junction and proximal gastric carcinoma. *Medicine (Baltimore)* 2019; **98**: e18449 [PMID: 31876727 DOI: 10.1097/MD.0000000000018449]

22 **Schizas D**, Moris D, Michalinos A, Kanavidis P, Oikonomou D, Papalampros A, Machairas A, Liakakos T. E-cadherin in gastric carcinomas: Relations with histological parameters and its prognostic value. *J BUON* 2017; **22**: 383-389 [PMID: 28534359]

23 **Hu L**, Li HL, Li WF, Chen JM, Yang JT, Gu JJ, Xin L. Clinical significance of expression of proliferating cell nuclear antigen and E-cadherin in gastric carcinoma. *World J Gastroenterol* 2017; **23**: 3721-3729 [PMID: 28611525 DOI: 10.3748/wjg.v23.i20.3721]

24 **Reddy P**, Liu L, Ren C, Lindgren P, Boman K, Shen Y, Lundin E, Ottander U, Rytinki M, Liu K. Formation of E-cadherin-mediated cell-cell adhesion activates AKT and mitogen activated protein kinase *via* phosphatidylinositol 3 kinase and ligand-independent activation of epidermal growth factor receptor in ovarian cancer cells. *Mol Endocrinol* 2005; **19**: 2564-2578 [PMID: 15928314 DOI: 10.1210/me.2004-0342]

25 **Tahara T**, Shibata T, Nakamura M, Yamashita H, Yoshioka D, Okubo M, Maruyama N, Kamano T, Kamiya Y, Fujita H, Nagasaka M, Iwata M, Takahama K, Watanabe M, Hirata I, Arisawa T. Chronic aspirin use suppresses CDH1 methylation in human gastric mucosa. *Dig Dis Sci* 2010; **55**: 54-59 [PMID: 19184424 DOI: 10.1007/s10620-008-0701-4]

26 **Xing X**, Tang YB, Yuan G, Wang Y, Wang J, Yang Y, Chen M. The prognostic value of E-cadherin in gastric cancer: a meta-analysis. *Int J Cancer* 2013; **132**: 2589-2596 [PMID: 23169395 DOI: 10.1002/ijc.27947]

27 **Curtin BF**, Gamble LA, Schueler SA, Ruff SM, Quezado M, Miettinen M, Fasaye GA, Passi M, Hernandez JM, Heller T, Koh C, Davis JL. Enhanced endoscopic detection of occult gastric cancer in carriers of pathogenic CDH1 variants. *J Gastroenterol* 2021; **56**: 139-146 [PMID: 33206267 DOI: 10.1007/s00535-020-01749-w]

28 **Pilonis ND**, Tischkowitz M, Fitzgerald RC, di Pietro M. Hereditary Diffuse Gastric Cancer: Approaches to Screening, Surveillance, and Treatment. *Annu Rev Med* 2021; **72**: 263-280 [PMID: 33217247 DOI: 10.1146/annurev-med-051019-103216]

29 **Memni H**, Macherki Y, Klayech Z, Ben-Haj-Ayed A, Farhat K, Remadi Y, Gabbouj S, Mahfoudh W, Bouzid N, Bouaouina N, Chouchane L, Zakhama A, Hassen E. E-cadherin genetic variants predict survival outcome in breast cancer patients. *J Transl Med* 2016; **14**: 320 [PMID: 27852262 DOI: 10.1186/s12967-016-1077-4]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 278-294

DOI: 10.4251/wjgo.v14.i1.278

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Digestive cancer incidence and mortality among young adults worldwide in 2020: A population-based study

Jian Li

ORCID number: Jian Li 0000-0002-5807-2360.

Author contributions: The manuscript including tables and figures was drafted and revised by Li J.

Institutional review board statement: The study was approved by the Ethics Committee of The Third Hospital of Mianyang.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: All data are available in corresponding online databases.

STROBE statement: The author has read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Supported by the Scientific Research Projects of Health Commission of Mianyang City, No. 202012.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer

Jian Li, Department of General Surgery, The Third Hospital of Mianyang, The Mental Health Center of Sichuan, Mianyang 621000, Sichuan Province, China

Corresponding author: Jian Li, MM, Surgical Oncologist, Department of General Surgery, The Third Hospital of Mianyang, The Mental Health Center of Sichuan, No. 190 East Section of Jiannan Road, Youxian District, Mianyang 621000, Sichuan Province, China.
654747973@qq.com

Abstract

BACKGROUND

Digestive cancer has traditionally been thought of as a disease that mainly occurs in elderly individuals, and it has been ignored in young adults by both patients and physicians.

AIM

To describe the worldwide profile of digestive cancer incidence, mortality and corresponding trends among 20–39-year-olds, with major patterns highlighted by age, sex, development level, and geographical region.

METHODS

I performed a population-based study to quantify the burden of young adult digestive cancers worldwide. Global, regional, sex, and country-specific data estimates of the number of new cancer cases and cancer-associated deaths that occurred in 2020 were extracted from the GLOBOCAN Cancer Today database. To assess long-term trends in young adult digestive cancer, cancer incidence data and mortality data were obtained from the Cancer in Five Continents Plus database and the World Health Organization mortality database, respectively. The associations between the human development index (HDI) and digestive cancer burden in young adults were evaluated by linear regression analyses.

RESULTS

In 2020, there were an estimated 19292789 new cancer cases, resulting in 9958133 deaths worldwide, which equated to an age-standardized incidence rate (ASIR) of 5.16 and age-standardized mortality rate (ASMR) of 3.04, accounting for 12.24% of all new cancer cases and 25.26% of all cancer deaths occurring in young adults. The burden was disproportionally greater among males, with male: female ratios of 1.34 for incidence and 1.58 for mortality. The ASIRs were 2.1, 1.4, and 1.0 per 100000 people per year, whereas the ASMRs were 0.83, 1.1, and 0.62 per 100000

reviewed.

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 18, 2021
Peer-review started: March 18, 2021
First decision: July 3, 2021
Revised: July 4, 2021
Accepted: November 24, 2021
Article in press: October 24, 2021
Published online: January 15, 2022

P-Reviewer: Luchini C
S-Editor: Zhang H
L-Editor: A
P-Editor: Zhang H

people per year for colorectal, liver, and gastric cancer, respectively. When assessed by geographical region and HDI levels, the cancer profile varied substantially, and a strong positive correlation between the mortality-to-incidence ratio of digestive cancer and HDI ranking was found (R^2 = 0.7388, P < 0.001).

CONCLUSION

The most common digestive cancer types are colorectal, liver and gastric cancer. The global digestive cancer burden among young adults is greater among males and exhibits a positive association with socioeconomic status. The digestive cancer burden is heavy in young adults, reinforcing the need for primary and secondary prevention strategies.

Key Words: Digestive cancer; Incidence; Mortality; Young adults; GLOBOCAN; Mortality-to-incidence ratio

Core Tip: This study is the first to explore the global burden of digestive cancer among young adults. By assessing 6 major digestive cancer types, I provide up-to-date estimates across levels of sex, geographical region, and human development. Furthermore, this study investigates the long-term trends in digestive cancer in young adults, serving as the latest report to aid oncology studies and increase awareness of digestive cancer among this underserved subpopulation. Through continuous prevention, screening, and early detection programs, the digestive cancer burden in young adults can be reduced.

Citation: Li J. Digestive cancer incidence and mortality among young adults worldwide in 2020: A population-based study. *World J Gastrointest Oncol* 2022; 14(1): 278-294
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/278.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.278

INTRODUCTION

Cancer is a leading cause of morbidity and mortality and is an important barrier to increasing life expectancy across all age groups globally. According to estimates from the GLOBOCAN database, there were an estimated 19.3 million new cases and 10 million cancer deaths worldwide in 2020[1]. Cancer has traditionally been thought to be a disease that mainly occurs in elderly individuals, and studies have focused on cancers at older ages. Therefore, in relative terms, cancers in young adults have been ignored by both patients and physicians.

Digestive cancer represents approximately 30% of the global cancer incidence and 40% of all cancer-related deaths worldwide[1]. Studies on cancer trends have demonstrated that the incidence of digestive cancers has increased significantly in young adults over the last few decades[2-5]. Digestive cancer is difficult to diagnose in a timely manner because of the lack of symptoms and signs at an early stage. It is more serious among young adults, which can be attributed to the relatively lower health consciousness and lack of screening programs in this age group[6]. However, young adults represent the main proportion of contributors to the economy and their family care[7]. Thus, to improve digestive cancer-associated outcomes among young adults, it is important to investigate the accurate profile of disease burden in this age group. This will help in not only determining the screening population to identify cancers early but also in developing preventative programs against them.

Therefore, I here presented a comprehensive assessment of the digestive cancer burden in young adult globally. I reported the incidence and mortality estimates of young adult digestive cancer in 2020, describing variations by sex and geographical region as well as the correlation between young adult digestive cancer burden and human development level.

MATERIALS AND METHODS

Study population

I performed a population-based study to assess the global burden of young adult digestive cancer incidence and mortality in 2020 and to investigate incidence and mortality trends over specific periods for selected countries. The study population comprised young adults diagnosed with digestive cancers, classified according to the International Classification of Diseases (ICD), tenth revision (ICD-10), as esophagus (C15), stomach (C16), colorectum (including anus, C18–21), liver (C22), gallbladder (C23), and pancreas (C25).

Definition of young adult digestive cancer

Age is a continuous variable. Variation exists among individuals of the same age, and any predefined age range is an arbitrary rather than an unequivocal definition. However, to facilitate clarity, consensus, simplicity and data collection and comparison, it is necessary to define different populations by upper and lower age limits. Although 0-19 years is broadly used to define childhood and adolescent cancer, the age range used to define young adult cancer are not always consistent in the literature or guidelines. Nevertheless, two large groups, the Adolescent and Young Adult Health Outcomes and Patient Experience (AYA HOPE) study and the Adolescent and Young Adult Oncology Progress Review Group (AYAO PRG), were in favor of the upper limit of 39 years of age in their studies[8,9]. As the AYAO PRG states, to apply this age range is based on the biological and physiological maturity and relative stability during the 20s and 30s, and these individuals have not yet experienced the effects of hormonal and immune response decline or chronic medical conditions that can influence oncologic decision-making and care of older patients. Furthermore, cancer survivorship studies have similarly used age 39 to define the upper limit of young adults[10,11]. For these reasons, the young adult digestive cancer in this analysis encompasses individuals diagnosed between 20–39 years.

Data sources

GLOBOCAN held at the International Agency for Research on Cancer (IARC) provides estimates of the incidence and mortality of 36 cancer types for 185 countries or territories by sex and age group, with the most recent estimates applying to 2020 (https://gco.iarc.fr/today/). To quantify the digestive cancer burden in young adults, global, regional, and country-specific cancer incidence and mortality estimates for 2020 were obtained from the GLOBOCAN Cancer Today database. I reported the numbers of new cases, deaths, age-standardized incidence rates (ASIRs), and age-standardized mortality rates (ASMRs) for digestive cancer among adults aged 20-39 years globally. Estimates are also presented and compared based on cancer type, world area, sex, country, and human development index (HDI). To make comparisons of the cancer type spectrum with younger and older age groups, I extracted the incidence and mortality data of all cancer types that were classified as digestive, brain and central nervous system (CNS), breast, lung, hematological, head and neck, genitourinary, and others (eight cancer groups) among individuals aged 0-19 years, 40-59 years, and 60 years and older. To make comparisons of the cancer type spectrum within young adults with digestive cancer, I also extracted the incidence and mortality data of all six digestive cancer types in four 5-year bands.

Furthermore, I used the GLOBOCAN data to examine the correlations of young adult digestive cancer burden with HDI ranking, a socioeconomic development indicator that was created by the United Nations Development Program, to highlight the importance of national policy decisions beyond economic growth in assessing development outcomes. The latest edition is the Human Development Report 2020 (http://hdr.undp.org). The association between the mortality-to-incidence ratio (MIR) and HDI ranking was also examined. The MIR, which was calculated as the ratio of all-age death counts and all-age incidence counts of the mean estimates, was employed as a proxy for 5-year survival rates to measure the severity of a disease. Because the number of new cases and deaths of young adult digestive cancer in most countries was small, I focused primarily on heavily burdened countries and regions with HDI rankings.

To assess long-term trends in young adult digestive cancer, I used cancer incidence data from IARC's Cancer in Five Continents Plus database, which compiles high-quality recorded cancer incidence data from cancer registries worldwide, with 2012 being the last available year (https://ci5.iarc.fr/CI5plus/Default.aspx). Mortality data were obtained from the World Health Organization mortality database compiled by

IARC for countries with different available time periods (https://www-dep.iarc.fr/WHOdb/WHOdb.htm). To include countries with representative geographies and developmental levels that spanned globally, I used the 14-year period of 1999–2012 to estimate trends in incidence and the 32-year period of 1985-2016 to estimate trends in mortality. Because of the volatile age-standardized rates in most countries, I only analyzed the overall trends of available countries based on the number of cases and deaths in young adults.

Statistical analysis

Detailed descriptions of the applied methods used to generate incidence and mortality estimates for each country are available on the GLOBOCAN website. In the current report, I used the Pearson correlation method to assess the correlation between ASIR, ASMR, and MIR for young adult digestive cancer and HDI ranking. Statistical analyses and graphs were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, United States) and Excel 2013. For all analyses, *P* values less than 0.05 were considered statistically significant, and all *P* values reported in this study were two-sided.

RESULTS

Global cancer spectrum according to age

In 2020, there were an estimated 19292789 new cancer cases, resulting in 9958133 deaths worldwide. The spectrum of cancers differed among age groups. Common tumor types in ages 0-19 (children and adolescents) included hematological tumors and cancers of the CNS. Epithelial tumors, including digestive cancers, were the majority of malignancies in other age groups with heterogeneous profiles. As age increased, the incidence and mortality of digestive cancers increased. The new cases and cancer-associated deaths of digestive cancer among young adults were 14 and 17 times that among children and adolescents, but markedly less than that in middle age (40–59 years) and elderly (60+ years) (Figure 1A and B). For digestive cancer, young adults accounted for approximately 2.55% (131068/5142192) of new cases and 2.19% (79614/3628920) of cancer-associated deaths. Among young adults, the cancer profile varied obviously, with the proportion of hematological cancer decreased and breast cancer increased with increasing age. However, the difference in digestive cancers was not significant, with 12.24% (131068/1071113) of all new cancer cases and 25.26% (79614/315177) of all cancer deaths occurring in the digestive system (Figure 1C and D).

Global burden of digestive cancers

Among young adults, it is estimated that there were 131068 new digestive cancer cases and 79614 digestive cancer-associated deaths in 2020. The ASIR and ASMR were 5.16 and 3.04 per 100000 people per year, respectively. Colorectal, liver, and gastric cancer together accounted for 111137 (86.04%) of the total new cases and 64843 (81.45%) of the total deaths (Figure 2). The ASIRs were 2.1, 1.4, and 1.0 per 100000 people per year, whereas the ASMRs were 0.83, 1.1, and 0.62 per 100000 people per year for colorectal, liver, and gastric cancer, respectively. Notably, digestive cancers were more frequent among men (male: female ratios of 1.34 for incidence and 1.58 for mortality), with liver cancer being more than 3 times more common in men than in women with regard to both incidence and mortality. However, the incidence and mortality of gastric and gallbladder cancer were lower in men than in women (Figure 3).

Geographical variations of digestive cancer burden

Based on the estimates in the 21 world areas, the incidence rate of digestive cancer among young adults was the greatest in Australia and New Zealand, followed by Eastern Asia, whereas other parts of Oceania, Southern Europe, and the Caribbean had the lowest incidence. There was substantial variability in the incidence and mortality rates across the geographical regions. For instance, the ASIR for colorectal cancer was 1.3 per 100000 in Middle Africa, whereas it was 6.2 per 100000 in Australia and New Zealand. The incidence of liver cancer showed the greatest variation, with the highest incidence in Melanesia, which was 24 times that in Western Europe. Despite the incidence being highest in Australia and New Zealand, the region conversely had a lower mortality rate (1.8 per 100000 people per year). In contrast, Africa and Asia contributed the majority of deaths. With regard to the estimated number of incident cases and deaths, 65.64% and 67.59% of digestive cancers occurred in Asia, with the

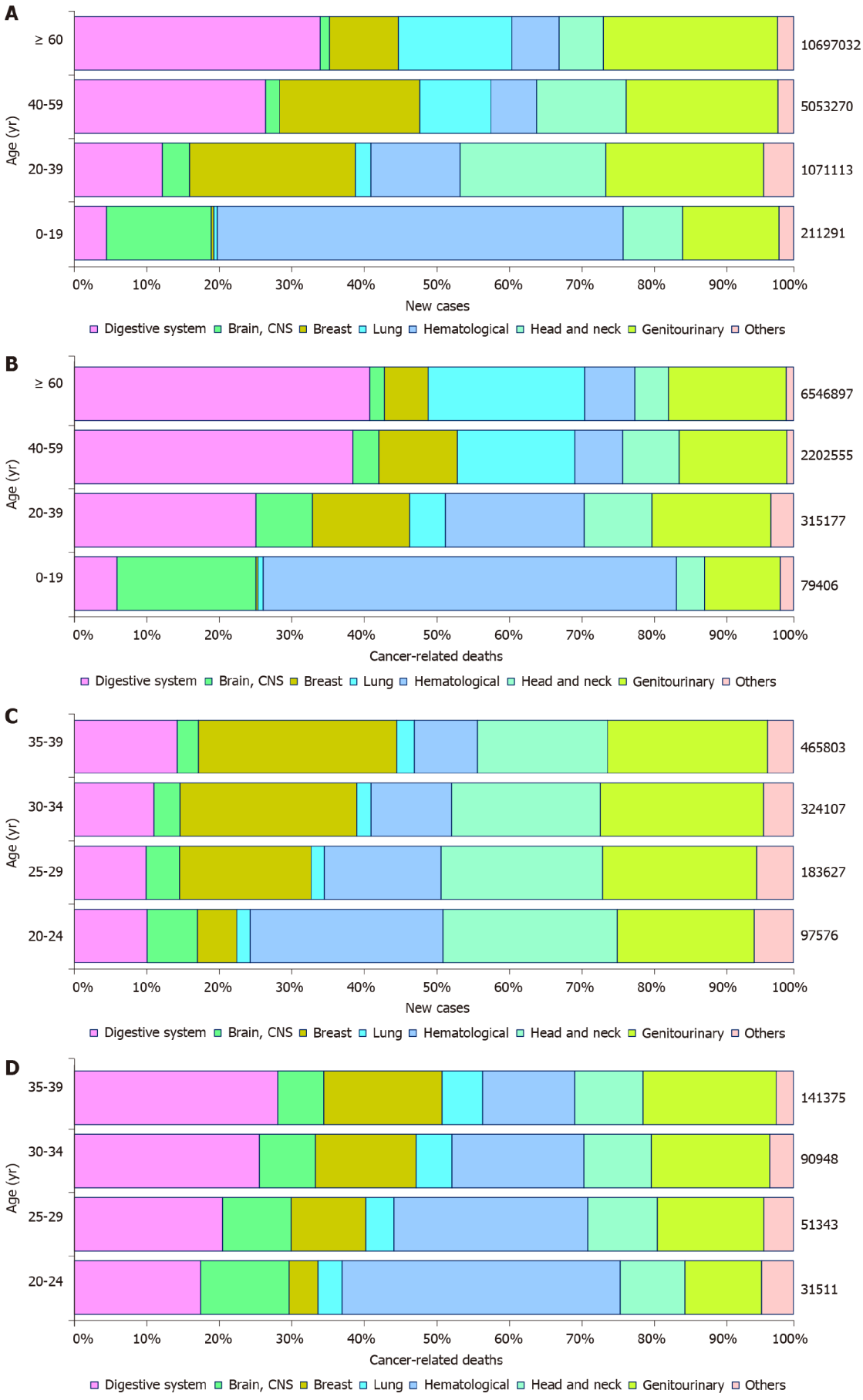


Figure 1 Cancer type distribution for estimated new cancer cases and cancer-related deaths. A and B: Cancer type distribution for estimated (A) new cancer cases and (B) cancer-related deaths in 2020 among younger, adolescent, young adult, and older age groups; C and D: Cancer type distribution for

estimated (C) new cancer cases and (D) cancer-related deaths in 2020 among young adults categorized into four 5-year bands. CNS: central nervous system.

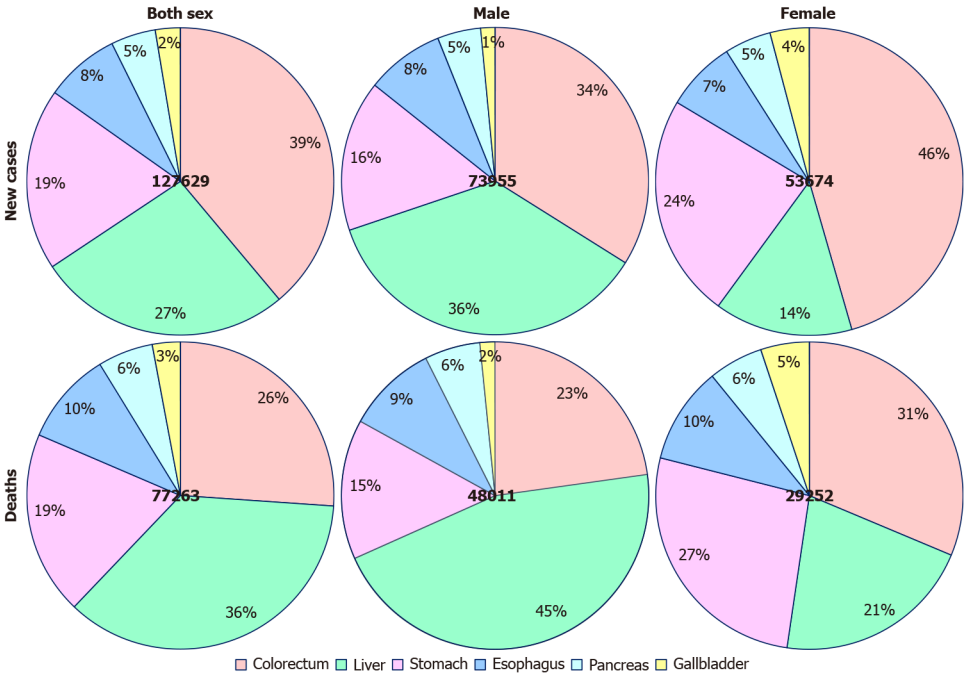


Figure 2 Proportion of six digestive cancer types for estimated new cases and cancer-related deaths among 20- to 39-year-olds in 2020 by sex.

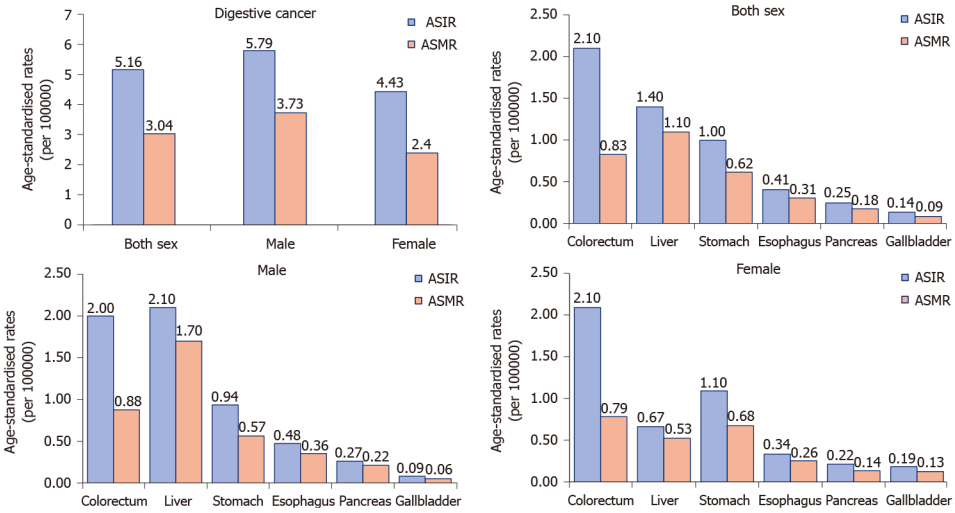


Figure 3 Estimated age-standardized incidence rate and age-standardized mortality rate among 20- to 39-year-olds in 2020 by sex and digestive cancer type. ASIR: Age-standardized incidence rate; ASMR: Age-standardized mortality rate.

majority occurring in Eastern Asia (Table 1, Supplementary Table 1).

Country-wise burden of digestive cancer in young adults

Country-wise, China and India were the two leading countries in terms of new cases, with 36723 and 17819 cases, respectively, in 2020. Together, they accounted for 41.6% of the total estimated new cases, followed by the United States, Pakistan, Bangladesh, and Indonesia, with estimated 6069, 3811, 3779, and 3462 new cases, respectively. In terms of death counts, China was the leading country, with 22345 deaths, followed by India, with an estimated 12166 deaths in 2020. Together, they accounted for 43.35% of the total deaths. Vietnam was ranked third in terms of death counts, with 2953 deaths, which was higher than the number of deaths in Bangladesh (2795), Pakistan (2520),

Table 1 Estimated new cases, deaths, age-standardized incidence rate, age-standardized mortality rate, and mortality-to-incidence ratio of digestive cancer among 20- to 39-year-olds worldwide in 2020 by world area

World areas	Incidence			Mortality			MIR
	Cases	%	ASIR	Deaths	%	ASMR	
Asia							
Eastern Asia	40966	31.26	7.70	24006	30.15	4.40	0.59
South-Central Asia	29100	22.20	4.20	20045	25.18	2.90	0.69
South-Eastern Asia	12048	9.19	5.40	7639	9.60	3.40	0.63
Western Asia	3919	2.99	3.90	2123	2.67	2.10	0.54
Americas and Caribbean							
Northern America	6690	5.10	6.10	2118	2.66	1.90	0.32
South America	6321	4.82	4.30	3626	4.55	2.50	0.57
Central America	2570	1.96	4.40	1472	1.85	2.50	0.57
Caribbean	485	0.37	3.60	293	0.37	2.20	0.60
Europe							
Central and Eastern Europe	3374	2.57	3.60	2136	2.68	2.20	0.63
Western Europe	2513	1.92	4.70	549	0.69	0.97	0.22
Southern Europe	1344	1.03	3.20	441	0.55	1.00	0.33
Northern Europe	1356	1.03	4.40	400	0.50	1.30	0.29
Africa							
Eastern Africa	7378	5.63	5.80	5724	7.19	4.50	0.78
Northern Africa	3722	2.84	4.70	2328	2.92	3.00	0.63
Western Africa	4751	3.62	4.40	3686	4.63	3.40	0.78
Middle Africa	2483	1.89	5.20	2037	2.56	4.30	0.82
Southern Africa	1092	0.83	4.50	691	0.87	2.80	0.63
Oceania							
Australia and New Zealand	721	0.55	7.80	165	0.21	1.80	0.23
Melanesia	227	0.17	6.60	132	0.17	3.80	0.58
Polynesia	5	0.00	2.70	3	0.00	1.80	0.60
Micronesia	3	0.00	2.00	0	0.00	0.00	0.00

ASIR: Age-standardized incidence rate; ASMR: Age-standardized mortality rate; MIR: Mortality-to-incidence ratio.

and the United States (1900). The top three countries in terms of ASIR were The Republic of the Gambia (16.9), Malawi (12.6), and Ghana (11.7). The ASMR mostly followed the patterns of ASIR, with The Republic of the Gambia (15.0), Malawi (1.4), and Ghana (8.9) as the three leading countries. In terms of individual cancer types, China had the largest counts of new colorectal, gastric, liver, and pancreatic cancer, and India had the largest number of new cases of esophageal and gallbladder cancer. The deaths of individual digestive cancer types mostly followed the patterns of new cases (Figure 4, Table 2, and Supplementary Tables 2-6).

Association between HDI ranking and young adult digestive cancer burden

The burden of young adult digestive cancer varied substantially according to the HDI ranking. The four HDI level-based analysis showed that colorectal cancer was the most frequent digestive cancer in the most developed regions (60.72% of all new digestive cancer cases, 43.55% of all digestive cancer-related deaths). At high and low HDI levels, the most common digestive cancers were colorectal and liver cancer, with more new cases of colorectal cancer and more deaths from liver cancer. The proportions of

Table 2 Estimated new cases, deaths, age-standardized incidence rate, age-standardized mortality rate, and mortality-to-incidence ratio of digestive cancer among 20- to 39-year-olds worldwide in 2020 by country

Country	Incidence			Mortality			MIR	HDI	HDI ranking
	Cases	%	ASIR	Deaths	%	ASMR			
China	36723	28.02	7.8	22345	28.07	4.6	0.61	0.761	87
India	17819	13.60	3.8	12166	15.28	2.6	0.68	0.645	130
United States of America	6069	4.63	6.2	1900	2.39	1.9	0.31	0.926	17
Pakistan	3811	2.91	5.4	2520	3.17	3.6	0.66	0.557	154
Bangladesh	3779	2.88	6.4	2795	3.51	4.7	0.74	0.632	134
Indonesia	3462	2.64	3.8	1645	2.07	1.8	0.48	0.718	110
Viet Nam	3440	2.62	9.9	2953	3.71	8.3	0.86	0.704	118
Brazil	3398	2.59	4.6	1801	2.26	2.4	0.53	0.765	84
Ethiopia	1966	1.50	5.9	1516	1.90	4.6	0.77	0.485	174
Mexico	1826	1.39	4.3	975	1.22	2.3	0.53	0.779	76
Russian Federation	1736	1.32	3.7	1193	1.50	2.4	0.69	0.824	49
Egypt	1647	1.26	5.1	1203	1.51	3.7	0.73	0.707	117
Philippines	1540	1.17	4.4	773	0.97	2.2	0.50	0.718	111
Iran, Islamic Republic of	1520	1.16	4.6	1003	1.26	3	0.66	0.783	70
Turkey	1445	1.10	5.2	775	0.97	2.8	0.54	0.82	54
Japan	1440	1.10	4.7	541	0.68	1.7	0.38	0.919	20
Korea, Republic of	1404	1.07	8.7	342	0.43	2.1	0.24	0.916	22
Thailand	1322	1.01	6.4	864	1.09	4.1	0.65	0.777	80
Germany	1212	0.92	5.3	195	0.24	0.79	0.16	0.947	4
Congo, Democratic Republic of	1171	0.89	5.1	965	1.21	4.2	0.82	0.48	174
Myanmar	1143	0.87	6.4	768	0.96	4.2	0.67	0.583	148
Ghana	1106	0.84	11.7	841	1.06	8.9	0.76	0.611	138
Nigeria	1001	0.76	1.8	719	0.90	1.3	0.72	0.539	161
South Africa	993	0.76	4.6	630	0.79	2.9	0.63	0.709	115
United Kingdom	985	0.75	4.9	300	0.38	1.5	0.30	0.932	14
Uganda	922	0.70	7.5	730	0.92	6	0.79	0.544	160
Colombia	889	0.68	5.1	550	0.69	3.2	0.62	0.767	83
Mozambique	812	0.62	9.6	649	0.82	7.7	0.80	0.456	181
Algeria	772	0.59	4.9	364	0.46	2.4	0.47	0.748	91
Tanzania, United Republic of	749	0.57	4.5	615	0.77	3.7	0.82	0.529	164
France	738	0.56	4.3	259	0.33	1.4	0.35	0.901	26
Malawi	663	0.51	12.6	548	0.69	10.4	0.83	0.483	174
Australia	644	0.49	8.1	140	0.18	1.8	0.22	0.944	7
Kenya	625	0.48	3.7	440	0.55	2.6	0.70	0.601	141
Canada	621	0.47	5.4	218	0.27	1.9	0.35	0.929	14
Argentina	593	0.45	4.2	291	0.37	2.1	0.49	0.845	46
Cameroon	572	0.44	7.3	473	0.59	6.1	0.83	0.563	153
Saudi Arabia	542	0.41	3.7	264	0.33	1.8	0.49	0.854	40

Morocco	528	0.40	4.3	293	0.37	2.4	0.55	0.686	121
Iraq	504	0.38	4.1	289	0.36	2.4	0.57	0.674	123
Uzbekistan	491	0.37	4.1	363	0.46	3	0.74	0.72	107
Madagascar	490	0.37	6.2	374	0.47	4.7	0.76	0.528	163
Ukraine	487	0.37	3.4	326	0.41	2.2	0.67	0.779	78
Burkina Faso	486	0.37	8.6	432	0.54	7.7	0.89	0.452	183
Afghanistan	484	0.37	4.4	368	0.46	3.3	0.76	0.511	169
Sudan	467	0.36	3.8	316	0.40	2.6	0.68	0.51	171
Peru	465	0.35	4.1	356	0.45	3.2	0.77	0.777	78
Mali	465	0.35	9.2	349	0.44	6.9	0.75	0.434	184
Italy	425	0.32	2.6	138	0.17	0.87	0.32	0.892	29
Spain	417	0.32	3.3	80	0.10	0.56	0.19	0.904	25

ASIR: Age-standardized incidence rate; ASMR: Age-standardized mortality rate; HDI: Human development index; MIR: Mortality-to-incidence ratio.

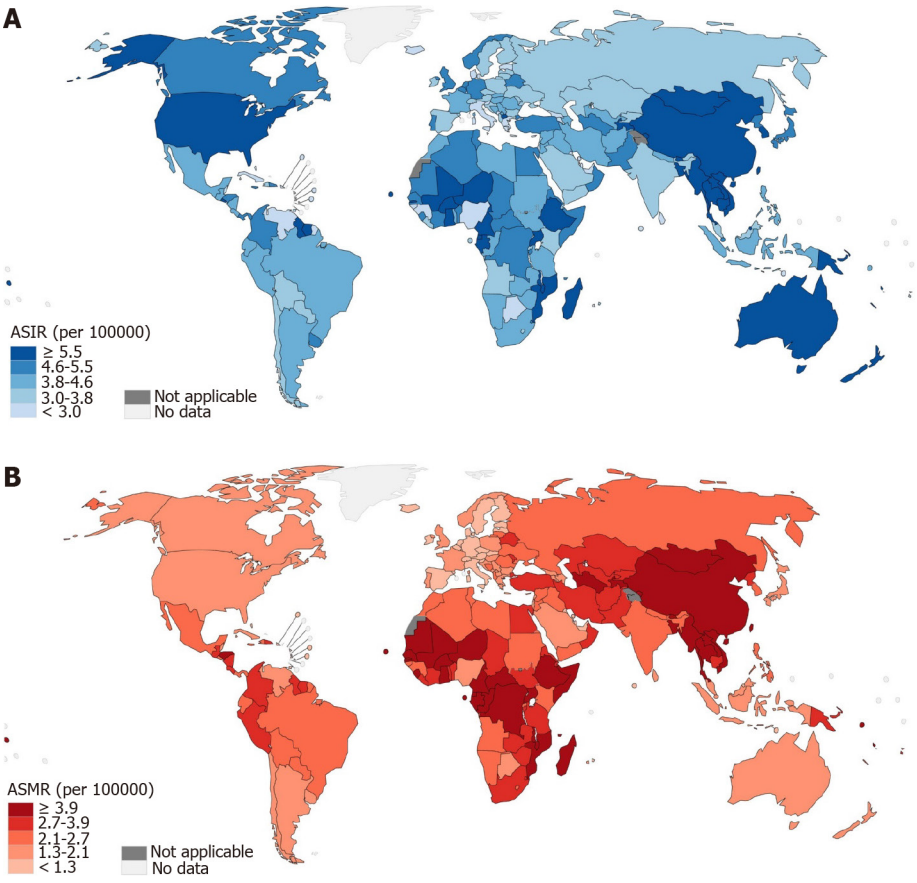


Figure 4 Global map depicting digestive cancer by country in terms of estimated age-standardized incidence rate and age-standardized mortality rate among 20- to 39-year-olds in 2020. A: Age-standardized incidence rate; B: Age-standardized mortality rate. ASIR: age-standardized incidence rate; ASMR: age-standardized mortality rate.

colorectal, liver, and stomach cancer were similar in the median HDI level both for new cases and for deaths (Figure 5). The Pearson correlation analysis reflected a weak positive correlation between the ASIR of digestive cancer and HDI ranking (R^2 = 0.0791, P = 0.0478), whereas a positive and significant correlation was observed between the ASMR of digestive cancer and HDI ranking (R^2 = 0.4252, P < 0.001). The results also demonstrated a strong positive correlation between the MIR of digestive cancer and HDI ranking (R^2 = 0.7388, P < 0.001). The positive correlation between MIR and HDI ranking was shared equally by individual digestive cancer types (Figure 6).

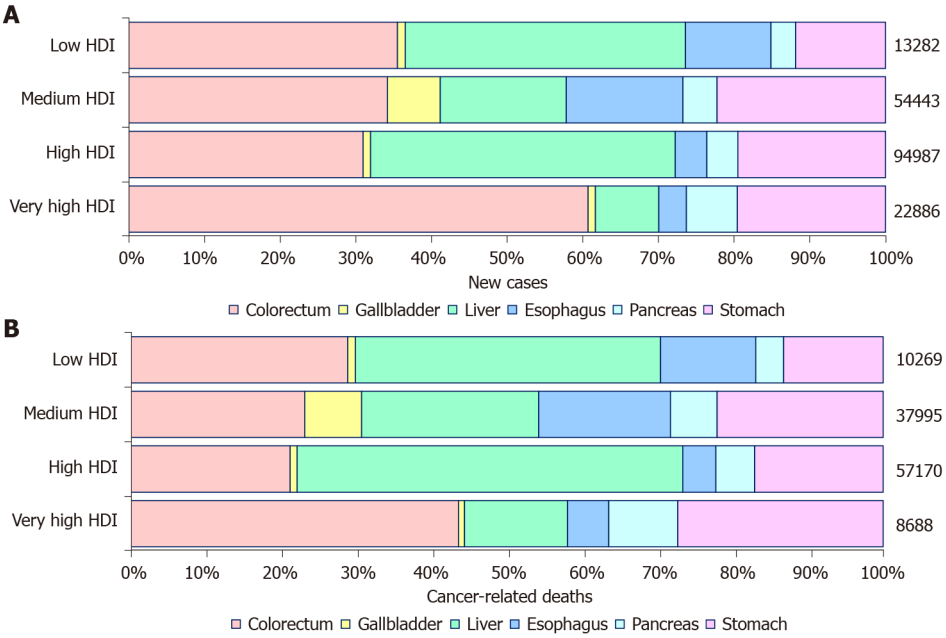


Figure 5 Digestive cancer type distribution for estimated new cancer cases and cancer-related deaths in 2020 among young adults by human development index level. HDI: Human development index.

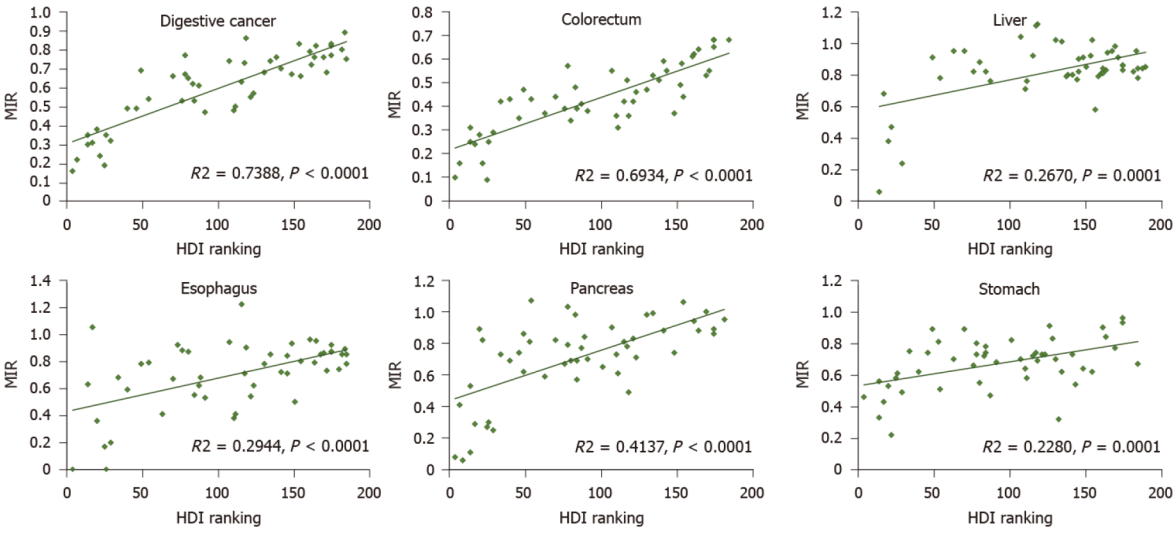


Figure 6 The association between human development index rankings and the mortality-to-incidence ratio of digestive cancer and its five subtype cancers. HDI: Human development index; MIR: Mortality-to-incidence ratio.

Except for both ASIR and ASMR of stomach cancer and ASMR of pancreatic cancer, the correlation was significant for individual digestive cancer types, with a negative correlation between HDI ranking and ASIR of colorectal and pancreatic cancer (Supplementary Figures 1 and 2).

Temporal variations in the incidence and mortality of digestive cancer

The 14-year (1999-2012) overall incidence trends showed a persistent slow increase for colorectal cancer, whereas obvious decreases were observed for liver and gastric cancer. The overall incidence trend curves of gallbladder, esophageal, and pancreatic cancer were volatile (Figure 7). The temporal variations (1985-2016) in mortality of colorectal and esophageal cancer were not obvious. The deaths resulting from liver cancer showed an unstable upward trend, especially in women. The number of deaths from gallbladder, pancreatic, and gastric cancer decreased from 1985 to 2016, especially for gastric cancer (Figure 8).

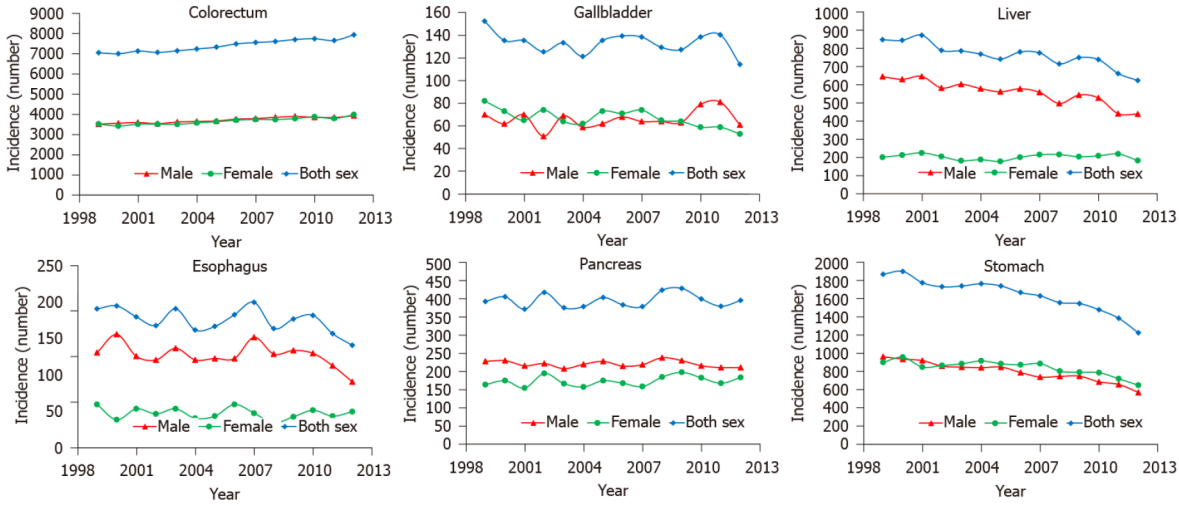


Figure 7 Time trends in new cases of six digestive cancers in young adults, both sexes, 1999-2012.

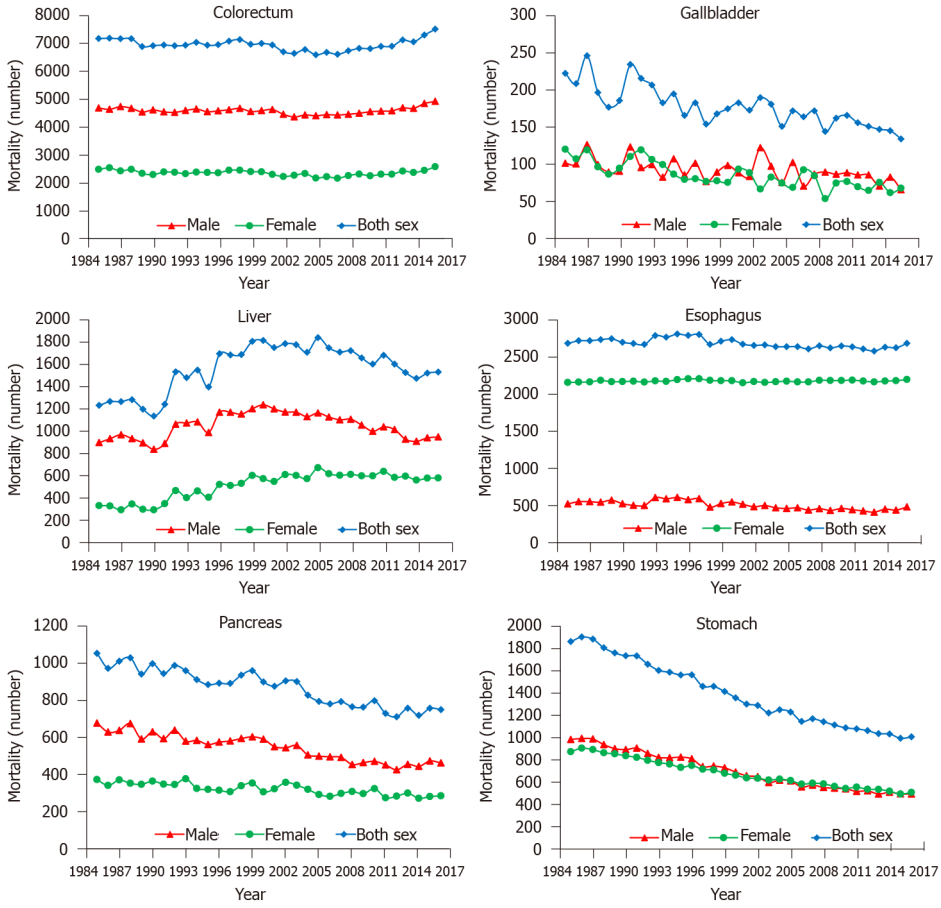


Figure 8 Time trends in deaths of six digestive cancers in young adults, both sexes, 1985-2016.

DISCUSSION

Worldwide, the incidence of digestive cancer in young adults in 2020 was 5.16 per 100000 people per year with an estimated 131068 new cases, and the corresponding mortality was 3.04 per 100000 people per year with an estimated 79614 deaths in 2020. Although only 2.55% of new digestive cancer cases and 2.19% of digestive cancer-associated deaths occurred in young adults, digestive cancer accounted for 12.24% of all new cancer cases and 25.26% of all cancer-associated deaths in this age group. This indicates that the disease burden is heavier and the outcome is much worse for digestive cancer than cancers arising from other systems in young adults[1,8]. Cancers

of the colorectum, liver, and stomach were the most common digestive cancer types, together accounting for 86.04% of the total estimated new cases and 81.45% of the total estimated cancer-associated deaths, which is consistent with that in the general population[12]. However, the burden of young adult digestive cancer varied substantially across levels of sex, geographical region, and human development, which was related to differences in cancer screening and detection modalities, genetic backgrounds, and carcinogenic exposures[6].

The distribution of digestive cancer between young males and females was clear, with higher incidence and mortality in men, mainly contributed by liver cancer, which was over 3 times more common in men than in women with regard to both incidence and mortality. The majority of primary liver cancer was hepatocellular carcinoma (HCC), which has been mainly associated with chronic infection with hepatitis B or C virus (HBV or HCV) as well as exposure to aflatoxin, excessive alcohol consumption, obesity, type 2 diabetes, and smoking. All of these risk factors are prevalent in males [12,13]. However, the incidence and mortality of gastric cancer were lower in men than in women. Many studies on gastric cancer in young adults have reported a higher female proportion, and the incidence in females gradually changes from a higher to a lower level than that in males at 40 years of age, which indicates that estrogen may play an important role in gastric cancer development in young females[14].

The burden of digestive cancer in young adults varies across geographical regions. This can be explained by the population size; therefore, most young adult digestive cancer cases and deaths occurred in Asia, especially in China and India, the world's two largest populations. This can also be explained by the development levels. A greater proportion of colorectal cancer occurred in developed countries, the incidence increased as the HDI ranking improved, and the incidence trends showed a persistent slow increase for colorectal cancer in young adults. These results are consistent with the established conclusion that colorectal cancer can be considered a marker of socioeconomic development, and incidence rates tend to rise uniformly as countries continue to undergo socioeconomic transition[2,15]. In contrast, liver cancer is generally more prominent in low- and middle-income countries (LMICs), with the aforementioned risk factors for HCC mainly prevalent in these countries and regions [12,13]. However, new cases of liver and gastric cancer showed a stable decrease from 1999 to 2012, reflecting the improvement in the environment and prevention of chronic infections in countries with a high incidence of these two cancer types[13,16]. Despite the increased incidence of colorectal cancer in young adults, deaths caused by this disease were stable. In addition, the number of deaths from gallbladder, pancreatic, and gastric cancer decreased from 1985 to 2016, especially for gastric cancer, which might be due to the improvement in the outcomes of these cancer types. In addition, variations in mortality and MIR across HDI rankings were significant, with mortality and MIR being higher in LMICs, implying that the worse outcomes are probably due to underdeveloped health systems, limitations in diagnostic capacity, and paucity of health insurance in less developed countries[6].

With the heavy burden and worse outcomes of digestive cancer in young adults and given that they are expected to have a long life remaining and they represent the main proportion of contributors to the economy and their family care, effective strategies are urgently needed to decrease the burden in these settings. For a long time, young adult patients with digestive cancer have been neglected by global health stakeholders[17,18]. However, in the past two decades, many studies and programs have been initiated to address this issue; landmarks of these programs are reports by AYAO PRG, National Comprehensive Cancer Network, and IARC[6,8,19]. Globally, however, there are still a lot of unknown fields about young adult oncology, and what is known is mainly recognized in high-income countries. Expanding these recommendations to LICMs remains a challenge, but it is more important for these countries, as young populations are the main contributors to productivity growth[6]. Therefore, considering the greater loss of life-years in this younger population, I need strategies to decrease digestive cancer burden in young adults. However, locally advanced or distant metastatic digestive cancer is more frequently diagnosed in young patients, which is influenced by highly aggressive growth patterns of these cancers in young individuals, physicians' lack of familiarity with these cancers, and diagnosis delay[17,20,21]. Currently, the treatment protocols offered to young adults with cancer are extrapolated from younger and older populations[22,23]. Although young adults can always tolerate more aggressive treatment because of fewer comorbidities, the outcomes are poor for young patients with digestive cancers[10]. Therefore, before the emergence of a treatment that can cure any patient with cancer, prevention and diagnosis at a relatively early stage are the two most effective ways to lower the burden of young adult digestive cancer.

Prevention is the most effective way to decrease the digestive cancer incidence in young populations because majority of them are caused by amenable risk factors[12]. Environmental and lifestyle factors, such as poor dietary habits, alteration of the microbiome, chronic infection, sedentary lifestyle, and obesity, contribute to most digestive cancer cases[24]. Given that liver cancer contributes a large proportion of young adult digestive cancer, a global HBV vaccination program of HBV-naive people would likely have a significant effect in decreasing cancer burden in young populations worldwide. In China, the full embedment of HBV vaccination into the neonatal immunization program and mandatory HCV screening for blood transfusions have largely prevented new HBV and HCV infections, which may decrease the incidence of liver cancer[25]. Similarly, the prevalence of chronic *Helicobacter pylori* (*H. pylori*) infection is extraordinarily high, infecting approximately half of the world's population[26]. Chronic *H. pylori* infection is considered the principal cause of gastric cancer[27]. Eradication of *H. pylori* and improvements in the preservation and storage of foods have led to a steady decline in the incidence and mortality rates of noncardia gastric cancer over the last half century in most populations[28]. Adequate treatment of these bacteria could decrease the cancer incidence of gastric cancer, particularly in East Asia, where the infection rate is the highest. As previously mentioned, the incidence of colorectal cancer in young adults has increased over the last decades, likely reflecting changes in lifestyle factors and diet, leading to decreased physical activity and an increased prevalence of obesity[29]. These unhealthy lifestyles are changeable, and this change has led to declines in colorectal cancer incidence in some high-incidence countries[12,30].

Although most of the young adult digestive cancers are caused by lifestyle and environmental factors, some of them are strongly correlated to hereditary factors. Approximately 20% of colorectal cancer and 5% to 10% of gastric cancer are associated with familial clustering, and most of them are associated with inherited cancer predisposition syndromes. A family history of hereditary diffuse gastric cancer (HDGC), familial adenomatous polyposis, and Lynch syndrome predisposes young people to develop gastric cancer and colorectal cancer[31,32]. HDGC is an autosomal dominant syndrome arising from germline mutations in the tumor suppressor gene CDH1 (encoding the cell-to-cell adhesion protein E cadherin). HDGC is characterized by the development of gastric cancers, predominantly the diffuse type, at a young age[31]. Genetic susceptibility also appears to be more prevalent among young colorectal patients, with a prevalence of germline mutations of 16%–33% among those diagnosed before age 50[32]. Therefore, for hereditary factors, which are unchangeable with current technological capabilities, the prevention approach is precursor lesion detection by endoscopic surveillance and prophylactic total gastrectomy or colectomy [31,32].

Another opportunity to improve the outcomes of young adult patients with digestive cancer is screening and ensuring timely diagnosis[7,17]. However, no age-specific screening tests are currently available for young adults, and the screening targets are limited to individuals 40 years or older[33,34]. Increased colonoscopy screening and the removal of precursor lesions have decreased the incidence of colorectal cancer in adults older than 50 years in some countries, whereas the incidence in young adults continues to increase[3,35]. Therefore, studies examining such programs to improve the effective screening and avoid potential risks in young populations are needed. For all cancers, including digestive cancer, patients with early-stage disease have a far better prognosis than those with advanced-stage cancers, whereas young patients experience more delays than patients in other age groups, leading to diagnosis at late stages[17,20,21]. Although the factors leading to diagnosis delay of young adult digestive cancer vary across countries and regions and may be associated with psychosocial factors, cultural norms, geographic accessibility, and limitations in diagnostic capacity, the results of this study indicate that screening and early diagnosis programs may significantly reduce the burden of young adult digestive cancer. For hepatobiliary neoplasms, screening is mainly based on ultrasound and alpha fetoprotein, which can be easily carried out[13]. In contrast, for cancers of the gastrointestinal tract, the effective approach is endoscopy, which has skill limitations and is expensive in some countries; therefore, overuse of endoscopy is associated with a low yield rate in young patients and is not cost-effective[34]. However, as previously mentioned, the young adult population has a long-life expectancy remaining and represents the most important creator of the social material wealth. Endoscopic screening among young people in regions with a higher incidence is worthwhile.

This study has several limitations which have also been described in many studies using data from the GLOBOCAN database[36-38]. First, although the estimates of

cancer burden were produced based on the best available data, they might not be accurate, particularly for countries where data are not available or of poor quality. Second, the number of digestive cancer cases was small in some countries, and the estimates may lack sufficient statistical power. Thus, the results should be interpreted with caution if they are used to inform cancer control policies. Third, the MIR was employed as a proxy indicator of the 5-year survival rate in this study; however, the MIR is not an exact measure of the survival rate and only serves as a proxy for the survival rate of digestive cancer in young adults in the absence of a country-wise exact measure. Fourth, due to concerns about generating misleading MIRs, I focused primarily on the heavily burdened countries and regions with HDI rankings, which makes the results incomplete and in the global context. Fifth, the HDI grading system was established in 2000, and it may not precisely reflect the current status of health care systems in different countries. The diagnosed stage and risk factors, such as smoking, obesity, and chronic infection, which play crucial roles in explaining the incidence and mortality rates, were not documented in this study. Therefore, the association between young digestive cancer burden and HDI ranking must be interpreted with caution in these countries. Despite these limitations, because of the small proportion of digestive cancer in young adults, individual studies with small sample sizes cannot reflect the whole picture with regard to the epidemiological characteristics. This study involved data retrieval from the GLOBOCAN database, the best currently available worldwide data, and these findings highlight the health burden of young adult digestive cancer and provide a direction to increase awareness and re-allocate resources for this neglected subpopulation.

CONCLUSION

In summary, the global digestive cancer burden among young adults differs from that in children and adolescents or individuals older in age, but it also varies significantly by sex, geographical region, and HDI level. Although the burden of young adult digestive cancer is much small when compared with digestive cancer in older populations, its effects remain substantial because young adults have a long-life expectancy remaining, and this age group represents the most important creator of the social material wealth. Estimating the burden of digestive cancer in young adults might help to raise awareness at both public and professional levels, inform recommendations for the aforementioned measures for prevention and timely diagnosis, and lead to improvements in outcomes of these specific cancer types across all resource levels.

ARTICLE HIGHLIGHTS

Research background

Studies on cancer trends have demonstrated that the incidence of digestive cancers has increased significantly in young adults over the last few decades. However, digestive cancer has traditionally been thought of as a disease that mainly occurs in elderly individuals, and studies have focused on cancers at older ages. Therefore, cancers in young adults have been relatively ignored by both patients and physicians.

Research motivation

Young adults represent the main proportion of contributors to the economy and their family care. Thus, it is important to investigate the specific issues unique to this age group of cancer patients.

Research objectives

To describe the worldwide profile of digestive cancer incidence, mortality and corresponding trends among 20–39-year-olds, with major patterns highlighted by age, sex, development level, and geographical region.

Research methods

I performed a population-based study to quantify the burden of young adult digestive cancers worldwide. Global, regional, sex, and country-specific data estimates of the number of new cancer cases and cancer-associated deaths that occurred in 2020 were

extracted from the GLOBOCAN Cancer Today database. To assess long-term trends in young adult digestive cancer, cancer incidence data and mortality data were obtained from the Cancer in Five Continents Plus database and the World Health Organization mortality database, respectively. The associations between the human development index (HDI) and digestive cancer burden in young adults were also evaluated by linear regression analyses.

Research results

A total of 131068 new digestive cancer cases and 79614 cancer-associated deaths occurred among young adults worldwide in 2020, which equated to an age-standardized incidence rate (ASIR) of 5.16 and age-standardized mortality rate (ASMR) of 3.04, accounting for 12.24% of all new cancer cases and 25.26% of all cancer deaths occurring in young adults. The burden was disproportionally greater among males, with male: female ratios of 1.34 for incidence and 1.58 for mortality. The ASIRs were 2.1, 1.4, and 1.0 per 100000 people per year, whereas the ASMRs were 0.83, 1.1, and 0.62 per 100000 people per year for colorectal, liver, and gastric cancer, respectively. When assessed by geographical region and HDI levels, the cancer profile varied substantially, and a strong positive correlation between the mortality-to-incidence ratio of digestive cancer and HDI ranking was found ($R^2 = 0.7388$, $P < 0.001$).

Research conclusions

The most common digestive cancer types were colorectal, liver and gastric cancer. The global digestive cancer burden among young adults was greater among males and exhibited a positive association with socioeconomic status. The digestive cancer burden is heavy in this age group, and the societal and economic effects remain great.

Research perspectives

Although the burden of young adult digestive cancer is much small when compared with digestive cancer in older populations, its effects remain substantial. Estimating the burden of young adult digestive cancer might help to raise awareness at both public and professional levels. Through continuous prevention, screening, and early detection programs, the digestive cancer burden in young adults can be reduced.

ACKNOWLEDGEMENTS

The author thanks the Health Commission of Mianyang City and the Science and Education Department of the Third Hospital of Mianyang for their support. The author also thanks the researchers, online database builders and cancer registries for their work in data sharing.

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Sung H**, Siegel RL, Rosenberg PS, Jemal A. Emerging cancer trends among young adults in the USA: analysis of a population-based cancer registry. *Lancet Public Health* 2019; **4**: e137-e147 [PMID: 30733056 DOI: 10.1016/S2468-2667(18)30267-6]

3 **Vuik FE**, Nieuwenburg SA, Bardou M, Lansdorp-Vogelaar I, Dinis-Ribeiro M, Bento MJ, Zadnik V, Pellisé M, Esteban L, Kaminski MF, Suchanek S, Ngo O, Májek O, Leja M, Kuipers EJ, Spaander MC. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. *Gut* 2019; **68**: 1820-1826 [PMID: 31097539 DOI: 10.1136/gutjnl-2018-317592]

4 **Lee J**, Lee MA, Kim IH, Roh SY. Clinical characteristics of young-age onset gastric cancer in Korea. *BMC Gastroenterol* 2016; **16**: 110 [PMID: 27600152 DOI: 10.1186/s12876-016-0528-y]

5 **Marcano-Bonilla L**, Mohamed EA, Mounajjed T, Roberts LR. Biliary tract cancers: epidemiology, molecular pathogenesis and genetic risk associations. *Chin Clin Oncol* 2016; **5**: 61 [PMID: 27829275 DOI: 10.21037/cco.2016.10.09]

6 **Fidler MM**, Gupta S, Soerjomataram I, Ferlay J, Steliarova-Foucher E, Bray F. Cancer incidence and mortality among young adults aged 20-39 years worldwide in 2012: a population-based study. *Lancet Oncol* 2017; **18**: 1579-1589 [PMID: 29111259 DOI: 10.1016/S1470-2045(17)30677-0]

7 **Magrath I**, Epelman S. Cancer in adolescents and young adults in countries with limited resources. *Curr Oncol Rep* 2013; **15**: 332-346 [PMID: 23832788 DOI: 10.1007/s11912-013-0327-3]

8 **Adolescent and Young Adult Oncology Progress Review Group**. Closing the gap: research and

care imperatives for adolescents and young adults with cancer. [cited 2021 Mar 18]. Available from: https://www.cancer.gov/types/aya/research/ayao-august-2006.pdf

9 **Smith AW**, Bellizzi KM, Keegan TH, Zebrack B, Chen VW, Neale AV, Hamilton AS, Shnorhavorian M, Lynch CF. Health-related quality of life of adolescent and young adult patients with cancer in the United States: the Adolescent and Young Adult Health Outcomes and Patient Experience study. *J Clin Oncol* 2013; **31**: 2136-2145 [PMID: 23650427 DOI: 10.1200/JCO.2012.47.3173]

10 **Hydeman JA**, Uwazurike OC, Adeyemi EI, Beaupin LK. Survivorship needs of adolescent and young adult cancer survivors: a concept mapping analysis. *J Cancer Surviv* 2019; **13**: 34-42 [PMID: 30604138 DOI: 10.1007/s11764-018-0725-5]

11 **Nichols HB**, Baggett CD, Engel SM, Getahun D, Anderson C, Cannizzaro NT, Green L, Gupta P, Laurent CA, Lin PC, Meernik C, Moy LM, Wantman E, Xu L, Kwan ML, Mersereau JE, Chao CR, Kushi LH. The Adolescent and Young Adult (AYA) Horizon Study: An AYA cancer survivorship cohort. *Cancer Epidemiol Biomarkers Prev* 2021; **30**: 857-866 [PMID: 33619021 DOI: 10.1158/1055-9965.EPI-20-1315]

12 **Arnold M**, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, Bray F. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology* 2020; **159**: 335-349. e15 [PMID: 32247694 DOI: 10.1053/j.gastro.2020.02.068]

13 **Sharma R**. Descriptive epidemiology of incidence and mortality of primary liver cancer in 185 countries: evidence from GLOBOCAN 2018. *Jpn J Clin Oncol* 2020; **50**: 1370-1379 [PMID: 32719873 DOI: 10.1093/jjco/hyaa130]

14 **Li J**. Gastric Cancer in Young Adults: A Different Clinical Entity from Carcinogenesis to Prognosis. *Gastroenterol Res Pract* 2020; **2020**: 9512707 [PMID: 32190044 DOI: 10.1155/2020/9512707]

15 **Fidler MM**, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. *Int J Cancer* 2016; **139**: 2436-2446 [PMID: 27522007 DOI: 10.1002/ijc.30382]

16 **Arnold M**, Karim-Kos HE, Coebergh JW, Byrnes G, Antilla A, Ferlay J, Renehan AG, Forman D, Soerjomataram I. Recent trends in incidence of five common cancers in 26 European countries since 1988: Analysis of the European Cancer Observatory. *Eur J Cancer* 2015; **51**: 1164-1187 [PMID: 24120180 DOI: 10.1016/j.ejca.2013.09.002]

17 **Bleyer A**. Young adult oncology: the patients and their survival challenges. *CA Cancer J Clin* 2007; **57**: 242-255 [PMID: 17626120 DOI: 10.3322/canjclin.57.4.242]

18 **Stevens MC**. The 'Lost Tribe' and the need for a promised land: the challenge of cancer in teenagers and young adults. *Eur J Cancer* 2006; **42**: 280-281 [PMID: 16426844 DOI: 10.1016/j.ejca.2005.11.007]

19 National Comprehensive Cancer Network. The NCCN adolescent and young adult (AYA) oncology clinical guidelines (version 1, 2021). [cited 2021 Mar 18]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/aya.pdf

20 **Zeng Y**, Ruan W, Liu J, Liang W, He J, Cui F, Pan H. Esophageal cancer in patients under 50: a SEER analysis. *J Thorac Dis* 2018; **10**: 2542-2550 [PMID: 29997914 DOI: 10.21037/jtd.2018.05.72]

21 **Tricoli JV**, Boardman LA, Patidar R, Sindiri S, Jang JS, Walsh WD, McGregor PM 3rd, Camalier CE, Mehaffey MG, Furman WL, Bahrami A, Williams PM, Lih CJ, Conley BA, Khan J. A mutational comparison of adult and adolescent and young adult (AYA) colon cancer. *Cancer* 2018; **124**: 1070-1082 [PMID: 29194591 DOI: 10.1002/cncr.31136]

22 **Muffly L**, Lichtensztajn D, Shiraz P, Abrahão R, McNeer J, Stock W, Keegan T, Gomez SL. Adoption of pediatric-inspired acute lymphoblastic leukemia regimens by adult oncologists treating adolescents and young adults: A population-based study. *Cancer* 2017; **123**: 122-130 [PMID: 27622953 DOI: 10.1002/cncr.30322]

23 **Albritton KH**, Wiggins CH, Nelson HE, Weeks JC. Site of oncologic specialty care for older adolescents in Utah. *J Clin Oncol* 2007; **25**: 4616-4621 [PMID: 17925557 DOI: 10.1200/JCO.2006.08.4103]

24 **Tasneem AA**, Luck NH. Digestive tract neoplasms in young individuals: Demographics, staging and risk factors. *Cancer Rep (Hoboken)* 2021; **4**: e1319 [PMID: 33295088 DOI: 10.1002/cnr2.1319]

25 **Wang FS**, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. *Hepatology* 2014; **60**: 2099-2108 [PMID: 25164003 DOI: 10.1002/hep.27406]

26 **Hooi JKY**, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JJY, Kaplan GG, Ng SC. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017; **153**: 420-429 [PMID: 28456631 DOI: 10.1053/j.gastro.2017.04.022]

27 **Plummer M**, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to Helicobacter pylori. *Int J Cancer* 2015; **136**: 487-490 [PMID: 24889903 DOI: 10.1002/ijc.28999]

28 **Howson CP**, Hiyama T, Wynder EL. The decline in gastric cancer: epidemiology of an unplanned triumph. *Epidemiol Rev* 1986; **8**: 1-27 [PMID: 3533579 DOI: 10.1093/oxfordjournals.epirev.a036288]

29 **Conti L**, Del Cornò M, Gessani S. Revisiting the impact of lifestyle on colorectal cancer risk in a gender perspective. *Crit Rev Oncol Hematol* 2020; **145**: 102834 [PMID: 31790930 DOI: 10.1016/j.critrevonc.2019.102834]

30 **GBD 2017 Colorectal Cancer Collaborators**. The global, regional, and national burden of colorectal cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic

analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019; **4**: 913-933 [PMID: 31648977 DOI: 10.1016/S2468-1253(19)30345-0]

31 **Gamble LA**, Heller T, Davis JL. Hereditary Diffuse Gastric Cancer Syndrome and the Role of CDH1: A Review. *JAMA Surg* 2021; **156**: 387-392 [PMID: 33404644 DOI: 10.1001/jamasurg.2020.6155]

32 **Valle L**, Vilar E, Tavtigian SV, Stoffel EM. Genetic predisposition to colorectal cancer: syndromes, genes, classification of genetic variants and implications for precision medicine. *J Pathol* 2019; **247**: 574-588 [PMID: 30584801 DOI: 10.1002/path.5229]

33 **Fan X**, Qin X, Zhang Y, Li Z, Zhou T, Zhang J, You W, Li W, Pan K. Screening for gastric cancer in China: Advances, challenges and visions. *Chin J Cancer Res* 2021; **33**: 168-180 [PMID: 34158737 DOI: 10.21147/j.issn.1000-9604.2021.02.05]

34 **Chang HS**, Park EC, Chung W, Nam CM, Choi KS, Cho E, Cho WH. Comparing endoscopy and upper gastrointestinal X-ray for gastric cancer screening in South Korea: a cost-utility analysis. *Asian Pac J Cancer Prev* 2012; **13**: 2721-2728 [PMID: 22938448 DOI: 10.7314/apjcp.2012.13.6.2721]

35 **Siegel RL**, Torre LA, Soerjomataram I, Hayes RB, Bray F, Weber TK, Jemal A. Global patterns and trends in colorectal cancer incidence in young adults. *Gut* 2019; **68**: 2179-2185 [PMID: 31488504 DOI: 10.1136/gutjnl-2019-319511]

36 **Heer E**, Harper A, Escandor N, Sung H, McCormack V, Fidler-Benaoudia MM. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *Lancet Glob Health* 2020; **8**: e1027-e1037 [PMID: 32710860 DOI: 10.1016/S2214-109X(20)30215-1]

37 **Sharma R**. An examination of colorectal cancer burden by socioeconomic status: evidence from GLOBOCAN 2018. *EPMA J* 2020; **11**: 95-117 [PMID: 32140188 DOI: 10.1007/s13167-019-00185-y]

38 **Sung WW**, Wang SC, Hsieh TY, Ho CJ, Huang CY, Kao YL, Chen WJ, Chen SL. Favorable mortality-to-incidence ratios of kidney Cancer are associated with advanced health care systems. *BMC Cancer* 2018; **18**: 792 [PMID: 30081855 DOI: 10.1186/s12885-018-4698-6]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 295-318

DOI: 10.4251/wjgo.v14.i1.295

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Prospective Study

Intertwined leukocyte balances in tumours and peripheral blood as robust predictors of right and left colorectal cancer survival

Ramón Cantero-Cid, Karla Marina Montalbán-Hernández, Jenny Guevara, Alejandro Pascual-Iglesias, Elisa Pulido, José Carlos Casalvilla, Cristóbal Marcano, Cristina Barragán Serrano, Jaime Valentín, Gloria Cristina Bonel-Pérez, José Avendaño-Ortiz, Verónica Terrón, Roberto Lozano-Rodríguez, Alejandro Martín-Quirós, Elvira Marín, Eva Pena, Laura Guerra-Pastrián, Eduardo López-Collazo, Luis Augusto Aguirre

ORCID number: Ramón Cantero-Cid 0000-0002-8353-4010; Karla Marina Montalbán-Hernández 0000-0002-1612-103X; Jenny Guevara 0000-0001-9158-1610; Alejandro Pascual-Iglesias 0000-0002-2214-5441; Elisa Pulido 0000-0002-9267-0954; José Carlos Casalvilla 0000-0002-2816-4811; Cristóbal Marcano 0000-0002-7544-9238; Cristina Barragán Serrano 0000-0002-3214-4469; Jaime Valentín 0000-0001-5853-4636; Gloria Cristina Bonel-Pérez 0000-0001-6546-3039; José Avendaño-Ortiz 0000-0003-2774-1821; Verónica Terrón 0000-0001-8940-3380; Roberto Lozano-Rodríguez 0000-0003-0093-9707; Alejandro Martín-Quirós 0000-0003-4630-7668; Elvira Marín 0000-0003-2206-2328; Eva Pena 0000-0001-7113-045X; Laura Guerra-Pastrián 0000-0002-8478-8522; Eduardo López-Collazo 0000-0003-1957-877X; Luis Augusto Aguirre 0000-0003-0903-9385.

Author contributions: Cantero-Cid R and Montalbán-Hernández KM contributed equally to the manuscript; Cantero-Cid R participated in the design of the study and performed patient recruitment, surgery, and sample collection; Montalbán-Hernández KM performed sample collection, assisted with sample analysis, and

Ramón Cantero-Cid, Karla Marina Montalbán-Hernández, Alejandro Pascual-Iglesias, Elisa Pulido, José Carlos Casalvilla, Jaime Valentín, Gloria Cristina Bonel-Pérez, José Avendaño-Ortiz, Verónica Terrón, Roberto Lozano-Rodríguez, Elvira Marín, Eduardo López-Collazo, Luis Augusto Aguirre, Tumor Immunology Laboratory, The Innate Immune Response Group, Hospital La Paz Institute for Health Research (IdiPAZ), Madrid 28046, Spain

Jenny Guevara, Cristóbal Marcano, Cristina Barragán Serrano, Digestive Surgery Service, La Paz University Hospital, Madrid 28046, Spain

Alejandro Martín-Quirós, Emergency Department and Emergent Pathology Research Group, Hospital La Paz Institute for Health Research (IdiPAZ), Madrid 28046, Spain

Eva Pena, Laura Guerra-Pastrián, Pathologic Anatomy Service, Hospital La Paz, Madrid 28046, Spain

Corresponding author: Luis Augusto Aguirre, PhD, Senior Researcher, Tumor Immunology Laboratory, The Innate Immune Response Group, Hospital La Paz Institute for Health Research (IdiPAZ), Paseo de la Castellana, 261, Madrid 28046, Spain. luis.augusto.aguirre@idipaz.es

Abstract

BACKGROUND

Colorectal cancer (CRC) accounts for 9.4% of overall cancer deaths, ranking second after lung cancer. Despite the large number of factors tested to predict their outcome, most patients with similar variables show big differences in survival. Moreover, right-sided CRC (RCRC) and left-sided CRC (LCRC) patients exhibit large differences in outcome after surgical intervention as assessed by preoperative blood leukocyte status. We hypothesised that stronger indexes than circulating (blood) leukocyte ratios to predict RCRC and LCRC patient outcomes will result from combining both circulating and infiltrated (tumour/peritumour fixed tissues) concentrations of leukocytes.

AIM

To seek variables involving leukocyte balances in peripheral blood and tumour tissues and to predict the outcome of CRC patients.

performed image analysis; Pulido E performed sample analysis and assisted with image analysis; Pascual-Iglesias A performed statistical analysis; Casalvilla JC assisted with sample analysis and with image analysis; Valentín J assisted with sample analysis and with image analysis; Bonel-Pérez GC assisted with sample analysis and with image analysis; Avendañ o-Ortiz J assisted with sample analysis; Terrón V assisted with sample analysis; Lozano-Rodrí guez R assisted with sample analysis; Martín-Quirós A participated in patient recruitment; Marín E performed sample analysis; Guevara J participated in patient recruitment, surgery, sample collection, and assisted with data analysis; Marcano C participated in patient recruitment, surgery, sample collection, and assisted with data analysis; Barragá n C participated in patient recruitment, surgery, and sample collection; Pena E was involved with sample collection and assisted with anatomo-pathological analyses; Guerra-Pastrián L was involved with sample collection and performed anatomo-pathological analyses; López-Collazo E participated in the design and oversight of the study and drafted the manuscript; Aguirre LA designed and supervised the study, assisted with data analysis, and drafted the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee for Clinical Research of the La Paz University Hospital (Madrid, Spain). Access number: PI-1958.

Informed consent statement: All study participants, or their legal guardian, provided written consent prior to study enrolment.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

Data sharing statement: There is no additional data available.

METHODS

Sixty-five patients diagnosed with colon adenocarcinoma by the Digestive Surgery Service of the La Paz University Hospital (Madrid, Spain) were enrolled in this study: 43 with RCRC and 22 with LCRC. Patients were followed-up from January 2017 to March 2021 to record overall survival (OS) and recurrence-free survival (RFS) after surgical interventions. Leukocyte concentrations in peripheral blood were determined by routine laboratory protocols. Paraffin-fixed samples of tumour and peritumoural tissues were assessed for leukocyte concentrations by immunohistochemical detection of CD4, CD8, and CD14 marker expression. Ratios of leukocyte concentration in blood and tissues were calculated and evaluated for their predictor values for OS and RFS with Spearman correlations and Cox univariate and multivariate proportional hazards regression, followed by the calculation of the receiver-operating characteristic and area under the curve (AUC) and the determination of Youden's optimal cutoff values for those variables that significantly correlated with either RCRC or LCRC patient outcomes. RCRC patients from the cohort were randomly assigned to modelling and validation sets, and clinician-friendly nomograms were developed to predict OS and RFS from the respective significant indexes. The accuracy of the model was evaluated using calibration and validation plots.

RESULTS

The relationship of leukocyte ratios in blood and peritumour resulted in six robust predictors of worse OS in RCRC: $CD8^+$ lymphocyte content in peritumour ($CD8_{pt}$, AUC = 0.585, cutoff < 8.250, P = 0.0077); total lymphocyte content in peritumour ($CD4CD8_{pt}$, AUC = 0.550, cutoff < 10.160, P = 0.0188); lymphocyte-to-monocyte ratio in peritumour (LMR_{pt}, AUC = 0.807, cutoff < 3.185, P = 0.0028); $CD8^+$ LMR in peritumour ($CD8MR_{pt}$, AUC = 0.757, cutoff < 1.650, P = 0.0007); the ratio of blood LMR to LMR in peritumour (LMR_b/LMR_{pt}, AUC = 0.672, cutoff > 0.985, P = 0.0244); and the ratio of blood LMR to $CD8^+$ LMR in peritumour ($LMR_b/CD8MR_{pt}$, AUC = 0.601, cutoff > 1.485, P = 0.0101). In addition, three robust predictors of worse RFS in RCRC were found: LMR_{pt} (AUC = 0.737, cutoff < 3.185, P = 0.0046); LMR_b/LMR_{pt} (AUC = 0.678, cutoff > 0.985, P = 0.0155) and $LMR_b/CD8MR_{pt}$ (AUC = 0.615, cutoff > 1.485, P = 0.0141). Furthermore, the ratio of blood LMR to $CD4^+$ LMR in peritumour ($LMR_b/CD4MR_{pt}$, AUC = 0.786, cutoff > 10.570, P = 0.0416) was found to robustly predict poorer OS in LCRC patients. The nomograms showed moderate accuracy in predicting OS and RFS in RCRC patients, with concordance index of 0.600 and 0.605, respectively.

CONCLUSION

Easily obtainable variables at preoperative consultation, defining the status of leukocyte balances between peripheral blood and peritumoural tissues, are robust predictors for OS and RFS of both RCRC and LCRC patients.

Key Words: Left colorectal cancer; Leukocyte ratios; Prognostic variables; Recurrence-free survival; Right colorectal cancer; Overall survival

Core Tip: This was a prospective study involving 65 patients with colorectal cancer, seeking to find robust predictors of survival after surgical intervention amongst the leukocyte balances in peripheral blood, tumour, and peritumoural tissues. A number of these variables are shown to predict overall survival and recurrence-free survival in both right-sided colorectal cancer and left-sided colorectal cancer patients, thus allowing the improvement of pre- and postoperative patient treatments.

Citation: Cantero-Cid R, Montalbán-Hernández KM, Guevara J, Pascual-Iglesias A, Pulido E, Casalvilla JC, Marcano C, Serrano CB, Valentín J, Bonel-Pérez GC, Avendaño-Ortiz J, Terrón V, Lozano-Rodríguez R, Martín-Quirós A, Marín E, Pena E, Guerra-Pastrián L, López-Collazo E, Aguirre LA. Intertwined leukocyte balances in tumours and peripheral blood as robust predictors of right and left colorectal cancer survival. *World J Gastrointest Oncol* 2022; 14(1):

Supported by the Foundation for the Hospital La Paz Institute for Health Research, No. PI698; Fundación Familia Alonso; Fundación Antolín Garcíarena and the European Union's Horizon 2020 research and innovation program under the Marie Sklodowaska-Curie-'laCaixa', No. 713673.

Country/Territory of origin: Spain

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

Received: May 16, 2021
Peer-review started: May 16, 2021
First decision: June 16, 2021
Revised: August 7, 2021
Accepted: November 30, 2021
Article in press: November 30, 2021
Published online: January 15, 2022

P-Reviewer: Wu ZQ, Xie Q, Zeng YY
S-Editor: Chang KL
L-Editor: Filipodia
P-Editor: Chang KL

295-318
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/295.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.295

INTRODUCTION

Despite the great medical and scientific achievements attained over the last decades in the fields of cancer understanding, early detection, and care, cancer continues to be a majorly threatening disease worldwide. Amongst the many pathologies gathered under this term, colorectal cancer (CRC) accounts for 9.4% of overall cancer deaths, ranking second just after lung cancer[1]. CRC treatments vary depending on tumour location and stage of diagnosis; standard colectomy (along with lymphadenectomy) without adjuvant therapy is the usual treatment in early stages I and II, while most patients in advanced stages III and IV follow with chemo- and/or radiotherapy to reduce the risk of recurrence[2]. However, a large proportion of these patients present with (synchronous; 15%-25%) or will develop (metachronous; 40%-75%) metastases, mainly in the liver[3], which constitutes the major cause of deaths[4]. Therefore, a 5-year relative survival rate is reduced from 90% in early-stage detection to 12% in advanced cases[2]. Thus, finding robust markers before surgery to predict patient outcomes constitutes a safe strategy in order to stratify those groups with a high risk of recurrence and design personalised pre- and postoperative therapies.

A wide variety of factors, mainly based on clinical and pathological features, have been tested as prognostic markers for CRC development, such as: weight loss, haemoglobin levels, tumour-nodes-metastasis classification (TNM) staging and tumour differentiation, mismatch-repair proficiency, lymph node involvement, or response to (neo-) adjuvant therapies[5-7]. Moreover, since a clear distinction between the behaviour of right-sided CRC (RCRC) and left-sided CRC (LCRC) patients is well established, much effort has been put into categorising putative prognostic markers according to their respective characteristics, though still with controversial results[8].

Currently, an increasing number of research and clinical trials are supporting evidence of the influence of the systemic inflammatory response in cancer progression [5]. A measure of this response has been assessed by combining the number of peripheral circulating leukocytes: lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocytes ratio (NLR), and platelet-to-lymphocyte ratio (PLR). These analyses have shown interesting prognostic associations in several cancer types including urothelial, nasopharyngeal, osteosarcoma, lung carcinomas[9-12], and CRC[13-16]. Nevertheless, few studies have been directed towards the prognostic value of intertwined relationships across circulating and tumour-infiltrated populations of leucocytes on solid tumour progression[17-19].

Herein, we aimed to delve deep into the prognostic value of leukocyte distribution ratios, in both blood and tumour tissues, for CRC patient outcomes after surgery. We hypothesised that stronger indexes than circulating (blood) leukocyte ratios to predict patient outcome will result from combining both circulating and infiltrated (tumour/peritumoural tissues) concentrations of leukocytes. We show six robust predictors for RCRC overall survival ($CD8_{pt}$, $CD4CD8_{pt}$, LMR_{pt}, $CD8MR_{pt}$, LMR_b/LMR_{pt}, $LMR_b/CD8MR_{pt}$), three for RCRC recurrence-free survival (LMR_{pt}, $CD8MR_{pt}$, LMR_b/LMR_{pt}, $LMR_b/CD8MR_{pt}$), and another one for LCRC overall survival ($LMR_b/CD4MR_{pt}$), all these being based on the ratios between blood and peritumoural tissue concentration of lymphocytes and monocytes. Moreover, we highlight the importance of these variables in designing *ad hoc* surgical strategies, due to the ease with which surgeons can build a protocol by taking samples of peripheral blood and peritumoural tissue during a preoperative colonoscopy.

MATERIALS AND METHODS

Patient selection

Sixty-five patients diagnosed with colon adenocarcinoma, with no records of previous neo-adjuvant therapy, were recruited at the Digestive Surgery Service of La Paz University Hospital (Madrid, Spain) from January 2017 to September 2019. They were surgically treated according to each patient's condition for right (caecum, ascending, or transverse colon) or left (descending or sigmoid colon) hemicolectomies followed by

anastomosis, with partial hepatectomy if synchronous metastasis was present. Patients' clinical records were followed-up until March 2021. Overall survival (OS) was then defined as the length of time since surgery until *exitus* or the end of the study, whilst recurrence-free survival (RFS) was considered the interval from surgery until relapse, either from disease-free or (synchronous/metachronous) metastases-free statuses. All patients signed written consent, in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and the Ethics Committee for Clinical Research of La Paz University Hospital (PI-1958), for further uses of blood samples and surgically resected organs for research purposes.

Exclusion criteria

Only patients with adenomas or rectum adenocarcinoma were excluded from the study.

Blood tests

Venous blood samples were collected in 10 mL EDTA-tubes in the hospital room, 24 h prior to surgery and routinely tested for white blood cell, lymphocyte (L), monocyte (M), neutrophil (N) and platelet (P) counts at the Central Laboratory (CORE) of the La Paz University Hospital. Preoperative blood LMR (LMR_b), NLR (NLR_b), and PLR (PLR_b) were then calculated for each patient by dividing the absolute counts of the respective populations in the peripheral blood (Table 1).

Tissue preparation

Samples from the middle part (avoiding both the epicentre and the edge) of the tumours, 5 cm-adjacent peritumoural (non-neoplastic), and liver (in case of synchronous metastases) tissues were taken at the time of surgery, upon *in situ* evaluation of morphological characteristics by pathologists. Histological types and grades were based on microscopic features. Microsatellite stability analyses were performed as previously described[20].

Organ samples were washed with PBS solution containing 56 μg/mL gentamicin (Braun, Melsungen, Germany; 636159), 2.5 μg/mL fungizome/anphotericin-B (Gibco, Amarillo, TX, United States; 15290-018), and 1% penicillin/streptomycin (Sigma-Aldrich, Saint Louis, MO, United States; P4333-100mL) and gently shaken for 30 min at room temperature. Then they were fixed in 4% paraformaldehyde for 16 h, washed with PBS for 24 h, and paraffin-embedded by standard procedures.

Tissue microarrays (TMA) recipient paraffin-blocks (24 mm × 2.0 mm) were prepared with a TMA builder kit (Histopathology Ltd., Baranya, 7632, Hungary; 20010.2) and filled with properly matched samples of previous patients' blocks, following manufacturer's protocol.

Immunohistochemistry and image analysis

Thin sections (5 μm thick) of TMAs were cut with a Leica (RM2255) ultrathin-microtome and allowed to completely adhere to slides for 30 min at 60°C, before staining with commercially available antibodies against assessed surface markers was performed by standardised protocols (see Supplementary Table 1 for a complete list of primary and secondary antibodies used). Briefly, sections were deparaffinised with xylene, rehydrated through graded (100% to 70%) ethanol, and blocked for endogenous peroxidase by immersion in 97% methanol. Next, sections were immersed in heated sodium citrate buffer (10 mmol/L, pH 6.0) for antigenicity recovery and then incubated in unspecific-binding blocking solution [TBS solution containing 1% BSA, 1% Triton X-100 (Thermo Scientific; Waltham, MA, United States, 85111) and 2.5% horse serum (Gibco; Amarillo, TX, United States, 26050088)]. Primary antibodies were then added at recommended dilutions and incubated overnight at 4°C in a humid chamber. After washing slides with TBS, matched HRP-secondary antibodies were added and incubated for 1 h at room temperature. Then, DAB chromogen (DAB substrate kit, Cell Marque; Rocklin, CA, United States, 1-957D-30) was added for a few seconds until colour change and gently washed with TBS and distilled water. Finally, sections were counterstained by immersion in haematoxylin, dehydrated through graded (70% to 100%) ethanol, and mounted with DPX medium (Sigma-Aldrich; Saint louis, MO, United States, 06522).

An average of four photographs per sample (in order to cover the whole field for each sample on the TMA sections) were taken with an Olympus BX-41 microscope and blind-analysed by two independent observers with ImageJ (v1.52p), for the calculus of the relative areas to each antibody corresponding surface marker expression (CD4, CD8, and CD14). For a detailed description of the image processing see Supple-

Table 1 Baseline characteristics of patients included in this study

Characteristics	Frequency	%
All patients (*n* = 65)		
Age (yr) ± SD	73.54 ± 9.51	
(range)	(52-92)	
Gender		
Female	29	44.62
Male	36	55.38
Tumour localisation		
Right colorectal cancer	43	66.15
Caecum	13	30.23
Ascending colon	23	53.49
Transverse colon	7	16.28
Left colorectal cancer	22	33.85
Descending colon	11	50.00
Sigma	11	50.00
Emergency surgery		
Yes	1	1.54
No	64	98.46
Surgical procedure		
Laparoscopic hemicolectomy	48	73.85
Open hemicolectomy	17	26.15
Development at surgery		
Non-metastasised	37	56.92
Metastases	28	43.08
Liver synchronous	13	46.43
Liver metachronous	8	28.57
Other organs	13	46.43
MMR status		
pMMR	56	86.15
dMMR	5	7.69
Unknown	4	6.15
TNM stage		
0	3	4.62
I	7	10.77
IIA	21	32.31
IIB	5	7.69
IIIA	2	3.08
IIIB	8	12.31
IIIC	5	7.69
IV	1	1.54
IVA	10	15.38
IVB	3	4.62

Adjuvant chemotherapy			
Yes		30	46.15
No		35	53.85
Blood leukocytes counting (× 103/μL), (normal range)	RCRC	LCRC	*P* value
White blood cells count (3.6-10.5)	7.41 (3.52-16.2)	8.19 (4.83-15.8)	0.271
Lymphocytes (1.1-4.5)	1.77 (0.46-4.46)	2.04 (0.32-4.87)	0.235
Monocytes (0.1-0.9)	0.54 (0.20-1.26)	0.50 (0.22-1.11)	0.493
Neutrophils (1.5-7.7)	4.86 (1.76-15.3)	5.34 (2.96-13.0)	0.480
Platelets (150-370)	275.65 (101.0-602.0)	272.41 (142.0-725.0)	0.910
LMR_b	3.54 (0.42-7.96)	4.64 (0.58-11.88)	0.046
NLR_b	3.65 (0.69-25.93)	4.61 (0.93-30.66)	0.481
PLR_b	188.58 (52.24-551.22)	213.93 (29.16-1187.50)	0.585

TNM: Tumour-nodes-metastasis classification; MMR: DNA mismatch repair; pMMR: Proficient MMR; dMMR: Deficient MMR; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocytes ratio; PLR: Platelet-to-lymphocyte ratio; RCRC: Right-sided colorectal cancer; LCRC: Left-sided colorectal cancer; SD: Standard deviation; b: Blood.

mentary Figure 1A. A percentage of the total tissue area (A) for the three surface markers, in each patient's tumour and peritumour samples, was reported as the mean of all their relative areas per field.

Total tumour and peritumour LMRs (respectively, LMR_t and LMR_{pt}) were calculated by dividing the sum of the areas for CD4 and CD8 by the area for CD14, *e.g.*, $LMR_t = (A(CD4_t)+A(CD8_t))/A(CD14_t)$. Individual subpopulation ratios were also analysed for both tumour and peritumour samples ($CD4MR_t$, $CD8MR_t$ and $CD4MR_{pt}$, $CD8MR_{pt}$, respectively), *e.g.*, $CD4MR_t = A(CD4_t)/A(CD14_t)$. Then, blood-to-tissue ratios for all previous tumour and peritumour subpopulation ratios (LMR_b/LMR_t, $LMR_b/CD4MR_t$, $LMR_b/CD8MR_t$ and LMR_b/LMR_{pt}, $LMR_b/CD4MR_{pt}$, $LMR_b/CD8MR_{pt}$, respectively) were also reported for each patient.

Nomogram construction and validation

All RCRC patients from the cohort were randomly divided into training (60%) and validation (40%) sets to establish and validate the clinician-friendly nomograms. For each nomogram to predict the probability of OS or RFS, the six or the three respectively significant predictive factors found early were used to formulate the nomograms with several R packages. The discriminatory ability of the nomogram was assessed by calculating the Harrell's concordance index (C-index).

Statistical analysis

Data are represented as mean ± standard deviation. Student's *t* test was used for pairwise comparisons. Mann-Whitney *U* analysis was applied for equal standard deviations, otherwise Welch's correction was used. The distribution of the variables was assessed by a nonparametric test. Spearman *r* correlations were used to evaluate the association between the variables and ratios with the OS and RFS observed in our patients. Survival and population ratio relationships were analysed using Cox proportional hazard ratios; statistically significant variables in univariate analysis were further evaluated with the Cox multivariate step-by-step backward method to identify those with independent prognostic value. The Kaplan-Meier method was used to calculate the differences in OS and RFS rates for RCRC and LCRC over time (months), and significance was compared using the log-rank (Mantel-Cox) test; median time (months) survival proportions and P accuracy were reported. We calculated the receiver-operating characteristic (ROC) curve and the area under the curve (AUC) to determine whether the different variables and ratios could be used to predict OS and RFS in our cohort. We indicated the sensitivity, the specificity, the positive and negative predictive values, and 95% confidence interval for AUC and P accuracy. Optimal cutoff values, as determined with Youden's index, Harrell's C-index, and P accuracy, were calculated with R software. *P* values of 0.05 or less were considered indicative of statistical significance, and all these were two-sided. All statistics were performed in either Prism 6.0 (GraphPad, San Diego, CA, United States) or SPSS

version 23 (IBM, NY, United States) software.

RESULTS

Cohort baseline characteristics

The cohort included in this study was exclusively recruited by one team of surgeons, from their assigned patients for surgically treated disorders of the digestive tract, thus only a fraction is constituted of the whole figure of CRC patients attended at La Paz University Hospital during the period of recruitment. Detailed clinicopathological characterisation of patients is shown in Table 1.

A total of 65 patients with a mean age of 73.5 years, of whom 43 (66.1%) presented with RCRC and 22 (33.8%) with LCRC, were finally enrolled. Of these, 29 (44.6%) were women and 36 (55.4%) were men. With the exception of one case, all had been programmed for surgery without an emergency condition. Forty-eight (73.8%) were hemicolectomised by minimally invasive laparoscopic procedure. They ranged from stages 0 to IV, based on TNM classification; 28 (43.1%) were presenting metastasis (either synchronous or metachronous at the time of surgery), and 30 (46.1%) received adjuvant therapy after surgery. Fifty-six (86.1%) of the tumours were found proficient for the mismatch-repair machinery at the histological level.

Patient progression follow-up

The survival analysis, with a median follow-up of 26 mo, showed no differences for OS between RCRC and LCRC patients (Figure 1A) but a trend towards poorer outcome for the latter (74.5% *vs* 40.8%, $P = 0.1875$). However, in the analysis of RFS (Figure 1B), we observed significantly better outcomes for RCRC compared to LCRC patients (60.4% *vs* 19.1%, $P = 0.0036$).

Leukocyte counts and ratios

We found no differences (Table 1) in total leukocyte counts nor in individual populations of circulating lymphocytes, monocytes, neutrophils, or platelets between RCRC and LCRC patient peripheral blood. However, though all mean counts for both groups were within the normal physiological ranges, RCRC patients showed a trend towards low circulating lymphocytes. Thus, their LMR_b was lower ($P = 0.0462$) than LCRC patients (Figure 2A). Neither NLR_b nor PLR_b showed differences between RCRC and LCRC patients (Figure 2B and C).

Tissues from 54 out of the total 65 patients included in the study, 34 from RCRC patients (63%) and 20 from LCRC patients (37%), could be assessed for leukocyte infiltration analyses. This fact was mainly due to the morphological characteristics of 11 tumours, which made it impossible to separate pieces for research purposes without affecting the global diagnostics by pathologists.

Figure 3 shows the staining pattern for CD4, CD8, and CD14 cells in tumour and peritumour samples from two representative patients of LCRC and RCRC. The distribution of total ($CD4^+$ plus $CD8^+$) lymphocytes, $CD4^+$ lymphocytes, $CD8^+$ lymphocytes, and $CD14^+$ monocytes, in all analysed tissues, is shown in Supplementary Figure 2. Higher total lymphocyte content in tumours than peritumours from LCRC patients (13.06 ± 2.123 *vs* 7.57 ± 1.794, $P = 0.0095$) seemed due to the proportional increase of $CD8^+$ lymphocytes (11.19 ± 2.158 *vs* 5.13 ± 1.757, $P = 0.0020$), as we detected no differences amongst infiltrated $CD4^+$ lymphocytes in these tissues. No differences were found for lymphocyte infiltration in right tumours with respect to right peritumoural tissues. Moreover, infiltrated-leukocyte content in right tumours showed no differences to right peritumours.

The analysis of resulting ratios for lymphocyte and monocyte counts in tumour (t) and peritumoural (pt) tissues showed a higher LMR_t with respect to LMR_{pt} (4.128 ± 1.363 *vs* 2.022 ± 0.3432, $P = 0.0023$) beside higher $CD8MR_t$ than $CD8MR_{pt}$ (4.121 ± 1.374 *vs* 1.218 ± 0.3297, $P = 0.0001$) in LCRC patients (Figure 2D-F). No differences were detected for these ratios amongst RCRC respective tissues. Consequently, the analysis of blood-to-tissue ratios (Figure 2G-I) showed LCRC patients exhibited lower LMR_b/LMR_t than LMR_b/LMR_{pt} (2.104 ± 0.601 *vs* 2.900 ± 0.5061, $P = 0.0282$) as well as lower $LMR_b/CD8MR_t$ than $LMR_b/CD8MR_{pt}$ (3.381 ± 1.083 *vs* 5.898 ± 1.138, $P = 0.0033$). There were no differences in these ratios for RCRC respective tissues.

Association of leukocyte balance and patient's outcome

In order to assess the degree to which leukocyte balance (*i.e.* both the concentration

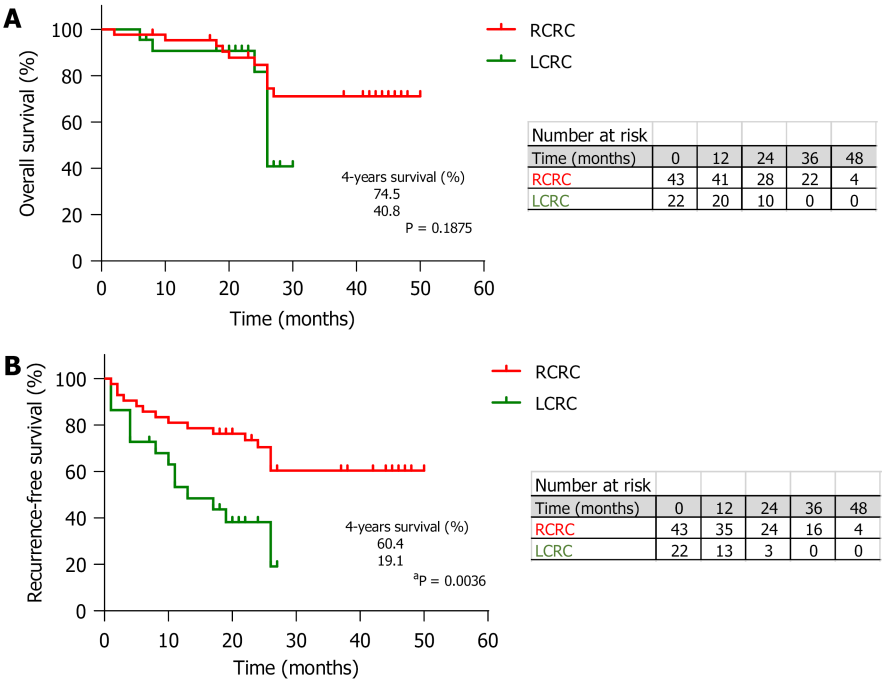


Figure 1 Survival rates of colorectal cancer patients. A, B: Kaplan-Meier curves for 4-year overall survival (A) and 4-year recurrence-free survival (B) observed in the cohort for both right-sided colorectal cancer (CRC) (orange) and left-sided CRC (green) patients. Survival proportions at 26 mo after surgery (median follow-up) of right-sided CRC and left-sided CRC patients are shown ($^bP < 0.01$, log-rank test). Number of cases at risk are tabled for both overall survival and recurrence-free survival. RCRC: Right-sided colorectal cancer; LCRC: Left-sided colorectal cancer.

and ratios of leukocytes for blood, tumour, and peritumours described above) was associated with RCRC and LCRC patient OS and RFS, we first conducted a Spearman correlation analysis (Table 2). We found that for RCRC LMR_b (r = -0.3039, P = 0.0476), LMR_{pt} (r = -0.4301, P = 0.0111), and $CD8MR_{pt}$ (r =-0.3596, P=0.0367) were negatively correlated with OS; LMR_t (r = -0.4775, P = 0.0043), LMR_{pt} (r = -0.3846, P = 0.0247), and CD8MRt (r = -0.4422, P = 0.0088) negatively correlated, but LMR_b/LMR_t (r = 0.3621, P = 0.0363) positively correlated with RFS. $LMR_b/CD8MR_t$ (r = 0.3364, P = 0.0517) also showed a trend towards being positively correlated. For LCRC, $CD14_{pt}$ (r = 0.5677, P = 0.009) and $LMR_b/CD4MR_{pt}$ (r = 0.4541, P=0.0443) positively correlated, but $CD4MR_{pt}$ (r = -0.473, P = 0.0352) negatively correlated with OS, whilst both $CD14_{pt}$ (r = 0.6018, P = 0.005) and $CD8MR_t$ (r = 0.4779, P = 0.331) positively correlated with RFS, and $CD4CD8_{pt}$ (r = 0.4425, P = 0.0507) also showed a trend towards being positively correlated.

Next, the effect of these variables on survival was assessed by Cox proportional hazards regression. For OS (Table 3), the univariate analysis revealed that besides previously found LMR_b (P = 0.043), LMR_{pt} (P = 0.024), and $CD8MR_{pt}$ (P = 0.031) in RCRC patients, NLR_b (P = 0.038) also significantly correlated with OS; $LMR_b/CD4MR_{pt}$ (P = 0.026) was also confirmed to be significantly correlated with OS of LCRC patients. After adjusting for confounding variables through the multivariate analysis, NLR_b (P = 0.038), $CD8MR_{pt}$ (P = 0.011), and $LMR_b/CD8MR_{pt}$ (P = 0.016) resulted in a significant association with OS of RCRC patients; $CD8_{pt}$ (P = 0.058) also showed a trend towards being associated.

Regarding RFS (Table 4), the univariate analysis showed that in addition to previously found LMR_t (P = 0.021) and LMR_b/LMR_t (P = 0.040) in RCRC, $LMR_b/CD8MR_t$ (P = 0.025) also significantly correlated with RFS, and $CD8MR_t$ (P = 0.052) showed a trend towards being associated. In addition to previously found $CD14_{pt}$ (P = 0.010), NLR_b (P = 0.020) and PLR_b (P = 0.018) were also significantly correlated with RFS in LCRC patients. After the multivariate analysis, several variables emerged as independent prognostic factors for RFS in RCRC patients: NLR_b (P = 0.039), PLR_b (P = 0.037), $CD14_t$ (P = 0.026), LMR_{pt} (P = 0.014), LMR_b/LMR_{pt} (P = 0.042), and $LMR_b/CD8MR_t$ (P = 0.006). In LCRC patients, NLR_b (P = 0.009), $CD8_{pt}$ (P = 0.020), $CD4CD8_t$ (P = 0.039), and $CD8MR_t$ (P = 0.019) were found, together with a trend observed for $CD4\ CD8_{pt}$ (P = 0.053).

Table 2 Association of leukocytes counts and ratios with overall and recurrence-free survival

Index	Total patients	4-year OS				4-year RFS			
	n (%)	Spearman *r*	95%CI	*P*		Spearman *r*	95%CI	*P*	
LMR_b									
Right	43 (66.2)	-0.3039	-0.5601 to 0.005346	0.0476	< 0.05	-0.1946	-0.4748 to 0.1214	0.211	NS
Left	22 (33.8)	0.1615	-0.2914 to 0.5553	0.4727	NS	-0.07447	-0.4912 to 0.3700	0.7419	NS
NLR_b									
Right	43 (66.2)	0.2262	-0.08868 to 0.5000	0.1446	NS	0.1062	-0.2094 to 0.4017	0.498	NS
Left	22 (33.8)	-0.06922	-0.4872 to 0.3746	0.7595	NS	0.1192	-0.3305 to 0.5247	0.5974	NS
PLR_b									
Right	43 (66.2)	0.2307	-0.08405 to 0.5035	0.1367	NS	0.06684	-0.2470 to 0.3680	0.6702	NS
Left	22 (33.8)	0.1615	-0.2914 to 0.5553	0.4727	NS	0.1192	-0.3305 to 0.5247	0.5974	NS
$CD4_t$									
Right	34 (63.0)	-0.05561	-0.3954 to 0.2976	0.7548	NS	0.05447	-0.2986 to 0.3944	0.7596	NS
Left	20 (37.0)	0.01892	-0.4387 to 0.4687	0.9369	NS	-0.0354	-0.4815 to 0.4253	0.8822	NS
$CD4_{pt}$									
Right	34 (63.0)	0.03708	-0.3144 to 0.3796	0.8351	NS	0.07371	-0.2809 to 0.4106	0.6787	NS
Left	20 (37.0)	-0.142	-0.5597 to 0.3334	0.5505	NS	0.3276	-0.1483 to 0.6803	0.1586	NS
$CD8_t$									
Right	34 (63.0)	-0.08526	-0.4202 to 0.2702	0.6316	NS	-0.2083	-0.1958 to 0.6531	0.2372	NS
Left	20 (37.0)	0.03784	-0.4233 to 0.4834	0.8741	NS	0.2832	-0.3863 to 0.3074	0.2263	NS
$CD8_{pt}$									
Right	34 (63.0)	-0.1186	-0.4476 to 0.2386	0.504	NS	-0.04486	-0.3863 to 0.3074	0.8011	NS
Left	20 (37.0)	0.3406	-0.1340 to 0.6881	0.1417	NS	0.3186	-0.1581 to 0.6749	0.171	NS
$CD4CD8_t$									
Right	34 (63.0)	-0.1372	-0.4626 to 0.2208	0.4392	NS	-0.1955	-0.5084 to 0.1630	0.268	NS
Left	20 (37.0)	0.03784	-0.4233 to 0.4834	0.8741	NS	0.2655	-0.2142 to 0.6420	0.2579	NS
$CD4CD8_{pt}$									
Right	34 (63.0)	-0.07044	-0.4079 to 0.2839	0.6922	NS	-0.009612	-0.3559 to 0.3389	0.957	NS
Left	20 (37.0)	0.3595	-0.1127 to 0.6993	0.1195	NS	0.4425	-0.01421 to 0.7464	0.0507	NS
$CD14_t$									
Right	34 (63.0)	0.1891	-0.1695 to 0.5034	0.2842	NS	0.2467	-0.1102 to 0.5472	0.1595	NS
Left	20 (37.0)	0.05677	-0.4076 to 0.4978	0.8121	NS	-0.1239	-0.5470 to	0.6028	NS

							0.3496		
$CD14_{pt}$									
Right	34 (63.0)	0.3003	-0.05262 to 0.5865	0.0844	NS	0.2596	-0.09658 to 0.5568	0.1382	NS
Left	20 (37.0)	0.5677	0.1533 to 0.8122	0.009	< 0.01	0.6018	0.2035 to 0.8292	0.005	< 0.01
LMR_t									
Right	34 (63.0)	-0.2929	-0.5812 to 0.06070	0.0927	NS	-0.4775	-0.7075 to -0.1559	0.0043	< 0.01
Left	20 (37.0)	0.07569	-0.3916 to 0.5119	0.7511	NS	0.4425	-0.01421 to 0.7464	0.0507	NS
LMR_{pt}									
Right	34 (63.0)	-0.4301	-0.6764 to -0.09719	0.0111	< 0.05	-0.3846	-0.6457 to -0.04285	0.0247	< 0.05
Left	20 (37.0)	0	-0.4538 to 0.4538	> 0.9999	NS	-0.0354	-0.4815 to 0.4253	0.8822	NS
CD4MRt									
Right	34 (63.0)	-0.2781	-0.5704 to 0.07674	0.1113	NS	-0.3173	-0.5987 to 0.03388	0.0675	NS
Left	20 (37.0)	0.04736	-0.4154 to 0.4907	0.8428	NS	0.1949	-0.2841 to 0.5960	0.4102	NS
CD4MRpt									
Right	34 (63.0)	-0.2781	-0.5704 to 0.07670	0.1112	NS	-0.2596	-0.5568 to 0.09650	0.1381	NS
Left	20 (37.0)	-0.473	-0.7631 to -0.02445	0.0352	NS	-0.0885	-0.5214 to 0.3806	0.7106	NS
CD8MRt									
Right	34 (63.0)	-0.2039	-0.5149 to 0.1545	0.2474	NS	-0.4422	-0.6845 to -0.1120	0.0088	< 0.01
Left	20 (37.0)	0.1135	-0.3588 to 0.5396	0.6337	NS	0.4779	0.03071 to 0.7657	0.0331	< 0.05
CD8MRpt									
Right	34 (63.0)	-0.3596	-0.6285 to -0.01393	0.0367	< 0.05	-0.2788	-0.5709 to 0.07601	0.1104	NS
Left	20 (37.0)	0.1893	-0.2894 to 0.5923	0.4241	NS	-0.03541	-0.4815 to 0.4253	0.8822	NS
LMR_b/LMRt									
Right	34 (63.0)	0.1075	-0.2492 to 0.4386	0.545	NS	0.3621	0.01678 to 0.6302	0.0353	< 0.05
Left	20 (37.0)	-0.05677	-0.4978 to 0.4076	0.8121	NS	-0.4248	-0.7366 to 0.03599	0.0619	NS
LMR_b/LMRpt									
Right	34 (63.0)	0.241	-0.1162 to 0.5430	0.1698	NS	0.2884	-0.06561 to 0.5779	0.0981	NS
Left	20 (37.0)	0.1325	-0.3420 to 0.5531	0.5778	NS	-0.0531	-0.4950 to 0.4106	0.8241	NS
$LMR_b/CD4MR_t$									
Right	34 (63.0)	0.1372	-0.2208 to 0.4626	0.4392	NS	0.2467	-0.1101 to 0.5473	0.1595	NS
Left	20 (37.0)	-0.09461	-0.5259 to 0.3754	0.6915	NS	-0.177	-0.5839 to 0.3010	0.4554	NS
$LMR_b/CD4MR_{pt}$									

Right	34 (63.0)	0.1446	-0.2136 to 0.4685	0.4146	NS	0.189	-0.1695 to 0.5034	0.2843	NS
Left	20 (37.0)	0.4541	0.0003499 to 0.7528	0.0443	< 0.05	0.1062	-0.3653 to 0.5343	0.6559	NS
$LMR_b/CD8MR_t$									
Right	34 (63.0)	0.04078	-0.3111 to 0.3828	0.8189	NS	0.3364	-0.01245 to 0.6123	0.0517	NS
Left	20 (37.0)	-0.03784	-0.4834 to 0.4233	0.8741	NS	-0.4071	-0.7267 to 0.05735	0.0748	NS
$LMR_b/CD8MR_{pt}$									
Right	34 (63.0)	0.1409	-0.2172 to 0.4655	0.4267	NS	0.1859	-0.1727 to 0.5009	0.2926	NS
Left	20 (37.0)	-0.1703	-0.5794 to 0.3073	0.4729	NS	-0.1416	-0.5595 to 0.3337	0.5515	NS

OS: Overall survival; RFS: Recurrence-free survival; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocytes ratio; PLR: Platelet-to-lymphocyte ratio; b: Blood; t: Tumour; pt: Peritumour; CD4MR: $CD4^+$-lymphocyte-to-monocyte ratio; CD8MR: $CD8^+$-lymphocyte-to-monocyte ratio; CI: Confidence interval; NS: Not significant.

Survival prognostic value of the studied variables and ratios

Taking into account all previous correlations, we then calculated the optimal cutoff values by ROC analyses for those variables significantly correlated with OS or RFS, using respectively cancer-specific death or relapse as the endpoints for both RCRC (Figures 4 and 6) and LCRC (Figure 5) patients after surgical intervention.

Regarding OS, ROC curve analysis of $CD8_{pt}$ (Figure 4A; AUC = 0.585, 95%CI: 0.376-0.793, P = 0.496) identified the optimal cutoff point at 8.250, which entails significantly worse outcomes for RCRC patients ranking below this (Figure 4B; 100% *vs* 48.2%, P = 0.0077). $CD4CD8_{pt}$ analysis (Figure 4C; AUC = 0.550, 95%CI: 0.334-0.766, P = 0.686) identified 10.16 as the optimal cutoff, with worse outcomes for RCRC patients ranking below this (Figure 4D; 92.3% *vs* 49.1%, P = 0.0188). LMR_{pt} analysis (Figure 4E; AUC = 0.807, 95%CI: 0.641-0.973, P = 0.013) identified 3.185 as the optimal cutoff, with worse outcomes for RCRC patients ranking below this (Figure 4F; 100% *vs* 42.7%, P = 0.0028). $CD8MR_{pt}$ analysis (Figure 4G; AUC = 0.757, 95%CI: 0.600-0.914, P = 0.039) identified 1.650 as the optimal cutoff, with worse outcomes for RCRC patients ranking below this (Figure 4H; 100% *vs* 35.7%, P = 0.0007). LMR_b/LMR_{pt} analysis (Figure 4I; AUC = 0.672, 95%CI: 0.479-0.865, P = 0.166) identified 0.985 as the optimal cutoff, with worse outcomes for RCRC patients ranking above this (Figure 4J; 91.6% *vs* 52.0%, P = 0.0244). $LMR_b/CD8MR_{pt}$ analysis (Figure 4K; AUC = 0.601, 95%CI: 0.419-0.782, P = 0.418) identified 1.485 as the optimal cutoff, with worse outcomes for RCRC patients ranking above this (Figure 4L; 100% *vs* 50.9%, P = 0.0101). Finally, $LMR_b/CD4MR_{pt}$ analysis (Figure 5A; AUC = 0.786, 95%CI: 0.564-1.000, P = 0.048) identified 10.57 as the optimal cutoff, with worse outcomes for LCRC patients ranking above this (Figure 5B; 66.6% *vs* 18.7%, P = 0.0416). In addition, ROC curve analyses (Supplementary Figure 3) of $CD4CD8_t$, PLR_b, $CD4CD8_{pt}$, and $CD8MR_t$, though they showed significant AUC (0.524, 0.619, 0.726 and 0.571, respectively), rendered optimal cutoff values with no significant differences for LCRC survival.

With respect to RFS, ROC curve analysis of LMR_{pt} (Figure 6A; AUC = 0.737, 95%CI: 0.554-0.920, P = 0.027) identified 3.185 as the optimal cutoff, with worse outcomes for RCRC patients ranking below this (Figure 6B; 92.3% *vs* 32.5%, P = 0.0046). LMR_b/LMR_{pt} analysis (Figure 6C; AUC = 0.678, 95%CI: 0.499-0.857, P = 0.098) identified 0.985 as the optimal cutoff, with worse outcomes for RCRC patients ranking above this (Figure 6D; 84.4% *vs* 40.6%, P = 0.0155). $LMR_b/CD8MR_{pt}$ analysis (Figure 6E; AUC = 0.615, 95%CI: 0.427-0.802, P = 0.286) identified 1.485 as the optimal cutoff, with worse outcomes for RCRC patients ranking above this (Figure 6F; 91.7% *vs* 40.7%, P = 0.0141). The ROC analyses in RCRC patients of $CD8MR_{pt}$, $CD8_{pt}$, and $CD4CD8_{pt}$ (Supplementary Figure 4), though they showed significant AUC (0.672, 0.528 and 0.506, respectively) did not render optimal cutoff values that significantly prognosticated the RCRC patient RFS. Similarly, ROC analyses of $CD4CD8_t$, PLR_b, $CD4CD8_{pt}$, $CD8MR_t$, and $LMR_b/CD4MR_{pt}$ (Supplementary Figure 5) with significant AUC (0.656, 0.635, 0.760, 0.781 and 0.563, respectively) did not provide optimal cutoff values with significant prognostication in LCRC patient RFS.

Table 3 Univariate and multivariate analyses for prognostic variables of overall survival after surgical interventions of right-sided colorectal cancer and left-sided colorectal cancer

	Total patients	Univariate analysis					Multivariate analysis				
	n (%)	HR	95%CI		*P*		HR	95%CI		*P*	
Variables			Low	High				Low	High		
LMR_b											
Right	34 (63.0)	0.565	0.325	0.982	0.043	< 0.05	0.133	0.000	71.041	0.529	
Left	20 (37.0)	1.141	0.867	1.502	0.346		1.141	0.867	1.502	0.346	
NLR_b											
Right	34 (63.0)	1.416	1.019	1.967	0.038	< 0.05	1.416	1.019	1.967	0.038	< 0.05
Left	20 (37.0)	1.043	0.944	1.152	0.410		1.126	0.987	1.284	0.078	
PLR_b											
Right	34 (63.0)	1.005	1.000	1.011	0.064		0.989	0.719	1.361	0.946	
Left	20 (37.0)	1.001	0.999	1.004	0.292		0.997	0.937	1.060	0.912	
$CD4_t$											
Right	34 (63.0)	0.923	0.638	1.334	0.670						
Left	20 (37.0)	0.843	0.493	1.439	0.531						
$CD4_{pt}$											
Right	34 (63.0)	0.959	0.817	1.124	0.604						
Left	20 (37.0)	0.813	0.454	1.456	0.487						
$CD8_t$											
Right	34 (63.0)	0.995	0.897	1.105	0.931		1.032	0.913	1.167	0.611	
Left	20 (37.0)	0.954	0.860	1.057	0.364						
$CD8_{pt}$											
Right	34 (63.0)	0.792	0.617	1.016	0.066		0.800	0.636	1.007	0.058	
Left	20 (37.0)	1.018	0.957	1.083	0.570		1.033	0.962	1.110	0.372	
$CD4CD8_t$											
Right	34 (63.0)	0.987	0.892	1.093	0.806						
Left	20 (37.0)	0.937	0.833	1.054	0.277						
$CD4CD8_{pt}$											
Right	34 (63.0)	0.891	0.761	1.043	0.150		1.073	0.821	1.401	0.606	
Left	20 (37.0)	1.015	0.953	1.082	0.641						
$CD14_t$											
Right	34 (63.0)	1.048	0.845	1.300	0.670		1.077	0.836	1.388	0.565	
Left	20 (37.0)	0.979	0.703	1.362	0.898						
$CD14_{pt}$											
Right	34 (63.0)	1.113	0.889	1.394	0.351		1.342	0.987	1.826	0.060	
Left	20 (37.0)	1.053	0.723	1.533	0.788		0.700	0.381	1.286	0.250	
LMR_t											
Right	34 (63.0)	0.635	0.365	1.103	0.107						
Left	20 (37.0)	1.000	0.908	1.101	0.997		0.976	0.555	1.716	0.933	
LMR_{pt}											
Right	34 (63.0)	0.416	0.194	0.889	0.024	< 0.05					

Left	20 (37.0)	1.030	0.712	1.490	0.876		0.031	0.000	4.228	0.166	
$CD4MR_t$											
Right	34 (63.0)	0.270	0.039	1.850	0.182						
Left	20 (37.0)	0.759	0.146	3.954	0.743						
$CD4MR_{pt}$											
Right	34 (63.0)	0.431	0.112	1.660	0.221						
Left	20 (37.0)	0.135	0.004	4.148	0.252						
$CD8MR_t$											
Right	34 (63.0)	0.712	0.364	1.394	0.321						
Left	20 (37.0)	1.001	0.910	1.100	0.986						
$CD8MR_{pt}$											
Right	34 (63.0)	0.223	0.057	0.872	0.031	< 0.05	0.024	0.001	0.430	0.011	< 0.05
Left	20 (37.0)	1.078	0.775	1.500	0.654						
LMR_b/LMR_t											
Right	34 (63.0)	0.957	0.603	1.519	0.853						
Left	20 (37.0)	1.163	0.935	1.446	0.175						
LMR_b/LMR_{pt}											
Right	34 (63.0)	1.253	0.824	1.907	0.292						
Left	20 (37.0)	1.196	0.845	1.695	0.313						
$LMR_b/CD4MR_t$											
Right	34 (63.0)	1.009	0.950	1.073	0.767		1.282	0.931	1.765	0.128	
Left	20 (37.0)	1.027	0.961	1.099	0.428						
$LMR_b/CD4MR_{pt}$											
Right	34 (63.0)	1.028	0.926	1.142	0.600		1.291	0.447	3.728	0.636	
Left	20 (37.0)	1.097	1.011	1.190	0.026	< 0.05	0.991	0.420	2.341	0.984	
$LMR_b/CD8MR_t$											
Right	34 (63.0)	0.973	0.747	1.269	0.842						
Left	20 (37.0)	1.103	0.940	1.294	0.229		1.971	0.258	15.069	0.513	
$LMR_b/CD8MR_{pt}$											
Right	34 (63.0)	1.053	0.880	1.260	0.572		0.484	0.268	0.873	0.016	< 0.05
Left	20 (37.0)	1.026	0.834	1.262	0.812		0.952	0.009	96.530	0.983	

LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocytes ratio; PLR: Platelet-to-lymphocyte ratio; HR: Hazard ratio; CI: Confidence interval; b: Blood; t: Tumour; pt: Peritumour; CD4MR: $CD4^+$-lymphocyte-to-monocyte ratio; CD8MR: $CD8^+$-lymphocyte-to-monocyte ratio.

Nomograms modelling and validation

In order to avoid conflicts in handling the different values of the predictive indexes for RCRC patients, clinician-friendly nomograms were developed for both OS (Figure 7A) and RFS (Figure 7B) of these patients. The six significant predictive variables found for OS and the three found for RFS were used to construct the respective nomograms, with data from the training set of RCRC patients. The calibration of these nomograms revealed C-indexes of 0.600 (95%CI: 0.561-0.639) and 0.605 (95%CI: 0.579-0.631), respectively (Supplementary Figure 6A-B). Moreover, the reliability of the nomograms was evaluated with the validation set of RCRC patients, showing a moderate accuracy, with C-indexes of 0.500 (95%CI: 0.475-0.525) and 0.570 (95%CI: 0.541-0.599) for OS and RFS, respectively (Supplementary Figure 6C-D).

Table 4 Univariate and multivariate analyses for prognostic variables of recurrence-free survival after surgical interventions of right-sided colorectal cancer and left-sided colorectal cancer

	Total patients	Univariate analysis					Multivariate analysis				
	n (%)	HR	95%CI		*P*		HR	95%CI		*P*	
Variables			Low	High				Low	High		
LMR_b											
Right	34 (63.0)	0.865	0.593	1.262	0.453						
Left	20 (37.0)	0.977	0.770	1.239	0.848		0.156	0.001	24.118	0.470	
NLR_b											
Right	34 (63.0)	1.135	0.841	1.532	0.407		2.760	1.050	7.254	0.039	< 0.05
Left	20 (37.0)	1.094	1.094	1.180	0.020	< 0.05	1.156	1.038	1.288	0.009	< 0.01
PLR_b											
Right	34 (63.0)	1.001	0.996	1.007	0.596		0.978	0.958	0.999	0.037	< 0.05
Left	20 (37.0)	1.002	1.000	1.004	0.018	< 0.05	0.987	0.954	1.022	0.468	
$CD4_t$											
Right	34 (63.0)	1.010	0.864	1.181	0.902		0.802	0.457	1.407	0.441	
Left	20 (37.0)	0.981	0.617	1.559	0.934						
$CD4_{pt}$											
Right	34 (63.0)	0.963	0.851	1.091	0.556		1.486	0.124	17.828	0.755	
Left	20 (37.0)	1.120	0.795	1.577	0.516						
$CD8_t$											
Right	34 (63.0)	0.962	0.872	1.062	0.446		1.821	0.451	7.347	0.400	
Left	20 (37.0)	1.032	0.971	1.097	0.310						
$CD8_{pt}$											
Right	34 (63.0)	0.906	0.780	1.053	0.199		1.117	0.848	1.472	0.431	
Left	20 (37.0)	1.050	0.988	1.115	0.116		1.098	1.015	1.189	0.020	< 0.05
$CD4CD8_t$											
Right	34 (63.0)	0.972	0.893	1.058	0.515						
Left	20 (37.0)	1.033	0.970	1.100	0.307		0.436	0.198	0.960	0.039	< 0.05
$CD4CD8_{pt}$											
Right	34 (63.0)	0.944	0.857	1.041	0.248						
Left	20 (37.0)	1.052	0.992	1.117	0.093		0.008	0.000	1.071	0.053	
$CD14_t$											
Right	34 (63.0)	1.123	0.949	1.329	0.178		1.467	1.048	2.054	0.026	< 0.05
Left	20 (37.0)	0.918	0.713	1.180	0.502						
$CD14_{pt}$											
Right	34 (63.0)	1.075	0.891	1.297	0.449		1.790	0.592	5.406	0.302	
Left	20 (37.0)	1.472	1.095	1.978	0.010	< 0.05					
LMR_t											
Right	34 (63.0)	0.555	0.337	0.915	0.021	< 0.05	0.641	0.213	1.926	0.428	
Left	20 (37.0)	1.084	0.999	1.176	0.052		0.165	0.003	9.203	0.380	
LMR_{pt}											
Right	34 (63.0)	0.691	0.458	1.042	0.078		0.312	0.123	0.793	0.014	< 0.05

Left	20 (37.0)	1.066	0.688	1.653	0.775					
$CD4MR_t$										
Right	34 (63.0)	0.588	0.220	1.572	0.290		0.876	0.382	2.010	0.756	
Left	20 (37.0)	0.950	0.381	2.370	0.912					
$CD4MR_{pt}$										
Right	34 (63.0)	0.734	0.327	1.648	0.454		7.229	0.515	101.544	0.142	
Left	20 (37.0)	0.583	0.199	1.709	0.325					
$CD8MR_t$										
Right	34 (63.0)	0.497	0.246	1.005	0.052					
Left	20 (37.0)	1.083	0.999	1.174	0.052		1.123	1.020	1.238	0.019	< 0.05
$CD8MR_{pt}$										
Right	34 (63.0)	0.584	0.333	1.023	0.060					
Left	20 (37.0)	1.191	0.815	1.741	0.365		0.293	0.026	3.345	0.323	
$LMRb/LMR_t$										
Right	34 (63.0)	1.311	1.013	1.697	0.040	< 0.05					
Left	20 (37.0)	0.958	0.730	1.258	0.760					
$LMRb/LMR_{pt}$										
Right	34 (63.0)	1.248	0.887	1.756	0.203		0.404	0.169	0.969	0.042	< 0.05
Left	20 (37.0)	1.030	0.787	1.347	0.830		1.132	0.859	1.493	0.378	
$LMRb/CD4MR_t$										
Right	34 (63.0)	1.012	0.965	1.060	0.632		1.056	0.973	1.146	0.192	
Left	20 (37.0)	0.991	0.937	1.048	0.742					
$LMRb/CD4MR_{pt}$										
Right	34 (63.0)	1.031	0.948	1.121	0.479		1.393	0.875	2.220	0.163	
Left	20 (37.0)	1.023	0.969	1.079	0.412		0.925	0.847	1.011	0.087	
$LMRb/CD8MR_t$										
Right	34 (63.0)	1.146	1.017	1.292	0.025	< 0.05	1.301	1.078	1.571	0.006	< 0.01
Left	20 (37.0)	0.941	0.775	1.143	0.542		1.036	0.591	1.816	0.903	
$LMRb/CD8MR_{pt}$										
Right	34 (63.0)	1.022	0.894	1.169	0.746		1.390	0.304	6.350	0.671	
Left	20 (37.0)	0.968	0.844	1.109	0.638		0.847	0.576	1.244	0.397	

LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocytes ratio; PLR: Platelet-to-lymphocyte ratio; HR: Hazard ratio; CI: Confidence interval; b: Blood; t: Tumour; pt: Peritumour; CD4MR: $CD4^+$-lymphocyte-to-monocyte ratio; CD8MR: $CD8^+$-lymphocyte-to-monocyte ratio.

DISCUSSION

The segment of the large intestine proximal to the splenic flexure, *i.e.* the right colon (comprising caecum, ascending colon, and proximal two-thirds of the transverse colon), derives from the embryonic midgut; whereas the left colon (comprising the distal third part of the transverse colon and descending and sigmoid colon) derives from the embryonic hindgut[21]. Distinct embryologic origin of right and left sides of the colon markedly determines important physiological differences, mainly: cell motility, vasculature, lymphatic drainage, extrinsic innervation, development of the endocrine components, and the expression and patterns of epigenetic marks of crucial molecular factors for cell development[21,22].

Since seminal contributions by Bufill *et al*[23], an increasing number of studies have supported the hypothesis that these differences in origin may explain why RCRC and LCRC constitute two distinct clinical entities, which arise through different

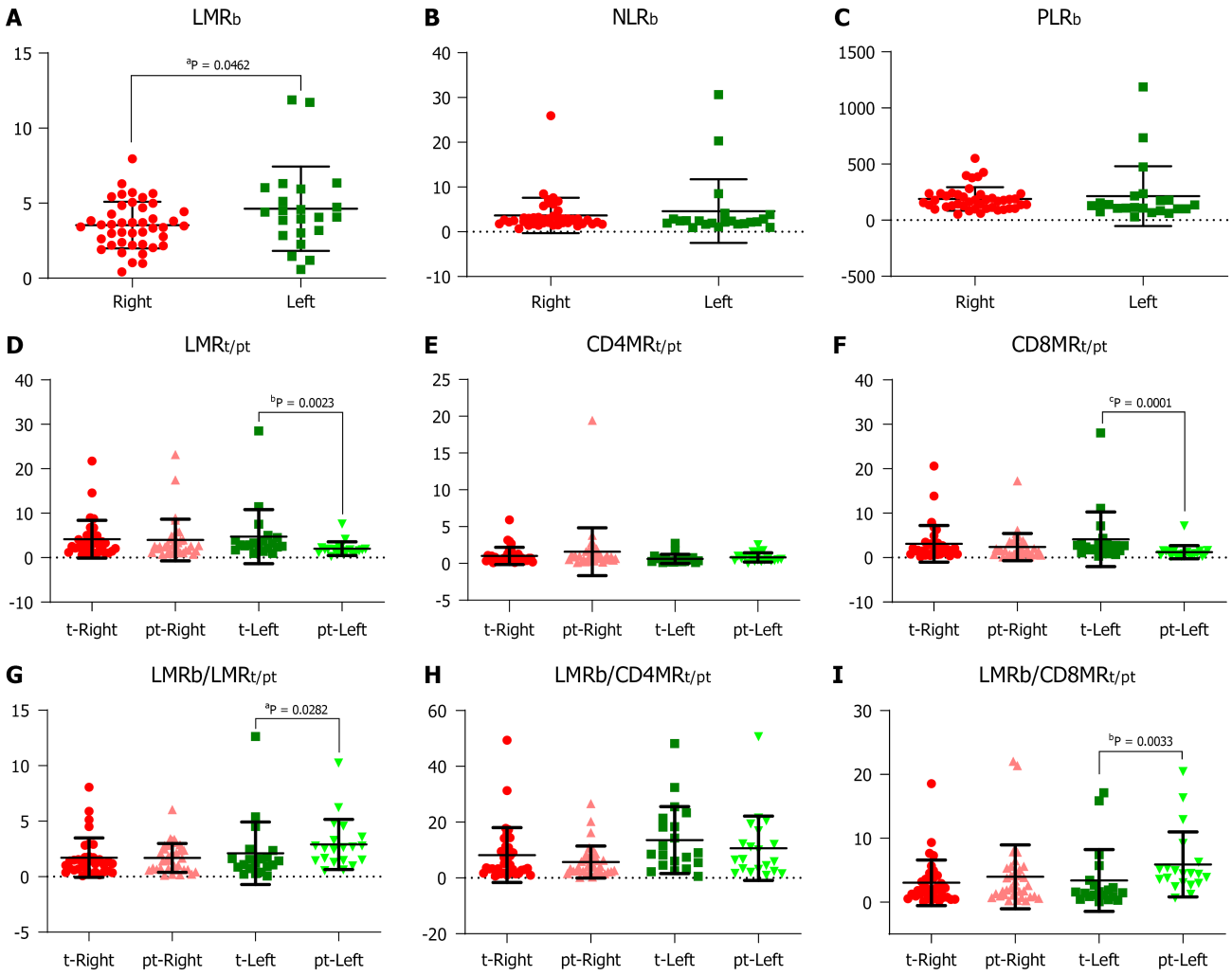


Figure 2 Leukocyte ratios in peripheral blood and tissues from colorectal cancer patients. A-C: Blood circulating leukocytes in right-sided colorectal cancer (CRC) patients (orange, n = 43) and left-sided CRC patients (green, n = 22) represented as lymphocyte-to-monocyte ratio $(LMR)_{blood\ (b)}$ (A), neutrophil-to-lymphocyte ratio (B), and platelet-to-lymphocyte ratio (C) ($^aP < 0.05$, unpaired t test, data are mean ± standard deviation); D-F: Tissue-infiltrated leukocytes in right-sided CRC tumours (tumour [t], orange, n = 34) and peritumours (peritumour [pt], light red, n = 34) and left-sided CRC tumours (t, green, n = 20) and peritumours (pt, light green, n = 20), represented as $LMR_{t/pt}$ (D), $CD4^+$-lymphocyte-to-monocyte ratio $(CD4MR)_{t/pt}$ (E), and $CD8^+$-lymphocyte-to-monocyte ratio $(CD8MR)_{t/pt}$ (F) ($^aP < 0.05$, $^bP < 0.01$, unpaired Mann-Whitney U test, data are mean ± standard deviation); G-I: Blood-to-tissue leukocyte ratios for right-sided CRC tumours (t, orange, n = 34) and peritumours (pt, light red, n = 34) and left-sided CRC tumours (t, green, n = 20) and peritumours (pt, light green, n = 20) represented as $LMR_b/LMR_{t/pt}$ (G), $LMR_b/CD4MR_{t/pt}$ (H), and $LMR_b/CD8MR_{t/pt}$ (I) ($^aP < 0.05$, $^bP < 0.01$, unpaired Mann-Whitney U test, data are mean ± standard deviation). PLR: Platelet-to-lymphocyte ratio.

pathogenetic mechanisms[22,24,25]. Thus, differential aspects such as incidence, presentation, microbiome composition, genetic burden, or immunogenicity could be explained on these grounds[26-31]. In a large study with more than 17000 CRC patients, Benedix *et al*[32] showed that RCRC represents a more distinct tumour entity than LCRC, mainly because of its higher incidence in women and older people, poor differentiation, locally advanced carcinomas, a distinct pattern of metastatic spread, and worse outcome.

Likewise, survival after surgical intervention to remove the tumour should constitute a prominent feature to differentiate both pathologies. In this line, controversial results arise throughout the literature. Thereby, some studies support RCRC patients having poorer overall and disease-free survival rates[8], whilst others call attention to the stage of the disease, with better rates for RCRC being limited to stage II and better rates for LCRC being limited to stage III[33]. In our cohort, perhaps due to the stage's heterogeneity of the patients, both OS and RFS were found side-dependent, with better outcomes in RCRC patients, reinforcing the idea that prognostic markers for the two pathologies should be studied separately.

A number of studies have stressed the importance of the systemic inflammatory response in CRC development and the search for variables involving its components as a valuable tool to drive prognosis[15,34]. Important prognostic records have been

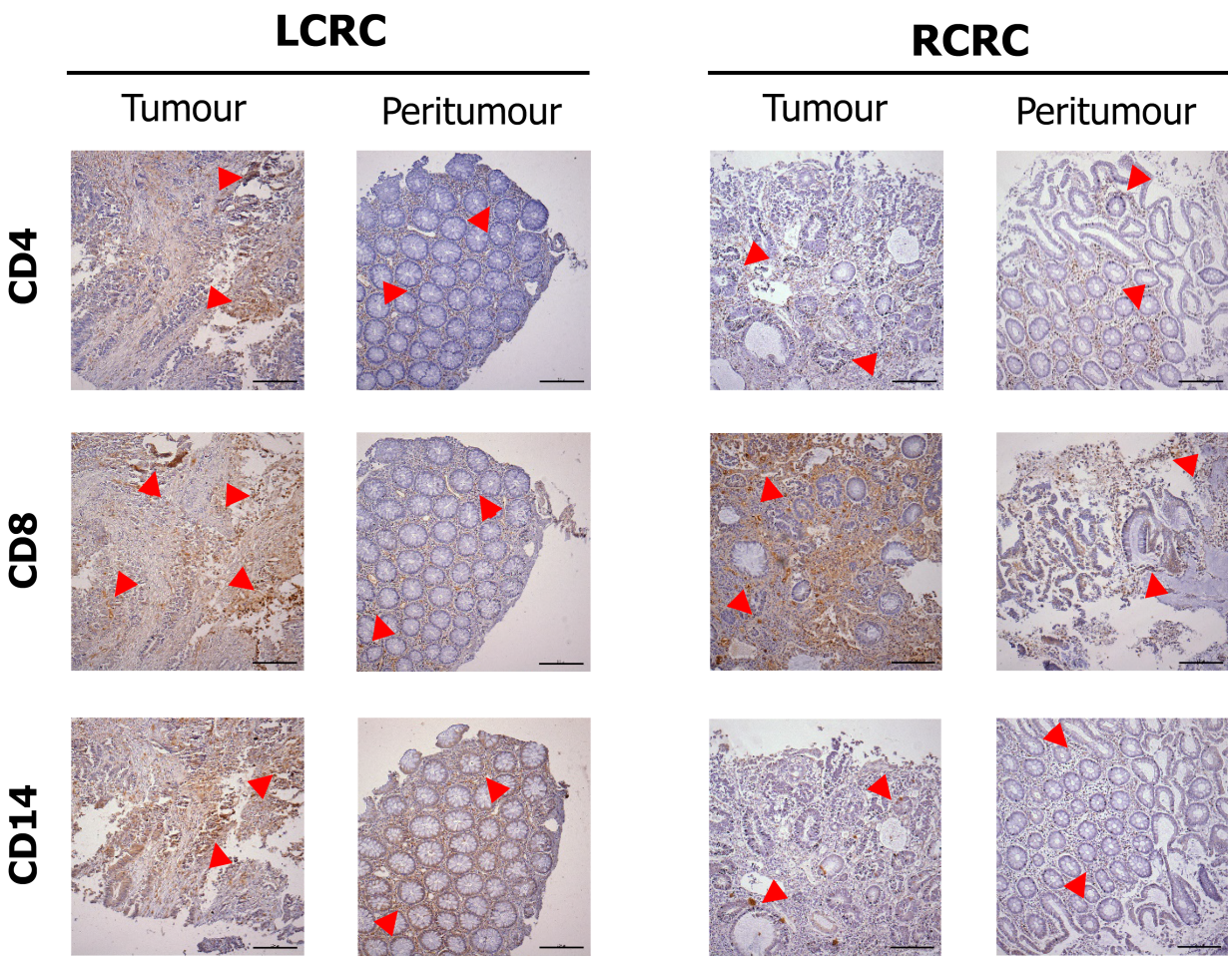
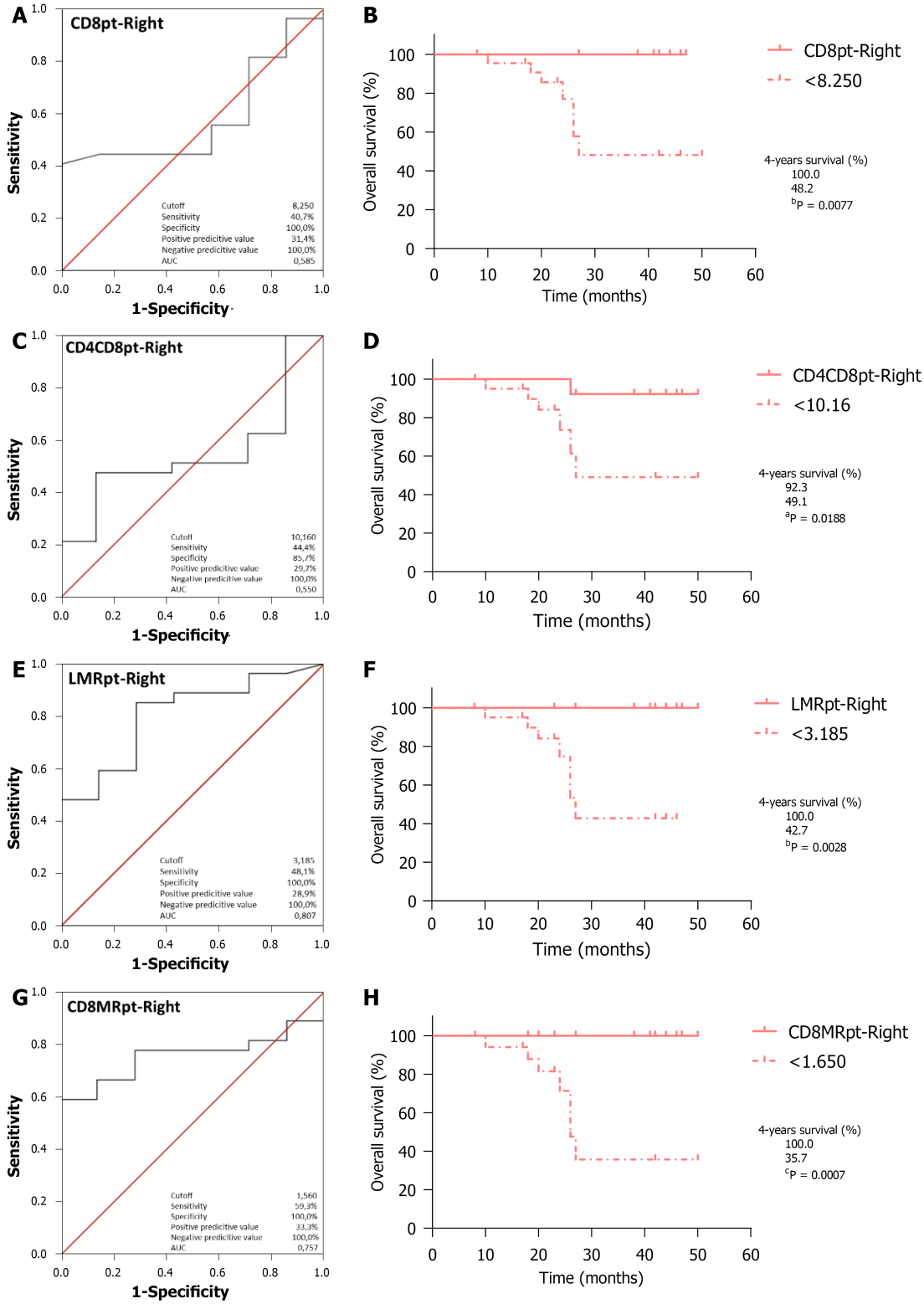


Figure 3 Leukocyte infiltration in tissues from right-sided colorectal cancer and left-sided colorectal cancer patients. Representative immunohistochemical images (× 100, scale bar = 200 μm) of $CD4^+$ lymphocytes, $CD8^+$ lymphocytes and $CD14^+$ monocytes in tumour and peritumour samples, from one left-sided colorectal cancer (LCRC) patient (left panel) and one right-sided colorectal cancer (RCRC) patient (right panel). Arrows show rich-marker zones in each sample.

obtained in several research works[16,35], which avail the use of blood leukocyte ratios as predictors in CRC progression after surgery. However, some studies have highlighted inherent failures to these analyses. Thus, Zhang *et al*[36] warn against the impact of the use of distinct factors, within different studies, to adjust possible confounders for multivariate hazard ratio determination, which can make the latter at risk of bias and heterogeneity, in turn making LMR fail to reach significance in survival. Likewise, sample size, race heterogeneity, and most of all the pre/post-operative dynamic changes in circulating leukocyte population can dramatically affect the observable effects of these variables in the multivariate models for survival progression[37]. In our correlative analyses, though all preoperative blood leukocyte ratios significantly rose at different stages, in the end we were unable to establish a predictor value for any of them, neither for RCRC nor for LCRC survival, perhaps due to a conjunction of previously discussed handicaps. Nonetheless, we do not discard the possibility for them to emerge as good predictors in the putative case those handicaps could be solved, thus improving the multivariate analyses.

Notably, we report tissue leukocyte ratios, both alone and combined with preoperative blood LMR_b, as six variables with a strong predictor value for RCRC overall survival ($CD8_{pt}$, $CD4CD8_{pt}$, LMR_{pt}, $CD8MR_{pt}$, LMR_b/LMR_{pt}, $LMR_b/CD8MR_{pt}$), three variables for recurrence-free survival (LMR_{pt}, $CD8MR_{pt}$, LMR_b/LMR_{pt}, $LMR_b/CD8MR_{pt}$), and another robust variable to predict LCRC overall survival ($LMR_b/CD4MR_{pt}$). In addition, to avoid conflicts when interpreting the different survival predictors of RCRC, physician-friendly nomograms are proposed for both OS and RFS. Albeit much effort has been made in describing and associating the leukocyte content of tumour tissues with CRC survival[38], most studies have been performed on disaggregated tumour and peritumour samples, and only a few of them have attempted to measure leukocyte expression in fixed samples of these tissues to associate them with circulating ratios[19] or to correlate them with patient survival[18,


A
CD8pt-Right
Sensitivity
1-Specificity
Cutoff 8,250
Sensitivity 40,7%
Specificity 100,0%
Positive predicitive value 31,4%
Negative predicitive value 100,0%
AUC 0,585
B
Overall survival (%)
Time (months)
CD8pt-Right
<8.250
4-years survival (%)
100.0
48.2
bP = 0.0077
C
CD4CD8pt-Right
Cutoff 10,160
Sensitivity 44,4%
Specificity 85,7%
Positive predicitive value 29,7%
Negative predicitive value 100,0%
AUC 0,550
D
CD4CD8pt-Right
<10.16
4-years survival (%)
92.3
49.1
aP = 0.0188
E
LMRpt-Right
Cutoff 3,185
Sensitivity 48,1%
Specificity 100,0%
Positive predicitive value 28,9%
Negative predicitive value 100,0%
AUC 0,807
F
LMRpt-Right
<3.185
4-years survival (%)
100.0
42.7
bP = 0.0028
G
CD8MRpt-Right
Cutoff 1,560
Sensitivity 59,3%
Specificity 100,0%
Positive predicitive value 33,3%
Negative predicitive value 100,0%
AUC 0,757
H
CD8MRpt-Right
<1.650
4-years survival (%)
100.0
35.7
cP = 0.0007

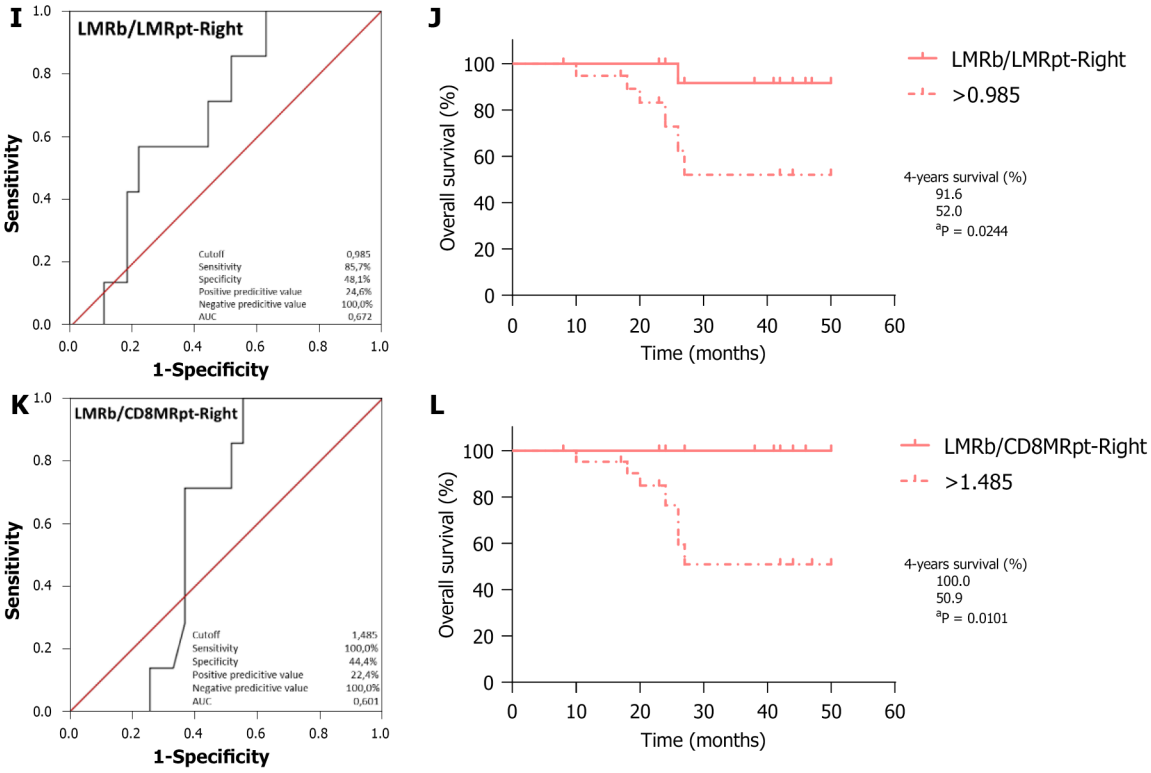


Figure 4 Receiver operating curve analyses for overall survival and Kaplan-Meier curves for optimal cutoff values in right-sided colorectal cancer patients for significant predictors. A-B: $CD8^+$-lymphocyte $(CD8)_{peritumour\ (pt)}$, worse below 8.25; C-D: $CD4^+$ plus $CD8^+$-lymphocyte $(CD4CD8)_{pt}$ worse below 10.16; E-F: Lymphocyte-to-monocyte ratio $(LMR)_{pt}$ worse below 3.185; G-H: $CD8^+$-lymphocyte-to-monocyte ratio $(CD8MR)_{pt}$ worse below 1.65; I-J: LMR_b/LMR_{pt} worse above 0.985; K-L: $LMR_{blood\ (b)}$/ $CD8^+$-lymphocyte-to-monocyte $ratio_{pt}$ worse above 1.485; survival proportions at 26 mo after surgery (median follow-up) are shown ($^aP < 0.05$, $^bP < 0.01$, log-rank test).

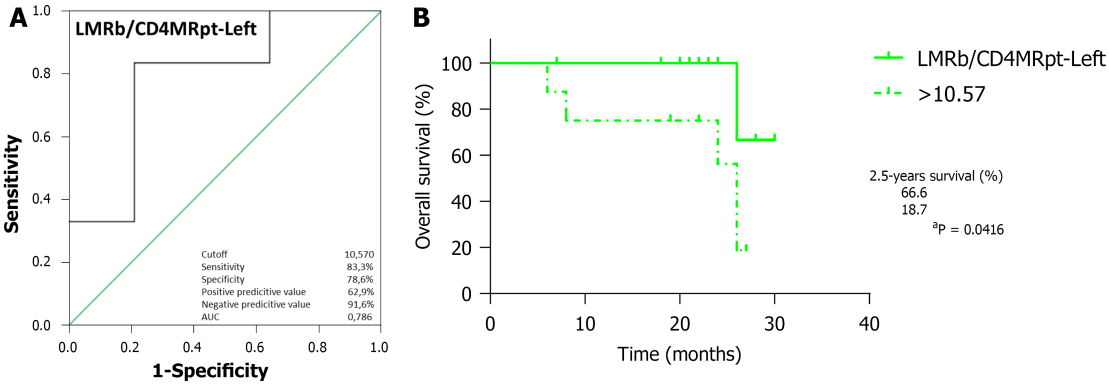


Figure 5 Receiver operating curve analysis for overall survival (A) and Kaplan-Meier curve (B) for optimal cutoff value in left-sided colorectal cancer patients for the significant predictor blood lymphocyte-to-monocyte ratio/peritumour $CD4^+$-lymphocyte-to-monocyte ratio. Worse above 10.57; survival proportions at 26 mo after surgery (median follow-up) are shown ($^aP < 0.05$, log-rank test). b: Blood; CD4MR: $CD4^+$-lymphocyte-to-monocyte ratio; LMR: Lymphocyte-to-monocyte ratio; pt: Peritumour.

39]. Hence, this could be the first study in which leukocyte measures in both blood and fixed tissues are put together into predictor indexes for CRC survival.

It is worth noting that, in addition to the well-established predictor value of blood leukocyte ratios, the 10 indexes involve leukocyte concentrations in peritumoural zones of the bowel but not in the tumour mass. A peritumour constitutes an easily obtainable tissue during a preoperative exploration of the patient (this could be the colonoscopy), which might be safely biopsied without affecting the tumour environment in an adenoma-like surgical extraction protocol. Therefore, on a routine basis, surgeons might access both preoperative peripheral blood parameters as well as non-neoplastic peritumoural tissue (without disturbing the tumour itself) and make use of the described ratios and nomograms to predict the patient's outcome after

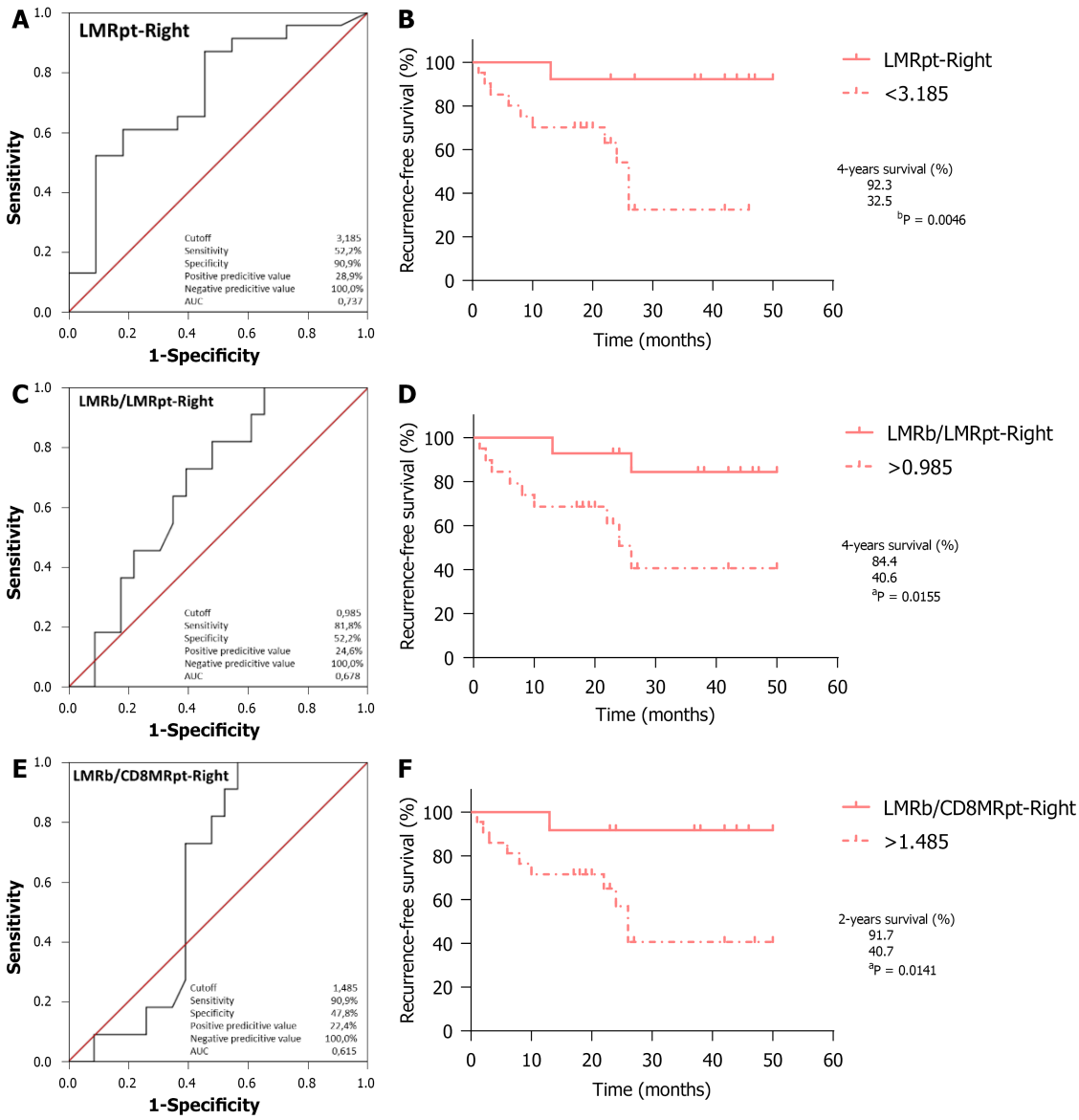


Figure 6 Receiver operating curve analyses for recurrence-free survival and Kaplan-Meier curves for optimal cutoff values in right-sided colorectal cancer patients for significant predictors. A-B: Peritumour lymphocyte-to-monocyte ratio (LMR) worse below 3.185; C-D: Blood (b) LMR/peritumour (pt) LMR worse above 0.985; E-F: Blood LMR/peritumour CD8$^+$-lymphocyte-to-monocyte ratio (CD8MR) worse above 1.485; survival proportions at 26 mo after surgery (median follow-up) are shown ($^aP < 0.05$, $^bP < 0.01$, log-rank test).

surgery. Thus, *ad hoc* surgical strategies can be designed to allow physicians to continue with surgery as programmed or delay the intervention until better scores are achieved after personalised treatments to correct the leukocyte levels in the patient.

Altogether, these indexes could be implemented in the first line of prognosis, making it easier to predict the outcome of patients after surgery depending on the tumour location and leukocyte distribution in both peripheral blood and biopsies of the peritumoural region.

Limitations

Our study is mainly limited by the cohort size. It might be expected that the extension of these variables to a greater cohort would reinforce our conclusions or even make foregoing unobserved interactions surface.

CONCLUSION

Herein we present important remarks on the value of combining circulating leukocyte ratios and tissue infiltrated leukocyte ratios on the sustaining of valuable prognosis tools for physicians in order to stratify patients regarding their putative outcome. In

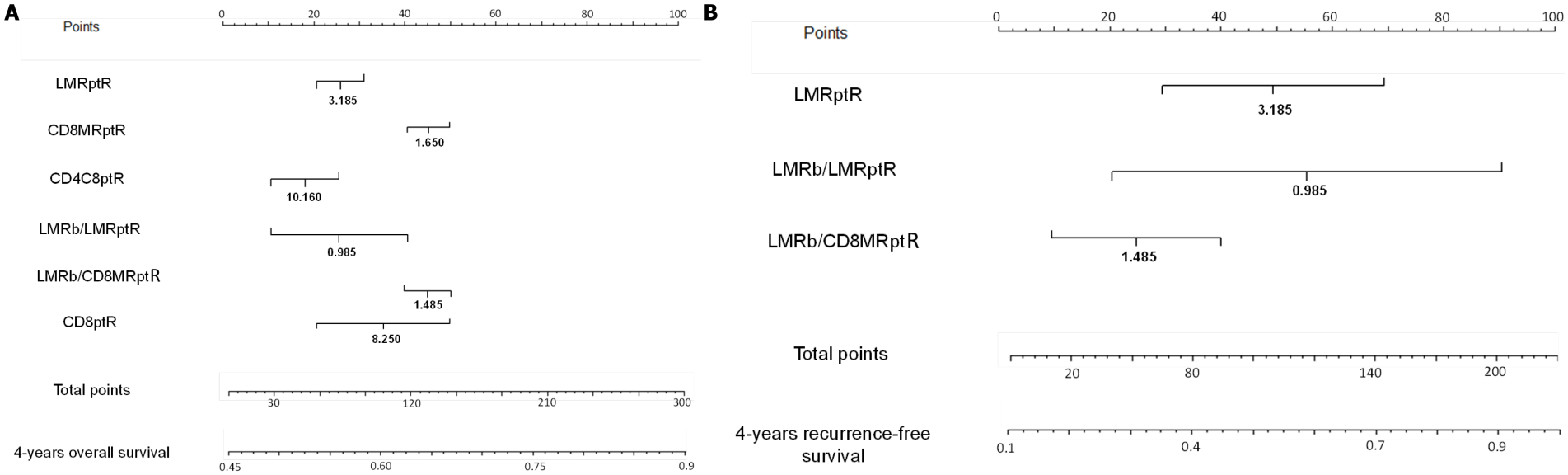


Figure 7 Nomograms for predicting overall survival and recurrence-free survival after surgical intervention of right-sided colorectal cancer patients. A: The 4-year probability of overall survival was estimated by summing the scores of peritumour (pt) lymphocyte-to-monocyte ratio (LMR), $CD8^+$-lymphocyte (CD8)$^+$-lymphocyte-to-monocyte ratio $(CD8MR)_{pt}$, $CD4^+$ plus $CD8^+$-lymphocyte $(CD4CD8)_{pt}$, blood (b) LMR/LMR_{pt}, $LMR_b/CD8MR_{pt}$, and $CD8_{pt}$; B: The 4-year probability of recurrence-free survival was estimated by summing the scores of LMR_{pt}, LMR_b/LMR_{pt}, and $LMR_b/CD8^+$-lymphocyte-to-monocyte $ratio_{pt}$. For each graph, locate the patient's values for each variable at one of the extremes of its corresponding axis, taking into account the correct position with respect to the optimal cutoff that is indicated; values higher than the cutoff go to the upper end and values lower than the cutoff go to the lower end. Then, draw a line straight upwards to the "Points" axis to determine the score associated to each variable. Add up all the scores, locate this sum in the "Total points" axis and draw a line straight down to the lowest axes of "4-year overall survival" or "4-year recurrence-free survival" to find the predictive probability of the patient for overall survival or recurrence-free survival outcome, respectively.

the era of personalised medicine, such indexes will provide benefits to improving both resources and well-being of CRC patients after surgery.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) points to 9.4% of cancer deaths worldwide, ranking second after lung cancer. Despite the wide variety of factors tested to predict their outcome, most patients with similar variables show big differences in survival. Moreover, right-sided CRC (RCRC) and left-sided CRC (LCRC) patients exhibit large differences in outcome after surgical intervention as assessed by preoperative blood leukocyte ratios [today, the most extended parameters used to assess a patient's overall survival (OS) and recurrence-free survival (RFS) after surgery]. However, few efforts have been

made to link tumour infiltrated leukocyte ratios to patient outcomes.

Research motivation

To determine whether both RCRC and LCRC patient outcomes could be accurately predicted based on the counting of infiltrated leukocytes in tumour and peritumoural tissues.

Research objectives

The aim of this study was to find stronger indexes than circulating (blood) leukocyte ratios to predict RCRC and LCRC patient outcomes.

Research methods

A prospective study was performed with CRC patients who had undergone surgical intervention to resect the tumours. Leukocyte concentrations in peripheral blood, tumour, and non-neoplastic peritumoural tissues were determined. Ratios of these parameters were evaluated as predictors for OS and RFS using Spearman correlations, Cox univariate and multivariate proportional hazards regression followed by the calculation of the receiver-operating characteristic and area under the curve (AUC) and the determination of Youden's optimal cutoff values for those variables that significantly correlated with either RCRC or LCRC patient outcomes. Clinician-friendly nomograms were developed to predict OS and RFS from the prediction indexes. The accuracy of the model was evaluated using calibration and validation analyses.

Research results

We obtained six robust predictors of worse OS in RCRC: $CD8^+$ lymphocyte content in peritumour ($CD8_{pt}$, AUC = 0.585, cutoff < 8.250, P = 0.0077), total lymphocyte content in peritumour ($CD4CD8_{pt}$, AUC = 0.550, cutoff < 10.160, P = 0.0188), lymphocyte-to-monocyte ratio in peritumour (LMR_{pt}, AUC = 0.807, cutoff < 3.185, P = 0.0028), $CD8^+$ LMR in peritumour ($CD8MR_{pt}$, AUC = 0.757, cutoff < 1.650, P = 0.0007), the ratio of blood LMR to LMR in peritumour (LMR_b/LMR_{pt}, AUC = 0.672, cutoff > 0.985, P = 0.0244), and the ratio of blood LMR to $CD8^+$ LMR in peritumour ($LMR_b/CD8MR_{pt}$, AUC = 0.601, cutoff > 1.485, P = 0.0101). In addition, three robust predictors of worse RFS in RCRC were found: LMR_{pt} (AUC = 0.737, cutoff < 3.185, P = 0.0046), LMR_b/LMR_{pt} (AUC = 0.678, cutoff > 0.985, P = 0.0155), and $LMR_b/CD8MR_{pt}$ (AUC = 0.615, cutoff > 1.485, P = 0.0141). Furthermore, the ratio of blood LMR to $CD4^+$ LMR in peritumour ($LMR_b/CD4MR_{pt}$, AUC = 0.786, cutoff > 10.570, P = 0.0416) was found to robustly predict poorer OS in LCRC patients. The developed nomograms to predict OS and RFS of RCRC patients showed C-indexes of 0.600 (95% confidence interval: 0.561-0.639) and 0.605 (95% confidence interval: 0.579-0.631), respectively.

Research conclusions

Easily obtainable variables at preoperative consultation, defining the status of leukocyte balances between peripheral blood and peritumoural tissue, have been shown to render indexes that accurately predict OS and RFS of CRC patients after surgical ablation of the tumours.

Research perspectives

We hope these indexes could be implemented in the first line of prognosis, making it easier to predict the outcome of patients after surgery depending on the tumour location and leukocyte distribution in both peripheral blood and biopsies of the peritumoural region.

ACKNOWLEDGEMENTS

We thank Dr. María Teresa Vallejo-Cremades and Onys Camps-Ortega from the Service of Image and Immunohistochemistry of IdiPAZ for helpful assistance, and Dr. Jair A. Tenorio for software support.

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Miller KD**, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL, Siegel RL. Cancer treatment and survivorship statistics, 2019. *CA Cancer J Clin* 2019; **69**: 363-385 [PMID: 31184787 DOI: 10.3322/caac.21565]

3 **Augestad KM**, Merok MA, Ignatovic D. Tailored Treatment of Colorectal Cancer: Surgical, Molecular, and Genetic Considerations. *Clin Med Insights Oncol* 2017; **11**: 1179554917690766 [PMID: 28469509 DOI: 10.1177/1179554917690766]

4 **Forster S**, Radpour R. Molecular Immunotherapy: Promising Approach to Treat Metastatic Colorectal Cancer by Targeting Resistant Cancer Cells or Cancer Stem Cells. *Front Oncol* 2020; **10**: 569017 [PMID: 33240813 DOI: 10.3389/fonc.2020.569017]

5 **Tan D**, Fu Y, Tong W, Li F. Prognostic significance of lymphocyte to monocyte ratio in colorectal cancer: A meta-analysis. *Int J Surg* 2018; **55**: 128-138 [PMID: 29807167 DOI: 10.1016/j.ijsu.2018.05.030]

6 **Mao Y**, Chen D, Duan S, Zhao Y, Wu C, Zhu F, Chen C, Chen Y. Prognostic impact of pretreatment lymphocyte-to-monocyte ratio in advanced epithelial cancers: a meta-analysis. *Cancer Cell Int* 2018; **18**: 201 [PMID: 30534002 DOI: 10.1186/s12935-018-0698-5]

7 **Peng J**, Li H, Ou Q, Lin J, Wu X, Lu Z, Yuan Y, Wan D, Fang Y, Pan Z. Preoperative lymphocyte-to-monocyte ratio represents a superior predictor compared with neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios for colorectal liver-only metastases survival. *Onco Targets Ther* 2017; **10**: 3789-3799 [PMID: 28794643 DOI: 10.2147/OTT.S140872]

8 **Guo D**, Li X, Xie A, Cao Q, Zhang J, Zhang F, Li W, Chen J. Differences in oncological outcomes and inflammatory biomarkers between right-sided and left-sided stage I-III colorectal adenocarcinoma. *J Clin Lab Anal* 2020; **34**: e23132 [PMID: 31755593 DOI: 10.1002/jcla.23132]

9 **Altan M**, Haberal HB, Akdoğan B, Özen H. A critical prognostic analysis of neutrophil-lymphocyte ratio for patients undergoing nephroureterectomy due to upper urinary tract urothelial carcinoma. *Int J Clin Oncol* 2017; **22**: 964-971 [PMID: 28600686 DOI: 10.1007/s10147-017-1150-x]

10 **Lu A**, Li H, Zheng Y, Tang M, Li J, Wu H, Zhong W, Gao J, Ou N, Cai Y. Prognostic Significance of Neutrophil to Lymphocyte Ratio, Lymphocyte to Monocyte Ratio, and Platelet to Lymphocyte Ratio in Patients with Nasopharyngeal Carcinoma. *Biomed Res Int* 2017; **2017**: 3047802 [PMID: 28321405 DOI: 10.1155/2017/3047802]

11 **Liu B**, Huang Y, Sun Y, Zhang J, Yao Y, Shen Z, Xiang D, He A. Prognostic value of inflammation-based scores in patients with osteosarcoma. *Sci Rep* 2016; **6**: 39862 [PMID: 28008988 DOI: 10.1038/srep39862]

12 **Biswas T**, Kang KH, Gawdi R, Bajor D, Machtay M, Jindal C, Efird JT. Using the Systemic Immune-Inflammation Index (SII) as a Mid-Treatment Marker for Survival among Patients with Stage-III Locally Advanced Non-Small Cell Lung Cancer (NSCLC). *Int J Environ Res Public Health* 2020; **17** [PMID: 33143164 DOI: 10.3390/ijerph17217995]

13 **Ying HQ**, Liao YC, Sun F, Peng HX, Cheng XX. The Role of Cancer-Elicited Inflammatory Biomarkers in Predicting Early Recurrence Within Stage II-III Colorectal Cancer Patients After Curable Resection. *J Inflamm Res* 2021; **14**: 115-129 [PMID: 33500648 DOI: 10.2147/JIR.S285129]

14 **Dell'Aquila E**, Cremolini C, Zeppola T, Lonardi S, Bergamo F, Masi G, Stellato M, Marmorino F, Schirripa M, Urbano F, Ronzoni M, Tomasello G, Zaniboni A, Racca P, Buonadonna A, Allegrini G, Fea E, Di Donato S, Chiara S, Tonini G, Tomcikova D, Boni L, Falcone A, Santini D. Prognostic and predictive role of neutrophil/lymphocytes ratio in metastatic colorectal cancer: a retrospective analysis of the TRIBE study by GONO. *Ann Oncol* 2018; **29**: 924-930 [PMID: 29324972 DOI: 10.1093/annonc/mdy004]

15 **Clarke SJ**, Burge M, Feeney K, Gibbs P, Jones K, Marx G, Molloy MP, Price T, Reece WHH, Segelov E, Tebbutt NC. The prognostic role of inflammatory markers in patients with metastatic colorectal cancer treated with bevacizumab: A translational study [ASCENT]. *PLoS One* 2020; **15**: e0229900 [PMID: 32142532 DOI: 10.1371/journal.pone.0229900]

16 **Ying HQ**, Deng QW, He BS, Pan YQ, Wang F, Sun HL, Chen J, Liu X, Wang SK. The prognostic value of preoperative NLR, d-NLR, PLR and LMR for predicting clinical outcome in surgical colorectal cancer patients. *Med Oncol* 2014; **31**: 305 [PMID: 25355641 DOI: 10.1007/s12032-014-0305-0]

17 **Wu Y**, Ye S, Goswami S, Pei X, Xiang L, Zhang X, Yang H. Clinical significance of peripheral blood and tumor tissue lymphocyte subsets in cervical cancer patients. *BMC Cancer* 2020; **20**: 173 [PMID: 32131750 DOI: 10.1186/s12885-020-6633-x]

18 **Hamada T**, Ishizaki H, Haruyama Y, Hamada R, Yano K, Kondo K, Kataoka H, Nanashima A. Neutrophil-to-Lymphocyte Ratio and Intratumoral CD45RO-Positive T Cells as Predictive Factors for Longer Survival of Patients with Colorectal Liver Metastasis after Hepatectomy. *Tohoku J Exp Med* 2020; **251**: 303-311 [PMID: 32779620 DOI: 10.1620/tjem.251.303]

19 **Guo G**, Wang Y, Zhou Y, Quan Q, Zhang Y, Wang H, Zhang B, Xia L. Immune cell concentrations among the primary tumor microenvironment in colorectal cancer patients predicted by clinicopathologic characteristics and blood indexes. *J Immunother Cancer* 2019; **7**: 179 [PMID: 31300050 DOI: 10.1186/s40425-019-0656-3]

20 **Cantero-Cid R**, Casas-Martin J, Hernández-Jiménez E, Cubillos-Zapata C, Varela-Serrano A, Avendaño-Ortiz J, Casarrubios M, Montalbán-Hernández K, Villacañas-Gil I, Guerra-Pastrián L, Peinado B, Marcano C, Aguirre LA, López-Collazo E. PD-L1/PD-1 crosstalk in colorectal cancer: are we targeting the right cells? *BMC Cancer* 2018; **18**: 945 [PMID: 30285662 DOI: 10.1186/s12885-018-4853-0]

21 **Wilson DJ**, Bordoni B. Embryology, Bowel. Treasure Island (FL): StatPearls, 2021. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31424831/

22 **Kostouros A**, Koliarakis I, Natsis K, Spandidos DA, Tsatsakis A, Tsiaoussis J. Large intestine embryogenesis: Molecular pathways and related disorders (Review). *Int J Mol Med* 2020; **46**: 27-57 [PMID: 32319546 DOI: 10.3892/ijmm.2020.4583]

23 **Bufill JA**. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. *Ann Intern Med* 1990; **113**: 779-788 [PMID: 2240880 DOI: 10.7326/0003-4819-113-10-779]

24 **Kwak HD**, Ju JK. Immunological Differences Between Right-Sided and Left-Sided Colorectal Cancers: A Comparison of Embryologic Midgut and Hindgut. *Ann Coloproctol* 2019; **35**: 342-346 [PMID: 31937074 DOI: 10.3393/ac.2019.03.17.1]

25 **Lee GH**, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? *Eur J Surg Oncol* 2015; **41**: 300-308 [PMID: 25468456 DOI: 10.1016/j.ejso.2014.11.001]

26 **Álvaro E**, Cano JM, García JL, Brandáriz L, Olmedillas-López S, Arriba M, Rueda D, Rodríguez Y, Cañete Á, Arribas J, Inglada-Pérez L, Pérez J, Gómez C, García-Arranz M, García-Olmo D, Goel A, Urioste M, González-Sarmiento R, Perea J. Clinical and Molecular Comparative Study of Colorectal Cancer Based on Age-of-onset and Tumor Location: Two Main Criteria for Subclassifying Colorectal Cancer. *Int J Mol Sci* 2019; **20** [PMID: 30813366 DOI: 10.3390/ijms20040968]

27 **Baek SK**. Laterality: Right-Sided and Left-Sided Colon Cancer. *Ann Coloproctol* 2017; **33**: 205-206 [PMID: 29354601 DOI: 10.3393/ac.2017.33.6.205]

28 **Baran B**, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference Between Left-Sided and Right-Sided Colorectal Cancer: A Focused Review of Literature. *Gastroenterology Res* 2018; **11**: 264-273 [PMID: 30116425 DOI: 10.14740/gr1062w]

29 **Ghidini M**, Petrelli F, Tomasello G. Right Versus Left Colon Cancer: Resectable and Metastatic Disease. *Curr Treat Options Oncol* 2018; **19**: 31 [PMID: 29796712 DOI: 10.1007/s11864-018-0544-y]

30 **Jung MK**, Shin US, Ki YJ, Kim YB, Moon SM, Sung SJ. Is the Location of the Tumor Another Prognostic Factor for Patients With Colon Cancer? *Ann Coloproctol* 2017; **33**: 210-218 [PMID: 29354603 DOI: 10.3393/ac.2017.33.6.210]

31 **Stintzing S**, Tejpar S, Gibbs P, Thiebach L, Lenz HJ. Understanding the role of primary tumour localisation in colorectal cancer treatment and outcomes. *Eur J Cancer* 2017; **84**: 69-80 [PMID: 28787661 DOI: 10.1016/j.ejca.2017.07.016]

32 **Benedix F**, Kube R, Meyer F, Schmidt U, Gastinger I, Lippert H; Colon/Rectum Carcinomas (Primary Tumor) Study Group. Comparison of 17,641 patients with right- and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. *Dis Colon Rectum* 2010; **53**: 57-64 [PMID: 20010352 DOI: 10.1007/DCR.0b013e3181c703a4]

33 **Huang ZS**, Wu JW, Li Y, Lin YH, Li XY. Effect of sidedness on survival among patients with early-stage colon cancer: a SEER-based propensity score matching analysis. *World J Surg Oncol* 2021; **19**: 127 [PMID: 33874958 DOI: 10.1186/s12957-021-02240-3]

34 **Patel M**, McSorley ST, Park JH, Roxburgh CSD, Edwards J, Horgan PG, McMillan DC. The relationship between right-sided tumour location, tumour microenvironment, systemic inflammation, adjuvant therapy and survival in patients undergoing surgery for colon and rectal cancer. *Br J Cancer* 2018; **118**: 705-712 [PMID: 29337962 DOI: 10.1038/bjc.2017.441]

35 **Guo G**, Chen X, He W, Wang H, Wang Y, Hu P, Rong Y, Fan L, Xia L. Establishment of inflammation biomarkers-based nomograms to predict prognosis of advanced colorectal cancer patients based on real world data. *PLoS One* 2018; **13**: e0208547 [PMID: 30513126 DOI: 10.1371/journal.pone.0208547]

36 **Zhang J**, Chen L, Zhou R, Sun H, Liao Y, Liao W. Pretreatment Lymphocyte Monocyte Ratio Predicts Long-Term Outcomes in Patients with Digestive System Tumor: A Meta-Analysis. *Gastroenterol Res Pract* 2016; **2016**: 9801063 [PMID: 27594882 DOI: 10.1155/2016/9801063]

37 **Li Z**, Zhao R, Cui Y, Zhou Y, Wu X. The dynamic change of neutrophil to lymphocyte ratio can predict clinical outcome in stage I-III colon cancer. *Sci Rep* 2018; **8**: 9453 [PMID: 29930287 DOI: 10.1038/s41598-018-27896-y]

38 **Idos GE**, Kwok J, Bonthala N, Kysh L, Gruber SB, Qu C. The Prognostic Implications of Tumor Infiltrating Lymphocytes in Colorectal Cancer: A Systematic Review and Meta-Analysis. *Sci Rep* 2020; **10**: 3360 [PMID: 32099066 DOI: 10.1038/s41598-020-60255-4]

39 **Forssell J**, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. *Clin Cancer Res* 2007; **13**: 1472-1479 [PMID: 17332291 DOI: 10.1158/1078-0432.CCR-06-2073]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 319-333

DOI: 10.4251/wjgo.v14.i1.319

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Effects of cognitive behavior therapy combined with Baduanjin in patients with colorectal cancer

Zheng-Gen Lin, Ren-Dong Li, Fu-Lu Ai, Song Li, Xin-An Zhang

ORCID number: Zheng-Gen Lin 0000-0003-1574-4590; Ren-Dong Li 0000-0003-3371-554X; Fu-Lu Ai 0000-0001-9121-3703; Song Li 0000-0002-9961-8622; Xin-An Zhang 0000-0002-7854-4134.

Author contributions: Lin ZG contributed to conception of the study; Li RD performed the experiments and wrote the manuscript; Ai FL contributed significantly to data analysis and manuscript preparation; Li S helped perform the data analysis with constructive discussions; Zhang XA contributed significantly to data analysis and manuscript preparation; Li S and Zhang XA contributed equally to the article and should be regarded as co-corresponding authors.

Institutional review board statement: This study was approved by the Institutional Review Board of Liaoning Cancer Hospital.

Clinical trial registration statement: Clinical Trial registration was not conducted for this study.

Informed consent statement: All patients gave informed consent.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest to disclose.

Zheng-Gen Lin, School of Social and Physical Education, Shenyang Sport University, Shenyang 110102, Liaoning Province, China

Ren-Dong Li, Physical Education Department, Shenyang University of Chemical Technology, Shenyang 110142, Liaoning Province, China

Fu-Lu Ai, Department of General Surgery (VIP Ward), Liaoning Tumor Hospital, Shenyang 110042, Liaoning Province, China

Song Li, Martial Arts and Dance Academy, Shenyang Sport University, Shenyang 110102, Liaoning Province, China

Xin-An Zhang, College of Kinesiology, Shenyang Sport University, Shenyang 110102, Liaoning Province, China

Corresponding author: Song Li, MSc, Additional Professor, Martial Arts and Dance Academy, Shenyang Sport University, No. 36 Jinqiansong East Road, Sujiatun District, Shenyang 110102, Liaoning Province, China. cuiluxiang@syty.edu.cn

Abstract

BACKGROUND

Cancer-related fatigue (CRF) is the most common concomitant symptom in the treatment of colorectal cancer (CRC). Such patients often present with subjective fatigue state accompanied by cognitive dysfunction, which seriously affects the quality of life of patients.

AIM

To explore the effects of cognitive behavior therapy (CBT) combined with Baduanjin exercise on CRF, cognitive impairment, and quality of life in patients with CRC after chemotherapy, and to provide a theoretical basis and practical reference for rehabilitation of CRC after chemotherapy.

METHODS

Fifty-five patients with CRC after radical resection and chemotherapy were randomly divided into either an experimental or a control group. The experimental group received the intervention of CBT combined with exercise intervention for 6 mo, and indicators were observed and measured at baseline, 3 mo, and 6 mo to evaluate the intervention effect.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at zhangxa2725@163.com.

CONSORT 2010 statement: The manuscript was checked according to the CONSORT 2010.

Supported by The Scientific Research Funds Project of Liaoning Education Department, No. LJC2019ST02.

Country/Territory of origin: China

Specialty type: Sport Sciences

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: June 10, 2021
Peer-review started: June 10, 2021
First decision: July 16, 2021
Revised: July 30, 2021
Accepted: September 10, 2021
Article in press: September 10, 2021
Published online: January 15, 2022

P-Reviewer: Eglinton TW, Liebig-Hoerl G
S-Editor: Wang JL

RESULTS

Compared with the baseline values, in the experimental group 3 mo after intervention, cognitive function, quality of life score, and P300 amplitude and latency changes were significantly better ($P < 0.01$). Compared with the control group, at 3 mo, the experimental group had significant differences in CRF, P300 amplitude, and quality of life score ($P < 0.05$), as well as significant differences in P300 latency and cognitive function ($P < 0.01$). Compared with the control group, at 6 mo, CRF, P300 amplitude, P300 latency, cognitive function and quality of life score were further improved in the experimental group, with significant differences ($P < 0.01$). The total score of CRF and the scores of each dimension were negatively correlated with quality of life ($P < 0.05$), while the total score of cognitive impairment and the scores of each dimension were positively correlated with quality of life ($P < 0.05$).

CONCLUSION

CBT combined with body-building Baduanjin exercise can improve CRF and cognitive impairment in CRC patients after chemotherapy, and improve their quality of life.

Key Words: Colorectal cancer; Cognitive behavior therapy; Baduanjin exercise; Cancer-related fatigue; Cognitive function; Quality of life

Core Tip: Cognitive behavior therapy combined with body-building Baduanjin exercise can improve cancer-induced fatigue and cognitive impairment in colorectal cancer patients after chemotherapy, and improve their quality of life. The quality of life of colorectal cancer patients may be related to cancer-induced fatigue and cognitive level. Cognitive behavior therapy combined with exercise intervention deserves to be promoted in cancer patients.

Citation: Lin ZG, Li RD, Ai FL, Li S, Zhang XA. Effects of cognitive behavior therapy combined with Baduanjin in patients with colorectal cancer. *World J Gastrointest Oncol* 2022; 14(1): 319-333
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/319.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.319

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies. According to the 2018 global cancer statistics, the global mortality rate of CRC accounted for 9.2% of the total number of cancer deaths, and the incidence rate accounted for 10.2% of the total number of cancers, ranking second and third, respectively[1]. CRC is mainly treated by surgical resection, combined with perioperative radiotherapy and chemotherapy and other comprehensive treatment methods. Cancer-related fatigue (CRF) is a subjective fatigue state caused by the adverse effects of cancer itself and chemotherapy, and is also affected by social objective factors and individual factors[2]. Cancer-related cognitive impairment (CRCI) is caused by chemotherapy in cancer patients, often referred to as chemotherapy brain or chemotherapy fog[3]. Due to chronic inactivity and the effects of CRF, cancer patients often develop cognitive impairments that ultimately affect their quality of life.

Cognitive behavioral therapy is concerned about the relationship between thought, feeling, and behavior; the main purpose is to reduce stress, in a rational way to solve the patients' severe psychological stress response, so that it can adapt to the changes brought by psychological stress[4]. Exercise therapy as a nondrug method has been pursued by clinicians in recent years. Aerobic exercise can improve the physical status of cancer patients, negative emotions such as anxiety and depression, and cognitive impairment and reduce the level of CRF[5]. Baduanjin is a medium-intensity aerobic exercise that has a good promoting effect on human digestion, respiration, circulation,

L-Editor: Wang TQ
P-Editor: Yu HG

and motor function[6]. So far, there have been few studies on the effect of cognitive behavior therapy (CBT) combined with exercise on cognitive function in patient with CRC. The present study used CBT combined with Baduanjin as the intervention for 6 mo in patients with CRC chemotherapy to explore the value of the combination therapy in the rehabilitation of CRC during chemotherapy.

MATERIALS AND METHODS

Inclusion and exclusion criteria

We recruited patients undergoing chemotherapy for CRC who were admitted to Liaoning Tumor Hospital between March and October 2018. The inclusion criteria were: (1) Subjects met the guidelines for diagnosis, staging, and treatment of CRC[7], and underwent surgery for CRC; (2) In the stable period after surgery (clinical stages I–III); (3) The score of the simple mini-mental state examination scale was 22–27 points; and (4) All patients with CRC successfully completed standardized chemotherapy regimen. The exclusion criteria were: (1) Complicated with serious cardiovascular and cerebrovascular diseases and mental diseases; (2) Presence of limb dysfunction; (3) Accompanied by pneumonia, asthma, and other respiratory diseases; and (4) Had participated in regular exercise during the past 6 mo. This study was approved by the Ethics Committee of Liaoning Tumor Hospital. All subjects participated voluntarily and gave signed informed consent.

Using a random number table method, 60 patients were randomly divided into either an experimental or a control group, with 30 cases in each group. During the study, a total of five patients withdrew from the study, including three in the experimental group who did not complete the treatment on time (n = 2) or whose condition deteriorated (n = 1), and two in the control group who were readmitted to the hospital for chemotherapy and withdrew from the study. Finally, 27 patients in the experimental group and 28 in the control group completed the study. There was no significant difference in patients' general baseline data or disease-related data between the two groups (P > 0.05; Table 1).

Intervention plan

The experimental group received CBT from psychotherapists. The patients provided details of their symptoms and illness for 1 h, three times a week. Doctors provided immediate monitoring and cognitive correction, stress management, cognitive restructuring, and relaxation counseling, and encouraged patients to adopt positive behavioral strategies. After discharge, doctors conducted home visits or telephone communication once a week and completed CBT for 6 mo.

During hospitalization, the experimental group received comprehensive and systematic guidance from professional coaches on the skills and exercise load of Baduanjin, until they could all regulate and master the exercise movements. The videos of Baduanjin were released upon discharge. After discharge, the patients were given weekly telephone supervision and follow-up visits. The patients were asked to write a daily exercise diary. The mean duration of exercise was ≥ 4 d/wk, ≥ 20 d/mo, 45–60 min/session, twice daily (once in the morning and once in the afternoon) for 6 mo. There was no exercise intervention in the control group.

Indicator testing and data processing

The cognitive potential P300 test and scale were evaluated at baseline, and 3 mo and 6 mo after intervention. The changes in cognitive function, CRF, and quality of life of subjects before and after intervention were compared and analyzed, and the effect of intervention was evaluated.

Cognitive function assessment

Functional assessment of cancer therapy-cognitive function (FACT-Cog)[8] comprises 37 items in four dimensions, including correction of cognitive impairment, cognitive ability, evaluation by others, and impact on quality of life; each item has a score of 0–4, with 5 grades. The lower the score, the worse the cognitive function, and the test has good reliability and validity[9].

Assessment of CRF

The Cancer Fatigue Scale (CFS) compiled by Okuyama *et al*[10] was designed for evaluating fatigue symptoms of cancer patients, consisting of 15 items and three

Table 1 Comparison of general information between the two groups of patients, *n* (%)

	Experimental group (*n* = 27)	Control group (*n* = 28)	*P* value
Mean age (range, yr)	52 (44-60)	51 (40-62)	0.516
Gender			
Male	19 (70.4)	21 (75)	0.70
Female	8 (29.6)	7 (25)	
Years of education			
< 9	8 (29.6)	9 (32.2)	0.747
9-12	15 (55.6)	13 (46.4)	
> 12	4 (14.8)	6 (21.4)	
Marital status			
Married	20 (74.1)	24 (85.7)	0.555
Unmarried	2 (7.4)	1 (3.6)	
Divorced	5 (18.5)	3 (10.7)	
Clinical stage			
I	3 (11.1)	2 (7.2)	0.763
II	11 (40.8)	10 (35.7)	
III	13 (48.1)	16 (57.1)	
Chemotherapy			
XELOX	12 (44.4)	10 (35.7)	0.509
FOLOX	15 (55.6)	18 (64.3)	
Mean BMI (kg/m^2)	22.56 (20.38-24.05)	22.71 (21.22-24.19)	0.561
Mean MMSE score	24 (22-27)	24 (22-27)	0.765

XELOX: Capecitabine combined with oxaliplatin; FOLOX: Oxaliplatin combined with calcium folinate and deoxyfluoruridine; BMI: Body mass index; MMSE: Mini-mental State Examination.

dimensions of physical fatigue, emotional fatigue, and cognitive fatigue. Each item was rated on a scale of 1–5, with higher scores indicating more fatigue[11]. Studies have shown that the coefficient of Cronbach's (a) in the total table is 0.84–0.88, and the sub-half reliability coefficient (r) is 0.32–0.67[12].

Quality of life assessment

Functional assessment of cancer therapy-colorectal (FACT-C) is specifically used in the assessment of CRC patients. It consists of generic and CRC-specific modules with five dimensions: Physiological status, social/family status, emotional status, functional status, and additional concern for CRC. The internal consistency coefficient of additional concern was 0.56, the retest correlation coefficient of other fields was ≥ 0.76, and the α coefficient of all fields and general modules was ≥ 0.80[13].

Cognitive P300 test

The amplitudes and latency of P300 were recorded using the 32-channel electroencephalography acquisition system produced by Neuroscan Corporation (Charlotte, NC, United States), and the position of electrode was recorded at CZ point, which is located in the central midline of the brain and is the most commonly used electrode placement for recording cognitive-related potentials in the International Electroencephalogram Society 10-20 standard. The test was completed in the Department of Neurology in hospital.

Statistical analysis

SPSS 20.0 was used for data analyses. Quantitative data are expressed as the mean ± SD. Repeatability measurement analysis of variance was used between groups and

within groups, and Pearson correlation analysis was conducted between variables. Stepwise multiple linear regression was conducted for the variables with high correlation, and $P < 0.05$ was considered significant, and $P < 0.01$ was considered highly significant.

RESULTS

Overall status of all subjects in chemotherapy phase of CRC at baseline

The quality of life, cognitive function, and CRF status of all subjects at baseline are shown in Table 2. Among all the quality of life dimensions, social/family status score was the highest, followed by emotional status, additional concern, physiological status, and functional status (Table 2). Among the cognitive status dimensions, others' evaluation score was the highest, followed by corrected cognitive impairment, cognitive ability, and impact on quality of life. The scores for CRF showed that the scores of each dimension from high to low were physical fatigue, emotional fatigue, and cognitive fatigue.

Changes in CRF before and after intervention

The total score for the CRF test and the scores of each dimension in each group before and after exercise intervention are shown in Figure 1 and Table 3. At baseline, there were no significant differences between the two groups in terms of overall fatigue and each dimension ($P > 0.05$). Compared with baseline values, there were significant differences in the total score ($P < 0.001$), body fatigue score ($P < 0.001$), emotional fatigue score ($P < 0.001$), and cognitive fatigue ($P = 0.013$) in the experimental group at 3 mo and 6 mo after intervention. Compared with the control group, there were significant differences in the total score ($P = 0.018$), body fatigue score ($P = 0.003$), emotional fatigue score ($P = 0.029$), and cognitive fatigue ($P = 0.022$) at 3 mo and 6 mo after exercise intervention in the experimental group ($P < 0.001$).

Changes in cognitive function in the two groups before and after intervention

Status of electrophysiological tests: Figure 2 shows the cognitive potential P300 test of each group before and after exercise intervention. At baseline, there was no significant difference in the latency or amplitude of P300 between the two groups ($P > 0.05$). Compared with baseline values, there were significant differences in latent period ($P < 0.001$) and amplitude of P300 ($P = 0.008$) in the experimental group at 3 mo; furthermore, after 6 mo of intervention, there were highly significant differences in latency and amplitude of P300 ($P < 0.001$). Compared with the control group, there were significant differences in latent period ($P = 0.002$) and amplitude of P300 ($P = 0.041$) at 3 mo; and after 6 mo of intervention, the latency of P300 in the experimental group was shortened and the amplitude of P300 increased, with highly significant differences ($P < 0.001$).

Cognitive scale scores: Figure 3A and Table 4 show the total score of cognitive function and scores of each dimension in each group before and after exercise intervention. At baseline, there were no significant differences between the two groups in FACT-Cog total score or the scores of the four dimensions ($P > 0.05$). Compared with baseline values, 3 mo after intervention, except for impact on quality of life ($P = 0.526$) and other's evaluation ($P = 0.013$), the P values of total FACT-Cog score and the scores of other two dimensions were all less than 0. 001; 6 mo after intervention, the P values of total FACT-Cog score and the scores of the four dimensions were all less than 0. 001. Compared with the control group, after 3 mo of intervention, there were significant differences in the total score ($P = 0.016$) and scores of corrected cognitive impairment ($P = 0.003$), cognitive ability ($P = 0.011$), and impact on quality of life ($P = 0.002$); after 6 mo of intervention, there were highly significant differences in the total score ($P = 0.002$) and scores of corrected cognitive impairment ($P < 0.001$), cognitive ability ($P = 0.002$), other's evaluation ($P = 0.002$), and impact on quality of life ($P < 0.001$).

Changes in quality of life in the two groups before and after intervention

The total score of quality of life and the scores of the five dimensions in each group before and after exercise intervention are shown in Figure 3B and Table 5. At baseline, there were no significant differences between the two groups in the total score of quality of life or the scores of the five dimensions ($P > 0.05$). Compared with baseline values, 3 mo after intervention, there were significant differences in total score ($P <$

Table 2 Quality of life, cognitive function, and cancer-related fatigue scores at baseline (*n* = 55)

Item	Scale score range	Actual score range	Actual score	Score percentage (%)
Quality of life				
FACT-C total score	0-144	36-131	81.65 ± 23.27	56.72
Physiological status	0-28	6-25	15.38 ± 4.47	54.93
Social/family status	0-28	9-28	19.05 ± 4.57	68.04
Emotional status	0-24	6-24	14.93 ± 4.08	62.21
Functional status	0-28	5-26	12.69 ± 4.46	45.32
Additional attention score	0-36	10-30	19.84 ± 4.87	55.11
Cognitive function				
FACT-Cog total score	38-132	47-108	80.15 ± 10.97	60.72
Corrected cognitive impairment	18-72	26-57	46.62 ± 4.98	64.75
Cognitive ability	0-28	7-22	14.76 ± 3.21	52.14
Other's evaluation	4-16	5-16	10.51 ± 2.28	65.69
Impact on quality of life	4-16	4-13	8.25 ± 2.08	51.75
CRF				
CFS total score	0-60	22-46	34.47 ± 6.59	57.45
Physical fatigue	0-28	11-28	18.09 ± 3.23	64.61
Emotional fatigue	0-16	6-15	8.85 ± 1.82	55.31
Cognitive fatigue	0-16	3-12	7.53 ± 2.05	47.06

FACT-C: Functional assessment of cancer therapy – colorectal; FACT-Cog: Functional assessment of cancer therapy-cognitive function; CRF: Cancer-related fatigue; CFS: Cancer fatigue scale.

Table 3 Comparison of cancer-related fatigue tests in different dimensions (mean ± SD)

Group	Physical fatigue	Emotional fatigue	Cognitive fatigue
Experimental group (*n* = 27)			
Baseline	17.56 ± 3.53	8.67 ± 1.78	7.37 ± 2.09
3 mo	15.78 ± 2.85[b,e]	7.59 ± 1.67[b,d]	6.59 ± 1.65[a,d]
6 mo	15.19 ± 2.66[b,e]	6.59 ± 1.47[b,c,e]	6.33 ± 1.66[b,e]
Control group (*n* = 28)			
Baseline	18.61 ± 2.82	9.04 ± 1.80	7.57 ± 2.06
3 mo	18.43 ± 3.71	8.61 ± 1.69	7.79 ± 2.08
6 mo	18.46 ± 3.31	8.68 ± 1.91	7.68 ± 1.83

Intra-group comparison before and after intervention:
[a]$P < 0.05$.
[b]$P < 0.01$ *vs* baseline.
[c]$P < 0.01$ *vs* 3 mo.
Comparison between experimental group and control group at different time:
[d]$P < 0.05$.
[e]$P < 0.01$, experimental group *vs* control group at baseline/3 mo/6 mo.

0.001), and scores of physiological status ($P < 0.001$), emotional status ($P < 0.001$), and additional attention ($P = 0.044$), while there were no significant differences in the score of social/family status ($P = 0.455$) or functional status ($P = 0.059$); 6 mo after intervention, there were significant differences in total score and the scores of the five dimensions ($P < 0.001$). Compared with the control group, after 3 mo of intervention,

Table 4 Comparison of cognitive function tests in different dimensions (mean ± SD)

Group	Corrected cognitive impairment	Cognitive ability	Others' evaluation	Impact on quality of life
Experimental group (n = 27)				
Baseline	47.19 ± 4.14	14.96 ± 3.47	10.93 ± 2.24	8.81 ± 2.08
3 mo	50.26 ± 3.96[b,e]	17.11 ± 3.64[b,d]	11.26 ± 2.33[a]	9.37 ± 2.66[e]
6 mo	54.22 ± 6.80[b,c,e]	18.30 ± 4.26[b,c,e]	12.22 ± 2.28[b,c,e]	10.81 ± 2.73[b,c,e]
Control group (n = 28)				
Baseline	46.07 ± 5.73	14.57 ± 2.87	10.11 ± 2.25	7.71 ± 1.94
3 mo	46.21 ± 5.37	14.68 ± 3.22	10.14 ± 2.17	7.32 ± 2.02
6 mo	46.25 ± 6.92	14.86 ± 3.50	10.21 ± 2.35	7.43 ± 2.01

Intra-group comparison before and after intervention:
[a]P < 0.05.
[b]P < 0.01 *vs* baseline.
[c]P < 0.01 *vs* 3 mo.
Comparison between experimental group and control group at different time:
[d]P < 0.05.
[e]P < 0.01, experimental group *vs* control group at baseline/3 mo/6 mo.

Table 5 Comparison of quality of life tests in different dimensions (mean ± SD)

Group	Physiological status	Social/family status	Emotional status	Functional status	Additional attention score
Experimental group (n = 27)					
Baseline	16.19 ± 3.48	19.85 ± 4.92	15.41 ± 4.41	13.78 ± 4.29	11.54 ± 4.36
3 mo	17.67 ± 3.96[b,d]	20.59 ± 4.73[d]	17.11 ± 4.47[b,d]	14.74 ± 4.78[e]	22.41 ± 6.08[a,d]
6 mo	18.70 ± 4.15[b,e]	21.48 ± 4.57[b,c,e]	17.48 ± 4.64[b,d]	16.00 ± 4.84[b,c,e]	24.85 ± 6.56[b,c,e]
Control group (n = 28)					
Baseline	14.61 ± 5.18	18.29 ± 4.14	14.46 ± 3.76	11.64 ± 4.44	19.14 ± 4.28
3 mo	14.89 ± 5.72	17.75 ± 4.40	14.32 ± 3.49	11.32 ± 4.28	18.71 ± 5.61
6 mo	14.25 ± 5.29	18.14 ± 4.61	14.46 ± 3.75	11.54 ± 4.36	19.11 ± 5.63

Intra-group comparison before and after intervention:
[a]P < 0.05.
[b]P < 0.01 *vs* baseline.
[c]P < 0.01 *vs* 3 mo.
Comparison between experimental group and control group at different time:
[d]P < 0.05.
[e]P < 0.01, experimental group *vs* control group at baseline/3 mo/6 mo.

there were significant differences in total score (P = 0.016) and the scores of physiological status (P = 0.039), social/family status (P = 0.025), emotional status (P = 0.012), functional status (P = 0.007), and additional attention (P = 0.023); after 6 mo of intervention, there were highly significant differences in total score (P = 0.002) and the scores of physiological status (P = 0.001), social/family status (P = 0.009), emotional status (P = 0.010), functional status (P = 0.001), and additional attention (P = 0.001).

Relationship between quality of life and CRF and cognitive function in the experimental group before and after intervention

Correlation between quality of life and CRF and cognitive function in the experimental group: Pearson correlation analysis was performed between the total scores of FACT-C and CFS and its three dimensions, and the total score of FACT-Cog and its four dimensions. As shown in Table 6, the total score of quality of life was negatively correlated with the total score of fatigue and the scores of the three dimensions, and the total score of quality of life and cognitive function was positively correlated with

Table 6 Correlation analysis between quality of life and cancer-related fatigue and cognitive dysfunction in experimental group

Item	FACT-C total score	
	r	*P* value
CFS total score	-0.733	< 0.000
Physical fatigue	-0.439	0.023
Emotional fatigue	-0.487	0.011
Cognitive fatigue	-0.642	< 0.000
FACT-Cog total score	0.753	< 0.000
Corrected cognitive impairment	0.663	< 0.000
Cognitive ability	0.624	0.001
Other's evaluation	0.186	0.342
Impact on quality of life	0.40	0.023

FACT-C: Functional assessment of cancer therapy – colorectal; FACT-Cog: Functional assessment of cancer therapy-cognitive function; CFS: Cancer fatigue scale.

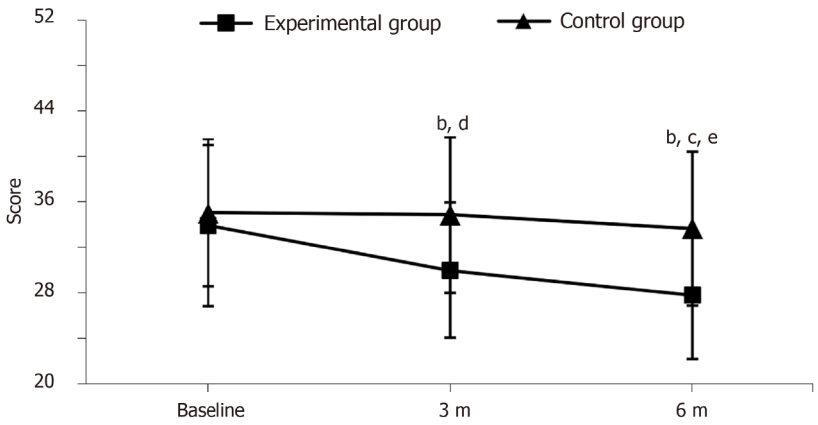


Figure 1 Total Cancer Fatigue Scale scores before and after intervention. Intra-group comparison before and after intervention: $^{b}P < 0.01$ *vs* baseline; $^{c}P < 0.01$ *vs* 3 mo. Comparison between experimental group and control group at different time points: $^{d}P < 0.05$, $^{e}P < 0.01$, experimental group *vs* control group at baseline/3 mo/6 mo.

the four dimensions.

Multivariate stepwise regression analysis of quality of life, CRF, and cognitive function in the experimental group: To analyze the relationship between quality of life and CRF and cognitive function, the difference between the total score of quality of life of patients undergoing CRC chemotherapy in the experimental group at 6 mo and the baseline data was used as the dependent variable. Five significant factors in the correlation analysis were taken as independent variables, and the five factors in the multivariate stepwise regression analysis were as follows: CRF total score, cognitive fatigue, FACT-Cog total score, corrected cognitive impairment, and cognitive ability.

The complex correlation coefficient $r = 0.80$ and the adjusted $R^2 = 0.603$ indicated that the dependent variable (total score of quality of life) of the stepwise fitting multiple linear regression equation could be explained by the independent variables (fatigue and cognition) by 60.3%. According to the standard regression coefficient, Table 7 shows that the factors affecting the quality of life of patients with CRC chemotherapy included total score of CRF and total score of FACT-Cog. Linear regression equation can be established according to the following model, with X_1 representing the total score of CRF and X_2 representing the total score of FACT-Cog: $Y = 4.923 - 0.585\ X_1 + 0.375\ X_2$, the results showed that the CRF score has a greater impact on quality of life than FACT-Cog score. The collinearity diagnosis results showed that all variables had a variance inflation factor (VIF) < 10, and there was no collinearity; therefore, it is of practical significance to establish the corresponding linear regression

Table 7 Stepwise regression analysis results of quality of life scores

Variable	Regression coefficient	SE	Standard regression coefficient	*t*	*P* value	Collinearity Statistics	
						Tolerance	VIF
Constant	4.923	1.429		3.209	0.004		
FACT-Cog Total score	0.375	0.149	0.464	2.512	0.019	0.447	2.235
CRF total score	-0.585	0.278	-0.388	-2.103	0.046	0.651	1.535

FACT-Cog: Functional assessment of cancer therapy-cognitive function; CRF: Cancer-related fatigue.

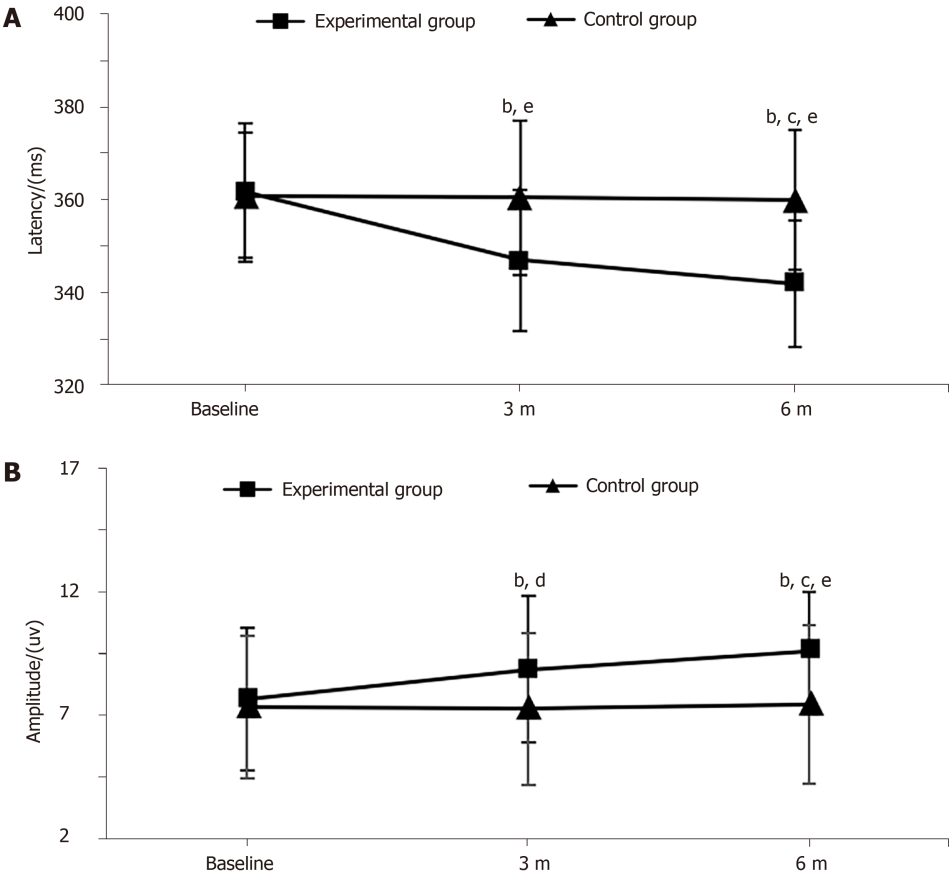


Figure 2 P300 latency and amplitude changes. A: P300 latency change; B: P300 amplitude change. Intra-group comparison before and after intervention: $^{b}P < 0.01$ *vs* baseline; $^{c}P < 0.01$ *vs* 3 mo. Comparison between experimental group and control group at different time points: $^{d}P < 0.05$, $^{e}P < 0.01$, experimental group *vs* control group at baseline/3 mo/6 mo.

model. VIF is a common measure for judging the severity of multicollinearity in multiple linear regression models. Usually, 10 is taken as the judgment boundary. When VIF < 10, there is no multicollinearity.

DISCUSSION

CRC has high clinical morbidity. With improvement in medical understanding, its fatality rate has decreased year by year. In recent years, people's health awareness has been gradually enhanced, but due to the neglect of early screening of CRC, often the best time for diagnosis and treatment is missed, resulting in adverse effects on recovery[14]. Patients with CRC generally need chemotherapy to inhibit the growth of cancer cells, and most patients with chemotherapy are accompanied by CRF. In addition to less exercise, bed rest, chemotherapy, and other internal and external factors, patients often appear with anxiety, depression, and other negative emotions and varying degrees of cognitive impairment. If the above factors are not effectively

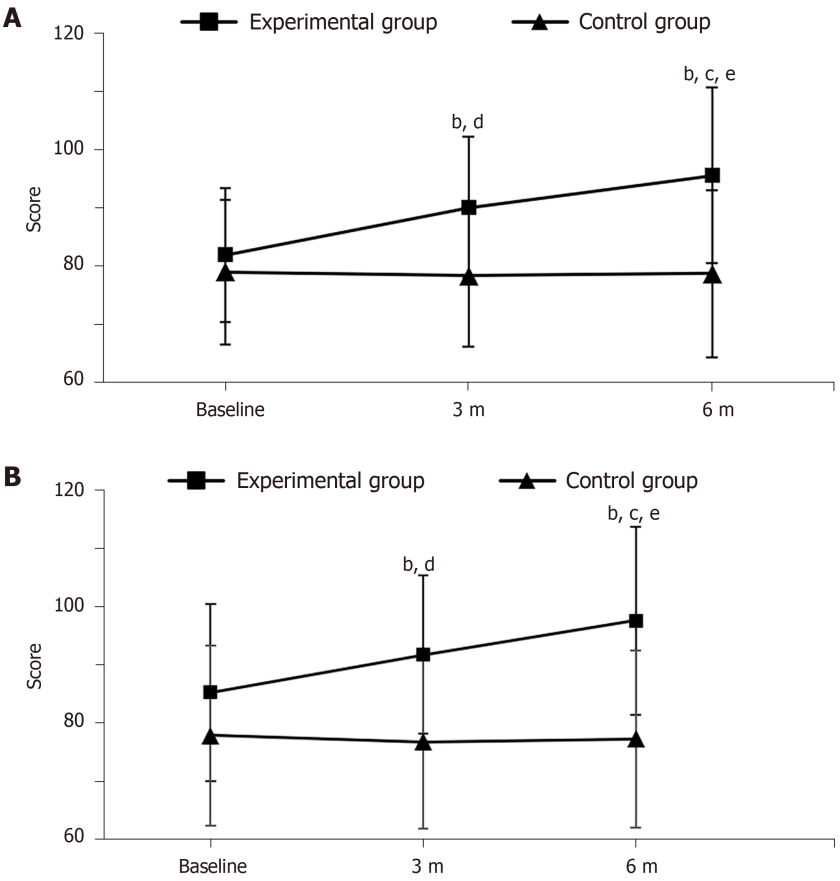


Figure 3 Functional assessment of cancer therapy. A: Functional assessment of cancer therapy-cognitive function total score change before and after intervention. Intra-group comparison before and after intervention: $^{b}P < 0.01$ *vs* baseline; $^{c}P < 0.01$ *vs* 3 mo. Comparison between experimental group and control group at different time: $^{e}P < 0.01$, experimental group *vs* control group at baseline/ 3 mo/ 6 mo; B: Functional assessment of cancer therapy – colorectal total score changes before and after intervention. Intra-group comparison before and after intervention: $^{b}P < 0.01$ *vs* baseline; $^{c}P < 0.01$ *vs* 3 mo. Comparison between experimental group and control group at different time: $^{d}P < 0.05$, $^{e}P < 0.01$, experimental group *vs* control group at baseline/ 3 mo/ 6 mo.

resolved or controlled, the cognitive level and quality of life of patients continue to decline, and even aggravate the condition of the patients, forming a vicious cycle. Studies have shown that exercise and psychological intervention could promote the improvement of quality of life in cancer patients[15], and exercise combined with psychotherapy may be an effective intervention to improve CRF in patients with CRC undergoing chemotherapy.

CBT focuses on the relationships between thought, feeling, and behavior, with the goal of reducing stress and fatigue, thereby improving the quality of life of patients. Fitness Qigong Baduanjin, a Chinese traditional Qigong exercise that focuses on a mind-body integration, is considered to be an effective exercise in promoting health. Numerous studies have shown that Baduanjin exercise could effectively relieve physical pain, improve physical function, relieve negative emotions such as anxiety and depression, and have a very good effect on the improvement of cognitive function. It is an effective adjunctive rehabilitation method for cognitive and psychological diseases, and is widely used in clinical practice[16]. A randomized controlled trial has shown that CBT combined with exercise can improve fatigue, sleep disturbance, anxiety, and depression in breast cancer patients[17].

Studies have shown that CRF is a common symptom in about 70% of cancer patients [18]. Fatigue in patients is often more serious than that in healthy groups, and is difficult to alleviate through sleep and rest, causing a major economic burden and mental pressure on patients and their families[19]. At present, there are many studies on the pathogenesis of CRF, among which, the explanation of CRF by 5-hydroxytryptamine (5-HT) disorder has been accepted by most researchers. This mechanism can be divided into two types of CRF: Peripheral and central fatigue[20]. Peripheral fatigue mainly refers to physical fatigue. After cancer patients receive chemotherapy, peripheral nerves can be stimulated to release neuroactive substances, and the vagus afferent nerve can be activated, thus inhibiting skeletal muscle activity. Decrease of skeletal muscle activity leads to physical fatigue. Central fatigue, including emotional

and cognitive fatigue, is mainly related to neural bundles and disorders in the brain, especially the increased concentration of 5-HT in the brain[21]. In the present study, the CFS was used to evaluate the degree of CRF, and it was found that body fatigue was most severe in patients undergoing chemotherapy for CRC, and this conclusion is consistent with the research of Jong *et al*[22]. Studies have confirmed that moderate physical activity has a good effect in improving CRF in patients with CRC, but most of the aerobic exercises used in existing studies are jogging, swimming, cycling, *etc.*, and the efficacy evaluation also focuses on a single time point and lacks periodic efficacy observation. In this study, Chinese traditional healthy Qigong Baduanjin was used to intervene patients with CRC for 6 mo, and the CFS was evaluated at 3 and 6 mo. The results showed that exercise intervention for 3 mo could improve the CRF of CRC patients significantly, among which the improvement of body fatigue was the most obvious, and still had a certain curative effect with the extension of intervention time. Therefore, Baduanjin is a good exercise intervention method for CRF, which is worthy of long-term adherence.

Cancer patients often have cognitive impairment, mainly for memory, attention, and event processing speed[23]. This may be caused by effects of chemotherapeutic drugs on the CNS and nerve cell damage directly, resulting in oxidative stress, inflammatory reaction, and changes in hormone levels, blood supply, and metabolism[24]. However, it should be noted that mild cognitive impairment is not easily detected by the patients themselves, and their families often focus on the recovery of disease symptoms, while the symptoms of CRCI, such as decline in memory and attention, are easily ignored. In this study, the FACT-Cog scale was used to evaluate the cognitive function of CRC patients from multiple dimensions, which showed that, there are different degrees of cognitive impairment in CRC patients; among the dimensions of the scale, the lowest score was for impact on quality of life and cognitive ability, while the highest score was for other people's evaluation. The reason may be that the cognitive impairment caused by chemotherapy for CRC makes the patients unable to have normal work and social skills, and affect the quality of life ultimately. Studies have shown that physical activities can improve cognitive function in patients, which may be because exercise stimulates the cranial nerves, activates the CNS, prevents brain atrophy, and increases the hippocampal volume, thereby promoting the remodeling of nerve cells and synapses[25]. Furthermore, Ferguson *et al*[26] found that CBT therapy could improve cognitive dysfunction and the quality of life effectively in breast cancer survivors following chemotherapy. In the present study, after 6 mo of Baduanjin and CBT intervention, the five main dimensions of FACT-Cog scale were improved significantly, and the results are consistent with those of previous studies. To further analyze the effect on the cognitive function of CRC patients, we used P300, which provides objective evidence for the theory that CBT combined with exercise intervention can improve related cognitive dysfunction in patients with CRC chemotherapy. P300 is an effective electrophysiological indicator reflecting cognitive function status. The latency of P300 is often used to reflect short-term memory, selective attention, and reaction speed, the ability to process events, and cognitive processing[27], and the amplitude of P300 reflects the resources invested in the brain when it senses incoming stimulus information, namely, the active control of attention and the ability of information processing. The decrease of P300 amplitude and the prolongation of latency indicate the decline of cognitive ability, and P300 latency is more sensitive to the occurrence of early CRCI than P300 amplitude response, so P300 amplitude and latency can be used as biological markers of cognitive physiological mechanism[28,29]. The present study was completed by auditory stimulation under the oddball paradigm, and the latency of P300 was significantly shortened and the amplitude of P300 was significantly increased in CRC patients receiving 3 mo of CBT combined with exercise intervention, and the difference became more significant with the duration of the intervention. In addition, the above changes were not observed in the control group, which was consistent with the scale evaluation results, confirming the effectiveness of combined intervention for CRCI in this type of disease.

In recent years, the quality of life of patients with CRC during chemotherapy has attracted much attention. Different from the traditional biomedical models, modern medical models do not take tumor elimination as the only goal, and their evaluation of survival rate of cancer patients is more systematic, especially paying attention to the quality of life of patients after surgery and chemotherapy[30]. The concept of quality of life is extremely complex, including physical, psychological, social function, mental state, *etc.* It reflects the gap between personal expectations and actual living conditions. Research has shown that CRC surgery and chemotherapy cause changes in normal defecation patterns and disorder of self-image, and lead to negative emotions such as anxiety and depression, and then affect the quality of life of patients seriously[31]. In

this study, the FACT-C scale was used valuate the quality of life of patients with CRC after chemotherapy, and then we found a general decline in quality of life. For the FACT-C scale, the decline of functional status is the most obvious, followed by physiological status and additional attention score, and the decline of social/family status is relatively small. The reason may be that after cancer chemotherapy, patients get the support and encouragement from family and friends, and get a great emotional release. Physical activities are closely related to the quality of life of CRC patients. A large number of studies have shown that the overall quality of life of patients who participate in physical activities for a long time is significantly higher than that of patients who do not[32]. Baduanjin exercise has been found effective in improving the quality of life and mental health, and reducing stress[33]. Ferguson *et al*[26] also found that 2 mo of brief cognitive-behavioral therapy intervention could improve the quality of life and verbal memory performance of breast cancer survivors effectively. In this study, we found that the scores of the four dimensions of the FACT-C scale were improved after 3 mo of Baduanjin combined CBT intervention in patients with CRC after chemotherapy, and the quality of life was further improved by 6 mo of intervention. Baduanjin exercise could improve the physical function and CBT could relieve the negative emotion effectively, and they cooperate with each other and play a benign promoting role together.

In this study, we analyzed the correlation between quality of life and CRF and cognitive function in patients undergoing chemotherapy for CRC, and found that patients with more severe CRF had lower quality of life. CRF causes many problems for cancer patients, including daily diet, daily life, leisure and entertainment, normal working ability, *etc*. The total score of cognitive function and the scores of each dimension are closely related to the total score of quality of life. The reason may be that chemotherapy and other factors lead to the cognitive decline. Cancer patients are often unable to concentrate and their memory declines, which seriously affects their normal social activities. Therefore, it will also have an impact on the quality of life of patients. In our study, the decrease in quality of life of subjects was mainly related to CRF, followed by CRCI. CRF and CRCI caused by cancer chemotherapy have varying impacts on the daily life of patients, leading to a decline in their quality of life. In the rehabilitation of patients with CRC chemotherapy, we should strengthen evaluation of their degree of fatigue, carry out health education in advance, and use CBT combined with Baduanjin as auxiliary rehabilitation therapy, which can prevent and slow down the decline of cognitive function, and ultimately improve quality of life.

CONCLUSION

Patients with CRC generally have obvious CRF (mainly body fatigue), cognitive impairment, and serious decline in quality of life, which affects their prognosis. CBT combined with Baduanjin exercise can improve fatigue and cognitive impairment of CRC patients undergoing chemotherapy, and improve their quality of life. The quality of life of CRC patients is closely related to their CRF and cognitive level. CBT combined with exercise intervention is worth promoting in the postoperative rehabilitation of cancer patients.

ARTICLE HIGHLIGHTS

Research background

Patients with colorectal cancer (CRC) after chemotherapy are often accompanied with cancer-related fatigue (CRF) and cancer-related cognitive dysfunction, which seriously affects the quality of life and recovery during chemotherapy, but there is no effective treatment.

Research motivation

This study sought to find an effective treatment for cognitive impairment and cancer-related fatigue after chemotherapy for CRC, and to provide a theoretical basis and practical reference for rehabilitation of CRC patients.

Research objectives

This study aimed to explore the effects cognitive behavior therapy (CBT) combined with Baduanjin exercise on CRF, cognitive impairment, and quality of life in patients

with CRC after chemotherapy.

Research methods

Patients with CRC were treated with CBT combined with Baduanjin (experimental group, n = 27) or usual care (control group, n = 28), and then the changes of cancer-related fatigue, cognitive function, quality of life, and P300 amplitude and latency were compared at baseline, 3 mo, and 6 mo.

Research results

Compared with the baseline values, the cancer-related fatigue, cognitive function, quality of life, and P300 amplitude and latency were significantly better in the experimental group at 3 mo ($P < 0.01$). The cancer-related fatigue, cognitive function, quality of life, and P300 amplitude and latency were significantly better in the experimental group than in the control group (experimental group *vs* control group at 3/6 mo; $P < 0.05$ or $P < 0.01$). The quality of life was negatively correlated with cancer-related fatigue and positively correlated with cognitive function.

Research conclusions

CBT combined with Baduanjin exercise can improve fatigue and cognitive impairment of CRC patients undergoing chemotherapy, and improve their quality of life. The quality of life of CRC patients is closely related to their CRF and cognitive level. CBT combined with exercise intervention is worth promoting in the postoperative rehabilitation of cancer patients.

Research perspectives

This study contributes to the rehabilitation of cognitive impairment and cancer-related fatigue in patients with colorectal cancer after chemotherapy. To confirm and validate the results of this study, a larger scale prospective study would be helpful.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Vogelzang NJ**, Breitbart W, Cella D, Curt GA, Groopman JE, Horning SJ, Itri LM, Johnson DH, Scherr SL, Portenoy RK. Patient, caregiver, and oncologist perceptions of cancer-related fatigue: results of a tripart assessment survey. The Fatigue Coalition. *Semin Hematol* 1997; **34**: 4-12 [PMID: 9253778]

3 **Wefel JS**, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. *Lancet Oncol* 2011; **12**: 703-708 [PMID: 21354373 DOI: 10.1016/S1470-2045(10)70294-1]

4 **Duijts SF**, Oldenburg HS, van Beurden M, Aaronson NK. Cognitive behavioral therapy and physical exercise for climacteric symptoms in breast cancer patients experiencing treatment-induced menopause: design of a multicenter trial. *BMC Womens Health* 2009; **9**: 15 [PMID: 19500403 DOI: 10.1186/1472-6874-9-15]

5 **Hu B**. Application of Wearable Technology in Clinical Walking and Dual Task Testing. *J Transl Int Med* 2019; **7**: 87-89 [PMID: 31637177 DOI: 10.2478/jtim-2019-0019]

6 **Zhou J**, Yu Y, Cao B, Li X, Wu M, Wen T, Xiong Y, Jia J, Zhao Y. Characteristic of Clinical Studies on Baduanjin during 2000-2019: A Comprehensive Review. *Evid Based Complement Alternat Med* 2020; **2020**: 4783915 [PMID: 33149753 DOI: 10.1155/2020/4783915]

7 **Lansdorp-Vogelaar I**, von Karsa L; International Agency for Research on Cancer. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition--Introduction. *Endoscopy* 2012; **44** Suppl 3: SE15-SE30 [PMID: 23012118 DOI: 10.1055/s-0032-1308898]

8 **Cheung YT**, Lim SR, Shwe M, Tan YP, Chan A. Psychometric properties and measurement equivalence of the English and Chinese versions of the functional assessment of cancer therapy-cognitive in Asian patients with breast cancer. *Value Health* 2013; **16**: 1001-1013 [PMID: 24041350 DOI: 10.1016/j.jval.2013.06.017]

9 **Cheung YT**, Shwe M, Chui WK, Chay WY, Ang SF, Dent RA, Yap YS, Lo SK, Ng RC, Chan A. Effects of chemotherapy and psychosocial distress on perceived cognitive disturbances in Asian breast cancer patients. *Ann Pharmacother* 2012; **46**: 1645-1655 [PMID: 23249868 DOI: 10.1345/aph.1R408]

10 **Okuyama T**, Akechi T, Kugaya A, Okamura H, Imoto S, Nakano T, Mikami I, Hosaka T, Uchitomi Y. Factors correlated with fatigue in disease-free breast cancer patients: application of the Cancer Fatigue Scale. *Support Care Cancer* 2000; **8**: 215-222 [PMID: 10789963 DOI:

10.1007/s005200050288]
11 **Finnegan-John J**, Molassiotis A, Richardson A, Ream E. A systematic review of complementary and alternative medicine interventions for the management of cancer-related fatigue. *Integr Cancer Ther* 2013; **12**: 276-290 [PMID: 23632236 DOI: 10.1177/1534735413485816]
12 **Okuyama T**, Akechi T, Kugaya A, Okamura H, Shima Y, Maruguchi M, Hosaka T, Uchitomi Y. Development and validation of the cancer fatigue scale: a brief, three-dimensional, self-rating scale for assessment of fatigue in cancer patients. *J Pain Symptom Manage* 2000; **19**: 5-14 [PMID: 10687321 DOI: 10.1016/s0885-3924(99)00138-4]
13 **Ward WL**, Hahn EA, Mo F, Hernandez L, Tulsky DS, Cella D. Reliability and validity of the Functional Assessment of Cancer Therapy-Colorectal (FACT-C) quality of life instrument. *Qual Life Res* 1999; **8**: 181-195 [PMID: 10472150 DOI: 10.1023/a:1008821826499]
14 **Cruzado J**, Sánchez FI, Abellán JM, Pérez-Riquelme F, Carballo F. Economic evaluation of colorectal cancer (CRC) screening. *Best Pract Res Clin Gastroenterol* 2013; **27**: 867-880 [PMID: 24182607 DOI: 10.1016/j.bpg.2013.09.004]
15 **Zhang P**, Mo L, Torres J, Huang X. Effects of cognitive behavioral therapy on psychological adjustment in Chinese pediatric cancer patients receiving chemotherapy: A randomized trial. *Medicine (Baltimore)* 2019; **98**: e16319 [PMID: 31277176 DOI: 10.1097/MD.0000000000016319]
16 **Chan JS**, Ho RT, Chung KF, Wang CW, Yao TJ, Ng SM, Chan CL. Qigong exercise alleviates fatigue, anxiety, and depressive symptoms, improves sleep quality, and shortens sleep latency in persons with chronic fatigue syndrome-like illness. *Evid Based Complement Alternat Med* 2014; **2014**: 106048 [PMID: 25610473 DOI: 10.1155/2014/106048]
17 **Zhang Q**, Li F, Zhang H, Yu X, Cong Y. Effects of nurse-led home-based exercise & cognitive behavioral therapy on reducing cancer-related fatigue in patients with ovarian cancer during and after chemotherapy: A randomized controlled trial. *Int J Nurs Stud* 2018; **78**: 52-60 [PMID: 28939343 DOI: 10.1016/j.ijnurstu.2017.08.010]
18 **Ahlberg K**, Ekman T, Gaston-Johansson F, Mock V. Assessment and management of cancer-related fatigue in adults. *Lancet* 2003; **362**: 640-650 [PMID: 12944066 DOI: 10.1016/S0140-6736(03)14186-4]
19 **Mohandas H**, Jaganathan SK, Mani MP, Ayyar M, Rohini Thevi GV. Cancer-related fatigue treatment: An overview. *J Cancer Res Ther* 2017; **13**: 916-929 [PMID: 29237952 DOI: 10.4103/jcrt.JCRT_50_17]
20 **Ryan JL**, Carroll JK, Ryan EP, Mustian KM, Fiscella K, Morrow GR. Mechanisms of cancer-related fatigue. *Oncologist* 2007; **12** Suppl 1: 22-34 [PMID: 17573453 DOI: 10.1634/theoncologist.12-S1-22]
21 **Scott K**, Posmontier B. Exercise Interventions to Reduce Cancer-Related Fatigue and Improve Health-Related Quality of Life in Cancer Patients. *Holist Nurs Pract* 2017; **31**: 66-79 [PMID: 28181972 DOI: 10.1097/HNP.0000000000000194]
22 **Jong MC**, Boers I, Schouten van der Velden AP, Meij SV, Göker E, Timmer-Bonte ANJH, van Wietmarschen HA. A Randomized Study of Yoga for Fatigue and Quality of Life in Women with Breast Cancer Undergoing (Neo) Adjuvant Chemotherapy. *J Altern Complement Med* 2018; **24**: 942-953 [PMID: 30247961 DOI: 10.1089/acm.2018.0191]
23 **Cruzado JA**, López-Santiago S, Martínez-Marín V, José-Moreno G, Custodio AB, Feliu J. Longitudinal study of cognitive dysfunctions induced by adjuvant chemotherapy in colon cancer patients. *Support Care Cancer* 2014; **22**: 1815-1823 [PMID: 24535240 DOI: 10.1007/s00520-014-2147-x]
24 **Gottschalk LA**, Holcombe RF, Jackson D, Bechtel RJ. The effects of anticancer chemotherapeutic drugs on cognitive function and other neuropsychiatric dimensions in breast cancer patients. *Methods Find Exp Clin Pharmacol* 2003; **25**: 117-122 [PMID: 12731457 DOI: 10.1358/mf.2003.25.2.723685]
25 **Cox EP**, O'Dwyer N, Cook R, Vetter M, Cheng HL, Rooney K, O'Connor H. Relationship between physical activity and cognitive function in apparently healthy young to middle-aged adults: A systematic review. *J Sci Med Sport* 2016; **19**: 616-628 [PMID: 26552574 DOI: 10.1016/j.jsams.2015.09.003]
26 **Ferguson RJ**, McDonald BC, Rocque MA, Furstenberg CT, Horrigan S, Ahles TA, Saykin AJ. Development of CBT for chemotherapy-related cognitive change: results of a waitlist control trial. *Psychooncology* 2012; **21**: 176-186 [PMID: 22271538 DOI: 10.1002/pon.1878]
27 **Higuchi Y**, Sumiyoshi T, Tateno T, Nakajima S, Sasabayashi D, Nishiyama S, Mizukami Y, Takahashi T, Suzuki M. Prolonged P300 Latency in Antipsychotic-Free Subjects with At-Risk Mental States Who Later Developed Schizophrenia. *J Pers Med* 2021; **11** [PMID: 33919276 DOI: 10.3390/jpm11050327]
28 **Hong JS**, Lee JH, Yoon YH, Choi JH, Shin JE, Kim SM, Park YG. The assessment of reliability of cognitive evoked potential in normal person. *Ann Rehabil Med* 2013; **37**: 263-268 [PMID: 23705123 DOI: 10.5535/arm.2013.37.2.263]
29 **Delle-Vigne D**, Kornreich C, Verbanck P, Campanella S. The P300 component wave reveals differences in subclinical anxious-depressive states during bimodal oddball tasks: An effect of stimulus congruence. *Clin Neurophysiol* 2015; **126**: 2108-2123 [PMID: 25703941 DOI: 10.1016/j.clinph.2015.01.012]
30 **Liu M**, Sun W, Cai YY, Wu HZ. Validation of Quality of Life Instruments for Cancer Patients - Colorectal Cancer (QLICP-CR) in patients with colorectal cancer in Northeast China. *BMC Cancer* 2018; **18**: 1228 [PMID: 30526549 DOI: 10.1186/s12885-018-5135-6]

31 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]

32 **Eyl RE**, Xie K, Koch-Gallenkamp L, Brenner H, Arndt V. Quality of life and physical activity in long-term (≥5 years post-diagnosis) colorectal cancer survivors - systematic review. *Health Qual Life Outcomes* 2018; **16**: 112 [PMID: 29859108 DOI: 10.1186/s12955-018-0934-7]

33 **Ho RTH**, Wan AHY, Chan JSM, Ng SM, Chung KF, Chan CLW. Study protocol on comparative effectiveness of mindfulness meditation and qigong on psychophysiological outcomes for patients with colorectal cancer: a randomized controlled trial. *BMC Complement Altern Med* 2017; **17**: 390 [PMID: 28789681 DOI: 10.1186/s12906-017-1898-6]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 334-347

DOI: 10.4251/wjgo.v14.i1.334

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

Exosomes as potential diagnosis and treatment for liver cancer

Xiao-Cui Wei, Li-Juan Liu, Fan Zhu

ORCID number: Xiao-Cui Wei 0000-0003-4606-2066; Li-Juan Liu 0000-0002-2549-5463; Fan Zhu 0000-0001-7031-2956.

Author contributions: Wei XC and Liu LJ equally contributed to this manuscript; Wei XC designed the research, drafted and revised the paper; Liu LJ analyzed the data, drafted and revised the paper; Zhu F was responsible for the design, edit, and revision of the article and supervised the manuscript; all authors read and approved the final manuscript.

Conflict-of-interest statement: All authors do not have any conflicts of interest relevant to this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Supported by National Natural Science Foundation of China, No. 81971943 and No. 81772196; and the Medical Science Advancement Program (Clinical Medicine) of Wuhan University, No. TFLC 2018003.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Provenance and peer review:

Xiao-Cui Wei, Li-Juan Liu, Fan Zhu, State Key Laboratory of Virology, Hubei Province Key Laboratory of Allergy and Immunology, Department of Pathogenic Biology, School of Medicine, Wuhan University, Wuhan 430071, Hubei Province, China

Corresponding author: Fan Zhu, PhD, Professor, State Key Laboratory of Virology, Hubei Province Key Laboratory of Allergy and Immunology, Department of Pathogenic Biology, School of Medicine, Wuhan University, No. 185 Donghu Road, Wuhan 430071, Hubei Province, China. fanzhu@whu.edu.cn

Abstract

BACKGROUND

Liver cancer is the fourth most significant cause of cancer-related death. Lack of early diagnosis strategy and a scarcity of efficient therapy constitute the main reasons for its lethality. Exosomes, which contain various bioactive molecules, are characterized by high biocompatibility, low immunogenicity, and high transport efficiency. As a result, exosomes have become a research hotspot and present significant potential for cancer diagnosis biomarkers, biotherapeutics, therapy targets, drug carriers and therapeutic agents.

AIM

To explore the potential of exosomes in the diagnosis and treatment of liver cancer.

METHODS

We conducted a systematic literature search *via* PubMed and Web of Science. The following keywords were used: "exosomal biomarkers", "exosomal therapy", "exosomal therapy", and "liver cancer" or "HCC". The duplicate data were deleted by EndNote software. Literature search focused on full-texts and references of each article were carefully checked. One author (Xiao-Cui Wei) screened the literature that met the following inclusion criteria: (1) Detection of exosomes or their contents in clinical samples (body fluid or tissue); or (2) Exosomes served as drug carriers or therapeutic factors. Two authors (Xiao-Cui Wei and Li-Juan Liu) independently reviewed all retained literature and analyzed the information.

RESULTS

A total of 1295 studies were identified using the systematic literature search. Of these, 835 duplicate studies were removed. A further 402 irrelevant studies were excluded due to being irrelevant, including other diseases, review articles, the literature containing neither clinical samples nor animal experiments, exosome-independent studies, methods for detecting exosomes, or articles in Chinese. Finally, 58 published papers were retained and analyzed in the study. It showed a

Invited article; Externally peer reviewed.

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 22, 2021
Peer-review started: March 22, 2021
First decision: June 14, 2021
Revised: July 3, 2021
Accepted: September 8, 2021
Article in press: September 8, 2021
Published online: January 15, 2022

P-Reviewer: de Melo FF
S-Editor: Ma YJ
L-Editor: Kerr C
P-Editor: Yu HG

list of potential exosomal biomarkers that were upregulated in the blood samples of patients with liver cancer. Those downregulated in exosomes might serve as possible biotherapeutics. Some exosomes derived from cells in vitro were used for cytology or animal experiments to explore the mechanism of these exosome contents in disease. These contents might serve as potential targets for liver cancer. Additionally, we also discussed that exosomes serve as drug carriers or therapeutic factors.

CONCLUSION
Exosomes might serve as potential biomarkers or therapeutic biotargets in liver cancer and have the potential to act as drug carriers and self-treatment factors for liver cancer patients.

Key Words: Exosomes; Liver cancer; Biomarker; Treatment; Drug delivery system

Core tip: We used a literature search to identify potential exosome diagnostic markers and novel therapeutic strategies for liver cancer. The latest literature was published in June 2021. Results were presented in tabular form, including 40 potential liver cancer biomarkers, 13 potential biotherapeutics, and 10 potential therapeutic targets for hepatocellular carcinoma. In addition, we also listed papers about exosomes as drug carriers and therapeutic factors.

Citation: Wei XC, Liu LJ, Zhu F. Exosomes as potential diagnosis and treatment for liver cancer. *World J Gastrointest Oncol* 2022; 14(1): 334-347
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/334.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.334

INTRODUCTION

Liver cancer is a common malignancy and the fourth leading cause of cancer death worldwide[1]. It is one of the most challenging cancers to treat. For patients with an early stage of liver cancer, surgical treatment is the standard of care. However, most patients with liver cancer are already in the advanced stage at the initial diagnosis, which results in a poor prognosis[2]. Currently, α-fetoprotein (AFP) is the most commonly used serum marker for liver cancer[3]. However, AFP has a sensitivity of 41%–64% and a specificity of 80%–94%, which is often missed diagnosis, especially in the early stages of liver cancer[4]. Therefore, it is vital to develop more sensitive and specific liver cancer biomarkers to improve patient survival.

Recent studies have shown that exosomes have potential as biomarkers for liver cancer[5]. Once considered cellular waste, exosomes are rich in bioactive molecules, such as proteins, lipids, and nucleic acids[6,7]. Almost all human cells can secrete exosomes. Tumor cells release more exosomes than normal cells, and the exosome contents of tumor cells are different from those of normal cells[8,9]. Additionally, the exosomal envelope protects proteins, nucleic acids, and other substances in exosomes from degradation by extramembrane enzymes[10]. The stability and abundance of exosome contents show the advantages of its unique liver cancer biomarkers.

Exosomes are widely involved in cell-to-cell communication. They can deliver their functional RNAs and proteins to recipient cells and affect their physiological functions [11]. Therefore, exosomes can also serve as drug delivery vehicles. Here, we summarize the potential of exosome contents in the diagnosis and treatment of liver cancer, provide new ideas for the diagnosis and treatment of liver cancer, and promote further research on the potential clinical applications of exosomes.

MATERIALS AND METHODS

Literature search

According to the conventional research methods of systematic review[12], a systematic literature search was conducted in PubMed and Web of Science using the following keywords: "exosomal biomarkers", "exosomal therapy", "exosomal therapy" and "liver cancer" or "HCC". The EndNote software was used to delete duplicate data[13]. The latest literature was published in June 2021. Literature search focused on full texts. Two reviewers independently screened the references of each article to remove the irrelevant studies according to our inclusion criteria. The inclusion criteria were as follows: (1) Detection of exosomes or their contents in clinical samples (body fluid or tissue); or (2) Exosomes served as drug carriers or therapeutic factors. Two authors (Xiao-Cui Wei and Li-Juan Liu) independently reviewed the full texts of all retained literature and analyzed the information.

Data extraction

The data collected from each study included the clinical sample, expression level, and application of exosomes divided into three major segments. The first part involved the exosomes isolated from the body fluid samples. The second part meant the data that were relevant to the detection of exosomal contents in the clinical tissue samples. The third part included the collection of data pertinent to the application of exosomes.

RESULTS

Literature selection

A total of 1295 studies were identified using the systematic literature search. After 835 duplicate studies were found and omitted, 460 were screened by two independent reviewers. A further 402 irrelevant studies were excluded, including review articles, other diseases, records containing neither clinical samples nor animal experiments, exosome-independent studies, methods for detecting exosome or articles in Chinese. Finally, 58 published papers were included in the study (Figure 1).

Exosomes are identified as potential biomarkers or potential biotherapeutics

In some literature, exosomes were isolated from liver cancer patients' blood samples. Then, the level of exosomal molecular contents was detected. Table 1[14-46] lists the potential biomarkers for liver cancer. In these studies, exosomal contents that were upregulated in blood exosomes might be potential exosomal biomarkers.

Table 2[22,35,39,47-56] includes potential biotherapeutics of exosomal contents for liver cancer. Those downregulated exosomal contents in blood liver cancer samples might serve as possible biotherapeutic drugs.

Exosomal contents are identified as potential therapeutic targets

The expression of exosomal contents was detected in liver cancer clinical tissue samples, and cytology or animal experiments were used to identify the role of exosomal contents. Upregulated exosomal contents might enhance hepatocellular carcinoma (HCC) progression, angiogenesis, and drug resistance, while downregulated exosomal contents might attenuate angiogenesis. In Table 3[57-66], all these abnormally expressed exosomal contents may become novel therapeutic targets for liver cancer.

Exosomes serve as drug carriers and therapeutic factors

Table 4[67-69] focuses on the carrier roles of exosomes in HCC. Drug-carrying exosomes were injected into tumor-prone mice to observe the effects of the drugs. These studies indicated that exosomes could serve as drug carriers that made cancer cells sensitive to antitumor drugs or enhanced their antitumor efficacy.

Table 5[70,71] shows the self-derived exosomes from dendritic cells as potential therapeutic factors. Data showed exosomes isolated from dendritic cells could inhibit tumor growth and improve the immune response. This indicated that exosomes serve as potential therapeutic factors.

Table 1 Potential biomarkers for liver cancer

Exosomal content	Sample	Expression	Isolation of exosomes	Content detection	Function	Ref.	Direction
HCC							
Proteins							
ANGPT2	Serum (n = 93)	Up	SBI	Immunoblotting and ELISA	Induces tumor angiogenesis	[14]	Potential targets
mRNAs							
hnRNPH1	Serum (n = 223)	Up	Total exosome isolation reagent (Thermo Fisher Scientific Co.)	qRT-PCR	Associated with the Child–Pugh classification, portal vein tumor emboli, lymph node metastasis, TNM stage, and OS	[15]	
LDH-C4	Serum (n = 212)	Up	exoRNeasy Serum/Plasma Midi Kit (Qiagen)	qRT-PCR	Related to treatments and recurrence prediction of HCC patients	[16]	
miRNAs							
miR-10b-5p	Serum (n = 37)	Up	Ultracentrifugation	qRT-PCR	Respectively, associated with early diagnosis and prognosis of HCC	[17]	
miR-1247-3p	Serum (n = 135)	Up	Ultracentrifugation	qRT–PCR	Shows a positive correlation with lung metastasis in HCC patients	[18]	Potential targets
miR-125b	Serum (n = 218)	Up	SBI	qRT-PCR	Discriminate HCC patients with a high risk of recurrence and poor prognosis	[19]	
miR-182	Serum and ascitic fluid	Up	exoRNeasy Serum/Plasma Midi Kit (Qiagen)	qRT-PCR	Up-regulated in NASH-induced liver cirrhosis with HCC compared to NASH-induced liver cirrhosis without HCC	[20]	
miR-21	Serum (n = 79)	Up	SBI	qRT-PCR	Related to TNM stage and other prognostic factors	[21]	
	Plasma (n = 150)	Up	SBI	qRT-PCR	Significantly higher in patients with HCC compared with cirrhotic patients and the control group	[22]	
	Serum (n = 90)	Up	Total Exosome Isolation Reagent (Invitrogen)	qRT-PCR	Positively correlated with cirrhosis and tumor stage	[23]	
	Serum (n = 95)	Up	Ultracentrifugation	qRT–PCR	Shows a positive correlation with survival in HCC patients	[24]	Potential targets
miR-215-5p	Serum (n = 37)	Up	Ultracentrifugation	qRT-PCR	Respectively, associated with early diagnosis and prognosis of HCC	[17]	
miR-224	Serum (n = 139)	Up	Total Exosome Isolation Kit	qRT–PCR	Related to tumor size and differentiate HCC patients from healthy controls	[25]	Potential targets
miR23-a/b	Serum (n = 50)	Up	Ultracentrifugation	qRT–PCR	A promising target for future treatment of HCC	[26]	Potential targets
miR-301a	Serum and ascitic fluid (n = 52)	Up	exoRNeasy Serum/Plasma Midi Kit (Qiagen)	qRT-PCR	Up-regulated in NASH-induced liver cirrhosis with HCC compared to NASH-induced liver cirrhosis without HCC	[20]	
miR-373	Serum and ascitic fluid (n = 52)	Up	exoRNeasy Serum/Plasma Midi Kit (Qiagen)	qRT-PCR	Up-regulated in NASH-induced liver cirrhosis with HCC compared to NASH-induced liver cirrhosis without HCC	[20]	
miR-4661-5p	Serum (n = 720)	Up	SBI	qRT-PCR	Associated with the prognosis of patients with HCC	[27]	

miR-638	Serum (n = 54)	Up	Ultracentrifugation	qRT–PCR	Promising for surveillance of HCC recurrence	[28]	Potential targets
miR-665	Serum (n = 40)	Up	SBI	qRT–PCR	Associated with tumor size, invasion, and clinical stage of HCC patients	[29]	Potential targets
miR-92a-3p	Plasma (n = 42)	Up	Ultracentrifugation	qRT–PCR	Shows a positive correlation with metastasis in HCC patients	[30]	Potential targets
miR-92b	Serum (n = 121)	Up	SBI	qRT-PCR	Prediction of posttransplant HCC early recurrence	[31]	
miR-93	Serum (n = 108)	Up	Total Exosome Isolation Reagent (Invitrogen)	qRT–PCR	Correlated with stage, tumor size and predict patients' survival rate of HCC patients	[32]	Potential targets
miRNA-96	Plasma (n = 150)	Up	SBI	qRT-PCR	Significantly higher in patients with HCC compared with cirrhotic patients and the control group	[22]	
lncRNAs							
lncRNA-ATB	Serum (n = 79)	Up	SBI	qRT-PCR	Related to TNM stage and other prognostic factors	[21]	
DANCR	Serum (n = 183)	Up	SBI	Digital droplet PCR (DDPCR)	Positively associated with HCV-HCC recurrence	[33]	
lncRNA FAL1	Serum (n = 60)	Up	SBI	qRT-PCR	Play an oncogenic role in HCC	[34]	Potential targets
lnc-FAM72D-3	Serum (n = 180)	Up	Ultracentrifugation	qRT-PCR	Functions as an oncogene in HCC	[35]	Potential targets
lncRNA Jpx	Plasma (n = 100)	Up	SBI	qRT-PCR	Promising biomarkers for female patients with HCC	[36]	
LINC00161	Serum (n = 112)	Up	Total Exosome Isolation Kit (Invitrogen)	qRT-PCR	A significant prediction of tumor growth and metastasis in HCC	[37]	
	Serum (n = ?)	Up	-	qRT-PCR	Promote HCC tumorigenesis	[38]	Potential targets
lncRNA-RP11-583F2.2	Serum (n = 120)	Up	exoRNeasy Serum/Plasma Midi Kit (Qiagen)	qRT-PCR	Up-regulated in the serum of hepatocellular carcinoma patients as compared with hepatitis C virus patients and normal good health control	[39]	
ENSG00000248932.1 ENST00000440688.1 ENST00000457302.2	Serum (n = 600)	Up	SBI	qRT-PCR	Potential fingerprints for the tumorigenesis prediction	[40]	
circRNAs							
circ_0070396	Plasma (n = 273)	Up	exoEasy Maxi Kit (QIAGEN)	qRT-PCR	Discriminate HCC individuals from patients with chronic hepatitis B and liver cirrhosis	[41]	
circAKT3	Serum (n = 224)	Up	SBI	qRT-PCR	Associated with HCC recurrence and mortality	[42]	
circ-DB	Plasma (n = 40)	Up	Ultracentrifugation	qRT-PCR	Promote the tumor growth	[43]	Potential targets
circPTGR1	Serum (n = 129)	Up	SBI	qRT-PCR	Promote HCC progression	[44]	Potential targets
circUHRF1	Serum (n = 643)	Up	SBI	qRT-PCR	Drive resistance to anti-PD1 immunotherapy	[45]	Potential targets
HB							
miRNAs							
miR-21	Serum (n = 64)	Up	SBI	qRT-PCR	Significantly higher in patients with HB	[46]	

Up: Upregulated; SBI: Exo-Quick exosome precipitation solution; HCC: Hepatocellular carcinoma; HB: Hepatoblastoma; qRT-PCR: Quantitative reverse transcription polymerase chain reaction.

DISCUSSION

Liver cancer is a global disease with high morbidity and mortality[72]. Despite the continuous development of novel treatment options, the 5-year survival rate of liver cancer patients is still low because of the delayed diagnosis[73,74]. Scientists are still trying to find new markers for early diagnosis and individualized treatments.

Over the past decade, exosomes have received widespread attention. Many studies have found that the differential expression of exosome proteins and RNAs has diagnostic significance for various cancers. Previous studies have suggested that exosomes may serve as liquid biopsies to help diagnose malignancies such as breast, pancreatic and lung cancer, and glioblastoma[75-78]. Here, we listed exosomal contents that have been identified as possible biomarkers for liver cancer in recent years. We found multiple research reports about miR-21[21-24,46] and LINC00161[37, 38]. There are five papers on exosomal miR-21. These studies indicate that expression level of miR-21 in serum exosomes of liver cancer patients is higher than that of healthy people, suggesting that it is the most likely marker for early liver cancer screening. Among the contents of liver cancer serum with downregulated exosomal expression, miR-122 has been reported most often. These studies suggest that miR-122 may be the most likely biotherapeutic drug for liver cancer[22,47].

In addition to serving as disease markers in patients' serum, exosomes are involved in the occurrence, development and prognosis of various cancers[79]. Bai *et al*[80] have shown that exosomes secreted by gastric cancer cells deliver miR-135b to tumor cells and promote angiogenesis by negatively regulating intracellular forkhead box O1. This study provides a potential target for antiangiogenic therapy. Huang and his collaborators demonstrated that colon cancer cells secrete Wnt4-rich exosomes delivered to normoxic cells to activate β-catenin signaling and enhance their metastatic behavior. They found that β-catenin inhibitors ICG-001 can inhibit this metastatic behavior, which provides a new target for treating metastatic colon cancer[81]. In this paper, we listed the previous studies on the mechanism of exosomal contents involved in the development of liver cancer. Therefore, developing drugs targeting these exosomal contents may be a potential therapy for liver cancer.

As drug carriers, exosomes have the characteristics of stability in circulation, good biocompatibility, low immunogenicity, and low toxicity[82,83]. Liang *et al*[84] have shown that exosomes loaded with 5-fluorouracil and miR-21 inhibitors can effectively improve cancer cell drug resistance and colon cancer treatment efficiency. Zhang and his group also found that HEK293T-cell-derived exosomes deliver exogenous si-c-Met to gastric cancer cells and enhance gastric cancer cell sensitivity to cisplatin[85]. In this paper, we reviewed recent studies on the therapeutic effect of exosomes as carriers in HCC.

In addition to being carriers, some researchers have reported the therapeutic effect of exosomes. As early as 1998, Zitvogel *et al*[86] found that dendritic-cell-derived exosomes (DEXs) could activate tumor-specific cytotoxic T lymphocyte response and inhibit tumor growth *in vivo*. DEXs have been used in several clinical trials. Researchers have processed DEXs derived from melanoma patients, loaded them with melanoma antigens, and observed an enhanced antimelanoma immunity after self-inoculation[87]. Another trial indicated that DEX therapy increases natural killer cells (NKs) lytic activity in patients with non-small cell lung cancer (NSCLC)[88]. Besse's group has conducted phase II clinical trials in NSCLC and confirmed the capacity of DEXs to boost the NK cell arm of antitumor immunity in patients with advanced NSCLC[89]. In addition to injecting DEXs, Dai and colleagues have found that the immunotherapy of colorectal cancer (CRC) with ascites-derived exosomes in combination with granulocyte–macrophage colony-stimulating factor can serve as a choice for immunotherapy of advanced CRC[90]. In liver cancer, however, there have been no such clinical trials.

Although exosomes present good application value, there are still problems with their clinical application. Firstly, the separation and purification of exosomes are complex. Secondly, the contents in exosomes are not unique. Thirdly, not all exosomes secreted by cells are suitable for use as carriers. Although there are currently small-scale clinical trials, the actual application of exosomes in the clinical diagnosis and treatment of liver cancer still needs more in-depth studies.

Table 2 Potential therapeutic drugs

Exosomal content	Sample	Expression	Isolation of exosomes	Content detection	Function	Ref.
HCC						
miRNAs						
miR-122	Serum (n = 75)	Down	SBI	qRT-PCR	Reflect the liver damage and residual liver function levels	[47]
	Plasma (n = 150)	Down	SBI	qRT-PCR	Significantly lower in patients with HCC compared with cirrhotic patients and the control group	[22]
miRNA-1298	Serum (n = 120)	Down	exoRNeasy Serum/Plasma MidiKit (Qiagen)	qRT-PCR	Down-regulated in patients of hepatocellular carcinoma compared with patients of hepatitis C virus and normal good health control	[39]
miR-320a	Serum (n = 209)	Down	SBI	qRT-PCR	Associated with lymph node metastasis, vein invasion, TNM stage, and survival of HCC patients	[48]
miR-320d	Serum (n = 150)	Down	Total Exosome Isolation Kit (Invitrogen)	qRT-PCR	Associated with clinicopathological parameters and prognosis of HCC patients	[49]
miR-638	Serum (n = 147)	Down	Total Exosome Isolation Kit (Invitrogen)	qRT-PCR	Influence liver carcinogenesis	[50]
miR-718	Serum (n = 59)	Down	Ultracentrifugation	qRT-PCR	Significantly different expression of HCC cases with recurrence after LT compared with those without recurrence	[51]
miR-744	Serum (n = 20)	Down	Ultracentrifugation	qRT–PCR	Facilitates the propagation and drug resistance of HCC cells	[52]
miR-9-3p	Serum (n = ?)	Down	Ultracentrifugation	qRT-PCR	A potential therapeutic target for HCC	[53]
lncRNAs						
lnc-EPC1-4	Serum (n = 180)	Down	Ultracentrifugation	qRT-PCR	Function as a tumor suppressor gene	[35]
SENP3-EIF4A1	Serum (n = 6)	Down	SBI	qRT-PCR	Block HCC progression	[54]
circRNAs						
circ-0051443	Plasma (n = 120)	Down	SBI	qRT-PCR	Suppress HCC progression	[55]
HB						
miRNAs						
miR-34s	Serum (n = 152)	Down	SBI	qRT-PCR	Significantly lower in patients with HB compared with the control group	[56]

Down: Downregulated; SBI: Exo-Quick exosome precipitation solution; HCC: Hepatocellular carcinoma; HB: Hepatoblastoma; qRT-PCR: Quantitative reverse transcription polymerase chain reaction.

CONCLUSION

Exosomes are composed of a lipid bilayer membrane structure, which has the advantages of rich content, high stability, ability to reflect the state of disease, and cellular communication. These features make them a research hotspot for liver cancer for potential biomarkers, biotherapeutics, therapeutic targets, drug carriers, and therapeutic factors.

Table 3 Potential therapeutic targets

Exosomal content	Sample	Expression	Content identification	Animal model (Yes/No)	Function	Ref.
HCC						
Proteins						
ENO1	Cancer cells-exosomes, tissue (n = 94)	Up	IHC staining	Y	Promotes HCC growth, metastasis, and further patient deterioration	[57]
miRNAs						
miR-125a/b	TAMs-exosomes Tissue (n = 6)	Down	qRT-PCR	N	A possible therapeutic target in HCC	[58]
miR-150-3p	Fibroblasts-exosomes, tissues (n = 82)	Down	qRT–PCR	N	Abrogate HCC migration and invasiveness	[59]
miR-32-5p	Bel/5-FU-exosomes, tissue (n = 72)	Up	qRT–PCR	Y	Induce multidrug resistance in HCC	[60]
miR-320a	Cancer cells-exosomes, tissue (n = 6)	Down	qRT–PCR	Y	Mediates HCC tumor progression	[61]
miR-3682-3p	Cancer cells-exosomes, tissue (n = 8)	Down	qRT–PCR	Y	Attenuate angiogenesis and provides novel potential targets for liver cancer therapy	[62]
miR-378b	Cancer cells-exosomes, tissue (n = 105)	Up	qRT–PCR	Y	Enhance HCC cell progression and angiogenesis	[63]
lncRNAs						
ASMTL-AS1	Cancer cells-exosomes, tissues (n = 70)	Up	qRT–PCR	Y	Aggravate the malignancy in residual HCC	[64]
PCED1B-AS1	Cancer cells-exosomes, tissues (n = 45)	Up	qRT–PCR	Y	Induce immunosuppression in HCC	[65]
circRNAs						
circRNA Cdr1as	Cancer cells-exosomes, tissues (n = 42)	Up	qRT–PCR	Y	Promote the progression of HCC by sponging miR-1270 to upregulate AFP level	[66]

IHC: Immunohistochemistry; TAMs: Tumor-associated macrophages; Up: Upregulated; Down: Downregulated; HCC: Hepatocellular carcinoma; HB: Hepatoblastoma; qRT-PCR: Quantitative reverse transcription polymerase chain reaction.

Table 4 As a carrier for drug treatment

Drugs	Source of exosomes	Animal model (Yes/No)	Clinical sample (Yes/No)	Functions	Ref.
Norcantharidin	BMSCs-exosomes	Y	N	Induce cell cycle arrest, reduced tumor cell proliferation, increased apoptosis	[67]
siGRP78	BMSCs-exosomes	Y	N	Sensitize Sorafenib resistant cancer cells to Sorafenib	[68]
miR-214	hCEC-exosomes	N	Y (n = 6)	Enhances the anti-tumor efficacy of oxaliplatin and sorafenib on HCC cells	[69]

BMSCs: Bone marrow mesenchymal stem cell; hCEC: Human cerebral endothelial cell; HCC: Hepatocellular carcinoma.

Table 5 Exosomes from dendritic cells as potential therapeutic factors

Cargos	Source of exosomes	Animal model (Yes/No)	Clinical sample (Yes/No)	Functions	Ref.
Exosomes plus microwave ablation	DCs-exosomes	Y	N	Inhibit tumor growth and improve the immune microenvironment	[70]
Exosomes	DCs-exosomes	Y	N	Elicited strong antigen-specific immune responses and resulted in tumor growth retardation and prolonged survival rates in mice with ectopic	[71]

DCs: Dendritic cells.

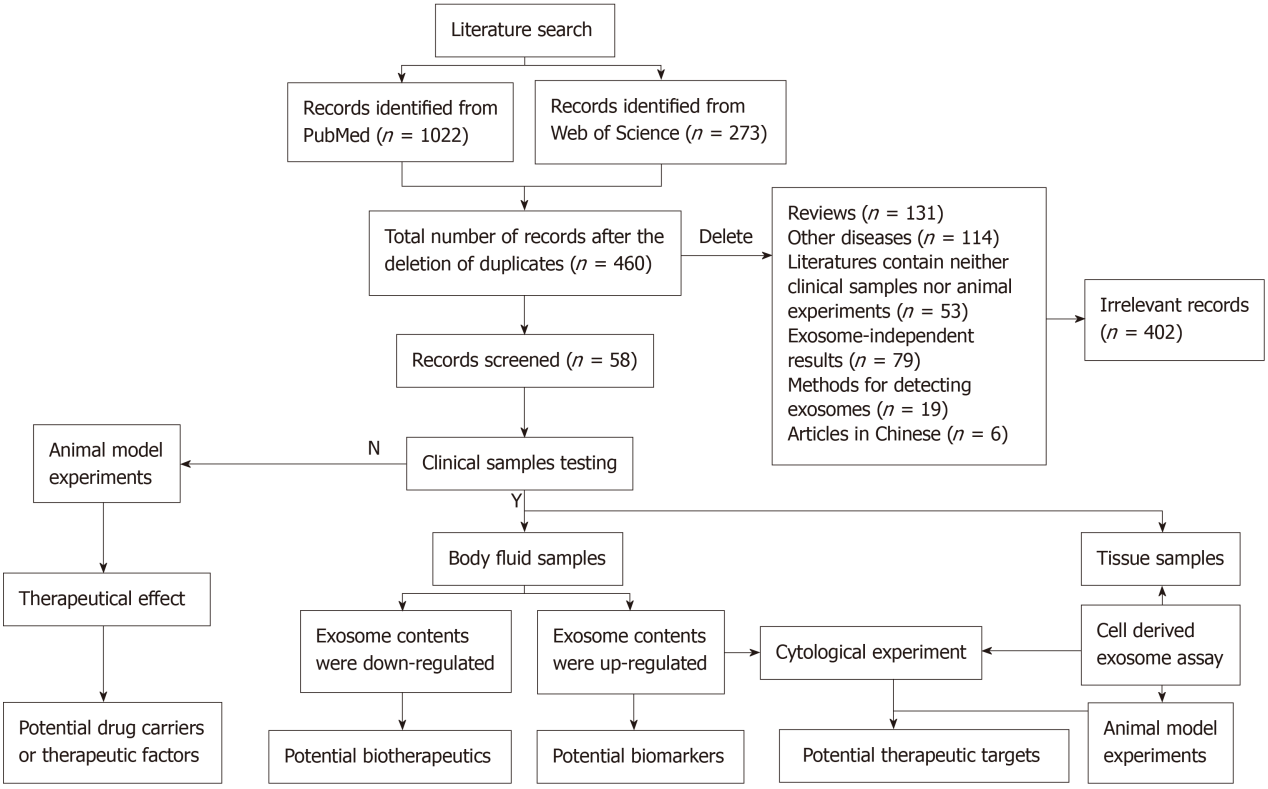


Figure 1 Flow diagram of the study search and selection in this review.

ARTICLE HIGHLIGHTS

Research background

Liver cancer is one of the most common malignant tumors with high morbidity and mortality because of lacking early diagnosis and treatment. Exosomes have been a newly discovered cellular communication tool with high biocompatibility, low immunogenicity, and high transport efficiency. They show great potential for cancer diagnosis and therapy.

Research motivation

This review aimed to consolidate the evidence on exosomes as biomarkers for the diagnosis and therapeutics for liver cancer in a systematic fashion.

Research objectives

The main result that the authors are concerned about is discovering the great potential of exosomes in the diagnosis and treatment of liver cancer.

Research methods
A systematic literature search was performed using PubMed and Web of Science. The latest literature was published in June 2021.

Research results
Fifty-eight studies were included in this systematic review. Blood-derived exosomes could be biomarkers or biotherapeutics. Cell-derived exosomes, which were used to explore underlying mechanisms of differentially expressed exosome contents in clinical tissue samples, might serve as potential therapeutic targets for liver cancer. Exosomes might also serve as drug carriers or therapeutic factors.

Research conclusions
Existing studies show that exosomes have great potential for clinical application as potential novel diagnostic and therapeutic markers of liver cancer.

Research perspectives
This present review might be helpful as a reference for clinical research on exosomes in liver cancer.

ACKNOWLEDGEMENTS
We are grateful to Wang Ying for her skillful statistical analysis guidance.

REFERENCES
1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics, 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Hollebecque A**, Malka D, Ferté C, Ducreux M, Boige V. Systemic treatment of advanced hepatocellular carcinoma: from disillusions to new horizons. *Eur J Cancer* 2015; **51**: 327-339 [PMID: 25559615 DOI: 10.1016/j.ejca.2014.12.005]
3 **Johnson PJ**. Role of alpha-fetoprotein in the diagnosis and management of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1999; **14** Suppl: S32-S36 [PMID: 10382636 DOI: 10.1046/j.1440-1746.1999.01873.x]
4 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50 [PMID: 12834318 DOI: 10.7326/0003-4819-139-1-200307010-00012]
5 **Liu H**, Li B. The functional role of exosome in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2018; **144**: 2085-2095 [PMID: 30062486 DOI: 10.1007/s00432-018-2712-7]
6 **Wortzel I**, Dror S, Kenific CM, Lyden D. Exosome-Mediated Metastasis: Communication from a Distance. *Dev Cell* 2019; **49**: 347-360 [PMID: 31063754 DOI: 10.1016/j.devcel.2019.04.011]
7 **Roma-Rodrigues C**, Fernandes AR, Baptista PV. Exosome in tumour microenvironment: overview of the crosstalk between normal and cancer cells. *Biomed Res Int* 2014; **2014**: 179486 [PMID: 24963475 DOI: 10.1155/2014/179486]
8 **Henderson MC**, Azorsa DO. The genomic and proteomic content of cancer cell-derived exosomes. *Front Oncol* 2012; **2**: 38 [PMID: 22649786 DOI: 10.3389/fonc.2012.00038]
9 **Bebelman MP**, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. *Pharmacol Ther* 2018; **188**: 1-11 [PMID: 29476772 DOI: 10.1016/j.pharmthera.2018.02.013]
10 **Li W**, Li C, Zhou T, Liu X, Li X, Chen D. Role of exosomal proteins in cancer diagnosis. *Mol Cancer* 2017; **16**: 145 [PMID: 28851367 DOI: 10.1186/s12943-017-0706-8]
11 **Ratajczak J**, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 2006; **20**: 847-856 [PMID: 16453000 DOI: 10.1038/sj.leu.2404132]
12 **Muka T**, Glisic M, Milic J, Verhoog S, Bohlius J, Bramer W, Chowdhury R, Franco OH. A 24-step guide on how to design, conduct, and successfully publish a systematic review and meta-analysis in medical research. *Eur J Epidemiol* 2020; **35**: 49-60 [PMID: 31720912 DOI: 10.1007/s10654-019-00576-5]
13 **Bramer WM**, Milic J, Mast F. Reviewing retrieved references for inclusion in systematic reviews using EndNote. *J Med Libr Assoc* 2017; **105**: 84-87 [PMID: 28096751 DOI: 10.5195/jmla.2017.111]
14 **Xie JY**, Wei JX, Lv LH, Han QF, Yang WB, Li GL, Wang PX, Wu SB, Duan JX, Zhuo WF, Liu PQ, Min J. Angiopoietin-2 induces angiogenesis *via* exosomes in human hepatocellular carcinoma. *Cell Commun Signal* 2020; **18**: 46 [PMID: 32183816 DOI: 10.1186/s12964-020-00535-8]

15 **Xu H**, Dong X, Chen Y, Wang X. Serum exosomal hnRNPH1 mRNA as a novel marker for hepatocellular carcinoma. *Clin Chem Lab Med* 2018; **56**: 479-484 [PMID: 29252188 DOI: 10.1515/cclm-2017-0327]

16 **Cui Z**, Li Y, Gao Y, Kong L, Lin Y, Chen Y. Cancer-testis antigen lactate dehydrogenase C4 in hepatocellular carcinoma: a promising biomarker for early diagnosis, efficacy evaluation and prognosis prediction. *Aging (Albany NY)* 2020; **12**: 19455-19467 [PMID: 33035196 DOI: 10.18632/aging.103879]

17 **Cho HJ**, Eun JW, Baek GO, Seo CW, Ahn HR, Kim SS, Cho SW, Cheong JY. Serum Exosomal MicroRNA, miR-10b-5p, as a Potential Diagnostic Biomarker for Early-Stage Hepatocellular Carcinoma. *J Clin Med* 2020; **9** [PMID: 31968558 DOI: 10.3390/jcm9010281]

18 **Fang T**, Lv H, Lv G, Li T, Wang C, Han Q, Yu L, Su B, Guo L, Huang S, Cao D, Tang L, Tang S, Wu M, Yang W, Wang H. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. *Nat Commun* 2018; **9**: 191 [PMID: 29335551 DOI: 10.1038/s41467-017-02583-0]

19 **Liu W**, Hu J, Zhou K, Chen F, Wang Z, Liao B, Dai Z, Cao Y, Fan J, Zhou J. Serum exosomal miR-125b is a novel prognostic marker for hepatocellular carcinoma. *Onco Targets Ther* 2017; **10**: 3843-3851 [PMID: 28814883 DOI: 10.2147/OTT.S140062]

20 **Muhammad Yusuf AN**, Raja Ali RA, Muhammad Nawawi KN, Mokhtar NM. Potential biomarkers in NASH-induced liver cirrhosis with hepatocellular carcinoma: A preliminary work on roles of exosomal miR-182, miR-301a, and miR-373. *Malays J Pathol* 2020; **42**: 377-384 [PMID: 33361718]

21 **Lee YR**, Kim G, Tak WY, Jang SY, Kweon YO, Park JG, Lee HW, Han YS, Chun JM, Park SY, Hur K. Circulating exosomal noncoding RNAs as prognostic biomarkers in human hepatocellular carcinoma. *Int J Cancer* 2019; **144**: 1444-1452 [PMID: 30338850 DOI: 10.1002/ijc.31931]

22 **Wang S**, Yang Y, Sun L, Qiao G, Song Y, Liu B. Exosomal MicroRNAs as Liquid Biopsy Biomarkers in Hepatocellular Carcinoma. *Onco Targets Ther* 2020; **13**: 2021-2030 [PMID: 32210570 DOI: 10.2147/OTT.S232453]

23 **Wang H**, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. *Biomed Res Int* 2014; **2014**: 864894 [PMID: 24963487 DOI: 10.1155/2014/864894]

24 **Zhou Y**, Ren H, Dai B, Li J, Shang L, Huang J, Shi X. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. *J Exp Clin Cancer Res* 2018; **37**: 324 [PMID: 30591064 DOI: 10.1186/s13046-018-0965-2]

25 **Cui Y**, Xu HF, Liu MY, Xu YJ, He JC, Zhou Y, Cang SD. Mechanism of exosomal microRNA-224 in development of hepatocellular carcinoma and its diagnostic and prognostic value. *World J Gastroenterol* 2019; **25**: 1890-1898 [PMID: 31057302 DOI: 10.3748/wjg.v25.i15.1890]

26 **Liu Y**, Tan J, Ou S, Chen J, Chen L. Adipose-derived exosomes deliver miR-23a/b to regulate tumor growth in hepatocellular cancer by targeting the VHL/HIF axis. *J Physiol Biochem* 2019; **75**: 391-401 [PMID: 31321740 DOI: 10.1007/s13105-019-00692-6]

27 **Cho HJ**, Baek GO, Seo CW, Ahn HR, Sung S, Son JA, Kim SS, Cho SW, Jang JW, Nam SW, Cheong JY, Eun JW. Exosomal microRNA-4661-5p-based serum panel as a potential diagnostic biomarker for early-stage hepatocellular carcinoma. *Cancer Med* 2020; **9**: 5459-5472 [PMID: 32537885 DOI: 10.1002/cam4.3230]

28 **Yokota Y**, Noda T, Okumura Y, Kobayashi S, Iwagami Y, Yamada D, Tomimaru Y, Akita H, Gotoh K, Takeda Y, Tanemura M, Murakami T, Umeshita K, Doki Y, Eguchi H. Serum exosomal miR-638 is a prognostic marker of HCC *via* downregulation of VE-cadherin and ZO-1 of endothelial cells. *Cancer Sci* 2021; **112**: 1275-1288 [PMID: 33426736 DOI: 10.1111/cas.14807]

29 **Qu Z**, Wu J, Ji A, Qiang G, Jiang Y, Jiang C, Ding Y. Exosomal miR-665 as a novel minimally invasive biomarker for hepatocellular carcinoma diagnosis and prognosis. *Oncotarget* 2017; **8**: 80666-80678 [PMID: 29113334 DOI: 10.18632/oncotarget.20881]

30 **Yang B**, Feng X, Liu H, Tong R, Wu J, Li C, Yu H, Chen Y, Cheng Q, Chen J, Cai X, Wu W, Lu Y, Hu J, Liang K, Lv Z, Zheng S. High-metastatic cancer cells derived exosomal miR92a-3p promotes epithelial-mesenchymal transition and metastasis of low-metastatic cancer cells by regulating PTEN/Akt pathway in hepatocellular carcinoma. *Oncogene* 2020; **39**: 6529-6543 [PMID: 32917956 DOI: 10.1038/s41388-020-01450-5]

31 **Nakano T**, Chen IH, Wang CC, Chen PJ, Tseng HP, Huang KT, Hu TH, Li LC, Goto S, Cheng YF, Lin CC, Chen CL. Circulating exosomal miR-92b: Its role for cancer immunoediting and clinical value for prediction of posttransplant hepatocellular carcinoma recurrence. *Am J Transplant* 2019; **19**: 3250-3262 [PMID: 31162867 DOI: 10.1111/ajt.15490]

32 **Xue X**, Wang X, Zhao Y, Hu R, Qin L. Exosomal miR-93 promotes proliferation and invasion in hepatocellular carcinoma by directly inhibiting TIMP2/TP53INP1/CDKN1A. *Biochem Biophys Res Commun* 2018; **502**: 515-521 [PMID: 29859935 DOI: 10.1016/j.bbrc.2018.05.208]

33 **Wang SC**, Li CY, Chang WT, Cheng WC, Yen CH, Tu WY, Lin ZY, Lin CC, Yeh ML, Huang CF, Huang JF, Dai CY, Chuang WL, Chen YL, Yu ML. Exosome-derived differentiation antagonizing non-protein coding RNA with risk of hepatitis C virus-related hepatocellular carcinoma recurrence. *Liver Int* 2021; **41**: 956-968 [PMID: 33346937 DOI: 10.1111/Liv.14772]

34 **Li B**, Mao R, Liu C, Zhang W, Tang Y, Guo Z. LncRNA FAL1 promotes cell proliferation and migration by acting as a CeRNA of miR-1236 in hepatocellular carcinoma cells. *Life Sci* 2018; **197**: 122-129 [PMID: 29421439 DOI: 10.1016/j.lfs.2018.02.006]

35 **Yao Z**, Jia C, Tai Y, Liang H, Zhong Z, Xiong Z, Deng M, Zhang Q. Serum exosomal long noncoding RNAs lnc-FAM72D-3 and lnc-EPC1-4 as diagnostic biomarkers for hepatocellular carcinoma. *Aging (Albany NY)* 2020; **12**: 11843-11863 [PMID: 32554864 DOI: 10.18632/aging.103355]

36 **Ma X**, Yuan T, Yang C, Wang Z, Zang Y, Wu L, Zhuang L. X-inactive-specific transcript of peripheral blood cells is regulated by exosomal Jpx and acts as a biomarker for female patients with hepatocellular carcinoma. *Ther Adv Med Oncol* 2017; **9**: 665-677 [PMID: 29344104 DOI: 10.1177/1758834017731052]

37 **Sun L**, Su Y, Liu X, Xu M, Chen X, Zhu Y, Guo Z, Bai T, Dong L, Wei C, Cai X, He B, Pan Y, Sun H, Wang S. Serum and exosome long non coding RNAs as potential biomarkers for hepatocellular carcinoma. *J Cancer* 2018; **9**: 2631-2639 [PMID: 30087703 DOI: 10.7150/jca.24978]

38 **You LN**, Tai QW, Xu L, Hao Y, Guo WJ, Zhang Q, Tong Q, Zhang H, Huang WK. Exosomal LINC00161 promotes angiogenesis and metastasis *via* regulating miR-590-3p/ROCK axis in hepatocellular carcinoma. *Cancer Gene Ther* 2021; **28**: 719-736 [PMID: 33414518 DOI: 10.1038/s41417-020-00269-2]

39 **Matboli M**, Labib ME, Nasser HE, El-Tawdi AHF, Habib EK, Ali-Labib R. Exosomal miR-1298 and lncRNA-RP11-583F2.2 Expression in Hepato-cellular Carcinoma. *Curr Genomics* 2020; **21**: 46-55 [PMID: 32655298 DOI: 10.2174/1389202920666191210111849]

40 **Lu Y**, Duan Y, Xu Q, Zhang L, Chen W, Qu Z, Wu B, Liu W, Shi L, Wu D, Yang Y, Sun D, Chen X. Circulating exosome-derived bona fide long non-coding RNAs predicting the occurrence and metastasis of hepatocellular carcinoma. *J Cell Mol Med* 2020; **24**: 1311-1318 [PMID: 31811749 DOI: 10.1111/jcmm.14783]

41 **Lyu L**, Yang W, Yao J, Wang H, Zhu J, Jin A, Liu T, Wang B, Zhou J, Fan J, Yang X, Guo W. The diagnostic value of plasma exosomal *hsa_circ_0070396* for hepatocellular carcinoma. *Biomark Med* 2021; **15**: 359-371 [PMID: 33666515 DOI: 10.2217/bmm-2020-0476]

42 **Luo Y**, Liu F, Gui R. High expression of circulating exosomal circAKT3 is associated with higher recurrence in HCC patients undergoing surgical treatment. *Surg Oncol* 2020; **33**: 276-281 [PMID: 32561093 DOI: 10.1016/j.suronc.2020.04.021]

43 **Zhang H**, Deng T, Ge S, Liu Y, Bai M, Zhu K, Fan Q, Li J, Ning T, Tian F, Li H, Sun W, Ying G, Ba Y. Exosome circRNA secreted from adipocytes promotes the growth of hepatocellular carcinoma by targeting deubiquitination-related USP7. *Oncogene* 2019; **38**: 2844-2859 [PMID: 30546088 DOI: 10.1038/s41388-018-0619-z]

44 **Wang G**, Liu W, Zou Y, Wang G, Deng Y, Luo J, Zhang Y, Li H, Zhang Q, Yang Y, Chen G. Three isoforms of exosomal circPTGR1 promote hepatocellular carcinoma metastasis *via* the miR449a-MET pathway. *EBioMedicine* 2019; **40**: 432-445 [PMID: 30630697 DOI: 10.1016/j.ebiom.2018.12.062]

45 **Zhang PF**, Gao C, Huang XY, Lu JC, Guo XJ, Shi GM, Cai JB, Ke AW. Cancer cell-derived exosomal circUHRF1 induces natural killer cell exhaustion and may cause resistance to anti-PD1 therapy in hepatocellular carcinoma. *Mol Cancer* 2020; **19**: 110 [PMID: 32593303 DOI: 10.1186/s12943-020-01222-5]

46 **Liu W**, Chen S, Liu B. Diagnostic and prognostic values of serum exosomal microRNA-21 in children with hepatoblastoma: a Chinese population-based study. *Pediatr Surg Int* 2016; **32**: 1059-1065 [PMID: 27601233 DOI: 10.1007/s00383-016-3960-8]

47 **Suehiro T**, Miyaaki H, Kanda Y, Shibata H, Honda T, Ozawa E, Miuma S, Taura N, Nakao K. Serum exosomal microRNA-122 and microRNA-21 as predictive biomarkers in transarterial chemoembolization-treated hepatocellular carcinoma patients. *Oncol Lett* 2018; **16**: 3267-3273 [PMID: 30127924 DOI: 10.3892/ol.2018.8991]

48 **Hao X**, Xin R, Dong W. Decreased serum exosomal miR-320a expression is an unfavorable prognostic factor in patients with hepatocellular carcinoma. *J Int Med Res* 2020; **48**: 300060519896144 [PMID: 32339037 DOI: 10.1177/0300060519896144]

49 **Li W**, Ding X, Wang S, Xu L, Yin T, Han S, Geng J, Sun W. Downregulation of serum exosomal miR-320d predicts poor prognosis in hepatocellular carcinoma. *J Clin Lab Anal* 2020; **34**: e23239 [PMID: 32125733 DOI: 10.1002/jcla.23239]

50 **Shi M**, Jiang Y, Yang L, Yan S, Wang YG, Lu XJ. Decreased levels of serum exosomal miR-638 predict poor prognosis in hepatocellular carcinoma. *J Cell Biochem* 2018; **119**: 4711-4716 [PMID: 29278659 DOI: 10.1002/jcb.26650]

51 **Sugimachi K**, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, Shinden Y, Iguchi T, Eguchi H, Shirabe K, Ochiya T, Maehara Y, Mimori K. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *Br J Cancer* 2015; **112**: 532-538 [PMID: 25584485 DOI: 10.1038/bjc.2014.621]

52 **Wang G**, Zhao W, Wang H, Qiu G, Jiang Z, Wei G, Li X. Exosomal MiR-744 Inhibits Proliferation and Sorafenib Chemoresistance in Hepatocellular Carcinoma by Targeting PAX2. *Med Sci Monit* 2019; **25**: 7209-7217 [PMID: 31553714 DOI: 10.12659/MSM.919219]

53 **Tang J**, Li Y, Liu K, Zhu Q, Yang WH, Xiong LK, Guo DL. Exosomal miR-9-3p suppresses HBGF-5 expression and is a functional biomarker in hepatocellular carcinoma. *Minerva Med* 2018; **109**: 15-23 [PMID: 28750499 DOI: 10.23736/S0026-4806.17.05167-9]

54 **Wang J**, Pu J, Zhang Y, Yao T, Luo Z, Li W, Xu G, Liu J, Wei W, Deng Y. Exosome-transmitted long non-coding RNA *SENP3-EIF4A1* suppresses the progression of hepatocellular carcinoma. *Aging (Albany NY)* 2020; **12**: 11550-11567 [PMID: 32602848 DOI: 10.18632/aging.103302]

55 **Chen W**, Quan Y, Fan S, Wang H, Liang J, Huang L, Chen L, Liu Q, He P, Ye Y. Exosome-

transmitted circular RNA hsa_circ_0051443 suppresses hepatocellular carcinoma progression. *Cancer Lett* 2020; **475**: 119-128 [PMID: 32014458 DOI: 10.1016/j.canlet.2020.01.022]

56 **Jiao C**, Jiao X, Zhu A, Ge J, Xu X. Exosomal miR-34s panel as potential novel diagnostic and prognostic biomarker in patients with hepatoblastoma. *J Pediatr Surg* 2017; **52**: 618-624 [PMID: 28277300 DOI: 10.1016/j.jpedsurg.2016.09.070]

57 **Jiang K**, Dong C, Yin Z, Li R, Mao J, Wang C, Zhang J, Gao Z, Liang R, Wang Q, Wang L. Exosome-derived ENO1 regulates integrin α6β4 expression and promotes hepatocellular carcinoma growth and metastasis. *Cell Death Dis* 2020; **11**: 972 [PMID: 33184263 DOI: 10.1038/s41419-020-03179-1]

58 **Wang Y**, Wang B, Xiao S, Li Y, Chen Q. miR-125a/b inhibits tumor-associated macrophages mediated in cancer stem cells of hepatocellular carcinoma by targeting CD90. *J Cell Biochem* 2019; **120**: 3046-3055 [PMID: 30536969 DOI: 10.1002/jcb.27436]

59 **Yugawa K**, Yoshizumi T, Mano Y, Itoh S, Harada N, Ikegami T, Kohashi K, Oda Y, Mori M. Cancer-associated fibroblasts promote hepatocellular carcinoma progression through downregulation of exosomal miR-150-3p. *Eur J Surg Oncol* 2021; **47**: 384-393 [PMID: 32883551 DOI: 10.1016/j.ejso.2020.08.002]

60 **Fu X**, Liu M, Qu S, Ma J, Zhang Y, Shi T, Wen H, Yang Y, Wang S, Wang J, Nan K, Yao Y, Tian T. Exosomal microRNA-32-5p induces multidrug resistance in hepatocellular carcinoma *via* the PI3K/Akt pathway. *J Exp Clin Cancer Res* 2018; **37**: 52 [PMID: 29530052 DOI: 10.1186/s13046-018-0677-7]

61 **Zhang Z**, Li X, Sun W, Yue S, Yang J, Li J, Ma B, Wang J, Yang X, Pu M, Ruan B, Zhao G, Huang Q, Wang L, Tao K, Dou K. Loss of exosomal miR-320a from cancer-associated fibroblasts contributes to HCC proliferation and metastasis. *Cancer Lett* 2017; **397**: 33-42 [PMID: 28288874 DOI: 10.1016/j.canlet.2017.03.004]

62 **Dong SS**, Dong DD, Yang ZF, Zhu GQ, Gao DM, Chen J, Zhao Y, Liu BB. Exosomal miR-3682-3p Suppresses Angiogenesis by Targeting ANGPT1 *via* the RAS-MEK1/2-ERK1/2 Pathway in Hepatocellular Carcinoma. *Front Cell Dev Biol* 2021; **9**: 633358 [PMID: 33869178 DOI: 10.3389/fcell.2021.633358]

63 **Chen W**, Huang L, Liang J, Ye Y, He S, Niu J. Hepatocellular carcinoma cells-derived exosomal microRNA-378b enhances hepatocellular carcinoma angiogenesis. *Life Sci* 2021; **273**: 119184 [PMID: 33577844 DOI: 10.1016/j.lfs.2021.119184]

64 **Ma D**, Gao X, Liu Z, Lu X, Ju H, Zhang N. Exosome-transferred long non-coding RNA ASMTL-AS1 contributes to malignant phenotypes in residual hepatocellular carcinoma after insufficient radiofrequency ablation. *Cell Prolif* 2020; **53**: e12795 [PMID: 32722884 DOI: 10.1111/cpr.12795]

65 **Fan F**, Chen K, Lu X, Li A, Liu C, Wu B. Dual targeting of PD-L1 and PD-L2 by PCED1B-AS1 *via* sponging hsa-miR-194-5p induces immunosuppression in hepatocellular carcinoma. *Hepatol Int* 2021; **15**: 444-458 [PMID: 33219943 DOI: 10.1007/s12072-020-10101-6]

66 **Su Y**, Lv X, Yin W, Zhou L, Hu Y, Zhou A, Qi F. CircRNA Cdr1as functions as a competitive endogenous RNA to promote hepatocellular carcinoma progression. *Aging (Albany NY)* 2019; **11**: 8183-8203 [PMID: 31581132 DOI: 10.18632/aging.102312]

67 **Liang L**, Zhao L, Wang Y. Treatment for Hepatocellular Carcinoma Is Enhanced When Norcantharidin Is Encapsulated in Exosomes Derived from Bone Marrow Mesenchymal Stem Cells. *Mol Pharm* 2021; **18**: 1003-1013 [PMID: 33527831 DOI: 10.1021/acs.molpharmaceut.0c00976]

68 **Li H**, Yang C, Shi Y, Zhao L. Exosomes derived from siRNA against GRP78 modified bone-marrow-derived mesenchymal stem cells suppress Sorafenib resistance in hepatocellular carcinoma. *J Nanobiotechnology* 2018; **16**: 103 [PMID: 30572882 DOI: 10.1186/s12951-018-0429-z]

69 **Semaan L**, Zeng Q, Lu Y, Zhang Y, Zreik MM, Chamseddine MB, Chopp M, Zhang ZG, Moonka D. MicroRNA-214 enriched exosomes from human cerebral endothelial cells (hCEC) sensitize hepatocellular carcinoma to anti-cancer drugs. *Oncotarget* 2021; **12**: 185-198 [PMID: 33613846 DOI: 10.18632/oncotarget.27879]

70 **Zhong X**, Zhou Y, Cao Y, Ding J, Wang P, Luo Y, Liu H, Zhu Z, Jing X. Enhanced antitumor efficacy through microwave ablation combined with a dendritic cell-derived exosome vaccine in hepatocellular carcinoma. *Int J Hyperthermia* 2020; **37**: 1210-1218 [PMID: 33100037 DOI: 10.1080/02656736.2020.1836406]

71 **Lu Z**, Zuo B, Jing R, Gao X, Rao Q, Liu Z, Qi H, Guo H, Yin H. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *J Hepatol* 2017; **67**: 739-748 [PMID: 28549917 DOI: 10.1016/j.jhep.2017.05.019]

72 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

73 **Anwanwan D**, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta Rev Cancer* 2020; **1873**: 188314 [PMID: 31682895 DOI: 10.1016/j.bbcan.2019.188314]

74 **De Stefano F**, Chacon E, Turcios L, Marti F, Gedaly R. Novel biomarkers in hepatocellular carcinoma. *Dig Liver Dis* 2018; **50**: 1115-1123 [PMID: 30217732 DOI: 10.1016/j.dld.2018.08.019]

75 **Corcoran C**, Friel AM, Duffy MJ, Crown J, O'Driscoll L. Intracellular and extracellular microRNAs in breast cancer. *Clin Chem* 2011; **57**: 18-32 [PMID: 21059829 DOI: 10.1373/clinchem.2010.150730]

76 **Skog J**, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008; **10**: 1470-1476

[PMID: 19011622 DOI: 10.1038/ncb1800]

77 **Cui S**, Cheng Z, Qin W, Jiang L. Exosomes as a liquid biopsy for lung cancer. *Lung Cancer* 2018; **116**: 46-54 [PMID: 29413050 DOI: 10.1016/j.lungcan.2017.12.012]

78 **Melo SA**, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015; **523**: 177-182 [PMID: 26106858 DOI: 10.1038/nature14581]

79 **Kalluri R**. The biology and function of exosomes in cancer. *J Clin Invest* 2016; **126**: 1208-1215 [PMID: 27035812 DOI: 10.1172/JCI81135]

80 **Bai M**, Li J, Yang H, Zhang H, Zhou Z, Deng T, Zhu K, Ning T, Fan Q, Ying G, Ba Y. miR-135b Delivered by Gastric Tumor Exosomes Inhibits FOXO1 Expression in Endothelial Cells and Promotes Angiogenesis. *Mol Ther* 2019; **27**: 1772-1783 [PMID: 31416776 DOI: 10.1016/j.ymthe.2019.06.018]

81 **Huang Z**, Yang M, Li Y, Yang F, Feng Y. Exosomes Derived from Hypoxic Colorectal Cancer Cells Transfer Wnt4 to Normoxic Cells to Elicit a Prometastatic Phenotype. *Int J Biol Sci* 2018; **14**: 2094-2102 [PMID: 30585272 DOI: 10.7150/ijbs.28288]

82 **Das CK**, Jena BC, Banerjee I, Das S, Parekh A, Bhutia SK, Mandal M. Exosome as a Novel Shuttle for Delivery of Therapeutics across Biological Barriers. *Mol Pharm* 2019; **16**: 24-40 [PMID: 30513203 DOI: 10.1021/acs.molpharmaceut.8b00901]

83 **Peer D**, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol* 2007; **2**: 751-760 [PMID: 18654426 DOI: 10.1038/nnano.2007.387]

84 **Liang G**, Zhu Y, Ali DJ, Tian T, Xu H, Si K, Sun B, Chen B, Xiao Z. Engineered exosomes for targeted co-delivery of miR-21 inhibitor and chemotherapeutics to reverse drug resistance in colon cancer. *J Nanobiotechnology* 2020; **18**: 10 [PMID: 31918721 DOI: 10.1186/s12951-019-0563-2]

85 **Zhang Q**, Zhang H, Ning T, Liu D, Deng T, Liu R, Bai M, Zhu K, Li J, Fan Q, Ying G, Ba Y. Exosome-Delivered c-Met siRNA Could Reverse Chemoresistance to Cisplatin in Gastric Cancer. *Int J Nanomedicine* 2020; **15**: 2323-2335 [PMID: 32308384 DOI: 10.2147/IJN.S231214]

86 **Zitvogel L**, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 1998; **4**: 594-600 [PMID: 9585234 DOI: 10.1038/nm0598-594]

87 **Escudier B**, Dorval T, Chaput N, André F, Caby MP, Novault S, Flament C, Leboulaire C, Borg C, Amigorena S, Boccaccio C, Bonnerot C, Dhellin O, Movassagh M, Piperno S, Robert C, Serra V, Valente N, Le Pecq JB, Spatz A, Lantz O, Tursz T, Angevin E, Zitvogel L. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. *J Transl Med* 2005; **3**: 10 [PMID: 15740633 DOI: 10.1186/1479-5876-3-10]

88 **Morse MA**, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, Hsu DH, Le Pecq JB, Lyerly HK. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J Transl Med* 2005; **3**: 9 [PMID: 15723705 DOI: 10.1186/1479-5876-3-9]

89 **Besse B**, Charrier M, Lapierre V, Dansin E, Lantz O, Planchard D, Le Chevalier T, Livartoski A, Barlesi F, Laplanche A, Ploix S, Vimond N, Peguillet I, Théry C, Lacroix L, Zoernig I, Dhodapkar K, Dhodapkar M, Viaud S, Soria JC, Reiners KS, Pogge von Strandmann E, Vély F, Rusakiewicz S, Eggermont A, Pitt JM, Zitvogel L, Chaput N. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *Oncoimmunology* 2016; **5**: e1071008 [PMID: 27141373 DOI: 10.1080/2162402X.2015.1071008]

90 **Dai S**, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. *Mol Ther* 2008; **16**: 782-790 [PMID: 18362931 DOI: 10.1038/mt.2008.1]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 348-361

DOI: 10.4251/wjgo.v14.i1.348

ISSN 1948-5204 (online)

META-ANALYSIS

Increased risk of colorectal neoplasia in inflammatory bowel disease patients with post-inflammatory polyps: A systematic review and meta-analysis

De-Gao He, Xi-Jie Chen, Juan-Ni Huang, Jun-Guo Chen, Min-Yi Lv, Tian-Ze Huang, Ping Lan, Xiao-Sheng He

ORCID number: De-Gao He 0000-0001-9091-3281; Xi-Jie Chen 0000-0003-0307-9247; Juan-Ni Huang 0000-0001-7339-2613; Jun-Guo Chen 0000-0003-0844-2412; Min-Yi Lv 0000-0003-0155-9204; Tian-Ze Huang 0000-0003-2915-3687; Ping Lan 0000-0001-5206-5533; Xiao-Sheng He 0000-0002-8346-4972.

Author contributions: He DG, Chen XJ and Huang JN contributed equally to this work; He DG, Chen XJ and He XS designed study; He DG, Chen XJ, performed literature search and review, He DG, Chen XJ, Huang JN, Chen JG, Lv MY and Huang TZ collected data; He DG, Chen XJ, Huang JN and He XS wrote and edited the manuscript; He DG and Lan P did statistical analysis; He DG, Chen XJ, Huang JN, Chen JG, Lv MY and Huang TZ contributed to methodological quality assessment; Lan P and He XS reviewed manuscript; All authors have read and approved the final manuscript.

Conflict-of-interest statement: Authors have no conflicts of interest to declare.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009

De-Gao He, Xi-Jie Chen, Jun-Guo Chen, Min-Yi Lv, Tian-Ze Huang, Ping Lan, Xiao-Sheng He, Department of Colorectal Surgery, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangdong Institute of Gastroenterology, Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, Guangzhou 510655, Guangdong Province, China

Juan-Ni Huang, Department of Geriatrics, the first Affiliated Hospital, Guangzhou Medical University, Guangzhou 510120, Guangdong Province, China

Corresponding author: Xiao-Sheng He, MD, PhD, Doctor, Department of Colorectal Surgery, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangdong Institute of Gastroenterology, Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, No. 26 Yuancun Er Heng Road, Guangzhou 510655, Guangdong Province, China.
hexsheng@mail.sysu.edu.cn

Abstract

BACKGROUND

Inflammatory bowel disease (IBD) patients with post-inflammatory polyps (PIPs) may carry an increased risk of colorectal neoplasia (CRN) including dysplasia and cancer. Current guidelines recommend active colonoscopy follow-up for these patients. However, the evidence for guidelines is still poor. In addition, some recent high-quality reports present a different view, which challenges the current guidelines. We hypothesize that IBD patients with PIPs are at increased risk of CRN.

AIM

To evaluate the risk of CRN in IBD patients with and without PIPs.

METHODS

A systematic search of PubMed, Embase, Cochrane Library, and Web of Science was performed to identify studies that compared the risk of CRN in IBD patients with and without PIPs. In addition, we screened the reference lists and citation indices of the included studies. Quality assessment was performed using the Newcastle–Ottawa Scale. Pooled odds ratio (OR) was calculated using the random-effects model to explore the final pooled effect size of the included studies and determine whether PIPs increase the risk of CRN. Sensitivity analysis, subgroup analysis, and assessment of publication bias were performed to examine the sources of heterogeneity.

Checklist.

Supported by The National Key R&D Program of China, No. 2017YFC1308800; National Natural Science Foundation of China, No. 81970482; Natural Science Foundation of Guangdong Province, China, No. 2019A1515011313; Sun Yat-Sen University 5010 Project, No. 2010012; and the Fundamental Research Funds for the Central Universities, No. 19ykpy05, and National Key Clinical Discipline.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: August 6, 2021
Peer-review started: August 6, 2021
First decision: August 29, 2021
Revised: September 8, 2021
Accepted: November 24, 2021
Article in press: November 24, 2021
Published online: January 15, 2022

P-Reviewer: Sitkin S

RESULTS

Twelve studies with 5819 IBD patients, including 1281 (22.01%) with PIPs, were considered eligible for this meta-analysis. We found that IBD patients with PIPs were at an increased risk of CRN as compared to those without PIPs [OR 2.01; 95% confidence interval (CI): 1.43–2.83]. The results were similar when colorectal cancer was used as the study endpoint (OR 2.57; 95%CI: 1.69–3.91). Furthermore, the risk of CRN was still increased (OR 1.80; 95%CI: 1.12–2.91) when restricted to ulcerative colitis patients. Heterogeneity was high among the included studies (I^2 = 75%). Subgroup analysis revealed that the high heterogeneity was due to the study design. Sensitivity analysis showed that the main statistical outcomes did not essentially change after excluding any one of the included studies. No significant publication bias was found in the funnel plots.

CONCLUSION

IBD patients with PIPs have an increased risk of CRN as compared with those without PIPs, which support the current guidelines. However, a high-quality randomized controlled trial is warranted.

Key Words: Colorectal neoplasia; Inflammatory bowel disease; Ulcerative colitis; Post-inflammatory polyps; Pseudopolyps; Meta-analysis

Core Tip: Inflammatory bowel disease (IBD) patients with post-inflammatory polyps (PIPs) may carry an increased risk of colorectal neoplasia (CRN). Current guidelines recommend active colonoscopy follow-up for these patients. However, the evidence is still poor. We found that IBD patients with PIPs have a higher risk of CRN than those without PIPs. The results were similar when colorectal cancer was used as the endpoint of the study. Our findings not only confirm the viewpoint of the guidelines, but may also improve the degree of evidence. We expect that our study will provide a reference for the development of surveillance strategies for IBD patients.

Citation: He DG, Chen XJ, Huang JN, Chen JG, Lv MY, Huang TZ, Lan P, He XS. Increased risk of colorectal neoplasia in inflammatory bowel disease patients with post-inflammatory polyps: A systematic review and meta-analysis. *World J Gastrointest Oncol* 2022; 14(1): 348-361
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/348.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.348

INTRODUCTION

Post-inflammatory polyps (PIPs), commonly known as pseudopolyps in the past, are islets of mucosa that develop after severe ulceration and disruption of mucosal integrity in the setting of chronic inflammation, such as inflammatory bowel disease (IBD)[1-3]. PIPs can be classified into four pathologic types: (1) Ragged mucosal remnant; (2) Granulation tissue polyps; (3) Mixed polyps; and (4) "hyperplastic-adenomatous" polyps[1,4-6]. Whether different types of PIPs lead to the same risk of colorectal neoplasia (CRN) in IBD patients awaits further study. The prevalence of PIPs in IBD patients was reported to range from 10% to 40%[7-24]. This discrepancy may be due to the differences in diagnostic criteria, study year, and study population. PIPs are found more often in ulcerative colitis (UC) than in Crohn's disease (CD)[25], and can be even 2-fold more in some studies[6,13]. In addition, the incidence can increase with the extent and duration of colitis[9,10,16,26].

Colorectal cancer (CRC) is a serious complication in long-standing IBD and significantly increases the mortality rate due to this disease. Guidelines for Colorectal Cancer Screening and Surveillance in Moderate and High-Risk Groups (update from 2002)[27] recommend surveillance intervals among IBD patients according to risk stratification. Several risk factors such as duration and severity of disease, young age at IBD diagnosis, family history of CRC, whether accompanied by primary sclerosing

S-Editor: Liu M
L-Editor: A
P-Editor: Liu M

cholangitis and stricture, categorize IBD patients into low-, medium-, and high-risk groups. Patients with PIPs are included in the medium-risk group and colonoscopy is recommended every three years. Similarly, the AGA technical review on the diagnosis and management of CRN in IBD[28] recommend that patients with multiple inflammatory pseudopolyps should undergo more frequent colonoscopy surveillance.

Both guidelines recommend the presence of PIPs as a risk factor for CRC in IBD patients. However, the literature cited in the guidelines comes from small case-control studies, indicating an insufficient level of evidence[14,29]. In addition, different opinions have been raised in some recent high-quality literature[30-32]. To comprehensively assess the impact of the presence of PIPs, we aimed to conduct a meta-analysis to quantify the impact of co-existing PIPs on the risk of CRN in IBD patients. We hypothesized that IBD patients with PIPs had an increased risk of CRN.

MATERIALS AND METHODS

Our study followed PICOS principles and was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[33].

Search strategy

A systematic literature search strategy was designed to determine all published or unpublished studies comparing the risk of CRN in IBD patients with and without PIPs. A comprehensive literature search of the following databases was conducted: PubMed, Embase, Cochrane Library, and Web of Science (English literature only, each from inception to October 22, 2020). We used medical subject headings and all free texts to retrieve the following keywords: 'ulcerative colitis', 'crohn's disease', 'inflammatory bowel disease', 'post-inflammatory polyps', 'colorectal neoplasms', 'colonic neoplasms', 'rectal neoplasms' and 'dysplasia'. All searches were performed separately by two researchers strictly following our pre-designed search strategy. After consulting with the senior investigators (He XS and Lan P) regarding queries, we reached an agreement for the retrieval results. Also, citations from each article on the topic were manually searched for other potentially eligible studies.

Study selection

According to the inclusion and exclusion criteria, two investigators (He DG and Chen XJ) independently reviewed all the searched literature and resolved any discrepancies through discussion to reach a consensus. Studies meeting the following criteria were included: (1) A comparison of IBD patients with and without PIPs, with CRN as the endpoint; (2) A detailed number of IBD patients with and without PIPs, as well as the number of CRN cases among these patients; and (3) For republished studies, we included the most recent publications.

Studies were excluded if (1) They were review articles, case reports, letters, or laboratory studies; (2) Important data of interest were missing; (3) Republished literature; and (4) Not English literature. Twelve eligible studies were finally included in our meta-analysis[14,29,31,32,34-41].

Data extraction

Raw data included in the study were extracted by two researchers (He DG and Huang JN) according to our pre-designed data extraction form. After discussing the deviations in detail with the senior investigator (He XS), we agreed on each study. The information extracted from the article were: study author, published year, published journal, study design, study type, surveillance period, sources of patients, race, age at IBD diagnosis, IBD type, quantitative data on the number of IBD patients, and the number IBD patients with concomitant PIPs, male gender and the presence of neoplasia or cancer.

Outcome measures

The primary outcome of the current study was whether PIPs increased the risk of CRN (including dysplasia and cancer) in IBD patients. The secondary outcome was whether PIPs increased the risk of CRC in IBD patients.

Methodological quality assessment of the included studies

The quality of the included studies was assessed using the Newcastle-Ottawa Scale

(NOS), designed specifically for non-randomized studies in meta-analysis[42]. The included studies were evaluated based on population selection (4 options), comparability (2 options), outcome of interest (cohort study) or determination of exposure (case-control study) (3 options). One point was assigned for each option, for a total of 9 points (Supplementary Table 1). A high-quality study required a score of 7 or above.

Statistical analysis

The primary data extracted from the included studies were dichotomous variables. The pooled odds ratio (OR) and 95% confidence interval (CI) were used to represent the pooled effect size. Study heterogeneity was assessed by the Q test and the I^2 statistic (which could quantify the level of heterogeneity). An I^2 value more than 50% or P value less than 0.10 in the Q-test represented substantial heterogeneity. When study heterogeneity was present ($I^2 > 50\%$ or $P < 0.10$), the random-effects model was used to assess the pooled effect, otherwise, the fixed-effects model was used. Subgroup analysis was performed as stratified based on IBD subtype and study type. Publication bias in the included studies was assessed by funnel plots. If potential biases were detected, we performed further sensitivity analyses to assess the robustness of pooled effect estimates and the likely impact of biases. We also performed a sensitivity analysis to investigate the impact of each study on the overall risk estimate by omitting one study in turn. A two-sided P value less than 0.05 was considered statistically significant. All the statistics were analyzed by Review Manager 5.4 (Cochrane Collaboration, Oxford, UK) and STATA 15.1 (StataCorp, College Station, Texas, USA). The statistical methods used in this study were reviewed by Ping Lan from the Department of Colorectal Surgery, the Sixth Affiliated Hospital, Sun Yat-sen University.

RESULTS

Study selection

In the initial search, a total of 618 related articles were obtained according to the research strategy. We also obtained 7 articles from the reference lists and citation indices of the included studies[29,31,34,38,41,43,44]. Twenty articles[14,29-32,34-41,45-51] that might be eligible for inclusion were reviewed. Among them, 12 articles met our inclusion criteria (Figure 1)[14,29,31,32,34-41]. Five studies were excluded due to duplication[45-49], 2 studies due to incomplete data of interest[30,50], and one due to lack of a control group[51].

Study characteristics

The baseline characteristics of the included studies are described in Table 1. Overall, the 12 included studies were published between 2004 and 2020. A total of 5819 IBD patients, including 1821 (22.01%) IBD patients who also had PIPs, were identified. Of these studies, 5 studies were cohort studies[31,32,34-36], 6 were case-control studies [14,29,37,38,40,41] and one was a cross-sectional study[39]. One was a prospective study[39] and the others were retrospective studies[14,29,31,32,34-38,40,41]. In addition, 7 studies contained data that could be used to independently analyze UC patients[14,29,34-37,39], one study contained data that could be used to independently analyze CD patients[36], and in 5 studies we were unable to distinguish the data between UC patients and CD patients[31,32,38,40,41]. In addition, all studies included raw data with the endpoint as CRN, and 6 studies reported on the number of IBD patients who developed CRC[29,31,34,36,38,41].

Quality assessment of the included studies

The quality of each study was evaluated separately using the NOS. The results showed that the scores of the studies ranged from 5 to 9 points, and most scored 7 or above[14,29,31,32,34,35,37,38,40,41]. Table 2 shows the NOS results for the included studies.

Quantitative summary (meta-analysis)

PIPs were associated with a higher risk of CRN in IBD patients (OR 2.01; 95%CI: 1.43–2.83) (Figure 2). When CRC was used as an endpoint, patients with PIPs had an approximately 2.5-fold increased risk compared to those without PIPs (OR 2.57; 95%CI: 1.69–3.91) (Figure 3). When UC patients were analyzed separately, the pooled OR was 1.80 (95%CI: 1.12–2.97), suggesting that the existence of PIPs was related to a higher risk of CRN in UC patients (Figure 4). Limited data prevented us from performing a meta-analysis in CD patients, but the study by Ma *et al*[36] indicated that

Table 1 Characteristics of the included studies, *n* (%)

Ref.	Magazine	Design	Study type	Surveillance period	Sources of patients	Race	Age at IBD diagnosis(years)	IBD type	IBD with/without PIPs	Male gender (%)	Outcome
de Jong *et al*[31], 2020	Inflamm Bowel Dis	Retrospective	Cohort	January 2012 - December 2017	The Netherlands	Caucasian	28.5 (± 11.8)/28.9 (± 12.4)[1]	Mixed IBD	154/365	284 (48.2)	Neoplasia
Gu *et al*[35], 2019	Journal of Digestive Diseases	Retrospective	Cohort	June 1986 -July 2018	China	Asian	29.5-54.0	UC	57/189	120 (48.8)	Neoplasia
Mahmoud *et al* [32], 2019	Gastroenterology	Retrospective	Cohort	January 1997 - January 2017	America, The Netherlands	Caucasian	NA	Mixed IBD	462/1120	835 (52.8)	Neoplasia
Ünal *et al*[34], 2019	Turkish Journal of Gastroenterology	Retrospective	Cohort	1993-2016	Turkey	Asian	40.5 ± 15	UC	100/701	475 (59.3)	Neoplasia
Ma *et al*[36], 2017	Laboratory Investigation	Retrospective	Cohort	2006-2016	NA	NA	NA	Mixed IBD	102/220	NA	Neoplasia
Jegadeesan *et al* [37], 2016	Inflamm Bowel Dis	Retrospective	Case–control	1998-2011	America	Caucasian	37 (30.5–50.5)/38 (28.2–23.4)[2]	UC	138/329	251 (53.7)	Neoplasia
Lutgens *et al*[38], 2015	Clinical Gastroenterology and Hepatology	Retrospective	Case–control	1990-2011	Belgium, The Netherlands	Caucasian	NA	Mixed IBD	259/271	276 (52.1)	Cancer
Badamas *et al*[40], 2014	Gastroenterology	Retrospective	Case–control	2007-2013	NA	NA	30.3 (± 15.6)/29.3 (± 13.2)	Mixed IBD	90/93	93 (50.8)	Neoplasia
Freire *et al*[39], 2014	Scand J Gastroenterol	Prospective	Cross-sectional	April 2011 -December 2013	Portugal	Caucasian	33.3 ± 11.6	UC	33/43	30 (39.5)	Neoplasia
Baars *et al*[41], 2011	Am J Gastroenterol	Retrospective	Case – control	January 1990 – July 2006	The Netherlands	Caucasian	NA	Mixed IBD	147/366	266 (47.1)	Cancer
Velayos *et al*[29], 2006	Gastroenterology	Retrospective	Case – control	January 1976 - December 2002	America	Caucasian	25 (6–76)/27 (8–66)	UC	184/192	266 (70.7)	Cancer
Rutter *et al*[14], 2004	Gut	Retrospective	Case – control	January 1988 - January 2002	Britain	Caucasian	33 (6–65)	UC	95/109	117 (57)	Neoplasia

[1]Data expressed as mean ± SD.

[2]Data expressed as median (range).

IBD: Inflammatory bowel disease; PIPs: Post-inflammatory polyps; UC: Ulcerative colitis; NA: Not available.

no association was observed between PIPs and CRN in these patients (OR 1.27; 95%CI: 0.24–6.75). However, there was high heterogeneity among studies (I^2 = 75%); therefore, the Mantel-Haenszel random-effects model was used to test the results. Furthermore, the sources of heterogeneity were assessed by subgroup analyses, sensitivity analysis, and assessment of publication bias.

Table 2 Assessment of the quality of studies

Ref.	Selection				Comparability	Outcome			
	1	2	3	4	5	6	7	8	Score
de Jong *et al*[31], 2020	b[1]	a[1]	a[1]	a[1]	a b[2]	a[1]	a[1]	a[1]	9
Gu *et al*[35], 2019	b[1]	a[1]	d	a[1]	a b[2]	a[1]	a[1]	a[1]	8
Mahmoud *et al*[32], 2019	b[1]	a[1]	a[1]	a[1]	a b[2]	a[1]	a[1]	a[1]	9
Ünal *et al*[34], 2019	b[1]	a[1]	d	a[1]	a b[2]	a[1]	a[1]	a[1]	8
Ma *et al*[36], 2017	d	a[1]	a[1]	a[1]	a b[2]	d	c	d	5
Jegadeesan *et al*[37], 2016	a[1]	a[1]	b	a[1]	a b[2]	a[1]	a[1]	a[1]	8
Lutgens *et al*[38], 2015	a[1]	b	b	a[1]	a b[2]	a[1]	a[1]	a[1]	7
Badamas *et al*[40], 2014	a[1]	a[1]	b	a[1]	a b[2]	a[1]	a[1]	b	7
Freire *et al*[39], 2014	a[1]	b	b	a[1]	a b[2]	e	a[1]	a[1]	6
Baars *et al*[41], 2011	a[1]	a[1]	b	a[1]	a b[2]	e	a[1]	a[1]	7
Velayos *et al*[29], 2006	a[1]	a[1]	b	a[1]	a b[2]	a[1]	a[1]	a[1]	8
Rutter *et al*[14], 2004	a[1]	a[1]	b	a[1]	a b[2]	a[1]	a[1]	a[1]	8

[1]Data expressed as mean ± SD.
[2]Data expressed as median (range). The quality of studies was assessed using the Newcastle-Ottawa Scale (Supplementary Table 1). A high-quality study required a score of 7 or above.

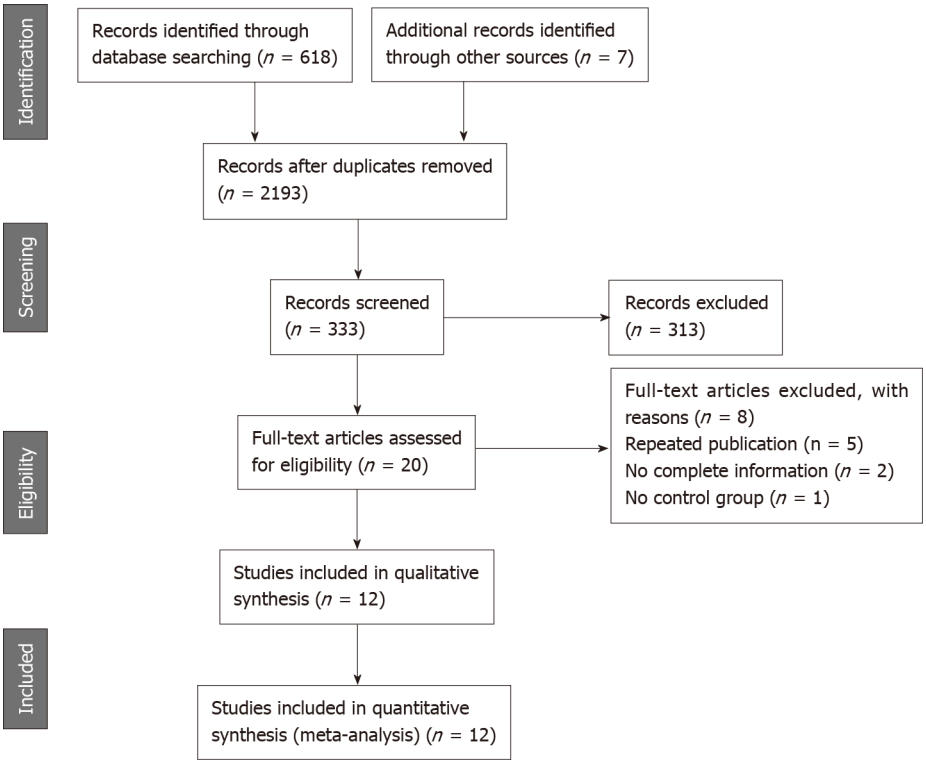


Figure 1 PRISMA flow diagram.

Subgroup analysis

Subgroup analysis based on study types (cohort, case-control, cross-sectional) showed a significant reduction in heterogeneity (Figure 5). In other words, different study types may be a source of heterogeneity. An increased risk of CRN was found in cohort studies (OR 1.73; 95%CI: 1.12–2.66)[31,32,34-36] and case-control studies (OR 2.31; 95%CI: 1.45–3.67)[14,29,37,38,40,41]. Nevertheless, no association between PIPs and

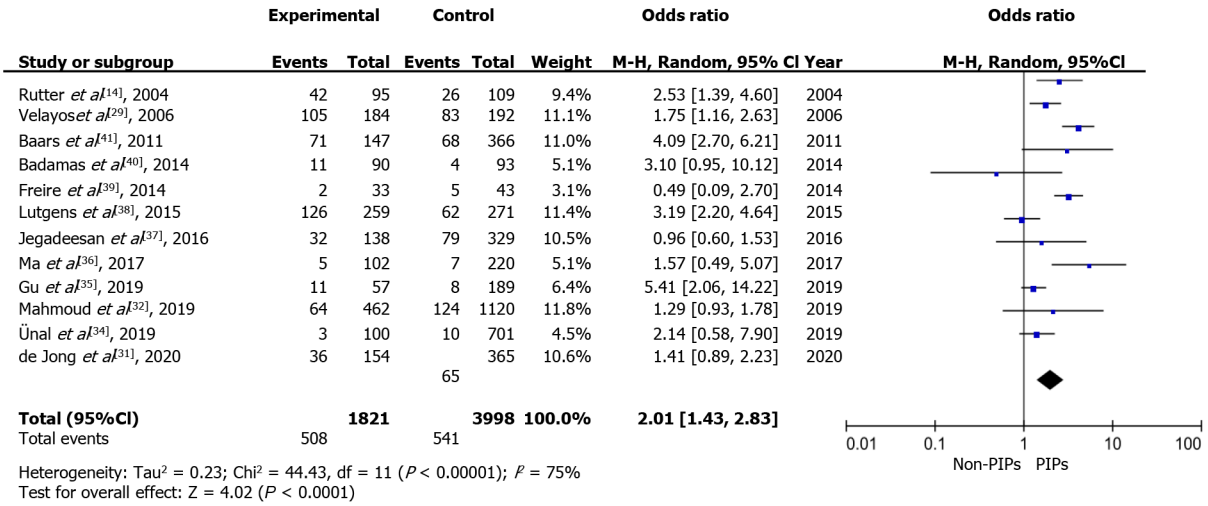


Figure 2 Risk of the development of colorectal neoplasia in inflammatory bowel disease patients with post-inflammatory polyps. M-H: Mantel-Haenszel; CI: Confidence interval; PIPs: Post-inflammatory polyps.

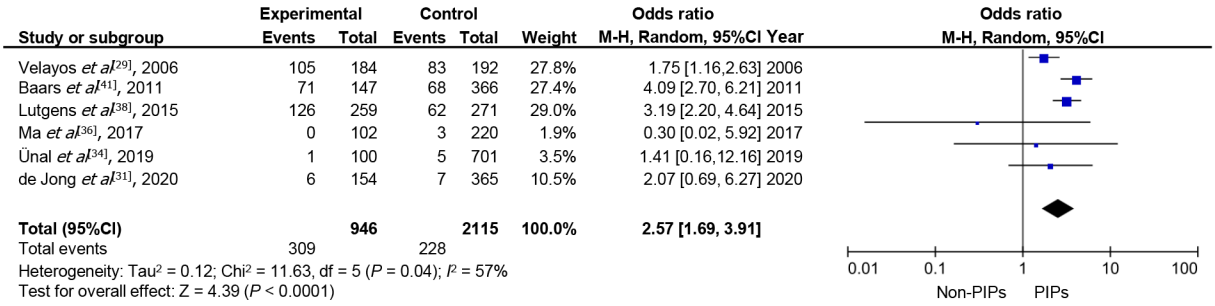


Figure 3 Risk of the development of colorectal cancer in inflammatory bowel disease patients with post-inflammatory polyps. M-H: Mantel-Haenszel; CI: Confidence interval; PIPs: Post-inflammatory polyps.

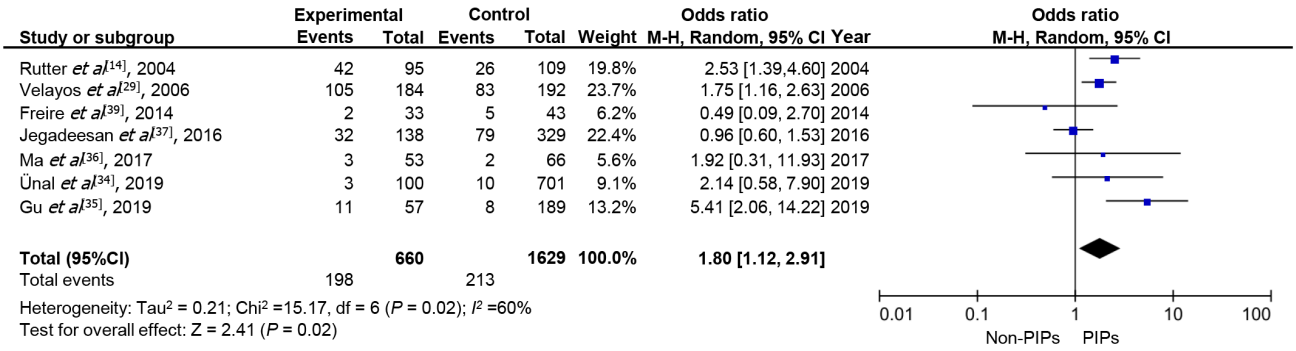


Figure 4 Risk of the development of colorectal neoplasia in ulcerative colitis patients with post-inflammatory polyps. M-H: Mantel-Haenszel; CI: Confidence interval; PIPs: Post-inflammatory polyps.

CRN was found in cross-sectional studies (OR 0.49; 95%CI: 0.09–2.70)[39].

Sensitivity analysis

Sensitivity analysis was performed with the pooled OR and 95%CI. The results were essentially unchanged in the statistical outcomes of all the indicators after excluding any one study. The results are shown in Table 3.

Assessment of publication bias

A funnel plot with 12 studies was used to evaluate publication bias (Figure 6). It can be seen that the scatter point distribution was basically symmetrical, indicating no significant publication bias in the current meta-analysis.

Table 3 Results of the sensitivity analysis in the impact of each study on the overall risk estimate

Ref.	Odds Ratio	95% Confidence Interval	Heterogeneity (I^2)
de Jong *et al*[31], 2020	2.0980003	[1.443344, 3.0495887]	76%
Gu *et al*[35], 2019	1.8818157	[1.3357893, 2.6510394]	75%
Mahmoud *et al*[32], 2019	2.1335025	[1.4797615, 3.0760586]	73%
Ünal *et al*[34], 2019	2.0057204	[1.406724, 2.8597751]	77%
Ma *et al*[36], 2017	2.0393412	[1.4283487, 2.9116926]	77%
Jegadeesan *et al*[37], 2016	2.1974959	[1.5660043, 3.0836365]	71%
Lutgens *et al*[38], 2015	1.8946518	[1.3246907, 2.7098444]	73%
Badamas *et al*[40], 2014	1.9649121	[1.3789148, 2.7999403]	77%
Freire *et al*[39], 2014	2.1011214	[1.4932369, 2.9564707]	76%
Baars *et al*[41], 2011	1.8324436	[1.326488, 2.5313833]	67%
Velayos *et al*[29], 2006	2.0466876	[1.3896827, 3.014307]	77%
Rutter *et al*[14], 2004	1.9644971	[1.35726, 2.8434114]	77%
Combined	2.011362	[1.4304829, 2.82812]	75%

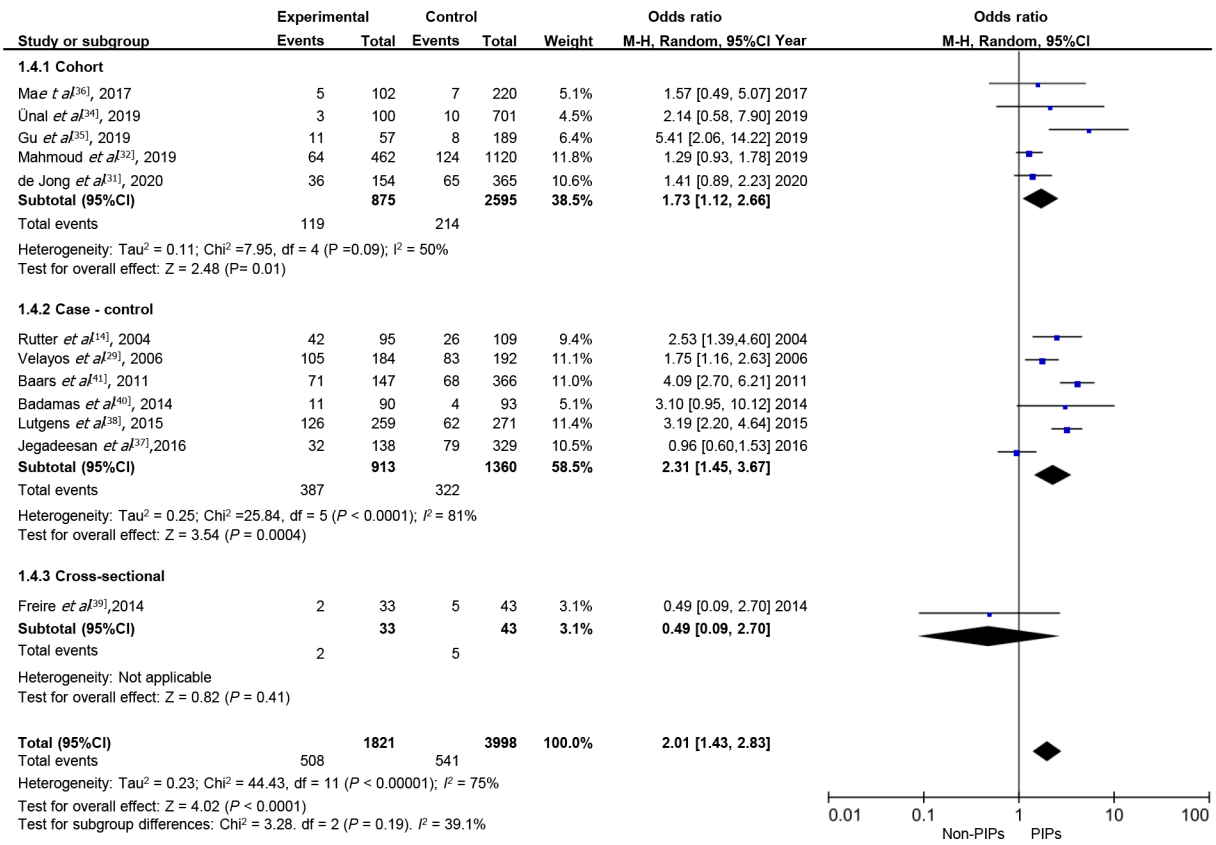


Figure 5 Risk of the development of colorectal neoplasia in inflammatory bowel disease patients with post-inflammatory polyps in cohort, case-control and cross-sectional studies. M-H: Mantel-Haenszel; CI: Confidence interval; PIPs: Post-inflammatory polyps.

DISCUSSION

Currently, the leading guidelines from Europe and the United States recommend more frequent colonoscopy for IBD patients with PIPs, in order to detect CRN in a timely manner[27,28]. However, several high-quality studies have recently shown that the presence of PIPs is not an independent risk factor for CRN in IBD patients[30-32]. The current meta-analysis of 12 observational studies including 1821 (22.01%) patients with

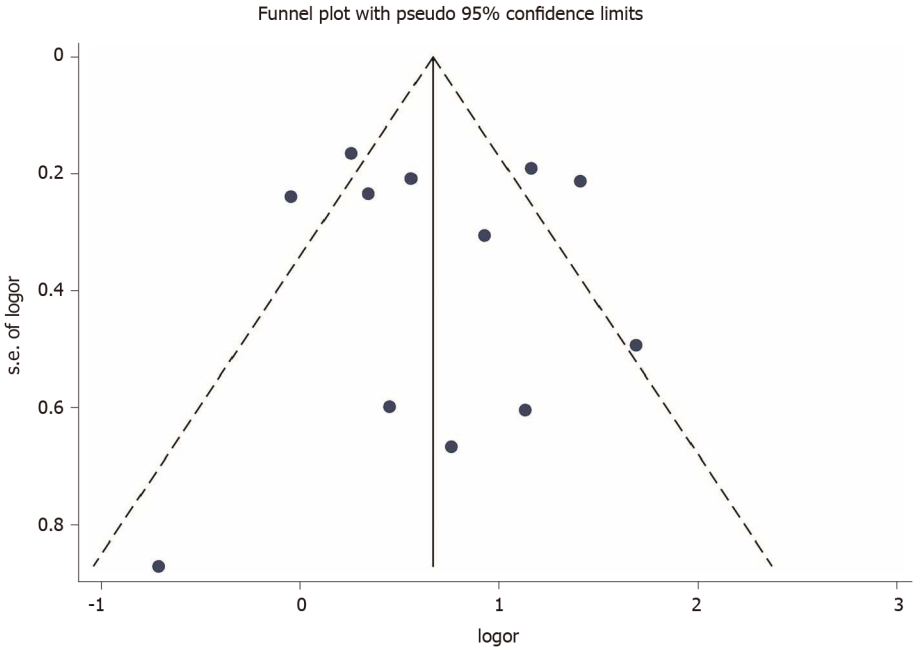


Figure 6 Funnel plots of the included studies.

PIPs indicated that IBD patients with PIPs had an approximately 2-fold increased risk of CRN. It is worth mentioning that we included two abstracts[36,40] with required data in our analysis, which may have introduced bias. However, even when we excluded these abstracts, we came to the same conclusion (OR 1.99; 95%CI: 1.37-2.88). Even when excluding dysplasia, the risk of CRC was still increased by approximately 2.5-fold in IBD patients with PIPs as compared with those without PIPs. In addition, UC patients with PIPs had an approximately 1.8-fold increased risk of CRN as compared with those without PIPs. Limited data on the risk of CRN in CD patients with PIPs may be related to the low incidence of PIPs among CD patients.

The reasons and mechanisms for the increased risk of CRN in IBD patients with concomitant PIPs remain unclear. One possible reason for this association is that PIPs are thought to be markers of previous episodes of severe inflammation. The incidence of PIPs increases with the severity of colitis, which may accelerate the development of CRN[9,32,52]. Another possible reason is that multiple PIPs may weaken the ability of endoscopy to detect dysplastic lesions[29,31]. However, both of these reasons only indicate that PIPs are indirect signs of increased risk of CRN in IBD patients. At present, it is generally believed that PIPs are benign and do not directly cause malignant transformation, even though there have been occasional reports of malignant transformation of PIPs[53,54]. However, Jawad *et al*[55] reported that PIPs may be the source of precancerous mutations following the analysis of DNA extracted from 30 different PIPs samples in which 4 identifiable mutations were found. In addition, Lozyns'ka[56] found 21.4% chromosomal anomalies in PIPs samples from IBD patients. Taken together, these findings suggest that we may have to re-think whether PIPs can directly lead to malignant transformation and the therapeutic strategy for PIPs may change in the future.

It is interesting to note that most included studies from the last two years[31,32,34] had a contrary conclusion to previous studies[14,29,41], although the quality of relevant studies was relatively high. Recent studies reported that no independent association between PIPs and CRN was found[30-32,34], which was contrary to our findings. This may be explained by the differences in the included study population and changes in the treatment patterns of IBD patients in recent years[57-60]. On the one hand, PIPs are the complication of prior extensive colon inflammation, leading to colectomy, and higher rates of early colectomy may result in a lower risk of CRN in these patients. On the other hand, with the widespread application of biological agents (such as infliximab, mesalazine, azathioprine, *etc*.) in the treatment of IBD, the risk of CRN in IBD patients may decline. Recent studies might have included more better-treated IBD patients, leading to a lower risk of CRN in their study population. However, our study included 5819 IBD patients since 1976, indicating that our results may be less influenced by these factors.

Our findings may have implications for clinical practice as they might provide an individual reference for endoscopic surveillance strategies in IBD patients. The evidence cited in the current guidelines is inadequate. Our study, which included 5,819 IBD patients from 12 studies, confirms the viewpoint of the guidelines for more aggressive colonoscopy in IBD patients with PIPs and further improves the degree of evidence. Above all, we agree that executing an evidence-based risk stratification model to determine surveillance intervals is cost-effective and in line with the concept of individualized treatment. In addition, it is necessary to identify the time period of higher incidence of CRN in IBD patients with PIPs. In this time period, patients with PIPs will receive more frequent endoscopic surveillance to detect CRN early. Finally, PIPs may weaken the endoscopic recognition of dysplasia. Improving endoscopic techniques to recognize PIPs and dysplasia may further reduce the incidence of CRN in IBD patients with PIPs. Perhaps these proposals could be considered in the IBD surveillance guidelines in the future.

High heterogeneity was found in our study. When subgroup analysis was conducted according to different study types (Figure 5), heterogeneity decreased to an acceptable level. In addition, sensitivity analysis and assessment of publication bias were performed to determine the sources of heterogeneity. We reanalyzed the included studies after excluding two studies with a score below 7[36,39]; however, the results were similar to those before the exclusion of these studies (OR 2.14; 95%CI: 1.50–3.06; I^2=78%). Also, when we excluded one of the included studies in turn, there were no significant changes in the results of the pooled effect size (Table 3) and heterogeneity. Furthermore, as shown in the funnel plot (Figure 6), there was no significant publication bias in our included studies. In general, we found that study type may be one of the sources of heterogeneity, and other potential heterogeneity may arise due to internal factors (such as IBD type, study population, statistical methods) within each included study.

Several limitations of our study should be considered. Firstly, heterogeneity was pronounced in our study, which may be related to the study types. Secondly, the studies included did not have standardized reports on PIPs. The reports on PIPs were mainly from endoscopy reports by endoscopists, which may have resulted in misclassification of PIPs. However, the reports on PIPs by a qualified endoscopist were usually reliable, and the relevant pathology reports confirmed the findings. Thirdly, some confounding factors, such as the degree of colitis at the time of the colonoscopy, duration of colitis, and endoscopy interval were not well controlled in the included studies, which prevented us from analyzing their impact on the results. Therefore, controlling these confounding factors and performing higher quality studies is the direction of our efforts in the future. Despite these limitations, to the best of our knowledge, this is currently the most comprehensive and high-quality meta-analysis with the largest population investigating the risk of CRN in IBD patients with PIPs.

CONCLUSION

In summary, IBD patients with PIPs have a higher risk of CRN than those without PIPs. Our best evidence-based study advocates the current guideline that IBD patients with PIPs require more intensive surveillance.

ARTICLE HIGHLIGHTS

Research background

Inflammatory bowel disease (IBD) patients with post-inflammatory polyps (PIPs) may carry an increased risk of colorectal neoplasia (CRN). Current guidelines recommend more aggressive colonoscopy follow-up in these patients. However, the guidelines are based on a low degree of evidence and several recent high-quality studies have shown that the presence of PIPs is not an independent risk factor for CRN in IBD patients.

Research motivation

Whether the risk of CRN in IBD patients with PIPs is increased will have a significant impact on the surveillance strategies of IBD patients.

Research objectives

The current study aimed to evaluate the risk of CRN in IBD patients with and without PIPs.

Research methods

A systematic literature search was performed to identify studies that compared the risk of CRN in IBD patients with and without PIPs. Pooled odds ratio (OR) was calculated using the random-effects model to explore the final pooled effect size of the included studies and determine whether PIPs increase the risk of CRN. Sensitivity analysis, subgroup analysis, and assessment of publication bias were performed to determine the sources of heterogeneity.

Research results

We found that IBD patients with PIPs had an approximately 2-fold increased risk of CRN [OR 2.01; 95% confidence interval (CI): 1.43–2.83]. The results were similar when colorectal cancer was used as the study endpoint (OR 2.57; 95%CI: 1.69–3.91).

Research conclusions

IBD patients with PIPs have a higher risk of CRN than those without PIPs, which support current guidelines that IBD patients with PIPs require more frequent surveillance.

Research perspectives

Our findings not only confirm the viewpoint of the guidelines, but may also improve the degree of evidence. We expect that our study can provide a reference for the development of surveillance strategies for IBD patients.

REFERENCES

1 **Politis DS**, Katsanos KH, Tsianos EV, Christodoulou DK. Pseudopolyps in inflammatory bowel diseases: Have we learned enough? *World J Gastroenterol* 2017; **23**: 1541-1551 [PMID: 28321155 DOI: 10.3748/wjg.v23.i9.1541]

2 **Gallart-Esquerdo A**. [Anatomicopathological study of rectocolic inflammatory pseudo-polyposis following mucohemorrhagic rectocolitis (grave ulcerative colitis)]. *Rev Bras Gastroenterol* 1958; **10**: 1-4 [PMID: 13568179]

3 **Goldgraber MB**. Pseudopolyps in ulcerative colitis. *Dis Colon Rectum* 1965; **8**: 355-363 [PMID: 5830658 DOI: 10.1007/bf02627260]

4 **Munyer TP**, Montgomery CK, Thoeni RF, Goldberg HI, Margulis AR. Postinflammatory polyposis (PIP) of the colon: the radiologic-pathologic spectrum. *Radiology* 1982; **145**: 607-614 [PMID: 7146388 DOI: 10.1148/radiology.145.3.7146388]

5 **Lumb G**. Pathology of ulcerative colitis. *Gastroenterology* 1961; **40**: 290-298 [PMID: 13764247]

6 **Kelly JK**, Gabos S. The pathogenesis of inflammatory polyps. *Dis Colon Rectum* 1987; **30**: 251-254 [PMID: 3829873 DOI: 10.1007/bf02556166]

7 **Schneider R**, Dickersin GR, Patterson JF. Localized giant pseudopolyposis. A complication of granulomatous colitis. *Am J Dig Dis* 1973; **18**: 265-270 [PMID: 4695612 DOI: 10.1007/bf01070986]

8 **Baars JE**, Nuij VJ, Oldenburg B, Kuipers EJ, van der Woude CJ. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis* 2012; **18**: 1634-1640 [PMID: 22069022 DOI: 10.1002/ibd.21925]

9 **De Dombal FT**, Watts JM, Watkinson G, Goligher JC. Local complications of ulcerative colitis: stricture, pseudopolyposis, and carcinoma of colon and rectum. *Br Med J* 1966; **1**: 1442-1447 [PMID: 5933046 DOI: 10.1136/bmj.1.5501.1442]

10 **Jalan KN**, Walker RJ, Sircus W, McManus JP, Prescott RJ, Card WI. Pseudopolyposis in ulcerative colitis. *Lancet* 1969; **2**: 555-559 [PMID: 4185531 DOI: 10.1016/s0140-6736(69)90260-8]

11 **Chuttani HK**, Nigam SP, Sama SK, Dhanda PC, Gupta PS. Ulcerative colitis in the tropics. *Br Med J* 1967; **4**: 204-207 [PMID: 6053989 DOI: 10.1136/bmj.4.5573.204]

12 **Geboes K**, Vantrappen G. The value of colonoscopy in the diagnosis of Crohn's disease. *Gastrointest Endosc* 1975; **22**: 18-23 [PMID: 1205096 DOI: 10.1016/s0016-5107(75)73675-1]

13 **Margulis AR**, Goldberg HI, Lawson TL, Montgomery CK, Rambo ON, Noonan CD, Amberg JR. The overlapping spectrum of ulcerative and granulomatous colitis: a roentgenographic-pathologic study. *Am J Roentgenol Radium Ther Nucl Med* 1971; **113**: 325-334 [PMID: 5098634 DOI: 10.2214/ajr.113.2.325]

14 **Rutter MD**, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC, Forbes A. Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* 2004; **53**: 1813-1816 [PMID: 15542520 DOI: 10.1136/gut.2003.038505]

15 **Ray G**. Inflammatory bowel disease in India--changing paradigms. *Int J Colorectal Dis* 2011; **26**: 635-644 [PMID: 21063715 DOI: 10.1007/s00384-010-1084-5]

16 **Teague RH**, Read AE. Polyposis in ulcerative colitis. *Gut* 1975; **16**: 792-795 [PMID: 1205273 DOI: 10.1136/gut.16.10.792]

17 **Reinglas J**, Gonczi L, Kurt Z, Bessissow T, Lakatos PL. Positioning of old and new biologicals and small molecules in the treatment of inflammatory bowel diseases. *World J Gastroenterol* 2018; **24**: 3567-3582 [PMID: 30166855 DOI: 10.3748/wjg.v24.i32.3567]

18 **Teh LB**, Koh D, Ng HS, Kwok KC, Lim TC, Ho MS, Seah CS. Ulcerative colitis in Singapore: a clinical study of sixty-one patients. *Ann Acad Med Singap* 1987; **16**: 474-479 [PMID: 2893576]

19 **Wright V**, Watkinson G. The arthritis of ulcerative colitis. *Br Med J* 1965; **2**: 670-675 [PMID: 14337733 DOI: 10.1136/bmj.2.5463.670]

20 **Wang Y**, Ouyang Q; APDW 2004 Chinese IBD working group. Ulcerative colitis in China: retrospective analysis of 3100 hospitalized patients. *J Gastroenterol Hepatol* 2007; **22**: 1450-1455 [PMID: 17716349 DOI: 10.1111/j.1440-1746.2007.04873.x]

21 **Baars JE**, Kuipers EJ, van Haastert M, Nicolaï JJ, Poen AC, van der Woude CJ. Age at diagnosis of inflammatory bowel disease influences early development of colorectal cancer in inflammatory bowel disease patients: a nationwide, long-term survey. *J Gastroenterol* 2012; **47**: 1308-1322 [PMID: 22627504 DOI: 10.1007/s00535-012-0603-2]

22 **Chang DK**, Kim JJ, Choi H, Eun CS, Han DS, Byeon JS, Kim JO. Double balloon endoscopy in small intestinal Crohn's disease and other inflammatory diseases such as cryptogenic multifocal ulcerous stenosing enteritis (CMUSE). *Gastrointest Endosc* 2007; **66**: S96-S98 [PMID: 17709044 DOI: 10.1016/j.gie.2007.01.016]

23 **Luo YY**, Chen J. [Clinical and colonoscopic characteristics of pediatric inflammatory bowel disease]. *Zhonghua Erke Zazhi* 2009; **47**: 129-133 [PMID: 19573460]

24 **Kosugi I**, Tada T, Tsutsui Y, Sato Y, Mitsui T, Itazu I. Giant inflammatory polyposis of the descending colon associated with a Crohn's disease-like colitis. *Pathol Int* 2002; **52**: 318-321 [PMID: 12031089 DOI: 10.1046/j.1440-1827.2002.01348.x]

25 **Buck JL**, Dachman AH, Sobin LH. Polypoid and pseudopolypoid manifestations of inflammatory bowel disease. *Radiographics* 1991; **11**: 293-304 [PMID: 2028064 DOI: 10.1148/radiographics.11.2.2028064]

26 **Bacon HE**, Carroll PT, Cates BA, Mcgregor RA, Ouyang LM, Villalba G. Non-specific ulcerative colitis, with reference to mortality, morbidity, complications and long-term survivals following colectomy. *Am J Surg* 1956; **92**: 688-695 [PMID: 13372871 DOI: 10.1016/s0002-9610(56)80140-2]

27 **Cairns SR**, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, Eaden JA, Rutter MD, Atkin WP, Saunders BP, Lucassen A, Jenkins P, Fairclough PD, Woodhouse CR; British Society of Gastroenterology; Association of Coloproctology for Great Britain and Ireland. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 2010; **59**: 666-689 [PMID: 20427401 DOI: 10.1136/gut.2009.179804]

28 **Farraye FA**, Odze RD, Eaden J, Itzkowitz SH. AGA technical review on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* 2010; **138**: 746-774 [PMID: 20141809 DOI: 10.1053/j.gastro.2009.12.035]

29 **Velayos FS**, Loftus EV Jr, Jess T, Harmsen WS, Bida J, Zinsmeister AR, Tremaine WJ, Sandborn WJ. Predictive and protective factors associated with colorectal cancer in ulcerative colitis: A case-control study. *Gastroenterology* 2006; **130**: 1941-1949 [PMID: 16762617 DOI: 10.1053/j.gastro.2006.03.028]

30 **Al Bakir I**, Curtius K, Kabir M, Saunders B, Suzuki N, Thomas-Gibson S, Wilson A, Moorghen M, Graham T, Hart A. Long-term outcomes following endoscopic resection of neoplastic lesions in ulcerative colitis: A large single-centre retrospective study. *United Eur Gastroent* 2019; **7**: 80 [DOI: 10.1177/2050640619854671]

31 **de Jong ME**, Gillis VELM, Derikx LAAP, Hoentjen F. No Increased Risk of Colorectal Neoplasia in Patients With Inflammatory Bowel Disease and Postinflammatory Polyps. *Inflamm Bowel Dis* 2020; **26**: 1383-1389 [PMID: 31677385 DOI: 10.1093/ibd/izz261]

32 **Mahmoud R**, Shah SC, Ten Hove JR, Torres J, Mooiweer E, Castaneda D, Glass J, Elman J, Kumar A, Axelrad J, Ullman T, Colombel JF, Oldenburg B, Itzkowitz SH; Dutch Initiative on Crohn and Colitis. No Association Between Pseudopolyps and Colorectal Neoplasia in Patients With Inflammatory Bowel Diseases. *Gastroenterology* 2019; **156**: 1333-1344.e3 [PMID: 30529584 DOI: 10.1053/j.gastro.2018.11.067]

33 **Shamseer L**, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015; **350**: g7647 [PMID: 25555855 DOI: 10.1136/bmj.g7647]

34 **Ünal NG**, Özütemiz Ö, Tekin F, Turan İ, Osmanoğlu N. Colorectal cancer and dysplasia risk of ulcerative colitis patients in a tertiary referral center in Turkey. *Turk J Gastroenterol* 2019; **30**: 139-147 [PMID: 30460897 DOI: 10.5152/tjg.2018.18221]

35 **Gu YB**, Xu WM, Guo YG, Zhong J, Du P. Risk factors of colorectal stricture associated with developing high-grade dysplasia or cancer in ulcerative colitis: A multicenter long-term follow-up study. *J Dig Dis* 2019; **20**: 26 [DOI: 10.1111/1751-2980.12808]

36 **Ma YR**, Ko HBM, Zhu H, Harpaz N, Polydorides AD. Gastrointestinal Pathology. *Lab Invest* 2017; 97: 157-210 [PMID: 28134861 DOI: 10.1038/labinvest.2016.168]

37 **Jegadeesan R**, Navaneethan U, Gutierrez NG, Venkatesh PG, Hammel JP, Sanaka MR, Shen B. Pattern of Inflammation on Surveillance Colonoscopy Does Not Predict Development of Colitis-associated Neoplasia. *Inflamm Bowel Dis* 2016; **22**: 2221-2228 [PMID: 27542134 DOI: 10.1097/MIB.0000000000000862]

38 **Lutgens M**, Vermeire S, Van Oijen M, Vleggaar F, Siersema P, van Assche G, Rutgeerts P, Oldenburg B; Dutch Initiative on Crohn and Colitis. A rule for determining risk of colorectal cancer in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2015; **13**: 148-54. e1 [PMID: 25041864 DOI: 10.1016/j.cgh.2014.06.032]

39 **Freire P**, Figueiredo P, Cardoso R, Manuel Donato M, Ferreira M, Mendes S, Silva MR, Cipriano MA, Ferreira AM, Vasconcelos H, Portela F, Sofia C. Predictive value of rectal aberrant crypt foci for intraepithelial neoplasia in ulcerative colitis - a cross-sectional study. *Scand J Gastroenterol* 2014; **49**: 1219-1229 [PMID: 25157637 DOI: 10.3109/00365521.2014.951390]

40 **Badamas J**, Limketkai BN, Lazarev M. Risk of dysplasia according to severity of pseudopolyposis in inflammatory bowel disease. *Gastroenterology* 2014; **146**: S-436 [DOI: 10.1016/S0016-5085(14)61568-7]

41 **Baars JE**, Looman CW, Steyerberg EW, Beukers R, Tan AC, Weusten BL, Kuipers EJ, van der Woude CJ. The risk of inflammatory bowel disease-related colorectal carcinoma is limited: results from a nationwide nested case-control study. *Am J Gastroenterol* 2011; **106**: 319-328 [PMID: 21045815 DOI: 10.1038/ajg.2010.428]

42 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]

43 **Okugawa Y**, Toiyama Y, Kusunoki M, Goel A. Re: Cumulative burden of inflammation predicts colorectal neoplasia risk in ulcerative colitis: a large single-centre study. *Gut* 2019; **68**: 575 [PMID: 29535153 DOI: 10.1136/gutjnl-2018-316205]

44 **Lakatos L**, Mester G, Erdelyi Z, David G, Pandur T, Balogh M, Fischer S, Vargha P, Lakatos PL. Risk factors for ulcerative colitis-associated colorectal cancer in a Hungarian cohort of patients with ulcerative colitis: results of a population-based study. *Inflamm Bowel Dis* 2006; **12**: 205-211 [PMID: 16534422 DOI: 10.1097/01.MIB.0000217770.21261.ce]

45 **Xu W**, Ding W, Gu Y, Cui L, Zhong J, Du P. Risk Factors of Colorectal Stricture Associated with Developing High-Grade Dysplasia or Cancer in Ulcerative Colitis: A Multicenter Long-term Follow-up Study. *Gut Liver* 2020; **14**: 601-610 [PMID: 31816674 DOI: 10.5009/gnl19229]

46 **Ando T**, Ishiguro K, Maeda O, Watanabe O, Miyake N, Nakamura M, Miyahara R, Ohmiya N, Goto H. Clinicopathological characteristics of colorectal cancer in patients with ulcerative colitis. *Gastrointest Endosc* 2010; **71**: AB345-AB346 [DOI: 10.1016/j.gie.2010.03.919]

47 **Lutgens M**, Siersema PD, Vleggaar F, Broekman M, Van Bodegraven AA, Dijkstra G, Hommes DW, De Jong D, Stokkers PCF, Van Der Woude CJ, Oldenburg B. Risk factors for inflammatory bowel disease associated colorectal carcinoma. *J Crohns Colitis* 2011; **5**: S12

48 **Lutgens MW**, Siersema PD, Vleggaar FP, Broekman M, Van Oijen MG, Van Bodegraven AA, Dijkstra G, Hommes DW, De Jong DJ, Ponsioen C, Van Der Woude CJ, Oldenburg B. Risk factors for advanced neoplasia of the rectal stump in IBD patients. *Gastroenterology* 2011; **140**: S351-S352 [DOI: 10.1016/S0016-5085(11)61433-9]

49 **Mahmoud R**, Shah S, Ten Hove J, Torres J, Mooiweer E, Castaneda D, Glass J, Elman J, Kumar A, Axelrad J, Ullman T, Colombel JF, Oldenburg B, Itzkowitz S. Post-inflammatory polyps do not predict colorectal neoplasia in patients with inflammatory bowel disease: A multinational retrospective cohort study. *J Crohns Colitis* 2018; **12**: S156-S157

50 **Lewis AE**, Kirchgesner J, Dray X, Svrcek M, Beaugerie L. Clinical Significance of Pseudopolyps for Patients With Inflammatory Bowel Disease. *Am J Gastroenterol* 2019; **114**: S179-S180 [DOI: 10.14309/01.ajg.0000590752.06064.e8]

51 **Abou Rached A**, Saniour J, Shehab R, Abou Fadel C, Yaghi C, Khairallah S. The association between the severity of histological lesions with the disease location and presence of colonic lesions in patients with ulcerative colitis. *J Crohns Colitis* 2019; **13**: S235-S236 [DOI: 10.1093/ecco-jcc/jjy222.398]

52 **Flores BM**, O'Connor A, Moss AC. Impact of mucosal inflammation on risk of colorectal neoplasia in patients with ulcerative colitis: a systematic review and meta-analysis. *Gastrointest Endosc* 2017; **86**: 1006-1011.e8 [PMID: 28750838 DOI: 10.1016/j.gie.2017.07.028]

53 **Dukes CE**. The surgical pathology of ulcerative colitis. *Ann R Coll Surg Engl* 1954; **14**: 389-400 [PMID: 13159102]

54 **Kusunoki M**, Nishigami T, Yanagi H, Okamoto T, Shoji Y, Sakanoue Y, Yamamura T, Utsunomiya J. Occult cancer in localized giant pseudopolyposis. *Am J Gastroenterol* 1992; **87**: 379-381 [PMID: 1539578]

55 **Jawad N**, Graham T, Novelli M, Rodriguez-Justo M, Feakins R, Silver A, Wright N, McDonald S. PTU-124 Are pseudopolyps the source of tumorigenic mutations in ulcerative colitis? *Gut* 2012; **61**: A236 [DOI: 10.1136/gutjnl-2012-302514c.124]

56 **Lozyns'ka MR**. [The spectrum of cytogenetic chages in polyps of the large intestine with different histologic structure]. *Tsitol Genet* 2008; **42**: 43-49 [PMID: 19253754]

57 **Liatsos C**, Kyriakos N, Panagou E, Karagiannis S, Salemis N, Mavrogiannis C. Inflammatory polypoid mass treated with Infliximab in a Crohn's disease patient. *J Crohns Colitis* 2010; **4**: 707-708 [PMID: 21122587 DOI: 10.1016/j.crohns.2010.09.006]

58 **Cohen-Mekelburg S**, Schneider Y, Gold S, Scherl E, Steinlauf A. Advances in the Diagnosis and Management of Colonic Dysplasia in Patients With Inflammatory Bowel Disease. *Gastroenterol Hepatol (N Y)* 2017; **13**: 357-362 [PMID: 28690452]
59 **Shah SC**, Itzkowitz SH. Management of Inflammatory Bowel Disease-Associated Dysplasia in the Modern Era. *Gastrointest Endosc Clin N Am* 2019; **29**: 531-548 [PMID: 31078251 DOI: 10.1016/j.giec.2019.02.008]
60 **Choi YS**, Suh JP, Lee IT, Kim JK, Lee SH, Cho KR, Park HJ, Kim DS, Lee DH. Regression of giant pseudopolyps in inflammatory bowel disease. *J Crohns Colitis* 2012; **6**: 240-243 [PMID: 22325179 DOI: 10.1016/j.crohns.2011.08.007]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 362-365

DOI: 10.4251/wjgo.v14.i1.362

ISSN 1948-5204 (online)

LETTER TO THE EDITOR

Liquid biopsy: Precise diagnosis and therapy for cholangiocarcinoma

Si-Qin Wang, Chu-Xing Chai, Bi-Rong Wang, Feng Zhu, Dan Shang, Min Li

ORCID number: Si-Qin Wang 0000-0002-8034-7471; Chu-Xing Chai 0000-0002-7361-0200; Bi-Rong Wang 0000-0003-3195-0407; Feng Zhu 0000-0003-0647-9537; Dan Shang 0000-0003-4585-2744; Min Li 0000-0002-0047-2804.

Author contributions: Wang SQ, Chai CX and Wang BR contributed equally to this work and drafted the manuscript; Chai CX and Li M revised the manuscript; Wang SQ, Chai CX, Wang BR and Zhu F collected the references; Li M and Shang D designed the work; all authors approved the final version.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Supported by Wuhan Municipal Health Commission, No. WX14B22; and National Natural Science Foundation of China, No. 81874208 and No. 81700425.

Country/Territory of origin: China

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Si-Qin Wang, Feng Zhu, Dan Shang, Department of Vascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Chu-Xing Chai, Min Li, Department of Hepatobiliary Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Bi-Rong Wang, Department of Breast and Thyroid Surgery, Wuhan Fourth Hospital; Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Corresponding author: Dan Shang, MD, PhD, Associate Professor, Department of Vascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan 430022, Hubei Province, China.
danshang@hust.edu.cn

Abstract

The following letter to the editor highlights the review titled "Liquid biopsy in cholangiocarcinoma: Current status and future perspective" in *World J Gastrointest Oncol* 2021; 13: 332-350. It is necessary to realize individualized therapy to improve the clinical prognosis of patients with cholangiocarcinoma.

Key Words: Liquid biopsy; Cholangiocarcinoma; Diagnosis; Therapy; Precision medicine

Core Tip: Cholangiocarcinoma (CCA) is an aggressive biliary malignancy, and existing clinical tools cannot improve survival rates. The major goal of this letter is to stress the fascinating promise and challenge of liquid biopsy in the diagnosis and therapy of patients with CCA.

Citation: Wang SQ, Chai CX, Wang BR, Zhu F, Shang D, Li M. Liquid biopsy: Precise diagnosis and therapy for cholangiocarcinoma. *World J Gastrointest Oncol* 2022; 14(1): 362-365

Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: June 12, 2021
Peer-review started: June 12, 2021
First decision: July 16, 2021
Revised: July 19, 2021
Accepted: November 26, 2021
Article in press: November 26, 2021
Published online: January 15, 2022

P-Reviewer: Hosoe N, Rompianesi G
S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

URL: https://www.wjgnet.com/1948-5204/full/v14/i1/362.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.362

TO THE EDITOR

We read with great interest the review titled "Liquid biopsy in cholangiocarcinoma: Current status and future perspective" by Rompianesi *et al*[1], and we believe that liquid biopsy (LB) has opened new avenues for personalized medicine in patients with cholangiocarcinoma (CCA). This review summarizes the present challenges of diagnosing, managing and monitoring CCA and the unique advantage of LB for these challenges. The authors conclude that a growing body of research supports the idea that LB can overcome the difficulties of traditional tools and might be particularly helpful in detecting early cancer, identifying therapeutic targets, predicting treatment response, and monitoring the genetic profile of CCA.

CCA is an aggressive biliary malignancy originating from cholangiocytes along the biliary tree, excluding the gall bladder and the Vater ampulla[2]. CCA is usually asymptomatic in the early stages. Therefore, the majority of CCA patients are generally diagnosed at an advanced stage. Because there are limited therapeutic options, advanced CCA has a dismal prognosis[3,4]. Even for patients with localized early disease who can benefit from surgery, the high recurrence rate may cause an inferior clinical outcome[5]. Despite recent advances in systemic chemotherapy, targeted therapy, and immunotherapy, the prognosis of patients with advanced unresectable CCA remains disappointing because of tumour heterogeneity and the variability of treatment response[6]. As the recognition of the importance of precision medicine by clinicians is growing, there is an urgent need for new, accurate tools for early cancer detection, monitoring of the tumour molecular profile, real-time assessment of therapeutic efficacy, and identification of therapeutic targets and resistance mechanisms in CCA.

Tumours can release their contents along with genetic material into body fluids such as blood, urine, saliva, bile, and cerebrospinal fluid[7]. LB is a novel, minimally invasive, and safe method for detecting tumour components in body fluids, including circulating tumour cells, circulating tumour DNA (ctDNA), circulating cell-free RNA, extracellular vesicles, and tumour-educated platelets[8]. Advances in the detection and characterization of ctDNA have enabled LB to be rapidly translated into the management of patients with advanced solid tumours. With the development of next-generation sequencing and oncology genomics assessment, researchers can identify and analyse a wealth of cancer genetic markers that contribute to the occurrence, progression and heterogeneity of cancer[9]. Analysing genetic markers or the molecular profile of solid cancers traditionally relies on tissue biopsy. However, limited accessibility to tumour samples and tumour heterogeneity present challenges for acquiring representative tumour samples throughout the disease course[10]. As a less invasive approach, LB can be used to track spatial and temporal heterogeneity and monitor dynamic changes in tumour biology at the molecular and genetic levels[11].

LB samples (in most cases, blood) are easy to obtain, and LB can be repeated in patients, enabling real-time molecular monitoring of CCA. LB approaches can also be used to detect abnormalities before imaging examinations. As previously reported, the detection of ctDNA precedes the radiological detection of early tumour recurrence by 3–5 mo in several cancers[11,12]. Furthermore, LB can be used to guide clinical treatment and monitor the treatment response. Among patients with biliary tract cancers who received systemic treatment after ctDNA analysis and drug matching, the matched targeted regimens showed longer progression-free survival and a better disease control rate than unmatched methods[9]. Characterized, therapeutically relevant ctDNA alterations can also be found in CCA patients after gene-targeted therapy[13]. Furthermore, since ctDNA may include DNA shed into the bloodstream from both primary and metastatic tumours, the genomic alterations of ctDNA can reflect the cancer heterogeneity of the whole body better than those found in tissue biopsy[14,15]. Cancer heterogeneity may be part of the reason for the unfavourable outcomes of several gene-targeted trials in CCA[16].

There remain several challenges for the clinical application of LB. The low concentration of ctDNA and difficulty in identifying ctDNA in peripheral blood may limit the accuracy of detection. There are also high sensitivity and specificity requirements of detection methods. Since various ctDNA assays are available, more comprehensive cross-platform comparisons are needed to standardize the preanalytical and analytical

procedures. Detectable genomic mutations are not always relevant to cancer biology or therapy, so ctDNA analysis and sequencing data should be carefully interpreted. The use of machine learning tools and artificial intelligence technology may efficiently aid the analysis of increasingly complex cancer LB data[17].

In conclusion, it is necessary to realize individualized therapy to improve the clinical prognosis of patients with CCA[5]. As an easy method for assessing genetic material and molecular profiling, LB can play an important role in early cancer detection, tumour heterogeneity assessment, therapy selection, and prognostic stratification in CCA. Although challenges exist for the clinical application of LB, its potential represents a movement towards precision medicine and individualized therapy. The scarcity of clinical data suggests that larger and deeper studies to define and validate the diagnostic and therapeutic roles of LB in CCA are needed.

REFERENCES

1 **Rompianesi G**, Di Martino M, Gordon-Weeks A, Montalti R, Troisi R. Liquid biopsy in cholangiocarcinoma: Current status and future perspectives. *World J Gastrointest Oncol* 2021; **13**: 332-350 [PMID: 34040697 DOI: 10.4251/wjgo.v13.i5.332]

2 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]

3 **Cillo U**, Fondevila C, Donadon M, Gringeri E, Mocchegiani F, Schlitt H, Ijzermans J, Vivarelli M, Zieniewicz K, Olde Damink S, Groot Koerkamp B. Surgery for cholangiocarcinoma. *Liver Int* 2019; **39**: 143-155 [PMID: 30843343 DOI: 10.1111/liv.14089]

4 **Banales JM**, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, Cardinale V, Carpino G, Andersen JB, Braconi C, Calvisi DF, Perugorria MJ, Fabris L, Boulter L, Macias RIR, Gaudio E, Alvaro D, Gradilone SA, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Rizvi S, Heimbach J, Koerkamp BG, Bruix J, Forner A, Bridgewater J, Valle JW, Gores GJ. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 557-588 [PMID: 32606456 DOI: 10.1038/s41575-020-0310-z]

5 **Marin JJG**, Prete MG, Lamarca A, Tavolari S, Landa-Magdalena A, Brandi G, Segatto O, Vogel A, Macias RIR, Rodrigues PM, Casta A, Mertens J, Rodrigues CMP, Fernandez-Barrena MG, Da Silva Ruivo A, Marzioni M, Mentrasti G, Acedo P, Munoz-Garrido P, Cardinale V, Banales JM, Valle JW, Bridgewater J, Braconi C; working group 6 of the COST-action 18122 (Euro-Cholangio-NET) as part of the European Network for the study of Cholangiocarcinoma (ENSCCA). Current and novel therapeutic opportunities for systemic therapy in biliary cancer. *Br J Cancer* 2020; **123**: 1047-1059 [PMID: 32694694 DOI: 10.1038/s41416-020-0987-3]

6 **Roy D**, Lucci A, Ignatiadis M, Jeffrey S. Cell-free circulating tumor DNA profiling in cancer management. *Trends Mol Med* 2021; **27**: 1014-1015 [PMID: 34312074 DOI: 10.1016/j.molmed.2021.07.001]

7 **Bradley SH**, Barclay ME. "Liquid biopsy" for cancer screening. *BMJ* 2021; **372**: m4933 [PMID: 33397684 DOI: 10.1136/bmj.m4933]

8 **Alix-Panabières C**, Pantel K. Liquid Biopsy: From Discovery to Clinical Application. *Cancer Discov* 2021; **11**: 858-873 [PMID: 33811121 DOI: 10.1158/2159-8290.CD-20-1311]

9 **Okamura R**, Kurzrock R, Mallory RJ, Fanta PT, Burgoyne AM, Clary BM, Kato S, Sicklick JK. Comprehensive genomic landscape and precision therapeutic approach in biliary tract cancers. *Int J Cancer* 2021; **148**: 702-712 [PMID: 32700810 DOI: 10.1002/ijc.33230]

10 **Kilgour E**, Rothwell DG, Brady G, Dive C. Liquid Biopsy-Based Biomarkers of Treatment Response and Resistance. *Cancer Cell* 2020; **37**: 485-495 [PMID: 32289272 DOI: 10.1016/j.ccell.2020.03.012]

11 **Biswas D**, Ganeshalingam J, Wan JCM. The future of liquid biopsy. *Lancet Oncol* 2020; **21**: e550 [PMID: 33271107 DOI: 10.1016/S1470-2045(20)30687-2]

12 **Wang Y**, Li L, Cohen JD, Kinde I, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tie J, Gibbs P, Tomasetti C, Kinzler KW, Papadopoulos N, Vogelstein B, Olsson L. Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer. *JAMA Oncol* 2019; **5**: 1118-1123 [PMID: 31070668 DOI: 10.1001/jamaoncol.2019.0512]

13 **Mody K**, Kasi PM, Yang JD, Surapaneni PK, Bekaii-Saab T, Ahn DH, Mahipal A, Sonbol MB, Starr JS, Roberts A, Nagy R, Lanman R, Borad MJ. Circulating Tumor DNA Profiling of Advanced Biliary Tract Cancers. *Jco Precision Oncology* 2019; 3 [DOI: 10.1200/po.18.00324]

14 **Balasaheb Mali S**, Dahivelkar S. Liquid biopsy = Individualized cancer management: Diagnosis, monitoring treatment and checking recurrence and metastasis. *Oral Oncol* 2021; **123**: 105588 [PMID: 34744021 DOI: 10.1016/j.oraloncology.2021.105588]

15 **Pectasides E**, Stachler MD, Derks S, Liu Y, Maron S, Islam M, Alpert L, Kwak H, Kindler H, Polite

B, Sharma MR, Allen K, O'Day E, Lomnicki S, Maranto M, Kanteti R, Fitzpatrick C, Weber C, Setia N, Xiao SY, Hart J, Nagy RJ, Kim KM, Choi MG, Min BH, Nason KS, O'Keefe L, Watanabe M, Baba H, Lanman R, Agoston AT, Oh DJ, Dunford A, Thorner AR, Ducar MD, Wollison BM, Coleman HA, Ji Y, Posner MC, Roggin K, Turaga K, Chang P, Hogarth K, Siddiqui U, Gelrud A, Ha G, Freeman SS, Rhoades J, Reed S, Gydush G, Rotem D, Davison J, Imamura Y, Adalsteinsson V, Lee J, Bass AJ, Catenacci DV. Genomic Heterogeneity as a Barrier to Precision Medicine in Gastroesophageal Adenocarcinoma. *Cancer Discov* 2018; **8**: 37-48 [PMID: 28978556 DOI: 10.1158/2159-8290.CD-17-0395]

16 **Hezel AF**, Deshpande V, Zhu AX. Genetics of biliary tract cancers and emerging targeted therapies. *J Clin Oncol* 2010; **28**: 3531-3540 [PMID: 20547994 DOI: 10.1200/JCO.2009.27.4787]

17 **Im YR**, Tsui DWY, Diaz LA Jr, Wan JCM. Next-Generation Liquid Biopsies: Embracing Data Science in Oncology. *Trends Cancer* 2021; **7**: 283-292 [PMID: 33317961 DOI: 10.1016/j.trecan.2020.11.001]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 January 15; 14(1): 366-368

DOI: 10.4251/wjgo.v14.i1.366

ISSN 1948-5204 (online)

LETTER TO THE EDITOR

Comment on "Outcomes of curative liver resection for hepatocellular carcinoma in patients with cirrhosis"

Xiao-Long Tang, Yan-Dong Miao, Deng-Hai Mi

ORCID number: Xiao-Long Tang 0000-0001-9229-6424; Yan-Dong Miao 0000-0002-1429-8915; Deng-Hai Mi 0000-0002-8643-4496.

Author contributions: Tang XL and Miao YD designed, performed research and analyzed data; Tang XL wrote the comment; and Mi DH revised the comment.

Conflict-of-interest statement: No conflict of interest exists in submitting this manuscript, and all authors approve the manuscript for publication. All the authors listed have approved the manuscript that is enclosed.

Supported by The Special Plan for Condition Construction of Gansu Provincial Scientific Research Institutes, No. 20JR10RA432.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0

Xiao-Long Tang, Yan-Dong Miao, Deng-Hai Mi, The First Clinical Medical College, Lanzhou University, Lanzhou 730000, Gansu Province, China

Xiao-Long Tang, The Second Department of Gastrointestinal Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, Sichuan Province, China

Deng-Hai Mi, Dean's Office, Gansu Academy of Traditional Chinese Medicine, Lanzhou730000, Gansu Province, China

Corresponding author: Deng-Hai Mi, MD, Chief Doctor, Dean, Professor, The First Clinical Medical College, Lanzhou University, No. 1 Donggang West Road, Chengguan District, Lanzhou 730000, Gansu Province, China. mi.dh@outlook.com

Abstract

The present letter to the editor is in response to the research "Outcomes of curative liver resection for hepatocellular carcinoma in patients with cirrhosis" by Elshaarawy *et al* in *World J Gastroenterol* 2021; 13(5): 424–439. The preoperative assessment of the liver reserve function in hepatocellular carcinoma (HCC) patients with cirrhosis is crucial, and there is no universal consensus on how to assess it. Based on a retrospective study, Elshaarawy *et al* investigated the impact of various classical clinical indicators on liver failure and the prognosis after hepatectomy in HCC patients with cirrhosis. We recommend that we should strive to explore new appraisal indicators, such as the indocyanine green retention rate at 15 min.

Key Words: ICG-R15; Hepatectomy; Cirrhosis; Hepatocellular carcinoma

Core Tip: Inappropriate hepatectomy might result in liver failure and even death for hepatocellular carcinoma (HCC) patients with cirrhosis. The main highlight of our comment is to emphasize the urgency of discovering and confirming new markers before hepatectomy in HCC patients with cirrhosis.

Citation: Tang XL, Miao YD, Mi DH. Comment on "Outcomes of curative liver resection for hepatocellular carcinoma in patients with cirrhosis". *World J Gastrointest Oncol* 2022; 14(1):

Grade D (Fair): D
Grade E (Poor): 0

Received: June 21, 2021
Peer-review started: June 21, 2021
First decision: July 29, 2021
Revised: August 9, 2021
Accepted: December 25, 2021
Article in press: December 25, 2021
Published online: January 15, 2022

P-Reviewer: Huang Z, Imai N
S-Editor: Wang JL
L-Editor: A
P-Editor: Wang JL

366-368
URL: https://www.wjgnet.com/1948-5204/full/v14/i1/366.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i1.366

TO THE EDITOR

In "Outcomes of curative liver resection for hepatocellular carcinoma in patients with cirrhosis", Elshaarawy *et al*[1] evaluated many classical predictors for liver failure and the prognosis in cirrhosis patients experiencing a radical resection of hepatocellular carcinoma (HCC) through univariate and multivariate analysis. They discovered that the preoperative model for end-stage liver disease (MELD) score, tumor diameter, length of hospital stays after radical resection of liver cancer, and hospital stay length were meaningful independent predictors of liver decompensation. The preoperative MELD score, various grades of posthepatectomy liver failure, and postoperative HCC recurrence after resection were meaningful independent predictors of the patients' outcome. This study provides helpful information and is valuable for doctors to enhance the preoperative assessment of HCC patients with cirrhosis. Despite intensely appreciating this work, we believe that the research would have been much more attractive if the writer had adopted the indocyanine green retention rate at 15 min (ICG-R15). For more details about this viewpoint, we look forward to an assessment and a communication with the writers.

With the dramatic advancement of surgical techniques, the procedures of hepatectomy are getting progressively radical. Inappropriate surgery might result in liver failure and even death. It is worthwhile for surgeons to concentrate on identifying the meaningful markers of postoperative liver decompensation and the prognosis. In recent years, the ICG-R15 has gained expanded attention in assessing liver function and has been widely employed for the preoperative assessment of hepatic functional reserve. Thus, it is more attractive if the writer can further strengthen the relevant study.

Indocyanine green retention (ICG) is specifically absorbed by hepatocytes after injection, is secreted by hepatocytes into bile, and is promptly excreted through the biliary tract[2]. ICG has no chemical reaction in the body and is eliminated only through the liver. Therefore, it can be a good way to determine the liver's functional reserve. The ICG-R15 can vary in reply to the current liver functional anomalies when there are no irregularities in many of the traditional biochemical markers. Hence, it supplies the required standards to prevent surgical trauma, blood loss through the liver, and other complications associated with acute liver failure. Recently, Kokudo *et al*[3] reported that ICG-R15 might improve the clinicians' capability to stratify patients at risk for surgical liver failure. Likewise, in a comparative analysis of 185 patients, Wang *et al*[4] found that the ICG-R15 is more reliable than the MELD score and the Child-Pugh score in indicating hepatic functional reserve before hepatectomy.

A precise assessment of the liver's functional reserve is very essential for the proper therapy of HCC patients with cirrhosis. A proper therapy is critical to the patient's recovery. Although no universal consensus is presently available on the assessment of liver functional reserve, we believe that we should vigorously look for more novel and valuable markers to adapt to the advancement of surgical techniques.

ACKNOWLEDGEMENTS

We thank the professors of the School of Foreign Languages at Lanzhou University for their assistance in the linguistic embellishment of this paper.

REFERENCES

1 **Elshaarawy O**, Aman A, Zakaria HM, Zakareya T, Gomaa A, Elshimi E, Abdelsameea E. Outcomes of curative liver resection for hepatocellular carcinoma in patients with cirrhosis. *World J Gastrointest Oncol* 2021; **13**: 424-439 [PMID: 34040703 DOI: 10.4251/wjgo.v13.i5.424]
2 **Inagaki Y**, Kokudo T, Kamiya M, Uno SN, Sato M, Kaneko J, Kokudo N, Urano Y, Hasegawa K. A novel liver-specific fluorescent anti-cancer drug delivery system using indocyanine green. *Sci Rep* 2019; **9**: 3044 [PMID: 30816163 DOI: 10.1038/s41598-019-39269-0]

3 **Kokudo T**, Hasegawa K, Shirata C, Tanimoto M, Ishizawa T, Kaneko J, Akamatsu N, Arita J, Demartines N, Uldry E, Kokudo N, Halkic N. Assessment of Preoperative Liver Function for Surgical Decision Making in Patients with Hepatocellular Carcinoma. *Liver Cancer* 2019; **8**: 447-456 [PMID: 31799202 DOI: 10.1159/000501368]

4 **Wang YY**, Zhao XH, Ma L, Ye JZ, Wu FX, Tang J, You XM, Xiang BD, Li LQ. Comparison of the ability of Child-Pugh score, MELD score, and ICG-R15 to assess preoperative hepatic functional reserve in patients with hepatocellular carcinoma. *J Surg Oncol* 2018; **118**: 440-445 [PMID: 30259515 DOI: 10.1002/jso.25184]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com