SCIENCE CHINA
Life Sciences

•COVER ARTICLE•

May 2023 Vol.66 No.5: 893–1066
https://doi.org/10.1007/s11427-023-2305-0

Biomarkers of aging

Aging Biomarker Consortium[1], Hainan Bao[2†], Jiani Cao[3†], Mengting Chen[4,5,6†], Min Chen[7,8,9†], Wei Chen[10†], Xiao Chen[11†], Yanhao Chen[12†], Yu Chen[13†], Yutian Chen[14†], Zhiyang Chen[15†], Jagadish K Chhetri[16†], Yingjie Ding[2,17†], Junlin Feng[18†], Jun Guo[19†], Mengmeng Guo[20†], Chuting He[17,21,22,23†], Yujuan Jia[24†], Haiping Jiang[3,17,22,23†], Ying Jing[25,26,27†], Dingfeng Li[28†], Jiaming Li[2,17†], Jingyi Li[17,21,22,23†], Qinhao Liang[29†], Rui Liang[30†], Feng Liu[31†], Xiaoqian Liu[3,17,22,23†], Zuojun Liu[32†], Oscar Junhong Luo[33†], Jianwei Lv[34†], Jingyi Ma[35†], Kehang Mao[36†], Jiawei Nie[37†], Xinhua Qiao[38†], Xinpei Sun[39†], Xiaoqiang Tang[40†], Jianfang Wang[41†], Qiaoran Wang[2,17†], Siyuan Wang[42†], Xuan Wang[43†], Yaning Wang[44,45†], Yuhan Wang[17,21,22,23†], Rimo Wu[46†], Kai Xia[47,48†], Fu-Hui Xiao[49,50†], Lingyan Xu[51†], Yingying Xu[2†], Haoteng Yan[25,26,27†], Liang Yang[52†], Ruici Yang[53†], Yuanxin Yang[54†], Yilin Ying[55,56†], Le Zhang[57,58†], Weiwei Zhang[59†], Wenwan Zhang[18†], Xing Zhang[60†], Zhuo Zhang[61,62†], Min Zhou[63†], Rui Zhou[64†], Qingchen Zhu[18†], Zhengmao Zhu[65,66†], Feng Cao[59*], Zhongwei Cao[67*], Piu Chan[16*], Chang Chen[38*], Guobing Chen[68,69*], Hou-Zao Chen[70*], Jun Chen[71*], Weimin Ci[2*], Bi-Sen Ding[67*], Qiurong Ding[12*], Feng Gao[60*], Jing-Dong J. Han[36*], Kai Huang[7,8,9,72*], Zhenyu Ju[15*], Qing-Peng Kong[49,50*], Ji Li[4,5,6*], Jian Li[19*], Xin Li[3,17,22,23*], Baohua Liu[73*], Feng Liu[74*], Lin Liu[65,66,75,76*], Qiang Liu[28*], Qiang Liu[77,78*], Xingguo Liu[52*], Yong Liu[29*], Xianghang Luo[63*], Shuai Ma[17,21,22,23*], Xinran Ma[51*], Zhiyong Mao[13*], Jing Nie[35*], Yaojin Peng[3,17,23*], Jing Qu[3,17,22,23*], Jie Ren[2,17,22*], Ruibao Ren[37,79*], Moshi Song[17,21,22,23*], Zhou Songyang[31,80*], Yi Eve Sun[10*], Yu Sun[18,81*], Mei Tian[82*], Shusen Wang[30*], Si Wang[25,26,27*], Xia Wang[20*], Xiaoning Wang[83*], Yan-Jiang Wang[84*], Yunfang Wang[43*], Catherine CL Wong[42*], Andy Peng Xiang[47,48*], Yichuan Xiao[18*], Zhengwei Xie[39,85*], Daichao Xu[54*], Jing Ye[55,56*], Rui Yue[41*], Cuntai Zhang[57,58*], Hongbo Zhang[44,45*], Liang Zhang[18,22*], Weiqi Zhang[2,17,22*], Yong Zhang[46,86*], Yun-Wu Zhang[87*], Zhuohua Zhang[88,89*], Tongbiao Zhao[3,17,22,23*], Yuzheng Zhao[61,62*], Dahai Zhu[46,86*], Weiguo Zou[53*], Gang Pei[90*] & Guang-Hui Liu[17,21,22,23,27*]

†Contributed equally to this work
*Corresponding authors (Feng Cao, email: fengcao8828@163.com; Zhongwei Cao, email: zhongweicao@scu.edu.cn; Piu Chan, email: pbchan@hotmail.com; Chang Chen, email: changchen@ibp.ac.cn; Guobing Chen, email: guobingchen@jnu.edu.cn; Hou-Zao Chen, email: chenhouzao@ibms.cams.cn; Jun Chen, email: cjbiochem@bjmu.edu.cn; Weimin Ci, email: ciwm@big.ac.cn; Bi-Sen Ding, email: dingbisen@scu.edu.cn; Qiurong Ding, email: qrding@sibs.ac.cn; Feng Gao, email: fgao@fmmu.edu.cn; Jing-Dong J. Han, email: jackie.han@pku.edu.cn; Kai Huang, email: huangkai1@hust.edu.cn; Zhenyu Ju, email: zhenyuju@163.com; Qing-Peng Kong, email: kongqp@mail.kiz.ac.cn; Ji Li, email: liji_xy@csu.edu.cn; Jian Li, email: lijian@bjhmoh.cn; Xin Li, email: xinli@ioz.ac.cn; Baohua Liu, email: ppliew@szu.edu.cn; Feng Liu, email: liuf001@csu.edu.cn; Lin Liu, email: liulin@nankai.edu.cn; Qiang Liu, email: liuq2012@ustc.edu.cn; Qiang Liu, email: qliu@tmu.edu.cn; Xingguo Liu, email: liu_xingguo@gibh.ac.cn; Yong Liu, email: liuyong31279@whu.edu.cn; Xianghang Luo, email: xianghangluo@csu.edu.cn; Shuai Ma, email: mashuai@ioz.ac.cn; Xinran Ma, email: xrma@bio.ecnu.edu.cn; Zhiyong Mao, email: zhiyong_mao@tongji.edu.cn; Jing Nie, email: niejing@smu.edu.cn; Yaojin Peng, email: yaojin.peng@ioz.ac.cn; Jing Qu, email: qujing@ioz.ac.cn; Jie Ren, email: renjie@big.ac.cn; Ruibao Ren, email: rbren@sjtu.edu.cn; Moshi Song, email: songmoshi@ioz.ac.cn; Zhou Songyang, email: songyanz@mail.sysu.edu.cn; Yi Eve Sun, email: yi.eve.sun@gmail.com; Yu Sun, email: sunyu@sibs.ac.cn; Mei Tian, email: tianmei@fudan.edu.cn; Shusen Wang, email: shusen@vip.163.com; Si Wang, email: wangsi@xwh.ccmu.edu.cn; Xia Wang, email: xiawang@mail.tsinghua.edu.cn; Xiaoning Wang, email: xnwang88@163.com; Yan-Jiang Wang, email: yanjiang_wang@tmmu.edu.cn; Yunfang Wang, email: wangyf2011126@126.com; Catherine CL Wong, email: catclw321@126.com; Andy Peng Xiang, email: xiangp@mail.sysu.edu.cn; Yichuan Xiao, email: ycxiao@sibs.ac.cn; Zhengwei Xie, email: xiezhengwei@hsc.pku.edu.cn; Daichao Xu, email: xudaichao@sioc.ac.cn; Jing Ye, email: yj11254@rjh.com.cn; Rui Yue, email: ryue@tongji.edu.cn; Cuntai Zhang, email: ctzhang0425@163.com; Hongbo Zhang, email: zhanghongbo@mail.sysu.edu.cn; Liang Zhang, email: zhangliang01@sibs.ac.cn; Weiqi Zhang, email: zhangwq@big.ac.cn; Yong Zhang, email: yongzhang@ibms.pumc.edu.cn; Yun-Wu Zhang, email: yunzhang@xmu.edu.cn; Zhuohua Zhang, email: zhangzhuohua@sklmg.edu.cn; Tongbiao Zhao, email: tbzhao@ioz.ac.cn; Yuzheng Zhao, email: yuzhengzhao@ecust.edu.cn; Dahai Zhu, email: dhzhu@pumc.edu.cn; Weiguo Zou, email: zouwg94@sibcb.ac.cn; Gang Pei, email: peigang@tongji.edu.cn; Guang-Hui Liu, email: ghliu@ioz.ac.cn)

[1]*Aging Biomarker Consortium (ABC), Beijing 100101, China;*
[2]*CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, Beijing 100101, China;*
[3]*State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;*
[4]*Department of Dermatology, Xiangya Hospital, Central South University, Changsha 410008, China;*
[5]*Hunan Key Laboratory of Aging Biology, Xiangya Hospital, Central South University, Changsha 410008, China;*
[6]*National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, China;*
[7]*Clinic Center of Human Gene Research, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China;*
[8]*Hubei Clinical Research Center of Metabolic and Cardiovascular Disease, Huazhong University of Science and Technology, Wuhan 430022, China;*
[9]*Hubei Key Laboratory of Metabolic Abnormalities and Vascular Aging, Huazhong University of Science and Technology, Wuhan 430022, China;*
[10]*Stem Cell Translational Research Center, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China;*
[11]*Department of Nuclear Medicine, Daping Hospital, Third Military Medical University, Chongqing 400042, China;*
[12]*CAS Key Laboratory of Nutrition, Metabolism and Food Safety, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200031, China;*
[13]*Shanghai Key Laboratory of Maternal Fetal Medicine, Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Frontier Science Center for Stem Cell Research, Shanghai Key Laboratory of Signaling and Disease Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China;*
[14]*The Department of Endovascular Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China;*
[15]*Key Laboratory of Regenerative Medicine of Ministry of Education, Institute of Ageing and Regenerative Medicine, Jinan University, Guangzhou 510632, China;*
[16]*National Clinical Research Center for Geriatric Diseases, Xuanwu Hospital, Capital Medical University, Beijing 100053, China;*
[17]*University of Chinese Academy of Sciences, Beijing 100049, China;*
[18]*CAS Key Laboratory of Tissue Microenvironment and Tumor, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai 200031, China;*
[19]*The Key Laboratory of Geriatrics, Beijing Institute of Geriatrics, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology of National Health Commission, Beijing 100730, China;*
[20]*School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, China;*
[21]*State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;*
[22]*Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China;*
[23]*Beijing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China;*
[24]*Department of Neurology, First Affiliated Hospital, Shanxi Medical University, Taiyuan 030001, China;*
[25]*Beijing Municipal Geriatric Medical Research Center, Xuanwu Hospital, Capital Medical University, Beijing 100053, China;*
[26]*Aging Translational Medicine Center, International Center for Aging and Cancer, Xuanwu Hospital, Capital Medical University, Beijing 100053, China;*
[27]*Advanced Innovation Center for Human Brain Protection, and National Clinical Research Center for Geriatric Disorders, Xuanwu Hospital Capital Medical University, Beijing 100053, China;*
[28]*Department of Neurology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230036, China;*
[29]*College of Life Sciences, TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan 430072, China;*
[30]*Research Institute of Transplant Medicine, Organ Transplant Center, NHC Key Laboratory for Critical Care Medicine, Tianjin First Central Hospital, Nankai University, Tianjin 300384, China;*
[31]*MOE Key Laboratory of Gene Function and Regulation, Guangzhou Key Laboratory of Healthy Aging Research, School of Life Sciences, Institute of Healthy Aging Research, Sun Yat-sen University, Guangzhou 510275, China;*
[32]*School of Life Sciences, Hainan University, Haikou 570228, China;*
[33]*Department of Systems Biomedical Sciences, School of Medicine, Jinan University, Guangzhou 510632, China;*
[34]*School of Life Sciences, Xiamen University, Xiamen 361102, China;*
[35]*The State Key Laboratory of Organ Failure Research, National Clinical Research Center of Kidney Disease, Division of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China;*
[36]*Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Center for Quantitative Biology (CQB), Peking University, Beijing 100871, China;*
[37]*Shanghai Institute of Hematology, State Key Laboratory for Medical Genomics, National Research Center for Translational Medicine (Shanghai), International Center for Aging and Cancer, Collaborative Innovation Center of Hematology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China;*
[38]*National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of*

Sciences, Beijing 100101, China;
[39]*Peking University International Cancer Institute, Health Science Center, Peking University, Beijing 100101, China;*
[40]*Key Laboratory of Birth Defects and Related Diseases of Women and Children of MOE, State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu 610041, China;*
[41]*Institute for Regenerative Medicine, Shanghai East Hospital, Frontier Science Center for Stem Cell Research, Shanghai Key Laboratory of Signaling and Disease Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China;*
[42]*Clinical Research Institute, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China;*
[43]*Hepatobiliary and Pancreatic Center, Medical Research Center, Beijing Tsinghua Changgung Hospital, Beijing 102218, China;*
[44]*Key Laboratory for Stem Cells and Tissue Engineering, Ministry of Education, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China;*
[45]*Advanced Medical Technology Center, The First Affiliated Hospital, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China;*
[46]*Bioland Laboratory (Guangzhou Regenerative Medicine and Health Guangdong Laboratory), Guangzhou 510005, China;*
[47]*Center for Stem Cell Biology and Tissue Engineering, Key Laboratory for Stem Cells and Tissue Engineering, Ministry of Education, Sun Yat-sen University, Guangzhou 510080, China;*
[48]*National-Local Joint Engineering Research Center for Stem Cells and Regenerative Medicine, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China;*
[49]*CAS Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China;*
[50]*State Key Laboratory of Genetic Resources and Evolution, Key Laboratory of Healthy Aging Research of Yunnan Province, Kunming Key Laboratory of Healthy Aging Study, KIZ/CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China;*
[51]*Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China;*
[52]*CAS Key Laboratory of Regenerative Biology, Joint School of Life Sciences, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences; Guangzhou Medical University, Guangzhou 510530, China;*
[53]*State Key Laboratory of Cell Biology, Shanghai Institute of Biochemistry and Cell Biology, Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 200031, China;*
[54]*Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 201210, China;*
[55]*Department of Geriatrics, Medical Center on Aging of Shanghai Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China;*
[56]*International Laboratory in Hematology and Cancer, Shanghai Jiao Tong University School of Medicine/Ruijin Hospital, Shanghai 200025, China;*
[57]*Gerontology Center of Hubei Province, Wuhan 430000, China;*
[58]*Institute of Gerontology, Department of Geriatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;*
[59]*Department of Cardiology, The Second Medical Centre, Chinese PLA General Hospital, National Clinical Research Center for Geriatric Diseases, Beijing 100853, China;*
[60]*Key Laboratory of Ministry of Education, School of Aerospace Medicine, Fourth Military Medical University, Xi'an 710032, China;*
[61]*Optogenetics & Synthetic Biology Interdisciplinary Research Center, State Key Laboratory of Bioreactor Engineering, Shanghai Frontiers Science Center of Optogenetic Techniques for Cell Metabolism, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China;*
[62]*Research Unit of New Techniques for Live-cell Metabolic Imaging, Chinese Academy of Medical Sciences, Beijing 100730, China;*
[63]*Department of Endocrinology, Endocrinology Research Center, Xiangya Hospital of Central South University, Changsha 410008, China;*
[64]*Department of Nuclear Medicine and PET Center, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, China;*
[65]*Department of Genetics and Cell Biology, College of Life Science, Nankai University, Tianjin 300071, China;*
[66]*Haihe Laboratory of Cell Ecosystem, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China;*
[67]*State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu 610041, China;*
[68]*Department of Microbiology and Immunology, School of Medicine, Jinan University, Guangzhou 510632, China;*
[69]*Guangdong-Hong Kong-Macau Great Bay Area Geroscience Joint Laboratory, Guangzhou 510000, China;*
[70]*Department of Biochemistry and Molecular Biology, State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100005, China;*
[71]*Peking University Research Center on Aging, Beijing Key Laboratory of Protein Posttranslational Modifications and Cell Function, Department of Biochemistry and Molecular Biology, Department of Integration of Chinese and Western Medicine, School of Basic Medical Science, Peking University, Beijing 100191, China;*
[72]*Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China;*

[73]*School of Basic Medical Sciences, Shenzhen University Medical School, Shenzhen 518060, China;*
[74]*Metabolic Syndrome Research Center, The Second Xiangya Hospital, Central South Unversity, Changsha 410011, China;*
[75]*Institute of Translational Medicine, Tianjin Union Medical Center, Nankai University, Tianjin 300000, China;*
[76]*State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300350, China;*
[77]*Department of Neurology, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin 300052, China;*
[78]*Tianjin Institute of Immunology, Tianjin Medical University, Tianjin 300070, China;*
[79]*International Center for Aging and Cancer, Hainan Medical University, Haikou 571199, China;*
[80]*Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, China;*
[81]*Department of Medicine and VAPSHCS, University of Washington, Seattle, WA 98195, USA;*
[82]*Human Phenome Institute, Fudan University, Shanghai 201203, China;*
[83]*Institute of Geriatrics, The second Medical Center, Beijing Key Laboratory of Aging and Geriatrics, National Clinical Research Center for Geriatric Diseases, Chinese PLA General Hospital, Beijing 100853, China;*
[84]*Department of Neurology and Center for Clinical Neuroscience, Daping Hospital, Third Military Medical University, Chongqing 400042, China;*
[85]*Beijing & Qingdao Langu Pharmaceutical R&D Platform, Beijing Gigaceuticals Tech. Co. Ltd., Beijing 100101, China;*
[86]*The State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing 100005, China;*
[87]*Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, School of Medicine, Xiamen University, Xiamen 361102, China;*
[88]*Key Laboratory of Molecular Precision Medicine of Hunan Province and Center for Medical Genetics, Institute of Molecular Precision Medicine, Xiangya Hospital, Central South University, Changsha 410078, China;*
[89]*Department of Neurosciences, Hengyang Medical School, University of South China, Hengyang 421001, China;*
[90]*Shanghai Key Laboratory of Signaling and Disease Research, Laboratory of Receptor-Based Biomedicine, The Collaborative Innovation Center for Brain Science, School of Life Sciences and Technology, Tongji University, Shanghai 200070, China*

Received January 19, 2023; accepted February 27, 2023; published online April 11, 2023

Aging biomarkers are a combination of biological parameters to (i) assess age-related changes, (ii) track the physiological aging process, and (iii) predict the transition into a pathological status. Although a broad spectrum of aging biomarkers has been developed, their potential uses and limitations remain poorly characterized. An immediate goal of biomarkers is to help us answer the following three fundamental questions in aging research: How old are we? Why do we get old? And how can we age slower? This review aims to address this need. Here, we summarize our current knowledge of biomarkers developed for cellular, organ, and organismal levels of aging, comprising six pillars: physiological characteristics, medical imaging, histological features, cellular alterations, molecular changes, and secretory factors. To fulfill all these requisites, we propose that aging biomarkers should qualify for being specific, systemic, and clinically relevant.

aging, senescence, biomarker, clock

Citation: Aging Biomarker Consortium, Bao, H., Cao, J., Chen, M., Chen, M., Chen, W., Chen, X., Chen, Y., Chen, Y., Chen, Y., et al. (2023). Biomarkers of aging. Sci China Life Sci 66, 893–1066. https://doi.org/10.1007/s11427-023-2305-0

CONTENTS

Introduction

Do we truly know how old we are biologically, that is, more accurately describing the status of our body than our chronological ages? Are some people at higher risk of certain types of age-related diseases, i.e., cardiovascular disorders or neurodegenerative diseases, and how can they be identified? Or how do we know if any of the claimed geroprotective treatments are effective? To answer these questions, we need to establish biomarkers for aging. In a broad aspect, these biomarkers are defined as scientifically measured parameters of the physiological aging process, to measure age-related changes and to predict the transition into a pathological status.

As a biological measurement to qualify aging, a biomarker must be specific, systemic, and serviceable. (i) Specific: aging is such a heterogeneous process that it proceeds at different rates in different individuals and varies in different organs, even in the same individual. Therefore, it is impossible to have one biomarker for the entire organism but different ones or even different sets of biomarkers for different organs for evaluation; *vice versa*, each biomarker should be able to capture a unique aging signal of the relevant organ. Moreover, aging biomarkers should be predictive of the risk of disease development, which requires a specific threshold for the transition from physiological aging to pathological disorder. (ii) Systemic: aging involves almost every organ system, comprising numerous interconnected biological processes. Moreover, changes in one organ may elicit compensatory mechanisms or systemic feedback across the body. Therefore, biomarkers should be able to reflect such systemic changes with age, and a collection of biomarkers from multiple dimensions is required for this aspect. (iii) Serviceable: biomarkers collected through non-invasive or minimally invasive methods are particularly suited for translation into clinical practice. As aging is a gradually deteriorating process over time, longitudinal studies are needed, and again, non-invasive measurements are preferred. In larger cohort studies, cost and convenience should be considered when choosing biomarkers. In all, being specific, systemic, and serviceable are as critical to the broad spectrum of aging biomarkers as the three primary colors.

Over the years, various data types and modeling techniques have been used to develop a broad spectrum of aging biomarkers. Based on the nature of these parameters used for aging biomarkers, the collection of alterations with age can be categorized into 6 classes, or 6 pillars, although biomarkers in different categories are often interconnected with each other. There are higher-order types of changes that reflect physiological and functional changes, such as physiological characteristics, imaging traits, and histological features. Additionally, there are more causal or mechanistic driver types of biomarkers, such as cellular alterations and molecular changes. Finally, there are biomarkers serving in between, such as hormones and secretory factors that are detectable in body fluids, such as blood, urine, saliva, and cerebrospinal fluid (CSF), among which those act in a paracrine manner are of particular interest. The latter three types, as they may also serve as hallmarks or drivers of aging, may be targeted to intervene in the aging process.

However, the predictions of biological ages based on different variables are often inconsistent. In certain cases, a thoughtful set of aging biomarkers could be more reliable and useful to solve this problem. To clarify these confusions, we compile current knowledge of aging biomarkers to provide a comprehensive reference for researchers in academia and industry and medical practitioners in geriatrics and gerontology. We cover biomarkers across the dimensions of cellular, organ, organismal and population aging, each organized according to the 6 pillars of classification. Additionally, we demonstrate how this broad spectrum of biomarkers is utilized in various prediction models, or aging clocks, and applied in cohort studies. Finally, we discuss an important aspect of aging studies, that is, the ethical and social implications of aging biomarkers (Figure 1).

Biomarkers of cellular aging

At the foundation of the hierarchy of aging biomarkers are

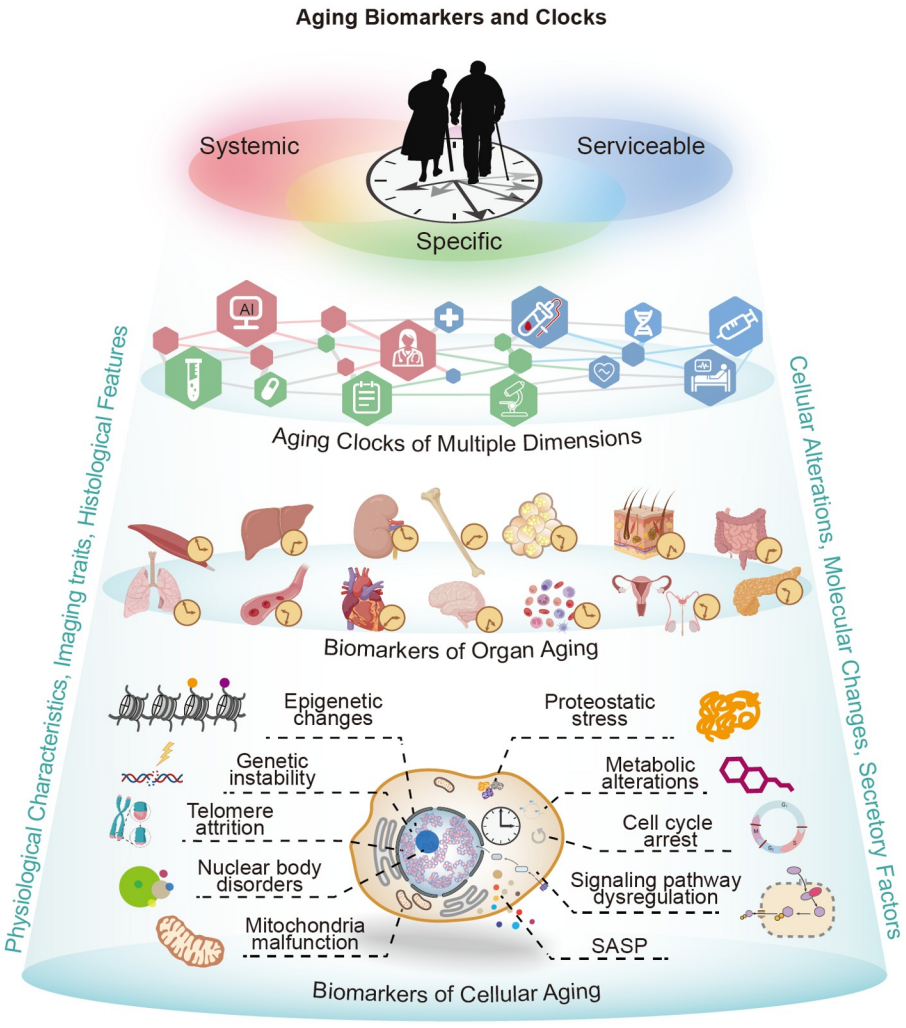

Figure 1 Aging biomarkers reflect biological ages at the cellular, organ, and organismal levels. Three criteria, i.e., being specific, systemic, and serviceable, determine the spectrum of biomarkers as three primary colors. The complex collection of aging biomarkers is supported by six pillars: physiological characteristics, imaging traits, histological features, cellular alterations, molecular changes, and secretory factors. Abbreviation: SASP, senescence-associated secretory phenotype.

those for cellular aging. Cells are the building blocks of organs and organisms, and cellular aging serves as the driving force of organ and organismal aging. Thus, biomarkers at this level not only monitor a basic cellular process underlying the aging process, but may also exert impacts higher up on the entire organ or organism. However, there are only a few widely accepted biomarkers of cellular aging, such as senescence-associated beta-galactosidase (SA-β-gal) activity, although their functions are still not well understood. We propose the following ten aspects of cellular aging biomarkers: epigenetic changes, genetic instability, telomere shortening, nuclear body disorders, cell cycle arrest, mitochondrial malfunction, proteostatic stress, metabolic alterations, signaling pathway rerouting, and senescence-associated secretory phenotype (SASP). We cover each aspect in a separate section. In particular, we will introduce these biomarkers for how they reflect changes associated with age and how they drive aging as well. Therefore, these biomarkers will broaden our understanding of the basic mechanism of aging and may serve as potential targets for aging interventions.

Epigenetic alterations

Over the past decades, great efforts have been made to categorize molecular hallmarks of aging, many of which also qualify as aging biomarkers (López-Otín et al., 2013; López-Otín et al., 2023). Epigenetics is defined as reversible heritable mechanisms that occur without alterations in the DNA sequence (Jaenisch and Bird, 2003), and epigenetic alterations have been reported to be crucial in aging and age-related diseases. These aging-associated epigenetic biomarkers include altered genomic DNA methylation, aberrant histone modifications, loss of heterochromatin, reorganized

three-dimensional (3D) genome architecture, and deregulated RNA modifications. They lead to altered local accessibility to genetic material, aberrant transcription such as retrotransposon elements, and genomic instability, thereby contributing to aging and age-related diseases (Li et al., 2019d; Pal and Tyler, 2016; Wang et al., 2022a; Zhang et al., 2020d) (Figure 2). Therefore, understanding epigenetic biomarkers in aging will help to address the fundamental questions—"how old are we?" and "why do we age?", which provides new avenues to develop strategies to delay aging.

Altered DNA methylation

Global and local DNA methylation changes in the genome during aging are among the most extensively studied and best characterized epigenetic biomarkers during aging (Field et al., 2018; Horvath and Raj, 2018). Hypermethylation of CpG islands within promoters leads to transcriptional repression; conversely, hypomethylation allows gene expression (Horvath, 2013; Salameh et al., 2020). A global decrease in DNA methylation is observed during aging in a variety of species, which may be attributed to the progressive decline in levels of the DNA methyltransferase 1 (DNMT1) (Jung and Pfeifer, 2015). In contrast, *de novo* methylation increases with age due to the upregulation of other DNMTs, such as DNMT3A and DNMT3B (Yagi et al., 2020), suggesting that changes in DNA methylation patterns during aging can be a good indicator of aging. Using machine learning methods and based on DNA methylation patterns at certain CpG sites, Horvath (2013) and Hannum et al. (2013) developed the first-generation epigenetic biomarker of aging, DNAmAge, also known as the "epigenetic clock", which is able to estimate the age of most tissues and cell types, and predict outcomes of aging, including mortality risk and age-related diseases. Since then, multiple epigenetic clocks have been reported with greater accuracy, precision, and broader application prospects in aging research. For example, by combining multiple clinical biomarkers, PhenoAge can predict a variety of aging outcomes, including all-cause mortality, cancers, healthspan, physical functioning, and Alzheimer's disease (AD) (Levine et al., 2018). GrimAge, a more predictive epigenetic clock for identifying clinical phenotypes, is based on seven DNA methylation surrogates and a DNA methylation-based estimator of smoking pack-years (Lu et al., 2019; McCrory et al., 2021). A single-cell age clock (scAge) has recently developed using single-cell methylation data, which is able to discriminate the age of cells in heterogeneous issues and recapitulate the chronological age of tissues in mice (Trapp et al., 2021). Consequently, the altered methylation pattern contributes to the deregulation of aging-associated genes (Field et al., 2018; Li et al., 2022h; Zhang et al., 2020d). Recently, Liu et al. showed that reduced DNA methylation levels of human endogenous retrovirus (HERV) elements are associated with the resurrection of endogenous retroviruses during aging, which acts as both biomarkers and drivers of aging in multiple aging cell models, as well as various organs and species (Dasgupta and Adams, 2023; Liu et al., 2023b; Thuault, 2023; Zhou et al., 2023; Zlotorynski, 2023). In summary, these epigenetic clocks are promising models using aging biomarkers for predicting biological age and assessing the efficacy of aging interventions.

Aberrant histone modifications

Changes in histone modifications such as methylation and acetylation are widely studied during cellular senescence (Pal and Tyler, 2016; Wang et al., 2022a; Zhang et al.,

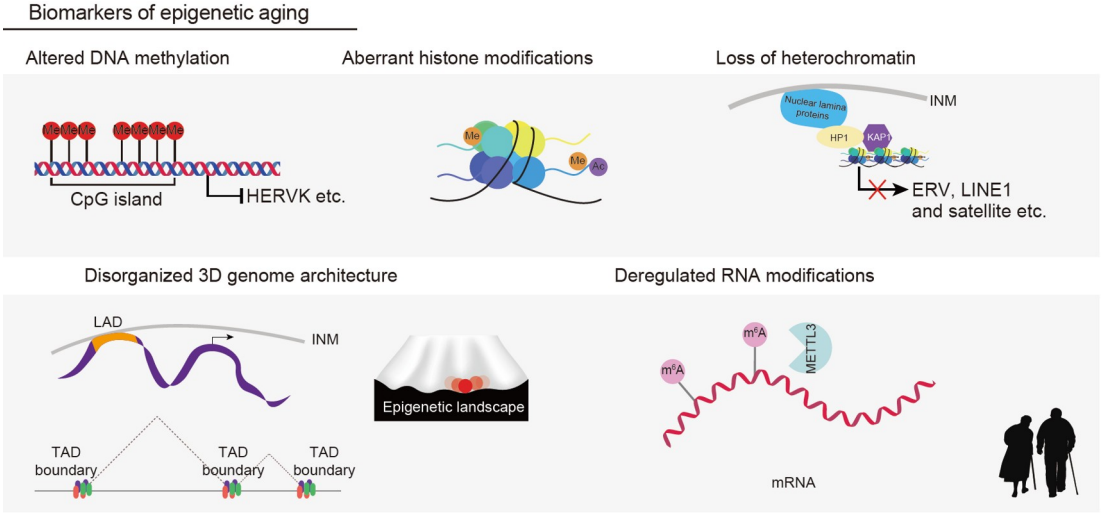

Figure 2 Biomarkers of epigenetic aging. Epigenetic biomarkers include altered genomic DNA methylation, aberrant histone modifications, loss of heterochromatin, reorganized 3D genome architecture, and deregulated RNA modifications. Abbreviations: LINE1, long interspersed nuclear element 1; INM, inner nuclear membrane; LAD, lamina-associated domain; TAD, topologically associating domains; Me, methylation; Ac, acetylation; ERV, endogenous retrovirus; HERVK, human endogenous retrovirus type K.

2020d). H3K4me3, a marker associated with active transcription, has also been shown to increase with age in yeast, *C. elegans*, and a mouse model of AD but decrease in aged human hematopoietic stem cells (HSCs) and neurons (Adelman et al., 2019; Cao et al., 2020; Cheung et al., 2010; Cruz et al., 2018; Pu et al., 2018). Reduced H3K27me3 was observed in prematurely aged cells from Hutchinson-Gilford progeria syndrome (HGPS, $LMNA^{G608G/+}$) patients and in aged *C. elegans* (Guillermo et al., 2021; McCord et al., 2013). Global loss of H3K9me3 has been reported in aged *Drosophila* intestinal stem cells (ISCs), in aged nematode somatic tissues, as well as in various human mesenchymal progenitor cells (hMPCs), including those with pathogenic mutations of Werner syndrome (WS, $WRN^{-/-}$) (Jeon et al., 2018; Zhai et al., 2021; Zhang et al., 2015). Reduced expression of the H3K9me3 methyltransferase SUV39H1 was observed in senescent cells, and inactivation of SUV39H1 in wild-type hMPCs led to a reduction in overall H3K9me3 and induction of cellular senescence (Zhang et al., 2015).

Similar to changes in histone methylations observed during aging, decreased levels of H3K56ac and increased levels of H4K16ac are also associated with replicative aging in yeast and human fibroblasts (Dang et al., 2009; O'Sullivan et al., 2010). Sirtuin family members, evolutionarily conserved nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylases, have been implicated in aging and age-related diseases (Bi et al., 2020; Diao et al., 2021; Simon et al., 2019; Sun et al., 2020a). Impaired deacetylation of H3K56 upon Sirt6 deletion promoted HSC proliferation through aberrant activation of Wnt signaling (Wang et al., 2016a). Ectopic expression of Sirt7 alleviated prematurely senescent phenotypes and extended the lifespan in a Hutchinson-Gilford progeria mouse model (Sun et al., 2020a). It has also been reported that global histone hypoacetylation, particularly a decrease in H4K12ac, occurs in repetitive DNA elements in aged mouse brains (Peleg et al., 2010). Furthermore, via genome-wide CRISPR/Cas9-based loss-of-function screens, Wang et al. (2021e) identified KAT7, a histone acetyltransferase, as a senescence driver in both HGPS and WS hMPCs. Inactivation of KAT7 decreased H3K14ac levels in the promoter region of $p15^{INK4b}$, a cyclin-dependent kinase inhibitor that mediates cell cycle arrest, leading to suppression of $p15^{INK4b}$ transcription and ultimately alleviating cellular senescence. Thus, substantial changes in histone modifications occur during aging and age-related diseases, providing new insights into the development of aging intervention strategies. In the future, more research is needed to refine the regulatory mechanisms between these modifications and aging.

Loss of heterochromatin

Heterochromatin domains tightly pack DNA into an inaccessible and transcriptionally inactivated structure (Dixon et al., 2012). Heterochromatin is associated with specific proteins, such as heterochromatin protein 1 (HP1), and specific histone modifications, such as H3K9me3 (Becker et al., 2016; Maison and Almouzni, 2004). Accompanied by global reduction of H3K9me3, loss of heterochromatin has been reported to be associated with cellular senescence from yeast to humans and with the onset of age-related diseases (Pal and Tyler, 2016; Tsurumi and Li, 2012; Villeponteau, 1997). Loss of heterochromatic mating-type loci or ribosomal DNA (rDNA) leads to genomic instability, sterility, and aging in yeast (Kennedy et al., 1997; Kobayashi, 2011; Sinclair and Guarente, 1997). Diminished heterochromatin-associated inner nuclear membrane (INM) proteins LAP2 and LBR and reduced heterochromatin structure underneath the nuclear envelope were also observed in HGPS patients, stem cell models of Werner syndrome, and prematurely senescent stem cells with deficiency of zinc finger protein with KRAB and SCAN domains 3 (ZKSCAN3), Ddb1- and Cul4-associated factor 11 (Dcaf11), DiGeorge syndrome critical region gene (DGCR8, a protein critical for microRNA (miRNA) biogenesis), SIRT3 and SIRT7, as well as circadian locomotor output cycles protein kaput (CLOCK) and brain and muscle arnt-like 1 (BMAL1, two core components of the molecular circadian clock machinery) (Bi et al., 2020; Deng et al., 2019; Diao et al., 2021; Goldman et al., 2004; Hu et al., 2020; Le et al., 2021; Liang et al., 2022; Liang et al., 2021; Scaffidi and Misteli, 2006; Zhang et al., 2015). Recently, Zhao et al. (2022a) found that apolipoprotein E (APOE), a component of lipoprotein particles that function in the homeostasis of cholesterol and other lipids, participated in autophagy-lysosomal pathway-mediated degradation of nuclear lamina proteins and a heterochromatin-associated protein KRAB-associated protein 1 (KAP1), thereby destabilizing heterochromatin and driving senescence. Knockdown of HP1 promoted cellular senescence, whereas replenishment of HP1 rescued premature senescent phenotypes (Bi et al., 2020; Deng et al., 2019; Diao et al., 2021; Hu et al., 2020; Liang et al., 2022; Liang et al., 2021; Zhang et al., 2015). As an intrinsic feature of cellular senescence, heterochromatin erosion leads to the derepression of repetitive sequences such as ERV, long interspersed nuclear element 1 (LINE1) and satellite repeats, and activation of innate immune signaling via the cyclic guanosine mono phosphate (GMP)-adenosine monophosphate (AMP) syn thase (cGAS)-stimulator of interferon genes (STING) pathway (Bi et al., 2020; De Cecco et al., 2019; Liang et al., 2022; Liang et al., 2021; Simon et al., 2019; Zhang et al., 2015). Treatment of senescent hMPCs with reverse transcriptase inhibitors, vitamin C, gallic acid (GA), or low-dose chloroquine (CQ) increased the levels of heterochromatin-associated marks, including H3K9me3 and HP1, leading to rejuvenation of cellular and tissue senescence (Bi et al., 2020; De Cecco et al., 2019; Geng et al., 2019; Li et al.,

2022e; Li et al., 2016a; Shan et al., 2022; Simon et al., 2019). In addition, by probing chromatin accessibility at single-cell resolution in the brains of young, middle-aged, and old mice, Zhang et al. (2022e) revealed increased chromatin accessibility within specific heterochromatin domains and activated expression of LINE1 elements in excitatory neurons of old mice. Taken together, the loss of constitutive heterochromatin has frequently been recognized as a biomarker of tissue and cellular aging.

Disorganized 3D genome architecture

Advances in various high-throughput sequencing technologies, such as high-throughput chromosome conformation capture (Hi-C), DNA adenine methyltransferase identification (DamID), and assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), enable mapping of epigenetic changes during senescence at higher-order structural levels, such as 3D genome architecture and chromatin accessibility (Bonev and Cavalli, 2016; Dixon et al., 2012; Kempfer and Pombo, 2020). Using prematurely senescent hMPC models, Liu et al. revealed an overall increase in chromatin entropy and epigenetic instability with aging (Liu et al., 2022b; Liu et al., 2022c; Zhao and Chen, 2022). Decompartmentalization, which is characterized by decreased DamID signals in lamina-associated domains (LADs) but increased signals in inter-LADs (iLADs) and the switch of topologically associating domains (TADs) from compacted and transcriptionally silenced (B) to open and transcriptionally active (A) compartments, especially in boundary regions, was observed in senescent hMPCs, which led to heterochromatin loss and activation of repressive compartments (Liu et al., 2022b; Liu et al., 2022c). In addition, a gain of A-B interactions was also observed in replicatively senescent human fibroblasts (Sati et al., 2020). Using a system called "ICE" (inducible changes to the epigenome), Yang et al. (2023) found that the act of faithful DNA repair led to erosion of the epigenetic landscape, deregulation of cellular function, and promotion of aging in mice, which can be reversed by OSK-mediated epigenetic reprogramming. Collectively, the reorganized 3D genome architecture is manifested as an increase in entropy, of which the extent may serve as a potential biomarker for aging, although there is still a long way for such measurement to be employed in clinical practice.

Deregulated RNA modifications

More than 170 types of RNA modifications have been identified, among which N6-methyladenosine (m^6A) messenger RNA (mRNA) methylation is a well-known epitranscriptional regulatory mechanism that regulates mRNA metabolism, thereby affecting central biological processes (Deng et al., 2018; Huang et al., 2020b; Zhao et al., 2017). The promoted translation of cyclin-dependent kinase inhibitor (CDKI) $p21^{CDKN1A}$ via the methyltransferases (known as "writers") METTL3/METTL14-mediated m^6A methylation has been reported in H_2O_2-induced cellular senescence of TP53-deficient human colon carcinoma cells (HCT116) (Li et al., 2017b). A global reduction in RNA m^6A abundance was detected in sulforaphane (SFN)-induced senescence of cancer cells, and human peripheral blood mononuclear cells (PBMCs) from aged cohorts (Lewinska et al., 2017; Min et al., 2018). Knockdown of METTL3 or METTL14 accelerated cellular senescence by destabilizing *AGO2* mRNA in human fibroblasts and reduced the production of miR-34a-5p in tumor necrosis factor-alpha (TNFα) induced cellular senescence of human nucleus pulposus cells (NPCs) (Min et al., 2018; Zhu et al., 2021a). Wu et al. demonstrated that METTL3 was reduced in prematurely senescent hMPCs, and knockout of METTL3 also accelerated hMPC senescence, resulting from markedly reduced m^6A modifications and destabilized *MIS12* mRNA, a key regulator of cell proliferation (Wu et al., 2020c; Wu et al., 2022b). Another study showed that knockdown of METTL3 led to accelerated senescence by upregulating the expression of polo-like kinase 1 (PLK1), a critical cell cycle modulator, in an m^6A-dependent manner (Luo et al., 2021). Moreover, knockout of obesity-associated protein (FTO), one of the "erasers" of m^6A, also accelerated hMPC senescence, and downregulation of FTO led to increased overall m^6A levels during ovarian aging in mice (Jiang et al., 2021; Sun et al., 2021; Zhang et al., 2022c). Taken together, these studies demonstrate that deregulation of m^6A modification represents a novel epi-transcriptional biomarker in aging, and more investigation of m^6A dynamics at the genome-wide scale is required to reveal its potential impact on aging.

Summary and perspectives

In summary, although great progress has been made in identifying epigenetic biomarkers to predict biological age, it is important to highlight that there is still prediction bias due to the small number of sample sets. In the future, combining multiple layers of epigenetic biomarkers using large-scale datasets from different cells, tissues, populations, species, and diseases will be demanded to provide a more precise prediction of biological age and to evaluate the outcomes of clinical aging interventions.

Genetic instability

Genetic instability is one of the fundamental biomarkers of aging. DNA, as the carrier of genetic information, undergoes various types of alterations with increasing age and further negatively affects normal cellular activities and tissue homeostasis, both of which are closely linked to biological aging. Many aspects of DNA-associated alterations con-

tribute to genetic instability, including but not limited to changes in DNA damage, DNA damage response (DDR) and repair, mutations, replication stress, transposition, chromosome aberrations, telomere shortening, micronuclei, and DNA fragments. Many of the aforementioned aspects have been demonstrated to serve as biomarkers of aging, which can be employed to reflect or predict cellular biological status. Here, we will briefly summarize the research progress on the following biomarkers of genetic instability and their relations with aging.

DNA damage and repair

According to the DNA damage theory of aging (Gensler and Bernstein, 1981), DNA lesions are recognized as the primary cause of aging. Supporting this idea, increased DNA damage in senescent cells, progeria cells, and aged individuals have been reported by a great number of studies (d'Adda di Fagagna et al., 2003; Liu et al., 2005; Sedelnikova et al., 2008; Sedelnikova et al., 2004; Wang et al., 2009; Zhang et al., 2020b), making DNA damage a universal biomarker of aging. The frequency of γH2AX foci, a well-known biomarker of double-strand breaks, is upregulated in senescent mouse embryonic fibroblasts and multiple organs, including liver, lung, dermis, crypt of the small intestine, and spleen lymphocytes, in aged mice (Wang et al., 2009). γH2AX and 53BP1 foci, which commonly colocalize with each other, are also found in replicative senescent cells (d'Adda di Fagagna et al., 2003). The incidence of γH2AX foci increases with age in human lymphocytes, and a higher level of γH2AX foci is detected in fibroblasts derived from Werner syndrome patients (Sedelnikova et al., 2008).

Impaired DNA repair is one of the causes of DNA damage accumulation in aged cells, evidenced by reduced expression (Chen et al., 2017; Chen et al., 2020b; Ju et al., 2006; Redwood et al., 2011; Zhang et al., 2020b) or delayed recruitment kinetics of DNA repair factors (Liu et al., 2005; Redwood et al., 2011; Sedelnikova et al., 2008; Zhang et al., 2020b) and a decline in repair fidelity (Li et al., 2016b; Vaidya et al., 2014). Normally, γH2AX foci are cleared upon completion of the repair process. However, a study delineated that senescence-associated γH2AX foci might represent unrepairable DNA lesions (Sedelnikova et al., 2004). Moreover, a kind of large and persistent DNA damage foci containing γH2AX, 53BP1, MDC1, NBS1, MRE11 and phosphorylated ataxia telangiectasia-mutated (ATM) and CHK2, namely, DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), is observed in senescent cells and irradiated mouse tissues and found to be critical for maintaining senescence-associated phenotypes (Rodier et al., 2011).

In addition to double-strand breaks, the levels of oxidative DNA damage, measured by the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG), also significantly increase with age in the liver, kidney, intestine, brain, and testes of rats (Fraga et al., 1990), in accordance with the oxidative stress theory of aging (Harman, 1956). Moreover, the levels of repair products of oxidative damage show an age-related decline in urine, suggesting impaired excision repair in aged rats (Fraga et al., 1990). Employing a plasmid reactivation assay, the capacity of base excision repair, which is responsible for mending oxidative DNA damage, has also been found to decline with increasing age in mesenchymal stem cells and skin fibroblasts (Xu et al., 2015; Zhang et al., 2020b). Intriguingly, the alteration of DNA repair during the process of aging seems to be tissue type- and cell type-specific, which has been discussed in another review article (Chen et al., 2020c). Elucidating the age-related, context-dependent regulatory mechanisms of DNA repair with the aid of *in vivo* research models (Chen et al., 2022; Kass et al., 2016; Vaidya et al., 2014; Wang et al., 2020a) is still an important subject in the future.

The presence of large amounts of unrepaired or misrepaired DNA damage also contributes to other biomarkers of aging. DNA damage has been reported to be one of the major causes of apoptosis (Wang, 2001), which underlies the age-related loss of tissue homeostasis (Park et al., 2008). In addition, independent of the state of cell cycle arrest, persistent DNA damage induces the secretion of inflammatory factors, such as interleukin-6 (IL-6) (Rodier et al., 2009), which is one of the components of the SASP, modulating the microenvironment in aged individuals. Although DNA damage and repair play a profound role in aging, whether targeting DNA repair can prevent age-related diseases and contribute to longevity requires further investigation.

DNA damage response

The DNA damage response is a molecular cascade mastering the cellular responsive activities once DNA damage occurs. The DNA damage response activates cell cycle checkpoints, initiates DNA repair, and determines the fate of damaged cells. Due to the failure to fix damaged DNA, factors involved in the DNA damage response are often overactivated in aged cells (Bartek et al., 2001; d'Adda di Fagagna et al., 2003; Kang et al., 2017; Park et al., 2015; Park et al., 2017), which can possibly be understood as a compensatory mechanism. DNA damage, particularly double-strand breaks, causes the phosphorylation and activation of CHK2, eventually leading to checkpoint activation and cell cycle arrest (Bartek et al., 2001). The phosphorylation of CHK2 and its upstream kinase, ATM, increases in senescent cells (Kang et al., 2017). Enhanced ATM and ATR activation have also been reported in ISCs in the aged *Drosophila* (Park et al., 2015). Moreover, increased DNA damage activates DNA-PKcs in the aged skeletal muscle (Park et al., 2017). In addition, telomere uncapping, another senescence-associated event, triggers the DNA damage response, including acti-

vation of CHK1 and CHK2 (d'Adda di Fagagna et al., 2003). Notably, CHK2 activation has also been found to play a role in replicative senescence (Gire et al., 2004).

Apart from DNA repair regulation, several moonlighting functions of DNA damage response factors have been reported. ATM regulates V-ATPase assembly to control lysosomal pH and influences the normal function of the lysosome/autophagy system, leading to impaired removal of dysfunctional mitochondria, elevated reactive oxygen species (ROS) levels and nuclear disorganization (Kang et al., 2017). Inhibition of ATM alleviates senescence through re-acidification of the lysosome/autophagy system and metabolic reprogramming (Kang et al., 2017). DNA-PKcs regulates the inflammatory responses in dendritic cells (DCs), mediating the development of airway diseases (Mishra et al., 2015a), and also regulates AMPK through inhibiting the chaperone function of heat shock protein 90 (HSP90) alpha, ultimately driving the metabolic and fitness decline in aged skeletal muscle (Park et al., 2017). Further efforts are clearly required to better understand the role of the overactivated DNA damage response, one of the important biomarkers of aging, in the onset of organ dysfunction and individual aging.

Mutations

Mutation is another broadly reported biomarker of aging. The frequency of spontaneous mutation, which has been determined with the LacZ transgenic mice, increases almost linearly with age in the spleen (Ono et al., 1995). Another independent study also reports that the spontaneous mutation exhibits an age-related increase in the liver, while in the brain, only an increase during early life is observed (Dollé et al., 1997), which has also been confirmed by another study (Stuart et al., 2000). In contrast to these two studies, another group reported age-dependent accumulation of spontaneous mutations in multiple tissues, including the brain, while the rates of increase differ among tissues (Ono et al., 2000).

Age-related changes in mutations are tissue-type specific. Mutation frequencies in the heart and small intestine both increase with increasing age, while the mutation spectra exhibit a striking difference between these two organs. Moreover, the mutation frequency is significantly higher in the small intestine than in the heart (Dollé et al., 2000). Another study showed that approximately 40 novel mutations per year are acquired in adult stem cells of the small intestine, colon, and liver, although a tissue-specific mutational signature was observed (Blokzijl et al., 2016). A study in 2021 showed that, in contrast to the accumulated mutations examined in the intestine, liver, and lung of naturally aged mice, no age-related increase in mutations is evident in the heart (De Majo et al., 2021), implicating an efficient cardiac DNA repair system throughout life. Although the tissue-specific mutational frequency and signature have been reported, knowledge is lacking about whether there exists a cell type-specific feature of mutations with age. Given that the cellular composition varies among tissues, there is a pressing need for uncovering age-related alterations in mutations at single-cell resolution in the future with the aid of single-cell omics technologies.

The difference in mutation between germline and soma is another interesting topic. The mutation frequency in a mixed population of seminiferous tubule cells derived from young adult mice is significantly lower than that of somatic cells, and there is a decrease in mutation frequency during spermatogenesis, both indicating a protected state of germ cells in young mice (Walter et al., 1998). However, in old mice, the mutation frequency significantly increases in spermatogenic cells, and the mutation frequency has also been found to be elevated during the process of spermatogenesis, suggesting a deprotected state of the germ line in old mice (Walter et al., 1998).

Impaired DNA repair is believed to cause mutations, and genomic instability plays a vital role in the development of premature aging. Nevertheless, accumulation of mutations has not been reported in short-lived mice with defects in transcription-related repair (Dollé et al., 2006), assayed with a lacZ reporter system (Boerrigter et al., 1995). Although the phenomenon may arise from the non-transcribed lacZ-reporter gene being insensitive to impaired transcription-related repair, there exists a possibility that DNA repair deficiency may contribute to aging phenotypes independently of inducing deleterious mutations, at least in some contexts.

Chromosome aberrations

Chromosome aberration, which refers to changes in the number or structure of chromosomes, is another well-known biomarker of aging and age-related diseases, such as cancer, in both animal models and humans.

Translocations and insertions have been reported to be increased with age in mouse peripheral blood lymphocytes, while there is no significant change in the frequencies of dicentrics and acentric fragments (Walter et al., 1998). In human blood cells, both megabase-range and small-scale structural variants display a positive correlation with age (Forsberg et al., 2012). Moreover, the frequency of chromosome nondisjunction increases with age in both sexes (Wojda et al., 2007). A study demonstrated that the frequency of mosaic chromosomal abnormalities, including aneuploidy and copy-neutral loss of heterozygosity, shows an age-related increase by approximately 8-fold, in human blood and buccal samples collected from 75–79-year-old individuals compared with that from individuals younger than 50 years old (Jacobs et al., 2012). The frequencies of duplications, deletions, and uniparental disomy have also been reported to rapidly rise in the elderly (Laurie et al., 2012).

Different chromosomes may exhibit different susceptibilities to age-related chromosome aberrations. A longitudinal human study showed that both numerical and structural chromosome aberrations exist in aged skin fibroblasts (Mukherjee and Thomas, 1997). Intriguingly, the aneuploidy of chromosomes 1, 4, 6, 8, and 10, the majority of which harbor senescence-associated genes, are more frequently affected by age than the other chromosomes analyzed (Mukherjee and Thomas, 1997). The chromosome-specific fashion of aneuploidy has also been reported by another study, in which chromosomes 7, 18, and Y are the most severely affected by age in non-neuronal nuclei of the cortex in mice (Busuttil et al., 2004). Female mosaic X events also increase with age (Machiela et al., 2016). Interestingly, the frequency of chromosome nondisjunction involving chromosome X or Y reaches a peak in male centenarians, while the frequency of X-containing chromosome nondisjunction markedly decreases in female centenarians (Wojda et al., 2007), implicating a sex specificity in chromosome aberrations during the process of aging.

Notably, compared with cancer-free individuals, clonal mosaicism is at least 27 times more commonly detectable in individuals whose DNA is collected at least one year prior to being diagnosed with hematological cancers, indicating a strong link between chromosome aberrations and increased susceptibility to cancer in aged people (Jacobs et al., 2012). Consistently, mosaic loss of chromosome Y is also linked with higher cancer risk (Forsberg et al., 2014). Several studies have also uncovered that mosaic loss of the Y chromosome in the blood contributes to cardiac failure (Sano et al., 2022), solid tumors (Forsberg et al., 2014), and AD (Dumanski et al., 2016), suggesting that chromosome aberrations in blood cells might serve as a profound biomarker of multiple human age-related diseases.

Micronuclei

Micronuclei are small, membrane-bound, DNA-containing compartments that originate due to errors during mitosis (Ohsugi et al., 2008). Micronuclei have long been recognized as biomarkers of a suite of human diseases (Fenech et al., 2020). Given that the formation of micronuclei depends on cell division (Ohsugi et al., 2008), whether and how micronuclei are regulated during cellular aging requires further investigation. It has been shown that the frequency of micronuclei increases with age from newborns to 40 years old but decreases in older individuals in a Yugoslavian population, possibly due to a gradually declining cellular proliferating capacity with age (Milosevic-Djordjevic et al., 2002). Micronuclei frequencies have also been reported by two other studies to exhibit a biphasic character with increasing age in humans (Orta and Günebakan, 2012; Wojda et al., 2007), although one of the studies found no significant change in proliferative indexes with age (Orta and Günebakan, 2012).

As we reviewed above, the loss of sex chromosomes is frequently reported as an age-related event of aneuploidy. Interestingly, the sex chromosomes are documented to be excluded from the nucleus and be incorporated into the micronuclei (Guttenbach et al., 1994; Hando et al., 1994). In lymphocytes, the frequency of X-bearing or Y-bearing micronuclei in aged individuals is twice as high as that in young individuals (Guttenbach et al., 1994). Another study also found that the frequency of autosome-containing micronuclei is not significantly changed in aged human males, while the percentage of Y chromosome-containing micronuclei is markedly increased in lymphocytes with age (Catalán et al., 1998). The causes and biological consequences of sex chromosome exclusion into micronuclei in aged individuals remain to be addressed.

DNA fragments

Senescent cells also extrude DNA fragments, which are lamin A/C negative but strongly γH2AX positive and H3K27me3 positive, into the cytoplasm (Ivanov et al., 2013). This kind of cytosolic DNA, termed cytoplasmic chromatin fragments (CCFs), is apparently different from micronuclei, which are encapsulated by the nuclear envelope. Lamin B depletion in senescent cells (Shimi et al., 2011) is associated with nuclear-to-cytoplasm chromatin blebbing and the formation of CCFs (Ivanov et al., 2013). Extranuclear double-strand DNA accumulation has also been reported in cells derived from patients with ataxia-telangiectasia syndrome and Hutchinson-Gilford progeria syndrome (Lan et al., 2019), both of which are severe premature aging disorders. CCFs can activate the cGAS-STING pathway, mediating pro-inflammatory responses in senescent cells (Dou et al., 2017; Han et al., 2020c; Yang et al., 2017) and further promoting paracrine senescence (Glück et al., 2017). Since increased cytoplasmic DNA burden leads to senescence and inflammation, targeted removal of extranuclear DNA holds great potential in alleviating innate immune responses and senescence-associated phenotypes (Lan et al., 2019). Interestingly, a defect in DNA-degrading activity is partially responsible for DNA fragment-mediated senescence, and activation of autophagy with metformin or rapamycin reduces CCFs and represses senescence (Han et al., 2020c).

Summary and perspectives

As we mentioned above, multiple cellular alterations associated with genomic instability have been reported as biomarkers of aging, although many of them are not absolutely universal and are regulated in a cell type-, tissue type- or sex-specific manner, of which the underlying mechanisms thus far remain ambiguous. Most of the biomarkers we reviewed here are related to changes in nuclear DNA. However, mi-

tochondrial DNA alterations are also tightly connected to aging (Kujoth et al., 2005; Ross et al., 2013; Sun et al., 2016). A recent study also showed that mitochondrial DNA replication defects have deleterious effects on nuclear genomic stability (Hämäläinen et al., 2019), providing an unexpected association between the nucleus and mitochondria, which has been neglected for a long time. Moreover, whether these genetic instability-related biomarkers mentioned above can be used for measuring the process of aging, predicting disease susceptibility, and directing personalized treatment for age-associated diseases warrants further study (Figure 3).

Telomere attrition

Among many biological processes related to aging, telomeres stand out because they follow a simple and important path: they shorten during aging. In eukaryotes, telomeres are nucleoprotein complexes at the linear chromosome ends that play a vital role in protecting the integrity of the genome from nucleolytic degradation, DNA damage response, and unnecessary DNA recombination. The basic composition of telomeres is tens of kilobases of G-rich tandem repeat DNA sequences ending with a 50- to 400-nt single-stranded 3′ overhang and organized in a peculiar chromatin structure. In humans, the chromatin structure of telomeres involves the shelterin protein complex and the noncoding RNA TERRA (de Lange, 2005; Gilson and Géli, 2007). The shelterin complex comprises six proteins, including telomeric repeat-binding factor 1 (TRF1, encoded by the *TERF1* gene), telomeric repeat-binding factor 2 (TRF2), TPP1 (or ACD, recruiting telomerase), protection of telomeres 1 (POT1, encoded by *POT1*), TRF1 interacting nuclear factor 2 (TIN2, encoded by *TINF2*), and TRF2 interacting protein (RAP1 or TERF2IP). They carry out multiple functions, including telomere replication regulation, telomere capping, and higher-order structure determination of telomeric chromatin.

Uncapping shelterin complexes from telomeres leads to the activation of the DNA damage response and unwanted DNA repair at telomeres (Mendez-Bermudez et al., 2020; Ye et al., 2014). Thus, shelterin deficiency leads to telomere uncapping and even telomere collapse. TRF2 suppresses ATM phosphorylation at telomeres (Denchi and de Lange, 2007) and allows replication through telomeric chromatin

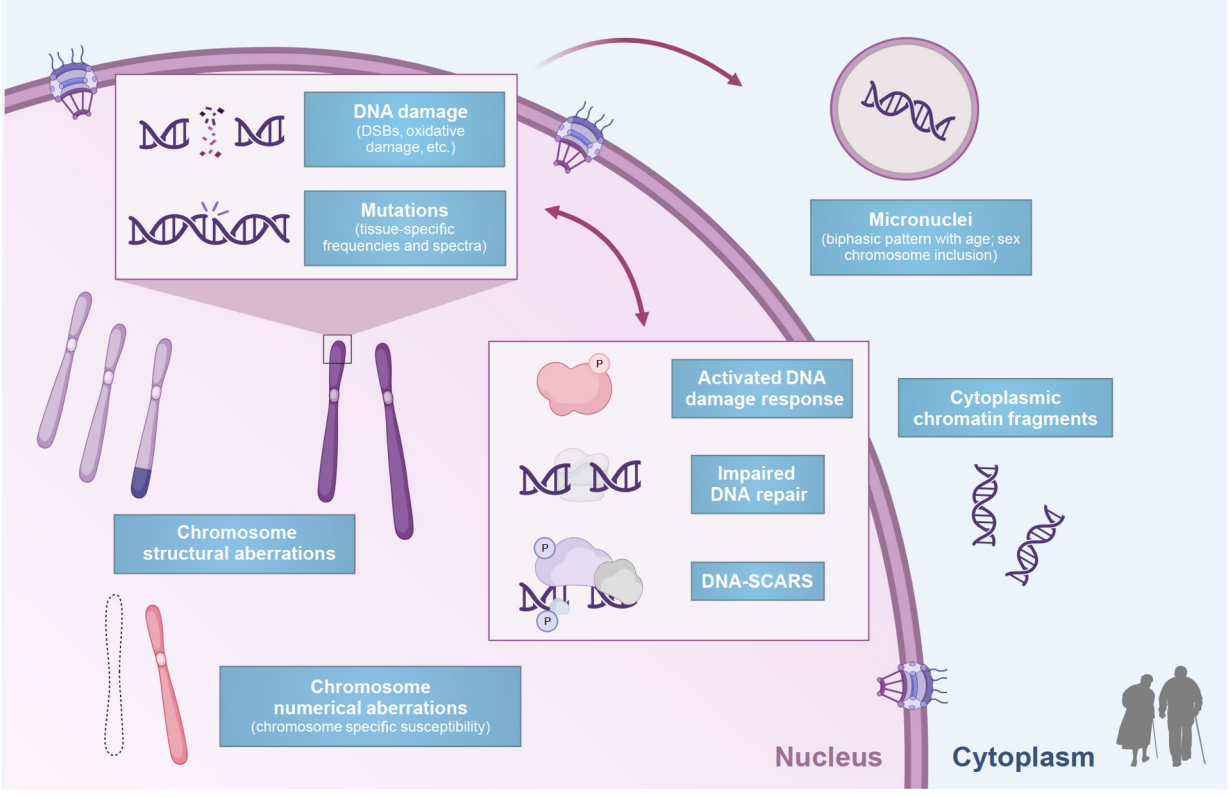

Figure 3 Biomarkers of age-related genetic instability. Destabilization of the genome represents a universal biomarker of aging. Increased DNA damage, mutations, and chromosome aberrations (both structural and numerical) are frequently reported in senescent cells and aged individuals, and impaired DNA repair might be a possible causal factor. Accumulation of DNA lesions leads to persistent activation of the DNA damage response. Unrepaired damaged DNA and activated damage response factors together constitute the damage-associated nuclear substructure, namely, DNA-SCARS. Damaged nuclear DNA is also present in the cytoplasm in the form of micronuclei or cytoplasmic chromatin fragments, both of which are altered during the process of aging. Notably, many of the biomarkers are regulated in a context-dependent way, and the underlying mechanisms thus far remain elusive. Abbreviations: DSBs, double-strand breaks; DNA-SCARS, DNA segments with chromatin alterations reinforcing senescence.

(Ye et al., 2010). Inactivation of TPP1/POT1 activates the Ataxia telangiectasia and Rad3-related (ATR) signaling pathway (Jones et al., 2014; Wang et al., 2011; Xin et al., 2007). TRF1 removal activates ATR kinase (Zimmermann et al., 2014). RAP1 and TRF2 form a heterodimer and repress the homology-directed repair pathway and non-homologous end joining (NHEJ) (Chen et al., 2007b; Ghilain et al., 2021; Lototska et al., 2020; Rai et al., 2016; Zhang et al., 2019d). TIN2 deletion produces a complex response that involves ATM kinase signaling and c-NHEJ, which are partly due to the loss of TRF2 from telomeres (Jones et al., 2014; Takai et al., 2011; Wang et al., 2011; Xin et al., 2007). Several loss-of-function models for shelterin components indicate a decline in tissue regenerative capacity and accelerated aging (Alder et al., 2015; Morgan et al., 2019; Uryga et al., 2021; Ying et al., 2022).

Telomere DNA length

Human telomeres consist of short tandem repeats (5′-TTAGGG-3′) that range from 8 to 15 kb at birth. Due to end replication problems, telomeres shorten after each replication cycle. During early development, telomere DNA is elongated by telomerase to counteract dramatic telomere shortening by approximately 50–200 nucleotides after each replication cycle due to high cellular proliferation (Anifandis et al., 2021). However, the embryonic development stage ended with telomerase inactivation in most somatic cells, and telomere DNA length (TL) can be seen as a counting machine for the number of cellular divisions. When programmed telomere shortening leads to a critically short TL stage (the Hayflick limit), it induces a permanent DDR, triggering irreversible cell cycle arrest, known as replicative senescence. Therefore, TL can be considered a biomarker to gauge aging (Chakravarti et al., 2021).

Many studies indicate that the dynamics of TLs are not only a mitotic clock at the cellular level but also at the level of individual aging. As humans age, the average TL in most tissues declines with age (Daniali et al., 2013). This occurs in high-proliferative tissues such as the skin, gastrointestinal tract, and hematopoietic system, as well as in low-proliferative tissues such as the heart, brain, and fat (Blackburn et al., 2015). Since telomere shortening is intrinsically linked to cell division and non-proliferative tissues are mainly composed of long-lived post-mitotic cells (LLPMC), the mechanism of age-related telomere changes in non-proliferative tissues is still elusive (Jacome Burbano and Gilson, 2020). Mitochondrial dysfunction and ROS accumulate in these tissues. Due to its G-richness, telomeric DNA is particularly sensitive to oxidation by ROS, which incurs telomere damage, possibly causing telomere attrition and uncapping over decades (Robin et al., 2020; Wagner et al., 2017). These data suggest that telomere structural changes are associated with proliferative and non-proliferative tissue aging.

Since the dynamics of TL are a potential marker of various types of tissue aging, numerous clinical and epidemiological studies have addressed the question of whether TL shortening in blood cells can reflect tissue aging or even individual aging. Indeed, TL shortening in peripheral leukocytes or PBMCs reflects systemic influences on TL distribution across human tissues (Demanelis et al., 2020). The average TL loss in PBMCs ranges from approximately 1,000 bp per year during birth and 1 year of age to approximately 100 bp per year during childhood and approximately 50 bp per year in adulthood, suggesting that TLs in PBMCs are an aging-predicting marker (Aubert et al., 2012).

At the organismal level, the dynamics of TL are influenced by genetic variants and nongenetic parameters throughout the human lifespan. Heritability contributes to human TL variation ranging from 30% to 80%. TL homeostasis responds to lifestyle (Epel and Prather, 2018) and social factors or environmental changes (Garrett-Bakelman et al., 2019). Even pathogen infection, for example, by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can cause significant telomere shortening (Mongelli et al., 2021), suggesting that the degree of telomere attrition is a sensitive biomarker to account for the accumulation of stress exposure. Therefore, TL as a general aging biomarker represents an exciting opportunity to predict and track frailty, loss of resilience and age-related diseases early. For instance, regarding lifestyle, social and environmental stress are major contributors to aging heterogeneity, and individual TL variation may indicate a danger of aging acceleration. Recent studies have compared TL variation and the methylation clock in PBMCs to track chronological age. Interestingly, they can be complementary in aging evaluation since they appear to reflect different biological mechanisms determining the aging trajectory (Franzago et al., 2022; Pearce et al., 2022) (Table S1 in Supporting Information).

The methods to detect telomere

The above examples support the use of TL as a relevant biomarker for an early prediction of aging. A reproducible TL measurement will be important for future clinical applications. Many methods exist, including quantitative polymerase chain reaction (Q-PCR), terminal restriction fragment (TRF) analysis, a variety of quantitative fluorescence *in situ* hybridization (Q-FISH) methods, single telomere length analysis (STELA), telomere shortest length assay (TeSLA), peptide nucleic acid hybridization analysis of single telomere (PHAST) assay and single-molecule real-time (SMRT) sequencing. Although these methods have been improved in recent decades, there are still major technical impediments in clinical settings, including accuracy, reproducibility, timing and technical difficulty. Moreover, a relevant biological assessment of telomere status requires

information beyond the classical average TL to allow the prediction and prognosis of age-related diseases. For example, TL distribution is emerging as a more potent biomarker than just the median or average TL, since the shortest TL at individual chromosome ends, not the average TL, can trigger senescence (Abdallah et al., 2009; Kaul et al., 2011). The longest telomeres are used as a signature of adult stem cell compartments (Kim et al., 2013), but it remains difficult to accurately detect telomeres longer than 20 kb. Metaphase images with abnormal telomere phenotypes can provide compelling evidence to reveal the status of telomere dysfunction, for example, telomere fusion with normal TL distribution.

A real-time Q-PCR-based TL measurement is a rapid method with small amounts of sample (~20 ng), providing an average of total TL normalized to a single copy gene, but it does not provide information about the telomere length distribution (Cawthon, 2002). Moreover, this method is poorly standardized across laboratories due to the use of various normalization methods. Q-PCR is also inaccurate for quantifying TL in cancer studies because of the common occurrence of aneuploidy and mutation of the gene used for standardization. Several methods have been developed based on Q-PCR, such as Ω-qPCR (Xiong and Frasch, 2021) and the single telomere absolute-length rapid (STAR) assay (Luo et al., 2020). These methods can determine the absolute TL. Moreover, the STAR assay can quantify individual telomere molecules at a relatively broad spectrum, from the shortest telomere to longer ones of hundreds of kb in individual cells, and can be applied for tumor TL detection. It is a promising method of TL detection in the future both in the clinic and in fundamental studies.

The TRF assay is considered the gold standard for telomere analysis. The TRF assay requires a Southern blot procedure to separate telomere fragments after restriction enzyme digestion. As it is a laborious process and requires a large amount of starting genomic DNA, this assay is not suitable for clinical applications (Harley et al., 1990). STELA and its modified approach (Universal STELA (U-STELA) and TeSLA) were developed based on the TRF assay, which combines Southern blot and PCR amplification after adapter ligation to measure the TL of individual chromosome ends and provide valuable information about TL distribution (Baird et al., 2003; Bendix et al., 2010; Lai et al., 2017). These approaches are capable of detecting the shortest telomeres. As many studies on telomere biology have revealed that critically short telomeres, rather than average telomere lengths, are causative of age-related pathologies, STELA or U-STELA and TeSLA are crucial for clinics to predict pathological aging early. However, these assays are too labor-consuming for routine clinical and large population studies.

The Q-FISH method hybridizes the fluorescently labeled $(CCCTAA)_3$ peptide nucleic acid (PNA) probe to fixed interphase cells or various tissue biopsies and quantifies TL by counting the number and intensity of the fluorescence signals to determine the TL (Lansdorp et al., 1996). The distinct disadvantage of Q-FISH is that the fluorescence signals of telomeres, also called "telomere spots", are dependent on the aggregation of higher-order telomere structures, which leads to a reduction in the overall telomere count that could result in the incorrect estimation of TL distribution. Metaphase Q-FISH can detect TL distribution and the abnormal phenotype of individual chromosome ends but can only be performed on actively dividing cells (Lansdorp et al., 1996). Based on a similar principle to Q-FISH but modified for the flow cytometry technique, flow FISH measures the median TL in individual cells in suspension after hybridization with telomeric PNA probes. Combined with different flow cytometry methods, flow FISH can be adapted for higher throughput and enhanced reproducibility, and it is the first TL method to have been validated for clinical diagnostic purposes (Baerlocher et al., 2006). Another advantage of flow FISH is measuring the distinct cell populations by antibody staining (specific cell populations can be cell sorted prior to flow FISH); for example, flow FISH is currently the fastest and most sensitive method available to measure the average TL in subgroups of human blood cells. The disadvantage of this technique is that PNA probes may bind non-specifically due to the fixation and hybridization efficiency in various labs.

The PHAST assay is designed to pull down PNA probes that are hybridized to the telomere sequence and pass through a microfluidic channel for analysis by light-sheet fluorescence (Luo et al., 2020). PHAST requires very specialized equipment and is not suitable for measuring telomere lengths longer than 15 kb.

Recently, SMRT sequencing developed a high throughput TL measurement at nucleotide resolution using the PacBio high fidelity (HiFi) sequencing platform on purified genomic DNA containing telomeric sequence after hybridizing and ligating the telomeric single-stranded G-rich overhangs at the 3′ ends of chromosomes (Tham et al., 2023). This assay not only provides a high-throughput and accurate assay to detect TL at a high resolution but also reveals the presence of telomeric variant sequences (TVSs) interspersed within the long tracts of canonical telomeric repeat regions, which will provide more information for the clinic and research of telomere biology.

To conclude, all the methods above for detecting TL distribution have advantages and disadvantages. The strengths and limitations of the major methods developed to measure TL in cells and tissues are presented in Table S1 in Supporting Information. With the development of the technique for fundamental study and the requirement of large clinical studies, a single-cell approach is highly pre-

ferable to identify cells with aberrantly long or short telomeres in the future.

Telomere as an aging biomarker

Telomere shortening, being a marker of biological aging, could be used as a sentinel to intercept individuals at risk of developing age-related diseases. Indeed, the level of telomere attrition is associated with the incidence and mortality of diabetes, cardiovascular disease (CVD), depression, and cognitive decline (Blackburn et al., 2015). Telomere shortening also has the potential to predict the prognosis of various cancers independent of chronological age (Hampton, 2011; Mender et al., 2020; Tamura et al., 2016; Tian et al., 2019). The severity of telomere attrition is a risk factor for age-related diseases. It predicts poor immune function and fragility (Cohen et al., 2013). Patients within the higher percentiles of short telomeres have a higher risk of developing severe COVID-19 pathologies (Sanchez-Vazquez et al., 2021).

TINF2 mutations are the second most common genetic cause of dyskeratosis congenita (DKC), a congenital disease of human premature aging syndrome, and affect approximately 15% of DKC patients, suggesting that TIN2 function is crucial to act against pathological aging. Although few studies have detected shelterin levels during human aging, published data have shown that TRF2 (telomeric repeat-binding factor 2) declines with tissue aging in clinical biopsies (Robin et al., 2020; Tian et al., 2019) and that TRF2 expression is abnormally elevated in cancer biopsies (Biroccio et al., 2013; Cherfils-Vicini et al., 2019). All these results suggest that TRF2 is a potential marker for the aging trajectory. Further studies are required to understand the expression level of TRF2 as well as other shelterin members in normal and pathological aging in a large epidemiological cohort or clinical samples from patients with age-related diseases.

A telomere dysfunction assay that is useful for DNA damage studies is TIF analysis (telomere dysfunctional induced foci). This method is based on PNA-telomere probe Q-FISH generally conducted on interphase cells *in vitro* or in tissue sections and involves an antibody that recognizes the DNA damage response, such as γH2AX or 53BP1. The co-localization of telomeres with DDR antibody signals suggests damage at many telomeres. Although this assay does not provide information about TL, it is useful as a biomarker to detect the number of cells with telomere dysfunction (short or uncapped) that appear damaged, which is a crucial marker to predict the tendency to cause cell senescence or malignant proliferation. An increase in TIF, dissociated from TL variation, is characteristic of early stages of B-cell chronic lymphocytic leukemia (B-CLL) (Augereau et al., 2011), indicating that TIFs can be interesting TL-independent biomarkers of some age-related diseases.

Summary and perspectives

Here, we have summarized the possible telomere-related biomarkers related to the aging trajectory and age-related diseases. We highlighted the underlying cause of telomere dysfunction, either in the form of telomere shortening, telomere DNA damage, or telomere-specific protein depletion, to characterize the aging trajectory. This provides the groundwork to develop important biomarkers for aging interventions not only for predicting the early risk of developing age-related diseases but also to promote healthy aging. Carefully designed clinical studies should be conducted in the future to test and validate telomere biomarkers and targets as useful tools in the fight against the adverse consequences of aging.

Nuclear body disorders

Nuclear bodies are macromolecular condensates within the nucleus of eukaryotic cells. These dot-like structures further compartmentalize the nuclear space and perform specialized functions similar to organelles (Sabari et al., 2020). However, unlike membranous organelles such as the Golgi apparatus and lysosomes, nuclear bodies are usually formed through liquid-liquid phase separation (LLPS) of their protein or nucleic acid components without lipid membranes via a nucleation mechanism (Brangwynne et al., 2009; Shimobayashi et al., 2021; Wang et al., 2021a). Thus, nuclear bodies are also considered membraneless organelles (MLOs) with the nucleus (Lyon et al., 2021). To date, at least 18 nuclear bodies have been documented in human cells, including nucleoli, Cajal bodies, promyelocytic leukemia (PML) bodies, nuclear speckles, paraspeckles, nuclear gems (Sabari et al., 2020). Nuclear bodies show diverse functions but cooperate in a series of biological processes, such as gene expression regulation, RNA processing, and maturation (Hirose et al., 2022). Similar to the alteration of nuclear architecture during normal aging (Haithcock et al., 2005), some nuclear bodies, such as the nucleolus, have been implicated in the aging process and become potential biomarkers of aging (Buchwalter and Hetzer, 2017; Kasselimi et al., 2022; Papandreou et al., 2022; Ren et al., 2017; Ren et al., 2019; Tiku et al., 2017).

Architecture of nuclear bodies

Nuclear bodies are usually composed of proteins and nucleic acids represented by various RNAs (Hirose et al., 2022). Many proteins in nuclear bodies contain intrinsically disordered regions (IDRs), such as RNA-binding proteins, which enrich charged and aromatic amino acid residues to interact with other proteins or RNAs (Shin and Brangwynne, 2017). In addition, some folded domains, such as small ubiquitin-like modifier (SUMO) and SUMO interacting motif (SIM), also contribute to multivalent interactions for

the formation of LLPS (Shen et al., 2006), suggesting that nuclear bodies can be organized by multiple mechanisms.

Compartmentalization is the main function of nuclear bodies. To further separate distinct biological processes or reactions, most nuclear bodies contain substructures. The "scaffold and client theory" is now widely accepted as a model for the organization of a nuclear body (Banani et al., 2016). Scaffold proteins are prone to undergo LLPS and are essential for nuclear body formation, while client proteins cannot undergo LLPS and are usually adaptor proteins or enzymes for the specific functions of nuclear bodies (Banani et al., 2017).

The nucleolus is the first discovered nuclear body and acts as a central hub for nuclear functions, including ribosome biogenesis, genome organization, stress response, and telomere maintenance (Brown and Gurdon, 1964; Iarovaia et al., 2019). As the largest condensate in the nucleus, the nucleolus shows a three-layer substructure from core to shell: a fibrillar center (FC) containing rDNA genes, a dense fibrillar compartment (DFC) for pre-rRNA processing after rDNA transcription at the FC-DFC border (Yao et al., 2019), and a granular compartment (GC) for late pre-rRNA processing and ribosomal protein assembly (Lafontaine et al., 2021). Recent studies suggest that the nucleolus also functions as a quality control compartment for misfolded proteins (Frottin et al., 2019). Given that the loss of proteostasis is one hallmark of aging (López-Otín et al., 2013), the role of the nucleolus in the aging process can also be linked from the perspective of proteostasis.

PML body (also known as ND10) is named after its concentrated PML protein (Ishov et al., 1999). SUMOylated PML interacts with its associated proteins that have SIM through SUMO-SIM multivalent interactions (Shen et al., 2006). PML and its direct interactors constitute the outer cage-like structure, which surrounds an inner core with various client proteins (Lallemand-Breitenbach and de Thé, 2018). The core region of the PML body concentrates proteins including enzymes for biochemical processes such as SUMOylation and chromatin regulation (Lallemand-Breitenbach and de Thé, 2018). Cells utilizing alternative lengthening of telomeres (ALT) have a special PML nuclear body (ALT-associated PML body, APB) containing PML protein, telomere DNA and some telomere binding proteins (Yeager et al., 1999). Since telomere attrition is another hallmark of aging (López-Otín et al., 2013), the PML nuclear body might be associated with the aging process by regulating telomere stability.

Senescent cells often undergo dramatic alterations to chromatin organization (Criscione et al., 2016b). Senescence-associated heterochromatin foci (SAHF) were first discovered in oncogene-induced senescence (OIS) of human fibroblasts (Narita et al., 2003) (Figure 4). These heterochromatic domains contain chromatin-repressive proteins, such as HP1, high-mobility group A (HMGA) proteins (Narita et al., 2006), the histone variant macroH2A (Zhang et al., 2005b) and trimethylated histone H3 Lys9 (H3K9me3) (Zhang et al., 2007). Senescence-specific spatial clustering of heterochromatin contributes to the formation of SAHF

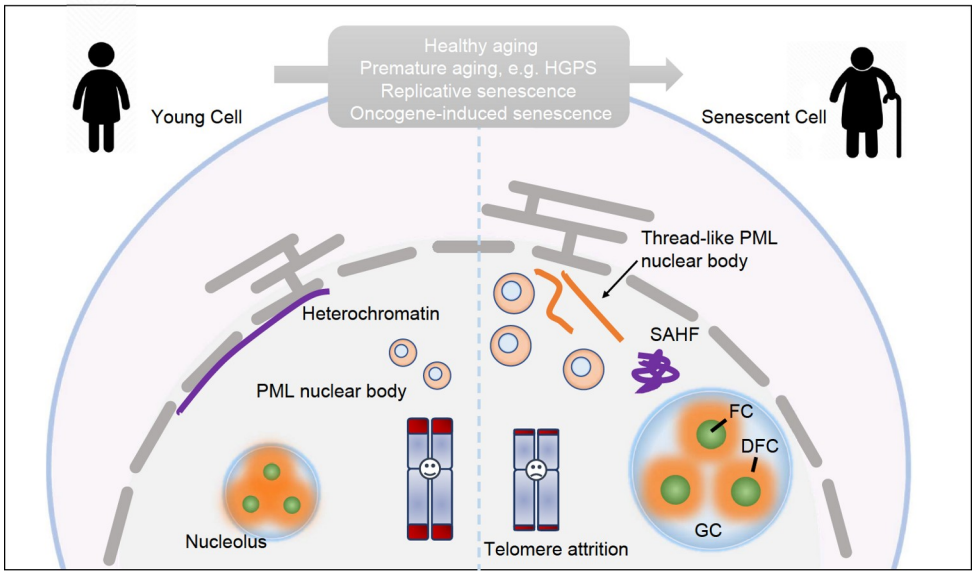

Figure 4 Changes in nuclear bodies as biomarkers of aging. Some nuclear bodies are changed during cell senescence. The nucleolus is the largest nuclear body and has a three-layer structure including the FC, DFC and GC. The nucleoli become larger in the fibroblasts from HGPS patients and healthy old individuals. In oncogene-induced senescenc of human fibroblasts, both the number per cell and the size of PML nuclear bodies are increased. Some of the classical dot-like PML nuclear bodies also changed into thread-like structures in the fibroblasts from HGPS patients. The PML nuclear body is also required for p53-PML-p300 complex formation, which promotes the induction of replicative cell senescence. In some senescent cells, such as oncogene-induced senescence of human fibroblasts, heterochromatin is detached from the nuclear membrane and organized into SAHF. Telomeres are also regarded as nuclear bodies. Telomere attrition is a hallmark of cell senescence and aging.

(Chandra et al., 2015). However, SAHF is not a universal biomarker for all types of senescent cells (Di Micco et al., 2021).

Morphology of nuclear bodies

As a subcellular structure mainly formed by the mechanism of LLPS, nuclear bodies often show a round or dot-like morphology (Brangwynne et al., 2009). However, this is not always the case. For example, nuclear speckles and paraspeckles have irregular shapes, such as small granules or distorted spheres (Galganski et al., 2017; Wang and Chen, 2020), which are consistent with their names as speckles. The size of nuclear bodies also varies within a range from 0.2–2 μm for diameters (Hirose et al., 2022). The nucleolus is the largest round nuclear body, ranging from 1–10 μm in different cell types and growth states (Lafontaine et al., 2021). Cajal bodies, nuclear gems, PML nuclear bodies, and histone locus bodies are quite similar round structures, all approximately 0.2–1 μm in size (Hirose et al., 2022).

The nucleolus undergoes morphological changes with the senescence onset (Kasselimi et al., 2022). And its size inversely correlates with longevity in animals, including nematodes, fruit flies and mice (Tiku et al., 2017). Primary fibroblasts from pre-maturely aged patients with HGPS (Eriksson et al., 2003), as well as from old healthy individuals, have expanded nucleoli, indicating that nucleolar size is an aging biomarker (Buchwalter and Hetzer, 2017). In the fibroblasts from HGPS patients, the classical dot-like PML nuclear bodies are reorganized into thread-like structures, suggesting that the morphological alteration of PML nuclear bodies might be a biomarker for late senescence (Wang et al., 2020c) (Figure 4).

Function of nuclear bodies

The core function of nuclear bodies is compartmentalization. By sequestrating or excluding specific factors, nuclear bodies cooperate to realize the flow of genetic information based on Central Dogma. Because the nucleus is vital for the biogenesis of various RNAs, most nuclear bodies are associated with chromatin and function in gene transcription as well as RNA maturation (Hirose et al., 2022). For example, the nucleolus transcribed pre-rRNA from rDNA loci within its FC-DFC interface and further processing for mature rRNA occurs in DFCs (Yao et al., 2019). The Cajal body associates with U snRNA gene loci (Smith et al., 1995) with the assistance of nascent snRNA (Frey and Matera, 2001), thus reinforcing snRNA transcription and producing small nuclear ribonucleoproteins (snRNP) for the spliceosome (Wang et al., 2016b). In addition, Cajal bodies also have a role in snoRNA (Meier, 2017) and histone gene expression (Wang et al., 2016b). The histone locus body associates with the replication-dependent histone gene and recruits factors for efficient histone mRNA biogenesis (Tatomer et al., 2016). The PML nuclear body interacts with specific chromatin regions, such as telomeres, and contains chromatin regulators, thereby affecting gene transcription via chromatin remodeling (Corpet et al., 2020). Nuclear speckle plays important roles in both gene transcription and pre-mRNA processing by accumulating transcription regulators and splicing factors (Galganski et al., 2017).

Dysregulation of nuclear bodies has been implicated in aging. In the nucleolus, CpG hypermethylation of ribosomal DNA is an evolutionarily conserved marker of aging across mammalian species from humans to dogs, providing a useful concept of the "rDNA clock" to gauge individual age (Wang and Lemos, 2019). Oncogenic and replicative stress lead to defects in ribosome biogenesis in nucleoli, which induces RPL11-mediated p53 activation and cellular senescence (Nishimura et al., 2015). Oncogene-induced stable p53-PML-p300 complex formation depends on the PML nuclear body, which mediates p53 acetylation and promotes the induction of replicative senescence (Pearson et al., 2000) (Figure 4).

Numbers of nuclear bodies

To date, at least 18 nuclear condensates have been documented, some of which are common among cell types, including the nucleolus, Cajal body, PML body, nuclear speckle (Sabari et al., 2020). Some nuclear bodies are cell-type specific, such as paraspeckles, which are common in cancer cells but absent in embryonic stem cells (Chen and Carmichael, 2009).

The number of each nuclear body varies greatly among cell types and under specific stress conditions. For example, there are an average of 3 nucleoli in each nucleus of HeLa, MCF10A, and CHO cells (Farley et al., 2015). Usually, 10–20 paraspeckles are detected in human cells (Fox et al., 2002). In hyperproliferating cells such as cancer cells, RNA biogenesis and processing are very vigorous. Therefore, the number and size of nuclear bodies in cancer cells are usually greater than those in normal somatic cells. In addition, because of the dynamic nature of LLPS, nuclear bodies are prone to be affected by environmental stimuli, such as osmosis and heat shock (Mähl et al., 1989). During cell senescence, the number of some nuclear bodies is altered. Overexpression of the oncogene RasV12 in human fibroblast WI38 cells increases both the number and size of PML nuclear bodies from 12.1±3.6 bodies per nucleus of (229±50.9) nm diameter to 30±10 bodies per nucleus of (601.7±124.4) nm diameter (Pearson et al., 2000). Therefore, the number of certain nuclear bodies in the cell might be an ideal biomarker for cell senescence (Figure 4).

Locations of nuclear bodies

As the largest membranous organelle, the nucleus can be divided into several subdomains, including the nuclear en-

velope, nuclear lamina, chromatin, and nucleoplasm. As the largest nuclear body and easily seen in phase-contrast microscopy, the nucleolus is often considered a special subregion in the nucleus (Lafontaine et al., 2021). Most nuclear bodies scatter in the nucleoplasm, and some of them, such as the Cajal body, PML nuclear body, and histone locus body, are associated with the chromatin (Banani et al., 2017). One of the PML isoforms, PML-II, can localize to the nuclear envelope, linking to the nuclear lipid droplet formation (Ohsaki et al., 2016).

Nuclear body-related diseases

The PML body is involved in the ubiquitin-mediated proteolytic system, and its dysfunction leads to acute promyelocytic leukemia, neurodegenerative disease and antiviral defects (Lallemand-Breitenbach and de Thé, 2018; Scherer and Stamminger, 2016). Spinal muscular atrophy disease gene product (SMN) is one of the key protein components of nuclear Gems, which promote the maturation of snRNP (Liu et al., 1997). Mutation of SMN causes a neurological disease of spinal muscular atrophy (Lefebvre et al., 1995). Malfunctions of other nuclear bodies, such as the Cajal body, nucleolus, and paraspeckle, have been well documented in cancer and neurogenerative diseases (Hirose et al., 2022). Nevertheless, more pathological aging-related phenotypes of nuclear bodies require further investigation.

Summary and perspectives

Although the study of nuclear bodies has made great progress in recent years, more types of nuclear bodies need to be identified. In addition, the following questions need to be answered in the future. Are there any new nuclear bodies related to aging and diseases? How do these nuclear bodies communicate in young and old cells? Is there any "nuclear body grammar" that specific protein or nuclear acid sequence is important for macromolecules targeting to a specific nuclear body? Can some nuclear bodies be used as biomarkers or therapeutic targets? The answers to these questions will benefit the study of cell senescence and healthy aging research.

Cell cycle arrest

The cell cycle is the series of events that drive proliferation-competent cells to divide into two new daughter cells. The typical cell cycle is composed of G1, S, G2, and M phases (Panagopoulos and Altmeyer, 2021). Cell cycle progression is regulated by cyclins and CDKs, CDKIs, and retinoblastoma tumor suppressor protein (RB). Three major cell cycle checkpoints exist, including the G1/S and G2/M transition checkpoints and the spindle assembly checkpoint (SAC), to ensure a proper cell cycle progression (Barnum and O'Connell, 2014; Satyanarayana and Kaldis, 2009). Cyclins (such as cyclins D and E) and CDKs (such as CDK2, 4 and 6) can form cyclin-CDK complexes and phosphorylate RB, which leads to the release of transcription factor E2F from the RB-E2F complex and subsequent translocation to the nucleus, thereby transcriptionally activating downstream target genes involved in DNA replication and thus positively driving cell cycle progression from G1 to S phase transition. In contrast, CDKIs such as $p21^{CIP1}$ and $p16^{INK4a}$ can inhibit CDK activity; therefore, RB cannot be phosphorylated and remains in its hypophosphorylated state, which leads to sequestration of E2F in the RB-E2F complex, thereby repressing E2F target gene transcription, blocking the cell cycle in G1 phase and inhibiting entry into S phase (Hume et al., 2020). Hence, proper cell cycle progression requires precise coordination between the cyclin-CDK complex and CDKI.

Regardless of diverse intracellular and extracellular senescence stimuli, one of the most defining hallmarks of cellular senescence is a stable cell cycle arrest in the G1 or possibly G2 phase, which blocks the damaged cells from proliferation (Gire and Dulić, 2015; Salama et al., 2014). Cell cycle arrest in cellular senescence is mainly regulated by the p53/$p21^{CIP1}$ and $p16^{INK4a}$/RB pathways (Beauséjour et al., 2003; Shay et al., 1991). Under senescence stressors, p53 is activated via DDR-dependent or DDR-independent pathways and then upregulates the expression of its downstream target gene $p21^{CIP1}$. As a CDKI, $p21^{CIP1}$ suppresses the formation of cyclin E-CDK2 and cyclin A-CDK2 complexes to halt the cell cycle and initiate senescence. Then, $p16^{INK4a}$ is induced to repress the formation of the cyclin D-CDK4/6 complex. When $p21^{CIP1}$ and $p16^{INK4a}$ are chronically activated to coordinately inhibit CDK activity, E2F target gene transcription is inhibited, and the cell cycle is blocked in G1 phase (Roger et al., 2021). Prolonged overexpression of p53, $p21^{CIP1}$, $p16^{INK4a}$, or RB is sufficient to induce cellular senescence (Coppé et al., 2011; Li et al., 2022f; McConnell et al., 1998). In contrast, the inactivation or depletion of these CDKIs can lead to the bypass of cellular senescence (Bond et al., 2004; Brown et al., 1997; Noh et al., 2019; Reyes et al., 2018). Evidence suggests that $p21^{CIP1}$ is mainly activated early during the induction of senescence, whereas $p16^{INK4a}$ is induced later and maintains cellular senescence (Gil and Peters, 2006). Both pathways also crosstalk (Yamakoshi et al., 2009; Zhang et al., 2006).

$p53/p21^{CIP1}$ pathway in cellular senescence

p53 plays a critical role in the modulation of cellular senescence via multiple mechanisms (Kastenhuber and Lowe, 2017). Constitutive DDR signaling caused by telomere attrition and oxidative or oncogenic stress activates p53 and its downstream effector $p21^{CIP1}$, which induces cellular senescence. Inactivation of p53-mediated signaling disrupts the onset of cellular senescence (Beauséjour et al., 2003; Brown et al., 1997; Reyes et al., 2018).

The expression level and activity of p53 are tightly regulated at different levels by different factors. At the post-transcriptional level, noncoding RNAs, including miRNAs and long noncoding RNAs (lncRNAs), can regulate p53 abundance and activity. For example, miR-504 reduces p53 mRNA stability, thereby decreasing its protein level and activity (Hu et al., 2010). Inactivation of the Gld2/miR-122/CPEB/Gld4 pathway enhances p53 mRNA translation and promotes cellular senescence (Burns et al., 2011). In addition, various post-translational modifications, such as ubiquitination, phosphorylation, acetylation, sumoylation, and neddylation, also play important roles in regulating p53 levels and activity (Kruse and Gu, 2009). For example, the E3 ubiquitin ligase MDM2 regulates p53 ubiquitination and promotes protein degradation in collaboration with murine double minute X (MDMX) (Wade et al., 2010). Conversely, miR-605 reduces MDM2 mRNA stability and its protein level, thereby triggering p53-mediated senescence (Xiao et al., 2011). p53 phosphorylation at serine-15 by ATM kinase promotes its protein stability and is the common change during replicative senescence or DNA damage-induced senescence (Webley et al., 2000). Forkhead box O4 (FOXO4) can maintain senescent cell viability by binding p53 and inhibiting p53-mediated apoptosis in favor of $p21^{CIP1}$-induced cell cycle arrest, and disruption of the FOXO4-p53 interaction causes senescent cell-intrinsic apoptosis (Baar et al., 2017).

$p21^{CIP1}$ is the first identified transcriptional target of p53 (el-Deiry et al., 1993). $p21^{CIP1}$ is encoded by the *CDKN1A* gene and is required for p53-induced cell cycle arrest at either G1/S or G2/M checkpoints (Al Bitar and Gali-Muhtasib, 2019; Rufini et al., 2013). $p21^{CIP1}$ suppresses cyclin E-CDK2 and cyclin A-CDK2 complex formation, thereby inhibiting RB phosphorylation and preventing subsequent E2F dissociation and formation of the dimerization partner, RB-like, E2F and multi-vulval class B (DREAM) complex, which ultimately leads to cell cycle arrest in G1 phase (Gomatou et al., 2021; McConnell et al., 1998). The p53-dependent induction of $p21^{CIP1}$ is crucial for the initiation of cellular senescence (Hernandez-Segura et al., 2017). $p21^{CIP1}$ can also be activated by transforming growth factor (TGF)-β/SMAD and phosphatidylinositol 3-kinase (PI3K)/FOXO signaling pathways in a p53-independent manner and plays a key role in developmental senescence, a transient programmed cellular senescence that occurs during mammalian embryonic development (Muñoz-Espín et al., 2013; Storer et al., 2013).

The expression level of $p21^{CIP1}$ is also regulated by divergent mechanisms. The transcription factor Sp1 can activate $p21^{CIP1}$ transcription (Huang et al., 2006), whereas c-Myc, ID1, CTIP-2, CUT, and retinoid X receptor suppress $p21^{CIP1}$ transcription (Jung et al., 2010). miRNAs such as miR-17-92, miR-106a-363, and miR-106b-25 or RNA-binding proteins such as HuD, HuR, RBM28, Msi-1, PCBP1/CP1/hnRNP E1, TAX, and AUF1 modulate $p21^{CIP1}$ mRNA stability and protein levels at the post-transcriptional level (Borgdorff et al., 2010). Diverse post-translational modifications of the $p21^{CIP1}$ protein, such as phosphorylation by Akt1/PKB, PKA, PKC, PIM-1, and GSKβ and ubiquitination by E3 ubiquitin ligases, also modulate $p21^{CIP1}$ expression levels (Al-Khalaf and Aboussekhra, 2013; Jung et al., 2010).

$p16^{INK4a}$/RB pathway in cellular senescence

The *INK4a*/*ARF*/*INK4b* gene cluster encodes $p16^{INK4a}$, $p14^{ARF}$ (or $p19^{Arf}$ in mice), and $p15^{INK4b}$, respectively (Gil and Peters, 2006). $p16^{INK4a}$ and $p15^{INK4b}$ are CDKIs and block cell cycle progression by inhibiting CDK4/6 activity (Gil and Peters, 2006; Kim and Sharpless, 2006; Kotake et al., 2011). $p16^{INK4a}$ is often used as a unique and specific biomarker for senescence *in vitro* and *in vivo* (Baker et al., 2011; Burd et al., 2013).

In most primary cells, the *INK4a*/*ARF*/*INK4b* locus is tightly regulated at multiple levels. At the transcriptional level, transcription factors such as Sp1, Ets, AP1 (JunB subunit), PPARγ, HBP-1, CTCF, and FOXA1 activate $p16^{INK4a}$ expression (Gan et al., 2008; Li et al., 2013; Ohtani et al., 2001; Passegué and Wagner, 2000; Salama et al., 2014; Wang et al., 2007), whereas ITSE (INK4a transcription silence element), YB1, ID1, and AP-1 (c-Jun subunit) transcriptionally repress $p16^{INK4a}$ expression in various stimuli-induced cellular senescence (Huang et al., 2011; Kotake et al., 2013; Li et al., 2011a). Additionally, in oxidative stress-induced cellular senescence, the extracellular signal-regulated kinases ERK1/2 and the stress-activated protein kinases p38 also upregulate $p16^{INK4a}$ expression (Jenkins et al., 2011; Shin et al., 2013). At the post-transcriptional level, the RNA-binding proteins hnRNP A1 and A2 promote $p16^{INK4a}$ mRNA stability (Zhu et al., 2002), while AUF1 enhances $p16^{INK4a}$ mRNA turnover (Guo et al., 2010). The lncRNA VAD inhibits the incorporation of the repressive histone variant H2A.Z at *INK4* gene promoters to promote $p16^{INK4a}$ expression in RAF-induced senescence (Lazorthes et al., 2015). The lncRNA UCA1 disrupts the hnRNP A1-$p16^{INK4a}$ mRNA interaction, thereby promoting $p16^{INK4a}$ mRNA stability and protein levels (Kumar et al., 2014). miR-24 suppresses $p16^{INK4a}$ expression at the post-transcriptional level (Lal et al., 2008). At the translational level, $p16^{INK4a}$ mRNA 5′-UTR contains a cellular internal ribosome entry site (IRES) that can enhance mRNA translation efficiency, in part through YBX1 (Bisio et al., 2015). $p16^{INK4a}$ protein is also subjected to various post-translational modifications; for example, phosphorylation at serine-140 and methylation at arginine-138 alter the affinity of $p16^{INK4a}$ for CDK4 (Lu et al., 2017), and its N-terminal ubiquitination promotes $p16^{INK4a}$ protein degradation (Ben-Saadon et al., 2004).

The epigenetic regulation of *INK4a*/*ARF*/*INK4b* locus transcription also plays a key role in the regulation of $p16^{INK4a}$ expression. The $p16^{INK4a}$ promoter is methylated by the methyl-transferase DNMT3b to silence its expression, while DNMT1 maintains existing methylation (Velicescu et al., 2002). DNMT1 inhibitors cause demethylation of the $p16^{INK4a}$ promoter and a senescence-like phenotype (Pan et al., 2013; Venturelli et al., 2013; Zhu et al., 2017). The repressive histone variant macroH2A1 is enriched in the inactive $p16^{INK4a}$ locus but replaced by H2A.Z in the active $p16^{INK4a}$ locus (Barzily-Rokni et al., 2011). In most primary cells, the *INK4a*/*ARF*/*INK4b* locus is tightly regulated by Polycomb group (PcG) proteins that function as epigenetic modifiers and transcriptional repressors (Li et al., 2011a). PcG proteins form two distinct protein complexes called Polycomb Repressive Complex 1 (PRC1) and 2 (PRC2). During cellular senescence, many PcG proteins, such as BMI1, MEL18, CBX4, CBX7, CBX8, EZH1/2, and SUZ12, are down-regulated, leading to the loss of H3K27me3 at the *INK4a*/*ARF*/*INK4b* locus, which results in the upregulation of $p16^{INK4a}$. On the other hand, ectopic expression of these PcG proteins delays or bypasses the onset of cellular senescence (Agherbi et al., 2009; Bracken et al., 2007; Dietrich et al., 2007; Gil et al., 2004; Kotake et al., 2007; Luis et al., 2011; Maertens et al., 2009). The antisense lncRNA for $p16^{INK4a}$, ANRIL, is required to recruit PRC1 and PRC2 complexes to the $p16^{INK4a}$ promoter to repress its transcription (Yap et al., 2010). Histone H3K27me3 site-specific demethylase JMJD3 has the opposite effect to PcG proteins, which can bind to the *INK4a*/*ARF*/*INK4b* locus and specifically catalyze H3K27me3 demethylation, thus releasing the inhibition of PRC complexes on *INK4a*/*ARF*/*INK4b* gene cluster transcription and promoting cellular senescence (Agger et al., 2009; Barradas et al., 2009). Other epigenetic regulators, such as ZRF and MLL1, also regulate *INK4a*/*ARF*/*INK4b* locus expression at the epigenetic level (Kotake et al., 2009; Ribeiro et al., 2013).

PcG proteins themselves are also subjected to diverse regulation. At the post-transcriptional level, miRNAs, including miR-26b, 181a, 210, and 424, downregulate the PcG proteins CBX7, EED, EZH2, and SUZ12, leading to $p16^{INK4a}$ upregulation and promotion of cellular senescence (Puvvula et al., 2014). In proliferating cells, the lncRNA PANDA recruits PRC complexes to repress $p16^{INK4a}$ transcription. Conversely, the loss of PANDA promotes cellular senescence (Puvvula et al., 2014). In addition, various post-translational modifications of PcG proteins also affect PRC complex function. For example, the ubiquitin-specific proteases USP7 and USP11 de-ubiquitinate BMI1 and MEL18 to enhance their protein stability, thereby downregulating $p16^{INK4a}$ transcription to delay cellular senescence (Maertens et al., 2010). EZH2 can be phosphorylated by CDK1, Akt, and AMP-activated protein kinase (AMPK), leading to alterations in PRC complex stability and enzymatic activity, thereby affecting target gene transcription (Chen et al., 2010; Kaneko et al., 2010; Liu et al., 2012b; Wan et al., 2018; Wei et al., 2011). The phosphorylation of CBX2, CBX7, CBX8, BMI1, and MEL18 by various protein kinases also affects PRC complex function and $p16^{INK4a}$ transcription, thereby playing an important role in regulating cellular senescence (Elderkin et al., 2007; Kawaguchi et al., 2017; Voncken et al., 2005; Wu et al., 2013; Zhan et al., 2018).

Summary and perspectives

In summary, stable cell cycle arrest mediated by $p53/p21^{CIP1}$ and $p16^{INK4a}$/RB pathways is one of the most defining hallmarks of cellular senescence. Both pathways are complex, as they involve many upstream regulators and downstream effectors (Figure 5). In humans, senescent cells accumulate in multiple tissues during aging. And a widely accepted biomarker for cellular senescence, SA-β-gal activity has been explored to monitor cellular aging in living mice (Sun et al., 2022a). $p16^{INK4a}$ is also the most widely used biomarker of cellular senescence *in vivo* (Idda et al., 2020). $p21^{CIP1}$ can also be useful in the detection of senescent cells in tissues. However, p53 and RB activation also occur in other forms of cell cycle arrest (Rodier and Campisi, 2011). $p21^{CIP1}$ is not usually maintained once the senescence program has been established (Stein et al., 1999). Even $p16^{INK4a}$ is not expressed by all senescent cells (Hernandez-Segura et al., 2018) and is also expressed in certain non-senescent cells (Sharpless and Sherr, 2015). Therefore, multiple biomarkers are required to precisely identify senescent cells *in vitro* and *in vivo*, such as the cell cycle regulators $p53/p21^{CIP1}$ and $p16^{INK4a}$/RB, DNA replication markers EdU or BrdU, cell proliferation markers Ki-67 or PCNA, colony formation assay or cell growth curve, SA-β-gal staining, DNA damage marker γH2AX, Lamin B1, and/or the SASP (Gorgoulis et al., 2019).

Mitochondrial malfunction

Mitochondria are important intracellular organelles that play essential roles in multiple cellular activities, including energy supply, calcium homeostasis, cell signaling, apoptosis regulation, and many biosynthetic pathways. Mitochondria contain their own genome, termed mitochondrial DNA (mtDNA), encoding 37 genes, including 13 genes coding for proteins, 2 genes coding for ribosomal RNAs (16S and 12S rRNAs), and 22 genes coding for transfer RNAs. ROS, which are primarily generated at complexes I and III of the mitochondrial respiratory chain, cause oxidative damage to mtDNA. The resulting mtDNA mutations produce defective respiratory chain components and thus generate more ROS, which leads to the vicious cycle of ROS and the accumulation of mtDNA mutations. The cell damage caused by

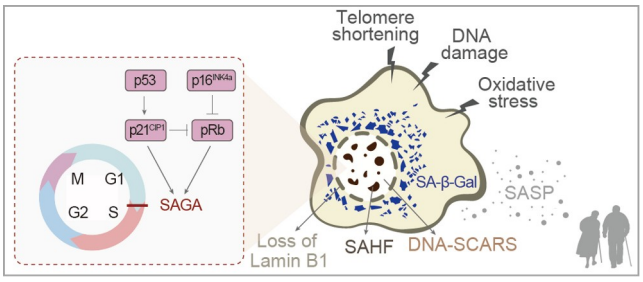

Figure 5 Cell cycle arrest as a biomarker of aging. Various internal or external senescence stressors, such as telomere shortening, DNA damage, oxidative stress, oncogene activation, tumor suppressor gene inactivation, chemotherapeutic drugs, UV light, radiation, and viral infections, activate the p53/p21^{CIP1} and/or p16^{INK4a}/RB pathways to block the cell cycle in G1 phase and inhibit entry into S phase, therefore achieving senescence-associated stable cell cycle arrest. Abbreviations: SAGA, senescence-associated growth arrest.

ROS and mtDNA mutations finally accelerates the aging process by compromising mitochondrial functions (Alexeyev, 2009), which is regarded as one of the hallmarks of aging (Ross et al., 2013). Oxidative damage theory is one of the main theories of the aging mechanism (Harman, 1972; Miquel et al., 1980). In general, age-related abnormalities of mitochondria, including increased ROS generation, accumulated mtDNA mutations and content, altered mitochondrial dynamics, decreased mitochondrial unfolded protein response (UPR^{mt}), and reduced activity of the respiratory chain, have been linked to aging (Li et al., 2022b).

ROS

Increased oxidative stress has been reported to be associated with aging (Kokoszka et al., 2001; Kurosu et al., 2005) and shown to reduce the lifespan of *C. elegans* (Dilberger et al., 2019). Moreover, increased ROS production is regarded as a biomarker of aging in monocytes (Jacinto et al., 2018). Spontaneous bursts of mitochondrial flash (mitoflash) frequency have been negatively correlated with and validated as a powerful predictor of the lifespan of *C. elegans* (Shen et al., 2014). Notably, the activated mitoflash can dramatically enhance the reprogramming of these cells from old individuals (Ying et al., 2016).

mtDNA mutations

The accumulation of mtDNA mutations has been considered to be an important contributor to aging and age-related diseases (Kong et al., 2022; Larsson, 2010; Linnane et al., 1989; Payne and Chinnery, 2015). mtDNA mutations have been reported to induce the aging of multiple organs in mice, such as the ovary, heart, and liver (Giorgi et al., 2018; Kauppila et al., 2017; Kujoth et al., 2005; Niemann et al., 2017; Trifunovic et al., 2004; Yang et al., 2020; Zhang et al., 2018a). Especially for some tissues with strong energy demand, including heart, liver and skin tissues, excessive mtDNA mutations result in mitochondrial dysfunction by compromising oxidative phosphorylation and accelerating aging phenotypes (Herbst et al., 2007). During the aging process, the accumulation of mtDNA mutations has been observed in aged rodent and human tissues (Baines et al., 2014; Cortopassi and Arnheim, 1990; Greaves et al., 2012; Greaves et al., 2006; Lob and Hugonnaud, 1978; Pikó et al., 1988; Taylor et al., 2003). Moreover, a larger number and higher frequency of mtDNA mutations, including point, deletion, and insertion mutations, have been identified in aged tissues (Corral-Debrinski et al., 1992; Fayet et al., 2002; Larsson, 2010; Yen et al., 1991). Notably, low-frequency (less than 0.5%) mtDNA point mutations accumulate in human oocytes during aging, which is linked with impaired blastocyst formation (Yang et al., 2020). The accumulation of mtDNA mutations has been shown to decrease fertility by reducing the amount of NADH in oocytes. Therefore, low-frequency mtDNA point mutations may be used as a potential biomarker of oocyte aging. In addition to mtDNA mutations, mitochondrial content (Tao et al., 2017; Vyas et al., 2020; Welle et al., 2003) is also altered in aged individuals. Different mtDNA copy numbers have been observed in different tissues during the aging process (Ding et al., 2015; He et al., 2014b; Knez et al., 2016; Mengel-From et al., 2014; van Leeuwen et al., 2014), implying that mtDNA copy number is tissue-specific for aging.

Mitochondrial dynamics

Mitochondrial dynamics, including mitochondrial fission, fusion, transport, biogenesis, and mitophagy, maintain the metabolic function and mtDNA integrity as well as their regulatory roles in mitochondrial turnover and many signaling pathways. Several studies have shown that mitochondrial dynamics are required for lifespan extension in different long-lived conditions (Burkewitz et al., 2015; Weir et al., 2017; Zhang et al., 2019e), demonstrating their important roles in the aging process. Indeed, altered mitochondrial dynamics, such as defective mitochondrial fission, have been linked with cellular senescence (Yu et al., 2020a). Due to direct regulation by mitochondrial fusion-fission dynamics, changes in mitochondrial morphology have also been reported to be associated with aging. The enlargement of mitochondria has been observed in aged *Drosophila* heart muscle (Sohal, 1970). Abnormal, fragmented mitochondrial networks are implicated in age-related diseases such as Parkinson's disease (PD), AD, and Huntington's disease (HD) (Manczak and Reddy, 2012; Rappold et al., 2014; Shirendeb et al., 2012), and are also observed in healthy aged hearts (Stotland and Gottlieb, 2016). In addition, mitochondrial morphology differs across muscle fiber types or tissues during aging (Mishra et al., 2015b; Navarro and Boveris, 2004; Wyckelsma et al., 2017). For example, the oxidative soleus muscle contains mitochondrial fragmentation, while the glycolytic white gastrocnemius is fea-

tured by mitochondrial elongation in aged rats (Faitg et al., 2019). In brief, the impact of aging on mitochondrial dynamics is tissue-specific.

Mitophagy

Mitophagy is a specific form of autophagy that regulates the turnover of dysfunctional or damaged mitochondria, thus maintaining a healthy mitochondrial population for producing energy. The compromise of mitophagy results in mitochondrial dysfunction and proteostasis imbalance and thus exacerbates aging (Babbar et al., 2020; Fang et al., 2019; Fang et al., 2014; Fivenson et al., 2017; Onishi et al., 2021). During the aging process, compromised mitophagy has been observed in aged tissues such as the ovary and heart (Jin et al., 2022; Stotland and Gottlieb, 2016). In *C. elegans*, the inhibition of mitophagy leads to the accumulation of mitochondria in neurons, muscles, and the small intestine during aging (Palikaras et al., 2015). In addition, it has been shown that mitophagy regulates the aging process of *Drosophila* and mice via the modulation of the morphology and quantity of the mitochondria (Koehler et al., 2017; Liu et al., 2021a). As such, compromised mitophagy could also be considered a biomarker for aging.

Mitochondrial unfolded protein response (UPR^{mt})

UPR^{mt} is triggered by mitochondrial dysfunction. The activation of UPR^{mt} has often been proposed as an important pathway in lifespan extension induced by mitochondrial disruption (Houtkooper et al., 2013). For instance, the mitochondrial chaperone HSP60 and mitochondrial protease lon peptidase 1 (LONP1), two components of the UPR^{mt}, have been found to be upregulated in long-lived mouse models (Ozkurede and Miller, 2019), showing a correlation between lifespan extension and UPR^{mt} activation. During physiological aging, UPR^{mt} has been reported to decline with age. Lower LONP1 activity has been shown in old rat livers, demonstrating an impaired UPR^{mt} during aging (Bakala et al., 2003). *LONP1* expression has also been reported to decrease with age in mouse skeletal muscle (Bota et al., 2002), which could be restored by caloric restriction that extends mouse lifespan (Lee et al., 1999). Likewise, the expression of the UPR^{mt} genes *Hsp60* and *Hsp10* decreased in muscle stem cells from old mice (Yokoyama et al., 2002). These studies point to a causal effect of UPR^{mt} decline on aging, and UPR^{mt} decline could thus be regarded as a biomarker for aging.

Mitochondrial respiratory function

Disorders of mitochondrial respiratory function, such as defective electron transport chain and decreased mitochondrial membrane potential ($\Delta\psi m$), have been associated with aging (Conley et al., 2000; Migliavacca et al., 2019; Petersen et al., 2003). The respiration rate declines with age in mouse spleen lymphocytes (Rottenberg and Wu, 1997). Furthermore, the enzymatic activities of mitochondrial complexes are also decreased in aging rats (Navarro and Boveris, 2004). $\Delta\psi m$ is controlled by electron transport and proton leaks and determines the synthesis rate of ATP and ROS. A lower level of $\Delta\psi m$ was observed in aged mouse lymphocytes (Rottenberg and Wu, 1997) and p53-induced senescence (Sugrue et al., 1999). In addition, higher heterogeneity of $\Delta\psi m$, showing an increased ratio of cells with lower $\Delta\psi m$, has been observed in hepatocytes of old rats (Hagen et al., 1997). Therefore, a lower $\Delta\psi m$ may be considered a biomarker for aging. Due to the possible effect of accumulated oxidative damage and mitochondrial dysfunction, alterations in mitochondrial ultrastructure were observed in mice and *Drosophila* during aging (Brandt et al., 2017). Mitochondrial respiratory function decreases during the aging process, providing another biomarker for aging.

Taken together, all of the above referred mitochondrial biomarkers of aging and their corresponding test methods are summarized in Figure 6 and Table S2 in Supporting Information.

Loss of proteostasis

The maintenance of proteostasis is pivotal for the proper function of cells and organisms (Costa-Mattioli and Walter, 2020). A progressive decline in proteostasis with aging increases the risk of abnormal protein aggregate accumulation (Sala and Morimoto, 2022). Loss of proteostasis is recognized as a hallmark of aging and various age-related diseases (López-Otín et al., 2013; López-Otín et al., 2023). The proteostasis network, which includes the following core members, contributes to proteostasis: (i) molecular chaperones and cochaperones promote efficient protein folding and assembly and prevent the aggregation of misfolded proteins; and (ii) the autophagy-lysosomal system and the ubiquitin-proteasome system are two major quality control pathways that are critical to proteostasis (Dikic, 2017) and collaborate to ensure protein homeostasis (Hipp et al., 2019). In general, large aggregates can be degraded in the lysosome as mediated through autophagy, and terminally misfolded substrates can be degraded by the proteasome (Hartl et al., 2011). (iii) Stress response pathways, such as the heat shock response (HSR) and UPR of the endoplasmic reticulum (UPR^{ER}) and mitochondria (UPR^{mito}), are activated by protein misfolding (Labbadia and Morimoto, 2015).

Recently increasing evidence indicates that proteostasis is closely associated with aging and longevity. A single-nucleus transcriptome atlas of primate hippocampal aging shows that loss of proteostasis is an obvious characteristic of aging (Zhang et al., 2021e). A severe proteome imbalance develops during *C. elegans* aging, and in the long-lived *daf-2* mutant, this proteome imbalance is reduced, whereas in the

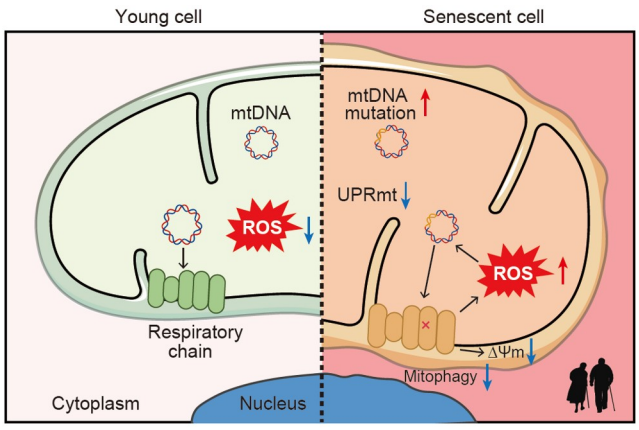

Figure 6 Mitochondrial biomarkers of aging. Several mitochondrial biomarkers are changed during aging. The red cross in the respiratory chain indicates the disorder of the respiratory chain.

short-lived *daf-16* mutant, this proteome imbalance is enhanced (Walther et al., 2015). The relative size of organismal chaperone networks has been reported to be directly linked to species longevity. The shortest-lived vertebrate (*Nothobranchius furzeri*) carries few chaperones and is therefore widely used for establishing fragile protein homeostasis models (Draceni and Pechmann, 2019). Moreover, the longest-lived rodent (the naked mole-rat) is characterized by an above-average number of chaperones and shows the distinct ability to maintain proteostasis via proteasome-mediated degradation and autophagy during aging (Draceni and Pechmann, 2019; Rodriguez et al., 2016). In addition, the UPR^{ER} rate is decreased in aged *C. elegans* (Ben-Zvi et al., 2009), whereas an increased UPR^{mito} rate extends worm lifespan (Li et al., 2022g). All these results suggest that chaperone status, the activities of the autophagy-lysosomal system, the ubiquitin-proteasome system, and the functionality of UPR^{ER} and UPR^{mito} can be used as biomarkers of aging.

Regulation of proteostasis by PTMs

Post-translational protein modifications (PTMs) increase the functional diversity of the proteome and are essential for all eukaryotic organisms. Enzymatic and nonenzymatic PTMs in aging are key regulatory mechanisms in the decline of proteostasis; these PTMs are mainly phosphorylation, ubiquitination, SUMOylation, acetylation, carbonylation, and oxidative posttranslational modifications (OxiPTMs) of cysteine residues. Examples of PTM targets involved in the loss of proteostasis in aging or age-related diseases are listed in Table S3 in Supporting Information.

(1) Protein phosphorylation. Protein phosphorylation affects proteostasis in aging by activating or inactivating a protein and/or the tendency of a protein to misfold and aggregate. The study on osteoarthritis in young and old cynomolgus monkeys demonstrated that phosphorylated IRE1 alpha was increased due to the loss of molecular chaperone expression with aging. Dephosphorylation at the Thr172 residue of AMPKα disrupted the maintenance of efficient cellular homeostasis and accelerated the aging process (Salminen et al., 2016). α-Synuclein is the key protein implicated in PD and other synucleinopathies, and phosphorylation at serine 129 (pS129) is a pathological hallmark of PD and a principal component in Lewy bodies (Chen and Feany, 2005). Acinus is a multifunctional nuclear protein that plays a role in the regulation of basal, starvation-independent autophagy. Phosphorylation of acinus at conserved serine 437 enhances protein stability, extending the lifespans of flies by elevating levels of basal autophagy (Nandi and Krämer, 2018).

(2) Protein ubiquitination and SUMOylation. Ubiquitination is the process of attaching ubiquitin (76-amino acid protein), a small functional regulatory protein, to a targeted molecule (Swatek and Komander, 2016). Ubiquitin ligase CHIP is a key regulator of proteostasis. CHIP deficiency leads to decreased ubiquitination levels of the insulin receptor (INSR) and a reduced lifespan of worms and flies (Tawo et al., 2017). Enhancing K63-linked ubiquitination of beclin 1 by small molecules restored proteostasis by activating autophagy in cells in which mutant tau, α-synuclein, or huntingtin had accumulated (Xu et al., 2020).

Protein SUMOylation is a widespread posttranslational modification by which a SUMO is covalently attached to target proteins. Increasing the SUMOylation rate of the germline protein CAR-1 disrupted proteostasis and shortened the lifespan in *C. elegans*, indicating that CAR-1 SUMOylation may be used as an aging biomarker of the germline (Moll et al., 2018). The disruption of lamin A (LMNA) SUMOylation may be related to the loss of nuclear proteostasis and may cause early aging in laminopathies (Ghosh et al., 2022a).

(3) Protein acetylation. Lysine acetylation is a normal and versatile protein posttranslational modification. Lysine acetyltransferase and lysine deacetylase catalyze the addition or removal of acetyl groups on histone and nonhistone targets, respectively (Shvedunova and Akhtar, 2022). HDAC6, a cytosolic histone deacetylase, is a suppressor of age-dependent ectopic fat accumulation (EFA). Loss of HDAC6 led to EFA and reduced animal longevity on a high-fat diet (Yan et al., 2017). Human positive cofactor 4 (PC4) accumulates and is activated during aging and accelerates the aging process by disrupting proteostasis, with PC4 interacting with the Sin3-HDAC complex and inhibiting the deacetylation activity of this complex to regulate the mTOR signaling pathway (Chen et al., 2021b). Clinical research has revealed significant acetylated tau pathology in the distribution pattern of AD and other major tauopathies. The acetylation of tau at lysine 280 may contribute to tau-mediated neurodegeneration (Irwin et al., 2012). Heat shock factor 1 (HSF1) is

central to heat shock regulation and plays a pivotal role in guarding proteostasis. The HSF1 acetylation rate is increased in aged mice and impairs the cellular stress response (Jurivich et al., 2020).

(4) Protein carbonylation. Protein carbonylation is a major hallmark of oxidative damage to proteins and an important process in the context of proteostasis. Proteins are oxidatively modified by a large number of reactive species, including reactive oxygen species, lipid peroxidation-derived aldehydes, and reducing sugars (Fedorova et al., 2014). Protein carbonylation is significantly increased in rat kidneys with aging (Goto et al., 1999). Muscle stem cell replication and differentiation are compromised with age due to the accumulation of oxidized proteins, which contribute to sarcopenia. A modified proteomic analysis showed that proteins involved in protein quality control and glycolytic enzymes are the main targets of carbonylation (Baraibar et al., 2016).

OxiPTMs

Common OxiPTMs of cysteine thiols (-SH) include *S*-nitros(yl)ation (-SNO), *S*-glutathionylation (-SSG), *S*-sulfhydration (-SSH), *S*-sulfenylation (-SOH), *S*-sulfinylation (-SO_2H) and *S*-sulfonylation (-SO_3H) (Shi and Carroll, 2020).

(1) *S*-Nitros(yl)ation. *S*-nitrosylation or *S*-nitrosation is a reversible posttranslational modification of protein cysteine thiols by nitric oxide (NO) or its derivatives (Zhang et al., 2012). Many aspects of proteostasis are specifically regulated by *S*-nitrosation. For instance, the increase in *S*-nitrosation of the pivotal ER sulfhydryl oxidase Ero1α leads to decreased activity and accelerates senescence that is accompanied by disrupted proteostasis and a compromised UPR^{ER} (Qiao et al., 2022). *S*-nitrosoglutathione reductase (GSNOR) deficiency promotes excessive *S*-nitrosylation of Drp1 and Parkin, thereby impairing mitochondrial dynamics and mitophagy and driving cell senescence (Rizza et al., 2018). hmtThrRS *S*-nitrosation decreases its aminoacylation and editing activities, thereby regulating protein synthesis (Zheng et al., 2020). *S*-nitrosation of ATG4B (a core autophagy protein) resulted in attenuation of autophagy in the hippocampus of diabetic Goto-Kakizaki (GK) rats and caused neurotoxicity (Li et al., 2017c).

(2) *S*-sulfhydration. *S*-sulfhydration (protein persulfidation) is a posttranslational modification of cysteine thiols mediated by hydrogen sulfide (H_2S), which is an essential regulatory signaling molecule (Gupta et al., 2022; Zivanovic et al., 2019). It has been reported that *S*-sulfhydration of the HMG-CoA reductase degradation protein (Hrd1, an E3 ubiquitin ligase) played an important role in regulating lipid droplet formation in the context of diabetes (Yu et al., 2020b). *S*-Sulfhydration of ubiquitin-specific peptidase (USP8) enhances the deubiquitination of Parkin and promotes mitophagy (Sun et al., 2020b). Interventions based on diet or pharmacology to increase persulfidation are linked with increased longevity (Zivanovic et al., 2019).

In addition to PTMs, the cellular redox balance provides a stable microenvironment for various intracellular biomacromolecules to perform normal functions and is thus another key factor in the regulation of proteostasis in aging and age-related diseases. The accumulation of protein aggregates in cells is considered a feature of cellular senescence that can be induced by oxidative proteostasis (Höhn et al., 2017). It has been reported that the decline in redox-stress response capacity (RRC) is a dynamic characteristic of aging; i.e., it affects the ability of cells to generate appropriate reactive oxygen species and thus activate cell signaling pathways, to maintain redox homeostasis, and to degrade damaged proteins, i.e., to maintain proteostasis (Meng et al., 2017). In a promising finding, increasing the redox-stress signaling threshold (RST, identified as the maximum level below which redox stress shows benefits) by starvation, exercise or heat stress during development improved RRC to maintain protein homeostasis and delay aging (Meng et al., 2022). Another study showed that reduction stress in the ER is an important driving force of cellular senescence, since under reductive stress conditions, protein synthesis, protein folding, and UPR^{ER} activity are all disrupted. Specific elevation in the degree of ER oxidation successfully delays cellular aging (Qiao et al., 2022). Notably, it has been proposed that precision redox is the key for antioxidant pharmacology, and the avenue to precision redox medicine is opening (Meng et al., 2021; Sies et al., 2022). Therefore, RRC, and RST and precision redox status effectively reflect proteostatic status and are therefore effective biomarkers of aging.

Summary and perspectives

Overwhelming evidence supports the loss of proteostasis as one of the key characteristics in aging and as an effective biomarker of aging, and proteostasis failure contributes to the development of aging and age-related diseases. Protein PTMs are involved in proteostasis in aging by regulating protein activity, localization or interaction with other cellular molecules and are also therefore necessary for cellular homeostasis. oxiPTMs competing for specific protein thiol groups are important to protein stability, and the roles of oxiPTMs depend on the cellular redox state, which is either optimal or stressful. Enlargement of RST is a way to maintain proteostasis by increasing the redox-stress signaling threshold. The ability to maintain proteostasis in RRC is a dynamic characteristic of aging. The methods and indices for quantitatively assessing the relationship between biomarkers of proteostasis (PTMs levels, RRC, and RST) and aging progression need to be established in future studies.

Metabolic dysregulation

Cellular metabolism, a fundamental activity to sustain life, is

deeply intertwined with most, if not all, biological processes, including aging and senescence (Amorim et al., 2022; Wang et al., 2022g; Wei et al., 2023). Aging has been characterized with a number of hallmarks, among which quite a few roots from abnormal metabolic activities (López-Otín et al., 2023). For example, while nutrient-sensing and mitochondrial function are primarily an intrinsic part of cell metabolism, others have a more complicated and close relationship with cellular metabolism, such as the cause of DNA damage by the imbalance of redox metabolism, alteration of mTORC activity (a conserved signaling pathway in aging) with amino acids and glucose (Liu and Sabatini, 2020), and most recently, creation of aging intervention environment through cell-cell metabolite exchange (Correia-Melo et al., 2023). Since cellular metabolism elicits a profound influence on the aging process, metabolites are naturally thought to be good biomarkers for aging. Indeed, much effort has been paid to measure the difference of metabolites between humans with distinct ages, and a database named MetaboAge compiles manually curated human aging-related metabolome studies (Bucaciuc Mracica et al., 2020). At present, a handful of metabolites have been consistently reported to change the aging process (Table S4 in Supporting Information), thus they are the promising biomarker candidates for aging. In the following sections, we summarize the metabolic pathway, aging-related functions, and potentials as aging biomarkers of these metabolites.

The level of hundreds of metabolites have been documented to change with age (Bucaciuc Mracica et al., 2020; López-Otín et al., 2016; Panyard et al., 2022), and dozens of them are able to promote or resist the aging process (Amorim et al., 2022; Finkel, 2015). However, due to the metabolic heterogeneity of individuals and different metabolite assays between studies, only a small number of metabolic features has been consistently validated in multiple studies. Among them, seven metabolites are strongly associated with age and/or elicit the geroprotective effects across species, therefore can be considered as the candidates of metabolic biomarkers for aging.

NAD^+

NAD^+ is one of star molecules that strongly affect the aging process in different model organisms. Besides the interconversion with NADH, NAD^+ is mainly synthesized from precursors through the salvage pathway, the Preiss-Handler pathway, and the *de novo* pathway in the mammalian cells, and is catabolized through a number of NAD^+-consuming enzymes such as Poly (ADP-ribose) polymerase (PARP), SIRT, and CD38 (Chini et al., 2021). NAD^+ regulates the aging process via several mechanisms (Covarrubias et al., 2021). At first, as a core enzyme I, NAD^+ is involved in more than a hundred redox reactions and hence NAD^+ deficiency can lead to metabolic dysfunction and aging. Furthermore, NAD^+ plays an important role in macrophage activation and neuronal survival, and NAD^+ depletion promotes the functional decline of innate immunity and causes neurodegenerative diseases, including AD and Parkinson's disease. In addition, replenishment of NAD^+ in aging mice may improve health by maintaining the homeostasis of stem cell pools (Zhang et al., 2016). However, a study showed that an increase of NAD^+ level in oncogene-induced senescent cells might enhance the senescence-associated secretory phenotype (Nacarelli et al., 2019). Thus, more effort should be made to understand the function of NAD^+ in cellular aging (senescence) beyond organismal aging. NAD^+ is compartmentalized in cells with a typical concentration of 50–300 μmol L^{-1} while its abundance in blood is much low. A decrease in NAD^+ is consistently associated with age (Zou et al., 2020), thus making it a promising biomarker for aging.

Alpha-Ketoglutarate

Alpha-Ketoglutarate is a key intermediate metabolite of the tricarboxylic acid cycle. It can be synthesized from glutamate through reversible conversion in mitochondrial by glutamate hydrogenase or transamination reactions in both cytosol and mitochondria (Figure 7). Alpha-Ketoglutarate can extend the health span in several model organisms including worms (Chin et al., 2014), fruit flies (Su et al., 2019), and mice (Asadi Shahmirzadi et al., 2020; Zhang et al., 2021j). In worms, this metabolite can bind to the ATP synthase, which is also known as the complex V of mitochondrial electron transport chain, and partial inhibition of mitochondrial respiratory activity can decrease the aging rate. In addition, the intervention geroprotective effect of α-ketoglutarate may be mediated by TOR signaling (Chin et al., 2014; Su et al., 2019). Although the geroprotective effect of α-ketoglutarate is consistently observed, the level of α-ketoglutarate during aging and cellular senescence appears to be dependent on the biological contexts. Some studies indicated that the α-ketoglutarate abundance increased in chemical-induced senescence (Fernandez-Rebollo et al., 2020; Wu et al., 2017), and others reported that it declined in the blood and follicular fluid of aged mice (Wang et al., 2020e; Zhang et al., 2021j).

Tryptophan

Tryptophan is one of the essential amino acids in human. While a small fraction of free tryptophan is used for the biosynthesis of proteins, such as the important neurotransmitter serotonin and neuromodulator tryptamine, the majority is catabolized into kynurenine via indoleamine-2,3-dioxygenase, tryptophan-2,3-dioxygenase, and a recently identified enzyme, IL4I1 (Figure 7). Kynurenine and its derivatives are key regulators of immune cells, such as macrophages, dendritic cells, T cells, and innate lymphoid cells, and they can be further catabolized into quinolinic acid,

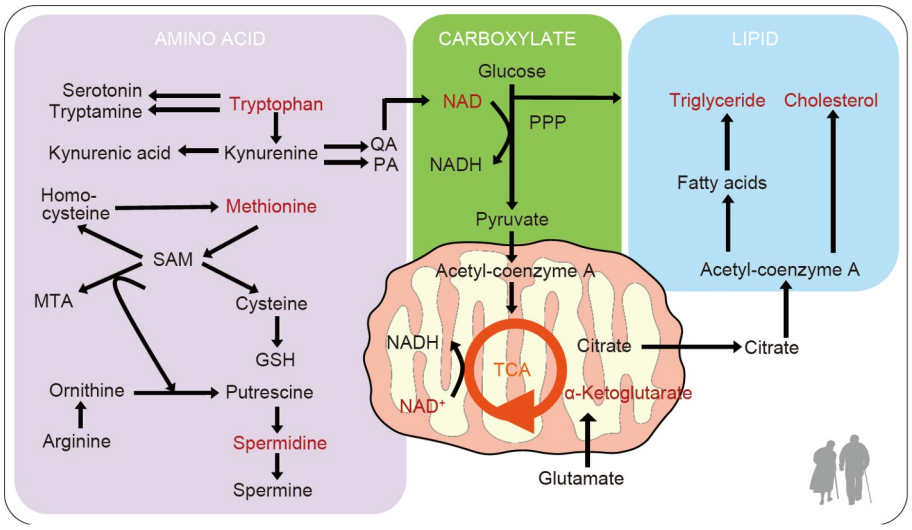

Figure 7 Altered metabolic pathways as biomarkers of aging. Seven metabolites that have consistent effects on the aging process across species are colored in red. They can be divided into four groups, including amino acids and derivatives, core coenzymes in central carbon metabolism, lipids, and intermediate metabolites in tricarboxylic acid cycle. Abbreviation: QA, quinolinic acid; PA, picolinic acid; SAM, *S*-adenosyl-methionine; MTA, 5′-methyl-thioadenosine; GSH, glutathione; NAD^+, oxidative nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycle.

a precursor of NAD^+. The immunological effect of kynurenine and its derivatives contributes to the decrease of immune function and neurodegeneration with aging, but it remains unknown if other underlying mechanisms link the tryptophan metabolism with aging (Salminen, 2022; van der Goot and Nollen, 2013). The tryptophan abundance decreases in the blood (Panyard et al., 2022; Yu et al., 2012).

Methionine

Methionine is an essential amino acid necessary for the initiation of mRNA translation into proteins. As a common sulfur-containing amino acid, methionine is interconverted into homocysteine through the methionine cycle, and homocysteine synthesizes cysteine via the transsulfuration pathway (Figure 7). In addition, methionine is involved in the biosynthesis of polyamines. Just as caloric restriction being a promising approach to achieving longevity across phylogenetically diverse species, methionine restriction can also delay the progress of aging from yeast to mice (Bárcena et al., 2019; Bárcena et al., 2018). Although the exact reason is not yet fully understood, a few mechanisms have been proposed, such as reduction of translation rate, modulation of autophagy, and antioxidant defense (Bárcena et al., 2019; Parkhitko et al., 2019). In aged mice, the methionine decreases in serum but increases in brain compared with young mice (Ding et al., 2021a; Houtkooper et al., 2011).

Spermidine

Polyamines include three related metabolites, putrescine, spermidine, and spermine, which contain two or more amine groups. All of them are catabolic product of arginine (Figure 7). Polyamines have been known to exhibit a geroprotective effect for a long time (Eisenberg et al., 2009). Nutritional supplementation of spermidine brings about the protective effect on cardiovascular and immune cells in mice and rats (Eisenberg et al., 2016; Puleston et al., 2019; Zhang et al., 2019a). Recent studies show that the polyamines levels declined with age (Liu et al., 2022d; Panyard et al., 2022).

Triglycerides

Triglycerides are primary forms of fatty acid storage in organisms, mainly in adipocytes and hepatocytes. Triglycerides are made by esterifying a glycerol with three fatty acids, and under energy-demanding conditions, they are degraded into fatty acids which are translocated into mitochondria to be oxidized through the β-oxidation pathway and release energy. The level of triglycerides is generally elevated with age (Auro et al., 2014; Bucaciuc Mracica et al., 2020). However, this elevation might not be specific to aging, because higher triglyceride level is often associated with obesity, metabolic syndrome, and cardiovascular conditions (Wishart et al., 2018).

Cholesterol

As fatty acids, cholesterol is taken from diet or synthesized from acetyl coenzyme A with NADPH providing the reducing power. Cholesterol is consumed as one of the major constituents of the cell membrane and a precursor of steroid hormones. The cholesterol concentration in blood often raises with age, but it is also strongly associated with other factors such as exercise, nutrient conditions and genetic polymorphisms, for example, cholesterol is more abundant in serum of familial hypercholesterolemia patients (Bucaciuc Mracica et al., 2020; Wishart et al., 2018).

Aberrant signaling pathways

Cellular senescence is a cell fate program that limits the proliferation of impaired cells by enforcing stable cell cycle arrest (Tchkonia et al., 2013). Senescence can be triggered by diverse forms of stress stimuli, including DNA damage signals, chronic oxidative stress, and telomere dysfunction (Correia-Melo et al., 2014; Herbig et al., 2004; Sahin et al., 2011; von Zglinicki et al., 2005). Senescent cells can secrete a wide range of cytokines and chemokines, known as the SASP (Coppe et al., 2008; Freund et al., 2010; Kuilman et al., 2008; Kuilman and Peeper, 2009). Cytokines, such as TNFα, can in turn initiate, promote or sustain senescence (Acosta et al., 2013; Acosta et al., 2008; Kandhaya-Pillai et al., 2017; Kuilman et al., 2008; Li et al., 2017a; Orjalo et al., 2009). Engagement of senescence requires integration of multiple signaling pathways. To date, experimental evidence collectively suggests that although many stimuli can induce senescence response, they converge on two main pathways, p53 pathway and pRb pathway (Campisi, 2005). However, this could be just the tip of the iceberg. Gene expression profile reveals that the characteristics of the replicative senescence response are highly cell-type specific (Shelton et al., 1999), suggesting multiple pathways to induce cellular senescence. Among these pathways, TNFα signaling has been strongly linked to senescence (Kandhaya-Pillai et al., 2017; Li et al., 2017a). TNFα is a pleiotropic pro-inflammatory cytokine known to mediate a broad range of biological functions. It stimulates the proliferation of normal cells, exerts cytolytic activity against tumor cells, and causes inflammatory and immunoregulatory effects (Aggarwal, 2003). TNFα acts as a potent inducer of inflammatory responses and plays a crucial role in the pathogenesis of numerous chronic inflammatory diseases and age-related diseases (Holtmann and Neurath, 2004). In addition, persistent presence of low-level circulating TNFα can lead to chronic activation of the immune system, called inflammaging (Frasca and Blomberg, 2016). In this section, we will discuss signaling pathways induced by TNFα as biomarkers for cellular senescence.

TNFα signaling

The biological effects of TNFα are mediated by two distinct cell surface receptors, TNFR1 and TNFR2 (Rothe et al., 1992; Screaton and Xu, 2000; Vandenabeele et al., 1995). Both receptors have significant homology in their extracellular domains but differ structurally in their cytoplasmic domains. TNFR1 contains a death domain (DD), whereas TNFR2 lacks DD. The cytoplasmic DD of TNFR1 is critical for signal transduction of TNFα, which can recruit other DD-containing molecules. Upon ligation of TNFα, TNFR1 undergoes trimerization and induces association of the receptors DD and subsequent recruitment of two DD-containing proteins, TNF receptor-associated death domain (TRADD) and receptor-interacting serine/threonine-protein kinase 1 (RIPK1), to form a transient membrane signaling complex named TNFR1 signaling complex (TNF-RSC) or complex I (Micheau and Tschopp, 2003; Yang et al., 2022b) (Figure 8). In TNF-RSC, TRADD recruits the E3 ubiquitin ligases cIAPs via the adaptor protein TRAF2, which in turn induces K63-linked ubiquitination on RIPK1 (Bertrand et al., 2008; Hsu et al., 1996). The linear ubiquitination assembly complex (LUBAC), which is recruited by binding with K63-linked ubiquitin chains in TNF-RSC, leads to M1-linked ubiquitination on RIPK1 (Gerlach et al., 2011; Ikeda et al., 2011; Tokunaga et al., 2011). The K63-linked ubiquitin chains on RIPK1 facilitate the recruitment of TAK1 complex (TAB2/TAB3/TAK1) (Bertrand et al., 2008), while the M1-linked ubiquitin chains on RIPK1 promote the recruitment of IKK complex (NEMO/IKKα/IKKβ) (Ikeda et al., 2011). Subsequently, activated TAK1 phosphorylates and activates IKKα/IKKβ, which further phosphorylates IκB to induce its proteasomal degradation and thus promotes the activation of NF-κB signaling (Hayden and Ghosh, 2012). In addition, activated TAK1 also phosphorylates mitogen-activated protein kinase kinases (MAPKKs) to mediate the activation of MAPK signaling (Sabio and Davis, 2014) (Figure 8). The M1-linked ubiquitin chains also recruit A20 to TNF-RSC, which is a suppressor of NF-κB and MAPK activation by deubiquitinating K63-linked ubiquitin chains on RIPK1 to terminate the signaling (Wertz et al., 2004) (Figure 8).

Activation of NF-κB pathway

The senescence program involves a complex interplay between cell-intrinsic and cell-extrinsic processes that influence the senescence-associated cell cycle arrest, and the surveillance of senescent cells by the immune system (Kuilman and Peeper, 2009). Emerging evidence indicates that the SASP can reinforce the senescence arrest and mediate cross-talk between senescent cells and immune cells within their microenvironment (Acosta et al., 2008; Krizhanovsky et al., 2008; Krtolica et al., 2001; Kuilman et al., 2008). NF-κB activation has been proposed as a master regulator in senescence that is required for SASP production and contributes to cell cycle arrest (Chien et al., 2011; Mongi-Bragato et al., 2020; Salminen et al., 2012b). NF-κB transcription factors contain both the Rel family proteins (RelA/p65, c-Rel and RelB) and NF-κB components (p50/p105 and p52/p100), which are dimerized with each other in the cytoplasm and inhibited by binding to IκB proteins (Hoffmann and Baltimore, 2006). After activation, the NF-κB complexes translocate into the nucleus and transactivate the expression of special sets of target genes. p65 subunit of NF-κB complex has been found more significantly enriched into the chromatin of senescent fibroblasts as compared to the young counterparts. Phosphorylation of p65 Ser536 re-

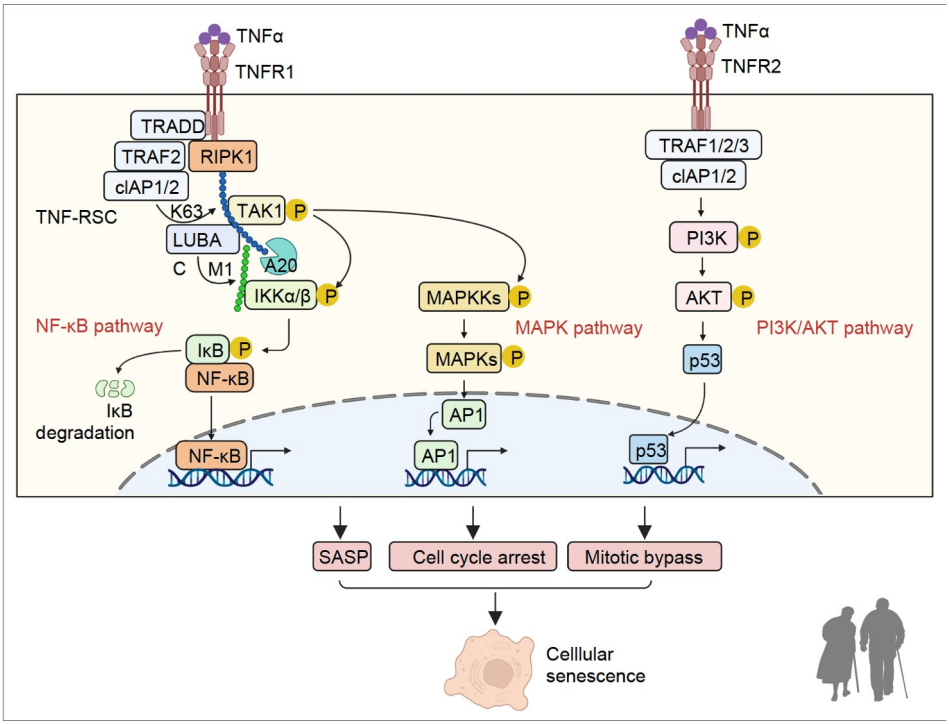

Figure 8 Activation of TNFα signaling pathway as a biomarker of cellular senescence. TNFα can activate different pathways in cellular senescence, including NF-κB activation by RIPK1, MAPKs-dependent kinase cascade, and PI3K-AKT kinase cascade. Together, these signaling events contribute to cellular senescence and their activation may represent biomarkers of cellular senescence. Abbreviations: TNFR1, TNF receptor 1; TRADD, TNFR1-associated death domain protein; RIPK1, receptor-interacting protein kinase 1; TRAF1/2/3, TNF receptor-associated factor 1/2/3; cIAP1/2, cellular inhibitors of apoptosis 1 and 2; TAK1, transforming growth factor β-activated kinase 1; IKKα/β, IκB kinase α/β; IκB, inhibitor of NF-κB; NF-κB, nuclear factor-κB; MAPKs, mitogen-activated protein kinases; AP-1, activator protein 1; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B (PKB), also known as AKT; p53, tumor protein p53.

sidue, a transactivating modification of p65, is correlated with increased expression and secretion of SASP in senescence (Chien et al., 2011). In cultured fibroblasts, NF-κB suppression causes escape from immune recognition by natural killer (NK) cells and cooperates with p53 inactivation to bypass senescence. In a mouse lymphoma model, NF-κB inhibition bypasses treatment-induced senescence (Chien et al., 2011). Interestingly, inhibition of NF-κB signaling could overcome the growth arrest induced by p53-p21^{CIP1} pathway (Rovillain et al., 2011). Thus, NF-κB pathway has a causative role in the induction of SASP and its activation may represent a biomarker of cellular senescence.

Evidence further supporting the role of NF-κB pathway in senescence is that many TNF-RSC components are involved in senescence. For example, A20 has been shown to play an important role in TNFα-induced senescence, which is a ubiquitin-editing enzyme that restricts NF-κB signaling (Wertz et al., 2004). A20 prevents the occurrence of multiple inflammatory diseases. Interestingly, A20 also has a self-protective effect on the senescence of nucleus pulposus cells induced by TNFα (Peng et al., 2020). Downregulation of A20 in nucleus pulposus cells exacerbated the senescence phenotype, including increased senescence-associated beta-galactosidase activity, increased expression of senescence-associated proteins, increased synthesis of extracellular matrix (ECM), and G1 cycle arrest (Peng et al., 2020). NEMO is a key activator of TNFα-induced NF-κB signaling and apoptosis (Liu et al., 2017b). NEMO also plays an important role in radiation-induced senescence (Dong et al., 2015). Irradiation caused vascular endothelial cells to gain a senescence-like phenotype through the NEMO/NF-κB pathway, suggesting that NEMO may be a critical switch that regulates cellular senescence and apoptosis caused by exposure to radiation. TNF-RSC components may also regulate senescence in a TNFα signaling-independent manner. TRADD is a central adaptor in the TNF-RSC, which mediates both cell death and pro-inflammatory signals (Aggarwal, 2003). Although TRADD is usually considered a cytoplasmic protein, it may also have a function in the nucleus (Morgan et al., 2002; Wesemann et al., 2004). Indeed, dynamic TRADD shuttling from the cytoplasm into the nucleus regulates the interaction of p19^{Arf} with the ubiquitin ligase for ARF (Chio et al., 2012). p19^{Arf} is an instrumental mediator of cellular senescence (Yetil et al., 2015). Primary cells lacking TRADD were less susceptible to HRas-induced senescence and showed a reduced level of accumulation of the p19^{Arf} protein that is independent of TNFα signaling (Chio et al., 2012).

Activation of MAPK pathway

In addition to NF-κB pathway, activation of MAPK pathway is another outcome of TNFα signaling. MAPKs are capable of sensing changes in diverse cellular conditions, and in turn elicit adaptive responses including senescence (Anerillas et al., 2020). MAPKs modulate the levels and function of many proteins in the senescence-regulatory axes, including factors in the $p21^{CIP1}$/p53 and $p16^{INK4a}$/RB pathways (Anerillas et al., 2020; Debacq-Chainiaux et al., 2010; Deschênes-Simard et al., 2013; Freund et al., 2011; Sun et al., 2018a). Through these actions, MAPKs implement key traits of senescence, including growth arrest, cell survival, and SASP (Debacq-Chainiaux et al., 2010; Deschênes-Simard et al., 2013; Freund et al., 2011; Sun et al., 2018a). Although MAPKs encompass a large number of kinases, the best-known MAPKs that are mostly linked to cellular senescence are ERKs (ERK1 and ERK2), p38s (p38α, p38β, p38γ, and p38δ), and JNKs (JNK1, JNK2, and JNK3) (Debacq-Chainiaux et al., 2010; Deschênes-Simard et al., 2013; Freund et al., 2011; Lee et al., 2010; Sun et al., 2018a; Vizioli et al., 2020). In senescence, multiple MAPK pathways are activated by various stress signaling. MAPKs regulate senescence by either regulating the transcription of senescence-associated genes or controlling gene expression programs post-transcriptionally by phosphorylation and thereby modulating the activity of RNA-binding proteins implicated in senescence (Lafarga et al., 2009; Ziaei et al., 2012). In addition, the MAPK substrate MK2 is involved in the translation of SASP factors and links MAPK pathway with mTOR pathway, which is also activated in senescent cells (Herranz et al., 2015). MAPKs also cooperate with NF-κB pathway to regulate senescence. It is well known that p38 MAPK activates MSK1/2, which can phosphorylate the p65 subunit of the NF-κB complex and thus potentiate NF-κB signaling (Kefaloyianni et al., 2006; Vermeulen et al., 2003). p38 MAPK has been shown to control SASP via the transcriptional activation of NF-κB signaling. Depletion of p65 subunit of NF-κB complex significantly reduces the secretion of pro-inflammatory cytokines induced by p38 MAPK activity in senescence (Alimbetov et al., 2016; Freund et al., 2011; Rodier et al., 2009). Thus, MAPK signaling governs both transcriptional and translational programs in senescent cells and activation of MAPKs may also represent an important biomarker of cellular senescence.

Activation of PI3K/AKT pathway

In addition to NF-κB activation, the interaction of TNFα with TNFR2 also activates the reciprocal PI3K/AKT signaling pathway (Fu et al., 2021; Takahashi et al., 2022) (Figure 8). The PI3K/AKT oncogenic signaling modules are frequently mutated in sporadic human cancer. Although this pathway has been shown to play critical roles in driving tumor growth and proliferation, multiple lines of evidence indicate that its activation in normal human cells can also promote cellular senescence (Alimonti et al., 2010b; Astle et al., 2012; Jung et al., 2019b). Loss of PTEN, the major negative regulator of the PI3K/AKT pathway, induces senescence in mouse embryonic fibroblasts and mouse prostate epithelium (Chen et al., 2005; Jung et al., 2019b). Constitutively activation of AKT induces senescence in human endothelial cells, mouse embryonic fibroblasts, and mouse primary keratinocytes (Alimonti et al., 2010a; Chen et al., 2005; Nogueira et al., 2008). PI3K/AKT pathway activation-induced senescence requires mTORC1-dependent accumulation of p53, which involves increased p53 synthesis and stabilization mediated by inactivation of MDM2 (Astle et al., 2012). Transcriptomic and metabolomic profilings revealed that there were numerous escape routes beyond p53 pathway in AKT-induced senescence. NF1-mediated suppression of RAS-ERK signaling maintains AKT-induced senescence, which is a unique hallmark for AKT-induced senescence (Chan et al., 2020). Thus, activation of PI3K/AKT activity induces senescence in both p53-dependent and independent manners, which may represent a biomarker of cellular senescence.

Activation of cGAS-STNG pathway

Studies on cellular senescence have revealed that although the causes of and phenotypes generated by cellular senescence were manifold, persistent genotoxic stress, particularly DNA damage was thought to be the common mechanism critical for the establishment and maintenance of senescence phenotypes (d'Adda di Fagagna, 2008). DNA damage can trigger the activation of NF-κB, MAPK, and PI3K-AKT pathways during senescence. However, the link between DNA and these pathways was not clear. cGAS is a DNA sensor that triggers innate immune responses through the production of the second messenger cyclic GMP-AMP (cGAMP), which binds and activates the adaptor protein STING. Several studies have provided strong evidence that cGAS-STING pathway also has an essential role in promoting cellular senescence (Bi et al., 2020; Dou et al., 2017; Glück et al., 2017; Liu et al., 2023b; Yang et al., 2017). Mechanistically, cGAS senses micronuclei as the result of genomic DNA damage to promote senescent phenotypes by producing a range of cytokines and chemokines, such as IFN-β, IL-1β, IL-6, and IL-8, which are known to feedback to the secreting cells to reinforce senescence signaling. Given that the cGAS-STING pathway provides a critical paracrine signal that is necessary for sustaining cellular senescence, activation of this pathway may also represent a biomarker of cellular senescence.

Summary and perspectives

Emerging studies indicate that activation of TNFα signaling, including NF-κB pathway, MAPK pathway, and PI3K-AKT pathway, controls the cellular phenotypes of senescence,

including generation of SASP, cell cycle arrest, and mitotic bypass (Figure 8). In response to stimuli capable of triggering cellular senescence, NF-κB, MAPKs, and PI3K-AKT pathways function as sensors to identify the extent of damage, and help to determine whether the response ought to be cell proliferation, apoptosis, senescence, or others. If cells adopt a senescence response, these pathways participate directly in the various traits of cellular senescence by either contributing to implementing the gene expression programs that enable growth arrest and production and secretion of SASP factors transcriptionally and post-transcriptionally, or counteracting the apoptotic phenotype to ensure the long-term survival of senescent cells.

SASP

Cellular senescence represents a special cell state implicated in a number of pathophysiological processes and an array of age-related disorders. Due to the discovery of contribution of senescent cells to human morbidity, clinical interest in therapeutically targeting senescence to achieve healthy aging and prevent or ameliorate age-related diseases, including but not limited to senotherapy, continues to grow. Appropriate identification and accurate detection of senescent cells, both *in vitro* and *in vivo*, is theoretically essential, albeit technically challenging. Several biomarkers of cellular senescence have been identified, including SA-β-gal, $p16^{INK4a}$, and $p21^{CIP1}$, but very few markers have high sensitivity and specificity (Huang et al., 2022c).

The SASP and its intracellular regulation

Senescent cells actively synthesize and secrete a plethora of soluble factors, including pro-inflammatory cytokines, chemokines, angiogenic factors, growth modulators, and matrix metalloproteinases (MMPs), collectively termed the SASP, or alternatively, senescence messaging secretome (SMS) (Figure 9) (Acosta et al., 2008; Cai et al., 2022d; Coppe et al., 2008; Kuilman and Peeper, 2009). To date, it is well established that the SASP constitutes a typical hallmark of senescent cells and mediates the vast majority of their *in vivo* effects, particularly in mammalians. The SASP can reinforce senescence in autocrine and paracrine manners (Acosta et al., 2013), and trigger immune responses to eliminate senescent cells in tissue microenvironments (Krizhanovsky et al., 2008; Muñoz-Espín and Serrano, 2014). Although SASP factors are physiologically essential in mediating developmental senescence, wound healing, cellular reprogramming, and tissue plasticity (Demaria et al., 2014; Mosteiro et al., 2016; Muñoz-Espín et al., 2013; Storer et al., 2013), they cause persistent and chronic inflammation in diverse tissues and organs, a phenomenon known as inflammaging (Franceschi and Campisi, 2014), explaining an array of senescence-caused deleterious and pro-aging consequences.

Interestingly, the SASP composition and strength can vary substantially, with the overall profile depending on the duration of senescence, nature of the senescence stimulus, specific cell type and duration after senescence initiation (Hernandez-Segura et al., 2017). Although senescence-associated markers result from altered transcription of cells exposed to stress, the senescence phenotype is variable and dynamic, changing at varying intervals after senescence induction, with methodologies for readily identifying senescent cells largely lacking. The heterogeneity of the SASP, or even the senescence program, can be technically exemplified by characterizing numerous whole-transcriptome datasets that are publicly available, while transcriptomic signatures associated with specific senescence-inducing stresses or senescent cell types have been identified. Identification of novel transcriptomic signatures to detect specific subtypes of senescent cells or to discriminate among diverse senescence-driving programs represents an attractive strategy to determine the individual biological roles of senescent cells and to develop optimal drug targets for interventions in translational and clinical medicine.

The complexity of the SASP, typically appraised by a list of secreted proteins, has been largely underestimated, as a small handful of factors cannot explain this varying phenotype. In fact, many studies reported that expression of the SASP is subject to regulation by an intricate but well-ordered signaling network, which comprises but is not limited to ATM, γH2AX, Zscan4, TAK1, p38, MAPK, mTOR, IL-1α, NF-κB, c/EBPβ, JAK2/STAT3 and GATA4 (Borghesan et al., 2020; Hernandez-Segura et al., 2018; Song et al., 2020a; Song et al., 2020b; Sun et al., 2018b; Sun et al., 2022b). Despite these advances, increasing lines of data suggest that SASP regulation is indeed multilayered, including contributions from pre-transcriptional signaling cascades such as the cGAS-STING pathway, epigenetic factors governing DNA and histone modifications as well as the super-enhancer landscape in senescent cells (Criscione et al., 2016a; Glück et al., 2017; Sati et al., 2020; Tasdemir et al., 2016; Yang et al., 2017; Zhang et al., 2021a). Continued inputs into the SASP biology are being generated, such as those contributed by the SASP Atlas, a comprehensive proteomic database of soluble factors and exosome-delivered SASP cargoes derived from multiple cell types exposed to various senescence inducers (Basisty et al., 2020). Some candidate biomarkers that coincidently overlap with aging markers in human plasma, including growth differentiation factor 15 (GDF15), stanniocalcin 1 (STC1), and serine protease inhibitors (SERPINs), which largely correlate with age, were identified in a human cohort of the Baltimore Longitudinal Study of Aging (BLSA) (Basisty et al., 2020). Furthermore, pathogen-related factors, such as lipopolysaccharide (LPS) or SARS-CoV-2 S1 antigen, can remarkably amplify the

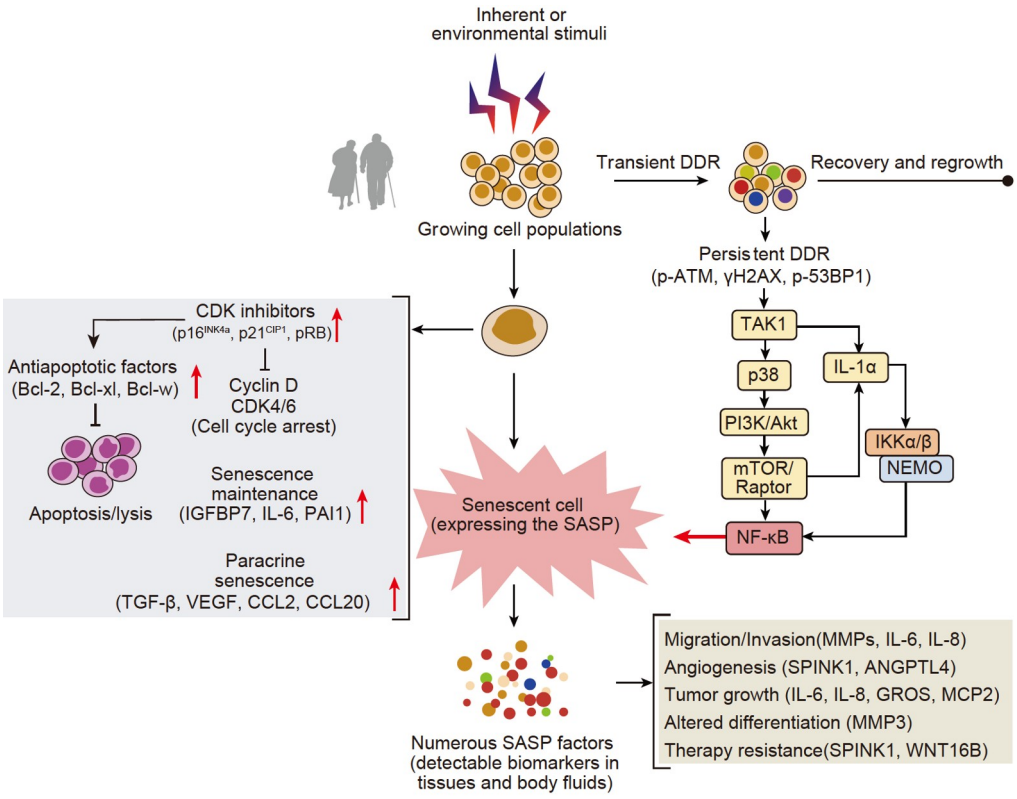

Figure 9 The SASP as a biomarker of aging. Cellular senescence is a cell state triggered by stressful insults and certain physiological signals, usually characterized by a prolonged and irreversible cell cycle arrest with a highly secretory capacity, macromolecular damage (DNAs, proteins and lipids) and an altered metabolic landscape. In the full-spectrum of the SASP, a few secreted factors function in a cell-autonomous manner, such as IGFBP7, IL-6, and PAI1, which maintain and reinforce the senescent state through a positive feedback loop sustaining the DDR, while others (TGF-β, VEGF, CCL2, CCL20) cause paracrine senescence of neighboring cells. Once released into the tissue microenvironment, a handful of SASP components can mediate loss of tissue homeostasis and organ dysfunction, thus accelerating organismal aging and contributing to incidence of age-related pathologies. As a technical challenge, detecting a senescence-associated cell-cycle arrest and the SASP requires quantification of multiple factors and special features. Abbreviations: BCL2, B-cell leukemia/lymphoma-2; IGFBP7, insulin-like growth factor binding protein 7; PAI1, plasminogen activator inhibitor-1; TNF-β, tumor necrosis factor β; VEGF, vascular endothelial growth factor; CCL2, C-C motif chemokine ligand 2; IL-1β, interleukin-1β; ATM, the ataxia telangiectasia mutated; mTOR, mammalian target of rapamycin; NEMO, NF-κB essential modulator; MMP3, matrix metalloproteinases; MCP2, monocyte chemotactic protein 2; SPINK1, serine peptidase inhibitor kazal type 1.

SASP cascade of senescent cells, thus increasing risks of the cytokine storm and enhancing clinical mortality in the elderly and those with chronic or fundamental pathologies associated with a high burden of senescent cells in the case of COVID-19, a pandemic currently causing unprecedented public health concerns worldwide (Camell et al., 2021; O'Driscoll et al., 2021).

Understanding of the distinctive heterogeneity of the SASP

Appropriate understanding of the temporal and spatial regulation of the SASP allows further insights into the mechanisms supporting SASP heterogeneity and exploration of targets that may be exploited to modulate the SASP composition. A recent study, which took advantage of single-cell isolation and a nanofluidic PCR platform to determine the contributions of single individual cells to the overall expression profile of senescent human fibroblast populations, disclosed substantial intercellular variability of SASP expression (Wiley et al., 2017). Many genes encoding SASP factors show remarkable variability, despite the presence of a subset of highly induced genes that account for the increased expression observed at the population level. Of note, inflammatory genes in clustered genomic loci display a higher correlation with senescence compared to non-clustered loci, suggesting co-regulation of these genes by genomic location (Wiley et al., 2017). Thus, the data provide several lines of new clues regarding how genes are regulated in senescent cells and imply that single markers are insufficient to identify senescent cells, especially *in vivo*.

Alternatively, another study demonstrated that the transition from the early transforming TGF-β-dependent secretome to a pro-inflammatory secretome is subject to modulation by Notch1 activity fluctuations, while the changing composition of the SASP can determine the beneficial and/or detrimental properties of senescence programs, tipping the balance toward either an immunosuppressive environment or a pro-inflammatory state (Ito et al., 2017). Moreover, the interferon type 1 (IFN-I) response arises as a

relatively late event and is driven partially by the de-repression of LINE-1 (abbreviated as L1) retrotransposable elements (De Cecco et al., 2019). Triggered by cytoplasmic L1 cDNA but subject to downregulation by inhibitors of the L1 reverse transcriptase, the IFN-I response is a phenotype of late senescence and contributes to the long-term maintenance of the SASP (De Cecco et al., 2019). The authors further proposed that activation of retrotransposons was an important contributor to sterile inflammation, a hallmark of aging, and that L1 reverse transcriptase was a relevant target for future intervention of age-related diseases. In addition, senescent cells communicate with their surrounding microenvironment via juxtacrine NOTCH/JAG1 signaling, release of ROS, cytoplasmic bridges, and extracellular vesicles (EVs) (Biran et al., 2017; Han et al., 2020b; Ito et al., 2017; Kuilman et al., 2010; Takasugi et al., 2017), supporting that a thorough characterization of the senescent secretome in diverse biological contexts may help identify senescence, more specifically, the SASP-associated molecular signatures.

Factors that hold the potential to serve as biomarkers of the SASP

Identifying secreted factors produced by senescent cells with the potential to be developed as biomarkers indicative of the SASP appears to be a challenging task, since the profile of secreted factors is usually determined by a number of elements. The comprehensive soluble SASP atlases of human senescent fibroblasts induced by ionizing radiation, oncogenic HRAS or Atazanavir, a protease inhibitor of HIV, and radiation-induced renal senescent epithelial cells suggested that total 17 soluble SASP factors were commonly shared by these senescent cells, whereas a few other factors varied depending on the tissue type and insult nature (Basisty et al., 2020). An unbiased analysis based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) proteome assessment and Gene Ontology (GO) evaluated the secretomes of senescent bone marrow and adipose mesenchymal stromal cells (MSC) exposed to different stimuli and found that they exhibited uniform senescent phenotypes featured by four subcategories of SASP components: ECM and cytoskeleton and/or cell junctions, metabolic activities, redox factors as well as gene expression regulators (Özcan et al., 2016). Together with seven proteins exclusively expressed in all the analyzed senescent phenotypes, three key signaling paths including MMP2/TIMP2, IGFBP3/PAI-1 and Peroxiredoxin 6/ERP46/PARK7/Cathepsin D, were identified, while these paths were likely involved in the paracrine circuit inducing senescence of adjacent cells and might confer apoptosis resistance on senescent cells. A recent study based on stable isotope labeling with amino acids (SILACs) disclosed 343 SASP factors secreted by senescent human fibroblasts at two-fold or higher levels compared with their quiescent counterparts, with 44 of these proteins involved in hemostasis, a process rarely linked with senescence (Wiley et al., 2019).

Though the SASP components fulfill diverse *in vivo* functions, many of them are indeed associated with the chronic inflammation, a phenomenon frequently observed in the course of organismal aging (Guerrero et al., 2021; Tchkonia et al., 2021). Several constituents of the SASP are immunomodulatory, such as IL-1α, IL-1β, IL-6, IL-7, IL-8, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and TNFα, which, once released into the extracellular space, will actively recruit various immune cells including macrophages, granulocyte, neutrophils, and T lymphocytes (Kale et al., 2020) (Table S5 in Supporting Information). Several SASP factors can modulate the cellular response to therapies. The SASP components C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, IL-1α, IL-6, and TGF-β, released by human fibroblasts upon OIS can reinforce senescence in an autocrine manner and also induce senescence of neighboring cells via a paracrine mechanism (Acosta et al., 2013; Acosta et al., 2008; Faget et al., 2019). Of note, similar paracrine signaling pathways can be mediated by small EVs of the SASP (ev-SASP) released by human fibroblasts displaying OIS, and in MCF7 mammary cancer cells after treatment with the CDK4/6 inhibitor palbociclib (Borghesan et al., 2019). The single-cell RNA-seq (scRNA-seq) data presented at the National Cancer Institute Workshop on Radiation, Senescence, and Cancer (August 10–11, 2020, National Cancer Institute, Bethesda, MD) demonstrated that cells in the case of therapy-induced senescence (TIS) in culture and those isolated from kidneys of aged mice can display different characteristics, and only a small number of $p16^{INK4a+}$ senescent cells in the kidney are responsible for the production of SASP factors of profibrotic activities (Prasanna et al., 2021).

Summary and perspectives

Identifying novel and distinct transcriptomic signatures to detect specific types of senescent cells is an attractive strategy for the evaluation of diverse biological roles of senescent cells and the development of effective drug targets. At the core of senescence and SASP heterogeneity, the characterization of whole-transcriptome datasets has rendered a fingerprint of 55 senescence-associated gene transcriptomes in human fibroblasts, allowing specific targeting of senescent cells that are either tumorigenic, immune-suppressive, or potentially implicated in other age-related conditions (Hernandez-Segura et al., 2017). However, limitations still remain, particularly for studies involving biospecimens, mainly due to the absence of specific markers. To this end, a multi-marker approach was recently proposed (Gorgoulis et al., 2019), which may be employed to assess the efficacy of

senolysis, an emerging and promising therapeutic approach currently entering clinical trials for the intervention of various age-related pathologies.

Given the distinct heterogeneity of SASP, the National Institutes of Health has recently identified five broad areas to advance senescence-related studies, including identification and characterization of senescent cells, establishment of senescence atlases, development of biomarkers, optimization of model systems, and design of imaging tools (Roy et al., 2020). However, there remain several open questions, which together pose a major challenge in this field. Biomarkers, model systems, and imaging techniques all need validation projects, in which pathways of cellular homeostasis are perturbed to induce, modulate and fine-tune senescence, while assessment of the impact of those perturbations on SASP development and on the physiological integrity of diverse tissues and organs is essential to address the pathological factors responsible for chronological aging. Advances in these aspects may help discover more accurate senescence-associated signatures to address key questions: what initiates and regulates the SASP? What phenotypes do deep senescent cells acquire? What is the best timepoint to target the SASP during cellular senescence? Answers to these intriguing questions will help develop new panels of markers for subtypes of both senescence and the SASP and guide the evolving field of senotherapy, thus allowing to surf in the next wave of tides in the current epoch of precision medicine.

Biomarkers of organ aging

Aging comprises many biological processes that may not change in concert. This is the case at the cellular level, as described in the previous chapter, and likely also be true higher up in the hierarchy of aging dimensions, i.e., at the organ level. In this chapter, we dive into individual organs with their own aging biomarkers (Figure 10), with each organized in a framework highlighting the six pillars of aging biomarkers: physiological characteristics, imaging traits, histological features, cellular alterations, molecular changes, and secretory factors. In addition to being specific to the organ of interest, some of these biomarkers are commonly shared by several organs or interconnected via compensatory mechanisms, system feedbacks, and peripheral immune functions involving the gut and circulating immune cells. Ideally, the aging biomarkers should be accessible for measurement over the entire lifespan. Therefore, we propose that good organ aging biomarkers should be specific, systemic, and serviceable.

Brain aging

The human brain, a three-pound organ, contains the most recently evolved structure, the neocortex, which greatly enhances cognitive function and separates homo sapiens from the rest of the animal kingdom. Through sensory, motor, and autonomic nervous systems, the brain serves as a supreme commanding center of the human body. The central nervous system (CNS), through the peripheral nervous system, tracks blood vessels and innervates all organs. Therefore, like blood supplies, neural control is also everywhere. The brain is an organ prone to aging, which increases the risks of a range of neurodegenerative diseases. During the aging process, the brain changes at biochemical, cellular, structural, and functional levels, and some of the characteristic changes can be used as biomarkers to reflect and evaluate the aging process of the brain. This section focuses on those markers (Table S6 in Supporting Information).

Physiological characteristics

Along the aging trajectory, progressive functional decline occurs in multiple organs including the brain. At the cellular and molecular level, ten hallmarks of brain aging have been postulated (Mattson and Arumugam, 2018): mitochondrial dysfunction, intracellular accumulation of oxidatively damaged molecules, dysregulated energy metabolism, impaired cellular "waste disposal" mechanisms, impaired adaptive stress response signaling, compromised DNA repair, aberrant neuronal network activity, dysregulated neuronal calcium homeostasis, stem cell exhaustion, and inflammation. In recent years, several promising molecular biomarkers indicative of brain aging have been identified, e.g., DNA and histone methylation markers, proteomic markers, which will be discussed below. Moreover, these hallmarks of brain aging are interconnected, causing alterations of brain structures and functions. Clinically, brain aging is manifested by changes in brain morphology, pathological accumulation of abnormal proteins, and altered physiological functions, which display enormous changes in human lifespan and could be used as better indicators for future risk assessment of experience-based age-associated health issues, e.g., neurodegeneration, disability, and poor quality of life. Brain morphological changes during aging demonstrated by neuroimaging, primarily consisting of loss in brain volume, gray and white matter degradation, enlargement of cerebral ventricles, or cortical thinning due to shrinking of neuronal cells, dendritic degeneration, demyelination, metabolic deficit, microglial activation, and formation of white matter lesions, resulting from small vessel diseases (Blinkouskaya et al., 2021). Although accumulation of pathological deposition of Amyloid-beta (Aβ), tau, and α-synuclein in the brain has been considered as markers of neurodegenerative diseases including Alzheimer's and Parkinson's diseases, as well as physiological markers of brain aging (Sengupta and Kayed, 2022; Zhang et al., 2021k). At the phenotypical and functional levels, brain aging is characterized by a decline in

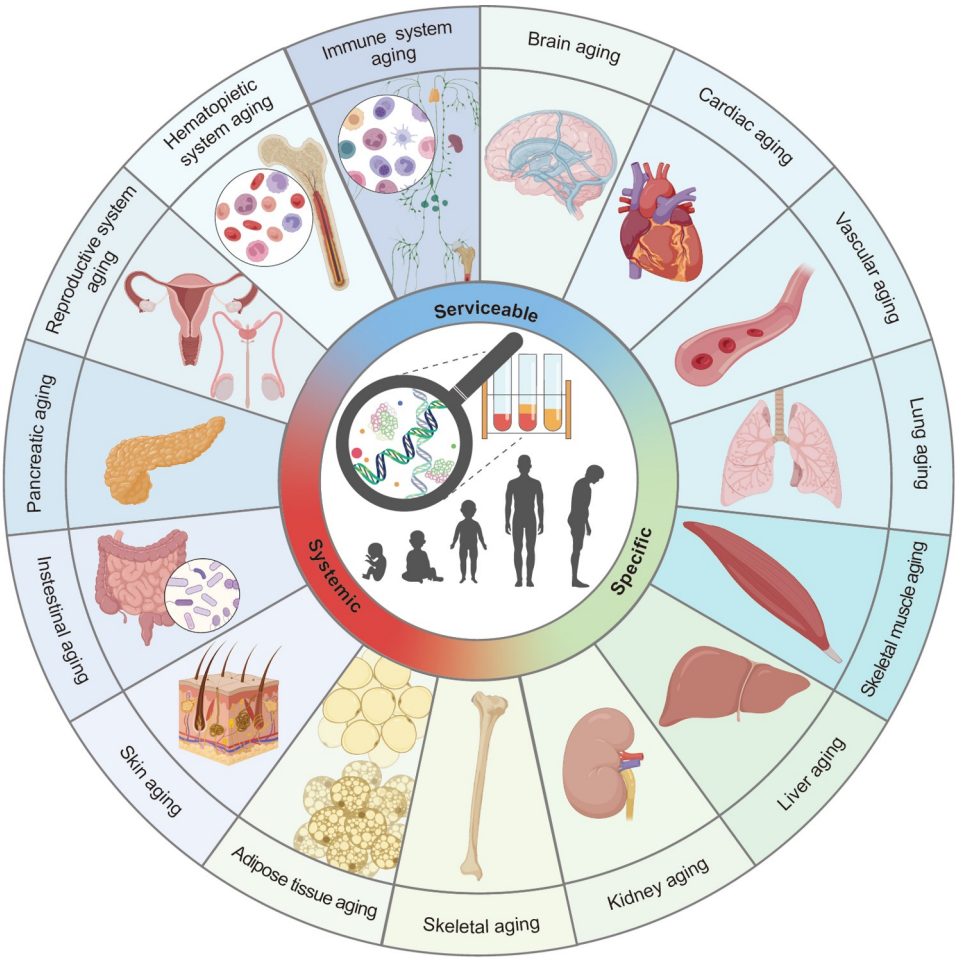

Figure 10 Biomarkers of organ aging. On the rim of the circle, aging of the 15 organs or organ systems across the body reviewed here is presented; while in the center, aging biomarkers are depicted as biological measurements that can evaluate the aging of these organs, and they should fulfill the three criteria: being specific, systemic, and pragmetic.

learning and memory, decision-making speed, sensory functions, and motor coordinations. All of these aging-associated changes have a huge impact on the basic activities of individuals' daily life and contribute to geriatric syndromes such as frailty (Xue, 2011). A recently introduced concept of "intrinsic capacity" by the World Health Organization (WHO) (Chhetri et al., 2021), considers individual's capacities in locomotion, cognition, sensory, psychology, as well as vitality, and could be utilized to measure chronological aging as well as brain aging at functional levels.

Whilst signs of age-related neurodegeneration may appear beyond the fifth decade of life, the rate of brain aging like any other organs varies among individuals. Environmental factors (e.g., stress, diet, socioeconomic status, smoking, and alcohol intake) an individual experienced throughout earlier life can impact cognitive function later in life. People with genetic risk factors (e.g., APOE4) or environmental risk exposures may have a higher rate of brain aging than others. Similarly, exposure to severe trauma or acute infection may also accelerate the process of brain aging. The aging process, including brain aging, involves a vicious chain of events (concomitantly influenced by environmental factors), from molecular to cellular and physiological levels, ultimately impacting the function of an individual. These changes could be largely indistinguishable initially, due to homeostatic or compensatory mechanisms of the resilient central nervous system. Ultimately, the system's ability to maintain homeostasis reaches the threshold, signs of brain aging would manifest. Nonetheless, some of the biological changes are detectable early and may serve as reliable markers to predict brain aging (Figure 11).

Similar to generic aging biomarkers, brain aging biomarkers should be able to predict the rate of brain aging (should provide information on the brain age status at any given time), monitor the aging process, and also distinguish between physiological and pathological brain aging. Those markers should allow for repeated testings without causing harm, and ideally work both in humans and animals (for prior validation). Moreover, these markers should be compatible for clinical practice to screen people with high risks for

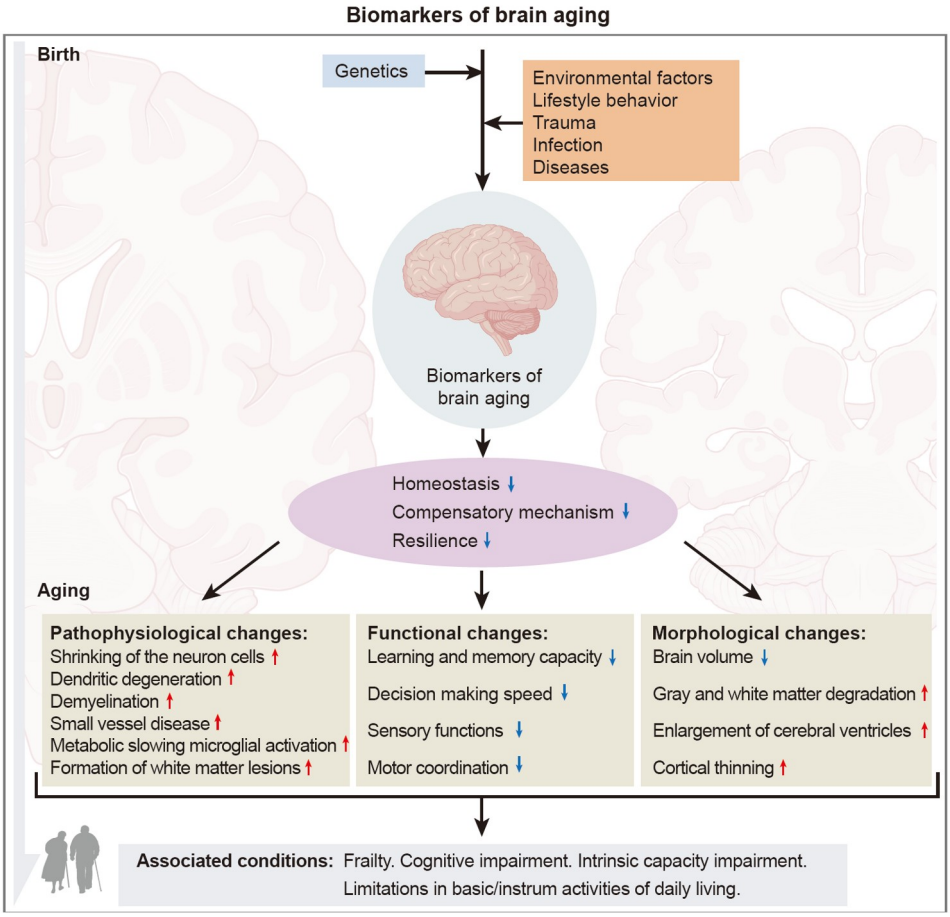

Figure 11 Biomarkers of brain aging at the level of physiological characteristics. Brain aging is not only exacerbated by the hallmark changes, but it is also influenced by a combination of genetics, environment, lifestyle, trauma, and diseases. The decline in homeostasis, compensatory mechanisms, and resilience can result in various pathophysiological, functional, and morphological changes in the brain, which can serve as potential biomarkers for brain aging. Frailty, cognitive decline, reduced intrinsic capacity, or limitations in activities of daily living and instrumental activities of daily living are some conditions associated with brain aging and the aging process as a whole, as they are the result of the interplay among multiple organ systems, including the brain.

timely interventions. Besides, it should also be noted that a group of biomarkers, in combination, may be needed to capture brain aging more effectively, as there is a heterogeneity in the aging process, thus, a single biomarker may not have high enough sensitivity or specificity to predict brain aging. Relevant clinical data from the central nervous system and the periphery- due to interactions between organ systems, regulated by the blood-brain-barrier (e.g., proteins, metabolites, and cells from blood or CSF) has been suggested to successfully capture brain aging (Higgins-Chen et al., 2021). Such composite biomarkers will enable us to understand the mechanism and markers of the brain from a holistic, systemic, and multidimensional perspective.

In the following sections, we will discuss potential biomarkers for brain aging, with a focus on neuroimaging, histological features, cellular alterations, molecular changes, and secretory factors.

Imaging traits

(1) Structural MRI. Magnetic resonance imaging (MRI) has been widely used to show age-related structural changes of the brain *in vivo*. Accumulating evidence indicated that the combination of several structural imaging markers, including brain atrophy, microvascular alterations, microbleeds, white matter lesions (WMLs), and impaired white matter integrity, can predict brain aging and neurodegenerative diseases (Beaman et al., 2022; Erten-Lyons et al., 2013; Grajauskas et al., 2019; Jung et al., 2021; Kavcic et al., 2008).

Brain atrophy is one of the most common changes in aged brain, which affects both grey and white matter. Atrophy can be seen across the entire brain, with the greatest decline in frontal lobe volumes during normal aging (DeCarli et al., 2005; Fox and Schott, 2004). While shrinkage is often localized, with differential atrophy patterns occurring in different neurodegenerative diseases, e.g., medial temporal lobe atrophy on MRI is a hallmark for AD (Scheltens et al., 2021). MRI-based microbleeds are estimated to range from 8.8% to 35%, with prevalence rate increasing with age (Barnaure et al., 2017; Poels et al., 2010; Romero et al., 2014). Microbleeds caused by hypertensive angiopathy is usually seen in

basal ganglia, thalamus, brainstem and cerebellum. Microbleeds caused by cerebral amyloid angiopathy (CAA) is distributed in the cerebral cortex (Ding et al., 2017; Haller et al., 2018; Park et al., 2013). Increasing loads of microbleeds might predict further cognitive decline due to disruptions of connections between different brain regions (Heringa et al., 2014; Werring et al., 2004).

WMLs refer to patches of hyperintensity on T2-weighted imaging (T2WI) in the white matter, linking to microvascular alterations and ischemia in aged brain. WHLs commonly occur in the aging population, and increase with age (Habes et al., 2018; Sundaresan et al., 2019). WHLs have been stratified into two forms, i.e., periventricular white matter lesions (distributed around the ventricles) and deep white matter lesions (first in the frontal lobe, then in other cortical regions), with different underlying pathogenesis (Jung et al., 2021; Zeng et al., 2020). For the role of white matter integrity in aging, decreased fornix fractional anisotropy (FA) derived from diffusion tensor imaging (DTI) is one of the earliest MRI abnormalities, which predicted the decline in memory function, observed in elderly individuals with increased risks for AD (Kantarci et al., 2014).

(2) Functional MRI. Functional MRI (fMRI) can provide information on neuronal activity and vascular physiology, which is well suited for *in vivo* observations of brain pathophysiology at an early stage. Large amounts of studies showed decreased cerebral blood flow (CBF) with age in elderly, both regionally and globally, which is relevant to the cognitive decline, and declined in cerebrovascular health (Chen et al., 2011; De Vis et al., 2018; Tarumi et al., 2014). The spatial-temporal pattern of CBF decreased from the precuneus, posterior cingulate and temporal-parietal regions to broader areas with progression from health control to mild cognitive impairment (MCI) to AD, supporting the incorporation of CBF into the AD research framework (Zhang et al., 2021f). Oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen (CMRO2) are markers of cerebral oxygen homeostasis and metabolism that may offer insights into abnormal changes in brain aging (Lin et al., 2019; Peng et al., 2014). Resting CMRO2 based on blood oxygen level-dependent (BOLD) is found to be decreased in the parietotemporal and precuneus regions in elderly with AD (Lajoie et al., 2017).

In addition to well-documented changes in brain structure and function, normal aging and age-related neurodegenerative diseases are related to alterations of functional brain networks. Functional brain networks are commonly measured from resting state fMRI. Using a graph theoretical approach, decreased connectivity within and increased connectivity between functional brain systems were prominent in the elderly (Chan et al., 2014). Most studies show decreased functional connectivity between regions of the default mode network (DMN) in the elderly with memory decline, specifically between posterior cingulate cortex and parietal areas, compared with non-decliners, indicating that functional networks could be as an early marker of cognitive decline during aging and to guide early interventions of AD (Bernard et al., 2015; Sperling et al., 2009). Task state fMRI is needed to concurrently measure cognitive function in the elderly, through which changes may be much more easily detected than resting state MRI to distinguish normal elderly from MCI. Emerging studies showed decreased anterior-to-posterior functional connectivities and increased local efficiency in the cognitive task state between normal elderly and MCI, with no significant difference in the resting state (Wang et al., 2013a). This indicates task can amplify the sensitivity of detection, which could be an early biomarker to assess the risk of AD.

(3) PET. Positron emission tomography (PET) with multiple radiotracers can help to deeply understand age-related pathophysiological changes, especially abnormal neural activity, synaptic loss, Aβ deposition and tau accumulation, as well as the relationship between these changes and age-related cognitive decline.

^{18}F-fluorodeoxyglucose (FDG) PET imaging measures cerebral glucose metabolism, which is sensitive to neural activity alteration and synaptic dysfunction. Decreased ^{18}F-FDG uptake has been predominately reported in frontal, cingulate, and temporal lobes during normal aging, which suggests that these brain regions are vulnerable to aging (Bonte et al., 2017; Yoshizawa et al., 2014). Compared with normal elderly individuals, those with cognitive decline showed greater hypometabolism in these brain aging, indicating ^{18}F-FDG PET imaging could predict cognition progression during aging and risks to convert into AD (Apostolova et al., 2010). Posterior cingulate and temporoparietal hypometabolism on ^{18}F-FDG PET is a hallmark for AD (Frisoni et al., 2017; Scheltens et al., 2021). Moreover, ^{18}F-FDG PET imaging can identify the involvement of specific brain regions in various cognitive domains to help clarify the neural mechanisms of the age-related cognitive changes in brain aging study (Brugnolo et al., 2014).

Aβ amyloid plaque and neurofibrillary tangles composed of hyperphosphorylated tau are considered as neuropathological hallmarks of AD, leading to cognitive impairment in AD. These two proteins also appear in brains of the elderly with normal cognition, indicating a latent period of 10–25 years for AD (Masters et al., 2015). Aβ and Tau PET imaging can help to clarify the role of these aggregated proteins in age-related cognitive decline as well as AD onset and progression. Large prospective studies could provide answers regarding the clinical impact and utility of Aβ and tau imaging. Studies indicate that Aβ deposition occurs before the onset of cognitive impairment and reaches a plateau in the symptomatic stage of dementia (Hanseeuw et al., 2019; Jack et al., 2013). Aβ PET imaging in the elderly with

normal cognition revealed a significant association between Aβ and cognitive functions, especially in episodic memory (Vogel et al., 2017). Aβ deposition in normal elderly can lead to cognitive impairment, which is related to reduced cortical thickness (Vemuri et al., 2019), altered white matter integrity (Caballero et al., 2020) and decreased DMN functional connectivity (Lim et al., 2014).

Tau-PET imaging has characterized tau tracer retention *in vivo*, in keeping with Braak stages (Cho et al., 2016; Pascoal et al., 2021; Schwarz et al., 2016). Tau-PET imaging has revealed that tau pathology begins focally at the perirhinal cortex in cognitively unimpaired elderly, which influences memory performance by disrupting medial temporal lobe-cortical functional connectivity, independent of Aβ (Berron et al., 2020; Berron et al., 2021; Sanchez et al., 2021). When tau interacts with Aβ directly at the inferior temporal gyrus, tau deposits spreads catastrophically into neocortical regions (Lee et al., 2022c). Longitudinal tau-PET studies could track the progression of AD, which correlates with cognitive deficits and best predicts cognitive decline (Bucci et al., 2021; Harrison et al., 2019).

(4) Other imaging modalities. Developments in PET tracers for synaptic density, neuroinflammation, and α-synuclein have opened new ways to explore brain aging (Chen et al., 2018a; Meyer et al., 2020; Seibyl, 2022). Future longitudinal studies using multi-radiotracer PET imaging combined with other neuroimaging modalities, such as MRI morphometry, task-based fMRI, functional near-infrared spectroscopy (fNIRS), and magnetoencephalography, are essential to elucidate the neuropathological underpinnings and interactions in brain aging. In the near future, development of automated image analysis, multimodal analysis, and hybrid PET-MR imaging, as well as artificial intelligence into brain aging study will be highly demanded.

Histologic features

The histological architecture of the brain is complex but generally can be classified into two structures: grey matter and white matter. The grey matter consists of neuronal cell bodies, dendrites, unmyelinated axons, various glial cells, and some blood capillaries as well as microglia. The white matter is mainly composed of myelinated axons that form nerve fibers and oligodendrocytes that form myelin, as well as astrocytes and microglia. During aging, the brain is subjected to alternations at multiple levels. Primary morphological changes with aging include brain volume loss, gray and white matter atrophy, cortical thinning, gyrification loss, and ventricular enlargement (Blinkouskaya et al., 2021; Blinkouskaya and Weickenmeier, 2021). Herein, we briefly summarize histological changes in the brain during normal aging.

(1) Whole brain weight loss and atrophy. In early studies by measuring brain weight, it is found that the human brain weight increases mostly during the first 3 years of life, remains relatively stable at adolescent and young adult age, and then starts to decline at about 45–50 years of age (Dekaban, 1978). Some later studies suggest that brain weight decline accelerates after the age of 70, with an annual rate of weight loss between 2% and 5% (Ho et al., 1980; Svennerholm et al., 1997; Teissier et al., 2020).

The brain volume loss with aging has been determined by multiple neuroimaging studies. Hedman et al. (2012) compared 56 longitudinal MRI work and suggested that after 35 years of age, the brain volume started to lose at an annual rate 0.2%, which accelerated and increased to 0.5% by the age of 60, and kept a steady loss of more than 0.5% after that age. Although different studies report the change rates with substantial deviations, they consistently suggest that the brain volume declines with aging and brain atrophy accelerates along with advanced aging (Blinkouskaya et al., 2021; Resnick et al., 2003).

Initial studies by Brody concluded that about 50% of neurons are lost with aging and thus suggested that the progressive brain atrophy with aging was attributed to neuronal loss (Brody, 1955). However, Haug suggested that there was no significant loss of neurons in the human brain during aging (Haug et al., 1984). Indeed, later studies have also shown that neuron numbers in multiple brain regions are relatively stable over the entire human life span (Gómez-Isla et al., 1997; Peters, 1993; West et al., 1994). Now a decline of neuronal volumes (rather than their number or density) and a decrease in neuronal dendritic and axonal arborizations have been proposed as a major reason for brain atrophy with aging (Dickstein et al., 2007; Teissier et al., 2020).

(2) Grey matter atrophy. The global grey matter volume also decreases during aging (Good et al., 2001; Hedman et al., 2012; Resnick et al., 2003; Taki et al., 2011). One longitudinal MRI study estimated the annual rate of gray matter volume decline to be 0.424% in males and 0.298% in females (Taki et al., 2011).

Human brain surface folds to form multiple gyri. This gyrification increases human brain surface areas. A major part of gray matter distributes near the surface area of the brain and forms the cortex. Therefore, whole brain and grey matter atrophy would affect cortical thickness and gyrification. Indeed, multiple studies have shown global cortical thinning and decreased in degrees of gyrification during aging (Fjell et al., 2014; Lamballais et al., 2020; Lemaitre et al., 2012; Madan, 2021; Storsve et al., 2014). For example, Storsve et al. (2014) reported a mean cortical thickness decrease at a rate of 0.35 mm/year during aging. Madan (2021) determined a gyrification decreasing slope of 0.04291 per decade.

Same to global brain atrophy, grey matter atrophy during aging is likely caused by neuronal shrinkage and extensive dendritic regression rather than cell loss (Dickstein et al.,

2007; Fjell and Walhovd, 2010).

(3) White matter atrophy. The white matter volume also decreases during aging but has a late onset compared to that of gray matter (Blinkouskaya et al., 2021; Liu et al., 2017a; Resnick et al., 2003). The white matter volume keeps increasing in early and middle adulthood but then starts to decrease more rapidly than grey matter volume loss in later stages of life, with an estimated annual rate of 0.77% per year in the chronological age of 70s (Blinkouskaya et al., 2021; Driscoll et al., 2009; Salat et al., 2005). Moreover, multiple studies using DTI have demonstrated age-related decline in the composition of integrity of white matter (Bennett and Madden, 2014).

Myelinated nerve fibers in the white matter are also affected by normal aging but demonstrate complicated changes. Many myelinated nerve fibers are lost and thus causes disconnections of some parts of the brain. On the other hand, after losing the myelin sheath, some axons can be remyelinated (though incompletely) by oligodendrocytes, whose number increases during aging (Peters et al., 2008). Nevertheless, remyelinated nerve fibers may have shorter internodes segment as well as reduced conduction velocity, thereby compromising the integrity and function of neural circuits. Moreover, the sheath thickness of some intact nerve fibers is even increased with aging, probably due to sustained oligodendrocyte activity (Peters, 2009).

(4) Ventricle. The brain ventricular system is essential for the circulation of cerebrospinal fluid to supply nutrients to and drain wastes out of the brain. Multiple MRI-based studies have identified ventricle expansion with aging (Fjell and Walhovd, 2010; Resnick et al., 2003; Shook et al., 2014); and the increase of ventricular volume is believed to result from the shrinkage of the brain and grey matter (Pfefferbaum et al., 1994). By using both longitudinal and cross-sectional analyses, Resnick et al. (2000) find that the ventricular volume increases at a rate of 1.3–1.5 cm^3/year.

(5) Cerebrovascular changes. An arterial network covers the brain surface and penetrates into the brain parenchyma in the form of capillaries. This cerebrovascular system is affected by aging: a majority of studies in humans and rats show that the vascular density declines with age (Brown and Thore, 2011; Riddle et al., 2003). Moreover, the arterioles supplying the deep white matter have the longest course throughout the brain and often become tortuous during aging, though the biological meaning of these tortuous arterioles is unknown (Brown and Thore, 2011).

(6) Neurogenesis. Neurogenesis is a process of generation of new neurons from neural stem cells (Isaev et al., 2019). Proper neurogenesis requires support from all cells in the neurogenesis niches. Neurogenesis has been identified in the olfactory bulb, the hippocampus, and the sub-ventricular zone in the mammalian brain; and can partially compensate neuronal death in these regions (Galvan and Jin, 2007; Isaev et al., 2019). Neurogenesis is significantly impaired with aging, due to age-dependent stem cell exhaustion, though the detailed biological significance of this decline has yet to be specified (Ahlenius et al., 2009; Enwere et al., 2004; Zhang et al., 2021e).

Cellular alterations

Neurons, glial cells and endothelial cells are the basic building blocks of brain. Physiological brain aging is associated with changes in brain size, vasculature, neuronal connections and networks. It is well established that neuronal functions decline with aging. At cellular level, hallmarks of brain aging include loss of synapses, mitochondrial dysfunction, increased oxidative stress and damage, metabolic alterations, autophagy dysfunction, and dysregulated stress and inflammatory responses (Mattson and Arumugam, 2018). Senescence is a central hallmark of aging, a process in which cells cease to divide and undergo distinct phenotypic alterations, including profound chromatin and secretome changes, as well as tumor-suppressor activation (van Deursen, 2014). When senescence is triggered, cells increase in size and granularity. The levels of cell cycle inhibitors $p21^{CIP1}$ and $p16^{INK4a}$, are increased. The SA-β-gal, a lysosomal enzyme, becomes more active, serving as a marker for senescence characterization (Beauséjour et al., 2003; Dimri et al., 1995; Sikora et al., 2021). Neurons are post-mitotic cells. Neuronal senescence is likely regulated in a different manner from that of proliferating cells. Nevertheless, increased detection of SA-β-gal is also found in aged mouse, hippocampus and monkey, as well as in long-term cultured neurons, suggesting that neurons undergo senescence by increasing levels of SA-β-gal. Aging also changes neuronal morphology, particularly synaptic structures. In general, synaptic numbers are reduced in aged brains compared to young brains (Peters, 2006). The aged prefrontal cortex tends to lose more thin spines that confer neural plasticity. In contrast, the aging hippocampus tends to lose large, complex (perforated mushroom) synapses that help control established memories and learning circuits. Again, this suggests that there could be some differences in line of maturing thin versus mushroom spines with different classes of neurons. Alterations in mitochondrial morphology and mitochondrial function have long been considered hallmarks and potential drivers of aging. During the process of neuronal aging, mitochondrial function is reduced with the accumulation of damaged mitochondria and ROS in neurons (Chakrabarti et al., 2011). Meanwhile, the load of mtDNA mutations is increased. All these results in mitochondrial dysfunction, reduced ATP supply and increased ROS production, eventually leading to functional impairment of neurons and further damage of the brain. Mitochondrial dynamic proteins are regulated in an age-dependent manner. Phosphorylation of $Drp1^{S616}$ is downregulated in aged brains (Han et al., 2020a).

Brain aging is also correlated with increased neuroinflammation (Lynch, 2010). Inflammatory cytokine IL-6 levels are increased in brain of healthy aged animals. IL-6 in the brain has been suggested to reduce food intake, inhibit memory and learning, and cause neurodegeneration. Remarkably, increased expression of IL-6 correlated with neuron-specific upregulation of neuronal senescence marker REST protein (Sunderland et al., 2020), implying a role of IL-6 in brain aging.

In the past, most research into age-related alterations in the brain focused on neurons. Nonetheless, non-neuronal glial cells are the first cells responding to "stress" in the CNS. The major types of glial cells in the CNS are astrocytes, microglia, and oligodendrocytes. The diverse and important functions of different types of glial cells situate them in a position to be affected by brain aging. Indeed, a transcriptional study from human aged brain showed that glial cells, but not neurons, displayed robust changes in gene expression during aging. Studies showed that cellular senescence in glia cells (such as telomere shortening), might also interfere with their physiological function. Microglia, constituting 10% of brain cells, are actually immune cells of the brain (Crotti and Ransohoff, 2016). Microglia alterations under different brain conditions were more dramatic than those observed in other glial cells. In the process of aging, microglia are uniformly enlarged, accumulating non-degraded inclusion materials and with cytoplasmic disruptions (Streit and Xue, 2010). Aged human microglia become dystrophic manifested by abnormal cytoplasmic structures with beaded and fragmented processes (Streit, 2006; Streit et al., 2004). Consequently, these morphological changes are accompanied with alterations in their physiological functions, including enhanced pro-inflammatory responses, impaired motility, and compromised immune responses. Intrinsic and extrinsic aging risk factors can induce aberrant microglia activation. This activation results in increased neuroinflammation and synaptic pruning, leading to eventual neural circuit impairment and neurodegeneration. Therefore, aberrant microglia activation is associated with aging at certain conditions. Enlargement and accumulation of lysosomes are characteristic of aberrant microglia activation. Astrocytes, like neurons, are present in the brain throughout life. They are not replenished, nor do they divide except in the case of injury. Thus, they are particularly susceptible to age associated disturbances such as accumulation of a lifetime of stress. Glial fibrillary acidic protein (GFAP) is a biomarker for astrocyte reactivation. Levels of GFAP mRNA and protein were increased with age in hippocampus of human, rhesus monkey, rat, and mouse (David et al., 1997; Diniz et al., 2010; Nichols et al., 1993; Rodríguez et al., 2014). Transcriptomic profiles of astrocytes show increased expression of complement pathway and neuroinflammatory genes in all brain regions of mice (Boisvert et al., 2018; Clarke et al., 2018). In human, astrocyte gene expression profiles change across different ages and brain regions, particularly hippocampus and substantial nigra (Soreq et al., 2017). Remarkably, these two regions are predominantly affected by AD and PD, the top two most studied neurodegenerative diseases of the CNS. Single cell sequencing revealed the presence of an AD-associated astrocyte cluster in hippocampal astrocytes of aging mouse and human (Habib et al., 2020). Roles of oligodendrocytes are not well defined. In aged brains, myelination is decreased. Shorter internodes are formed. Myelin debris are aberrantly released. Results suggest a functional abnormality of oligodendrocyte.

In summary, characterization of cellular changes in aged brain is rather complex. Cell morphology and changes of cell organelles may vary in different brain regions. A combination of cellular and molecular analyses may prove to be eminent.

Molecular changes

As with other organs, brain also ages, and quantifying brain aging is critical to understand the mechanisms of brain aging and related diseases. Over the past decades, many molecular features have been identified and used as molecular biomarkers associated with or indicative of brain aging. Here, we provide an overview of those measurements that have been studied the most in the context of brain aging: DNA methylation marks, histone marks, non-coding RNAs (ncRNAs), as well as proteomic markers, and present how these biomarkers are related to the underlying brain aging processes. The mechanistic understanding of these biomarkers may help us to effectively intervene brain aging process or treat age-related diseases.

(1) DNA Methylation. The methylation of 5-position of cytosine base (5mC) is one of the most studied epigenetic marks. Global 5mC level shows an age-dependent increase in mouse brains (Chouliaras et al., 2012). DNA hydroxymethylation (5-hmC), a more recently discovered DNA modification and highly enriched in the brain (Li and Liu, 2011), also shows an increase with age in the hippocampus of mouse brains (Chouliaras et al., 2012). Methylation of cytosine is catalyzed by DNMTs, including DNMT1, DNMT3a, and DNMT3b. DNMT3a shows an age-related increase in the hippocampus of the mouse brain (Chouliaras et al., 2012). These findings suggest that global DNA methylation is increased with age in the brain and DNA methylation measurements are shown to be an age prediction tool.

Alterations of DNA methylation have been proposed as risk markers for age-related diseases, such as AD (Herdy et al., 2022; Kim-Ha and Kim, 2016). Increased methylation of senescence gene promoters is observed in neurons derived from AD patients (Herdy et al., 2022). The findings of these studies suggest that DNA methylation could serve as a new

diagnostic biomarker for brain aging and age-related diseases.

(2) Histone modification. Histone modifications are involved in brain aging by regulating chromatin plasticity (Peleg et al., 2010). An increase in repressive histone marks of H3K9me2, H3K9me3 and H3K27me3 and a decrease in activating histone marks of H3K36me3 and H3K27ac have been reported in cerebral cortex and hippocampus of aged mice (Akbarian et al., 2013). Aged mice display a specific deregulation of H4K12ac, thus fail to initiate gene expression program associated with memory consolidation, suggesting that reduction in H4K12ac may serve as an early biomarker of brain aging (Peleg et al., 2010). In addition to histone acetylation, changes in histone methylation marks were reported in the brain of aged mice, including decreased methylation in H3K27me3, H3R2me2, H3K79me3, and H4K20me2 (Gong et al., 2015). These findings support that both types of histone marks are deeply involved in brain aging and age-related cognitive functions (Geng et al., 2021). It should be emphasized that two recent separate studies demonstrated that downregulation of H3K9me3 and transcriptional de-repression of LINE1 are important features of brain aging in mice and monkeys (Zhang et al., 2021e; Zhang et al., 2022e). This may constitute one of the drivers for the upregulation of neuroinflammation associated with aging.

(3) ncRNAs. ncRNAs, comprising lncRNAs, miRNAs, transfer RNA-derived small RNAs (tsRNAs), and piwi-interacting RNAs (piRNAs), are highly expressed in the brain and play a regulatory role in brain aging (Danka Mohammed et al., 2017; Mao et al., 2019; Marttila et al., 2020; Yuan et al., 2021). Recent studies identify 336 differentially expressed lncRNAs in aged human brains, 80 differentially expressed miRNAs in mouse brains, and 8 differentially expressed tsRNAs in senescence-accelerated mouse models (Danka Mohammed et al., 2017; Marttila et al., 2020; Zhang et al., 2019c). Transcriptome-wide piRNA profiling identifies a total of 9,453 piRNAs, that are differentially expressed in the prefrontal cortex of human brains. Among them, seven intergenic and one intronic piRNAs are positively associated with brain aging, and two intronic piRNAs are negatively associated with brain aging (Mao et al., 2019). These findings suggest that dysregulated ncRNAs contribute to brain aging and could serve as potential diagnostic biomarkers for brain aging, although their regulatory functions in brain aging remain largely elusive.

(4) Proteomics. Large-scale proteomic analysis of human brains identifies 579 proteins associated with brain aging (Wingo et al., 2019). Similarly, proteomic analysis on mouse brain also identifies 390 proteins associated with hippocampal aging and 258 proteins associated with cortical aging in mouse brains (Li et al., 2020d). Bioinformatic analysis indicates that these dysregulated proteins are highly involved in a diverse range of functions, including synapses (SYT12, GLUR2), mitochondrial functions (FIS1, DRP1), oxidative stress (PRDX6, GSTP1, and GSTM1), transcriptional regulation, ribosome (RPL4, RPS3), cytoskeletal integrity, and GTPase function (Li et al., 2020d). Such changes predictably lead to functional decline of brain by decreasing ATP content, increasing DNA oxidative damage, and deteriorating synaptic function. Taken together, these systemic changes in protein levels are associated with brain aging, suggesting that proteomic markers could serve as molecular biomarkers for brain aging.

As we discussed above, epigenetic factors and proteomic markers are deeply involved in brain aging. An in-depth and mechanistic understanding toward these age-related changes is required in order to take advantage of these systemic changes to effectively intervene brain aging process and treat age-related diseases.

Secretory factors detectable in biofluids

Humoral biomarkers present in biological fluids such as plasma, urine, and cerebrospinal fluid, could be used as clinical indicators to reflect physiological and/or pathogenic processes including brain aging. Due to their natures of being non- or minimal-invasive, highly sensitive, and easily measurable with accuracy, measurement of secretory factors in the realm of IVD (*in vitro* diagnosis) is also an indispensable means for quantitatively detecting the aging process. Changes in brain homeostasis could be reflected in circulating fluids in a timely manner, because of the highly active communications between the CNS and the periphery. Biomarkers in body fluid have been actively pursued in the field of early detection of neurodegenerative diseases. Interestingly, although highly correlated with age, dementia is not necessarily an inevitable consequence of old age (Arosio et al., 2017), making normal brain aging markers still distinguishable from markers for neurodegeneration such as in the case of AD or PD. In this section, we briefly discuss how humoral markers can be used to monitor physiological and pathological brain aging.

(1) Hormonal homeostasis. At the early stage of human life cycle, growth hormone (GH) and insulin-like growth factor 1 (IGF-1) gradually increase their levels in the plasma, which are crucial for development and growth of the body. GH and IGF-1 began to decline with age, yet surprisingly, when people get older, plasma levels of GH and IGF-1 became positively correlated with brain aging (Duran-Ortiz et al., 2021). High plasma IGF-1 levels in elderly populations have been linked to dementia (Coschigano et al., 2000; Zhang et al., 2020f). In a study of centenarians, it was found that high plasma concentrations of the IGF-1 receptors, IGFBP2 and IGFBP6, perhaps reflecting low concentrations of the ligand, were related to longevity (Sebastiani et al., 2021). In addition, GH inhibition was also found to improve insulin sensitivity (Vijayakumar et al., 2010). Various experimental

models have confirmed that insulin has a positive effect on balancing brain energy supplies. Therefore, heightened insulin resistance could be viewed as an indicator for brain aging. Other hormones, including gastrin, prolactin, leptin, starch cellulose and adiponectin, regulate appetite and energy homeostasis, and are also potential markers of brain aging (Cunnane et al., 2020).

(2) Metabolic homeostasis. NAD^+ is a coenzyme used for redox reaction and is the core of energy metabolism. The aging process of the body is accompanied by a decrease of blood NAD^+ levels or NAD^+/NADH ratio. More and more evidence demonstrated that NAD^+ was the main metabolite for maintaining a healthy nervous system (Covarrubias et al., 2021). Chronic low-grade inflammation has been considered a key driver of brain aging and related diseases, and those inflammatory processes are NAD^+-dependent. Abnormal and continuously activated innate immunity as well as reduced adaptive immune responses consume a large amount of NAD^+. Increased levels of CD38 in circulating immune cells are indicative of large consumptions of NAD^+ (Chatterjee et al., 2018; Covarrubias et al., 2020), and are somehow also closely related to brain aging. Not surprisingly, SASP also depends on the level of NAD^+. Therefore, NAD^+ as a brain aging marker needs to be considered in conjunction with inflammatory backgrounds.

(3) Protein factors and lipid markers. Through the parabiosis experiment, it is observed that young blood can elicit an aging-reversal process, while aged blood can accelerate aging. Under this condition, several humoral markers related to brain aging were also discovered. High levels of CCL11 and β2-microglobulin in blood and CSF are associated with decreased levels of neurogenesis (Shi et al., 2018; Smith et al., 2015; Villeda et al., 2011). Metallopeptidase inhibitor TIMP2 is related to the recovery of hippocampal neural activities (Castellano et al., 2017). Cytokine GDF11 is also associated with brain aging, and high expression of which can enhance brain vitality as well as adult neurogenesis of the ependymal/subependymal zones surrounding the lateral ventricles (Frohlich and Vinciguerra, 2020; Katsimpardi et al., 2014). Protein composition in human CSF changes during the aging process. Protein levels of a immune signal hub gene TREM2 have been detected to increase gradually in the CSF with increasing age, which reflects the function of microglia and is also related to AD (Deczkowska et al., 2020). Moreover, Fgf17 in the CSF of young individuals can promote the rejuvenation of the nervous system of elderly individuals (Iram et al., 2022). Interestingly, people are increasingly aware of the role of "exercise" as a "drug" to promote brain rejuvenation. Tony Wyss-Coray group found that the blood produced anti-inflammatory factors as well as complement signal inhibitory factors such as Clusterin (CLU), factor H (FH), glycoprotein pigment epithelium-derived factor (PEDF), and leukemia inhibitory factor receptor (LIFE), all increase their levels in the plasma after exercise (De Miguel et al., 2021), which could be beneficial against brain aging. And the effect of long-term exercise on the brain itself is demonstrated by another study as evidenced by the increased thickness of the cortex and reduced inflammation in the entire CNS in aged mice (Sun et al., 2023).

With recent advancement in research, it is gradually recognized that elimination of aged cells may be crucial to anti-tissue aging process, whereas accumulation of aged cells is related to brain aging. The biomarkers released from the lysis of aged cells into the body fluids may also become specific biomarkers for brain aging. Aged cells can synthesize a large amount of oxidized lipids, which are bioactive metabolites produced by the oxidation of polyunsaturated fatty acids, e.g., Dihomo-15d-PGJ2 is found to be unique to aged cells. This molecule accumulates in aged cells and is released when cells die, which can be detected in both the blood and the urine (Wiley et al., 2021).

(4) Markers found in neurodegenerative diseases. By studying 391 cognitively normal subjects aged between 23 and 91 years from many countries, Lue et al. (2019) described the relationship between age and three plasma and CSF core biomarkers of AD (Aβ40, Aβ42 and t-Tau), provided the normal range of Aβ and t-Tau in plasma, and clarified the dynamic changes of these biomarkers during normal development. In addition, research showed that uric acid (UA) played an antioxidant role in oxidative stress, and the dynamic decline in serum is positively correlated with cognitive impairment. In populations with high level of sUA, Aβ42 no longer correlates with tau pathology and the link between Aβ42-Tau and cognitive impairment is also disrupted (Butterfield and Halliwell, 2019; Huang et al., 2022b), suggesting sUA might be neuroprotective. In another study, Cofilin 2, a serum marker was found to be able to distinguish AD from healthy subjects, as well as AD from vascular dementia (Sun et al., 2019).

Summary and perspectives

It is well known that brain aging is the biggest risk factor for various neurodegenerative diseases including AD and PD, which brings tremendous burden to families and society, and also represents a huge public health challenge. It is worth mentioning that even in the case of familial AD or PD, where individuals do carry disease-causing genetic mutations, and the mutant genes are expressed in the brain, people usually do not manifest pathology at young age, suggesting that in order for the disease gene to elicit obvious pathological changes, something else age-related need to corroborate with the disease gene. Therefore, perhaps a major common strategy for disease prevention or delaying disease onset is to intervene in brain aging. To achieve this goal, it is necessary to discover biomarkers for brain aging, which is specific, systemic, and pragmatic. Humoral biomarkers, physiological

and medical imaging biomarkers are particularly easily applicable for longitudinal studies with large populations. From the above review, it is obvious that cellular, molecular and histological biomarkers may be reflected at humoral, physiological and medical imaging levels. According to the Chinese Taoism script, Tao-Te-Ching, it is said that "tao generates one, one generates two, two generates three, and three generates all things", we therefore propose the "Three-Elements" for brain aging biomarkers, coming from Physiological, Medical Imaging, and Humoral measurements, to monitor brain aging and also serve as reliable outcome measures for future anti-brain aging interventions.

Cardiac aging

Age is widely accepted as an independent risk factor for CVD (Obas and Vasan, 2018). It is critical to understand the normal structural remodeling and functional changes that accompany cardiac aging in the atria, ventricles, valves, pericardium, the cardiac conduction system, and vasculature (Tracy et al., 2020) (Figure 12A; Table S7 in Supporting Information).

Physiological characteristics

With regard to the vasculature, aging is associated with a decrease in elastic fibers, an increase in fractured fibers (Komutrattananont et al., 2019), collagen deposition (Kleefeldt et al., 2019), and the calcification and thickening of the arterial wall (Jakovljevic, 2018). These changes are linked to impairment of atrial conduction and function, such as atrial compliance and active atrial contraction, which are associated with hypertension and increased mortality risk.

Due to increased oxidative stress, the number of cardiomyocytes would decrease and the fibroblasts would senesce with age in the left ventricle (LV). Hence, the remaining cells undergo compensatory hypertrophy, and the wall thickens. The major age-associated structural change in the LV is concentric hypertrophy, including increased wall thickness and LV mass and decreased LV cavity size (Nakayama et al., 2016; Yoneyama et al., 2017). These changes lead to a less compliant ventricle, resulting in ventricular diastolic dysfunction with age, both of which are age-associated contributors to heart failure with preserved ejection fraction (Pagel et al., 2021).

LV systolic dysfunction is not affected by age, at least in rest situations. However, ventricular contractile function declines in older participants when faced with a high demand of cardiac output or when using more sensitive indicators of cardiac function, such as LV longitudinal strain and strain rate (Støylen et al., 2020). The major age-related morphological changes in cardiac valves are stenosis and regurgitation. Older valves have increased deposits of calcium and connective component and declining sulfated glycosaminoglycan content, which contribute to valvular stiffness, progressing into valve stenosis (Coffey et al., 2021). The age-associated changes in the pericardium include increased fibrosis and deposition of pericardial adipose tissue (AT). The adipose tissue synthesizes and secrets proinflammatory adipokines which may be related to atrial fibrosis and conduction abnormalities in the old people (Bernasochi et al., 2017). As age progresses, the release of noradrenaline and adrenaline from tissues into the circulation system increases, while the clearance rate of catecholamines decreases. The sympathetic nervous system is activated during senescence. This activation is enlarged in the heart failure situation, with a nearly 50-fold increase of noradrenaline releasing in the sinus node and LV, which may be associated to the arrhythmia and cardiac structural remodeling (Toledo et al., 2019). Due to the dysfunction of the sinoatrial (SA) node, atrioventricular node, and the his-purkinje system, the prevalence of cardiac conduction system related diseases increases with age, resulting in bradycardia, palpitations, dizziness, syncope, fatigue, and sudden cardiac death.

Imaging traits

With the advancement of age, echocardiographic indicators of atrial function, such as the left atrial expansion index, peak systolic strain, strain rate, and left atrial emptying fraction (LAEF) decrease and left atrial stiffness index and atrial conduction time (PA-TDI) increase (Abou et al., 2017; Olsen et al., 2021). Color Doppler imaging studies have determined that the average early-diastolic velocity (EDV) decreases and the late-diastolic velocity (LDV) increases as age progresses, leading to the decline of EDV/LDV ratio, an indicator of cardiac diastolic function (Seo et al., 2020). Cardiac magnetic resonance (CMR) is a specialized application of MRI which produces high-quality images of cardiovascular system to assess cardiac function and structure. The T2 relaxation time of a quantitative MRI-based T2-mapping decreases significantly in aged hearts (Lee et al., 2022b). As LV compliance reduces with aging, the early radial displacement, radial velocity, and circumferential strain rate decrease, while the late circumferential strain rate increases (Lin et al., 2021).

Histologic features

A key feature of aged hearts is histological alternation, which also serves as the structural basis of deregulated heart function homeostasis and as the obstructive factor for post-injury myocardial repair. As previously mentioned, the aged hearts show increased stiffness, loss of cardiomyocytes, hypertrophic growth, chronic inflammation, and fibrosis (Figure 12B), as well as valvular stiffening, which is the most common age-related heart disease and is associated with a decline in proliferation and extensibility and an increase in collagen deposition, calcium deposition, and stenosis. Besides, amyloid deposits and lipofuscin accumu-

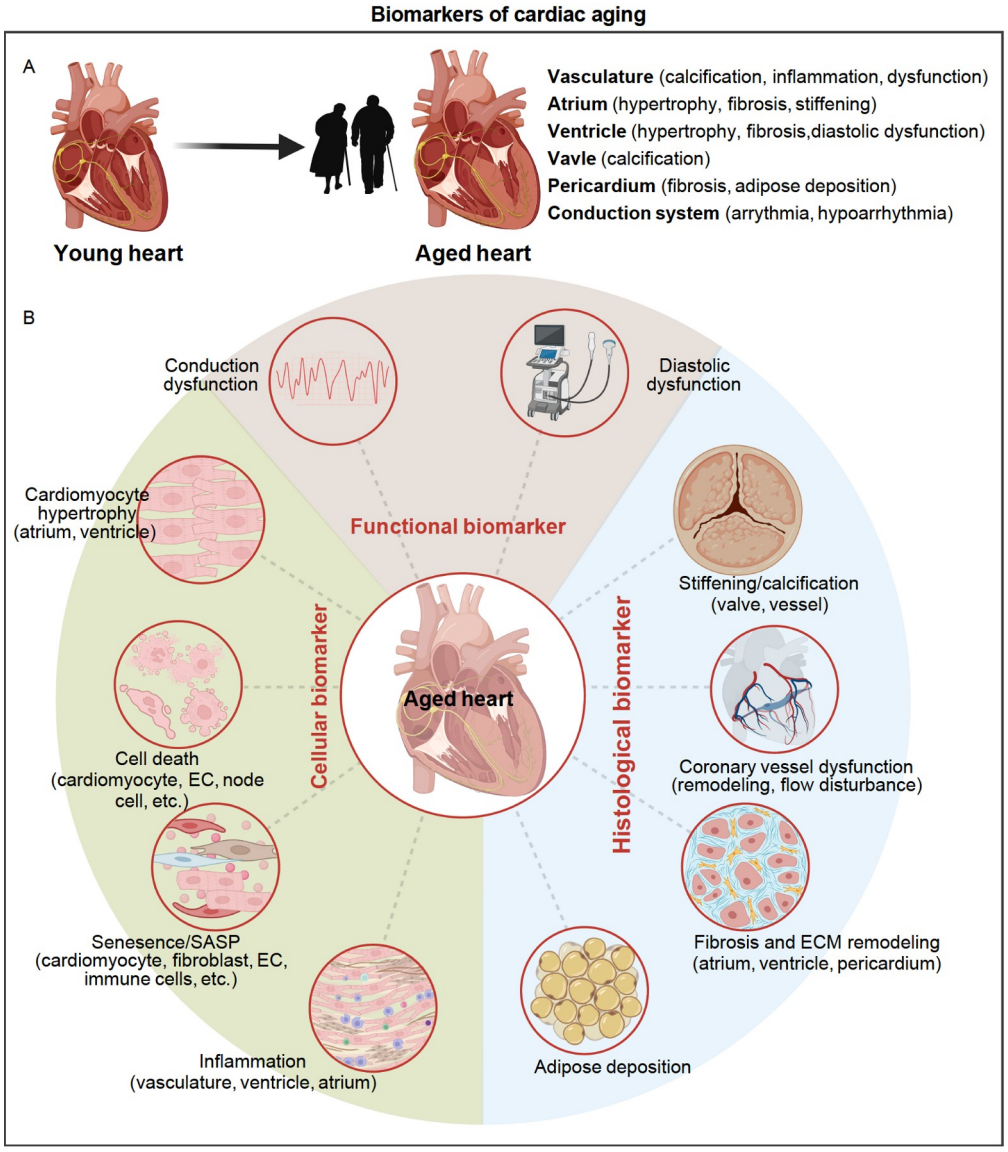

Figure 12 Biomarkers of cardiac aging. A, Aging-associated changes in structures of the heart. B, Functional, histological, and cellular biomarkers of cardiac aging. Abbreviations: EC, endothelial cell.

lation are also important features of cardiac aging. The direct results of these pathological hallmarks of aged hearts are impaired autonomic control and arrhythmias, the functional hallmarks of cardiac aging.

The self-renewal rate of cardiomyocytes in adult hearts is very low. When the cardiomyocytes undergo death (apoptosis, pyroptosis, necroptosis, ferroptosis) during aging, no other newly generated cardiomyocytes can be added to take over the functions of the lost ones. On one hand, the myocardial tissues undergo hypertrophic growth to support heart function, and on the other hand, fibroblasts and inflammatory cells proliferate to form scars for structure repair. Thus, myocardial hypertrophy and fibrosis are two typical features of aged hearts across species. In aged hearts, the heart rate is influenced not only by the loss of cells in the sinoatrial node but also by structural changes in the heart, including fibrosis and hypertrophy. This functional decline would slow the propagation of electric impulses throughout the heart, and impairs the conduction (Curtis et al., 2018). Additionally, the aged hearts also respond differently to cardiac injury compared with the young ones. For instance, aging reduces the clearance of dead cells and the inflammatory cells and fibroblasts are duller in response to ischemic injury, which leads to the formation of weak/loose scars and sensitivity to subsequent cardiac events, such as heart failure (Tracy et al., 2020). The vascular vessels within the myocardial tissue, namely coronary vessels, undergo remodeling and dysfunction. Coronary dysfunction is a key feature of aged hearts and is related to heart failure with preserved ejection fraction (Mishra and Kass, 2021). The deposition of lipids within the coronary vessels also leads to atherosclerosis and myocardial infarction (Tracy et al.,

2020). Aging also reduces the angiogenesis capability of vascular endothelial cells (Das et al., 2018), which is one of the mechanisms underlying reduced myocardial repair post-ischemia in aged hearts.

Cellular alterations

The cellular components or fractions in aged hearts are significantly different compared with the young ones, which is well elucidated by recent single-cell studies (Ma et al., 2021; Schaum et al., 2020). In addition to the loss of cardiomyocytes in the myocardial tissues of the aged hearts as discussed above, the enrichment of immune cells such as macrophages, neutrophils, dendric cells, and T cells is also influenced by aging. For instance, it has been reported the crucial roles of dendritic cells and macrophages in aged hearts in humans and rodents. These immune cells can secrete chemokines and cytokines, such as thymus and activation-regulated chemokine (TARC/CCL17) to facilitate cardiac aging via regulating T helper cells (Zhang et al., 2022f). Many cells within aged myocardial tissues show senescent signatures. Fibroblasts and endothelial cells expressed and secreted typical SASP such as IL-6, plasminogen activator inhibitor 1 (PAI-1), TNFα, CXCLs and MMPs (Mehdizadeh et al., 2022). Senescent vascular endothelial cells undergo an endothelial-to-mesenchymal transition (EndoMT) and fibrogenesis of the aged hearts, which is regulated by cytokines such as TGF-β, endothelin-1 (EDN1) and IL-1 (Piera-Velazquez and Jimenez, 2019). Cardiomyocytes also show senescence, but their hallmarks of senescence are much different from those of proliferative cells such as endothelial cells. In aged hearts, the senescent cardiomyocytes show hallmarks of DNA damage, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, hypertrophic growth, and atypical SASP (e.g., TGF-β2, growth-dependent factor 15 (GDF15) and EDN3), and contractile dysfunction (Tang et al., 2020). Besides, the accumulation of somatic mutations in cardiomyocytes during aging suggests age-associated DNA damage and widespread oxidative genotoxicity. This age-related accumulation of cardiac mutations provides an explanation of the influence of aging on cardiac dysfunction (Choudhury et al., 2022). Depletion of senescent cells reduces aging features of the heart, such as hypertrophy and fibrosis, and prolongs the lifespan of mice (Baker et al., 2016).

The cell changes are largely regulated by the related organelles and the crosstalk among the organelles and nucleus. Mitochondria are the key organelles for the homeostasis of myocardial cells. Cardiomyocytes have a high demand for energy, which evolutionarily results in a high number of mitochondria within cardiomyocytes (Picca et al., 2018). Although endothelial cells have low numbers of mitochondria compared with cardiomyocytes, mitochondria in endothelial cells also regulate endothelial functions such as angiogenesis and paracrine (angiocrine) functions in a crucial manner (Li et al., 2019e). Mitochondria regulate myocardial function not only via supporting energy and regulating oxidative stress but also modulate protein and histone post-translational modifications by supporting donors such as acyl-CoA. For instance, the deregulation of mitochondrial crotonyl-CoA caused by the Enoyl-CoA hydratase, short chain 1 (ECHS1) mutation is a key mechanism underlying aging-related cardiac hypertrophy and fibrosis (Cai et al., 2022b; Tang et al., 2021). The changes in mitochondrial structure and function are observed in the cells (cardiomyocyte, endothelial cell, fibroblast) of aged hearts. In the cells of young hearts, the mitochondria undergo fusion and fission to maintain homeostasis, which is important for normal cell function. The imbalance of these mitochondrial dynamics (fusion and fission) accelerates mitochondrial senescence in cardiac cells and is an important biomarker of cardiac aging and diseases (Picca et al., 2018; Song et al., 2017).

Molecular changes

Elucidation of the molecular pathways involved in cardiac aging helps understand the mechanism of cardiac aging and facilitates the prognosis and diagnosis of aging-related cardiovascular diseases. A variety of important molecular signaling pathways change during heart aging, such as oxidative stress and autophagy. In addition, several recent single-cell transcriptome studies provide important references for revealing the molecular mechanisms of cardiac aging (Choudhury et al., 2022; Emechebe et al., 2021; Ma et al., 2021; Zhang et al., 2022g).

ROS accumulates in the aged heart, leading to higher mitochondrial protein carbonylation along with increased mitochondrial DNA mutations and deletions, which is indicative of mitochondrial oxidative damage in the heart with age (Dai et al., 2012). In addition, as the elevation of ROS can cause oxidative DNA damage, single-cardiomyocyte sequencing has revealed the accumulation of somatic single-nucleotide variants (sSNVs) in aged human hearts, with a higher rate than those in neurons and lymphocytes, pointing to a higher age-related somatic mutation load in the heart (Choudhury et al., 2022). On the contrary, autophagy decreases with age in the heart (Taneike et al., 2010). As mTOR is a key regulator of autophagy (Marín-Aguilar et al., 2020), it is also considered as a critical driver of cardiac aging. For instance, mTOR phosphorylation increases with age in mouse hearts (Hua et al., 2011), whereas the inhibition of mTOR extends lifespan and ameliorates age-related cardiac diseases by promoting autophagy in mice (Dai et al., 2014; Flynn et al., 2013; Harrison et al., 2009). Likewise, sirtuins, a conserved family of NAD^+-dependent deacetylases, have been widely implicated in cardiac aging; the protein expression of multiple sirtuins, including SIRT1, SIRT2 and

SIRT3, has been found to decrease in the heart with age (Li et al., 2018b; Sakamoto et al., 2004; Tang et al., 2017).

In addition to the studies on individual molecular pathways, a variety of potential new biomarkers for cardiac aging have been recently identified with the development and application of single-cell or single-nucleus transcriptomic sequencing techniques. For instance, single-nucleus transcriptomic sequencing of primate ventricles has revealed that the inflammatory factor IL-7 increases during cardiac aging (Ma et al., 2021). Besides, single-nucleus transcriptomic sequencing analysis of primate hearts of different ages has identified forkhead box protein 1 (FOXP1) and forkhead box protein 2 (FOXP2) as key age-downregulated transcriptional regulators whose target genes are associated with various heart diseases (Ma et al., 2021; Zhang et al., 2022g). Consistently, FOXP1-deficient cardiomyocytes derived from human embryonic stem cells exhibit multiple cardiac aging phenotypes, including cellular hypertrophy and senescence (Zhang et al., 2022g). Furthermore, single-cell transcriptome analysis of endothelial cells sorted from mouse hearts at 3 months and 24 months of age has revealed increased expression of spectrin repeat containing nuclear envelope 2 (Syne2) and decreased expression of nestin (Nes) with age (Emechebe et al., 2021).

Secretory factors detectable in biofluids

Aging is strongly associated with an increased risk of various cardiac diseases. Secretory factors detectable in the biofluids such as blood and urine are potentially powerful tools as measurable and quantifiable biomarkers in the diagnosis, prognosis, and surveillance of cardiac aging and related diseases.

Several secretory factors detectable in the blood have been linked with cardiac aging. For example, the level of serum B-type natriuretic peptide (BNP) has been found to increase in aged individuals, while age-related impairment of left atrial strain positively correlates with even higher BNP levels as an independent factor (Yoshida et al., 2019). Likewise, the level of plasma high-sensitivity cardiac troponin T (hs-cTnT) also increases with age, and is often higher in men than women (de Lemos et al., 2010; Saunders et al., 2011). In addition, the levels of serum IL6 and C-reactive protein (CRP) are higher in elderly populations (Puzianowska-Kuźnicka et al., 2016). Consistent with the notion that age is a major contributing factor to the incidence of cardiovascular diseases, the evaluation of circulating secretory factors from individuals without prevalent cardiovascular diseases has revealed that all of the aforementioned biomarkers are positively correlated with increased risks of aging-related cardiovascular diseases (de Lemos et al., 2017; Kuh et al., 2019; Markousis-Mavrogenis et al., 2019; Saeed et al., 2018). Besides, a cohort study revealed that the circulating ceramide- and phospholipid-based risk score is positively correlated with the incidence of cardiovascular diseases (Hilvo et al., 2020).

Compared with the other types of biofluids, urine is the easiest to collect, the least invasive to the patients, and generally more stable, making it highly feasible for biomarker identification and application. As tested in the blood, increased urinary BNP is also associated with higher aging-related CVD risks (Campbell et al., 2020). Increased urinary fibrinopeptide A (FPA) is also associated with angina pectoris and myocardial ischemia (Sonel et al., 2000). In addition, hundreds of proteins and peptides have been identified in urine samples from the patients as putative biomarkers of coronary artery diseases, including CD14, alpha-1-antitrypsin (AAT), collagen types 1 and 3, granin-like neuroendocrine peptide precursor (ProSAAS), membrane-associated progesterone receptor component 1 (PGRMC1), sodium/potassium-transporting ATPase gamma chain (FXYD2) and fibrinogen-alpha chain (FGA) (Delles et al., 2010; Lee et al., 2015).

Summary and perspectives

The aging heart shows changes in function, structure, and cellular/extracellular components. The typical biomarkers of cardiac aging are summarized above and many molecular pathways (Sirtuin, mTOR, AMPK, and FOXO) are involved in cardiac aging. Some outstanding questions remain in the field of cardiac aging. First, some potential hallmarks of cardiomyocyte senescence have been summarized before (Tang et al., 2020), but it is still hard to define and detect cardiomyocyte senescence *in vivo*. Second, many biomarkers of cardiac aging overlap with the pathological characteristics of cardiac diseases in young adults. Further efforts are needed to define biomarkers of physiological aging of the heart in humans. Recent studies have reported that the loss of epigenetic information is a cause of the aging of mammalian tissues, including the heart (Liu et al., 2023b; Yang et al., 2023; Zhang et al., 2022g). It remains unknown what epigenetic marks contribute significantly to cardiac aging and can be used as the biomarker of cardiac aging. Finally, many strategies targeting aging biomarkers have been used to delay aging and disease. Depletion of senescent cells has been used to extend lifespan and reduce aging-related cardiac remodeling in mice (Baker et al., 2016). Further studies in large animals such as monkeys and pigs may promote clinical and translational science (Zou et al., 2022).

Vascular aging

Aging is the main risk factor for vascular disease and the related cardiovascular and cerebrovascular complications, which account for the majority of deaths worldwide (Hamczyk et al., 2020). In a rapidly aging society, biomarkers allowing early detection of individuals who are at high risk of developing vascular disease need to be devel-

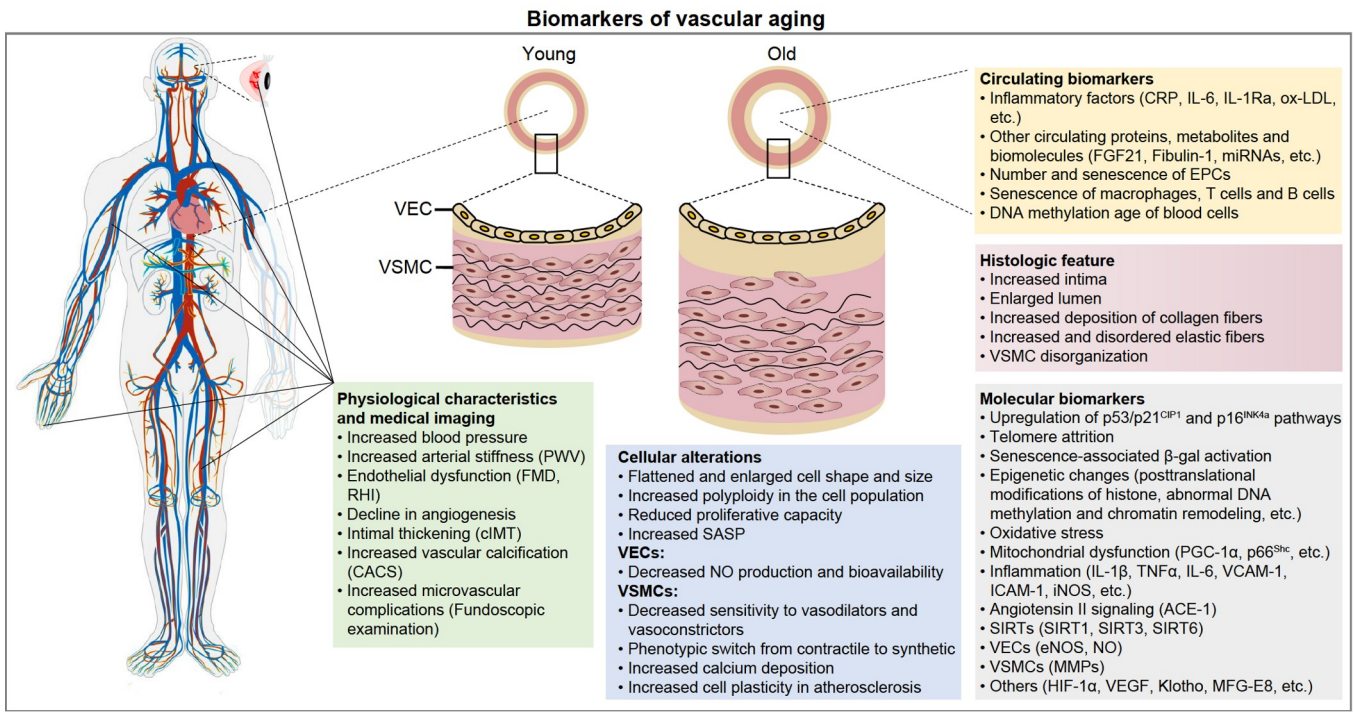

Figure 13 Biomarkers of vascular aging. Abbreviations: SIRT, sirtuin. ACE-1, angiotensin-converting enzyme-1; CACS, Coronary artery calcium scores; cIMT, carotid intima-media thickness; CRP, C-reactive protein; CT, computed tomography; eNOS, endothelial NO synthase; EPC, endothelial progenitor cell; FGF21, fibroblast growth factor 21; FMD, flow-mediated dilation; HIF-1α, hypoxia-inducible factor-1α; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-1Ra, IL-1 receptor antagonist; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; MFG-E8, milk fat globule epidermal growth factor 8; MMP, matrix metalloproteinase; NO, nitric oxide; ox-LDL, oxidized low-density lipoprotein; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1α; PWV, pulse wave velocity; RHI, reactive hyperemia index; SASP, senescence-associated secretory phenotype; SIRT, sirtuin; TNFα, tumor necrosis factor-α; VCAM-1, vascular adhesion molecule-1; VEC, Vascular endothelial cells; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells.

oped to improve primary prevention and reduce the health care and socioeconomic burdens associated with aging. (Figure 13; Table S8 in Supporting Information)

Physiological characteristics

Functionally, aging blood vessels show increased stiffness, decreased sensitivity to vasodilators and vasoconstrictors, and decreased angiogenesis (Ding et al., 2022; Zhang and Tao, 2018). The gold standard for measuring arterial stiffness is the carotid-femoral pulse wave velocity (PWV), the velocity at which the blood pressure wave moves from the carotid to the femoral, whereas the brachial-ankle and heart-ankle PWV are also popular in the clinic (Townsend et al., 2015; Van Bortel et al., 2012). An increase in PWV correlates with chronological age and is associated with an increased risk of CVD and all-cause mortality (Laurent et al., 2001; Mitchell et al., 2010). Elevated blood pressure is also a common feature of aging and the leading contributor to cardiovascular events and mortality (Poulter et al., 2015). Hypertension is often preceded and aggravated by arterial stiffening, whereas it also promotes arterial stiffening, indicating the existence of a positive feedback loop between them (Humphrey et al., 2016).

Vascular endothelial cells (VECs) sense and respond to stimuli from the blood system and regulate vascular smooth muscle cells (VSMCs), by producing NO (vasodilator) or angiotensin II (Ang II, vasoconstrictor) (Tian and Li, 2014; Ungvari et al., 2018). Endothelial health and function is a central mechanism of vascular aging and pathologies. For example, the age-related decrease in endothelium-dependent dilator responses contributes to the decline in angiogenesis, another main characteristic of vascular aging (Ungvari et al., 2018). Endothelial dysfunction also increases susceptibility to atherosclerosis, which is characterized by lipid-rich and inflammatory plaques accumulating in the subintimal space of medium and large arteries (Libby et al., 2019). Impaired endothelial function and disrupted endothelial integrity are considered as the major drivers and early events of atherosclerosis (Libby et al., 2019; Tian and Li, 2014). Endothelial dysfunction can be measured non-invasively by ultrasound using the flow-mediated dilation (FMD) technique (Celermajer et al., 1992; Thijssen et al., 2019). After transient vasoocclusion, brachial artery flow changes after infusion of vasoactive substances modulating nitric oxide release are measured. Whereas FMD examines the macrovascular endothelial function of the conduit brachial artery, peripheral arterial tonometry (PAT) may represent a measure of the function of the peripheral resistance of finger microvessels

after the natural logarithmic transformation of the reactive hyperemia index (RHI) (Kuvin et al., 2003; Thijssen et al., 2019). Several prospective studies have demonstrated that FMD and RHI are both independent predictors of future cardiovascular events (Matsuzawa et al., 2015), but these two methods are not closely related, suggesting that they reflect distinct aspects of vascular function (Hamburg et al., 2011). FMD decreases during aging and is an independent predictor of CVD outcomes (Skaug et al., 2013; Thijssen et al., 2019). RHI is associated with the coronary atherosclerotic burden (Heffernan et al., 2012), but may not reflect age-associated reductions in large artery endothelial function assessed via brachial artery FMD (Babcock et al., 2021).

Imaging traits

With age, carotid intima-media thickness (cIMT) increases (Homma et al., 2001) and is associated with the prevalence, morbidity, and mortality of CVD (Lorenz et al., 2007). It is possible to use ultrasound to measure cIMT in the vascular system as an estimate of the burden of subclinical atherosclerosis in major arteries (Bauer et al., 2012; de Groot et al., 2004). Even measured once (at baseline), cIMT is predictive of future CVD events in the general population after adjusting for a wide range of established CVD risk factors (Lorenz et al., 2007). However, increased cIMT can also reflect nonatherosclerotic processes (Homma et al., 2001; Inaba et al., 2012). In clinical trials, cIMT has been frequently used as a secondary outcome. In this context, absolute or annual cIMT progression is used instead of measuring cIMT on a single occasion based on at least two ultrasound scans over a year (Lorenz et al., 2015). More advanced atherosclerosis stages can be evaluated by quantifying various carotid plaque parameters, such as plaque presence, number, thickness, area, and volume (Naqvi and Lee, 2014), which outperform carotid IMT as a predictor of future CAD events (Inaba et al., 2012).

Vascular aging is also often accompanied by the active deposition of calcium phosphate crystals in both the intima (also called atherosclerotic intima calcification) and the media (also called Mönckeberg sclerosis) layers of blood vessels (Lanzer et al., 2014). Calcifications of both types often occur simultaneously, and imaging techniques sometimes have difficulty distinguishing between them (Kockelkoren et al., 2017). Coronary artery calcium scores (CACS) can be determined using computed tomography (CT), which is considered to be the gold standard technique (Wang et al., 2018c). Despite the fact that CACS could be frequently affected by medial calcification, it is often used for the assessment of atherosclerosis due to its good correlation with coronary plaque burden (Sangiorgi et al., 1998). CACS is highly correlated with chronological age (McClelland et al., 2006) and is the most important predictor of coronary heart disease and all atherosclerotic CVD outcomes combined (Ambale-Venkatesh et al., 2017; Raggi et al., 2008).

Fundoscopic examination is a physical examination technique that allows the visualization of the retina by using only a fundoscope and the naked eye (Gupta et al., 2017). It is recommended to detect diabetic retinopathy for the diagnosis and prevention of visual impairment in diabetic patients (Eppley et al., 2019; Song et al., 2022a). It also allows for the non-invasive identification of increased ocular microvascular abnormalities such as arterial and venous occlusive disease, retinal arteriolar macroaneurysm formation, and embolic events for the prevention and management of both the ocular and systemic complications of hypertension (DellaCroce and Vitale, 2008). In this context, fundoscopic examination could potentially be used for enhanced prognostication and risk reclassification of vascular aging through the assessment of microvascular complications that are tightly associated with vascular stiffness and atherosclerosis (Antonopoulos et al., 2021; Lovshin et al., 2018).

Age-related vasculature alteration is a prominent risk factor for various vascular diseases, including abdominal aortic aneurysm (AAA) and aortic dissection (Bossone and Eagle, 2021). AAA is conventionally defined as the dilation or widening of the aorta to greater than 3.0 cm, with most AAAs being asymptomatic until rupture, which leads to death in 65% of patients (Sakalihasan et al., 2005). The pathophysiological development of AAA involves various processes, including apoptosis and aging of VSMCs, inflammatory infiltration and oxidative stress in the vascular wall, and proteolytic fragmentation of the cellular ECM (Meng et al., 2023). Clinically, AAA is typically monitored with imaging tests (ultrasound or CT scan) over time to ensure that the aneurysm is not growing (Baman and Eskandari, 2022). Aortic dissection is a life-threatening event, during which a primary tear propagates along the aorta, causing catastrophic delamination of the inner (intima with most of the media) from the outer layers (leftover media with adventitia), with an increased susceptibility in elderly individuals (Bossone and Eagle, 2021; Horný et al., 2022). The delamination strength of the human aorta significantly decreases with age (Horný et al., 2022). CT is the gold standard for detecting aortic dissection and may show a dissection flap or aortic dilation; MRI may also be used as an alternative; transesophageal echocardiography (TEE) can be rapidly performed in emergency situations (Shi and Babu, 2021).

Histologic features

Vascular aging can be defined as morphological and functional alterations of the vasculature (Hamczyk et al., 2020; Zhang and Tao, 2018). Morphologically, aging blood vessels exhibit increased deposition of collagen fibers, increased and disordered elastic fibers, disorganized arrangement of VSMCs, enlarged lumens, increased intima, and eventually progressive calcification of the medial layer of the vascular

wall (Ding et al., 2022; Zhang and Tao, 2018).

Cellular alterations

Numerous senescence-associated morphological alterations and functional adaptations occur in constituent cells of the vasculature during aging. For example, most senescent VECs and VSMCs exhibit reduced proliferative capacity, become flattened and enlarged at cellular shape and size, and exhibit increased polyploidy in the cell population and increased SASP (Tian and Li, 2014; Yang et al., 2007). The integrity and functionality of the endothelium are crucial for vascular homeostasis; therefore, VEC senescence contributes to the onset of aging in the vasculature. Both basal and shear stress-stimulated NO production and endothelial NO synthase (eNOS) activity are reduced in senescent VECs (Sato et al., 1993). NO bioavailability is also reduced due to oxidative stress in senescent VECs (Zhang and Gao, 2021). NO can activate telomerase and delay the onset of senescence (Vasa et al., 2000). Senescent VECs attract monocytes to the endothelium and promote the proliferation and migration of VSMCs (Csiszar et al., 2012; Urbanek et al., 2016).

The responses of VSMCs to contractile and relaxation factors, including NO and β-adrenoreceptor agonists, are also decreased in aging (Ding et al., 2022). Another main characteristic of VSMC senescence is the phenotypic switch from contractile to synthetic. Senescent VSMCs secrete proinflammatory cytokines and MMPs. These SASP factors promote the chemotaxis of monocytes/macrophages and stimulate adjacent non-senescent VSMCs or VECs to release cytokines and express adhesion molecules, thus participating in or driving chronic vascular inflammation and diseases (Gardner et al., 2015). Senescent VSMCs also increase calcium deposition and promote the expression of calcification regulatory factors, leading to the mineralization of VSMCs and the calcification of blood vessels (Fakhry et al., 2017; Nakano-Kurimoto et al., 2009). In addition, the production of elastase in VSMCs and fibronectin in VECs contributes to fibrosis in vascular aging (Johnson, 2007; Leon and Zuckerman, 2005). In atherosclerosis, VSMCs are more plastic and can adopt alternative phenotypes, including phenotypes resembling foam cells, macrophages, mesenchymal stem cells, and osteochondrogenic cells, which could contribute both positively and negatively to disease progression (Basatemur et al., 2019).

Molecular changes

As aging includes various biological processes, it is difficult to attribute vascular aging to one or a few molecules. Similar to other types of cells, upregulation of the p53/p21^{CIP1} and p16^{INK4a} pathways, telomere attrition, and SA-β-gal activation are observed in VECs and VSMCs during aging (Hernandez-Segura et al., 2018; Minamino and Komuro, 2007; Wang et al., 2021c). These are identified as classical biomarkers of cellular senescence in the vasculature. Vascular aging could be pre-determined genetically. For example, a mutation at the LMNA or WRN locus could cause Hutchinson-Gilford progeria syndrome in children or Werner's syndrome in adults, respectively. Both syndromes are associated with premature death due to accelerated vascular aging and cardiovascular events (Burtner and Kennedy, 2010; Lebel and Monnat, 2018). Emerging evidence has also demonstrated that epigenetic changes during aging, including altered posttranslational acetylation and methylation of histones, abnormal DNA methylation, and chromatin remodeling, are closely associated with vascular aging (Wang et al., 2022a; Wang et al., 2021c; Wu et al., 2018; Zhang et al., 2018b). In addition, oxidative stress, which induces DNA damage and telomere shortening, is also a senescence biomarker (Kubben et al., 2016; Kurz et al., 2004). As a major source of ROS, mitochondrial integrity and function decline with aging, and several mitochondria-related molecules were found to be potential biomarkers of vascular aging, including peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α, p66^{Shc} and SIRT3 (Liberale et al., 2020; Wang et al., 2021c).

Another process involved in vascular aging is inflammaging. The NF-κB pathway and immune cells are major players in this process. NF-κB activation upregulates the expression of inflammatory cytokines and cellular adhesion molecules, including IL-1β, TNFα, IL-6, vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), inducible nitric oxide synthase (iNOS), monocyte chemo-attractant protein-1 (MCP-1), and cyclooxygenase-2 (COX-2) (Wang et al., 2021c). Angiotensin II signaling, which regulates many of the stimuli and signals that govern vascular aging, activates the NF-κB and p53/p21^{CIP1} pathways (Kunieda et al., 2006; Miyauchi et al., 2004). The activity and expression of angiotensin-converting enzyme-1 (ACE-1) are obviously increased during aging (Wang et al., 2003). SIRTs constitute a class of proteins involved in vascular aging, especially SIRT1, SIRT3, and SIRT6. Their activity declines in VECs and VSMCs during aging, and their overexpression counteracts vascular aging (Grootaert and Bennett, 2022). Furthermore, with cutting-edge technologies, a series of molecules have been found to be associated with or functionally contribute to vascular aging, including hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), Klotho, FOXO1A, FOXO3A, adrenoceptor beta 2 (ADRB2), milk fat globule epidermal growth factor 8 (MFG-E8), natriuretic peptide receptor A (NPRA), and APOE (Cai et al., 2022d; Long et al., 2022; Wang et al., 2021c; Zhang et al., 2020e).

Secretory factors detectable in biofluids

Aging affects the levels of proteins, metabolites, and other

biomolecules in the blood. As inflammation is highly associated with vascular aging, the protein levels of inflammatory factors in circulation, such as circulating CRP, IL-6, IL-1 receptor antagonist (IL-1Ra), and oxidized low-density lipoprotein (ox-LDL), are potential biomarkers of vascular aging (Durham et al., 2018; Gopcevic et al., 2021; Ishigaki et al., 2009). The circulating fibroblast growth factor 21 (cFGF21) concentration is positively correlated with age (Yang et al., 2022a), and high levels of cFGF21 are closely associated with an increased risk of CVDs (Zhang et al., 2021h). Circulating fibulin-1 is positively correlated with brachial-ankle PWV and is an independent risk factor for arterial stiffness (Luo et al., 2022a). Other circulating factors, including oxidative stress and miRNAs, have also been reported as potential biomarkers of vascular aging (Du et al., 2021; Gopcevic et al., 2021).

Aging also causes changes in the cells and in the blood. One of the well-identified factors that indicates vascular aging in the circulation is bone marrow-derived endothelial progenitor cells (EPCs). EPCs are known to participate in postnatal neovascularization and vascular repair. Aging reduces the number and promotes the senescence of EPCs, resulting in a decline in angiogenesis and vascular healing (Buffa et al., 2019). Moreover, the senescence of macrophages, T cells, and B cells is also associated with vascular aging. Downregulation of ATP-binding cassette transporter A1 (ABCA1) and abnormal polarization are characteristics of senescent macrophages (Sene et al., 2013). Senescent macrophages impair cholesterol efflux, increase cytokine expression and premature monocyte recruitment, and promote extracellular matrix degradation (Childs et al., 2016; Sene et al., 2013). The increase in the $CD8^{+}CD28^{-}$ T-cell proportion within the circulating CD8 subset is one of the most prominent changes during aging and T-cell senescence in humans (Weng et al., 2009). Senescent T cells are highly proinflammatory and release a large amount of interferon-γ (Franceschi et al., 2000; Leon and Zuckerman, 2005). It also induces direct lysis of ECs and VSMCs by releasing high levels of perforin and granzymes (Johnson, 2007). A major effect of aging on B cells is a significant decrease in the percentage of switched memory B cells ($IgD^{+}CD27^{+}$) and a significant increase in the percentage of naïve ($IgD^{+}CD27^{-}$) and double-negative memory ($IgD^{-}CD27^{-}$) B cells in the blood (Frasca et al., 2020). Senescent B cells display an enhanced ability to take up, process, and present antigens to T cells and contribute to local inflammation by secreting proinflammatory factors (Sage et al., 2019). In addition, by identifying the specific CpG sites undergoing age-related changes in methylation, the calculated DNA methylation age (DNAmAges, also called epigenetic clocks) of blood cells could be a good predictor of all-cause and CVD mortality (Hamczyk et al., 2020; Marioni et al., 2015).

Lung aging

The main function of the lung, with the largest surface area, is to exchange gas with the outside environment. And lung tissue homeostasis is critical for life and health. Accumulating evidence shows that lung function is facing progressive impairment with age, marked by physical, mechanical and structural changes that hamper gas exchange (Bowdish, 2019; Roman et al., 2016). The characteristics of aging lung are weaking of respiratory muscles (particularly the diaphragm), and stiffening of the chest wall (Schuliga et al., 2021). With the outbreak and prevalence of COVID-19, pneumonia-induced acute respiratory distress syndrome (ARDS), particularly in elderly people, results in global healthcare crises and severely strains health resources.

The lung is a complex and multicellular organ, including alveolar epithelial cells, vascular endothelial cells, airway epitheliums, fibroblasts, macrophages, platelets, neutrophils. The cellular and molecular regulatory processes of lung undergo changes over a lifetime. Gaining more insight into the lung-intrinsic changes that occur with aging is crucial for development of high effective clinical treatment.

Aging causes lung susceptibility to various respiratory diseases, such as chronic obstructive pulmonary disease (COPD), ARDS, interstitial pulmonary fibrosis (IPF), and pneumonia (Crook et al., 2021).

Advanced age is the main risk factor for chronic respiratory diseases. It is proved by the data that the mortality rate caused by COVID-19 is 20 times higher for the elderly people over 80 years old compared with people in their 50s (Strangfeld et al., 2021; Williamson et al., 2020). However, the fundamental mechanisms driving the aging process in the lung remain poorly understood. Here we mainly discuss the cellular alterations and molecular changes in the lung with advancing age (Figure 14; Table S9 in Supporting Information).

Physiological characteristics

On the whole, with the growth of age, lung function will deteriorate significantly (Skloot, 2017; Vaz Fragoso and Lee, 2012). Due to the weakening of chest wall function and respiratory muscle strength, the ability of aging lungs to clear mucus and foreign matters is impaired, which causes the elderly to be more susceptible to pneumonia. The ribs harden with age, and the shape of the thorax changes accordingly, affecting the normal implementation of lung function (Tolep et al., 1995). The curvature of the spine can reduce the volume of the thoracic cavity in the elderly. Although the elastic recoil force decreases with age due to the reduction of respiratory intensity and chest wall compliance, the total vital capacity does not change significantly with age (Enright et al., 1994; Sharma and Goodwin, 2006). According to statistics, lung function has declined since the age of 35.

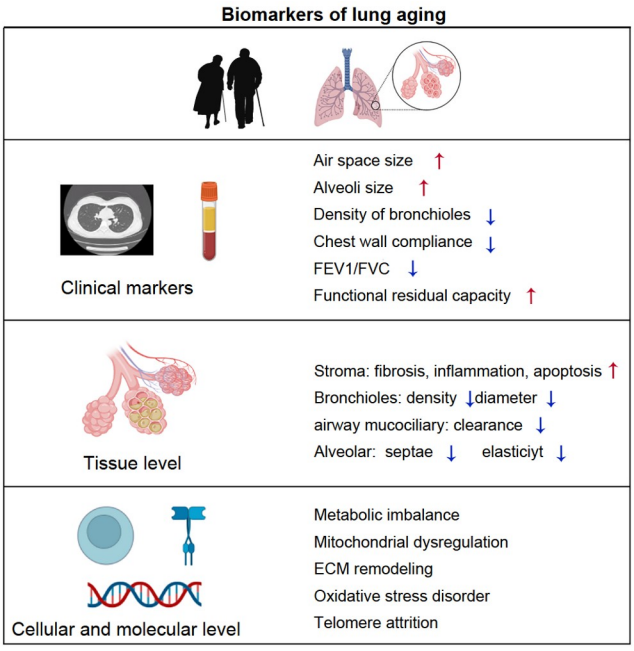

Figure 14 Biomarkers of lung aging. During the aging process of lung tissue, the molecular level, cell level and tissue level have changed in varying degrees. Understanding these alterations can help us to develop more efficient treatment methods for elderly lung tissue.

Forced expiratory volume (FEV_1) decreases by 30 mL per year and forced vital capacity (FVC) declines by about 20 mL per year (Stam et al., 1994; Verbeken et al., 1992; Xu et al., 1995). Due to the heterogeneity and high variability of the lung in the aging population, the current standard for lung function in the elderly still needs to be further clarified.

Imaging traits

Recognition of the lung CT characteristics of "normal" aging is more important to differentiate from clinically significant disease. Compared with young people, the prevalence of lung and airway cysts, reticular structures, air trapping, bronchiectasis and bronchial wall thickening in the elderly increased. The chest imaging features related to aging show vascular curvature and calcification, mediastinal lipomatosis, diaphragmatic bulge and protrusion, and musculoskeletal features, such as chest osteophyte and costal cartilage calcification (Copley, 2016; Ensor et al., 1983).

Histological features

Aging of lung is associated with mechanical, structural and physiological alterations that compromise lung function. The organization, concentration and form of ECM protein in the lung will change with age. Disordered and excessive ECM destroys the normal architecture of the lung. In the elderly lung, the number of alveolar attachments does not change, but the size of the alveoli and the surface of alveolar-capillary marked increase. Changes in alveolar depth and acinar airway lumen are related to advanced age (Quirk et al., 2016; Schuliga and Bartlett, 2019).

The fundamental mechanisms driving the aging process in the lung remains poorly understood. Here we mainly discuss the cellular alteration and molecular changes in the lung with advancing age.

Cellular alterations

Development of single-cell transcriptomic and *in vivo* lineage tracing technology provides a more comprehensive perspective for understanding the complexity of cellular in the lung (Schupp et al., 2021; Travaglini et al., 2020; Wang et al., 2021d). 144 cell subtypes have been identified during lung development through the integrated analysis of multiple omics (He et al., 2022). Although technological advances have made some progression in the study of lung tissue, the changes of cells in the process of aging are not completely clear. Pulmonary function would decrease with age in the absence of illness. So, it is fundamental to understand the cellular alteration in the aging process of lung tissue.

Aging results in increased transcriptional noise, suggesting deregulated epigenetic control. Single-cell transcriptional analysis showed that chronological aging would increase the gene characteristics related to cholesterol biosynthesis in type 2 alveolar epithelial cells (AT2) cells and lipid fibroblasts, leading to the increase of neutral lipid content in epithelium and fibroblasts with age (Angelidis et al., 2019). Compared with young mouse lung, ciliated cell marker gene signature is immensely upregulation in aged mouse. The increase of ciliated cells leads to the altered frequency of club to ciliated cells in aged mouse airways (Schneider et al., 2021). One of the key features of aging is the exhaustion or dysfunction of stem/progenitor cells. AT2 is the lung tissue resident progenitor cell with the potential to differentiate into AT1 after injury (Choi et al., 2020; Desai et al., 2014; Salahudeen et al., 2020). Some studies show the number of AT2 does not change with age, but the capacity of self-renew and differentiation reduces (Watson et al., 2020b). The number of basal cells and club cells decreases with age (Ortega-Martínez et al., 2016; Wansleeben et al., 2014). By clearing the senescent cells in the tissue microenvironment, the lung function of the aged mice can be improved to a certain extent (Childs et al., 2015; Xu et al., 2018b).

The human respiratory system is a crucial immune interface to respond to different stimuli from the outside environment. Chronic antigen stimulation and continuous accumulation of oxidative free radicals can lead to the production of pro-inflammatory cytokines during aging. Alveolar macrophages (AMs), the largest proportion of resident immune cells in lung tissue, play a key role in recognition, initiation and elimination of the host defense against external microbes (Baasch et al., 2021; Gorki et al., 2022; Zhang et al., 2021i). Single-cell transcriptome data and experimental

result demonstrate that the lung environment drives an age-associated resistance of AMs to proliferation that persisted during influenza A viral infection. This change is caused by the increase of hyaluronan in the extracellular matrix in the lung tissue of aged mice (McQuattie-Pimentel et al., 2021). The phagocytic capacity of AMs is age-related, resulting in impaired or delayed clearance of pulmonary pathogens (Li et al., 2017d; Wong et al., 2017). Neutrophil extracellular traps (NETs) play a fundamental role in immune regulation, pathogen clearance and disease pathology (Castanheira and Kubes, 2019; Hidalgo et al., 2022; Papayannopoulos, 2018). NETs-mediated pathogen destruction and age-related changes further lead to impaired bacterial clearance and may increase the susceptibility of the elderly to infection (Brinkmann and Zychlinsky, 2007). In healthy elderly people, the proportion of neutrophils in bronchoalveolar lavage fluid (BAL) increased and the percentage of macrophages decreased (Corberand et al., 1981). The reduction of $CD4^+$ and $CD8^+$ T cells in the elderly can damage the immunity to influenza vaccination and the cytotoxicity to influenza virus (Zhou and McElhaney, 2011).

Molecular changes

Moloney murine leukemia virus 1 (PIM1) and its target nuclear factor of activated T cells-1 (NFATc1) is identified as putative driving factors of sustained profibrotic gene signatures in IPF damaged aging fibroblasts (Pham et al., 2022). Microarray results demonstrate multiple genes undergo changes during age, such as IGF and TGFβ signalling pathways related to proliferation and differation (Watson et al., 2020b). With the increase of age, the clearance rate of mucociliary in respiratory system slows down, which determined by ciliary beat frequency. Increased oxidative stress activates protein kinase Cε (PKCε) signaling, thereby reducing ciliary beat frequency (Bailey et al., 2018).

After injury, vascular endothelial cells (ECs) play an active role in regulating lung stem cells by secreting angiocrine factors. Aging leads to reprogram of transcriptional signature in endothelial cells, which reduces the regeneration ability of aging lung after same insult as young mouse. Endothelial cells derived neuropilin-1 (NRP1)/hypoxia-inducible-factor 2α (HIF2α) suppresses anti-inflammatory and anti-thrombotic endothelial protein C receptor (EPCR) pathway. Blockade of NRP1 or HIF2α in ECs is able to restore regenerative capacity of aging organs (Chen et al., 2021d). Age-related alteration in endothelial vasodilation is attributed to diminishment of eNOS activity and NO production (Cho and Stout-Delgado, 2020). Persistent injury of lung results in suppression of CXCR7 expression and recruitment of endothelial growth factor receptor 1 (VEGFR1)-expressing macrophages. This recruitment activates Wnt/β-catenin-dependent persistent upregulation of Notch ligand Jagged1 in pulmonary capillary endothelial cells (PCECs), which in turn initiates exuberant Notch signaling in perivascular fibroblasts and aggravate fibrosis (Cao et al., 2016).

Secretory factors detectable in biofluids

Normal and accelerated aging would cause considerable differences in the outcomes of some chronic diseases, the later often represent higher mortality (Justice et al., 2018). How the pathological process related to aging drives the development of lung related diseases is not completely clear, the correlation between age-related biomarkers and the severity of IPF has been confirmed. The shorter length of leukocyte telomere, the lower the survival rate of patients with IPF (Stuart et al., 2014; Townsley et al., 2016). Some studies access the relationship between aging biomarkers and interstitial lung abnormalities (ILAs). The results show the increasing plasma concentration of GDF15, IL-6, TNFα and CRP is associated with increased odds of ILA presence (Oldham, 2021; Sanders et al., 2021; Zhang et al., 2019d). The concentration of Krebs von den lungen-6 (KL-6) produced by damaged or regenerating alveolar type Ⅱ pneumocytes in serum is significantly related to the prognosis of patients with COVID-19 (d'Alessandro et al., 2020). The severity of COVID-19 is positively correlated with the plasma myeloperoxidase (MPO)-DNA complexes concentration (Middleton et al., 2020).

Skeletal muscle aging

Skeletal muscle is the largest tissue by mass in the body and has a pivotal role in regulating posture, movements, respiration, and metabolism. Healthy muscle possesses the unique ability to regenerate and can fully recover its functions following routine damage or acute injuries, largely owing to the existence of muscle stem cells (MuSCs, also known as muscle satellite cells) (Sousa-Victor et al., 2022). Peaking in early adulthood, muscle mass and strength gradually decline after the age of 40 years, followed by a more prominent decline afterward with a faster decrease in strength (Dodds et al., 2014; Lang et al., 2010; McGregor et al., 2014), largely due to more substantial muscle fiber atrophy and loss (Wilkinson et al., 2018). Skeletal muscle aging is characterized by the loss of not only muscle mass but also muscle function, commonly referred to as age-related sarcopenia (Cruz-Jentoft and Sayer, 2019; Dennison et al., 2017). Sarcopenia leads to a variety of adverse outcomes that are highly associated with morbidity and healthcare costs (Dennison et al., 2017). It is anticipated that the prevalence of sarcopenia will become a major public health problem in the years to come. However, the progression of age-related sarcopenia is influenced by complex interactions between genetic and environmental lifestyle factors (Dennison et al., 2017). The development of sensitive and specific biomarkers for aging-related sarcopenia has been a daunting challenge

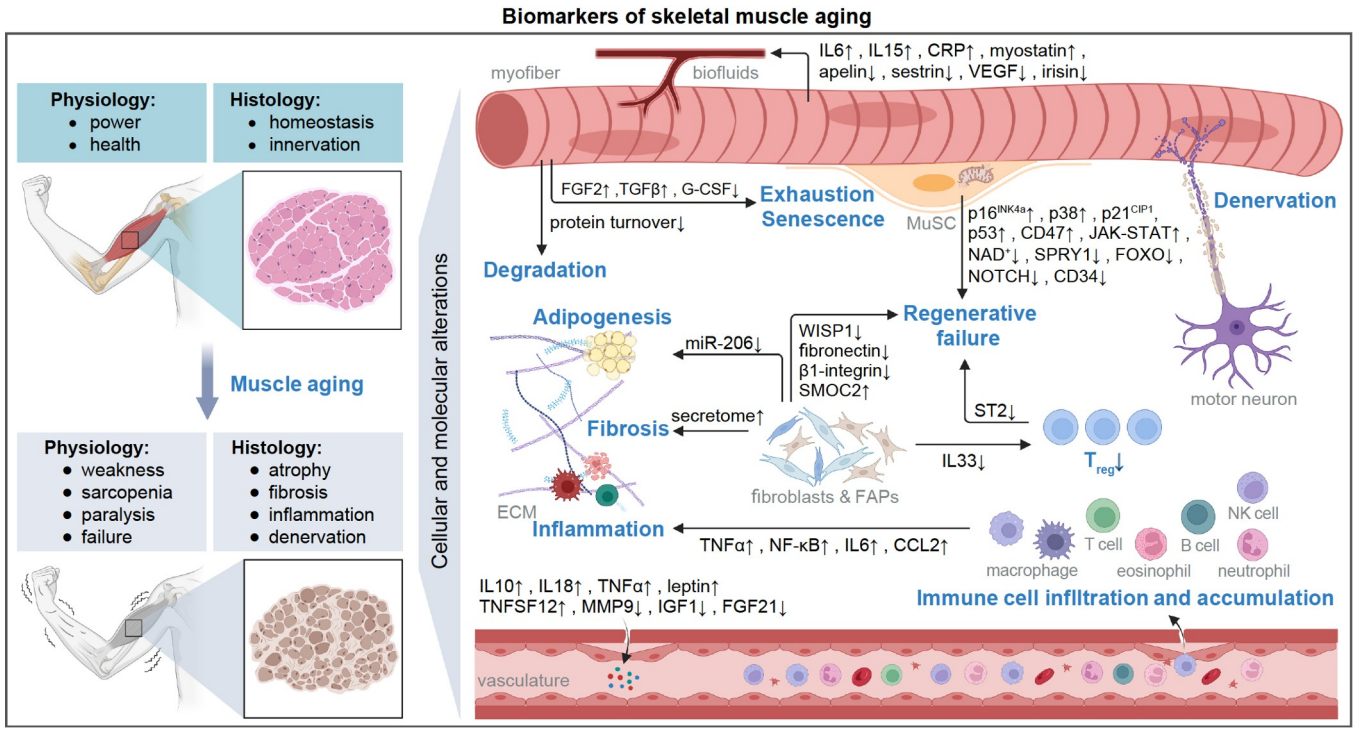

Figure 15 Biomarkers of skeletal muscle aging. Functional decline of skeletal muscle in aging results from the synergistic derangement of myofiber, MuSCs, fibroblasts and FAPs, vasculature, extramyofibril matrix (or ECM), as well as immune and neuron systems. Decreases of myofiber size and number due to aberrant protein turnover, impairment of innervation of motor neurons to myofibers, and MuSC exhaustion- or senescence-dependent failure of regeneration, are the main causal factors of aging-related muscle atrophy. MuSC senescence can be triggered by intrinsic molecular signaling dysregulations and mitochondrial dysfunction as well as disruption of extrinsic cellular interactions involving myofibers, FAPs, macrophages, regulatory T cells, etc. Aged skeletal muscle is also often accompanied by fibrosis and adipose deposition, mainly due to elevated matrix secretome and increased adipogenic conversion of FAPs. Aging-related alterations in ECM can induce chronic inflammation featured by pro-inflammatory cytokines accumulation, which putatively stems from recruitment of both tissue-resident immune cells and those infiltrated from circulation. In addition, aging-associated dynamic changes of various secretory factors either from myofibers or from other types of cells, particularly those detectable in the biofluids, in sarcopenic patients and mouse models, can be potentially employed for accurately monitoring aging-induced myopathies. Abbreviations: TNFSF12, TNF superfamily member 12; MMP9, matrix metallopeptidase 9; IGF1, insulin like growth factor 1; FGF2, fibroblast growth factor 2; Spry1, sprouty RTK signaling antagonist 1; FOXO, forkhead box, sub-group O; Notch, notch receptor; CD34, CD34 molecule; WISP1, WNT1 inducible signaling pathway protein 1; SMOC2, SPARC-related modular calcium binding 2; ST2, suppression of tumorigenicity 2.

(Calvani et al., 2015), given the poorly understood molecular and cellular mechanisms that involve a myriad of intrinsic and extrinsic factors in driving skeletal muscle aging (Tieland et al., 2018). Hence, it is of critical importance to unravel the molecular and cellular biomarkers of skeletal muscle aging for more accurately defining and monitoring sarcopenic states, ultimately offering new opportunities to translate our understanding of its pathophysiology into improved diagnosis, effective treatment, and preventive strategies for healthy muscle aging (Figure 15; Table S10 in Supporting Information).

Physiological characteristics

Advanced aging of skeletal muscle results in sarcopenia, meaning "loss of flesh" in the Greek phrase, which was first described as an age-associated decline in lean body mass (Rosenberg, 1997). Despite the ongoing debate on the best approach for defining sarcopenia, it is currently viewed as a progressive skeletal muscle disorder with accelerated loss of muscle mass and function (Cruz-Jentoft and Sayer, 2019; Sayer and Cruz-Jentoft, 2022). The clinical criteria for the diagnosis of sarcopenia follow the guidelines of the Asian Working Group for Sarcopenia (AWGS), European Working Group on Sarcopenia in Older People (EWGSOP2), International Working Group on Sarcopenia, and the Society of Sarcopenia, Cachexia and Wasting Disorders (SSCWD). The main features of sarcopenia include loss of muscle mass and strength, alterations in cellular composition and innervation, infiltration of ectopic fat (i.e., myosteatosis) with fibrotic signatures, along with declining regenerative capacity arising from the degenerative aging of MuSCs (Sousa-Victor and Muñoz-Cánoves, 2016). Consequently, sarcopenia is associated with deterioration of muscle quality, impairment of mobility, and poor physical performance (Correa-de-Araujo et al., 2017), which leads to many adverse health outcomes including falls, fractures, frailty, and even mortality in the elderly (Cruz-Jentoft et al., 2019). Among these, the physiological phenotypes of frailty in association with

muscle aging can be assessed based upon the following parameters: weak grip strength, slow gait speed, low-level physical activity, self-reported low energy level, and unintentional body weight loss (Fried et al., 2001). Conceivably, a combination of multiple biomarkers at molecular, cellular, and tissue levels will be necessary to precisely and collectively reflect the aging-dependent pathological changes of muscle that underlie these functional declines in physical performance.

Imaging traits

The diagnosis of muscle aging, or sarcopenia, requires measurement of both muscle mass and muscle strength, along with an evaluation of physical performance. Several imaging techniques have been available for estimating muscle mass and quality, including dual-energy X-ray absorptiometry (DXA), CT, MRI, and ultrasound (US) (Albano et al., 2020; Boutin et al., 2022; Chianca et al., 2022). With the advantage of being accurate, DXA is the only radiological tool that is most commonly used in clinics with accepted cutoff values for the diagnosis of sarcopenia (Cruz-Jentoft et al., 2019). As the reference standards, CT and MRI can be used for evaluating muscle quality and fatty infiltration, but the lack of consensus cutoff values for identifying sarcopenia has limited their application merely to research purposes (Csapo et al., 2014; Lenchik and Boutin, 2018). While the current application of US in sarcopenia is limited, all these imaging modalities can provide quantitative results and are reproducible and comparable over time. However, more advancements in imaging technologies based on new biomarkers of muscle aging are needed to overcome the major limitations of the current imaging approaches, e.g., variability in the results and inconsistent use of cutoff points, particularly in the assessment of muscle quality and function in relation to physical performance.

Histologic features

In parallel to the loss of muscle mass and function, the most apparent histological feature of muscle aging is the gradual decline of myofibers in both size and number, with type II (fast-twitch and glycolytic) myofibers deteriorating utmost, which has been considered as one of the prominent contributors to aging-induced sarcopenia (Frontera et al., 2000; Larsson et al., 1978; Lexell, 1995; Lexell et al., 1988). This is perhaps due to both aberrant protein turnover (protein synthesis, degradation, and sarcomere protein folding) (Murgia et al., 2017) and selective reductions of MuSCs (Verdijk et al., 2007) residing along type II myofibers. Another cause of muscle atrophy is the degeneration of the motor unit governing muscle contraction. In homeostatic young muscle, motor neurons directly interact with myofibers at a specialized central region called the neuromuscular junction (NMJ) (Li et al., 2018a; Pannérec et al., 2016; Tintignac et al., 2015). With aging, this interface becomes broken (Courtney and Steinbach, 1981; Jones et al., 2017; Larsson et al., 2019). Investigations into muscle degradation have provided clues suggesting that myofibers play directing roles in maintaining NMJ physiology in aging (Carnio et al., 2014; Li et al., 2011c; Masiero et al., 2009). However, it is as yet unknown whether NMJ degeneration is a causal factor of muscle aging or *vice versa*.

In the extramyofibril matrix, a robust aging-related alteration is the infiltration of adipose and connective tissue (Delmonico et al., 2009; Kragstrup et al., 2011). Overt fibrogenic activation of fibro-adipogenic progenitors (FAPs) (Brack et al., 2007; Brunet et al., 2023) and the failure of immune defense and clearance towards fibrogenic activity (Heredia et al., 2013) are the main causal factors, which ultimately lead to fibrosis. Besides, the vascular system, the intramuscular compartment in supporting and nourishing, undergoes significant changes during aging. For instance, microvasculature capillaries are in close proximity with MuSCs (Christov et al., 2007), providing factors to maintain MuSC stemness (Verma et al., 2018). While in aging, blood vessels in cardiac vasculature suffer increased permeability, tend to be stiff, and risk calcification and atherosclerosis (Harvey et al., 2016; Lacolley et al., 2018; Lacolley et al., 2017). It remains to be explored whether similar changes occur in aging skeletal muscle.

Cellular alterations

Apart from the syncytial myofibers, skeletal muscle contains various types of mononucleated cells. These populations undergo distinct fate decisions that exacerbate muscle weakness with aging (Almada and Wagers, 2016).

MuSCs are prerequisite precursors for muscle regeneration. Aging is accompanied by a dramatic decline of MuSCs, both in quantity and quality (Bengal et al., 2017; Sousa-Victor et al., 2022). Studies based on human and mouse models have revealed numerous hallmarks accompanying or driving MuSC senescence. Intrinsically, increased $p16^{INK4a}$ and deep quiescence (Sousa-Victor et al., 2014), increased p38 MAPK and reduced self-renewal (Bernet et al., 2014), decreased NAD^+ (Zhang et al., 2016) and mitochondrial fragmentation (Baker et al., 2022; Tezze et al., 2017), reduced autophagy (García-Prat et al., 2016) and functional heterogeneity (Tierney et al., 2018), loss of ciliation (Palla et al., 2022), decreased CD34 (García-Prat et al., 2020), FOXO (García-Prat et al., 2020; Jing et al., 2022) and Notch (Carlson et al., 2008; Conboy et al., 2003) signaling, elevated activation of CD47 (Porpiglia et al., 2022) and JAK-STAT signaling (Price et al., 2014; Tierney et al., 2014), have all been proven to be the prominent detriments to MuSC stemness and cause regenerative failure. Aging in the MuSC niche contributes another core source releasing secreted factors that drive MuSC senescence. Increased secretion of

FGF2 (Chakkalakal et al., 2012) and TGF-β (Carlson et al., 2008) from aged myofiber, together with loss of fibronectin (Lukjanenko et al., 2016) and β1-integrin (Rozo et al., 2016) in the niche, prohibits MuSC quiescence and leads to stem cell pool exhaustion. Notably, many of the above-mentioned regulators have been investigated as therapeutic targets in treating aging- or pathogen-induced myopathy in mouse models, and a few of them have been translated into human clinical trials (Elhassan et al., 2019).

Similar to many other organs, aged muscle accumulates increased adipose and connective tissue (Csapo et al., 2014; Zoico et al., 2010), a final outcome of fibrosis (Mann et al., 2011; Wynn, 2008). In young skeletal muscle, FAPs are indispensable intermediates promoting MuSC differentiation and muscle regeneration (Heredia et al., 2013; Joe et al., 2010; Uezumi et al., 2010; Wosczyna et al., 2019). During aging, however, FAPs become dysregulated, produce elevated matrix proteins, and tend to be the major cell type inducing fibrosis (Schüler et al., 2021). With the induction of matrix alterations, MuSCs initiate fibrogenic conversion in both morphology and the fibrotic gene program (Brack et al., 2007; Stearns-Reider et al., 2017). Importantly, aged FAPs gain increased adipogenic potential (Uezumi et al., 2011; Wosczyna et al., 2021), serving as the main source of adipose deposition, which hampers muscle regeneration and induces prolonged inflammation.

Aging-related chronic inflammation is predominantly driven by accumulated pro-inflammatory chemokines/cytokines, as well as their activating regulators such as IL-6, TNFα and NF-κB (Degens, 2010; Merritt et al., 2013; Peake et al., 2010; Schaap et al., 2006). Notably, both innate and adaptive immune cells are privileged in promoting muscle regeneration after injury (Arnold et al., 2007; Burzyn et al., 2013; Heredia et al., 2013; Ziemkiewicz et al., 2021). During aging, however, due to adipose infiltration and fibrosis in the extramyofibril matrix (Dennison et al., 2017; Kalinkovich and Livshits, 2017), as well as muscle cell disturbance (Wang et al., 2018d), myeloid cells (Wang et al., 2018d), especially macrophages (Chazaud, 2020; Wynn and Barron, 2010) and T cells (Kuswanto et al., 2016), exhibit prolonged and altered immune defense, ultimately leading to chronic inflammation and muscle fibrosis.

While plenty of elaborate mechanisms have been revealed towards understanding the cellular aging of muscle, it is still a narrow horizon for capturing the overall hallmarks. In the past ten years, the advanced single-cell transcriptomics analyses have enabled systemically dissecting and monitoring all cell type heterogeneity and their aging dynamics both in human (Barruet et al., 2020; De Micheli et al., 2020; Perez et al., 2022; Rubenstein et al., 2020) and model organisms (Dos Santos et al., 2020; Jing et al., 2022; Kim et al., 2020b; Petrany et al., 2020; Zhang et al., 2022d). With mammalian aging, skeletal muscle exhibits decrease in MuSCs, Schwann cells, and vascular cells but infiltration of various immune cells and fibroblasts (Kedlian et al., 2022), in parallel with physiological dysfunction and chronic inflammation. In addition, together with spatial transcriptomics (Wang et al., 2022f), new hallmarks of muscle aging driven by pro-inflammatory cytokines such as CCL2 (Kedlian et al., 2022; Moiseeva et al., 2023) may be defined.

Molecular changes

Numerous molecular changes have been uncovered during skeletal muscle aging, gaining mechanistic insights into the functional decline of MuSCs in their regenerative potential and ability to give rise to differentiated myocytes, as well as the decreased myofiber size resulting from the imbalance between anabolism and catabolism of muscular proteins. Elucidation of the molecular signatures of these dynamic processes may provide representative molecular biomarkers that can more accurately trace and monitor the progression of muscle aging.

MuSCs comprise a heterogeneous population. Specific subpopulations of MuSCs selectively decrease or increase during aging. It was reported that the $Pax7^{Hi}$ subpopulation of MuSCs is dramatically reduced in aged mice. Mechanistically, myofiber-secreted granulocyte colony-stimulating factor (G-CSF) is decreased during aging, which in turn compromises the asymmetric division of MuSCs and leads to the loss of the $Pax7^{Hi}$ subpopulation (Li et al., 2019c). A $CD47^{Hi}$ subpopulation of MuSCs has also been identified, which is increased in aged mice and found to impair muscle regeneration via thrombospondin-1/CD47 signaling (Kania et al., 1995; Porpiglia et al., 2022). Except for the subpopulation changes, MuSC quiescence is generally disrupted, and their self-renewal capabilities are remarkably compromised during aging. Active $p16^{INK4a}$/Rb axis in aging switches MuSCs from a reversible quiescent state into an irreversible cell cycle arrest (Sousa-Victor et al., 2014). The increased epigenetic marker H3K27me3 in aging might contribute to the disruption of MuSC quiescence (Liu et al., 2013). The elevated level of FGF2 produced by aged myofibers was shown to induce quiescent MuSCs into active cell division, which is responsible for reducing MuSC quiescence in homeostatic conditions and results in a state of persistent activation, leading to MuSC pool depletion (Chakkalakal et al., 2012). Notch signaling downregulated in aged MuSCs can stimulate their spontaneous differentiation and result in the depletion of MuSCs from the stem cell pool (Bjornson et al., 2012; Liu et al., 2018b).

Myofiber atrophy occurs during aging as a result of decreased anabolism and increased catabolism of proteins (Fealy et al., 2021). The ubiquitin proteasome system is one of the major pathways that regulate muscle protein degradation, and it plays a central role in controlling myofiber size by elevating protein catabolism via induced expression of mus-

cle specific E3 ubiquitin ligase TRIM63 (MuRF1) and F-box protein 32 (Fbxo32) with advanced age (Gumucio and Mendias, 2013). Dkk3 was reported to induce nuclear import of β-catenin and enhance its interaction with FoxO3, which in turn activates the transcription of E3 ubiquitin ligases Fbxo32 and Trim63, thereby driving muscle atrophy (Yin et al., 2018). Muscle contraction-induced apelin, a so-called exerkine, was shown to promote mitochondrial biogenesis and protein synthesis via activating AMPK, AKT and p70S6K in aged myofibers (Vinel et al., 2018). In addition, inhibition of 15-PGDH, a prostaglandin-degrading enzyme, leads to alterations in multiple pathways to improve aged muscle functions, including decreased proteolysis (Palla et al., 2021).

Because skeletal muscle tissue comprises multiple cell types and a complex extracellular matrix, many aging-related changes have been found to impact the MuSC crosstalk with the muscle niche. Among these, aging impairs the function of FAPs in mice. Decreased WISPI secretion by FAPs significantly affects MuSC expansion during muscle regeneration (Lukjanenko et al., 2019). The reduced extracellular matrix component fibronectin contributes to the loss of adhesion with MuSCs during aging, eliciting detrimental consequences for the function and maintenance of the MuSC pool (Lukjanenko et al., 2016). Decreased Sprouty1 expression also disrupts MuSC quiescence (Chakkalakal et al., 2012). Given the chronic inflammatory state of aging muscle, it would be of great significance to unravel age-related molecular signatures of intramuscular immune cells (Duggal et al., 2019), providing novel immunosenescence biomarkers for muscle aging. In addition, further in-depth investigations of cellular organelle stress responses, such as the UPR, in the regulation of age-dependent proteostasis and muscle regeneration (Cai et al., 2022d; He et al., 2021; Kaushik and Cuervo, 2015) may provide more valuable molecular biomarkers of muscle aging.

Secretory factors detectable in biofluids

Skeletal muscle is a secretory organ that secretes various hormone-like molecules called myokines (Whitham and Febbraio, 2016). Myokines are important for skeletal muscle health and can be potential biomarkers for muscle aging. Apelin, an exercise-induced myokine that is a ligand of the G-protein-coupled receptor APJ, has been observed to be lower in the skeletal muscle of old mice and elderly human serum (Alizadeh Pahlavani, 2022; Rai et al., 2017). Furthermore, several other myokines have been found to be decreased in the serum of elderly human subjects compared to young adults, including Sestrin1/Sestrin2 (Kwon et al., 2020; Rai et al., 2018), IGF-1 (Bando et al., 1991; Haden et al., 2000), Irisin (Huh et al., 2014; Miyamoto-Mikami et al., 2015) and VEGF (Ryan et al., 2006). Interestingly, higher serum levels of IL-6 (Haden et al., 2000; Hager et al., 1994; Palmeri et al., 2012; Wei et al., 1992) and Myostatin (Yarasheski et al., 2002) have been found in elderly human subjects, while higher serum levels of leptin have been observed in old rats (Mooradian and Chehade, 2000). All these myokines might be potential biofluid markers for muscular disorders associated with skeletal muscle aging.

Potential molecular biomarkers of sarcopenia have also been identified in the blood of older adults and sarcopenic patients. These include IL-15 (Yalcin et al., 2018), IL-6 (Rong et al., 2018; Rossi et al., 2019; Schaap et al., 2006; Volpato et al., 2014), CRP (Schaap et al., 2006; Volpato et al., 2014), Myostatin (Yarasheski et al., 2002), IL-10 (Rong et al., 2018), IL-18 (Li et al., 2019a), TNF-a (Volpato et al., 2014) and Leptin (Li et al., 2019a), which exhibit higher serum levels. Conversely, lower serum levels of MMP9 (Suzan et al., 2021), Irisin (Chang et al., 2017), IGF-1 (Naranjo et al., 2017; Volpato et al., 2014) and FGF21 (Li et al., 2019a) have been observed in sarcopenia patients.

Summary and perspectives

Prospectively, the integration of advanced single-cell genomics with mechanistic studies would provide broader avenues to identify molecular and cellular biomarkers in studying muscle aging and related diseases, paving the way to pre-clinical applications for healthy muscle aging. Facilitated by multi-omics approaches and lifestyle interventions like exercise, more extensive and careful investigations will be required to search for more aging-related biomarkers in body fluids, including not only myokines/cytokines, but also short peptides, small-molecule metabolites, or other entities. Ultimately, this will enable us to pinpoint genuine biomarkers from blood, urine, or muscle tissues, which can be collectively employed to accurately monitor the aging states of skeletal muscle for future translational applications.

Liver aging

Physiological characteristics

Similar to other organs, the liver undergoes a series of degenerative changes, including morphological structure and function, as the body ages (Maeso-Díaz and Gracia-Sancho, 2020; Sheedfar et al., 2013). The physiological features of aging include reduced liver volume, decreased perfusion, and functional atrophy (Wang, 2021). The relationship between the liver and aging was first explored by Popper in 1986, who reported that the typical features of the aged liver are browning and atrophy, mainly due to lipofuscin accumulation in hepatocytes (Popper, 1986). Wynne et al. (1989) recruited 65 subjects between the ages of 24 and 91 years and found that age was negatively correlated with liver volume, apparent liver blood flow, and liver perfusion. Morphologically, the livers of older people are not smaller than those of younger people. Instead, older people have fewer and larger hepatocytes (Wakabayashi et al., 2002; Watanabe and Ta-

naka, 1982). It has been reported that in healthy aged animal livers, a slight alteration in the hepatic sinusoidal cell phenotype causes a moderate increase in hepatic vascular resistance and thus reduces effective hepatic perfusion (Wakabayashi et al., 2002). Although the liver in elderly individuals exhibits some morphological changes, unlike other organs that age, the liver still exhibits normal functional reserve (Anantharaju et al., 2002; Kitani, 1992). However, the liver's regenerative capacity is significantly reduced in elderly individuals compared to young individuals (Furrer et al., 2011; Liu et al., 2018a; Pibiri, 2018). In addition, macrophage accumulation is reported in the normally aging liver (Bloomer and Moyer, 2021; Mohammed et al., 2021). Aging-related impairment of macrophage autophagy leads to proinflammatory cytokines, especially IL-6, which may be associated with age-related physiological dysfunction (Bloomer and Moyer, 2021). In addition, the expression of necrosis markers in hepatocytes and hepatic macrophages of senescent mice increases along with elevated levels of proinflammatory factors, whereas necrostatin-1s treatment decreases the expression of proinflammatory factors and slows cellular senescence in hepatic macrophages of senescent mice (Mohammed et al., 2021). Additionally, chronic liver inflammation drives liver fibrosis, and total collagen content, a marker of fibrosis severity, is significantly increased in the livers of aging mice relative to young mice (Mohammed et al., 2021; Noureddin et al., 2013; Shen et al., 2022). There is no doubt that aging not only predisposes patients to the development of liver fibrosis but also increases the risk of poor prognosis in various liver diseases and leads to increased mortality (Floreani, 2007; Mahrouf-Yorgov et al., 2011; Sheedfar et al., 2013) (Figure 16; Table S11 in Supporting Information).

Imaging traits

Autopsy studies show that aging is often accompanied by a decrease in liver weight, and this is confirmed by *in vivo* imaging studies (Meier et al., 2007; Tauchi et al., 1994; Wakabayashi et al., 2002). As determined by an ultrasound technique, compared to younger people, older adults have approximately 20%–40% less liver volume and 35%–50% less blood flow (Vollmar et al., 2002; Wynne et al., 1989). Wynne et al. (1989) selected 65 healthy volunteers aged 24–91 years to study age-related changes in liver volume and found a significant negative correlation between liver volume and age, with liver volumes of approximately 23.6 $mm^3\ kg^{-1}$ body weight at age 24 years and approximately 14.0 $mm^3\ kg^{-1}$ body weight at age 91 years. Pulsed echo Doppler showed a significant decrease in portal blood flow in elderly subjects (especially those ≥75 years old) (Wang, 2021). Zoli et al. (1999) selected healthy subjects of different ages to measure total hepatic flow (THF) and functional hepatic flow (FHF) by Doppler ultrasound and showed that THF and FHF were significantly negatively correlated with age, especially after 75 years. In addition, ^{18}F-FDG PET imaging has shown that liver FDG uptake increases with aging (Cao et al., 2021b; Wang, 2021). However, the reason for increased FDG uptake in the liver of elderly individuals has not been fully elucidated, which may be due to cumulative inflammatory changes in the liver caused by the long-term handling of various toxins (Cao et al., 2021b; Keramida and Peters, 2020; Wang, 2021). In both men and women, MRI T2* imaging shows that age is also associated with hepatic iron overload (Wang, 2021). Liver iron levels continue to increase until the age of 40 compared to adolescents, while from 40–70, liver iron levels remain stable or increase mildly (Sheng et al., 2020; Wang, 2021; Zacharski et al., 2000). However, the relationship between lipid accumulation in hepatocytes and aging is controversial. For instance, it has been reported that the lipid droplet content in hepatocytes is significantly higher in elderly individuals than in young individuals, leading to excessive steatosis and fibrosis, which in turn inhibit liver function (Chen et al., 2020a; Kuk et al., 2009). In contrast, Bedogni et al. (2005) showed an inverse relationship between age and fatty liver. Instead, liver fat accumulation appears to be more associated with obesity than age (Bedogni et al., 2005; Fan et al., 2005).

Histologic features

Although studies in general found preserved tissue architecture in aged livers (Jansen, 2002; Popper, 1986), features suggesting mild hepatic injury were observed in aged rats, including cytoplasmic vacuolation, nuclear pyknosis, cytoplasmic hypereosinophilia, diminished intercellular borders, and increased lipid accumulation (Maeso-Díaz et al., 2018).

Importantly, ultrastructural analysis of aged livers has revealed the most significant structural changes in the sinusoidal endothelium, which have been broadly documented in mice, rats, nonhuman primates, and humans (Cogger et al., 2014; Cogger et al., 2003; Ito et al., 2007; Le Couteur et al., 2001; Maeso-Díaz et al., 2018; McLean et al., 2003; Warren et al., 2005). The change in the sinusoidal endothelium is generally referred to as "pseudocapillarized", characterized by a reduction in the number and porosity of fenestrations, endothelium thickening, and deposition of perisinusoidal basal lamina and collagen. Pseudocapillarization consequently reduces the vasodilatory and angiocrine capacity of the sinusoidal endothelium, leading to increased hepatic vascular resistance and portal venous pressure. In addition, both secretion of von Willebrand factor (vWF) and expression of ICAM-1 are elevated in sinusoidal cells, resulting in the recruitment of more neutrophils and $CD68^+$ macrophages in the sinusoidal area (Ito et al., 2007; Licastro et al., 2005; Maeso-Díaz et al., 2018; Miyachi et al., 2017).

Liver endothelium fenestrations are important portals for

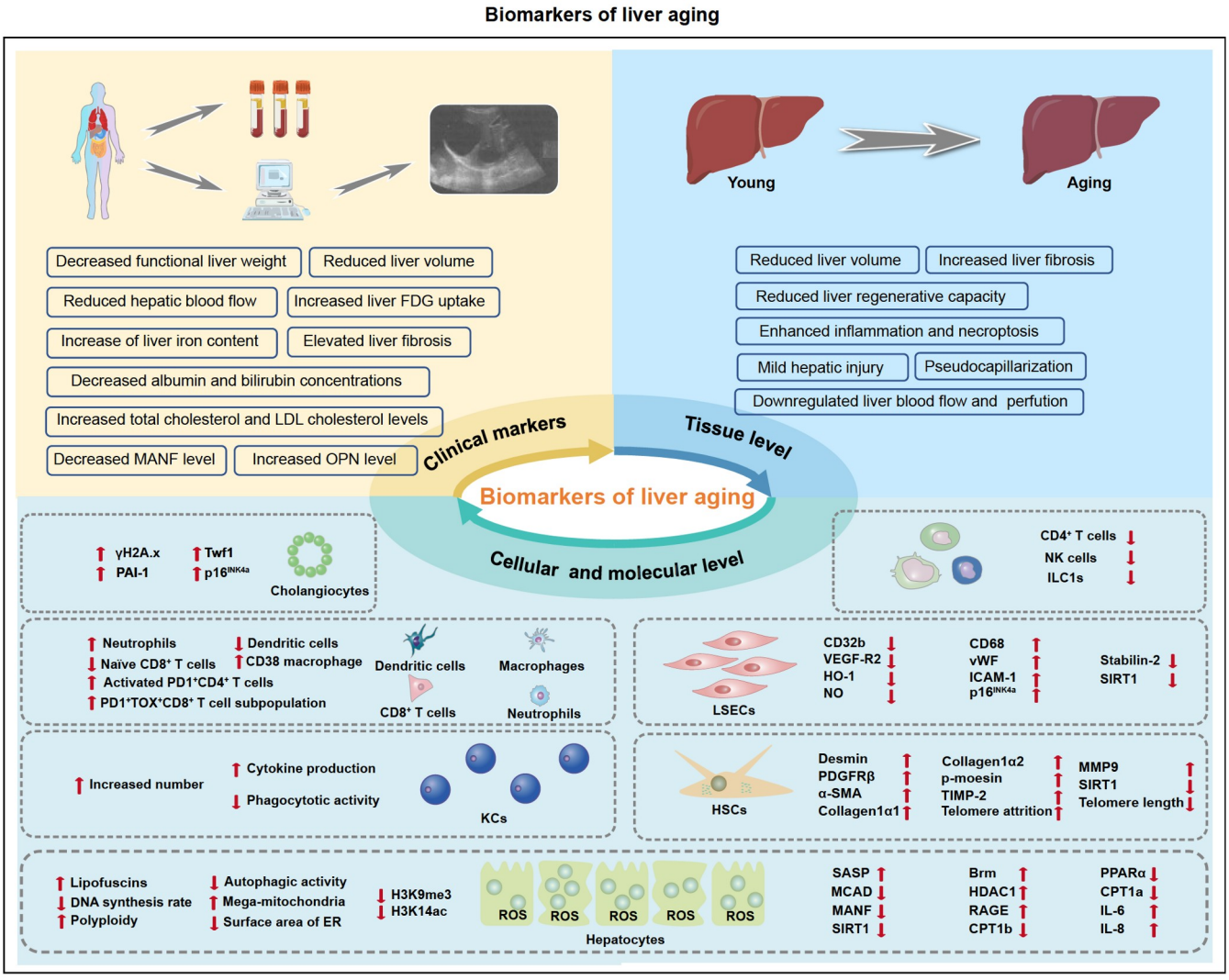

Figure 16 Biomarkers of liver aging. Abbreviations: α-SMA, actin alpha 2, smooth muscle, aorta; CD32b, Fc gamma receptor IIb; CPT1a, carnitine palmitoyltransferase 1A; H3K9me3, histone 3 Lys-9 trimethylation; H3K14ac, histone 3 lysine 14 acetylation; HDAC1, histone deacetylase 1; HO-1, heme oxygenase 1; MCAD, cadherin 15; OPN, steopontin; PDGFRβ, platelet derived growth factor receptor beta; PPARα, peroxisome proliferator activated receptor alpha; TIMP-2, TIMP metallopeptidase inhibitor 2; Twf1, Twinfilin-1; VEGF-R2, vascular endothelial growth factor receptor 2.

the uptake of lipoproteins, insulin, and carbohydrates. Loss of fenestrations in the aged liver can cause hyperlipidemia (e.g., increased plasma cholesterol and low-density lipoprotein (LDL) cholesterol) and hepatic insulin resistance (Maeso-Díaz et al., 2018; Mohamad et al., 2016), rendering a high susceptibility to cardiometabolic disease. In addition, aged hepatic endothelium is also the major reason for significantly worse effects after acute liver damage in comparison to young endothelium (Hide et al., 2020). These studies together highlight the vulnerability of liver sinusoidal cells to aging.

Cellular alterations

Liver tissue comprises several cell types, including hepatocytes, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and immune cells. The relative number of hepatocytes is decreased in aged liver, whereas polyploidy hepatocytes increase from less than 15% to approximately 42% (Kudryavtsev et al., 1993). Morphologically, in aged hepatocytes, the surface area of the endoplasmic reticulum is markedly reduced, which correlates with a decline in hepatic microsomal protein synthesis activity (Schmucker et al., 1990). In addition, mitochondria appear enlarged ("mega-mitochondria") with age-associated structural changes in the cristae and inner membrane (Sastre et al., 1996), accompanied by a reduced number and decreased function of mitochondria in hepatocytes (Daum et al., 2013; Hagen et al., 1997; Navarro and Boveris, 2004). Autophagic activity is also significantly impaired in aged hepatocytes (Uddin et al., 2012; Xu et al., 2013), resulting in elevated levels of protein misfolding, loss of proteostasis (Schneider et al., 2015; Zhang and Cuervo, 2008), and consequently, the formation

of protein aggregates such as lipofuscins (Le Couteur et al., 2001; Swanlund et al., 2008).

Aged hepatocytes also have reduced rates of DNA synthesis and repair, displaying higher genomic instability (Basso et al., 1998). Consistently, senescent hepatocytes increase with age (Aravinthan and Alexander, 2016) and have more lipid accumulation and production of reactive oxygen species (Basso et al., 1998; Ogrodnik et al., 2017). In addition, senescent hepatocytes release cytokines such as IL-6, TNFα, and IL-8, contributing to age-associated inflammation (Lasry and Ben-Neriah, 2015).

As mentioned above, due to the pseudocapillarization of the sinusoidal endothelium in aged livers, aged LSECs are dedifferentiated with less vasodilatory and angiocrine capacity, as evidenced by reduced NO bioavailability; decreased levels of cyclic guanosine monophosphate; and reduced expression of endothelial NO synthase protein, heme oxygenase-1, and several angiocrine receptors (stabilin-2, CD32b, and VEGF-R2) (Ito et al., 2007; Maeso-Díaz et al., 2018). In addition, aged LSECs exhibit a moderately proinflammatory state with increased CD68-positive cells and secretion of vWF and ICAM-1 (Ito et al., 2007; Maeso-Díaz et al., 2018). Some LSECs also become senescent with upregulation of $p16^{INK4a}$ and downregulation of SIRT1 (Maeso-Díaz et al., 2018). Interestingly, as the main carriers of the mannose receptor (MRC1) in the liver, the number of Mrc1-expressing LSECs increases with age, suggesting age-associated changes in the tissue immune response carried out by LSECs (Tabula Muris, 2020).

The number of HSCs in the aged liver is increased, as evidenced by enhanced desmin expression and a significant increase in the proliferative HSC-related growth factor PDGFRβ (Warren et al., 2011). In addition, aged HSCs display a moderately activated status, with increases in the expression of different activation markers, including α-SMA, collagen1α1, collagen1α2, and p-moesin, as well as changes in matrix remodeling genes, such as TIMP-2 and MMP9 (Maeso-Díaz et al., 2018). In contrast to HSC activation in liver diseases, HSC activation in aged livers is accompanied by an increased number and size of intracellular lipid droplets (Warren et al., 2011). Moreover, aged HSCs also have elevated patatin-like phospholipase domain-containing protein 3 and decreased cellular retinol-binding protein I expression, suggesting alterations in vitamin A metabolism (Maeso-Díaz et al., 2018). Some HSCs also become senescent, with telomere attrition in aged human liver tissues being reported (Verma et al., 2012).

Aging is also associated with significant alterations in immune cells. The number of liver Kupffer cells (KCs) increases with aging, and these cells display a basally activated status (Hilmer et al., 2007). These aged KCs show deficits in mitochondrial function (Salminen et al., 2012a), reduced phagocytotic activity, and increased cytokine production (Hilmer et al., 2007; Linehan et al., 2014; Partridge et al., 2018). In addition, aged KCs in elderly rodent livers exhibit a redistribution into the lymphoid collections (Singh et al., 2008; Stahl et al., 2018). Interestingly, proinflammatory M1-like macrophages accumulate in aged livers, together with high expression of the NAD-consuming enzyme CD38, which can be induced by the inflammatory cytokines secreted by senescent cells in aged tissues (Chini et al., 2020; Covarrubias et al., 2020). Additionally, the number of neutrophils is increased in the aged liver, whereas the number of dendritic cells is decreased (Mogilenko et al., 2021).

The abundance of naïve $CD8^+$ T cells is decreased; however, there exists a distinct age-associated $PD1^+TOX^+$ $CD8^+$ T-cell subpopulation across multiple tissues in aging, including the liver. These age-associated $CD8^+$ T cells constitute up to 60% of all $CD8^+$ T cells in these tissues, which exhibit a T-cell exhaustion phenotype (Blank et al., 2019) and produce a distinct set of proinflammatory cytokines upon TCR stimulation (Mogilenko et al., 2021). Activated $PD1^+CD4^+$ T cells also accumulate in the aged liver. However, both NK cells and group 1 innate lymphoid cells (ILC1s) are reduced in abundance in the aged liver (Mogilenko et al., 2021). Remarkably, communications between endothelial cells and various immune cell types (e.g., macrophages and T cells) are significantly altered in the aged liver (Ma et al., 2020), another indication of the importance of endothelial cells in the age-associated immune response.

Molecular changes

Hepatocytes are parenchymal cells that can comprise up to 70%–80% of the liver's total mass and are responsible for the majority of hepatic functions. With aging, there are various molecular alterations that occur in hepatocytes. Increased expression of markers commonly associated with cellular senescence, such as SA-β-gal activity, and $p21^{CIP1}$, $p16^{INK4a}$, and γ-H2AX, have been observed in aging hepatocytes (Irvine et al., 2014; Wang et al., 2014b), accompanied with the occurrence of SASP (Irvine et al., 2014). Studies of the aging phenotype have revealed several significant epigenetic changes involving liver pathophysiology. One such change is the increased expression of chromatin remodeling proteins Brm and HDAC1, which constitute the C/EBPα-Brm or HDAC1-C/EBPα-Brm complex with C/EBPα. The complex occupies and silences E2F-dependent promoters, leading to an age-dependent loss of liver regenerative potential (Iakova et al., 2003; Wang et al., 2008). In contrast with the upregulation of Brm and HDAC1, SIRT1 is downregulated in aging hepatocytes, potentially leading to aggravated alcoholic liver injury in older mice (Gong et al., 2014; Ramirez et al., 2017). A hepatic bivalent combination, marked as an H3K9me3/H3K14ac modification, was also found to be decreased in aging hepatocytes (Price et al., 2020). In addi-

tion, high circadian global protein acetylation was found to be lost in aged hepatocytes, while caloric restriction could rescue this aging-dependent decline by upregulating the NAD^+-SIRT1-AceCS1 pathway (Sato et al., 2017). In addition to the molecular changes at the epigenetic level, aging is generally accompanied by deregulated metabolic function, such as hepatic steatosis. Aging induces an aberrant advanced glycation end product receptor (RAGE)/PPARα axis in hepatocytes, which includes the upregulation of RAGE and downregulation of the fatty acid β-oxidation genes PPARα, carnitine palmitoyltransferase 1A (CPT1a), CPT1b, and Medium chain acyl-CoA dehydrogenase (MCAD), eventually leading to aging-associated hepatosteatosis (Wan et al., 2020). Moreover, a recent study also identified mesencephalic-astrocyte-derived neurotrophic factor (MANF) as experiencing aging correlated decline in flies, mice, and humans, while reduced expression in hepatocytes is associated with hepatosteatosis. Liver rejuvenation by heterochronic parabiosis in mice is dependent on MANF, whereas MANF supplementation ameliorates several hallmarks of liver aging, prevents diet-induced hepatosteatosis, and improves aging-related metabolic dysfunction (Sousa-Victor et al., 2019).

Compared with studies involving molecular changes in liver parenchymal cells, relatively little is known about the aging-related molecular alterations in liver nonparenchymal cells, such as LSECs, KCs, HSCs, and cholangiocytes. Aging results in the downregulation of the eNOS-NO-cGMP vasodilatory pathway, together with reduced angiocrine and antioxidant molecules (Stabilin-2, CD32b, VEGF-R2, HGF, Wnt2, and HO-1) in LSECs (Maeso-Díaz et al., 2018). A recent study suggests that there is an increased population of immune checkpoint protein programmed death-ligand 1 ($PD\text{-}L1^+$) cells in LSECs from aged mice, and PD-L1 expression correlates with higher levels of SASP (Wang et al., 2022d). Similarly, aging also affects HSCs in that increased expression of HSC activation markers, such as α-SMA, collagen1α1, collagen1α2, PDGFRβ, phosphorylated moesin, desmin, TIMP-2 and MMP9, has been described in aged rats (Maeso-Díaz et al., 2018). A study analyzing the lengths of telomeres in human donors demonstrated that age-related telomere length decrease is restricted to HSCs and KCs, while aging cholangiocytes and hepatocytes are able to resist telomere shortening (Verma et al., 2012). In addition to the decreased expression of SIRT1 in aging hepatocytes, the downregulation of SIRT1 is also present in the HSCs of older livers, which contributes to age-related alcoholic liver injury and fibrosis (Ramirez et al., 2017). Few studies have reported aging-related molecular changes in KCs. In addition to age-related telomere shortening in the KCs of the human liver (Wan et al., 2020), a recent study revealed an increase in the RNA expression of the inflammatory cytokine IL-6 in KCs of older rats. However, there were no age-related changes in the expression of other KC markers, including TNFα, Mrc1, Arg1, and IL-10 (Wang et al., 2008). The aging of cholangiocytes is generally seen in primary biliary cholangitis, primary sclerosing cholangitis, and other chronic liver diseases (Ferreira-Gonzalez et al., 2021). Aging cholangiocytes are characterized by increased expression of senescence markers ($p16^{INK4a}$ and γH2A.x), and SASP (IL6, IL8, CCL2, and PAI-1 secretion) (Tabibian et al., 2014). Twinfilin-1 (Twf1) is a cytosolic protein sequestering large amounts of actin monomers, which has recently been identified as a target of age-related microRNAs (miR-1a, miR-20a and miR30e) and is thus a mediator of the aging process in cholangiocytes (Maroni et al., 2019).

Secretory factors detectable in biofluids

Aging, at the serum level, is associated with a slight decrease in albumin and bilirubin concentrations and no or minimal changes in aminotransferase levels. The metabolism of cholesterol in the liver also decreases, leading to overall increases in total blood cholesterol and LDL cholesterol levels over time (Maeso-Díaz et al., 2018; Tietz et al., 1992). Recent investigations have also suggested that serum levels of osteopontin (OPN), a senescence-associated secretory phenotype factor, are elevated while, in contrast, serum MANF levels decline with liver aging (Gómez-Santos et al., 2020; Sousa-Victor et al., 2019).

Kidney aging

Physiological characteristics

(1) Functional changes. Renal function, shown as glomerular filtration rate (GFR), decreases progressively with age. A systematic review of 9 cross-sectional studies and 3 cohort studies found that in healthy individuals, the average annual decrease in estimated glomerular filtration rate (eGFR) ranged from 0.4 to 2.6 mL min^{-1} (Bolignano et al., 2014). After age 35, GFR declines by approximately 5%–10% per decade (Schmitt and Melk, 2017). Age-related decline in renal function may result in impaired renal functional reserve in the elderly, thereby increases susceptibility to acute kidney injury (AKI) (James et al., 2010) and chronic kidney disease (CKD) (Nitta et al., 2013).

In addition, the concentrating and diluting function of the renal tubules decreases during renal aging (O'Sullivan et al., 2017). It was found that the maximum urinary osmolality decreased by approximately 20% in the 60–79 age group compared to the 20–39 age group (Rowe et al., 1976). At the same time, the ability of aging kidneys to reabsorb sodium and excrete potassium is significantly reduced, leading to a predisposition to water-electrolyte disorders in the elderly (McGreevy et al., 2008; Mimran et al., 1992).

(2) Structural changes. On the macroscopic scale, renal aging manifests as increased volume, surface roughness,

focal scarring, and the appearance of simple renal cysts (Hommos et al., 2017). A study that evaluated kidney volume by MRI in 1,852 adults prior to kidney donation found that kidney volume decreased by approximately 16 cm^3 per decade after age 60 years (Roseman et al., 2017). In addition, Wang et al. (2014c) evaluated 1,344 potential kidney donors by contrast-enhanced CT imaging and found that cortical volume gradually decreased with age, while medullary volume increased before age 50 and gradually decreased after age 50. It was also found that the decrease in renal cortical volume was associated with an age-related decrease in GFR.

Histologic features and cellular alterations

The most prominent histologic change in the aging kidney is nephrosclerosis which is defined as the presence of two or more of the following histologic changes: glomerulosclerosis, tubular atrophy, interstitial fibrosis>5%, and arteriosclerosis. The prevalence of nephrosclerosis increases from 2.7% in patients aged 18–29 years to 73% in patients aged 70–77 years (Rule et al., 2010).

(1) Glomerulosclerosis. Aging causes glomerulosclerosis, which is often accompanied by enlargement of the surrounding glomeruli. A study of healthy kidney donors showed that the oldest age group, 70–75 years, had a 48% decrease in the number of non-sclerotic glomeruli compared to the youngest subgroup, 18–29 years (Denic et al., 2017).

Podocyte loss is a major determinant of glomerulosclerosis. A study of 89 normal kidney samples found that podocyte nuclear density decreased with age (Hodgin et al., 2015). Podocytes are terminally differentiated cells and no longer renewed during aging. Therefore, the loss of senescent podocytes is often accompanied by hypertrophy of the adjacent podocytes (Wang et al., 2014c). The hypertrophy of the remaining glomeruli eventually causes podocyte detachment, and overall glomerulosclerosis (Schmitt and Melk, 2017).

(2) Renal tubular atrophy and interstitial fibrosis. Studies of age-related morphologic changes in renal tubules have shown a decrease in the number of tubules, a decrease in tubular volume, and an increase in tubular atrophy with increasing age (Martin and Sheaff, 2007). The atrophy of renal tubules is accompanied by a marked increase in interstitial fibrosis. Proteomic analysis revealed age-related increases in the structural components of the interstitium, such as collagen VI, fibrillin-1 and fibronectin, and the matrix regulators TIMP3 and ADAMTS5. Moreover, alterations in interstitial composition preceded the apparent structural changes (Randles et al., 2021).

(3) Arteriosclerosis and sparse capillaries. Arterial changes in the aging kidney include small arteriosclerosis, fibrous intimal hyperplasia, and hyaline small arteriosclerosis (Martin and Sheaff, 2007). 3D reconstruction techniques reveal marked intimal thickening and narrowing of the interlobular artery veins accompanied by sclerotic glomeruli in aging kidneys (Uesugi et al., 2016). In addition, the number of peritubular capillaries decreases during renal aging (Uesugi et al., 2016), which may be due to a decrease in pericytes (Stefanska et al., 2015). Studies related to CKD have found a strong relationship between peritubular capillary thinning and tubular atrophy and interstitial fibrosis (Kida et al., 2014). However, a causal relationship between these two has not been established.

Molecular changes

Overall, the biology of kidney aging is complex, involving diverse changes to cells, tissues, organs, and the surrounding microenvironment. We will focus on the molecular changes of the cellular senescence, autophagy, and inflammation during kidney aging, but the readers should appreciate that this list is not exhaustive.

(1) Cellular senescence. Renal functional recovery after AKI is significantly worse in elderly patients. This decreased regenerative potential, which is a hallmark of the aging process, may be caused by cellular senescence. Halloran et al. (1999) hypothesize that the accumulation of senescent cells may be responsible for the insufficient repair capacity and functional loss in older kidneys. In support, recent study showed that constant removal of senescent cells attenuated age-related deterioration of renal function and glomerulosclerosis (Baker et al., 2016). Accumulation of senescent cells could also explain the increased prevalence of kidney diseases with aging. As comprehensively reviewed by Huang et al., the cellular senescence of virtually all renal cell types is involved in the pathogenesis of AKI and CKD (Huang et al., 2022c).

The common molecular changes of cellular senescence have also been identified in aging kidney, including upregulation of cell-cycle inhibitors (including $p16^{INK4a}$, $p21^{CIP1}$ and p53), SA-β-gal activity, telomere shortening, and SASP. Klotho is an aging intervention protein that is highly expressed in kidney tubular epithelia, and plays a role in phosphate hemostasis with implications for vascular calcification, hypoxia, cellular regeneration, and senescence (Mencke et al., 2017). Downregulation of α-Klotho is specific for kidney aging. Klotho knockout mice show arteriosclerosis and vascular changes as part of their aging phenotype (Kuro-o et al., 1997). Thus, developing reliable assays to monitor Klotho levels may help to predict the decline of renal function and the progression of CKD.

(2) Autophagy. Autophagy has been intensively studied in aging and in different disease models in the kidney (Lenoir et al., 2016). It was suggested that because of their longevity, podocytes, as well as tubular cells, might be particularly dependent on autophagy for effective “self-cleaning” from protein aggregates and defective organelles during the life-

span. Indeed, podocytes show a high rate of baseline autophagy; and podocyte-specific deletion of Atg5, a key component of the autophagy machinery, triggered an aging phenotype with accumulation of lipofuscin, oxidized proteins, and sequestosome 1-positive protein aggregates (Hartleben et al., 2010). Similar observations have been made when Atg5 was selectively ablated in tubular cells, resulting in impaired kidney function with a pro-aging phenotype (Liu et al., 2012a). Based on these data, age-associated disturbance of normal autophagy in podocytes or tubular cells would be expected to act as a pro-aging mechanism (Denic et al., 2016).

(3) Inflammation. Aging is associated with a subclinical systemic chronic inflammatory status called inflammaging (Franceschi et al., 2018), one of the most important traits of immunosenescence as reviewed everywhere (Sato and Yanagita, 2019). Immunosenescence involves a series of aging-induced alterations in the immune system and is characterized by two opposing hallmarks: defective immune responses and increased systemic inflammation. In the kidney, resident macrophages and fibroblasts are continuously exposed to components of the external environment, and the effects of cellular reprogramming induced by local immune responses, which accumulate with age, might have a role in the increased susceptibility to kidney disease among elderly individuals. Immunosenescence might be the mechanism of increased susceptibility to various kidney diseases (Couser, 2017; Jennette and Nachman, 2017) in the elderly.

Secretory factors detectable in biofluids

For over 70 years, eGFR has remained the primary index for detection and monitoring renal function. However, it does not accurately reflect renal tubular and interstitial lesions (Ix and Shlipak, 2021), and is not sensitive enough for identifying early-stage injury (Yan et al., 2021). Therefore, researchers have been working to find more early, comprehensive, and non-invasive biomarkers to reflect the extent of tubulointerstitial fibrosis and the progression of renal dysfunction. The following discussion will describe some of the biomarkers found so far.

(1) Tubule Injury and dysfunction markers. Tubular injury/atrophy is a very important aspect in the aging process of the kidney. Several biomarkers have been found to assess tubular injury. Kidney injury molecule-1 (KIM-1), a transmembrane glycoprotein released into the urine by injured proximal tubular cells (Han et al., 2002). It was found that urinary KIM-1 levels were positively correlated with decreased eGFR and histological changes of interstitial fibrosis and tubular atrophy (Malhotra et al., 2020). Studies in patients with diabetes have found that plasma KIM-1 is associated with progression of diabetic nephropathy and poor renal outcomes (Coca et al., 2017; Gutiérrez et al., 2022). α-1 microglobulin (A1M) is a low molecular weight protein freely filtered at the glomerulus and reabsorbed by proximal tubular epithelial cells (Åkerström et al., 2000; Weber and Verwiebe, 1992). Elevated urinary A1M was associated with decreased eGFR, the degree of interstitial fibrosis and tubular atrophy in patients with drug-induced interstitial nephritis and renal transplant recipients (Amer et al., 2013; Wu et al., 2010). In addition to proximal tubules, there are corresponding biomarkers for other types of renal tubules. Uromodulin (UMOD), produced exclusively in the thick ascending limb, is a biomarker of kidney tubular health. A prospective study looking at 2,652 patients with CKD found that lower serum urinary regulatory protein levels were independently associated with a higher risk of developing ESKD, even after adjustment for baseline eGFR, which remained significant (Lv et al., 2018). Meanwhile, urinary UMOD levels were negatively associated with the rate of decline in eGFR and the risk of eventual progression to CKD in patients after AKI (Puthumana et al., 2021). A novel marker of distal tubular function is epidermal growth factor (EGF), which is selectively expressed in distal renal tubular cells and critical for cell differentiation and regeneration in the repair process after renal tubular injury (Gesualdo et al., 1996; Lechner et al., 2007). The amount of EGF protein in urine (uEGF) showed significant correlation with interstitial fibrosis/tubular atrophy, eGFR, and rate of eGFR loss (Ju et al., 2015; Torres et al., 2008; Wu et al., 2020a). Addition of uEGF to standard clinical parameters improved the prediction of disease events in diverse CKD populations (Ju et al., 2015). Noteworthy, lower uEGF levels are associated with increased risk of rapid eGFR loss and incident of CKD in the general population (Norvik et al., 2021).

MMP-7, a secreted zinc- and calcium-dependent endopeptidase (Tan and Liu, 2012), has been reported to be involved in renal tubular injury as well as interstitial fibrosis through the activation of β-catenin signaling. It was found that urinary MMP-7 levels in CKD patients were strongly correlated with renal fibrosis scores (Zhou et al., 2017). Also, studies in patients with IgA nephropathy (IgAN) and diabetic nephropathy have found that circulating MMP-7 levels are strongly associated with GFR loss and renal interstitial fibrosis (Ihara et al., 2020; Zhang et al., 2017a). Additionally, Wang et al. (2017a) have discovered that urinary fibrinogen levels are significantly elevated in patients with proteinuric nephropathy. Higher urinary fibrinogen levels are associated with more severe interstitial fibrosis and renal tubular atrophy in patients. For the prediction of new-onset end-stage renal disease (ESRD), the addition of urinary fibrinogen to the traditional combination of urinary protein, blood pressure and baseline eGFR increased the area under the receiver operating curve from 0.73 to 0.76.

(2) Inflammatory biomarkers. Many inflammation-associated biomarkers in the circulation are also strongly associated with renal dysfunction and fibrosis. Tumor necrosis

factor receptors (TNFRs) are activated by TNFα, which has a significant role in inflammatory processes (Al-Lamki and Mayadas, 2015). Upon activation, TNFRs are shed from the cell surface into a soluble form (sTNFR) in the blood (Bell et al., 2007). Plasma sTNFR-1 and sTNFR-2 have been reported to be associated with decreased tubulointerstitial and glomerular lesions, and eGFR in patients with CKD and diabetic nephropathy (Coca et al., 2017; Gutiérrez et al., 2022; Srivastava et al., 2021). YKL-40, also known as chitinase 3-like 1 (CHI3L1), is a glycoprotein produced by macrophages, neutrophils, and other locally inflammatory cells (Schmidt et al., 2013). YKL-40 is an important mediator of inflammation after ischemic or reperfusion injury and activates pro-fibrotic signaling pathways in the context of ongoing injury and maladaptive repair (Coca et al., 2017). Patients with diabetic nephropathy who have higher plasma YKL-40 are at greater risk for kidney disease progression and eventual development of ESRD (Gutiérrez et al., 2022; Schrauben et al., 2021). Meanwhile, higher plasma YKL-40 levels in patients with CKD and ESRD are positively correlated with mortality (Lorenz et al., 2018; Srivastava et al., 2021).

MCP-1, also called CCL2, is expressed by endothelial cells, macrophages and fibroblasts and acts as a chemoattractant protein in response to tissue injury (Chow et al., 2006; Ix et al., 2017). In a multicenter, prospective cohort study including 1,538 hospitalized CKD patients with a median follow-up of 4.3 years, urinary MCP-1 levels at 3 months after hospitalization were associated with greater eGFR decline and increased incidence of the composite renal outcome (Puthumana et al., 2021). In a prospective, observational cohort study including 523 patients with diverse kidney disease, plasma MCP-1 levels were correlated with tubulointerstitial and glomerular lesions. Each doubling of plasma MCP-1 concentration was associated with increased risks of kidney disease progression and death (Srivastava et al., 2021).

Circulating soluble urokinase plasminogen activator receptor (suPAR), a soluble form of urokinase-type fibrinogen activator receptor, is expressed primarily on immune cells and endothelial cells and released into the circulation during inflammation (Mahdi et al., 2001). In a prospective cohort of adults with cardiovascular disease, higher levels of plasma suPAR were found to be associated with lower basal eGFR levels and subsequent decreases in eGFR (Hayek et al., 2015).

Activation of complement system plays an important role in the progression of renal disease (Thurman, 2015). Wendt et al. (2021) detected complement fragments in urine to observe their relationship with renal disease progression. Twenty-three different urinary peptides derived from complement proteins were identified, most of which C3-derived peptides were negatively correlated with eGFR. Also, this study revealed that using a combination of multiple complement peptide fragments in urine to assess kidney function was a better predictor than a single molecule.

(3) Other renal injury biomarkers. Besides various protein molecules have been used as biomarkers, accumulating studies show the power of metabolites in the plasma or urine to predict the decline of renal function. In a study of 1,921 subjects without CKD with a median follow-up of 19.6 years, the serum concentration of two metabolites, creatine and 3-indolyl sulfate, is significantly correlated with the decline of eGFR. Meanwhile, higher levels of 5-oxoproline and 1,5-anhydroglucitol (1,5-AG) were significantly associated with a lower risk of CKD (Yu et al., 2014). Recent studies have found that exosomes play a very important role in the development of renal fibrosis, and CKD as messenger cargoes for intercellular communication (Mahtal et al., 2022). Feng et al. (2018) reported a correlation between exosomal CCL2 and tubulointerstitial inflammation, C3 deposition and eGFR in IgAN. High CCL2 levels at the time of renal biopsy were associated with subsequent deterioration in renal function. In addition, mRNA, miRNA and protein in exosomes may be non-invasive biomarkers for chronic kidney disease (Eissa et al., 2016; Feng et al., 2018; Rossi et al., 2017). However, these findings need to be further investigated by large-scale cohort studies and randomized trials.

Summary and perspectives

So far, accumulating biomarkers have been identified to reflect various aspects of renal functional and structural changes in kidney diseases. However, more multi-center large-scale studies are needed to further verify their efficacy. Secondly, accumulating studies indicate that single biomarkers are hardly able to meet the criteria of comprehensive risk identification or diagnostic utility. More studies are needed to determine the optimal biomarkers combinations to monitor renal function, improve risk assessment for kidney outcomes, and reduce the burden of kidney disease (Figure 17; Table S12 in Supporting Information). Third, so far, Klotho is the only well-proved specific biomarker for both physiological and pathological kidney aging. Other commonly used biomarkers for aging, such as $p16^{INK4a}$, $p21^{CIP1}$, telomere-related biomarkers, also reflect the kidney aging, but not organ specific. CKD has been recognized as accelerated aging caused by various etiologies, therefore, biomarkers to predict renal dysfunction are different. Similarly, various factors contribute to the progression of physiological kidney aging by different mechanisms. The biomarkers found for kidney diseases could improve our understanding the etiology of nature kidney aging.

Skeletal aging

Physiological characteristics

Skeleton is involved in endocrine regulation and serves as an

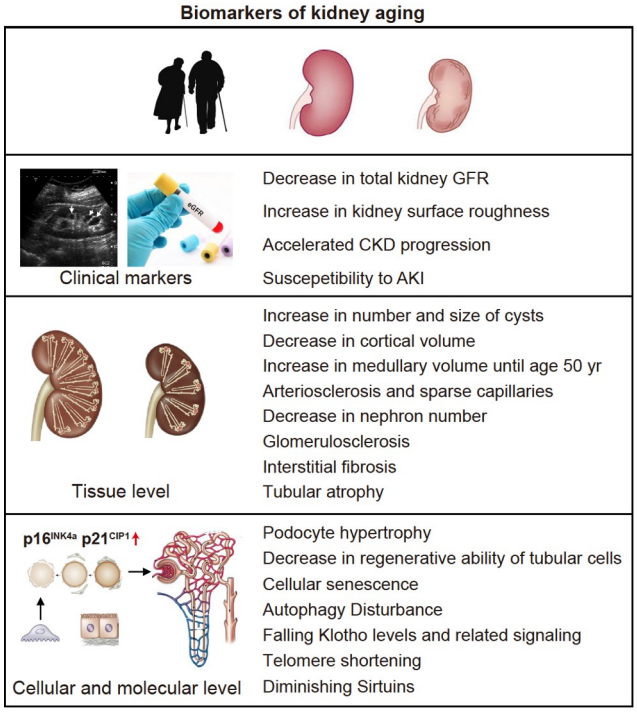

Figure 17 Biomarkers of kidney aging.

important repository for minerals, like 99% of total body calcium, which flow to and from skeleton is neutral, about five mmol is turned over a day (Song, 2017). Derangement of calcium leads to hypercalcemia and hypocalcemia, as risk factors for health (Song, 2017). Collagens can also be damaged by accumulation of advanced glycation end-products, another general feature of the aging process, causing osteoarthritis (OA), a disease in articular cartilage (Daneault et al., 2017; Rahmati et al., 2017). Skeleton material composition properties are monitored by parameters like mineral/matrix ratio, mineral maturity/crystallinity (MMC), nanoporosity, glycosaminoglycan (GAG) content, lipid content and pyridinoline content (Paschalis et al., 2016). Among these parameters, pyridinoline content shows the greatest deviation between healthy aging and postmenopausal osteoporosis.

The endocrine activity of skeleton forms a central component of a comprehensive biological system that mediates calcium-phosphate balance, energy metabolism and bone mineralization in response to dynamic and volatile physiological requirements. Endocrinological regulation of bone metabolism is highly influenced and tightly controlled by sub-categories of growth, gonadal and calcitropic hormones (Almeida et al., 2017; Brandhorst et al., 2015; Chotiyarnwong and McCloskey, 2020; Gallagher and LeRoith, 2011; Lowery and Rosen, 2018; Mazziotti et al., 2022; Mills et al., 2016; Quarles, 2012; Young et al., 2021) (Table S13 in Supporting Information). Hormonal activity begins to decline following the establishment of peak bone mass, as bone formation and resorption shifts from net formation during ontogeny, to equilibrium during early-to-middle adulthood, and net resorption during advanced age.

Beside the changes of minerals, extracellular matrix and hormones, aged bone marrow displays a signature of suppressed fatty-acids oxidation, like accumulation of free fatty acids (FFAs), polyunsaturated fatty-acids (PUFAs) and long-chain fatty-acids (LCFAs), and reduced acyl-carnitines (Connor et al., 2018). Elevated FFAs and decreased carnitine-conjugates are supposed to be resulted from suppressed β-oxidation which might activate oxidative phosphorylation pathway (Yu et al., 2017). The old bone marrow also shows a significant reduction in amino acid and nucleic acid pool (Connor et al., 2018; Navik et al., 2021). A diminished amino acids pool in old bone marrow could be the result of reduced synthesis of non-essential amino acids or from lowered autophagy. And downregulated nucleic acids may be a result of lipid peroxidation, which has been shown to generate hydroperoxides that undergo fragmentation to produce a broad range of intermediates (Kujoth et al., 2005).

Imaging traits

With aging, bone resorption increases due to a higher bone turnover rate, resulting in bone loss and decreased bone mineral density (BMD) (JafariNasabian et al., 2017). In Europe, it is estimated that 5.5 million men and 22 million women are suffering from osteoporosis (Hernlund et al., 2013). In the United States, an estimated 10 million people have osteoporosis, and this number is continuing to increase (Burge et al., 2007). At the same time, there are more than 8.9 million patients with osteoporotic fractures worldwide each year (Kimmel et al., 2022). Osteoporosis is characterized by systemic damage to bone mass, microstructure and strength, which increases fracture propensity and poses a significant economic threat to medicine and society (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). Several risk factors should be taken into account by screening, such as age, previous fragility fractures, low body mass index (BMI), glucocorticoid use, family history of fractures, and active smoking (Kanis, 2002). Measurement of BMD by double DXA is an effective method to diagnose osteoporosis, with T-score-2.5 or more below the average for young adults. N-terminal propeptide of type I procollagen (PINP) and C-telopeptide of type I collagen (CTX-I) are clinically recommended biomarkers of bone formation and bone resorption, which can be measured multiple times in a single person with high accuracy. In osteoporosis patients, PINP and CTX-I can be used to assess anabolic response and indicate possible secondary osteoporosis (Eastell and Szulc, 2017).

Imaging such as CT, MRI, FTIR also plays a vital role in the measurement of BMD and is also essential in the analysis of osteoporosis and OA.

Histologic features

Bone histomorphometry plays a vital role in studying the microstructure, morphology, and lesion characteristics and processes of the bone (Varela and Jolette, 2018). Histomorphometry has shown that bone formation rates decrease significantly with age (Kiebzak, 1991), as observed in both human and animal models. Age-related osteoporosis is characterized by a decrease in bone trabeculae quantity, average width, and their separation from each other. The average bone wall thickness and the number of cells in the bone marrow are also decreased, while the adipose tissue is increased (Singh et al., 2016). The rates of bone mineral deposition and osteoid deposition are consistent, and there is no significant decrease in peri-osteoclastic minerals (Liao and Cao, 2013). In a study of 43 healthy men between the ages of 20–80 years, it was found that the static histomorphometric parameters, such as cancellous bone volume and osteoblast-bone like interface, decreased by 40.0% and 19.2%, respectively, and the dynamic histomorphometric parameters, double and single labeled osteoid also decreased by 18.6% and 18.0%, respectively (Clarke et al., 1996). In addition, another histomorphometric analysis of rats has shown that the number of osteoclasts, the activity and number of osteoblasts that express type I collagen mRNA, were reduced in older rats (Ikeda, 1995). In cancellous bone, aging altered the relationship between osteoclasts and osteoblasts, manifesting significantly increased matrix/osteoblast-induced osteoclast formation and expansion of the osteoblast precursor pool (Cao et al., 2005). The periosteum contains undifferentiated mesenchymal stem cells with the potential for cartilage formation during fracture healing and cartilage repair. Analysis of rabbit animal model revealed that the chondrogenic potential of the periosteum decreases apparently with increasing age (O'Driscoll et al., 2001).

For OA, articular cartilage degeneration is the central pathological change. In the early phase after injury, chondrocytes proliferate and form clusters, as well as produce matrix remodeling enzymes (Varela-Eirin et al., 2018). When OA progresses, osteoarthritic chondrocytes show larger cell morphology and decreased proliferative ability, with reduced chondrogenic commitment (Singh et al., 2019). Chondrocytes in OA exhibit high levels of cellular senescence (Guo et al., 2021; Wang et al., 2022c). In aging cartilage, chondrocytes have a fibroblast-like shape, larger diameter, larger spreading areas (Sasazaki et al., 2008), and lower cytoskeletal protein renewal rate (Dominice et al., 1986), which can lead to cartilage degeneration.

Cellular alterations

Some aspects of aging clearly root in cell-intrinsic alterations, such as genomic instability, epigenetic alterations. All normal skeletal cells have a limited lifespan, which is controlled by the genomic stability and telomere length. Alterations in the methylation of DNA or acetylation and methylation of histones, like loss of H3K9me and H3K27me3, can induce epigenetic changes that contribute to aging process. Recent study has found that Lysine (K)-specific demethylase 4B (KDM4B), a H3K9me3 demethylase, whose ablation impaired skeletal stem/progenitor cell (SSPC) self-renewal and promotes stem cell exhaustion by inducing senescence-associated heterochromatin foci formation (Deng et al., 2021).

By means of fluorescence activated cell separation (FACS) and single-cell RNA sequencing multiple types of SSPCs originating from different skeletal sites, such as bone marrow, growth plate or periosteum were identified (Ambrosi et al., 2019). Although SSPCs from different sites have similar features with respect to cell surface markers, they are not identical, like differentiation capacity (Ambrosi et al., 2019). Recent studies have identified a hSSPC (Feng et al., 2022; Zhu et al., 2022b), and found that loss of SIRT1 expression but reactivation by trans-resveratrol or a small molecule compound restore the differentiation potential of aged hSSCs *in vitro* (Ambrosi et al., 2020; Chan et al., 2018). In the elderly, a progressive accumulation of senescent cells leads to elevated levels of pro-inflammatory mediator, a process known as "inflammaging". A low-grade anti-inflammatory drug can reverse a functional aging-associated decline of SSPCs (Josephson et al., 2019). Moreover, immune cells, including neutrophils and macrophages, secrete Grancalcin (GCA) to drive aging-related bone degeneration (Li et al., 2021a; Peng et al., 2022). In addition to aging environment affected by immune cells, aging SSPCs have been found to secrete colony stimulating factor 1 (CSF1) to promote the formation of osteoclasts and generate an inflammatory degenerative niche (Ambrosi et al., 2021).

Molecular changes

ROS is a typical aging hallmark of the skeletal system (Chandra and Rajawat, 2021), which is responsible for the elevated osteoclastic activity and reduced osteoblastic activity (Zhou et al., 2016). In contrast, FoxO transcription factors that promote ROS clearance show decreased expression with age (Alvarez-Garcia et al., 2017). Canonical Wnt signaling is a critical regulator of bone formation, the activity of which decreases in aged skeletal tissues (Almeida et al., 2007). The shift of bone marrow stromal cells (BMSCs) from osteogenic to adipogenic fate is also a striking characteristic during skeletal aging (Sebo et al., 2019). Expression of PPARγ, a transcription factor that is essential for adipogenic differentiation, is upregulated in BMSCs with age (Lecka-Czernik et al., 2010). PPARγ$^{+/-}$ mice show increased bone mass, while PPARγ$^{+/-}$ BMSCs show enhanced osteogenesis at the expense of adipogenesis (Akune et al., 2004). PGC-1α, an adipogenic inhibitor, shows

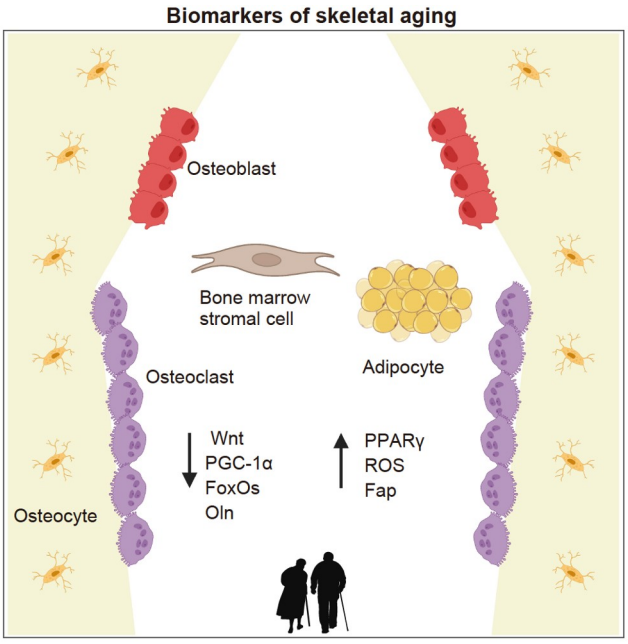

Figure 18 Biomarkers of skeletal aging. In aged skeletons, PPARγ, ROS and Fap are significantly increased, while canonical Wnt signaling, PGC-1α, FoxOs and Oln are decreased. Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ.

decreased expression with age in human and mouse skeletal stem cells (Yu et al., 2018). Fibroblast activation protein (Fap) is a serine protease whose activity significantly increases during aging (Wei et al., 2020). Genetic or pharmacological inhibition of Fap promotes bone formation and inhibits bone resorption by activating canonical Wnt signaling and dampening NF-κB signaling, respectively (Wei et al., 2020). Clec11a/Osteolectin (Oln) is a bone growth factor that inhibits Fap activity (Wei et al., 2020; Yue et al., 2016). In contrast to Fap, the plasma level of Oln significantly decreases with age, and $Oln^{-/-}$ mice show significantly decreased bone formation and premature osteoporosis (Wei et al., 2020; Yue et al., 2016) (Figure 18). Interestingly, Fap is also significantly increased in the synovium of knee joints during aging, which contributes to OA progression by degrading type II collagen of the articular cartilage (Fan et al., 2023). In contrast, Oln forms a protective layer in the superficial zone of articular cartilage to inhibit Fap activity, and significantly decreases during OA progression (Fan et al., 2023).

Secretory factors detectable in biofluids

Bone is not only a structural scaffold to support the body, but also an important endocrine organ (Guntur and Rosen, 2012). Senescent skeletal cells secret factors such as cytokines, chemokines, growth factors and proteases, which are known as SASPs (Pignolo et al., 2021). GH stimulates the production of IGF-1 to promote bone growth and development (Junnila et al., 2013). In humans, systemic and skeletal IGF-1 decline substantially with age (Bennett et al., 1984; Boonen et al., 1997). Systemic IGF-1 is also decreased in aged mice (Young et al., 2021). Conditional deletion of *Igf1* from BMSCs or megakaryocytes/platelets causes bone loss and defective fracture repair in the adult skeleton (Wang et al., 2023). Osteoclasts are responsible for resorbing the bone matrix and play important roles in bone remodeling. Macrophage-colony stimulating factor (M-CSF) secreted by BMSCs/osteoblasts promotes the proliferation of osteoclast precursors (macrophages). Differentiation of osteoclasts also requires the engagement of osteoblast-derived RANKL with its cognate receptor RANK (Boyle et al., 2003). Osteoprotegerin (OPG) is also secreted by osteoblasts, which functions as a decoy receptor of RANKL to negatively regulate its activity (Lacey et al., 1998). Aged skeletons show increased expression of M-CSF, RANKL and decreased expression of OPG, leading to enhanced osteoclast-mediated bone resorption (Chung et al., 2014). Decreased of bone formation markers such as Procollagen type I N-terminal propeptide (P1NP) and Osteocalcins (OCN), and increase of bone resorption marker, such as carboxy-terminal collagen cross-links (CTx), amino-terminal cross-linking telopeptide of type I collagen (NTx), deoxypyridinoline (DPD) and Tartrate-resistant acid phosphatase 5b (TRAcP5b) are typical hallmarks of skeletal aging (Kikuchi et al., 2021; Ryan and Elahi, 1998; Shahnazari et al., 2012; Takahashi et al., 1999; Tokida et al., 2021). Sclerostin (SOST) and Dickkopf-related protein 1 (DKK1) are secreted by osteocytes, both of which show increased expression with age and impair osteoblast formation by inhibiting Wnt signaling (Shahnazari et al., 2012). Aging and bone remodeling are also associated with dynamic change of circulating cytokines and proinflammatory factors (Michaud et al., 2013). For example, elevated levels of CRP, IL-1, IL-6, and TNFα can be detected in osteoporotic women (Koh et al., 2005; Zheng et al., 1997) (Table S13 in Supporting Information).

Summary and perspectives

The skeleton plays an important role in providing mechanical support and maintaining calcium ion homeostasis. New insights of the regulators controlling skeletal aging, like findings of cell-extrinsic and cell-intrinsic factors, pinpoint the pathways that could be targeted to reverse these aging-dependent changes. Additionally, large-scale omics profiling, particularly at the single-cell level, is uncovering clinically actionable conditions and novel molecular pathways for treating aging-related skeletal diseases. At the same time, it can be more easily to detect and identify more precise biomarkers of bone aging as technology evolves, facilitating an improved understanding of age-related skeletal changes and suggesting more effective targets for prevention.

Adipose tissue aging

Physiological characteristics

AT, commonly refers to as fat, are a highly flexible and heterogeneous organ composed of mature adipocytes, pre-adipocytes, progenitor cells, vascular endothelial cells and immune cells (Sakers et al., 2022). Though originally considered as an inert energy repository, AT is now well recognized as a metabolically active endocrine organ vital for whole-body energy balance, food intake, lipid and glycemic homeostasis, thermogenesis and immune responses.

There are three types of AT characterized of distinct anatomic location, morphology and function. In mice, the major function of white adipose tissues (WAT) is to store energy as triglyceride. The interscapular brown adipose tissues (BAT) and the subcutaneous WAT (sWAT)-dispersing beige fat dissipate energy as heat (Cohen and Kajimura, 2021). Adult humans possess various WAT depots, as well as functional BAT and beige fat in areas including ventral neck and supraclavicular area (Nedergaard et al., 2007; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zwick et al., 2018). White adipocytes feature single lipid droplets and low mitochondria numbers, while brown adipocytes contain multilocular lipid droplets, high mitochondria content that endow its brownish color, and express high levels of heat-producing uncoupling protein 1 (UCP-1). Beige adipocytes, which reside in dispersed fashion in sWAT and are indistinguishable with white adipocytes in basal state, adopt brown adipocyte-like features via a process called "browning" under stimulation conditions such as cold exposure, β3-adnergic signal input, mild hyperthermia, and exercise. This inducible thermogenic capacity of beige adipocytes bestows a great potential in increasing energy expenditure, thus emerging as a valuable therapeutic target for metabolic diseases (Harms and Seale, 2013).

Imaging traits

In clinic, BMI, skinfold anthropometry and bioelectrical impedance are used as indirect methods for assessing body fat content. To understand the characteristics and heterogeneity of fat depots, medical imaging to visualize body fat has been actively developed. Dual-energy DXA has weak ionizing radiation and measures whole-body or regional body composition in both rodents and humans, but cannot detect overlapping fat compartments or ectopic fat in organs like liver or muscle (Wang et al., 2014a). CT is a volumetric technique that is excellent for measuring regional adiposity, i.e., visceral AT and fat content in liver and muscles (Goodpaster et al., 2000; Kramer et al., 2017; Wang et al., 2014a). However, this technique has a drawback in potential exposure of significant radiation (Brenner and Hall, 2007). MRI and magnetic resonance spectroscopy (MRS) exploit the difference in the magnetic properties of hydrogen nuclei in water and fat to quantify the signal fat-fraction and/or the proton density fat-fraction in tissues (Reeder et al., 2011; Wu et al., 2020b). MRI/MRS can detect visceral fat and subcutaneous fat, as well as intramyocellular and intrahepatic lipids noninvasively with excellent reproducibility and better visibility of anatomical details without overt radioactivity (Addison et al., 2014; Sivam et al., 2012). However, complicated data analysis and high cost limit the routine use of this equipment in clinic (Ponti et al., 2019). Whilst CT and MRI/MRS are extremely informative in quantifying fat in depots and within organs, PET/CT provides vital information on metabolic activity of fat depots. Notably, ^{18}F-FDG PET/CT, which could reveal the existence of functional, aging-associated, and cold-activated BAT in adult human (Yoneshiro et al., 2011), is the current gold standard for BAT imaging study (Chen et al., 2016b). The downside of the ^{18}F-FDG PET/CT usage is its relatively complicated procedure and large dose of radiation due to ^{18}F-FDG intake (Law et al., 2018). Infrared thermography (IRT) collects thermal radiation from infrared radiation and converts it into false-color thermal images. Utilizing the heat-generating properties of thermogenic fat, IRT, i.e., infrared camera, has been wildly used for its non-invasive and low-cost feature to assess thermogenesis under different stimulations including cold, hyperthermia, glucocorticoids or caffeine exposure (Li et al., 2022i; Ramage et al., 2016; Symonds et al., 2012; Velickovic et al., 2019; Xu et al., 2022). Though controversy remains, a close correlation between IRT measurement and the accumulation of ^{18}F-FDG in mice and humans has been confirmed (Carter et al., 2011; Law et al., 2018), suggesting that IRT may be an easy and promising method for detecting brown and beige fat activation.

Histologic features

Fat mass increases with age but declines after approximately 60 years of age (Raguso et al., 2006). Fat redistributes from sWAT depots to intra-abdominal visceral WAT (vWAT) depots during and after middle age (DeNino et al., 2001; Hughes et al., 2004), while the quantity and functionality of brown and beige fat decline with age (Gohlke et al., 2019; Sellayah and Sikder, 2014). sWAT is associated with many of the metabolic benefits and vWAT adiposity leads to chronic inflammation and higher risks of various comorbidities (Mtintsilana et al., 2019; Siervo et al., 2016). Meanwhile, dysfunctional thermogenic AT may lead to impaired energy expenditure and energy substrates (glucose and free fatty acids) clearance. These histological changes of fat depots may contribute to the age-related metabolic diseases and underlie the deteriorating metabolic status and aggravating thermal dysregulation in the elderly, thus highlighting the importance of AT homeostasis in metabolic health and longevity (Berry et al., 2017; Cypess et al., 2009).

The abundant loose connective tissues around adipocytes

called stromal vascular fraction (SVF) harbor heterogeneous cell populations including adipose stem and progenitor cells (ASPCs), preadipocytes (PreAs) and adipogenesis-regulatory cells (Aregs). ASPCs is multipotent for adipogenic differentiation and cell renewal (Zuk et al., 2001). Lineage commitment of PreAs from ASPCs undergo growth arrest and differentiation into mature adipocytes (Gupta et al., 2010). Aregs regulate adipogenesis by blocking the adipogenic capacity of ASPCs (Schwalie et al., 2018). During aging, the replication potential and the capacity to differentiate into lipogenic lineages of ASPCs decrease gradually, damaging AT homeostasis (Schipper et al., 2008; Zhu et al., 2009). SVF contains heterogeneous cell populations such as mesenchymal progenitor/stem cells, endothelial cells, pericytes, and immune cells such as T cells and macrophages, which may produce inflammatory factors under pathophysiological conditions such as obesity and aging. Indeed, aging is characterized of a persistent low-grade, sterile and chronic pro-inflammatory status, a phenomenon refer to as inflammaging (Campisi et al., 2019). Increased infiltration of inflammatory immune cells is observed in WAT during aging, which accumulates in crown-like clusters in perivascular spaces and secrets pro-inflammatory cytokines and chemokines, contributing to WAT inflammaging. Furthermore, aging is associated with excess release, accumulation and modification of ECM components, including the production and secretion of multiple MMP proteins and fibronectin, which leads to AT fibrosis (De Luca et al., 2021).

Cellular alterations

Cell senescence is defined as an irreversible proliferative arrest driven by various mechanisms, such as telomere shortening, DNA damage and ROS, which cause cell cycle inhibition via $p16^{INK4}$ or cell cycle arrest via activation of $p53/p21^{CIP1}$. During aging, senescent cells accumulate in AT and secrete SASP-related factors consisting of cytokines, chemokines, proteases and growth factors, which impair AT function and dysregulate adipogenesis, leading to inflammation, fibrosis and insulin resistance (Campisi et al., 2019; Milanovic et al., 2018; Wiley et al., 2016).

Mitochondria are core to brown and beige adipocytes in maintaining intracellular glucose and lipid metabolism and heat generation. During aging, thermogenic fat exhibits mitochondrial dysfunction that manifests as accumulation of mtDNA mutations and deletion, oxidation of mitochondrial proteins, instability of macromolecular organization of the respiratory chain complex, changes in mitochondrial membrane lipid composition, alterations in mitochondrial dynamics, and defective mitochondrial autophagy (López-Otín et al., 2013), leading to a constitutive decline in mitochondrial oxidative phosphorylation (Hu et al., 2021). The mitochondrial free radical theory of aging proposes that the progressive mitochondrial dysfunction increases ROS production, which in turn causes further mitochondrial deterioration and global cellular damage.

The decline in tissue regenerative potential is pronounced characteristic of aging. AT expands to store excessive energy, which relies on ASPCs proliferation and differentiation to generate new adipocytes (Ghaben and Scherer, 2019). ASPCs isolated from old individuals show cellular senescence and loss of adipogenic potential due to telomere shortening and DNA damages (López-Otín et al., 2013; Palmer et al., 2019; Tchkonia et al., 2010). By scRNA-seq analysis of changes in different SVF populations during aging, an aging-dependent regulatory cell (ARC) population controlled by transcription factor Pu.1. was detected specifically in sWAT, which inhibited differentiation and proliferation of neighboring adipogenic precursors by secreting cytokines (Nguyen et al., 2021).

The crosstalk between immune cells and adipocytes is vital for AT function and systemic metabolism. Macrophages are the most abundant cell type in AT. Though total numbers of proinflammatory M1 macrophages and anti-inflammatory M2 macrophages in fat remains stable during aging, the ratio between M1 and M2 macrophages increases modulated by NF-κB, long-chain saturated fatty acids and hypoxia (Ou et al., 2022). T and B lymphocytes constitute the second most abundant immune cell population in AT. Aging induces a significant increase in inflammatory $CD4^+$ and $CD8^+$ T cells with enhanced pro-inflammatory cytokines such as IFN-γ, TNFα and IL-17, contributing to increased inflammaging (Lumeng et al., 2011). Furthermore, it is revealed that transition of $CD73^{hi}ST2^{lo}$ into $CD73^{lo}ST2^{hi}$ fat-resident Treg subsets (fTreg) and memory T cells (γδT) are increased during aging (Carrasco et al., 2022). Besides, B cells also increase in WAT with aging, which promoted antigen presentation and pro-inflammatory cytokines and pathogenic antibody secretion, overall result in inflammaging and aggravated insulin resistance (Zamboni et al., 2021).

AT fibrosis also underlies increased inflammation during aging (Eckel-Mahan et al., 2020). Recent scRNA-seq studies have identified a functionally distinct subpopulation of ASPCs, $PDGFR\beta^+LY6C^+$ in mouse vWAT that lacks inherent adipogenic capacity but exhibits fibrogenic and pro-inflammatory characteristic, thus are potentially predisposed to drive fibrosis and subsequent inflammaging (Hepler et al., 2018).

Molecular changes

Age-associated senescence of beige progenitor cells leads to impaired beige adipocyte differentiation and reduced browning upon cold stimulation. Targeting the p38/MAPK-$p16^{INK4a}$ pathway rejuvenates beige progenitors and restores browning (Berry et al., 2017), indicating anti-senescence modalities as a promising strategy inducing beiging and improving metabolic health in aging humans. Aside from $p16^{INK4a}$, SREBP1c may play a key role in senescence in

adipocytes by modulating DNA-damage responses via interaction with PARP1, independent of its lipogenic regulatory function. Genetic depletion of SREBP1c accelerates adipocyte senescence, leading to adipose tissue inflammation and insulin resistance (Lee et al., 2022a).

Aging leads to increased fat deposition and programmed loss of brown and beige adipocytes contributed by changes in core thermogenic regulators. For example, aging regulates Forkhead box factor A3 (Foxa3) levels by glucocorticoid signaling, consequently cooperates with C/EBPs for PPARγ transcription and lipid storage, while competing with CREB on *Pgc1α* promoter to inhibit its transcription and reduced thermogenic and mitochondrial functions, overall reduced energy expenditure (Ma et al., 2014; Ma et al., 2016). Meanwhile, aging leads to impaired epigenetic regulation such as enhanced 273 phosphorylation of Pparγ and caused insulin resistance (Xu et al., 2018a). Aged adipocytes also feature an increase in autophagic activity. Genetic ablation of Rubicon in adipocytes exacerbates metabolic disorders by promoting excess autophagic degradation of PPARγ coactivators SRC-1 and TIF2, highlighting a critical involvement of autophagy during aging (Yamamuro et al., 2020).

Impaired protein and metabolite secretion impact AT homeostasis during aging. For example, FGF21 has been proposed to be an aging intervention hormone, which induces browning of white fat, while FGF6 is shown to act as a proliferative factor for ASPC hyperplasia and prevents fibrosis and maintain AT homeostasis during aging (Liu et al., 2023a). Meanwhile, exercise-induced myokine Irisin promotes browning and improves age-associated metabolic dysfunction while its level declines under sarcopenia (Boström et al., 2012; Guo et al., 2023). On the other hand, cold exposure leads to increased hepatic acylcarnitine production and thermogenesis, which is impaired in aged mice (Simcox et al., 2017). Besides, mitochondrial proteomics analysis shows that mitochondrial lipoylation is disproportionally reduced in aged BAT, and enhancing mitochondrial lipoylation by α-lipoic acid supplementation effectively restores BAT function in old mice, thereby preventing age-associated obesity and glucose intolerance (Tajima et al., 2019).

It is noted that though detailed molecular changes may not be identical, obesity, which represents all major characteristics of age-associated impairment in AT homeostasis, is proposed as a state of accelerated aging and is itself a major risk factor for worsened age-associated metabolic diseases. The intervention strategies against obesity, such as caloric restriction and metformin, are also effective in preventing aging (Geng et al., 2022; Ou et al., 2022).

Secretory factors detectable in biofluids

Senescent cells express SASP factors that link their accretion with metabolic disorders. During aging, adipocytes chronologically accumulate lipids and pro-inflammatory factors such as IL-6, MCP-1 and TNFα under NF-κB signaling (Ahmed and Si, 2021). In addition to classic SASP factors, other forms of SASP were discovered. For example, aged sWAT shows a significant increase in pregnancy-associated plasma protein-A (PAPP-A) levels, which are also highly enriched on the surface of extracellular vesicles secreted by senescent pre-adipocytes (Conover and Bale, 2022).

AT, which synthesizes and secretes numerous bioactive molecules termed adipokines, has now been well recognized as an endocrine organ that regulates whole-body energy homeostasis (Fasshauer and Blüher, 2015). Dysregulated adipokine biosynthesis and secretion are regarded as a key feature of obesity and aging associated diseases (Arai et al., 2019; Tilg and Moschen, 2006). Leptin is a well-established adipokine that communicates with central nervous system to regulate appetite, satiety and energy expenditure. Leptin is associated with a reduced incidence of dementia and AD and with cerebral brain volume in asymptomatic older adults close to 80 years of age (Lieb et al., 2009). The functional decline of responsiveness to leptin in central nerve system with age may lead to negative consequences for cognitive function (Irving and Harvey, 2021), suggesting a pro-cognitive effect of leptin. Adiponectin, on the other hand, is associated with reduced inflammation and improved metabolic status in the elderly, which is positively correlated with longevity (Arai et al., 2019) by blocking NF-κB activation and inhibiting proinflammatory cytokine synthesis. Adiponectin also improves neuronal metabolism, muscle function and cardiovascular health that are highly related to aging, possibly via adiponectin receptors in these systems (Yamauchi and Kadowaki, 2013).

In aged animals, the differentiation of precursor cells of the metabolically beneficial beige adipocytes is impaired and transited toward an alternatively fibrogenic profile, which correlated with reduced adipocyte expression of PRDM16. PRDM16-expressing adipose cells secrete β-hydroxybutyrate (BHB), which blocks precursor fibrogenesis and facilitates beige adipogenesis. Dietary BHB supplementation in aged animals reduces adipose fibrosis and promoted beige fat formation (Wang et al., 2019), Moreover, sphingolipids, such as ceramides, accumulate and mediate metabolic impairment of thermogenic adipocytes during aging. Blocking ceramide synthesis in thermogenic adipocytes improves adipose biology and function (Chaurasia et al., 2021), suggesting that adipocyte-secreted metabolites may control beige fat remodeling.

AT from Ames dwarf (df/df) mice, an exceptionally long-lived animal model, provides additional information. Previous studies showed that miR-146a-5p increases during aging and this increase is not observed in long-living df/df mice. Intraperitoneal injections with miR-146a-5p mimetic increases cellular senescence and inflammation but decreases pro-apoptotic factors in vWAT of df/df mice, which is

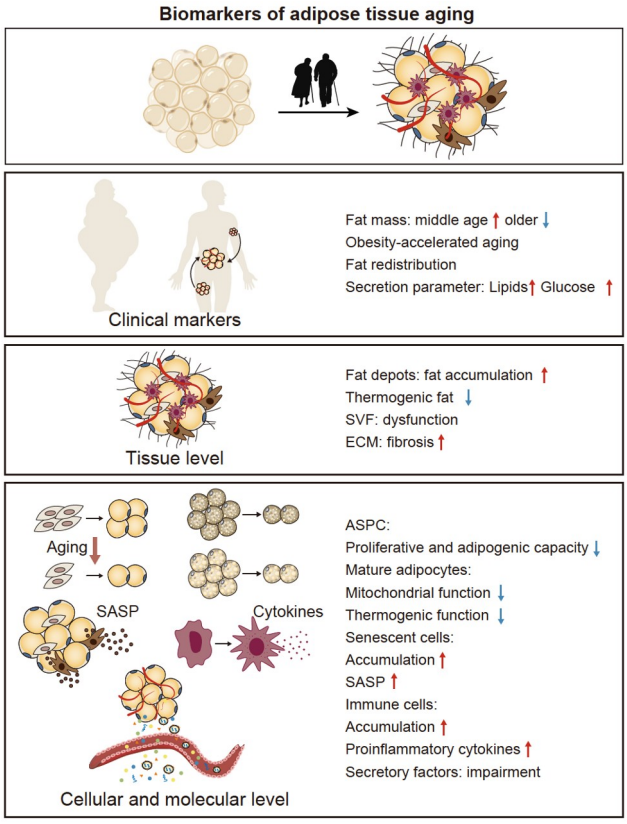

Figure 19 Biomarkers of adipose tissue aging. Fat mass increases with age but declines at older stages. Besides, aging is associated with fat redistribution from subcutaneous AT depots to intra-abdominal visceral depots, accompanied with hyperlipidemia and hyperglycemia, which is accelerated by obesity. Fat depots remodel during aging with fat accumulation, loss of thermogenic fat, dysfunction in stromal vascular fraction, and excessive accumulation of extracellular matrix. At the cellular level, aging leads to exhaustion and functional decline of adipose stem and progenitor cells, reduction in brown and beige adipocytes, accumulation of senescent cells producing SASP, dysregulation of immune cells, enhanced inflammation and fibrosis, and impaired secretory factors.

recapitulated in 3T3-L1 cells, indicating that miR-146a-5p can be a marker for cellular senescence (Nunes et al., 2022). Furthermore, 5-hydroxyeicosapentaenoic acid (5-HEPE), an ω-3 fatty acid metabolite, is increased in BAT and circulation of dwarf mice, which correlates with increased thermogenesis and insulin sensitivity. The levels of 5-HEPE are positively correlated with BAT activation and negatively correlated with body weight, insulin resistance and triglyceride levels in humans, thus represent a novel lipid secretory signature of AT in a mouse model of extreme longevity (Darcy et al., 2020) (Figure 19).

Skin aging

Skin aging is the most recognizable outcome of aging, which also directly reflects the degree of whole-body aging to some extent. Skin aging is a sophisticated, multifactorial process involving multiple steps in which chronologic aging and photoaging are closely intertwined (Kohl et al., 2011). The aged skin manifests structural, functional, cellular, and molecular changes as well as an accumulation of senescent cells (Franco et al., 2022). More importantly, these cells are accompanied by SASP, which induces senescence in adjacent cells through a process called paracrine senescence in the skin, further triggering age-related dysfunction of other tissues (Franco et al., 2022) (Figure 20).

Physiological characteristics

Like other tissues, the structural stability and physiological function of the skin are affected by aging. The most obvious sign is the appearance of wrinkles (Zhao et al., 2020a). Skin elasticity shows the clinical ravages of time, and the aged skin sags in the direction of gravity, resulting in bags under the eyes, etc. (Blair et al., 2020; Panwar et al., 2015). In addition, skin aging is accompanied by alterations in pigmentation, such as lighter color in unexposed areas due to reduced pigment synthesis, and colored patches in exposed areas due to increased pigment synthesis as a result of sunlight and ultraviolet (UV) rays (Kang et al., 2021). In addition, the irregular proliferation of epidermal keratinocytes leads to the occurrence of papules and maculopapules (seborrheic keratosis). Vascular changes are also common features of skin aging. Vascular proliferation in non-exposed areas forms senility angiomas, while exposed areas exhibit cutaneous vasodilation and reticulovascular proliferation, which is particularly prominent in highland populations (Kajiya et al., 2011). Hair follicle (HF) aging is another phenotypic trait of skin aging, usually manifesting as diffuse thinning, softening, graying, and gradual loss of luster (Fernandez-Flores et al., 2019). Moreover, there is a decrease in the secretion of sebaceous gland (SG) and sweat gland, usually resulting in xerosis (Ahmed et al., 2022).

The skin provides a barrier against the environment. However, it is more than just a barrier; it is also involved in physiological functions, including the maintenance of hydration and secretion, thermoregulation, immunological surveillance (Gravitz, 2018). It has been demonstrated that the aged skin had a reduced ability to transport water, hydrogen peroxide, drugs, and other substances due to impaired hydration (Ferreira et al., 2020). With age, impaired secretion of sweat glands along with reduced vasodilation of dermal arterioles and the loss of subcutaneous fat led to thermoregulation disorders (Ding et al., 2021b; Lazarus et al., 2019). Repair processes of collagen remodeling, cell proliferation, and wound metabolism in the elderly were shown to be delayed (Kenney et al., 2021; Mistry et al., 2021). Furthermore, a more pronounced inflammatory phenotype has been uncovered in aged skin wounds, featured by the persistence of neutrophils and a higher abundance of inflammatory macrophage subsets compared to younger counterparts (Kenney et al., 2021; Vu et al., 2022). Recent

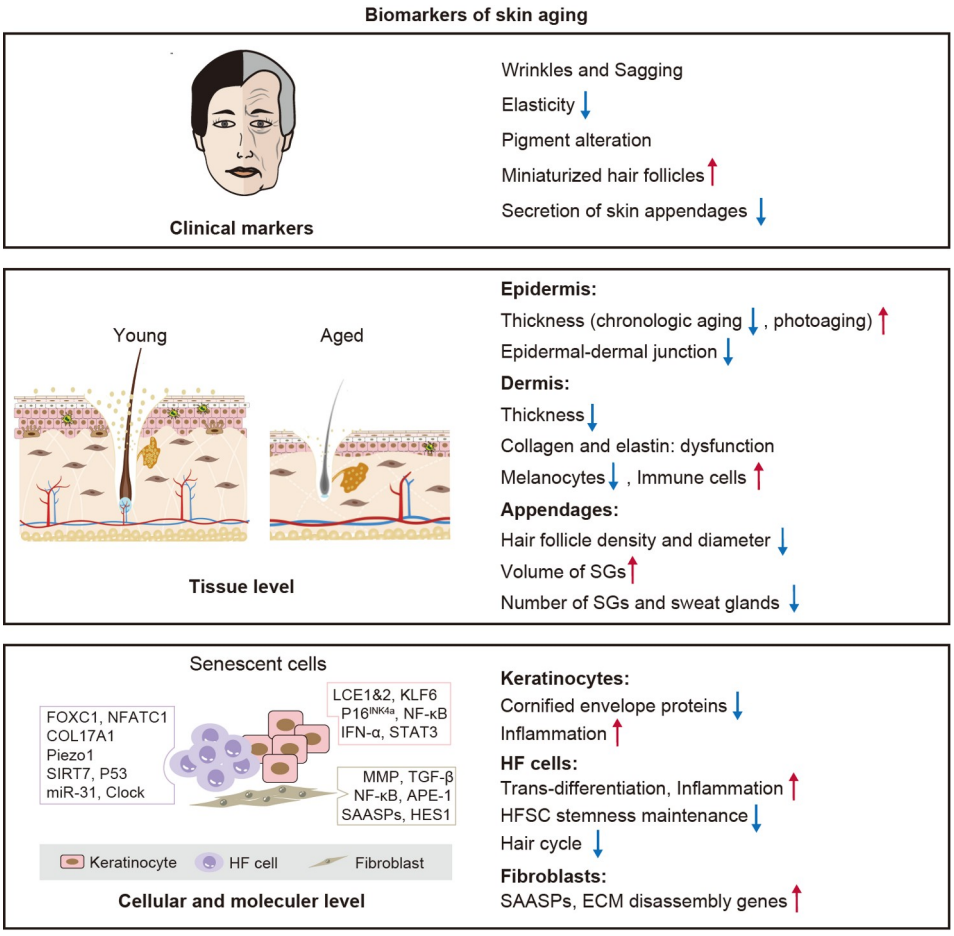

Figure 20 Biomarkers of skin aging. The skin mainly comprises epidermis, dermis, and skin appendages. Skin aging can be roughly divided into chronologic aging caused by intrinsic factors and photoaging caused by environmental factors. Aged skin is featured by the development of wrinkles and sagging, accompanied by decreased skin elasticity, which is due to a flattening of epidermal-dermal junction and a decrease/degradation of dermal collagen and elastin. Alterations in skin appendages, such as the increased miniaturized hair follicles and the reduced secretion of skin appendages, are common features during aging. Moreover, the amount and activity of melanocytes and immune cells in skin are dysregulated, resulting in uneven pigmentation and impaired immunological surveillance. Simultaneously, senescent cells accumulate in the skin with age. These cells exhibit the loss of proliferative potential, and several other markers for specific cell types, such as reduced cornified envelop proteins in keratinocytes, hampered stemness and delayed hair cycle in HF cells, as well as elevated ECM disassembly genes in fibroblasts. Notably, SASP from these senescent skin cells further triggers senescence in adjacent cells. This crosstalk among different cells eventually results in an aging phenotype. Abbreviations: FOXC1, forkhead box C1; COL17A, collagen type XVII; LCE1&2, late cornified envelope group I & 2; KLF6, krüppel-like factor 6; STAT3, signal transducer and activator of transcription 3; HES1, hairy and enhancer of split 1.

studies have also revealed an increase in immuno-suppressive activity in the aging process of the skin (Salminen, 2020; Salminen et al., 2022).

Imaging traits

The skin changes caused by aging can be detected by optical and ultrasonic imaging. Reflectance confocal microscopy (RCM) observes that skin thickness decreases and epidermal grooves increase; meanwhile, coarse collagen fibers and coiled collagen in the dermis increase (Segurado-Miravalles et al., 2018). Optical coherence tomography (OCT) shows uneven epidermal surface, significant light attenuation, and reduced papillary layer in the dermis (collagen degeneration) during aging (Mamalis et al., 2015). Dermatoscopic features of skin aging include severe xerosis, surface irregularity, atrophy, uneven pigmentation, telangiectasia, miniaturized HFs, and hair graying (Hu et al., 2019a; Ye et al., 2021). As observed by high-frequency ultrasound imaging, the echogenicity of the aged skin is diminished and its thickness decreases; besides, the aged dermis shows more irregularities in the subdermal interface (Vergilio et al., 2021).

Histologic features

There are differences between chronologic aging and photoaging in histological changes. During chronologic aging, one of the first alterations is thinning of the epidermal layer associated with the loss of epidermal rete ridges (Blume-Peytavi et al., 2016; Branchet et al., 1990; Czekalla et al., 2019; Lintzeri et al., 2022; Lock-Andersen et al., 1997), accompanied by a reduced count of melanocytes and Lan-

gerhans cells (Bhushan et al., 2002; Lavker, 1979), and acanthosis atrophy (El-Domyati et al., 2002; Lavker, 1979). The reduced epidermal-dermal junction is another major feature of skin aging (Lavker et al., 1986; Lavker et al., 1987). The dermis also thins gradually, mainly due to the reduction of dermal collagen fibers and elastin. There is an additional change in the dermis namely less compacted collagen bundles in the reticular dermis (Braverman and Fonferko, 1982; Lovell et al., 1987; Uitto, 2008). In addition, the number of dermal micro-vessels decreases (Toyoda et al., 2001), as well as the skin appendages (including HF, SG, and sweat gland) (Montagna and Carlisle, 1990), however, the volume of SGs increases. The aged skin is also accompanied by thinner subcutaneous tissue with fewer blood vessels and fat.

The skin epidermis of photoaging is characterized by irregularly thickened, which may be due to the irregular proliferation, differentiation and apoptosis of keratinocytes (Kanaki et al., 2016). However, studies have shown that severe photodamage can lead to epidermal atrophy (Bhawan et al., 1995; Fu et al., 2016). The changes of the dermis are the most pronounced of the photoaged skin, mainly including the deformation, thickening, and bifurcation of elastic fiber, the reduction of collagen fibers, and the cleavage of hyaluronic acid and other mucopolysaccharide components in the matrix (Fisher et al., 2002; Hughes et al., 2011; Lavker, 1979; Uitto, 2008), ultimately resulting in dry, loose, inelastic skin. Photoaging skin shows a decrease in the density of dermal micro-vessels, accompanied by an increase in the thickness of the vessel walls (Toyoda et al., 2001). In addition, the subcutaneous blood vessels in photoaged skin become varicose and dilated, increasing the vulnerability of the blood vessels.

Cellular alterations

The skin epithelium contains inter-follicular epidermis (IFE), HF, SG and sweat glands (Franco et al., 2022). It is a constantly self-renewing tissue mainly made by keratinocytes. The IFE is a stratified epithelium composed by basal, spinous, granular, and cornified layers. In aged human IFE, the normal stratified structure is maintained, but shows reduced epithelial extensions into dermis and/or overall thickness (Giangreco et al., 2010; Zou et al., 2021). In the basal layer, overall cell density and the abundance of IFE stem cells (IFESCs) expressing high MCSP and β1 integrin are reduced (Giangreco et al., 2010). Interestingly, overall cell proliferation in aged human IFE is not necessarily reduced (Giangreco et al., 2010), but may become further confined to the basal layer (Thuringer and Katzberg, 1959). Increased $p16^{INK4a+}$ senescent cells may be mostly melanocytes (Victorelli et al., 2019; Zou et al., 2021). Clonal expansion of IFESCs is prevalent during skin aging (Liu et al., 2019; Martincorena et al., 2015), reminiscent to that of clonal hematopoiesis (CH).

HF is a cyclic regenerating mini-organ driven by hair follicle stem cells (HFSCs). In late-stage HF aging, HFSCs are depleted through trans-differentiation or apoptosis, leading to HF miniaturization (Dries et al., 2021; Matsumura et al., 2016; Xie et al., 2022). In early-stage HF aging, HFSCs are maintained but with blunted regeneration responsiveness (Doles et al., 2012; Ge et al., 2020; Giangreco et al., 2008; Keyes et al., 2013; Zhao et al., 2022b). Hair graying is caused by loss of HF melanocytes and their stem cells (Arck et al., 2006; Nishimura et al., 2005), and is also associated with depletion of hair progenitors in human scalp HFs (Wu et al., 2022a). HF aging is also accompanied by changes in the distribution of nearby sensory neurons (Ge et al., 2020) and atrophy or dysfunction of SGs (Giangreco et al., 2008; Hou et al., 2022).

The skin dermis is mainly made by dermal fibroblasts and their ECM products (Lynch and Watt, 2018). The dermal fibroblasts are mostly non-proliferative during normal homeostasis and are gradually lost during aging (Marsh et al., 2018), leading to dermal atrophy with decreased tissue density, altered ECM composition, decreased contact between the cells and collagen fibers, and accumulation of $p16^{INK4a+}$ senescent cells (Farage et al., 2013; Haydont et al., 2019; Ogata et al., 2021; Quan et al., 2013; Zou et al., 2021).

Different types of immune cells in the skin undergo different changes during aging. In naturally aged IFE, Langerhans cells and their ability to migrate are reduced, the proportion of $CD4^+/CD8^+$ T cells is increased, and the proliferative capacity of monocytes is reduced, accompanied by impaired immune responsiveness and wound healing ability (Keyes et al., 2016; Koguchi-Yoshioka et al., 2021; Nestle et al., 2009). In HF aging, increased immune cell infiltration into HF and activation of inflammatory signaling pathways in HFSCs are observed (Doles et al., 2012; Wu et al., 2022a). In the dermis, increased infiltration of macrophages, T cells, and mast cells are also observed during photoaging (Bosset et al., 2003).

Molecular changes

Aged skin epidermis shows reduced levels of several calcium controlled cornified envelope proteins, such as loricrin, filaggrin and LCE1&2, implicating deregulated calcium gradient in IFE (Rinnerthaler et al., 2013). Aged epidermal keratinocytes also have reduced pro-growth transcription factor (TF) KLF6 and elevated $p16^{INK4a}$ (Adamus et al., 2014; Zou et al., 2021), implicating reduced growth potency. Several inflammatory signaling pathways, such as NF-κB and interferon-α pathways, are upregulated in aged IFE under normal homeostasis (Zou et al., 2021), consistent with the common theme of chronic inflammation (inflammaging) in other tissues. That said, aged epidermal keratinocytes are inefficient at activating STAT3 or up-regulating Skints under

wounding conditions, leading to reduced inflammatory response and delayed wound healing in aged skin (Keyes et al., 2016).

Aged HFs show sophisticated molecular changes depending on their hair cycle and aging status. Aging HFs at miniaturizing stage have reduced level of several key genes involved in HFSC maintenance, including *FOXC1*, *NFATC1* and *COL17A1*, leading to HFSC trans-differentiation and elimination (Matsumura et al., 2016; Zhang et al., 2021b). In aging HFs with hair shaft shrinkage, activated Piezo1 induces apoptosis via a calcium-TNFα pathway in HFSCs (Xie et al., 2022). In aging murine HFs with intact structure, HFSCs displays inflammaging feature mediated by JAK/STAT activation, along with upregulated NFATC1 expression and downregulated SIRT7 expression, leading to hampered hair cycle activation (Doles et al., 2012; Keyes et al., 2013; Li et al., 2020b). In human scalp HF, activation of P53 and inflammatory pathways are found in HFSCs and hair progenitors at early stage of hair graying (Wu et al., 2022a). Aging HFSCs also upregulate miR-31, which downregulates Clock to activate MAPK/ERK signaling to inhibit hair cycle activation and drive HFSC trans-epidermal elimination (Yu et al., 2021). Notably, miR-31 is also a pro-inflammatory microRNA that is up-regulated in IFE aging (Yan et al., 2015; Yu et al., 2021).

Aged dermis shows degradation of the dermal collagen network and elastin fibers, due to upregulation of MMP activity and downregulation of TGF-β signaling (Haydont et al., 2019; He et al., 2014a; Parkinson et al., 2015; Qin et al., 2017). These can be attributed to NF-κB and AP-1 activation induced by ROS, which is a key driver of dermal aging (Chiang et al., 2013; Shin et al., 2019; Vicentini et al., 2011). The fibroblasts of aged dermis also show increased senescence and production of skin aging-associated secreted proteins (SAASPs), which overlap with classical SASPs in matrix degradation and pro-inflammatory categories (Waldera Lupa et al., 2015). Single-cell transcriptional analysis confirms that aged dermis has elevated ECM disassembly genes and decreased growth control genes, with HES1 as potential core regulatory TF (Zou et al., 2021).

Secretory factors detectable in biofluids

Few studies have reported that specific biomarkers of skin aging can be detected in body fluids. Interestingly, patients with premature hair graying have higher serum levels of oxidative stress compared to healthy controls, even if they are of similar age (Acer et al., 2020). Similarly, signal pathways related to cytokines and growth factors play a driving role in skin photoaging (Fitsiou et al., 2021). Moreover, aging-related epidermal dysfunction partially increases cytokines in blood circulation, including TNFα, IL, EGF, and FGF (Hu et al., 2017).

In addition to circulation, the level of many age-related markers also changes in the skin wash fluid. This was mainly manifested by decreased markers involved in collagen synthesis (EGF and FGF), epidermal proliferation and wound healing (keratin-6), anti-inflammatory (IL-1Ra), and innate immunity (interferon alpha-2, IFN-α2) (Kinn et al., 2015). Additionally, age-associated upregulation of cortisol is also observed in the skin wash fluid (Kinn et al., 2015).

Intestinal aging

The intestine is the main digestive organ and the largest immune organ in the body, which absorbs nutrients and acts as a protective barrier against the external environment (Calleja-Conde et al., 2021). Aging induces significant shifts in the body, with decreased immune function and damage to the intestinal barrier, where harmful molecules, such as pathogens, escape the immune barrier and sneak into the bloodstream, causing serious damage to other organs (Bosco and Noti, 2021). Therefore, an in-depth understanding of the markers associated with aging-related intestinal failure would be beneficial for targeted prevention or treatment, reducing the occurrence of aging diseases and improving the quality of life for elderly people. Based on previous studies, we will describe the biomarkers of aging-related intestinal failure from different aspects, such as physiological characteristics, histologic features, and cellular and molecular levels.

Physiological characteristics

In terms of physiological characteristics, intestinal barrier failure has been described as a pathological hallmark of aging in both animal and human studies (Funk et al., 2020; Parrish, 2017). The function of intestinal digestion and absorption decreases during aging, and this physiological change significantly alters the intestinal flora structure (DeJong et al., 2020), such as a decrease in *Akkermansia muciniphila* (Akk) and short-chain fatty acid (SCFA)-producing bacteria, and a marked increase of potentially proinflammatory commensal microbes (Ragonnaud and Biragyn, 2021). Akk is a bacterium that degrades mucins and provides energy to beneficial microbes, as well as protects the integrity of the intestinal epithelium by activating epithelial cells and producing mucus. Its decrease in the gut of aging mice and, possibly, elderly people leads to leaky gut, which in turn leads to low levels of systemic inflammation, also known as “inflammaging” (Ragonnaud and Biragyn, 2021). In conclusion, during intestinal aging, changes in the physiological characteristics include a reduction in the diversity of the intestinal flora and a decrease in beneficial bacteria such as Akk bacteria, and a reduction in intestinal permeability, which triggers a range of health problems, including irregular intestinal transit, decreased appetite, leaky gut, intestinal inflammation and premature death of elderly

people (An et al., 2018; Nagpal et al., 2018).

Histologic features

Histologic features associated with intestinal aging include increased microbial metabolites, thinning of the mucus layer and an increase in villus length. In the intestine of healthy young people, beneficial commensal bacterial members of *Firmicutes* produce SCFAs. SCFAs (e.g., butyrate, propionate and acetate) have physiological functions such as providing energy to commensal microbes and colonocytes, inducing production of mucus, maintaining intestinal integrity, regulating the differentiation of $CD4^+$ T cells and the activation of $CD8^+$ T cells (Arpaia et al., 2013; Trompette et al., 2018). In aged mouse and human intestine, SCFAs levels are generally reduced and this reduction favors the survival of bacteria capable of degrading mucins in the intestine (Nagpal et al., 2018). In aged mice, decreased mucins lead to a thin, discontinuous mucus layer that allows direct interaction between microbes and intestinal epithelium, which triggers an inflammatory response (Elderman et al., 2017). Another study shows an increase in the length of intestinal villi in aged mice (Suzuki et al., 2022).

Cellular alterations

At the cellular level, physiological changes in aging-related intestinal failure are associated with deterioration in the function of ISCs. Upon aging, ISCs suffer from an accumulation of cellular and DNA damage (Liu and Rando, 2011). Thus, ISCs are highly prone to stem cell exhaustion, which is an integrative hallmark of aging (López-Otín et al., 2013). Abnormal proliferation of ISCs in *Drosophila* is associated with aging (Jasper, 2020). The expression of the ISC marker Lgr5 decreases in aged mice. Upon aging, the number of crypts where ISCs are located decreases, while the size of intestinal crypts, the number of Paneth cells and goblet cells increase in aged mice (Nalapareddy et al., 2017; Nalapareddy et al., 2022). There is a significant aging-induced reduction in the organoid-forming capacity of colonic crypts derived from biopsies of healthy human donors, which indicates a reduction in ISC function (Pentinmikko et al., 2019).

Molecular changes

Aging-induced aberrant proliferation of ISCs is associated with alterations in multiple signaling pathways, including different endogenous and exogenous signals such as Wnt, Notch and Sirt1/mTORC1. During aging in mice and humans, canonical Wnt signaling is reduced in ISCs, Paneth cells, and mesenchyme, which leads to decreased ISC regenerative potential upon aging. Addition of exogenous Wnt such as Wnt3a *in vitro* improves regeneration of aging ISCs (Nalapareddy et al., 2017). In addition to Wnt signaling, the expression levels of Notch1 receptor and target Olfactomedin-4 (*Olfm4*) gene in the Notch signaling pathway are reduced in the ISCs of aging mice (VanDussen et al., 2012). Upon aging, levels of Sirt1 and activity of mTORC1 also decline, and the treatment with the NAD^+ precursor nicotinamide riboside rejuvenates ISCs from aged mice and reverses an impaired ability to repair gut damage (Igarashi et al., 2019). Aging leads to a reduction in the number and function of ISCs in mice, which is associated with decreased fatty acid oxidation (FAO). Pharmacological activation of FAO or addition of FAO substrates (e.g., palmitate) enhances aging ISC organoid formation capacity (Mihaylova et al., 2018). Cdc42, a small RhoGTPase, has increased activity in proliferating TA cells and ISCs in aged mice, and inhibition of Cdc42 activity enhances ISC regeneration after radiation-induced injury and organoid formation in aging crypts and ISCs (Nalapareddy et al., 2021). The discovery of these markers strongly supports that the function of aging ISCs can be reversed by targeted mechanisms and also improves the understanding of the molecular alterations in the extrinsic physiology and intrinsic signaling of aging intestinal failure (Figure 21; Table S14 in Supporting Information).

Secretory factors detectable in biofluids

Clinically, aging-related secretory factors are detectable in body fluids, such as serum, plasma. Increased LPS can be detected in the plasma of elderly people. LPS is produced by the increased production of gram-negative bacteria in the gut and enters the blood circulation through the intestines, leading to the activation of chronic inflammatory factors (Bosco and Noti, 2021; Nagpal et al., 2018; Stevens et al., 2018). As a result, the elderly population tends to have high concentrations of inflammatory factors such as IL-6, IL-8, CRP and TNFα in their serum (Chambers and Akbar, 2020; Espinoza and Walston, 2005). High expression of inflammatory cytokines can reduce the expression of tight junction proteins in aged mouse and human intestine (e.g., Zonulin and Claudin), which increases intestinal permeability and induces long-term inflammation (An et al., 2018; DeJong et al., 2020; Mabbott, 2015). In addition, indole levels have been reported to decrease, while kynurenine increased in aged mouse and human fecal samples and mouse serum (Hohman and Osborne, 2022). Kynurenine, an alternative tryptophan-derived product, is a primary driver of the aging process and is associated with high mortality in humans (Kim et al., 2020a). Overall, an increase in bacterial metabolites and inflammatory factors, as well as a decrease in tight junction proteins, can be detected in the blood during aging.

Summary and perspectives

In this section, we review biomarkers of intestinal aging at the physiological characteristics, histologic features, cellular and molecular levels. Upon aging, the reduced intestinal

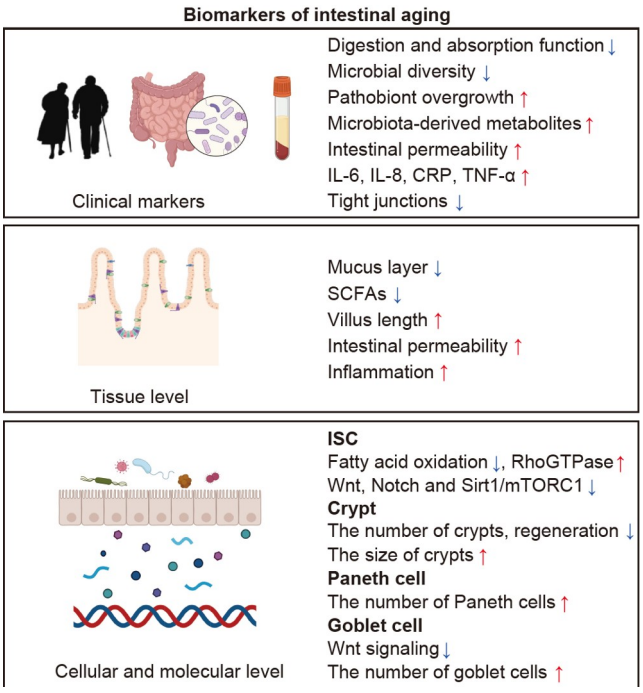

Figure 21 Biomarkers of intestinal aging. Abbreviations: mTORC1, mammalian target of rapamycin complex 1.

barrier integrity facilitates the entry of microbes/metabolites into the circulation, which promotes inflammation and induces disease. With the improvement of ISC organoid culture technology and maturation of single-cell sequencing technology, quantifying the phenotype and function of intestinal epithelial cells in the elderly and finding more biomarkers of intestinal aging will be the key to future research. These biomarkers could provide new intervention strategies to improve the quality of life and increase life expectancy in the global aging population.

Pancreatic aging

The pancreas lies in the upper abdomen behind the stomach and has a complex histology, with endocrine and exocrine cells coexisting in the same organ. It belongs to the gastrointestinal system that produces and secretes digestive enzymes into the intestine and is also an endocrine organ that makes and secretes critical metabolism-regulating hormones (Gyr et al., 1985). Exocrine pancreas, which comprises more than 95% of the pancreatic mass, primarily includes acinar and duct cells, with associated connective tissue, vessels, and nerves (Longnecker, 2014). Acinar cells make up about 85% of the pancreas and are arranged in acini, which empty into ducts and are functional units for digestive enzyme synthesis, storage, and secretion, including lipase, amylase, trypsin, chymotrypsin, and elastase (Longnecker, 2014; Madole et al., 2016). The centroacinar cells are an extension of the most peripheral of duct cells and partially cover the apical surface of each acinus. Downstream of the centroacinar cells are the intercalated ducts, which converge and form the intralobular ducts, then the interlobular ducts, and eventually drain into the main pancreatic duct (Figure 22). Endocrine pancreas, which comprises 1%–2% of pancreatic mass, is also called the islet of Langerhans (Figure 22), consists of α cells, β cells, delta cells, PP cells, and epsilon cells that make and secrete insulin, glucagon, somatostatin, and pancreatic polypeptide into the blood, respectively (Longnecker, 2014). Of these, β cells make up 60%–80% of the islet cell population and their dysfunction may cause diabetes mellitus (Noguchi and Huising, 2019).

Physiological characteristics

Both the exocrine and endocrine parts of the pancreas undergo aging (Figure 22; Table S15 in Supporting Information). Pancreatic exocrine function deteriorates with age, manifested as pancreatic exocrine insufficiency (Herzig et al., 2011), which may lead to maldigestion and malnutrition (Feibusch and Holt, 1982; Löhr et al., 2018). Secretin stimulation test (SST), or secretin test, is a standard test for pancreas exocrine function, measuring the pancreatic secretory volume, bicarbonate output, and pancreatic enzymes output (Dossin, 2011; Steer et al., 1995). Studies based on this test document that duodenal aspirates from elderly individuals contain significantly reduced volume of pancreatic secretions (Fikry, 1968; Matsumoto et al., 1989), reduced concentrations of pancreatic enzymes and bicarbonate (Matsumoto et al., 1989; Tiscornia et al., 1986; Vellas et al., 1988), as well as reduced activity of amylase and trypsin (Fikry, 1968). Fecal elastase test (FET) is to determine the elastase-1 (FE-1, an enzyme secreted by pancreas) content in feces, a highly sensitive marker in the diagnosis of severe and moderate exocrine pancreatic insufficiency (Herzig et al., 2011; Lüth et al., 2001). 10%–21.6% of the elderly have pancreatic exocrine insufficiency (EPI, with FE-1 <200 $\mu g \cdot g^{-1}$) and 5% of them have serious pancreatic exocrine insufficiency (SEPI, with FE-1 <100 $\mu g \cdot g^{-1}$), indicating that the incidence of EPI and SEPI increases significantly with age (Herzig et al., 2011; Lüth et al., 2001; Piciucchi et al., 2015). For pancreatic endocrine, its function is mainly reflected by glycemia control, glucose tolerance, and fasting or stimulated insulin/c-peptide secretion. Glycated hemoglobin test (Hemoglobin A1c, HbA1c) is a clinical test for glycemia control, reflecting the blood glucose fluctuation in the past 2–3 months (Sherwani et al., 2016). A couple of studies in different populations reported that the HbA1c level is higher in the elderly than that in the younger individuals (Arnetz et al., 1982; Yang et al., 1997). Especially for the elderly with type 2 diabetes (T2D), a less stringent HbA1c standard is suggested to maximize the treatment benefits and minimize harm (Lipska et al., 2016). Glucose tolerance and insulin secretion are usually measured through oral glucose

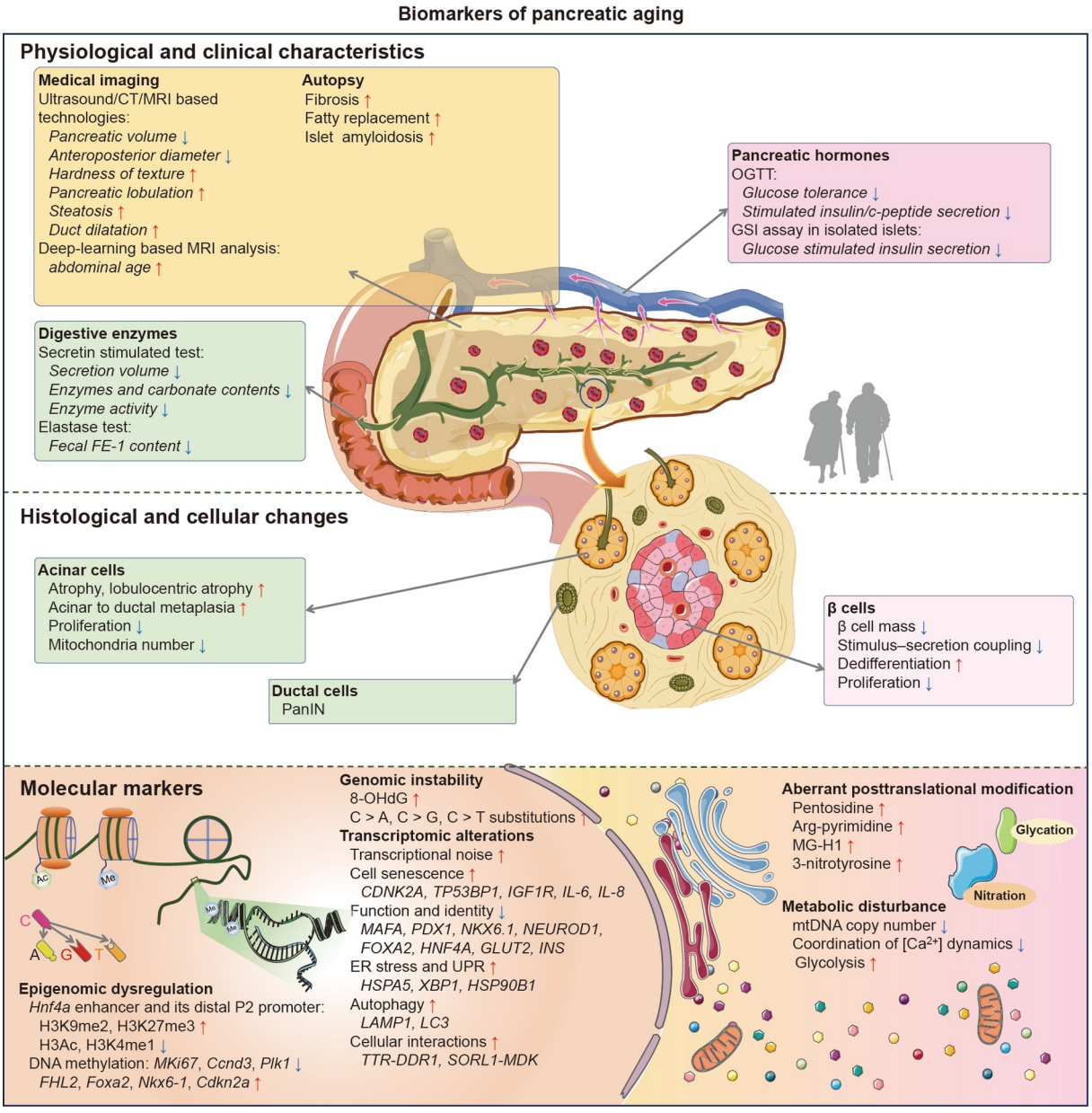

Figure 22 Biomarkers of pancreatic aging. The aged pancreases exhibit decreased pancreatic volume, hardened texture, increased lobulation, steatosis, and duct dilation, which can be detected by ultrasound/CT/MRI-based medical imaging technologies. Exocrine functional decay during aging can be detected by secretin stimulation test and fecal elastase test. Islet dysfunction during aging is reflected by decreased insulin secretion and impaired glucose tolerance during OGGT *in vivo*. At the histological level, increased prevalence of fibrosis, atrophy, lobulocentric atrophy, ADM, PanIN, and fatty replacement was observed in exocrine pancreas; and increased fibrosis, amyloidosis, decreased vascular density, and reduced β cell mass were observed in endocrine islet during aging. At the molecular level, markers indicating genomic instability, epigenomic dysregulation, transcriptomic alterations, aberrant posttranslational modification and metabolic disturbance are identified. Abbreviations: IGF1R, insulin like growth factor 1 receptor; HSP90B1, heat shock protein 90 beta family member 1.

tolerance test (OGTT). In humans, glucose tolerance gradually declines with age (Chang and Halter, 2003; Coordt et al., 1995). This age-related glucose intolerance is frequently accompanied by insulin resistance; meanwhile, a decline of glucose- or meal-stimulated insulin/c-peptide secretion with age also indicates β cell dysfunction in the elderly (Basu et al., 2003; Chang et al., 2006; Chen et al., 1985; Fritsche et al., 2002; Gumbiner et al., 1989; Kahn et al., 1990; Muller et al., 1996). Insulin secretion in isolated human islets also showed a declined glucose stimulation index (GSI) *in vitro*, compromised coordination of [Ca^{2+}] dynamics, and impaired insulin secretion dynamics in the elderly (Barker et al., 2015; Westacott et al., 2017). These assays act with the value as minimally invasive and promising biomarkers for diagnosis of metabolic diseases and assessment of the health status and aging of pancreas.

Imaging traits

Medical imaging is a promising source for biomarker development as it provides a macroscopic view of tissues of interest (O'Connor et al., 2017) and has advantages of non-invasiveness, readily availability in clinical care, and repeatability (Lambin et al., 2017; Morin et al., 2018). CT (Caglar et al., 2012), MRI (Sato et al., 2012; Wang et al., 2021b), ultrasonography (Glaser and Stienecker, 2000), endoscopic ultrasonography (EUS) (Rajan et al., 2005), endoscopic ultrasound elastography (EUS-elastography) (Janssen and Papavassiliou, 2014), and endoscopic retrocholangiography (ERCP) (Anand et al., 1989; Hastier et al., 1998), etc., have been commonly used to assess the age-related morphological changes of the human pancreas. Reduction of pancreatic volume, hardening of texture, and dilatation of the pancreatic ducts, as well as pancreatic lobulation (Sato et al., 2012) and pancreatic steatosis (Begovatz et al., 2015) are the main findings from these studies associated with aging, which are potential biomarkers for the macroscopic changes of pancreatic aging (Table S15 in Supporting Information).

Pancreatic atrophy is considered to be one of the characteristics of pancreatic aging. MRI analysis shows the pancreatic anteroposterior (AP) diameter reaches maximum values at the age of 30–39 years, followed by a gradual decrease, especially in the tail of the pancreas (Sato et al., 2012; Wang et al., 2021b). Accordingly, studies showed that the pancreatic volumes gradually increased to peak during the ages ranging from 20–49 years, and gradually decreased after 60 years of age by CT/MRI examinations (Caglar et al., 2012; Wang et al., 2021b; Yoon et al., 2020). Janssen and Papavassiliou (2014) found that the average strain values of pancreases were 110.2 in the young and middle-aged group and 80.0 in the elderly group by semi-quantitative EUS-elastography, indicating that the pancreas becomes significantly harder during aging. As regards dilatation of the pancreatic ducts, Glaser and Stienecker (2000) analyzed the results of pancreatic duct diameters measured by ultrasonography, and found that the mean diameter of the pancreatic duct was 1.5, 1.9 and 2.3 mm for the 18–29, 40–49 and ≥80 years old groups, respectively. Using ERCP, Anand et al. (1989) found that the mean diameter of the main pancreatic duct of the pancreatic body in those two groups was 2.36 and 2.86 mm in the two groups of people aged <40 and ≥40, respectively, indicating a significant broadening of the main pancreatic duct with age.

The development of deep-learning-based image segmentation facilitates analyzing medical images automatically and identifying pancreas changes, such as volumetric differences, at the pixel level among large populations, thus has been widely used in various medical imaging tasks (Cai et al., 2022a; Li et al., 2021c). For example, the deep-learning-based identification of CT biomarkers facilitates the diagnosis of T2D, such as measurements of pancreatic CT attenuation and visceral fat (Tallam et al., 2022). A 3D dual-contrast nnU-Net aided segmentation of pancreas on Dixon MRI images automates the assessment of pancreatic fat distribution with high reliability (Lin et al., 2023). In terms of pancreatic aging, a recent study by Le Goallec et al. (2022) built an abdominal age predictor by training convolutional neural networks to predict abdominal age from liver MRIs and pancreas MRIs, which is driven by both liver and pancreas anatomical features, as well as surrounding organs and tissues. They found that the accelerated abdominal aging is associated with biomarkers such as body impedance and blood pressure, clinical phenotypes such as chest pain, diseases such as cardiovascular diseases, and even environmental and socioeconomic factors (Le Goallec et al., 2022). It is foreseeable that deep learning (DL) technology has promising potential in the development of aging clocks by taking advantage of the availability of mass medical imaging data.

Histological features

Taking advantage of pancreas autopsies or pancreas tissue from organ donors, a mass of histological studies on pancreatic aging have been conducted, allowing the identification of microscopic histological changes during pancreatic aging.

In the exocrine pancreas, the aging-associated histological alterations mainly include increased fibrosis, fatty replacement, lobulocentric atrophy (LCA), presence of low-grade pancreatic intraepithelial neoplasia (PanIN). Pancreatic fibrosis can be caused by cell death, inflammation, or ductal obstruction, via cytokine-triggered transition of resident fibroblasts/pancreatic stellate cells to myofibroblasts and subsequent extracellular matrix production and deposition (Klöppel et al., 2003). The pattern of fibrosis in the aged pancreas was found to be multifocal and predominantly intralobular, termed patchy lobular fibrosis in the elderly (PLFE) (Detlefsen et al., 2005), which is present in 50% of those older than 60 years (Matsuda et al., 2017). Increased fibrosis is not only present in exocrine tissue, but also within or around islets, peri-vasculatures, and peri-ducts in the elderly (Chen et al., 2021a; Gupta and Kumar, 2018; Hastier et al., 1998; Li et al., 2011b). Pancreatic fatty replacement, also known as lipomatosis, is describing the partial replacement of the acinar parenchyma by adipose tissue. The proportion of fat in pancreas increases progressively with age in both rodents and human beings (Murakami et al., 2017; Walters, 1966). LCA represents a change that affects the center of the pancreatic lobules, which is a common phenomenon in the aging pancreas (Detlefsen et al., 2005). LCA often co-occurs with other important age-related alterations, such as fibrosis, acinar to ductal metaplasia (ADM) (Esposito and Häberle, 2022), and PanIN (Matsuda et al., 2017). Among them, the frequency of PanIN, a precursor of pancreatic ductal ade-

nocarcinoma is closely correlated with age, as low-grade PanINs are uncommon in patients younger than 40 years but become more obvious after 40 years of age (Matsuda et al., 2015).

In the endocrine islets, amyloidosis (Su et al., 2012) and reduced vasculature density (Chen et al., 2021a) have been reported as age-associated histological changes. Islet amyloidosis, or amyloid deposit, which mainly occurs in the pancreas of T2D patients and is strongly associated with islet dysfunction, affects 6% of non-diabetic individuals after 80 years of age (Su et al., 2012). Both the prevalence of islet amyloidosis and the frequency of affected islets increase with age (Cornwell and Westermark, 1980; Su et al., 2012). In addition, the instantaneous and dynamic hormone output of islets also relies on rich vascularization (Ballian and Brunicardi, 2007; Gorczyca et al., 2010). By comparing adult and aged mice, the decreased number of capillaries and arterioles, but not in perivascular cells, is also observed in the aged pancreas (Chen et al., 2021a). Chen et al. (2021a) also discovered a distinct age-dependent subset of endothelial cells, which supports β cell expansion but declines during aging. Due to the invasiveness of obtaining pancreas biopsies, these microscopic alterations can only be assessed as aging biomarkers in research so far. Luckily, some of the above alterations can be partially reflected by the measurements from medical imaging. For instance, pancreas atrophy can be reflected by the pancreas volume measurements from CT/MRI/US (Caglar et al., 2012; Sato et al., 2012; Wang et al., 2021b; Yoon et al., 2020), fibrosis by pancreas hardness measurements from EUS-elastography (Janssen and Papavassiliou, 2014), and fatty replacement by steatosis measurements from CT (Meier et al., 2007) or MRI (Li et al., 2011b; Sato et al., 2012).

Cellular alterations

Cellular alterations of each cell type in the pancreas are shown in detail (Table S15 in Supporting Information). Generally, the cellular proliferation rate in the pancreas decreases with aging, not only in the endocrine and exocrine cells but also endothelial cells and stellate cells (Chen et al., 2021a; Chen et al., 2009; Fitzner et al., 2012; Reers et al., 2009; Takahashi et al., 2012). Here, cell type-specific age-related characteristics which have been extensively documented are introduced (Figure 22; Table S15 in Supporting Information).

(1) Exocrine cells. A critical alteration of exocrine pancreatic aging is acinar atrophy, which is commonly accompanied by ADM, a critical contributor to lobulocentric atrophy and ductal cell hyperplasia (Detlefsen et al., 2005; Matsuda et al., 2017). Metaplasia between different cell types occurs through multiple mechanisms, including dominant proliferation or loss of one cell type, transdifferentiation or dedifferentiation of specific cells, and abnormal activation of progenitor cells (Tosh and Slack, 2002). Similarly, ADM is a process that acinar cells reprogram into ductal-like cells with ductal cell characteristics (Jiang et al., 2022; Pour et al., 1982). Early ADM cells express both ductal (e.g., CK19) and acinar markers (e.g., trypsin, amylase) and established ADM cells only express ductal markers, indicating a transdifferentiating process (Esposito and Häberle, 2022). Besides, the proliferation rate and mitochondria number in acinar cells were also found to decrease with age in mouse studies (Nagata, 2012; Oates and Morgan, 1986; Takahashi et al., 2012). In ductal cells, a key age-related pathological change is oncogenic transformation, which occurs possibly via the accumulation of gene mutations, epigenetic dysregulation, telomere dysfunction, or an altered stromal milieu (Arai and Takubo, 2007; DePinho, 2000; Risques and Kennedy, 2018). Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal form of pancreatic cancer, and one of its key risk factors is aging (Jentzsch et al., 2020; Matsuda, 2019). Except for the aforementioned PanIN, another main precursor of PDAC is intraductal papillary mucinous neoplasms (IPMN). The median ages at the time of diagnosis for IPMN and PDAC were both over 70 years (Khan et al., 2010; Latenstein et al., 2020).

(2) Endocrine cells. Different from age-related carcinogenesis in ductal cells, the neoplasms in islet endocrine cells are rarely associated with age. In contrast, the aging-related change in islet cells accounts for the strong association of T2D incidence with age (Halter, 2010). T2D represents 90 percent of diabetes cases and is generally associated with insulin resistance and compensatory hyperinsulinemia, which are early indicators of metabolic dysfunction. When the insulin secretion from islet β cells fails to compensate for the insulin resistance, hyperglycemia occurs and β cell dysfunction accelerates (Weir et al., 2020). Clinical studies have shown that insulin secretion in the elderly is impaired, which might contribute to the increasing incidence of diabetes with age. The aging-related cellular changes in β cells include the following aspects. (i) Decreased β cell mass and restricted proliferation rate. Some reports have indicated a modest age-associated reduction in β cell mass in non-diabetic subjects (Mizukami et al., 2014; Rahier et al., 2008; Saisho et al., 2013), which is possibly due to the restricted β cell proliferation rate and increase of senescent β cell in the elderly (Chen et al., 2009; Desgraz et al., 2011; Reers et al., 2009). The clearance of these senescent cells by senolytics is demonstrated to improve insulin secretion in aged mice (Aguayo-Mazzucato et al., 2019). (ii) β cell identity alterations. β cell dedifferentiation is a critical mechanism for β cell failure in type 2 diabetes (Talchai et al., 2012; Wang et al., 2020b), manifested with a decrease or loss of maturity-related genes and obtaining of progenitor gene expressions, as well as compromised β cell function. Recently, Song et al.

(2022b) found an almost 2-fold increase in the proportion of dedifferentiated cells in the elderly and middle-aged groups versus the young group. Earlier studies also reported that β cell maturity and identity genes such as PDX1 had lower expression in the elderly than in the younger individuals (Avrahami et al., 2015; Mizukami et al., 2014; Reers et al., 2009), supporting the notion of increased dedifferentiation level during aging. Single-cell sequencing analyses in human islets or non-human primates also revealed the age-associated increase of transcription noise and compromised β cell identity along with increased oxidative stress, increased unfolding protein response and ER stress, downregulated transcription factor expressions, and impaired proteostasis (Enge et al., 2017; Li et al., 2021b; Shrestha et al., 2022). Similar to the histological alterations, these aging-related cellular changes also need to acquire the pancreas tissue, therefore their application is restricted to aging biomarkers research. Developing non-invasive or minimally invasive biomarkers for pancreatic aging and establishing the association between these biomarkers with the histological and cellular alterations during aging are urgent for pancreatic aging prediction.

Molecular changes

Driven by intrinsic and external factors, pancreatic aging is caused by interlinked molecular changes over time. In recent years, with the aid of cutting-edge technologies, especially those based on omics (genomics, epigenomics, transcriptomics, proteomics and metabolomics), novel biomarkers of pancreatic aging have been identified (Table S15 in Supporting Information). More importantly, these approaches provide the potential for identifying previously unknown interconnections among different biomarkers associated with pancreatic aging.

(1) Genomic instability. Mutational loads are considered to be positively correlated with pancreatic aging. Cells harboring DNA sequence rearrangements, which accumulated in aged pancreas, led to an increased mutation frequency (Wiktor-Brown et al., 2006). The most common method to study somatic mutations is to perform whole genome sequencing or whole exome sequencing, which hinders the discovery of rare mutations. However, single-cell genomic sequencing or DNA sequencing of many cells originating from the same clone *in vitro* is capable of uncovering rare mutations but costly. Enge et al. (2017) developed a computational method for determining genetic variation associated with aging using scRNA-seq data collected from pancreata with donors spanning six decades of life. This identified genome instability as a novel age-dependent signature in endocrine cells, characterized by high rates of C>A, C>G and C>T substitutions for the transcribed strand related to oxidative stress in aged cells. This mutational signature may serve as a candidate biomarker of pancreatic aging. Consistently, 8-OHdG (Reiter et al., 1999; Shi et al., 2004), a major oxidative DNA damage marker, has been observed to be higher in aged rodent pancreas. Additionally, the free base of 8-OHdG, namely 8-hydroxyguanine (8-OHG), was detected to be elevated in plasma samples from diabetic patients and positively correlated with endocrine dysfunction (Shin et al., 2001). This may indicate the potential of 8-OHG as a plasma biomarker of pancreatic aging.

(2) Epigenomic dysregulation. Epigenomic changes are also of great predictive or diagnostic value in pancreatic aging and related diseases, as extensive studies have linked the dysregulation in histone modification or DNA methylation to pancreatic aging (Avrahami et al., 2015; Bacos et al., 2016; Horvath, 2013; Li et al., 2019b; Sandovici et al., 2011). For example, *Hnf4a*, a transcription factor involved in regulating insulin secretion, was epigenetically silenced with age (Sandovici et al., 2011). In aged rat islets, the *Hnf4a* enhancer and its distal P2 promoter were enriched with repressive marks H3K9me2 and H3K27me3, while lacking active marks H3Ac and H3K4me1, thus causing impaired promoter-enhancer interaction and its gene expression (Sandovici et al., 2011). Additionally, in mice, aging-associated methylation changes explained the decline in proliferative capacity and enhanced insulin secretion in β cells of old mice (16–20 months) (Avrahami et al., 2015). Promoters of genes involved in cell-cycle, such as Ki-67 (*MKi67*), cyclin D3 (*Ccnd3*), and *Plk1*, became *de novo* methylated, and their expression was decreased during β cell aging. Whereas, the differentially methylated regions associated with genes involved in β cell function, such as *Foxa2* and NK6 homeobox 1 (*Nkx6-1*), and cell cycle inhibitor *Cdkn2a* became demethylated with age, and their expression was increased (Avrahami et al., 2015). And the methylation level of the aging-associated differentially methylated promoters could be partially reversed via rejuvenation strategies (Chondronasiou et al., 2022). A cohort study of genome-wide analysis for DNA methylation in islets from donors aged 26–74 years identified loci with elevated DNA methylation levels with age (Bacos et al., 2016). Some age-associated DNA methylation changes in blood can mirror the changes in islets. For example, the methylation level of CpG sites located in promoter regions of four and a half LIM domains 2 (*FHL2*) was increased with age both in islets and blood (Bacos et al., 2016). The increased methylation hindered binding of repressive transcription factors, thereby enhancing *FHL2* expression (Bacos et al., 2016). Interestingly, high expression level of *FHL2* was closely correlated with high blood HbA1c level (Fadista et al., 2014; Krus et al., 2014; Taneera et al., 2012) and its level was much higher in blood samples of T2D patients compared to that of the healthy donors (Solimena et al., 2018). Accordingly, FHL2 deficiency improved a series of pancreatic functions, such as insulin secretory capacity of pancreatic islets and glucose

tolerance, further indicating the potential of *FHL2* methylation as a biomarker and target for therapeutic interventions on pancreatic aging (Habibe et al., 2022).

(3) Transcriptomic alterations. Increasing efforts have been devoted to characterize the aging-associated transcriptomic changes in pancreas, including (i) increased transcriptional noise. Single-cell transcriptomic analyses identified pancreatic α and β cells from older individuals with increased transcriptional noise in both human and non-human primates, implying the aggravated heterogeneity of aged pancreatic cells (Enge et al., 2017; Li et al., 2021b; Shrestha et al., 2022). (ii) Increased expression of cellular senescence markers. Extensive studies measured the mRNA and protein levels of *CDKN2A*, DNA damage response factor (*TP53BP1*), transmembrane tyrosine kinase (*IGF1R*), and the genes encoding SASP factors such as *IL-6* and *IL-8* to indicate the senescent state of aged pancreatic cells (Aguayo-Mazzucato et al., 2019; Arda et al., 2016; El-Far et al., 2020; Enge et al., 2017; Helman et al., 2016). (iii) Decreased expression of cellular function and identity genes. Decreased expression of key β cell transcription factors such as MAF bZIP transcription factor A (*MAFA*), pancreatic and duodenal homeobox 1 (*PDX1*), *NKX-6.1*, neuronal differentiation 1 (*NEUROD1*), *FOXA2* and hepatocyte nuclear factor 4 alpha (*HNF4A*), solute carrier family 2 member 2 (*GLUT2*), and insulin (*INS*) in aged mammalian β cells, is associated with the dedifferentiation of β cells that probably contributes to diabetic β cell failure (Aguayo-Mazzucato et al., 2019; Ihm et al., 2007; Odom et al., 2004; Shrestha et al., 2022; Talchai et al., 2012). (iv) Increased ER stress and unfolded protein response. In senescent β cells, protein synthesis was increased to meet the higher demand for glucose metabolism, which overloads the ER and triggers ER stress response and UPR pathways (Gonzalez-Teuber et al., 2019; Iwawaki et al., 2004; Kalwat et al., 2021; Lee and Lee, 2022). In aged human islets, a chronic state of ER stress was observed, which contributed to β cell secretory dysfunction and/or death (Shrestha et al., 2022). *HSPA5* and X-box binding protein-1 (*XBP-1*), encoding key regulators of ER stress and UPR, and *HSP90B1*, encoding a molecular chaperon, were transcriptionally increased in aged β cells when early adaptive events were present (Li et al., 2021b; Shrestha et al., 2022). The β cell-specific age-related upregulation of HSP90B1, verified to be a key insulin secretion regulator, may serve as a potential biomarker for β cell aging. (v) Increased autophagy. Autophagy-associated genes like lysosome-associated membrane protein 1 (*LAMP1*, lysosome marker) were transcriptionally increased in human and rat aged β cells, together with elevated LAMP1 and microtubule associated protein 1 light chain 3 alpha/beta (LC3A/B, autophagosome marker) protein levels, indicating that the extent of pancreatic autophagy is positively correlated with age (Shrestha et al., 2022; Wang et al., 2013b). (vi) Enhanced cellular interaction. Cell-cell communications were shown to be enhanced in aged islets, especially the interaction between the ligand transthyretin (*TTR*) expressed by α cells and its receptor discoidin domain receptor tyrosine kinase 1 (*DDR1*), or that between sortilin related receptor 1 (*SORL1*) upregulated in aged β cells and its ligand midkine (*MDK*) highly expressed in all four kinds of aged islet cells, implying that the altered microenvironments in aged pancreatic islets might give rise to disruption of proteostasis and activated UPR in aged β cells (Li et al., 2021b).

(4) Aberrant posttranslational modification. PTMs are increasingly being recognized as important markers of organ aging. Glycated and nitrated proteins, the non-enzymatically modified proteins, accumulated in aged pancreatic islets. Kehm et al. (2018) observed an accumulation of advanced glycation end products (AGEs), such as pentosidine, argpyrimidine and MG-H1, and nitrated proteins (3-nitrotyrosine, 3-NT) in the islet vascular system of old mice. Since the formation of AGEs has been reported to be associated with β cell aging and contributed to the decline in insulin secretory capacity of β cells, thereby rendering them potentially valuable protein biomarkers of pancreatic islet aging (Coughlan et al., 2011; Lim et al., 2008; Zhao et al., 2009). Moreover, other modifications, such as SUMOylation and *O*-GlcNAcylation have also been identified to play crucial roles in regulation of β cell function and viability during pathological processes (Akimoto et al., 2007; Li et al., 2020c). For instance, Akimoto et al. (2007) found a global increase in *O*-GlcNAcylated proteins in the diabetic rat model, which was accompanied by impeded glucose-stimulated insulin secretion. Despite these important discoveries, further research is warranted to uncover the roles of these PTMs in the physiological aging of the pancreas, with the goal of defining biomarkers and potential therapeutic targets for pancreatic aging.

(5) Metabolic disturbance. During aging, the endocrine pancreas undergoes metabolic changes, contributing to a deregulated glucose homeostasis. Mitochondria play essential roles in stimulus-secretion coupling in β cells. Depletion of mtDNA copy number is associated with impaired insulin secretion in pancreatic β cell lines (Wollheim, 2000). In isolated human islets, there was a negative correlation between mtDNA copy number and islet donor age, suggesting decline in mtDNA copy number might be an age-related characteristic of β cell (Cree et al., 2008). Meantime, in β cells, elevated mitochondrial respiration subsequently induced $[Ca^{2+}]$ influx through cell membrane localized voltage-gated $[Ca^{2+}]$ channels and consequently triggering insulin exocytosis (Westacott et al., 2017; Wollheim and Maechler, 2002). By examining β cell $[Ca^{2+}]$ and electrical communication during aging in mouse and human islets, Westacott et al. (2017) found that there was a decline in the coordination of $[Ca^{2+}]$ dynamics, gap junction coupling, and

insulin secretion dynamics with age, indicating an age-related decay in stimulus-secretion coupling in β cell. In addition, aged mouse islets show increased glycolysis and altered cytosolic NAD metabolism, affecting β cell function and identity, similar to that seen in diabetic islets (Murao et al., 2022). Metabolite profiling identified 1,5-AG, 5′-methylthioadenosine (5′-MTA), X-11315 as potential biomarkers of pancreatic aging and T2D in human serum, saliva, and urine samples (Mook-Kanamori et al., 2017; Pramodkumar et al., 2016; Wang et al., 2017e; Zhang et al., 2021c). It is worth mentioning that, for people aged 65 and over, via the ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) approach, the abundance of 5′-MTA was 34 percent higher in the urine of late-onset diabetic subjects, which therefore may be a promising biomarker of pancreatic aging and T2D in human urine (Tam et al., 2017).

Expanding molecular signatures of pancreatic aging have been identified across a number of species and different cell types. These studies have yielded important predictive and diagnostic biomarkers, and have laid the groundwork for the development of therapies for pancreatic aging and its associated diseases.

Summary and perspectives

The question of how to accurately evaluate pancreatic aging and predict the risk of associated diseases continues to fuel research efforts. Given the advancements in the development of non-invasive detection technologies, and the gradually deepening understanding of pancreatic aging, more and more diagnostic methods and biomarkers hold great potential for clinical utility to evaluate pancreatic aging. Nevertheless, efficient biomarkers to enable better judgement of the degree of pancreas degeneration and the development of effective intervention strategies are still needed. Additionally, combining existing evaluation methods to develop diagnostic panels could provide an efficient alternative to discovering new pancreatic aging biomarkers. As research progresses, we believe that imaging examinations, clinical biochemical assays, biomarker tests and other novel methods for understanding pancreatic aging will provide a wealth of beneficial opportunities for the global population.

Reproductive system aging

Aging is characterized by progressive physiological changes and a decline in function of organisms during adulthood (López-Otín et al., 2023). As one of the major functional systems, reproductive system aging not only compromises the fertility but also dampens the functions of other organs, leading to an array of age-associated diseases. Subfertility is one of the earliest clinical signs related to reproductive aging. Reproductive life span of women (age at natural menopause (ANM)) remains constant (50–52 years). A later age at ANM is associated with a higher risk of cardiovascular and other reproductive aging-associated chronic diseases (Yureneva et al., 2021). Women′s fecundity starts to decline gradually after the mid-20s and becomes more pronounced after 35 years of age (Ahmed et al., 2020; Broekmans et al., 2009; Kasapoğlu and Seli, 2020). In women aged ≥35 years, the incidences of infertility, aneuploidy, and birth defects dramatically increase. Since ovary plays a dominant role in the female reproductive system, reduction of female fertility over time is considered the natural consequence of ovarian aging (Busnelli et al., 2021). Although the male reproductive system ages more slowly, age-related decline in male fertility is also a hallmark of male reproductive aging (Bhasin et al., 2000). Other accompanying symptoms include a loss of libido, erectile dysfunction, as well as decreases in muscle mass and bone density, which are caused by sex hormone dyshomeostasis and known as late-onset hypogonadism (LOH) (Snyder, 2022). Moreover, the incidence of benign prostatic hyperplasia and prostate cancer also increases with age and reduces the quality of life in older men (Kaufman et al., 2019). Here, we summarize our latest understanding of the biomarkers of reproductive system aging, in particular of ovary and testis, two critical reproductive organs in female and male, respectively, which are expected to play a certain role in the diagnosis and intervention of female and male reproductive aging (Figure 23; Table S16 in Supporting Information).

Biomarkers of ovarian aging

(1) Physiological characteristics. Ovarian aging manifests as reproductive decline until menopause, accompanied by endocrine dysfunction and menstrual cycle irregularities. Subfertility is one of the earliest clinical signs in the cascade of events associated with reproductive aging. In addition, ovarian aging leads to declined secretion of estrogen and inhibin-B and elevated level of follicle-stimulating hormone (FSH), which can be easily assessed by blood test, although these alterations do not become prominent until menopause. Anti-Müllerian hormone (AMH) is produced by granulosa cells of small antral follicles, not controlled by the hypothalamus or gonadotropins, and independent of the menstrual cycle. AMH and antral follicle count (AFC) represent direct and accurate measurements of the ovarian reserve (Broer et al., 2014; Practice, 2015).

(2) Imaging traits. The ovarian aging process is dominated by decline in both the quantity and the quality of oocytes or follicle reserve (Broekmans et al., 2009; Li et al., 2012; Llarena and Hine, 2021; May-Panloup et al., 2016; Qiao et al., 2014; te Velde and Pearson, 2002). The nongrowing follicles (NGFs) are considered to represent the ovarian reserve. But direct measures of ovarian NGFs in women are relatively rare due to the technical challenges, given histo-

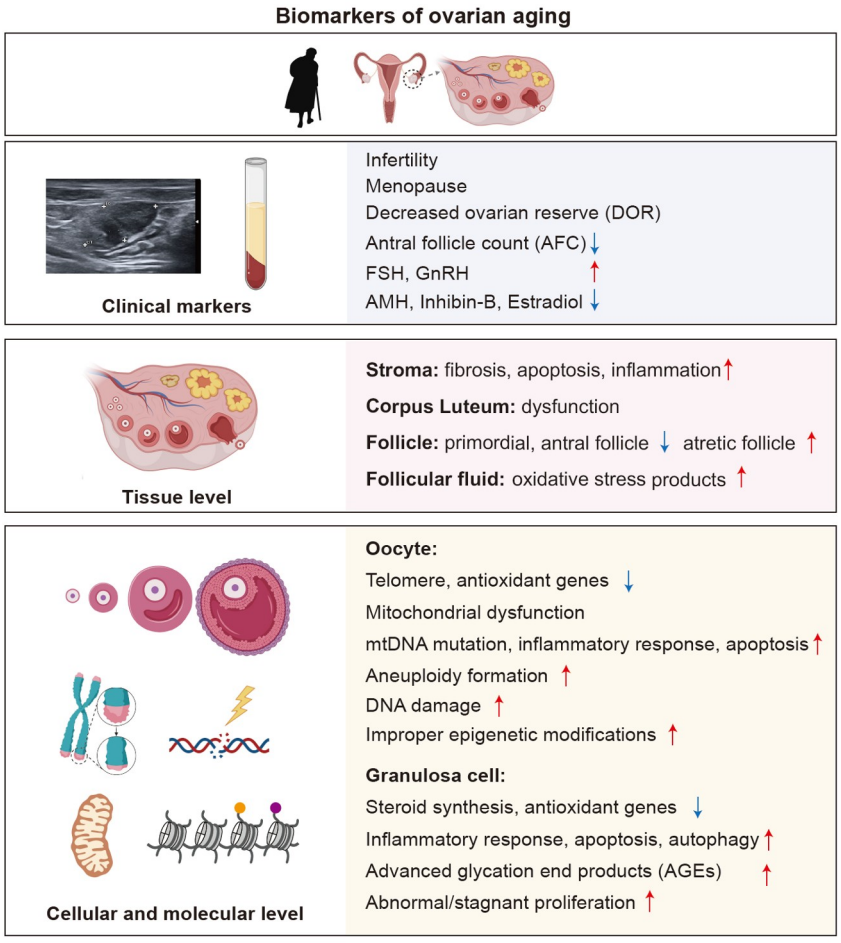

Figure 23 Biomarkers of ovarian aging. Clinical markers include age-associated infertility, menopause, and decreased ovarian reserve as shown by decreased antral follicle count revealed by 3D ultrasound imaging and serum AMH levels. Elevated serum FSH and reduced estradiol and inhibin-B levels can be found in peri-menopause to menopause. On the ovarian tissue level, elevated inflammation, fibrosis and apoptosis in the microenvironment, dysfunction corpus luteum, reduced number of primordial and antral follicles and elevated atretic follicles and oxidative stress products in the follicular fluid can be considered as major biomarkers indicative of ovarian aging. At the cellular and molecular levels, oocytes exhibit age-associated aneuploidy, reduced cohesin, reduced DNA damage repair, increased DNA damage, altered epigenetic modifications, shorter telomeres, reduced expression of antioxidant genes, elevated mtDNA mutation and mitochondria dysfunction, inflammatory response and apoptosis. Reduced steroid synthesis and expression of antioxidant genes, elevated inflammatory response, apoptosis, autophagy, and advanced glycation end products in granulosa cells surrounding the oocytes within the follicles or in follicular fluid provide convenient non-invasive biomarkers to indicate oocyte quality with increasing maternal age.

logical examination is invasive and time-intensive (Hansen, 2013). Antral follicle (2–10 mm diameter) count, visualized by transvaginal ultrasonography, is one of the most affordable and easy-to-perform diagnostic methods to measure ovarian reserve (Yureneva et al., 2021). An age-related decline in the AFC can be assessed by 3D ultrasound (Kupesic and Kurjak, 2002; Maheshwari et al., 2006; Scheffer et al., 1999). The number of visualized follicles has been correlated with ovarian reserve with histologic confirmation (Hansen et al., 2011), and AFC is considered as a marker of ovarian reserve (Hansen, 2013). Moreover, AFC is considered to be the best marker of ovarian response to stimulation in *in vitro* fertilization (IVF) cycles (Wang et al., 2021f). However, the accuracy of counting antral follicles depends on the experience level of the ultrasonographer.

(3) Histological features. The ovary consists of the germinal epithelium layer, the nonvascularized and thick fibrous-rich layer, the cortex containing ovarian follicles, and the medulla containing loose connective tissue and blood vessels. As women enter the perimenopausal period from the reproductive period, the ovary exhibits notable morphological and structural degeneration. Ovarian shrinkage, fibrosis and stiffness increase with age (Amargant et al., 2020).

Ovarian follicles are structural and functional units of the ovary, most primordial follicles (PMFs) undergo degeneration or atresia at any stage of ovarian folliculogenesis and approximately 300,000–400,000 PMFs are retained at menarche. By the age of 33 on average, approximately 90% of the ovarian NGFs are depleted (Hansen, 2013). Only 400–500 follicles reach the ovulatory phase during the reproductive span. At the age of about 51 years, the number of follicles decreases to 750–1,000 when menopause ensues

(Zhu et al., 2022c). Ovarian biopsies are not an adequate technique for the assessment of ovarian reserve due to the irregular distribution of ovarian NGFs within the ovarian cortex (Lass, 2004). Additionally, the depletion rate of NGFs accelerates in reproductive older women compared with younger women (Gougeon et al., 1994). Recently, a quantitative morphometric analysis using modern stereology techniques has been employed to define the ovarian reserve in the primate monkey (Tu et al., 2022).

(4) Cellular alterations. Unlike most somatic cell organs, germline stem cells have not been found in adult ovaries (Hainaut and Clarke, 2021). Absence of germline stem cells directly affects ovarian tissue homeostasis and function. Exhaustion and no-replenishment of oocytes due to the absence of neo-oogenesis result in depletion of follicle reserve and thus, menopause inevitably ensues. Therefore, depletion of the PMF pool caused by massive follicular atresia and periodic ovulation is the fundamental cause of ovarian aging.

The age-related decline in female fertility is also attributable to the oocyte quality (Bentov et al., 2011; Broekmans et al., 2009; Keefe et al., 2015; Navot et al., 1991). Many factors significantly contribute to the poor oocyte quality associated with maternal aging. Among them, mitochondrial dysfunction, ROS, recombination failure, cohesin deterioration and spindle assembly checkpoint dysregulation are the leading causes of oocyte aneuploidy (Charalambous et al., 2023; Mikwar et al., 2020; Zhu et al., 2022c).

Decreased mitochondrial biogenesis, impaired mitochondrial homeostasis, and free radical imbalance play critical roles in ovarian aging (Wang et al., 2017c). Mitochondrial genome, lacking of protective histones, is particularly susceptible to oxygen free radicals attack and to somatic mutation development (Busnelli et al., 2021). MtDNA mutation exacerbates female reproductive aging via impairment of the $NADH/NAD^+$ redox (Yang et al., 2020). Women aged ≥35 years with a poorer ovarian response are often characterized by a higher incidence of 4,977 bp deletion and a lower mtDNA copy number (Chan et al., 2005). Moreover, mtDNA T414G mutation of the human oocytes increases in an age-dependent manner (Barritt et al., 2000). Also, granulosa cells from women older than 38 years have been reported to contain higher levels of mtDNA deletions and damaged mitochondria (Busnelli et al., 2021), which results in a reduced capacity for steroid hormone biosynthesis and an increasing ROS generation (Liu et al., 2017c; Tatone et al., 2011).

Age-related increased ROS levels in the oocyte and other cells in the ovary and decreased antioxidant capacity result in oxidative stress, which decreases the oocyte quality and significantly accelerates the ovarian aging process (Hamatani et al., 2004; Lim and Luderer, 2011; Ntostis et al., 2021; Steuerwald et al., 2007; Wang et al., 2020d), supporting a rationale for antioxidant therapy to delay ovarian aging (Yan et al., 2022). Autophagy is also linked to oxidative stress-driven pathologies in the aging oocyte and surrounding ovarian environment (Peters et al., 2020). The link between accumulating ROS and mtDNA damage is well-known with age (Loeb et al., 2005). The mtDNA content of cumulus cells may be considered as a biomarker for IVF outcomes with age (Yang et al., 2021). Moreover, a single-cell transcriptomic atlas of young and aged non-human primate (NHP) ovaries reveals declined expression of GPX1 and GSR in early-stage oocytes or reduced expression levels of IDH1, PRDX4 and NDUFB10, for oxidoreductase activity, in granulosa cells that could provide specific molecular biomarkers to characterize oocyte quality and indicate ovarian aging in primates (Wang et al., 2020d).

Human oocyte aneuploidy is attributed to inherent meiotic spindle instability, increased merotelic attachments, and age-related changes in kinetochore and chromosome architecture (Das and Destouni, 2023). Age-related spindle abnormality and chromosome misalignment reduce the oocyte quality and contribute to the higher prevalence of aneuploidy in women with advanced age (Battaglia et al., 1996; Broekmans et al., 2009; Capalbo et al., 2013; Charalambous et al., 2023; Hunt and Hassold, 2008; Keefe et al., 2015). Meanwhile, actin cytoskeleton organization that supports the oocyte spindle is also notably downregulated in oocytes with age (Gou et al., 2022). Premature separation of sister chromatids and misaligned chromosomes detected in cytogenetic studies of human oocytes are associated with the age-related reduction of cohesin proteins, including Rec8, SA3, and SMC1β, which are not replenished with age (Charalambous et al., 2023; Chiang et al., 2010; Duncan et al., 2012; Jessberger, 2010; Liu and Keefe, 2008; Xu et al., 2005). Spindle abnormalities in human oocytes can be detected non-invasively using polarized light microscopy (Keefe et al., 2003). Moreover, biopsies of polar bodies from the oocytes of women undergoing IVF demonstrate high rates of premature sister chromatid separation with age (Capalbo et al., 2013; Christopikou et al., 2013).

Intercellular communications between oocytes and surrounding granulosa cells or cumulus cells (CCs) are critical for folliculogenesis in development as well as in maintaining ovarian function. Granulosa cells provide nutrients and mechanical support for oocytes and follicle development and homeostasis (Eppig, 1991; Wigglesworth et al., 2013). Senescence of granulosa cells, CCs and stromal cells can lead to inflammation and fibrosis (Secomandi et al., 2022; Tu et al., 2022). Moreover, ovulation is an intensely inflammatory process that generates ROS and leads to oxidative damage (Duffy et al., 2019). A unique population of macrophage-derived multinucleated giant cells, found in reproductively old mouse ovaries, is considered as functional drivers of inflammation and fibrosis in ovarian aging (Foley et al., 2021; Umehara et al., 2022). Additionally, the age-associated

increase in collagen and decrease in hyaluronan are conserved in the human ovary (Amargant et al., 2020). Also, accumulation of AGEs products at the level of the ovarian follicle might trigger early ovarian aging (Li et al., 2012; Pertynska-Marczewska and Diamanti-Kandarakis, 2017; Stensen et al., 2014; Tatone and Amicarelli, 2013; Tatone et al., 2008). Measurement of AGEs in granulosa cells or on the surface of follicular fluid-derived cells may be used as a biomarker of ovarian aging.

(5) Molecular changes. Early oocytes despite in a dormant state are particularly susceptible to DNA damage due to long exposure to chronic inflammation and oxidative stress in the ovarian microenvironment. The DDR pathway to guard the genome stability may represent the unifying link between oocyte quality and age (Das and Destouni, 2023). DDR has been identified to be the key regulator of ANM by genome-wide association studies (Stolk et al., 2012). Recently, 290 genetic determinants of ovarian aging are identified, and assessed using normal variation in ANM. Women in the top 1% of genetic susceptibility have an equivalent risk of premature ovarian insufficiency (POI) to those carrying monogenic *FMR1* pre-mutations. The identified loci implicate a broad range of DDR processes across the life-course to shape the ovarian reserve and its rate of depletion (Ruth et al., 2021). Earlier, genomic markers such as *FMR1* premutation can be regularly used to predict ovarian reserve (Wood and Rajkovic, 2013). Through whole-exome sequencing in a cohort of 1,030 patients with POI, another study detected 195 pathogenic/likely pathogenic variants in 59 known POI-causative genes and identified 20 novel POI-associated genes with a significantly higher burden of loss-of-function variants (Ke et al., 2023). Quantification of increased DNA oxidation (8-OHdG-positive) and damage (γH2AX-positive granulosa cells) by immunofluorescence microscopy also serve as biomarkers of ovarian aging (Wang et al., 2020d).

Progressive telomere shortening is associated with the age-related decrease in the quality of oocytes (Keefe et al., 2006). With human age, aneuploid embryonic cells possess significantly less telomere DNA than euploid embryonic cells at the cleavage stage (Treff et al., 2011). Polar body (PB) DNA is remarkably similar to that of the oocyte, PB telomere content thus provides a promising biomarker of oocyte aging (Keefe et al., 2015). Also, shortened telomere length and diminished telomerase activity are associated with biochemical primary ovarian insufficiency (Xu et al., 2017). Measurement of telomere length in peripheral blood leukocytes or granulosa cells could serve as biomarkers of ovarian aging and POI.

(6) Secretory factors detectable in biofluids. Markers of ovarian reserve include hormone levels and sonographically measured features of the ovaries. These markers can be useful as predictors of oocyte yield following controlled ovarian stimulation and oocyte retrieval.

AMH is a dimeric glycoprotein produced by granulosa cells of preantral (primary and secondary) and small antral follicles with a diameter of about 4 mm and regulates recruitment and maturation of follicles from the primordial follicle pool (PFP) (Broekmans et al., 2009; Visser et al., 2006). AMH prevents PFP depletion by reducing phosphorylation and maintaining activation of FOXO3a (Llarena and Hine, 2021). AMH levels are relatively stable across the menstrual cycle and no seasonal difference in AMH levels is observed (Long et al., 2018). With the decrease in the number of preantral follicles and small antral follicles, AMH serum levels become diminished and will invariably become undetectable near menopause, therefore, serum AMH levels are the best available biomarker of a woman's ovarian reserve and may provide an index of age at menopause (Birch Petersen et al., 2015; Freeman et al., 2012; Tehrani et al., 2022; Toner and Seifer, 2013). Similarly, serum AMH levels appear to be high in monkeys from young to middle reproductive age, but noticeably declined from middle (from the age of 11 or 12 years) to old age (Long et al., 2018; Tu et al., 2022). Nevertheless, AMH may not reflect oocyte health or chances for conception, age is thus the only current marker of oocyte quality in determining success rates with fertility treatments (Bishop et al., 2017; Cedars, 2022; Ulrich and Marsh, 2019). However, low serum AMH levels are associated with increased risk of embryo aneuploidy in women of advanced age (Jiang et al., 2018).

Elevated serum FSH levels at relatively late reproductive age and the cycle-specific nature of its measurement highlight its limitations as a marker of true ovarian reserve (Hansen, 2013). Clinical manifestations of subfertility as well as values of FSH>10 mIU mL^{-1} or AMH <1.0 ng mL^{-1} can serve as the criteria for diagnosing decreased ovarian reserve (DOR). The nature of DOR can be physiological when a woman is over 40 years old (Yureneva et al., 2021). An estimated 10% of the general female population will experience an accelerated loss of ovarian reserve, presumably from a more rapid rate of follicular atresia, leading to a loss of fertility in the mid-30s and early menopause by age 45 (Bishop et al., 2017; Ulrich and Marsh, 2019).

BMP15, GDF9, and c-KIT play critical roles in folliculogenesis through interaction between oocytes and cumulus cells (Gilchrist et al., 2008). Ovarian expressions of BMP15, GDF9, and c-KIT decrease with age (Park et al., 2020), and their expression levels in cumulus granulosa cells could serve as potential biomarkers of ovarian aging and predicting oocyte developmental potential (Li et al., 2014).

Follicular fluid (FF) is easily available during oocyte pick-up and may represent an optimal source on non-invasive biochemical predictors of oocyte quality. Evaluating levels of biochemical biomarkers in FF may be a noninvasive approach than extrapolating data from invasive methods like embryo biopsy (Molka et al., 2022). FF concentrations of

IGF1, GH and IL-6 are notably higher in women less than 35 years old with higher pregnancy than those of more than 35 years old with bad prognosis (Molka et al., 2022). Also, exosomes and exosomal miRNAs in ovarian follicle fluid may be potential biomarkers for evaluating oocyte quality associated with maternal age (Revelli et al., 2009; Zhang et al., 2021d). ROS levels from FF of women undergoing IVF correlate with chronological age, which may be associated with the down-regulated expression of antioxidant genes in granulosa cells, hence can be employed as a simple, non-invasive biomarker to assess ovarian aging (Wang et al., 2020d).

Biomarkers of testicular aging

(1) Physiological characteristics. The testis is a critical male reproductive organ that serves as the source of sperm and a major supplier of the sex hormone, indispensable processes for both male fertility maintenance and physiological homeostasis (Mäkelä et al., 2019). However, testicular function declines gradually as men age (Salonia et al., 2019). Previous studies have shown that aging is negatively correlated with sperm concentration, motility, normal morphological changes, and reproductive outcomes (Johnson et al., 2015; Matzkin et al., 2021; Sharma et al., 2015). In addition, the sperm of elderly men are more likely to bear genetic and epigenetic defects, leading to an elevated risk of pregnancy loss and birth defects in offspring (Laurentino et al., 2020; Potabattula et al., 2020). Aging also impairs testosterone production and causes male hypogonadism, which is characterized by low libido, erectile dysfunction, infertility, obesity, muscle weakness, osteoporosis, depressed mood, impaired cognition, and other symptoms (Kaufman et al., 2019; Mularoni et al., 2020; Xia et al., 2020a). Testicular aging therefore affects not only men's reproductive functions, but also their overall health status and quality of life (Matzkin et al., 2021).

Previous studies have shown that aging testes undergo profound alterations of germ cells and somatic cells, leading to reduced functionality, which also provide potential biomarkers for age-related male reproductive diseases (Jiang et al., 2014a; Santiago et al., 2019) (Figure 24).

(2) Imaging traits. Structural imaging methodologies including US, MRI and PET mainly provide evidence of morphological and functional changes of testes related to the aging process. Different studies have shown that testicular volume decline with advancing age in middle-aged and elderly men (Mahmoud et al., 2003; Well et al., 2007; Yang et al., 2011). Ultrasound, as the first choice for morphological evaluation of male genitalia, is often used to evaluate testicular volume. In addition to evaluating the changes of testicular morphology with age, MRI can be used to evaluate spermatogenic function. Wang et al. (2018a) report that younger men had significantly lower apparent diffusion coefficient and higher magnetization transfer ratio than those of older men, which may be explained by the age-related reduction in testicular spermatogenesis function and testosterone level. Besides, 2-deoxy-2-[^{18}F] FDG-PET can indirectly reflect hormone production and spermatogenesis, two important testicular functions, by providing data of glycolytic activity. In elderly men, the standardized uptake value of FDG decreases with age, consistent with the testicular function decline in aging (Kitajima et al., 2007; Well et al., 2007; Yang et al., 2011).

(3) Histological Features. The most common histologic pattern of human testicular aging is a mosaic of seminiferous tubular lesions ranging from complete spermatogenesis to complete sclerosis of the seminiferous epithelium (Perheentupa and Huhtaniemi, 2009). Other features include narrowing of tubular diameter, thickening of basal membrane associated with arrested spermatogenesis, interstitial fibrosis (Perheentupa and Huhtaniemi, 2009), and basement membrane and tunica albuginea thickening (Dakouane et al., 2005; Johnson, 1989; Johnson et al., 1984a). Further changes in the seminiferous tubules include thinning of the seminiferous epithelium and eventual obliteration of the seminiferous tubules (Regadera et al., 1985; Sasano and Ichijo, 1969). In addition to humans, similar alterations are also reported in monkey, such as fibrosis in the interstitium and increased thickness of the basement membrane in aged testes (Huang et al., 2022a). Compared with young mice, older mice have smaller testes (Gosden et al., 1982; Wolf et al., 2000) and thicker basement membrane (Gosden et al., 1982).

(4) Cellular alterations. (i) Germ cells. In most mammals, germ cell numbers decrease with increasing age, resulting in a reduced diameter of seminiferous tubules and epithelium vacuolization (Kimura et al., 2003; Paniagua et al., 1987). The loss of germ cells usually begins with the spermatids, but gradually affects less mature spermatocytes or spermatogonia until a completely sclerosed tubule forms (Jiang et al., 2014a; Kimura et al., 2003). However, there have been inconsistent findings about spermatogonia numbers during aging, significantly decreased (Nistal et al., 1987) or remained unchanged (Johnson et al., 1987). Morphological changes like multinucleated spermatocytes and spermatids due to the fusion of cell membranes of neighboring spermatocytes or spermatids in aged human testes have been reported (Miething, 1993; Nistal et al., 1986). In the ultrastructural level, intra-nuclear inclusions are seen in spermatocytes and spermatogonia, as well as spirals of endoplasmic reticulum in cytoplasm (Paniagua et al., 1987). Age-related alterations in spermatids include acrosome malformation, redundant nuclear membranes, intra-nuclear inclusions, excessive droplets in the cytoplasm, and irregular configuration of the nuclei (Paniagua et al., 1987).

(ii) Sertoli cells. Multiple alterations associated with aging

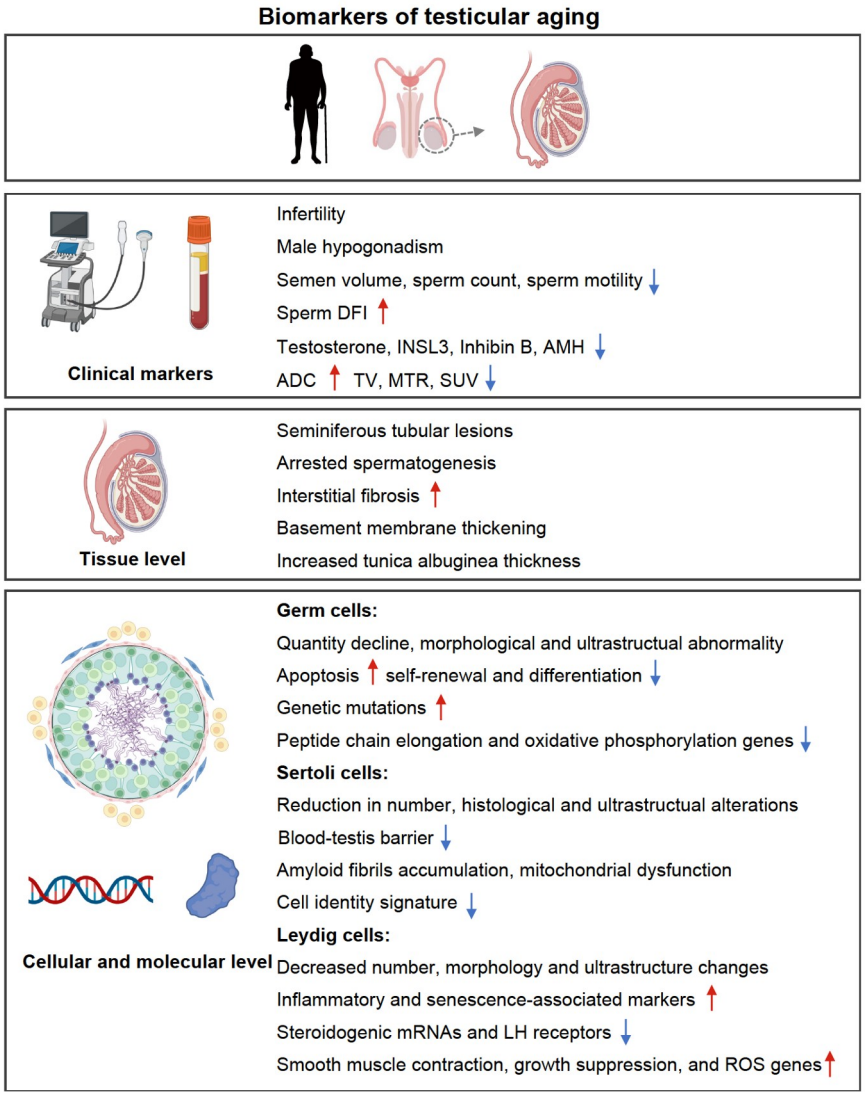

Figure 24 Biomarkers of testicular aging. Abbreviations: ADC, apparent diffusion coefficient; TV, testicular volume; MTR, magnetization transfer ratio; SUV, standardized uptake value; LH, luteotropic hormone.

have been observed in the Sertoli cell of different mammalian species. It has been consistently reported that the number of Sertoli cells declined with age in rat, monkey, and human (Huang et al., 2022a; Johnson et al., 1984b; Santiago et al., 2019). Moreover, Sertoli cells of aged individuals show multiple ultrastructural and histological alterations, such as irregularly shaped nuclei, lost typical localization, enlarged vesicles, mitochondrial metaplasia, loose and vesiculated endoplasmic reticulum, and irregular lysosomes (Bohl et al., 1991; Paniagua et al., 1987). Other Sertoli cell abnormalities such as dedifferentiation and multinucleation are also reported in aging males (Santiago et al., 2019). The cell junctions of aged Sertoli cells also lose characteristic appearance and degenerate, suggesting the presence of a damaged blood-testis barrier in the old individuals (Huang et al., 2022a; Jiang et al., 2014a).

(iii) Leydig Cells. Compared to other types of cells in the testes, there have been more contradictory findings about Leydig cells populations during aging. Some investigations show that the number of Leydig cells was decreased in aged testes (Mularoni et al., 2020; Neaves et al., 1985; Neaves et al., 1984), while others present the opposite results (Honoré, 1978; Ichihara et al., 1993). It is generally accepted that the capacity of Leydig cells to secret testosterone is declined during aging (Jiang et al., 2014b; Wang et al., 2017d). Many age-related changes in morphology and ultrastructure are observed in aged Leydig cells, such as cellular atrophy, multinucleation, intranuclear Reinke crystals, multiple vacuoles, as well as the accumulation of lipofuscin and lipid droplets (Paniagua et al., 1991). These cells also develop signs of dedifferentiation and a decreasing quantity of smooth endoplasmic reticulum and mitochondria during aging (Matzkin et al., 2021; Paniagua et al., 1991).

(5) Molecular changes. (i) Germ cells. The decreased

number of germ cells in aged testes is accompanied by increased apoptotic cells with age (Barnes et al., 1999). Age-related transcriptional signatures reveals imbalance of self-renewal and differentiation in aged spermatogonia (Huang et al., 2022a). Further analysis in older elongated spermatids reveals that upregulated genes are enriched in categories for protein targeting, whereas downregulated genes were enriched categories for peptide chain elongation and oxidative phosphorylation (Nie et al., 2022b). Despite being one of the cells with the lowest spontaneous mutation rates in all human tissues, germ cells show signs of genetic mutations with advancing age (Crow, 2000). It has been identified that the spermatogonia clusters displayed elongated DNA replication time and higher risks for replication errors (Aitken et al., 2020). Similar results have been observed in aging spermatocytes, such as DNA damage and defects in DNA methylation-related molecules (DNMT1 and Np95) (Selvaratnam et al., 2015; Takada et al., 2021).

(ii) Sertoli cells. It has been reported that aging could induce molecular changes of Sertoli cells. Due to the accumulation of amyloid fibrils and damaged mitochondria, Sertoli cells in aged testes develop high ROS levels (Desai et al., 2010). Sertoli cells in aged human testes show major metabolic changes, including dysregulation in lipid metabolic pathway as well as the decline of precursor metabolites and energy production (Nie et al., 2022b; Wang et al., 2022e). In accordance with disruption of the blood-testis barrier, the expression of junction components proteins such as ZO-1, Claudin 11, Jam2, Ocln and Ctnna are reduced in aged Sertoli cells (Huang et al., 2022a; Paniagua et al., 1985; Paul and Robaire, 2013). Moreover, single-nucleus transcriptomic profiling of young and aged NHP testes reveals decreased WT1 causes misregulation of inter-Sertoli cell contacts and a compromised cell identity signature (Huang et al., 2022a). Additionally, age-associated alterations in the cytoskeletal components of Sertoli cells have been observed, including F-actin, vimentin, and cytokeratin (Tanemura et al., 1994).

(iii) Leydig cells. Aged Leydig cells display dysregulation in signaling, function, and developmental identity. The expression of inflammatory marker cyclooxygenase-2 (COX2) and senescence-associated markers (p53, $p21^{CIP1}$) increase in aged Leydig cells (Chen et al., 2007a; Wang et al., 2005; Zhang et al., 2020a). Aging induces ROS production in Leydig cells, concomitant with upregulation of multiple genes, including *PRDX6*, *SOD2*, *MT2A*, *MT1X*, *NAMPT*, and *HIF1A* (Nie et al., 2022b). Consistent with the declined capacity of testosterone secretion, the age-related alterations of Leydig cells that have been reported include decreased expression of steroidogenic mRNAs (*Star*, *Cyp17a1*, *Cyp11a1*, *Hsd3b6*, *Hsd17b3*) and LH receptors (Amador et al., 1985; Curley et al., 2019). Aged Leydig cells also upregulate *PTEN*, *RHOB*, and *ROCK1/2*, which suppress cell survival and proliferation (Nie et al., 2022b). Additionally, the upregulated genes in older Leydig cells are associated with smooth muscle contraction (*ACTA2*, *MYH11*, *TPM1/2*, *MYL9*, and *FLNA*), indicating that aged Leydig cells acquire transcriptome features of peritubular myoid cells (Nie et al., 2022b).

(6) Secretory factors detectable in biofluids. Testicular function changes in aging processes can be assayed in blood and semen.

(i) Blood. It has been reported that serum total testosterone decreases by 0.4% per annum and serum free testosterone declines by 1.3% per annum (Wu et al., 2008). LC-MS/MS represents the gold standard and most accurate method for testosterone evaluation; however, standardized automated platform immuno-assay also works for total testosterone assessment (Huhtaniemi et al., 2012). LC-MS/MS remains the standard method for free testosterone determination. Alternatively, free testosterone can be derived from specific mathematical calculations, taking into account serum sex hormone binding globulin (SHBG) and albumin levels (http://www.issam.ch/freetesto.htm) (Vermeulen et al., 1999).

In addition, Insulin-like peptide 3 (INSL3) is a peptide hormone produced uniquely by the Leydig cells of the testes (Ivell and Anand-Ivell, 2009). And from 30–40 years onwards, serum INSL3 concentration in the blood appears to decline at approximately 15% per decade, serving as an accurate reflection of the reduced number and differentiation status of the Leydig cell population and hence also of their functional capacity to produce testosterone (Ivell et al., 2013; Toppari, 2021). INSL3 can be assessed by specific time-resolved fluorescent immunoassay (Ivell and Anand-Ivell, 2009; Ivell et al., 2022). This well-established and validated assay yields essentially identical values to a new LC-MS/MS procedure (Albrethsen et al., 2018). Inhibin B is a hormonal glycoprotein secreted by Sertoli cells. This hormone is often used as a marker for the impairment of spermatogenesis and can be detected by immunoassay (Chong et al., 2017). In men, older individuals present significantly lower serum Inhibin B already at the age of 40 (Haji et al., 1994). AMH is a dimeric glycoprotein, which is also secreted by the Sertoli cells and has the main role in male sexual differentiation (Xu et al., 2021). Men exhibit declining serum AMH levels with age after sexual maturity, as detected by immunoassay (Chong et al., 2017; Ramezani Tehrani et al., 2017).

(ii) Semen. Adverse alterations gradually appear in semen volume, sperm motility, and sperm function with advancing age. Daily sperm production is negatively correlated with male age and decreases by more than 30% in men older than 50 years (Neaves et al., 1984). One study found that men's semen volume decreases by 0.22 mL for every 5 years of age (Begueria et al., 2014) and that the sperm count begins to decrease significantly from the age of 41 years (Pino et al.,

2020; Verón et al., 2018). Similarly, sperm total motility and forward motility decrease with increasing male age (Johnson et al., 2015; Verón et al., 2018). Specifically, sperm motility decreases by 1.2% every 5 years (Begueria et al., 2014), and progressive motility is two-fold lower in men aged 50 years or older compared with those aged 40–50 years (Pino et al., 2020).

Sperm DNA fragmentation index (DFI) is a common parameter used to assess the quality of semen samples (Agarwal et al., 2020). Many studies have found that DFI increases with age (Evenson et al., 2020; Rosiak-Gill et al., 2019). Men over 45 years of age exhibit higher DFI and lower DNA stability (Deenadayal Mettler et al., 2020), and the DFI of 60-year-old men is more than double that of 20-year-old men (Chianese et al., 2014).

Semen analysis has been standardized by the WHO and disseminated by publication of the most updated version of the WHO Laboratory Manual for the Examination and Processing of Human Semen (WHO, 2021).

Summary and perspectives

In this section, we review the biomarkers for ovarian and testicular aging on clinical, tissue, cellular and molecular levels. Given that many aging biomarkers vary widely in accuracy, reliability, and invasiveness, combining multiple biomarkers of aging that meet comprehensive criteria can provide a more accurate assessment of aging. The emergence of new technologies, such as single-cell sequencing, spatial transcriptomics, bioinformatics, and artificial intelligence used for training of age prediction models (Zhang et al., 2022b), allows us to gain a more comprehensive understanding of the age-related changes in reproductive system, which helps identify more novel and precise sets of biomarkers and facilitate the development of early and accurate diagnosis of reproductive system aging in clinical practice. Yet, the final predictive relation among such sets of markers may only be derived from long-term follow-up studies.

Hematopoietic system aging

Aging of hematopoietic system contributes to a number of clinically significant pathologies, that include: clonal hematopoiesis (CH), increased incidence of hematological malignancies, elevated frequency of age-related chronic anemia, and dysfunction of immune system (Beerman et al., 2010). All cells of hematopoietic system arise from the most primitive hematopoietic stem cells (HSCs), therefore, the age-dependent cellular and molecular changes within this compartment contributes greatly to the functional decline of hematopoietic system (de Haan and Lazare, 2018; Morrison et al., 1996). CH, which most likely occurs at HSC level, is commonly seen in human aging and has been associated with hematopoietic disorders, cardiovascular diseases and overall mortality (Jaiswal and Ebert, 2019; Mitchell et al., 2022). Here, we mainly focus on the recent findings that implicate the biomarkers of HSC aging, the intrinsic and extrinsic factors associated with HSC aging, and the consequences of HSC aging in hematopoietic system (Figure 25).

Physiological characteristics

Hematopoietic system aging is characterized by a gradual disruption of hematopoietic homeostasis, leading to numerous changes including reduced production of red blood cells and lymphocytes as well as a relative increase in the production of myeloid cells (Konieczny and Arranz, 2018). These changes are associated with anemia, an increased risk of infection, poor response to vaccinations, increased risk of bone marrow failure and the development of hematological malignancies in older people (Belyavsky et al., 2021; Pang et al., 2011; Yamashita and Iwama, 2022). Defects in the function of HSCs during aging are considered to be the pivotal factor underlying this complex process (Groarke and Young, 2019). Most HSCs are at quiescent or dormant in a steady-state environment, which is conducive to maintaining their function and youthful stemness, while proliferations will lead to depletion. Old bone marrow contains an increased myeloid-dominant HSCs with a lower output of mature blood cells per HSC, while competitive transplantation assays have revealed a reduced self-renewal, regeneration potential as well as impaired homing ability for aged HSCs (Cho et al., 2020; Dykstra and de Haan, 2008; Wilkinson et al., 2020). The most common hematologic disease among elderly people is anemia, which is caused by deficiencies of iron, folate, and vitamin B12, as well as chronic inflammation. The cause of anemia has not been identified in one-third of cases, including patients with idiopathic cytopenia of undetermined significance (ICUS). 30% of ICUS patients have been found clonal expansion of HSCs with somatic mutations (Fujino et al., 2022). All cells of the immune system are derived from HSCs and immune aging stems from HSC aging (Shaw et al., 2010). Immunosenescence, the age-associated alterations in the immune system, has been considered to be involved in the vulnerability to infections among elderly people. The decline in B cell production as well as its antibody diversity may be the reason why the elderly is less effective when vaccinated and are more likely to develop autoimmune diseases (Hagen and Derudder, 2020; Kim et al., 2003). Naive CD4 and CD 8 T cells gradually decrease upon aging leading to dysfunction of adaptive immunity and a subsequent predisposition to cancers (Denkinger et al., 2015; Naylor et al., 2005; Qi et al., 2014). The number of NK cells is increased, but the cytotoxicity is weakened and cytokine secretion is reduced (Mocchegiani et al., 2009). Monocytes are important components of innate immunity and function by differentiating into antigen-presenting cells such as macrophages

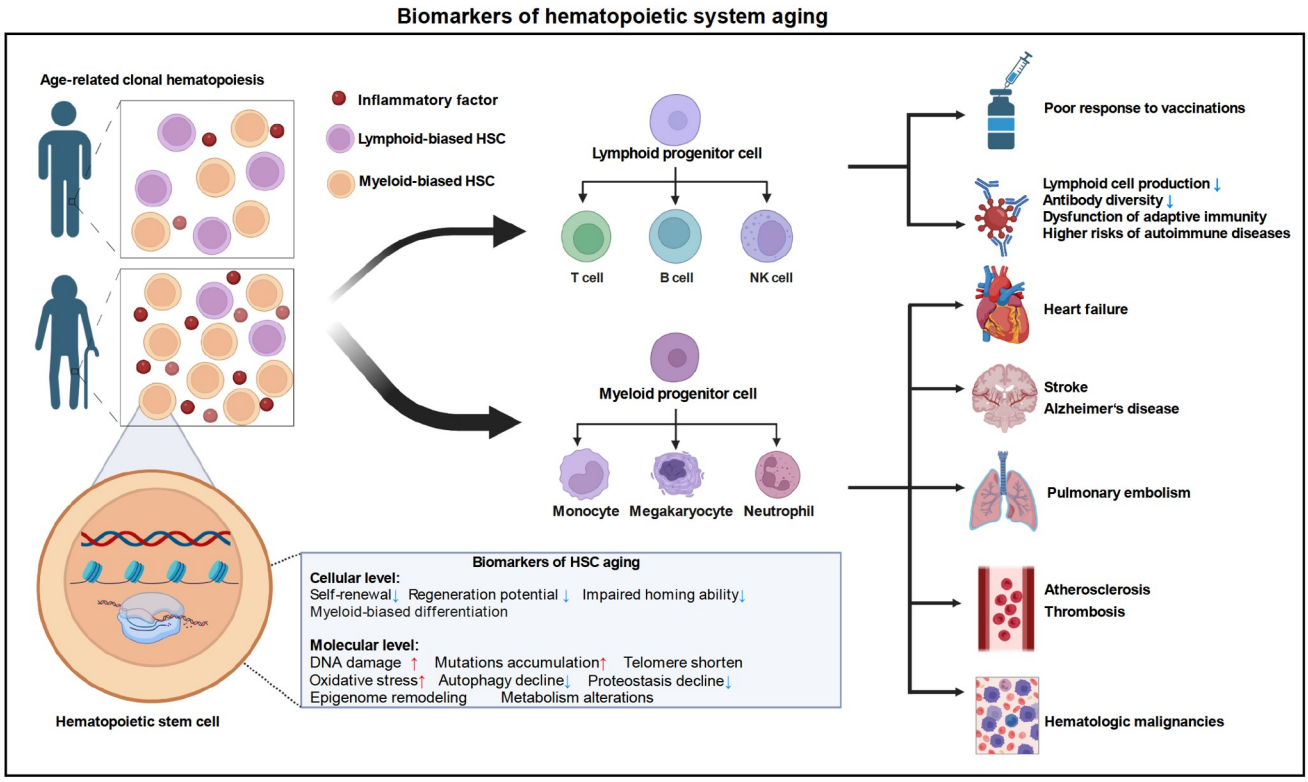

Figure 25 Biomarkers of hematopoietic system aging. Aged HSCs exhibit broad changes at cellular and molecular levels. The acquisition and accumulation of driver mutations in HSCs during aging lead to CH, which enhances myeloid cells output that is associated with an increased risk of hematological malignancies and diseases in several organs.

and dendritic cells. During hematopoietic aging, the number of circulating neutrophils and monocytes increases but their function decreases. It is manifested by a decrease in the migration of neutrophils to stimuli and a decrease in the phagocytic activity of macrophages, which affects the innate immune system (Mogilenko et al., 2022). A recent study defines pluripotent HSCs with platelet bias, where age-related platelet count decline and functional changes are critical for thrombotic diseases (Frisch et al., 2019; Jones, 2016).

Clonal hematopoiesis

HSCs undergo multiple divisions to sustain lifelong hematopoiesis, they are susceptible to accumulate mutations, which likely confer a survival advantage, resulting in higher chance of malignancy (Groarke and Young, 2019). Mutation in HSC and microenvironment aging lead to the imbalance of HSC expansion and downstream blood cell differentiation, that is, CH. DNA damage or DNA replication errors cause somatic mutations in HSCs, driving HSCs dysfunction (Li et al., 2022d). CH expanded stem cells usually have somatic mutations in genes of epigenetic regulators, which are also considered to be the driving mechanism of stem cell clonal dominance (Buscarlet et al., 2017; Xie et al., 2014). CH increases the risk of malignancy and is used as a biomarker of aging and cancer prognosis (Genovese et al., 2014; Jan et al., 2017). Transmitted through downstream blood lineages, CH leads to abnormal circulation, which is shown to be an independent risk factor for atherosclerotic cardiovascular diseases and affects the function of distant tissues (Jaiswal, 2020; Misaka et al., 2023; Sidlow et al., 2020). A series of large-scale population-based studies have found that CH increases with age, and the proportion of CH in older people is as high as 31% (Genovese et al., 2014; Gillis et al., 2017; Razavi et al., 2019; Xie et al., 2014). The most common mutated genes include epigenetic regulators (*DNMT3A*, *TET2*, *ASXL1*), RNA splicing factors (*SF3B1*, *SRSF2*, *U2AF1*, *PRPF8*), DNA repair and cell cycle regulatory genes (*PPM1D*, *TP53*). Recent population-wide genome analysis demonstrated that the GWAS signal of CH is enriched in the open chromatin region of hematopoietic stem and progenitor cells (HSPCs) and discovered novel locus fields associated with DNA damage repair (PARP1, ATM, CHEK2), HSCs migration/homing (CD164), and bone marrow tumorigenesis (SETBP1) (Kar et al., 2022), further illustrating the importance of CH as a marker of hematopoietic aging. Although CH is observed with increasing frequency with age, the vast majority of individuals with CH will not develop MDS or other hematologic malignancies. To distinguish cancer mutation-associated non-malignant CH clearly from other forms of CH, such as ICUS and clonal cytopenia of undetermined significance (CCUS), the concept of CH with

indeterminate potential (clonal hematopoiesis of indeterminate potential, CHIP) was introduced. Defined as at least 4% cancer-related clonal mutations are present in nucleated blood cells of individuals without obvious tumors (the variant allele fraction (VAF) threshold is set to 2%), CHIP can be used as an independent age-related risk factor for diseases such as cardiovascular and leukemia (Jaiswal, 2020). As bone marrow has a good correlation with VAF values in peripheral blood, the condition of HSCs in bone marrow can be reflected by measuring VAF values in peripheral blood (Hwang et al., 2018). Since the fitness effect of each mutation is specific, VAF can not only be used as an indicator to measure the size of the clone, but also can determine the fitness brought by a gene mutation to HSC (Venkataram et al., 2016) and the fitness effect of each mutation can be quantified by CH developmental models (Watson et al., 2020a). Without other hematologic abnormalities, people over 70 years old are prone to appear CHIP in peripheral blood with genetic mutations such as DNMT3A, TET2, ASXL1, PPM1D and JAK2. A WGS study on newborns to the elderly of HSCs has found that some older adults also show signs of clonal expansion without known driver gene mutations. The hematopoietic function of adults under 65 years of age is polyclonal and has high clonal diversity, while coming forth a sudden and general loss of clonal diversity after the age of 75, which proposes a new theory of human aging. The study concluded that clonal amplification after the age of 70 was more common than the statistical results of existing studies. Clones with more than 1% VAF are ubiquitous. The number of clone amplifications can reach to 10–20 per person accounting for 30%–60% of the entire hematopoiesis rather than just 3%–5%. These mutations are carried early in life (Mitchell et al., 2022). A GWAS and ExWAS study involving 27,331 CHIP mutation carriers revealed common and rare variants (mCA, mLOX, mLOY, and telomere length) in CH phenotypes, further illustrating that CHIP represents a complex set of phenotypes with common and unique germline genetic causes and different clinical significance (Kessler et al., 2022). We know that loss of HSCs diversity is associated with positive selection for clonal proliferation carrying driver mutations. Studies of oligoclonal competition in CH have found that although DNMT3A is the most common gene to drive clonal amplification preferentially initiated early in life (Huang et al., 2022f), the most common mutated gene after age 85 is not DNMT3A but TET2 (Buscarlet et al., 2017). Even the DNMT3A R882 hotspot mutation does not affect its amplification rate. On the contrary, U2AF1 and SRSF2-P95H mutations, although only initiated late in life, are the fastest drivers of CH and are considered risk factors for AML progression (Fabre et al., 2022). The emerging evidence suggests that mutations that drive faster clonal growth are associated with a higher risk of malignant progression.

Cellular alterations

HSCs maintain self-renewal and differentiation potential over lifetime in order to preserve the homeostasis of the hematopoietic system (Mejia-Ramirez and Florian, 2020). Young HSCs divide mainly asymmetrically, while aged HSCs undergo symmetrical division (Florian et al., 2018). This may explain the phenomenon that HSCs expand by 2 to10 fold in both murine and human during aging (Geiger et al., 2013; Pang et al., 2011). The expanded aged HSC compartment partially compensates the functional decline of reconstitution capacity at the population level as measured by total bone marrow transplantation assay (Harrison, 1983). While on a per cell basis, aged individual HSC exhibits impaired self-renewal in serial competitive transplantation assay (Dykstra et al., 2011). Similarly, HSCs from older donors show reduced reconstitution capacity when assayed by xenotransplantation in immunodeficient mice (Pang et al., 2011). In clinic, the age of the donor is a strong factor that limits the source of HSC for transplantation, as transplantation of HSCs from old donors always associates with worse survival of recipients (Wang et al., 2014d; Xu et al., 2019). Aged HSCs also display a biased differentiation capacity towards myeloid lineages with reduced lymphocyte output, which is strongly associated with CH (Zink et al., 2017). Aged HSCs highly express megakaryocyte-related genes, including *Selp*, *Vwf* and *CD41* (Flohr Svendsen et al., 2021; Frisch et al., 2019; Gekas and Graf, 2013; Grover et al., 2016). High level of CD61 is also associated with myeloid-biased differentiation in aged HSCs (Mann et al., 2018). In addition to the aforementioned characteristics, HSCs from aged mice also show deficiency in their homing to and engrafting the bone marrow of recipient mice (Morrison et al., 1996).

As mentioned above, HSCs reside mostly in a non-cycling state, termed as “quiescent”, and they need to enter cell cycle in order to either give rise to downstream progenitors or to self-renew for repopulation (Biermann and Reya, 2022). However, HSCs can only self-renew for a limited time (Bernitz et al., 2016; Wilson et al., 2008). Recent studies indicate that HSC functions are inversely correlated with the cell divisional history. In the 5-FU-induced myeloabative chemotherapy model, HSCs proliferate extensively and show reduced reconstitution capacity (Lerner and Harrison, 1990). After extended serial transplantation, young HSCs exhibit a strong feature of aging phenotype, which phenocopy the aged HSCs (Dykstra et al., 2011). Likewise, young HSCs that are processed with repeated inflammatory challenges also showed several reduced functional properties, which are usually considered as the markers of aged HSCs (Bogeska et al., 2022). The functional decline of HSCs with cell divisions also occurs under homeostatic conditions (Bernitz et al., 2016; Qiu et al., 2014). A study based on the H2B-GFP mouse model (H2B-GFP is diluted with each cell

division) has reported that HSC loss their reconstitution potential gradually along with cell division (Bernitz et al., 2016). Of note, HSCs may never return back to its identical state even if they return to quiescence phase after cell division, but rather replicate and give rise to the HSCs with impaired functionality (Qiu et al., 2014). The exact signaling underlying this phenomenon is unclear but it is strongly associated with the changes in transcription and metabolism during cell division (Hinge et al., 2020; Qiu et al., 2014; Umemoto et al., 2018). It seems plausible that cumulative stresses enforce HSCs to enter excessive replication during aging, however, they only self-renew phenotypically as they lose self-renewal potential along with each cell division. As a consequence, the aged HSC compartment expands but the function of individual stem cells is impaired.

Molecular changes

The aging process of HSCs is modulated by both transcriptional changes and epigenetic alterations, thus far known to be involved in a wide variety of physiological or pathological events including, DNA damage, mutations accumulation, telomere shorting, oxidative stress, proteostasis decline, metabolism and epigenome remodeling (Ju et al., 2007; Mejia-Ramirez and Florian, 2020; Raaijmakers, 2019). Aged HSCs have high level of DNA damage accumulation, as demonstrated by a significant increase in γH2AX foci and a higher tail moment in the comet assay (Beerman et al., 2014; Rossi et al., 2007; Rube et al., 2011). Increase in DNA damage may result in elevated DNA mutations in aged HSCs. Indeed, recent studies uncover that mutations associated with CH are accumulated in aged HSCs in both healthy murine and human, and consequently increase the myelopoiesis and age-related diseases in other organs (Ahmad et al., 2023; Chin et al., 2022; Mitchell et al., 2022). Transcriptional analyses reveal that aged HSC are distinct from young HSCs at transcriptomic level. The expression of genes involved in inflammation, protein quality control and stress response are enriched in aged HSCs, while genes associated with DNA repair and chromatin remodeling are down-regulated in aged HSCs (Chambers et al., 2007). Aged HSCs also exhibit up-regulated myeloid genes and down-regulated lymphopoiesis-related genes, which correspond to myeloid-biased differentiation capacity of aged HSCs (Sun et al., 2014).

The genomic alterations that occur in HSCs during aging have been extensively studied. Recent emerging evidence highlights that loss of epigenetic regulations may drive HSC aging and strongly contribute to neoplastic transformation in hematopoietic system (Zhang et al., 2020d). Studies have uncovered the functional importance of epigenetic regulation in HSC aging at several levels, including DNA methylation, histone modification, and changes in chromatin accessibility. The DNA methylome is generally stable in HSC during aging, however, DNA methylation is enriched specifically at the promoter regions of lymphoid-related genes, while genes responsible for myeloid output show reduced DNA methylation (Beerman et al., 2013). Interestingly, experimental evidence shows that a higher proliferative rate leads to DNA hypermethylation in HSCs, suggesting that the cell proliferation and DNA methylation are coordinated in modulation of HSC aging process (Beerman et al., 2013). Alterations of histone modifications also occur in HSC during aging. H3K4me3 peaks are more widespread in aged HSCs and are more enriched in genes associated with HSC identity (Sun et al., 2014). This change is correlated with the enforced self-renewal of aged HSCs (Sun et al., 2014). Upon aging, H4K16ac peaks are reduced and exhibit a loss of epipolarity redistribution, which is correlated with the functional decline of aged HSCs (Grigoryan et al., 2018). More recently, an integrated analysis of transcriptome and chromatin accessibility reveals that differentially open accessible regions (open DARs) in aged HSCs are preferably enriched in the regions that are activated in response to external stresses (Itokawa et al., 2022). However, an acute inflammatory stress in young HSCs does not exhibit the same persistent modulation of chromatin accessibility changes as that observed in the aged HSCs. This finding puts forward a conjecture that the signals generated following stress exposure may be epigenetically inscribed in HSCs during aging, thus endowing aged HSC to be more responsive to external stimuli (Itokawa et al., 2022). A previous study showed that HSCs gain long term epigenetic modifications in accessibility of specific myeloid lineage enhancers, that allows them to respond to secondary infections more efficiently (de Laval et al., 2020). This “epigenetic memory” mechanism endows young HSCs with enhanced function to rapidly differentiate in order to meet the need for more myeloid cells in the context of inflammation. However, the increased responsiveness of aged HSCs to external stimuli by the trained “epigenetic memory” may lead to reduced stemness and eventually cause HSC exhaustion. HSCs in human also undergo an age-associated epigenetic reprogramming (Adelman et al., 2019). Of note, the changes in epigenetic modifications and their targeting pathways in aged human HSCs are comparably altered in AML patients, indicating the epigenetic reprogramming of aged human HSCs may directly promote cancer formation in hematopoietic system (Adelman et al., 2019).

The genome is hierarchically organized in the nucleus and higher-order of chromatin structure including, chromosome territories, A/B compartments, topologically associating domains (TADs) and enhancer-promoter loops are involved in gene regulation (Szabo et al., 2019; Wang et al., 2016c; Zheng and Xie, 2019). The impact of epigenetic modifications of DNA and histone on HSC aging has been thoroughly investigated, the role of higher-order chromatin structure in regulating HSC aging still needs to be uncovered. Under-

standing the role of higher-order epigenome organization and how it interacts with gene regulation in HSCs during aging would facilitate the discovery of novel targets to control blood cancer formation.

Secretory factors detectable in biofluids

Increase in chronic inflammation is a driving force accelerating HSC and hematopoietic system aging (Caiado et al., 2023; Caiado et al., 2021). Recent studies have highlighted the emerging role of inflammation signals, particularly the secretory factors in both bone marrow microenvironment and the systemic circumstance on shaping the hematopoietic system. Upon aging, hematopoietic stem and progenitor cells niches undergo degeneration and remodeling. In aged mice, HSC-supporting niche cells close to the bone, including arterioles, transition zone vessels (TZV) and nestin-expressing MSC, are reduced (Ho et al., 2019; Raaijmakers, 2019). Whereas the niches distant from the bone, such as capillaries and nestin-positive stromal cells, are expanded (Ho et al., 2019; Raaijmakers, 2019). The increase in the sympathetic noradrenergic fibers triggers IL-6 secretion of MSCs and results in megakaryopoiesis of aged HSCs (Ho et al., 2019). In addition, the number of $LepR^{+}$ $Osteolectin^{+}$ osteogenic progenitors is lower in aged bone marrow niche. This results in reduced stem cell factor (SCF) secretion and contributes to reduction of common lymphoid progenitor (CLP) as well as impaired lymphopoiesis of aged mice (Shen et al., 2021). Furthermore, the accumulation of adipocytes and altered expression of extracellular vesicles in aged bone marrow also accelerates HSC aging and disturbs hematopoiesis (Ambrosi et al., 2017; Goldberg, 2021). Microbiota alterations are strongly associated with increased inflammation during aging (O'Toole and Jeffery, 2015; Trowbridge and Starczynowski, 2021). A recent study discovers that a microbiome-IL-1 axis functions as a self-sustaining driver of HSC aging (Kovtonyuk et al., 2022). Targeting IL-1 or antibiotic administration can reverse the myeloid-biased differentiation phenotype of aged HSC (Kovtonyuk et al., 2022).

Apart from changes in secretory factors originating from HSC bone marrow micro- and the systemic environment, HSPCs also secrete inflammatory factors in response to inflammatory stimuli (Zhao et al., 2014). For instance, HSPCs produce IL-6 in response to TLRs signaling activation, which acts on the adjacent HSPCs in a paracrine manner to promote HSPCs differentiation in order to meet the acute need of blood cell generation (Zhao et al., 2014). As aged HSCs exhibit higher responsiveness to inflammation, intrinsic changes in aged HSCs may also contribute greatly to the enlarged pool of secretory factors during aging (Chen et al., 2019b; Mann et al., 2018). Furthermore, the increased number of myeloid cells originated from aged HSCs due to the CH boosts the systemic inflammatory cytokine secretion, and may in turn negatively affect the aged hematopoietic system (Jaiswal and Libby, 2020; Yura et al., 2020).

The aging-associated hematological disorders are also strongly connected to increased level of inflammatory factors during aging. Several studies have shown the relationships between elevated chronic inflammation signals and CH. The age-dependent elevation of secretory factors, such as IFN-γ and TNFα, promote DNMT3a-mutant HSC expansion and leads to CH (Hormaechea-Agulla et al., 2021; Liao et al., 2022). Consistent with this finding, another study reveals that aging drives $Tet2^{+/-}$ CH via IL-1 signaling in aged mice (Caiado et al., 2023). Together, these observations point out the translational value of targeting secretory factors to treat age-dependent myeloid malignancies.

Based on the cognition of significance of inflammatory factors on hematopoietic system aging, interventions that could potentially reduce the inflammatory signals, including caloric restriction, changing the blood-borne factors via heterochronic parabiosis and senescent cell clearance, have been implicated to delay hematopoietic system aging. Caloric restriction has been known to attenuate chronic inflammatory diseases via reducing circulating monocytes and pro-growth factors (Jordan et al., 2019). Study has showed that prolonged fasting, refeeding cycles or reduced food intake by 30% could reduce circulating IGF-1 levels, which promotes HSC self-renew capacity in aged mice (Cheng et al., 2014; Tang et al., 2016). Heterochronic parabiosis, the pairing of a young and an aged mouse, has been widely used in aging research to evaluate the contribution of systemic factors that influence the aging processes (Ashapkin et al., 2020). The rejuvenating factors from the young systemic circulatory milieu alleviated age-associated lymphopoiesis decline in old mice after heterochronic parabiosis (Ma et al., 2022; Wang et al., 2022b). In line with this finding, another study also indicates that HSCs are one of the cell types showing high responsiveness to heterochronic parabiosis (Pálovics et al., 2022). Clearing of senescent cells via genetic modifications or senolytic drugs holds great promise to enhance regeneration potential and extend a healthy life span in aged mice (Baker et al., 2016; Baker et al., 2011; Chaib et al., 2022). Combating hematopoietic system aging by targeting senescent HSCs has been tested in mice. It will be interesting to know whether this approach could be applied to human to ameliorate aging-related hematopoietic changes (Chang et al., 2016). Although HSC aging is uncoupled from $p16^{INK4a}$-mediated senescence (Attema et al., 2009), pharmacological depletion of senescent cells in aged bone marrow and the whole body may decrease the secreted factors that have impacts on HSC function negatively.

Summary and perspectives

Here, we have summarized the aging-related disorders in the hematopoietic system, the biomarkers of HSC aging, and the

causal factors that contribute to HSC aging. Extensive efforts have been made in deciphering the mechanism underlying hematopoietic system aging, however, the possible approaches that potentially relieve aging-associated hematopoietic disorders are still limited. As many of the hematological pathologies are strongly associated with HSC aging, interventions that target HSC aging will be clinically important. Considering the role of CH in hematopoietic malignancies and in non-malignant aging-associated diseases, a deep understanding of the occurrence and development of CH will be beneficial for healthy aging.

Immune system aging

Physiological characteristics

Aging of the immune system is inevitable with physiological or pathologically induced aging, characterized by biomarkers denoting the degradation of immune organs and changes in the proportion and function of immune cells (Figure 26; Table S17 in Supporting Information). Aging disrupts the physiological balance of antigen recognition by the immune system. On the one hand, the immune response to foreign antigens, including pathogenic microorganisms and tumor antigens, is weakened with age, leading to a high incidence of cancer and a high mortality rate from viral infection. On the other hand, aging elevates the recognition of self-antigens, which contributes to the development of inflammation. It was recently reported that elderly people have an increased risk of developing and dying from viral infections, especially COVID-19, mainly caused by immune senescence (Chen et al., 2021c; Huang et al., 2020a). During aging, the efficiency of antigen presentation by DCs decreases, inhibiting the adaptive T-cell response and antibody response. Macrophages and neutrophils infiltrate infected tissues more severely, leading to destructive inflammation (Wong et al., 2022). Respiratory failure owing to severe lung injury is the major factor contributing to the clinical death of COVID-19 patients. The lung tissues of elderly patients with COVID-19 show a more serious senescent state, specifically characterized by the upregulation of cell aging markers, SASP expression, and DNA oxidative damage. In addition, the expression of the SARS-CoV-2 receptor ACE2 increases gradually with age, and virus invasion also induces the upregulation of ACE2 expression. As a consequence, the lung cells of elderly people are more vulnerable to SARS-CoV-2 infection, accelerating lung failure (Wang et al., 2021d).

Imaging traits

Medical imaging techniques like MRI and FDG-PET can assess changes in the structure and function of immune organs that occur with aging. Bone marrow produces various immune cells with distinct developmental stages and other stromal cells. Bone marrow includes both red marrow (containing 40% fat cells and 60% hematopoietic cells) and yellow marrow (containing about 95% fat cells and 5% non-fat cells) according to structural differences (Krishnaraj, 1997). The MRI technology used in bone marrow assessment relies on the ratio of fat cells to non-fat cells, which directly affects the signal intensity. The MRI results imply that the percentage of fat cells increases and a large part of red bone marrow is replaced by yellow bone marrow during aging (Blebea et al., 2007). These changes may contribute to physiological and histologic aspects of immune senescence.

Histological features

One of the most important hallmarks of the aging of the human immune system is the degeneration of the thymus. The human thymus weighs 10–15 grams at birth and develops during puberty to 30–40 grams. Thereafter, the cortex and medulla of the thymus begin to be replaced by adipose tissue, and at an old age, the entire thymus dwindles to roughly 10 grams (Kendall et al., 1980). Aging-related degeneration of the human thymus includes the deconstruction of its histological structure, reduction in thymus size and weight, and decrease in the number of thymocytes (Mittelbrunn and Kroemer, 2021). The thymus is one of the major organs for T-cell development, and functional degeneration of the thymus by aging could directly lead to a decline in naïve T-cell production, compensatory clonal expansion of memory T cells, and a reduction in the diversity of peripheral T cells. Thymus degeneration could also cause decreased functional activity of T cells, which in turn leads to a decline in immunity, with a possible deficiency in immune tolerance and enhancement of the autoimmune response.

The human body contains 300–500 lymph nodes, which weigh around 100 grams in total (Cakala-Jakimowicz et al., 2021). Through human aging, the number and volume of lymph nodes both gradually decrease, and age-related functional degeneration of lymph nodes starts to appear, e.g., fibrosis, vitrification, lipomatosis, reduction in the number of postcapillary vessels, and morphological and functional alteration of the venous capillary endothelial cell linings (Murakami, 2004). As degenerative changes accumulate, the functional zonal structure and composition of lymph nodes are gradually disordered: the number of lymphoid tissues in the cortex and medulla of lymph nodes decreases, the number and sizes of lymphoid follicle germinal centers decline, the number of follicular dendritic cells reduces (Phan et al., 2007; Szakal et al., 2002; Turner and Mabbott, 2017b), and the level of lymph node homeostasis-associated chemokines (e.g., CCL19, CCL21, and IL7) drops (Becklund et al., 2016; Chai et al., 2013; Masters et al., 2019; Textor et al., 2016). Eventually, these aging-induced structural and functional defects in lymph nodes ultimately lead to impaired cellular homeostasis of T and B cells, a reduced capability of antigen recognition by immune cells, and decreased humoral

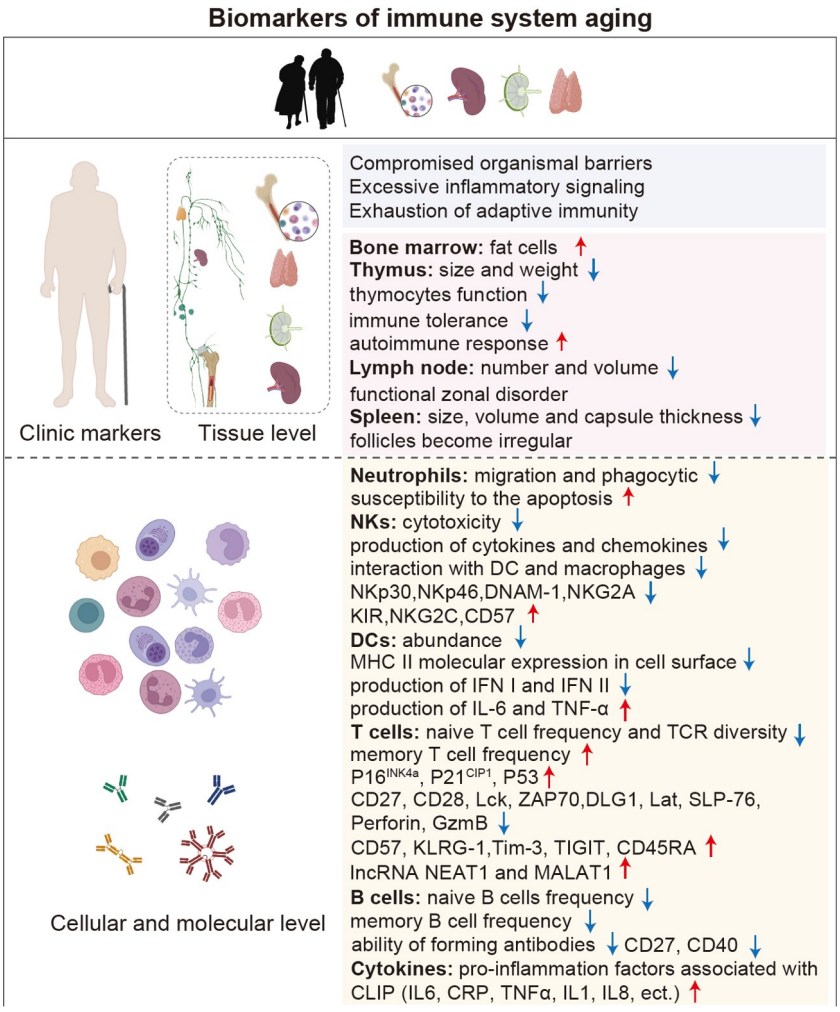

Figure 26 Biomarkers of immune system aging. Immunosenescence leads to compromised organismal barriers and the elevated susceptibility to infection and chronic diseases. The aged immune system is characterized by the degeneration of thymus, spleen and lymph node, the declined function the innate and adaptive immune cells, and excessive inflammatory signaling. Abbreviations: NKp30, natural cytotoxicity receptor in natural killer cells; DNAM-1, DNAX accessory molecule-1 (CD266); KIR, killer cell immunoglobulin like receptor; TCR, T-cell receptor; Lck, lymphocyte cell-specific protein-tyrosine kinase; ZAP70, zeta chain of T cell receptor associated protein kinase 70; DLG1, discs large homolog 1; SLP-76, SH2 domain-containing leukocyte protein of 76 kD; KLRG-1, lectin-like receptor subfamily G; TIGIT, T cell immunoreceptor with Ig and ITIM domains; NEAT1, nuclear paraspeckle assembly transcript 1; MALAT1, metastasis associated lung adenocarcinoma transcript 1.

immune responses.

Aging also causes a reduction in the size and volume of the human spleen. Structurally, the thickness of the spleen capsule is affected by age: the capsule thickness develops to its peak from birth to puberty and then starts thinning slowly (Alex et al., 2015). In addition, follicles become irregular, and somatic hypermutation in the germinal center of the spleen also decreases with age (Alex et al., 2015; Banerjee et al., 2002; Turner and Mabbott, 2017a).

Cellular alterations and molecular changes

In the aging process, many immune cell subsets in both the innate and the adaptive immune systems are altered. The major alterations in the immune cell populations and the relative molecular changes in aging subjects are discussed in this section.

Macrophages engulf and digest pathogens and act as tissue sentinels in the innate immune system, influencing adaptive immune responses and exerting tissue repair functions. Macrophages consist of a heterogeneous cell population that may differ in phenotype and behavior (Kohut et al., 2004), and the reported age-related alterations in macrophage functions differ according to the site (Mogilenko et al., 2022; van Beek et al., 2019).

With their ability to quickly capture and kill invading pathogenic microorganisms, neutrophils form a paramount part of the innate immune system. During aging, neutrophils display low migration and phagocytic ability and are more susceptible to apoptosis when activated, which reduces their recruitment to infection sites and phagocytosis of pathogens (Brubaker et al., 2013). Moreover, the persistence of inflammatory neutrophils may impede the resolution of in-

flammation, causing tissue lesions (Sendama, 2020; Stout-Delgado et al., 2009). Furthermore, the residencies of neutrophils in some organs increase with age, but the mechanisms and physiological function of higher neutrophil infiltration remain unclear.

DCs are important antigen-presenting cells that build a bridge between innate immunity and adaptive immunity. Elderly individuals have low circulating DCs (Agrawal and Gupta, 2011; Jing et al., 2009; Stervbo et al., 2015), and functional disturbance has also been demonstrated in aged dendritic cells, including decreased production of IFN I and II, increased production of IL-6 and TNFα, and lower MHC II molecular expression on the cell surface (Gupta, 2014; Jing et al., 2009), all of which are associated with compromised antiviral responses, pro-inflammatory bias and weakened activation of T cells (Agrawal and Gupta, 2011; Jing et al., 2009). Excessive NF-κB stimulation contributes to dendritic cell alteration during aging (Agrawal et al., 2009; Panda et al., 2010).

NK cells are a group of innate immune cells with cytotoxic properties. Age-related NK cells are characterized by compromised abilities in cytotoxicity, including the production of cytokines and chemokines and interactions with DCs and macrophages. Alterations in the expression of activation receptors, such as NKp30, NKp46, and DNAM-1, may impair the immune monitoring function of NK cells in the elderly (Campos et al., 2015; Campos et al., 2014). These changes probably result in an increased risk of morbidity and mortality in elderly individuals (Krishnaraj, 1997).

Immune senescence also results in the exhaustion of adaptive immunity, and the number of naïve T cells dramatically declines, while the number of memory T cells is increased. TCR diversity declines with age, and the reduced expressions of Lck, ZAP70, DLG1, Lat, and SLP-76 have been reported to be involved in the TCR signaling mechanism (Rodier et al., 2009; Zhao et al., 2020b). Senescent T cells present decreased SA-β-galactosidase and cytotoxic activity, low proliferative capacity, upregulation of the expression of cell cycle arrest genes such as $p16^{INK4}$ and $p21^{CIP1}$ and immune checkpoint-related molecules such as Tigit, Tim3, and CTLA-4, and decreased expression of functional molecules such as Perforin and GzmB. These changes may be associated with increased pro-inflammatory cytokine secretion and decreased new antigen recognition (Larbi et al., 2008; Mittelbrunn and Kroemer, 2021; Zhang et al., 2021g; Zhang et al., 2020g). The surface markers also change during aging, and senescent T cells lose the expression of the costimulatory molecules CD27 and CD28 but increase the expression of killer cell lectin-like receptor subfamily G (KLRG-1) and CD57 (Huff et al., 2019; Zhang et al., 2020g), so $CD27^{-}CD28^{-}CD57^{+}KLRG\text{-}1^{+}$ T cells can be used as an indicator of immunosenescence. Correspondingly, the naïve B-cell frequency and B-cell activation are impaired, and the ability to form antibodies and antibody affinity are reduced in elderly people, while memory B cells accumulate owing to increased antigen stimulation (Johnson et al., 2002; Pinti et al., 2016). The expression of costimulatory molecules (CD27, CD40) is reduced in senescent B cells (El-Naseery et al., 2020). Antibodies produced by B cells aimed at pathogenic microorganisms are important to resist bacterial and viral infections. Therefore, a decline in B-cell functions might contribute to weak anti-infection immunity in elderly individuals.

Secretory factors are detectable in body fluids such as blood, urine, and CSF. Peripheral blood is a common body fluid in which aging biomarkers are identified. Aging-related secretory factors in the blood are often linked to the chronic low-grade inflammatory phenotype (CLIP), which was first described by Krabbe et al. (2004). In contrast to classical acute inflammation, CLIP is characterized by a persistent low-grade inflammatory state, which typically develops during aging. CLIP is manifested by elevated levels (2 to 4-fold increase) of inflammatory cytokines in blood serum. In addition to the classical IL-6 and CRP, an increasing number of proinflammatory factors have been identified to be associated with CLIP initiation and development, including (i) cytokines and the corresponding soluble receptors or related molecules (e.g., IL-1, IL-8, IFN, and TNF); (ii) chemokines (e.g., CCL2, CCL3, and CCL5); (iii) adhesion molecules (e.g., VCAM-1, ICAM-1, and E-selectin); and (iv) acute phase reactants of inflammation (e.g., serum amyloid A and fibrinogen), many of which are also associated with SASP (Chen et al., 2019a). The accumulation of lncRNAs and miRNAs in peripheral blood was also found to be associated with immune aging (Table S17 in Supporting Information). Single-cell transcriptome analysis has revealed overexpression of lncRNA NEAT1 and MALAT1 by exhausted T cells and frailty-specific monocytes during aging (Luo et al., 2022b).

In addition to peripheral blood, urine samples are also commonly used for laboratory tests on age-related immune functions. A number of urinary or fecal measurements, including 8-OHdG, cell-free mitochondrial DNA, glucocorticoid metabolites, urinary C-peptide of insulin, thyroid hormones and neopterin, have been shown to be plausible biomarkers for inflammaging (Behringer et al., 2014; Johnston et al., 2021; Cooper et al., 2022; Sacco et al., 2021; Svoboda et al., 2008). EVs from urine and CSF have also been proven insightful for reflecting parental cell properties and remote cell-cell communication status. A study showed that SASP cytokines and immune defense factors could be identified in urinary EVs but not in urinary solutions. SASP-associated factors (e.g., IL-8, IP-10, GRO, and MCP-1) were detected at significantly higher levels in urinary EVs from old individuals (age >60) than young adults (Yeh et al., 2021).

The meninges and CSF surrounding the brain encompass immune cells and systems tightly regulated to respond to brain antigens under pathological conditions (Louveau et al., 2016). Several inflammaging cytokines and metabolic components have been found to change with age (Hu et al., 2019b; Huber et al., 2018; Solvang et al., 2022). Studies have revealed immunological changes in CSF in the pathological events of age-related neurodegenerative disorders such as AD and Lewy body dementia (Gate et al., 2020; Gate et al., 2021). More recently, aging-related single-cell transcriptome analysis has uncovered the upregulation of lipid transport genes (e.g., APOE, APOC2 and APOBR) in non-classical monocytes within the CSF of elderly healthy individuals, which highlights the critical role of lipid metabolism in innate immunity and immunoregulation of the age-related CSF system (Piehl et al., 2022).

Summary and perspectives

In this section, we summarize the biomarkers of immune system aging that are characterized by the degeneration of the thymus, spleen, and lymph nodes, decreased innate and adaptive immune responses, and the excessive inflammatory signaling. The aging of the immune system exhibits changes in function, structure, and cellular/extracellular components. Immunosenescence results in compromised organismal barriers and elevated susceptibility to infection and chronic diseases. Understanding these processes is required for developing more efficient interventions to modulate the senescence process and minimize the negative influence of immunosenescence.

Aging clocks and their applications

Beyond the cellular and organ levels, the heterogeneity of aging is also reflected at the organismal and population levels. Composite measures should be used to evaluate aging at these system levels, but it is not an easy task to decide which combination of biomarkers to use. Using various modeling techniques, especially with the application of artificial intelligence, biomarkers measured in large cohort studies have generated diverse prediction models of biological age, or aging clocks. In this chapter, we introduce aging clocks and their applications based on the nature of biomarkers used in these models, and delve into the challenges and complexities in interpreting these models, which are areas for further innovation.

Phenotypic clocks

The definition of aging phenotypes is broad and can be roughly divided into physical characteristics (e.g., facial, skin, and brain imaging) and functional capacities (e.g., cognitive and psychological evaluation scores) for building phenotypic aging clocks.

PhotoAgeClock is developed to predict chronological age using images of eye corners with deep neural network (DNN) and achieve the mean absolute error (MAE) of 2.3 years (Bobrov et al., 2018). Chen et al. (2015) first find human 3D facial imaging features could be as reliable aging markers, based on which a linear regression model had the MAE of around 6.1 years. Later with a larger cohort, Xia et al. (2020b) build a convolutional neural network (CNN) age predictor and reported an error between chronological/perceived age and predicted age of only ±2.79/2.90 years, and uncovered that heterogeneity of the aging rate reached its peak in middle age. Brain structural MRI data could also be applied to age prediction with the MAE of 4.16 years (Cole et al., 2017). More recently, a 3D CNN model (Yin et al., 2023) has been introduced with a more accurate MAE of 2.3 years. Their model can reveal neurocognitive trajectories in adults with MCI and AD and may serve as early indicators for AD.

Psychological function changes occurring throughout the human lifespan have been overlooked as a phenotypic biomarker. Zhavoronkov et al. (2020) trained a deep learning age predictor based on social and behavioral data from a large-scale study and obtained the MAE of 6.7 years for chronological age prediction, which contained actionable features that can be modified using interventions (Table S18 in Supporting Information).

Epigenomic clocks

In 1942, Waddington introduced the concept of epigenetics by describing the influence of the environment on the developmental process of the embryo(Waddington, 2012). In simple terms, epigenetics refers to all reversible, heritable processes that do not alter the sequence of the genome but are capable of regulating the expression of genes and causing changes in the phenotype. Living organisms are constantly influenced by the environment, which is reflected at the cellular level, and external influences constantly alter the intracellular phenotype. Aging is a process accompanied by the continuous accumulation of epigenetic changes that eventually lead to cellular, tissue, and organ degeneration (López-Otín et al., 2023). In turn, the features of the epiphenomena also change continuously with the aging process (Zhang et al., 2020d). Given that, it becomes possible to predict biological age by measuring the level of apparent epigenetic changes. Such a method of using epigenetic markers to predict biological age is also known as the "epigenetic clock".

Epigenetic modifications include many processes, such as DNA methylation and histone modifications. In this section, we will mainly focus on the changes in DNA methylation in

the aging process. DNA methylation refers to adding methyl groups to the cytosine at the fifth position or the adenine at the sixth position of the DNA. Methyl groups can be actively demethylated by ten-eleven translocation (TET) enzymes, thymine DNA glycosylase (TDG), and base excision repair (BER) (Moore et al., 2013). In eukaryotes, 5-methylcytosine is the most common form of methylation and usually occurs on a cytosine located in front of guanine, hence named "CpG sites" (Smith and Meissner, 2013). In mammals, approximately 75% of CpG sites are methylated (Tost, 2010). Genome-wide DNA methylation is erased and re-established during early embryonic development. Following embryonic implantation, tissue-specific DNA methylation is gradually established and remains stable for a considerable time (Cedar and Bergman, 2012). This suggests that DNA methylation plays a key role in maintaining cell fate determination and programmed embryonic development.

Age-related changes in DNA methylation have been observed in various species and tissues for decades. In 1967, Berdyshev et al. (1967) found that the level of DNA methylation gradually decreased with age in salmon. Later, similar changes in DNA methylation with age were found in mice, rats, and humans (Vanyushin et al., 1973; Wilson and Jones, 1983; Wilson et al., 1987). In rats, DNA methylation decreases in the brain, heart, and spleen, with no changes in the liver and lungs but a slight increase in the kidneys (Vanyushin et al., 1973). In mice, loss of DNA methylation sites was observed at a speed of approximately 4.7×10^4 per month (Wilson et al., 1987). A similar trend can also be observed in experimental primary cell models. DNA methylation is significantly reduced in mouse, hamster, and human fibroblasts. However, DNA methylation is relatively more stable in immortalized cells (Wilson and Jones, 1983). These observations indicate that DNA methylation changes along with the aging process in animal or cell models. A recent study found that some methylation sites that change along with aging are conserved across mammalian species by analyzing changes in methylation levels in more than 59 tissues from 128 mammalian species (Lu et al., 2021). One study on human twins found that epigenetic heterogeneity among twins increases with age (Talens et al., 2012). As we age, epigenetic modifications of DNA methylation tend to occur at some conserved loci (Bollati et al., 2009; Christensen et al., 2009; Rakyan et al., 2010). This suggests that it is possible to discover conserved DNA methylation sites and use them as a model to predict aging. And this epigenetic model is referred to as the DNA methylation-based epigenetic clock.

Early epigenetic clocks were generated from only a few samples and a small number of CpG loci with limited accuracy (Bocklandt et al., 2011; Koch et al., 2012; Koch and Wagner, 2011). Advanced epigenetic clocks emerged in recent years; these clocks incorporated data from a large number of tissues and organs, leading to the discovery of different epigenetic clocks, such as multi-tissue, tissue- or disease-specific, single CpG site prediction models. In 2013, Horvath discovered the first multi-tissue epigenetic clock (Horvath, 2013; Horvath, 2015). The Horvath clock was generated from 8,000 samples from 51 tissues and cell types. A total of 353 CpG sites were found to be strongly associated with aging, with a 0.96 correlation and a 3.6-year error. In the Horvath clock, different tissues show different aging rates. However, limitations are also evident. For example, it cannot be applied to cultured cells (Horvath et al., 2019; Horvath et al., 2018). Therefore, Horvath developed the Skin & Blood Clock, which is based on human fibroblasts, keratinocytes, buccal cells, endothelial cells, lymphoblastoid cells, skin, blood, and saliva samples (Horvath et al., 2018). The Skin & Blood Clock contains 391 CpGs. It can effectively predict the age of *in vitro* cultured neuron, glia, brain, liver, and even bone samples. An epigenetic clock developed by Zhang et al. (2019b) using blood and saliva as samples can also accurately predict age with samples from the mammary gland, liver, fat, and muscle. In addition to multi-tissue clocks, many tissue-specific clocks have been generated. In 2013, Hannum et al. (2013) collected blood samples from 656 individuals ranging in age from 19 to 101 and found 71 CpG loci highly correlated with aging. Weidner et al. (2014) identified 102 CpG loci from blood samples. From 508 human skin samples, Boroni et al. (2020) identified 2,266 CpG loci that are capable of accurately predicting age for both cultured skin cells and primary human skin tissue samples. To improve the accuracy of prediction in the young population, McEwen et al. (2020) developed the Pediatric Buccal Epigenetic Clock (PebBE) using buccal swab samples from the young population aged 0–20 years. To date, most epigenetic clocks are based on Illumina Infinium arrays. Their high price makes them unsuitable for large-scale clinical drug trials. Weidner et al. (2014) developed the 3-CpG clock based on blood samples that contain fewer CpG loci. For forensic purposes, Zbieć-Piekarska et al. (2015) used the less expensive pyrosequencing method to identify the five CpG loci that are most relevant to age (*ELOVL2*, *Clor132*, *TRIM59*, *KLF14*, and *FHL2*) as prediction models. The 5-CpG clock was able to predict age with high accuracy for 300 samples (R(2)=0.94, standard error of estimate=4.5 years). CpG clock based on these five genes was validated in multiple tissues (Cho et al., 2017; Dias et al., 2020; Jung et al., 2019a). It has been shown that even three of the five CpG loci (*ELOVL2*, *FHL2*, and *Clorf132*) are sufficient to predict effective age (Dias et al., 2020).

New training models were developed in order to generate more explainable epigenetic clocks that directly reflect biological phenotypes of the aging process. A series of studies (Horvath et al., 2014; Horvath et al., 2016; Levine et al., 2018) have classified traits such as BMI, obesity, physical fitness, Huntington's disease, sleep, and smoking as pro-

aging factors. Other studies have also linked the epigenetic clock to the mortality risk (Levine et al., 2018; Lu et al., 2019; Zhang et al., 2017c). Zhang et al. (2017c) analyzed DNA methylation in a population group with up to 14 years of follow-up and identified 10 CpG loci that were highly associated with mortality factors. Levine et al. (2018) combined chronological age and multiple clinical factors associated with mortality risk as a biological age score. They identified 513 CpG markers to develop the model PhenoAge. PhenoAge can effectively predict a variety of age-related phenotypes, including all-cause mortality, cancer, health span, and physical function, while Lu et al. (2019) developed GrimAge by combining the effects of smoking and age-related serum protein levels, which can effectively predict mortality risk and multiple age-related diseases.

Animal models play a key role in revealing the mechanisms of epigenetic changes with aging and the effects of intervention modalities on aging. Therefore, an accurate assessment of the epigenetic clock in non-human animal experimental models is needed. Stubbs et al. (2017) collected mouse liver, lung, heart, and brain samples from newborn up to 41 weeks and identified 329 unique CpG loci, thus constructing a model that could accurately assess DNA methylation age with a median absolute error of 3.33 weeks. Meer et al. (2018) collected multiple tissues from mice at 6, 10, 12, 20, and 30 months of age and derived a clock with 435 CpG loci. In addition to the multi-tissue clocks, tissue-specific clocks based on blood (Petkovich et al., 2017) and liver (Koch and Wagner, 2011) have also been developed in mice. DNA methylation clocks have also been established for a variety of model animals, including naked mole rats (Horvath et al., 2022), dogs, wolves (Thompson et al., 2017), humpback whales (Polanowski et al., 2014), and chimpanzees (Guevara et al., 2020).

Since there is a strong correlation between DNA methylation and aging, it is necessary to elucidate the underlying mechanisms of how epigenetic changes and the aging process interact reciprocally. Recently, Kabacik et al. (2022) provided a detailed analysis of the relationship between DNA methylation and the hallmarks of aging in human cells. Using the Skin & Blood Clock to measure the degree of epigenetic aging in primary cells across several human cell types, they found that nutrient sensing, mitochondrial function, stem cell exhaustion, and cell-cell communication affect epigenetic aging, while cell senescence, telomere attrition, and genomic instability do not. Lu et al. (2021) obtained an unprecedentedly large dataset from 121 eutherian species and 7 marsupial species to characterize senescence-associated CpG loci. They found that genes close to these senescence-associated CpG loci are also involved in developmental processes, such as HOX and PAX. This suggests that development and aging may share important yet unrevealed mechanisms throughout the life course of the organism.

Undoubtedly, the aging process is accompanied by epigenetic changes in DNA methylation, but whether and how epigenetic interventions affect the aging process remain unclear. Cellular reprogramming changes the apparent state of the entire cell. Ocampo et al. (2016) reported that short-term reprogramming cycles delay aging-related alterations, remodel the epigenetic status, and enhance regeneration in mice. This phenotypic remodeling is a shift from an aged state to a youthful state (Lu et al., 2020). Manipulating some key DNA methylation clock-related genes can also affect the aging process. For example, the *ELVOL2* gene, which functions as a long-chain fatty acid elongation catalyst, has a strong correlation with aging among DNA methylation clock-related genes (Zbieć-Piekarska et al., 2015). Recently, Li et al. (2022h) reported that DNA methylation caused impairment of *ELVOL2*, leading to lipid synthesis dysfunction, which in turn increased ER stress and mitochondrial dysfunction, ultimately leading to the appearance of aging-related symptoms. In contrast, back-compensation of *ELVOL2* restores mitochondrial function and attenuates the onset of age-related macular degeneration. These findings demonstrate that interventions on either the overall appearance or key epigenetic factors associated with aging can be used as interventions for aging (Figure 27). These studies indicate that DNA methylation, an important factor of the epigenetic clock, could not only be a marker of the aging process but also a direct regulator of it.

Transcriptomic clocks

Using gene expression levels to build aging clocks can link the aging process more directly to gene functions, making these clocks more interpretable. Transcriptomic data from multiple organs have been used to build aging clocks. Peters et al. (2015) performed a linear regression on peripheral blood mononuclear cell gene expression array data in several large cohorts, and obtained a model with the MAE of 7.8 years. This transcriptomic clock was found to have associations with some biomarkers and risk factors, including smoking status. In 2018, Fleischer et al. (2018) derived a clock using human dermal fibroblast RNA-seq data with a Median Absolute Error (MedAE) of 4.0 years. In order to reduce the noisiness of transcriptomic data, this study combined multiple linear discriminant analysis classifiers which could also be applied in detecting progeria samples. In the same year, Mamoshina et al. (2018) tested several supervised machine learning models, including neural networks, on human skeletal muscle transcriptome data for age prediction and obtained the MAE of 6.24 years.

Previous work mostly suffered from considerable variation between transcriptomic data, while Meyer and Schumacher (2021) demonstrated that a simple binarization and relative

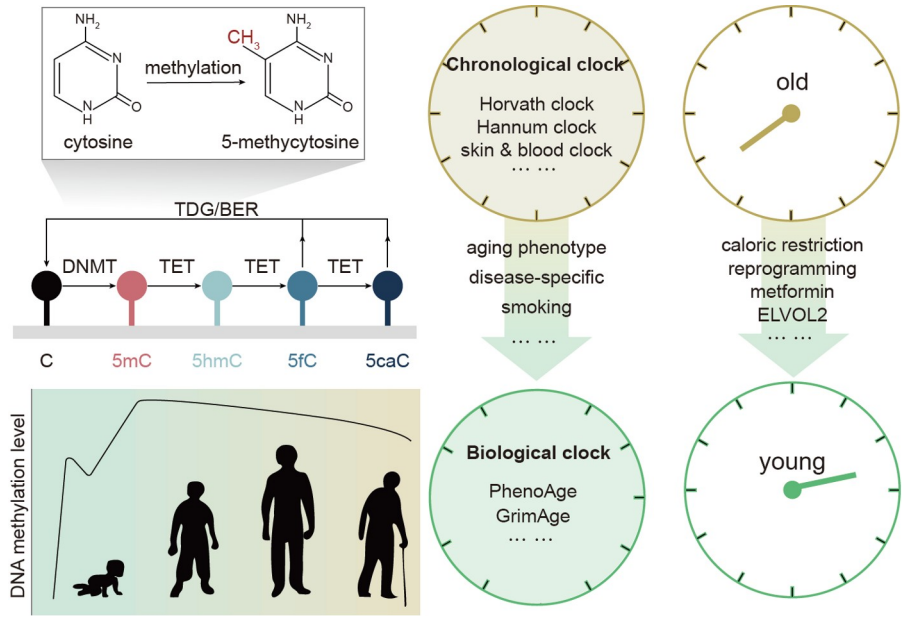

Figure 27 The DNA methylation clock theory of aging. In mammal, DNA can undergo reversible methylation or demethylation processes. Aging is accompanied by global DNA hypomethylation. Based on chronological age or biological age, different DNA methylation clocks are established by analyzing the methylation changes with age. These clocks are further used to predict the rate of aging. The methylation sites associated with aging can be altered by methods such as reprogramming in order to slow down or reverse the aging process.

age scaling of transcriptomic data to define a gene set could denoise the data and improve age prediction in *C. elegans*. Additionally, they demonstrated, using human fibroblast data from Fleischer et al. (2018) that an elastic net-based clock derived from binarized transcriptomic data improved age prediction to the MAE of 6.63 years. Holzscheck et al. (2021) constructed an artificial deep neural network according to well-described biological pathways, allowing the prediction of age from human epidermal skin transcriptome data with a MeAE of 4.71 years. Their model increased the interpretability of aging clocks and responded in expected ways to known perturbations of biological age in silico. Moreover, Xia et al. (2020b) predicted age with PBMC ribominus RNA-seq data using linear partial least squares regression (PLSR) which had the MAE of 5.68 years.

The recent development of the scRNA-seq method enables the characterization of transcriptomic data at the individual cell level and provides a new method for constructing aging clocks. Lu et al. (2022) developed a mixed-effect elastic net model to predict the age of individual $CD8^+$ T cells from three cohorts and showed a strong correlation with chronological age in cross-validation. Buckley et al. (2023) trained cell-type-specific aging clocks using single-cell transcriptomes from mice subventricular zone neurogenic region with high resolution and reached MAE ranging from 2.3 to 4.6 months across all cell types. Additionally, they reported that heterochronic parabiosis and exercise of these two interventions could reverse aging clocks in neurogenic regions, indicating the ability of their model to quantify transcriptomic rejuvenation (Table S19 in Supporting Information).

Proteomic clocks

Compared with DNA methylation or transcriptomic studies, the proteomic approach has been well recognized as a powerful technique which provides more insights into phenotypic traits changes across human aging (Bathke et al., 2019; Diz et al., 2012; Haider and Pal, 2013). In different biological fluids (i.e., plasma (Lehallier et al., 2019; Tanaka et al., 2018), serum (Di Narzo et al., 2017), saliva (Wang et al., 2018b), urine (Bakun et al., 2014), cerebrospinal fluid (Baird et al., 2012; Zhang et al., 2005a)) and tissues (i.e., skeletal muscle (Staunton et al., 2012), liver (Heinze et al., 2018)), thousands of proteins which dynamically vary along with age may directly regulate both of age-related health and disease biology. It is noteworthy that the generation of multiple proteomic datasets of human or animal lifespan during the past few years may hold a strong translational potential in aging research.

Since Zhang et al. (2005a) carried out the pioneering study to characterize proteomic signature in the cerebrospinal fluid of elderly and young individuals, increasing studies have been performed to map proteome alterations during aging. In the literature research led by Johnson et al. (2020), the authors investigated 36 proteomic analyses performed in different matrices. The results showed that a total of 1,128 proteins were reported by at least two or more analyses. Among them, a set of 32 proteins changing with age in five or more studies might show strong evidence of age asso-

ciation. As indicated in the enrichment analysis, the most common proteins were mainly involved in immune signaling, extracellular matrix organization, complement and coagulation cascades and metabolic signaling. Notably, in an analogous review conducted by Moaddel et al. (2021), the authors also summarized the findings of aging proteomes in multiple matrices and different species from 33 independent studies. It turned out that a list of 232 proteins was identified to be significantly associated with age across at least two studies and matrices. It is noteworthy that the most remarkable pathways with significant connection with age were essentially in accordance with those discovered in the previous review. Both two studies proposed important aging protein subsets in the plasma, which is an ideal source for aging biomarkers discovery largely owing to its high sensitivity to organ or cell function. Nevertheless, the robustness, reproducibility, as well as selectivity and sensitivity of these proteins remain to be validated. Besides, substantial biological work is still in great need to comprehensively elucidate the underlying mechanisms of aging.

On the basis of the age-associated proteomic datasets, a couple of aging clocks have been constructed. Tanaka et al. (2018) developed an aging clock based on circulating plasma proteins highly relevant to chronological age. The proteomic signature of 76 proteins was eventually identified to be possessed with favorable age prediction. Among them, Growth differentiation factor 15 (GDF15) demonstrated the strongest age association. Johnson et al. (2020) also established several novel proteomic clocks from 2019 to 2021. The first clock comprised of protein lists determined through literature searching among multiple analyses, which is capable of accurately predicting human age in an independent patient cohort derived from 3,301 subjects. Based on the first study, the second clock composing 491 proteins was developed by further data mining of the previous protein panel. The new clock denotes high-quality aging intervention protein targets with ultra-predictability (Pearson correlation $\geq$0.9) in 3 independent human cohorts (Johnson et al., 2021; Lehallier et al., 2020). There is a notable point that fewer protein inputs with more significant changes with age may provide the clock model with better predictability. It is encouraging that the established proteomic clocks settled for capturing the complex organismal mechanisms may have potential applications beyond measuring accelerated biological aging. Mostly, they can be used for enhancing the surveillance of multiple age-related diseases (Grassmann et al., 2018; Kim et al., 2021) and promoting the development of aging intervention drug candidates (Fahy et al., 2019; Schultz et al., 2020). So far, with close relevance to aging in liver and kidney pathophysiology, immune dysfunction, metabolic disorder and neurodegenerative diseases, several multiplex protein panels have revealed great application value in the clinical settings (Ganz et al., 2016; Kearney et al., 2018; Rutledge et al., 2022; Williams et al., 2019).

In spite of many remarkable achievements made in proteome-based aging clocks, there remain a few limitations. Firstly, the integrated proteomic datasets used for model development were usually generated from different proteomic platforms (Johnson et al., 2020; Moaddel et al., 2021). The measurements mainly include mass spectrometry (MS)-based platform, SomaLogic aptamer (SOMAscan)-based platform, and proximity extension assay (PEA, O-Link). Apart from the MS-based approach, the other two technologies are not possible to be tailored for quantifying the entire proteome. Thus, in-depth unbiased MS-based analysis pipelines (i.e., data-independent acquisition (DIA) methods (Bilbao et al., 2015)) which allow for reliable protein detection and quantification in large-scale cohorts are urgently needed. Another advantage of MS-based technology is that it may precisely identify the post-translational modifications of proteins, which also show significant alterations during the aging process (Baldensperger et al., 2020; Krištić et al., 2014; Meyer et al., 2018; Santos and Lindner, 2017). Secondly, besides chronological age, the clocks need to be further improved through incorporating other specific age-related molecular functions (Rutledge et al., 2022). With more inclusive insights into the biological features throughout aging progression, this will definitely enhance the accuracy and predictability of the existing models to a large extent. An interesting example is that in a recent study, Sayed et al. (2021) developed an inflammatory aging clock by tracking the patterns of predominant phenotypes of systemic inflammation like multimorbidity and immunosenescence. And they eventually proposed CXCL9 as a key biomarker for the early detection of immune system changes in the aging process. Thirdly, future well-constructed multi-center proteomic studies in aging research are required for more model validation to avoid any sex- or race-based differences, as well as inter- or intra-individual variabilities (Rivero-Segura et al., 2020). Lastly, it should be clearly realized that the widespread application of the models can be limited with relatively fixed protein panels as single indicators. On one hand, protein markers need to be further evaluated and continually updated in consideration of different stages of lifespan or aging disease progression. On the other hand, the integration of proteins with various signatures derived from other omics layers will create new possibilities for exploring to what extent the multi-omics markers can shed light on effective biomarkers or targets relevant to aging (Rivero-Segura et al., 2020; Rutledge et al., 2022).

Despite the gap between lab discovery and clinical application, protein-based aging clocks have distinctive advantages as they exert direct effects on multiple biological processes and form the vast majority of aging intervention drug targets. Committed efforts in the development and

improvement of realistic proteomic clocks will pave the way of outlining the roadmap to effective aging-associated interventions in the clinical practice.

Metabolomic clocks

Metabolism has strong associations with aging and the abundances of some metabolites are shown to be correlated with age (Chaleckis et al., 2016; Panyard et al., 2022; Yu et al., 2012), suggesting the potential to use these components to assess biological age. The first metabolomic age predictor was established based on the large-scale urine metabolite profiling using proton nuclear magnetic resonance (1H-NMR) spectroscopy, named the metabolic score, by applying a non-linear regression method (Table S20 in Supporting Information) (Hertel et al., 2016). The metabolic score was validated in two independent cohorts and was prognostic for weight loss in individuals who received bariatric surgery. Another study trained a metabolic age clock model based on the blood metabolome data from a larger cohort (van den Akker et al., 2020). Their results suggested that the blood metabolic age was associated with the risk of cardiovascular disease, mortality, and functionality in individuals with advanced age.

Robinson et al. (2020) measured metabolites in both urine and serum samples generated from different platforms, based on which the metabolic clock models were built. This study also demonstrated that the prediction performance of metabolic clocks was relatively stable across populations from different areas. Efforts have also been made to compare the performance of age prediction powers of metabolites from blood and urine samples. Rist et al. (2017) collected the metabolomic data from 301 healthy individuals aged 18–80 years, which were generated from multiple platforms. Metabolic clocks were built based on the blood and urine datasets both separately and in combined using different algorithms. The authors found that the overall performance of blood metabolic age predictors was superior to those based on urine metabolic data. A recent investigative study with a relatively small sample size shows the potential of CSF metabolites to predict age (Hwangbo et al., 2022). The authors subjected the prediction model based on healthy individuals to age-match patients with AD and Parkinson's Disease, which showed more significant prediction errors compared to the control, indicating that the association between the CSF metabolome and age differs with the neurodegenerative diseases.

The metabolic data also shows the ability to predict age-related diseases and mortality (Deelen et al., 2019; Fischer et al., 2014), which further underscores the potential utility of the metabolic clock in health monitoring. Future investigation on the development of stable targeted metabolic analysis methods on those metabolites being identified to be highly correlated with age in different studies might further promote the application of metabolomic clock into clinical use.

Circadian clocks

Nearly all physiological and behavioral activities, including sleep-wake cycles, body temperature, energy metabolism, hormone secretion and locomotor activity, are oscillated to be near 24 hours by an internal self-sustained circadian clock in the vast majority of living organisms (Panda et al., 2002). In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus serves as a master pacemaker that coordinates the endogenous circadian clocks across the body to the external environment (Hastings et al., 2018). Light is a predominant environmental cue, called *Zeitgeber*, that entrains the central clock in the SCN; SCN then synchronizes subsidiary oscillators in the peripheral via systemic cues like feeding activity, body temperature and hormones (Dibner et al., 2010; Mohawk et al., 2012). At the molecular level, the circadian oscillator is composed of a transcription- and translation-based negative feedback loop, wherein a heterodimer of CLOCK and BMAL1 promotes the transcription of Period (PER) and Cryptochrome (CRY) (Bunger et al., 2000; DeBruyne et al., 2007; Gekakis et al., 1998; Kume et al., 1999; Reick et al., 2001; Takahashi et al., 2008; van der Horst et al., 1999). PER and CRY dimerize to drive their own oscillation and rhythmic expression of genes involved in key cellular functions (Patke et al., 2020; Takahashi, 2017).

Aging is a gradual decline of physiological function, and the circadian clock is not an exempt. Disruption of circadian rhythmicity often leads to diseases, including metabolic disorders, diabetes, cardiovascular diseases and cancers (Acosta-Rodríguez et al., 2021; Bass and Lazar, 2016; Hood and Amir, 2017; Tabibzadeh, 2021). In humans, the most reliable observation of circadian disruption is the wakening activity and retiring to bed in elderlies which show a tendency to be earlier compared with younger adults (morning chronotype) (Carrier et al., 1997; Horne and Ostberg, 1976; Roenneberg et al., 2007). This is attributable to the loss of rhythmicity of biosynthesis and release of hormones, which regulate feeding and sleep activity, and oscillated the expression of clock genes in peripheral tissues (Gamble et al., 2014). Among these hormones, melatonin release is under the control of the SCN and plays an important role in regulating sleep onsets and core body temperature. Melatonin rhythmicity reduction is observed in old individuals (Kennaway et al., 1999; Reiter et al., 1980; Touitou et al., 1981; Zhao et al., 2002). The phase advance of melatonin rhythms also contributes to the earlier chronotype of the circadian sleep-wake cycle during aging. Their capacity to adapt to light/dark schedule changes (shift work or jet lag) is also impaired in old individuals (Monk et al., 2000; Monk et al., 1993). Analysis of the expression pattern of core clock genes in the orbitofrontal cortex reveals

dampened oscillation of *PER1*, *PER2* and *CRY1* in elderlies (Chen et al., 2016a). Aging also downregulates *BMAL1* transcripts in the peripheral blood cells in healthy women (Ando et al., 2010). Age-associated changes in core clock gene expression are also observed in nonhuman species (Bonaconsa et al., 2014; Kolker et al., 2003; Kolker et al., 2004; Yamazaki et al., 2002).

How aging perturbs the function of internal circadian clocks remains an open question. Transplant experiments suggest that the SCN is a critical regulator of diseases, aging and longevity (Li and Satinoff, 1998; Swaab et al., 1985). Studies have found a reduction in SCN volume in aged people compared with younger adults (Zhou and Swaab, 1999). The expression of two key neuropeptides, arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP), is consistently reduced in the SCN of aged humans and rodents (Cayetanot et al., 2005; Chee et al., 1988; Hofman and Swaab, 1994; Roozendaal et al., 1987; Zhou et al., 1995). The e*x vivo* bioluminescent imaging of cultured SCN slices of PER2::luciferase knock-in (PER2::LUC) mice reveals the reduced amplitude of PER2::LUC rhythms and a lengthening of the circadian period in aged mice under constant darkness condition (Nakamura et al., 2015). The most reliable change observed in aged SCN is loss of coherence in SCN neuronal network activity in old animals (Nakamura et al., 2011; Watanabe et al., 1995), which may contribute to the deterioration of daily rhythms of physiology and behavior. Aging affects the synchrony between the central pacemaker and peripheral oscillators (Sellix et al., 2012). Following 6-h phase advance of the light/dark schedule, the peripheral clocks of old mice showed slower rates of re-entrainment. By contrast, a more rapid SCN response is observed in aged mice compared to younger mice, suggesting a major consequence of aging is weakened control by the master clock over peripheral oscillators. Cellular senescence is a biomarker of aging and contributes to age-related diseases (Cai et al., 2022d; Guo et al., 2022). BMAL1 and CLOCK facilitate heterochromatin stabilization and prevent the senescence of mesenchymal stem cells (Liang et al., 2022; Liang et al., 2021). A recent study reveals that exercise can restore dysregulated circadian machinery in different cell types across tissues in aged mice (Sun et al., 2023). Particularly, the transcription of *Bmal1* is downregulated in various endothelial cells during aging, which was rescued by prolonged exercise, while overexpression of *Bmal1* delays the senescence of cardiac endothelial cells. Thus, small molecules that activate endogenous clocks are potential drugs for delaying age-related diseases (Chen et al., 2018b).

Chronotype varies owing to individual genetic predisposition, age and sex. Integrating the chronotype, circadian drug pharmacokinetics, age and sex could provide optimal interventions to promote healthy aging. Additionally, manipulating feeding time (time-restricted feeding) in the attempt to modulate the circadian clock shows powerful effects on mitigating age-related chronic diseases, though specific mechanisms are still far from being fully understood. Whether feeding behavior may serve as a potential biomarker of aging requires further investigation.

Longevity clocks

The heterogeneity of aging leads to differences in life expectancy and health state in different individuals of the same age. Almost all people want to know how many years of "good health" they have left. With this in mind, researchers have proposed the concept of biological age (Andrews et al., 2017), which is imperfectly associated with chronological age but can indicate individual health and longevity. If a person has a biological age younger than their chronological age, they are more likely to exhibit good health; conversely, those with a biological age older than their chronological age are more likely to show poor physical health or underlying chronic disease. Extensive efforts have been made over several decades to identify biomarkers and develop corresponding biological clocks to predict biological age, thus representing individual health and longevity potential.

Prediction of longevity by biological clocks

One challenge in establishing an accurate biological clock is to identify biomarkers/indices associated with biological age. At present, most biological clocks for health and longevity prediction are derivations of the aging clock, as most biological age related biomarkers/indices vary roughly linearly with chronological age. For example, frailty, measured by the frailty index (FI), reflects a state of increased risk of age-related negative health outcomes (e.g., physical disability, cognitive decline, and hospitalization). Several studies have shown that the FI-based biological clock can be utilized as a predictor of health and lifespan (Kim et al., 2017; Schultz et al., 2020). Research has also shown that the number of senescent cells (which accumulate with age and can be marked by $p16^{INK4a}$) in some tissues can reflect human biological age, with fewer $p16^{INK4a}$-positive cells representing a propensity for longevity in middle-aged individuals (Waaijer et al., 2012). Furthermore, various studies have suggested that blood biochemical markers (e.g., albumin and glucose) and cell counts can be used to construct a biological clock and quantify biological age (Putin et al., 2016; Pyrkov et al., 2021). Higher serum dehydroepiandrosterone sulfate (DHRAS) levels, which decrease with age, are considered a predictor of longevity in men (Enomoto et al., 2008). At the molecular level, TL (i.e., telomere length) declines progressively with age, and is thus considered a biomarker of chronological age (López-Otín et al., 2013). Coupled with evidence linking shorter TL with increased incidence of age-related diseases (e.g., cardiovascular disease, cancer) and

limited life expectancy (Barthel et al., 2017; Haycock et al., 2014; Heidinger et al., 2012), TL has been used to construct a biological clock to estimate the biological age and to predict longevity (Vaiserman and Krasnienkov, 2020; Zhang et al., 2014). Importantly, advances in multi-omics technologies in recent years have increased potential molecular biomarkers associated with chronological age as well as biological age and longevity. As a well-known epigenetic modification, DNA methylation has been widely used in the construction of aging clocks, represented by the epigenetic clocks of Horvath and Hannum based on tens to hundreds of age-related methylation sites (Hannum et al., 2013; Horvath, 2013). Studies have also suggested that epigenetic age is an indicator of biological age or a potential predictor of health and longevity, with epigenetic age (or biological age) minus chronological age (Xiao et al., 2018a; Xiao et al., 2019; Zhang et al., 2017c). Likewise, informative biomarkers extracted from transcriptomes, proteomes, and metabolomes have been applied to construct various aging clocks that can track individual biological age and assess longevity potential (Fleischer et al., 2018; Johnson et al., 2019; Tanaka et al., 2020). In addition, several attempts have been made to explore health- and longevity-related biomarkers based on the gut microbiota, given its close relationship with aging and age-related diseases (Claesson et al., 2012; Ghosh et al., 2022b). For instance, *Bacteroides* depletion is an important characteristic of healthy aging and a predictor of extended survival in older individuals (Wilmanski et al., 2021). Thus, these studies suggest that different types of biological clocks based on various molecular signatures have the potential to predict human longevity.

Training new efficient biological (or longevity) clocks: insights from longevous cohorts

Despite considerable advances in the construction of biological clocks, the biological functions of most current biomarkers in healthy human aging and longevity are poorly understood. The accuracy of biological clocks in predicting individual longevity potential also needs to be improved. As such, researchers have been driven to screen more representative causal and functional biomarkers to optimize longevity prediction and intervention guidance. Longevous people (e.g., centenarians) serve as paradigms of successful and extraordinary aging, living much longer than "normal" and managing to delay or even escape common age-related diseases, such as cardiovascular disease, neurodegenerative disease, and cancer (Atzmon et al., 2004; Engberg et al., 2009; Hitt et al., 1999). Therefore, long-lived people present an opportunity to search for reliable and causal molecular/biological indicators of biological age, health, and longevity potential.

Initial studies focused on identifying longevity-associated genetic mutations in long-lived cohorts that could serve as potential indicators of longevity. For example, several variations within the *FOXO3A* gene (e.g., rs13217795 (C), rs2802292 (G)) are highly prevalent in longevous cohorts and increase longevity potential by decreasing IGF-1 signaling (Willcox et al., 2008); and *vice versa*, some long-lived individuals display a depletion in the AD-susceptibility factor ε4 allele (i.e., rs7412 (C) + rs429358 (C)) of the *APOE* gene, thus achieving longevity by decreasing the risk of AD (Sebastiani et al., 2019). These findings indicate that genetic variations associated with longevity and age-related diseases may facilitate the construction of a biological clock that predicts health and longevity. Consistent with this, a recent predictive model based on longevity- and disease-related genetic markers successfully partitioned components of longevity and non-longevity (AUC=0.767) and predicted lifespan (explaining 8% of the variance in lifespan), with predictive ability improving (AUC=0.86 for longevity clas-

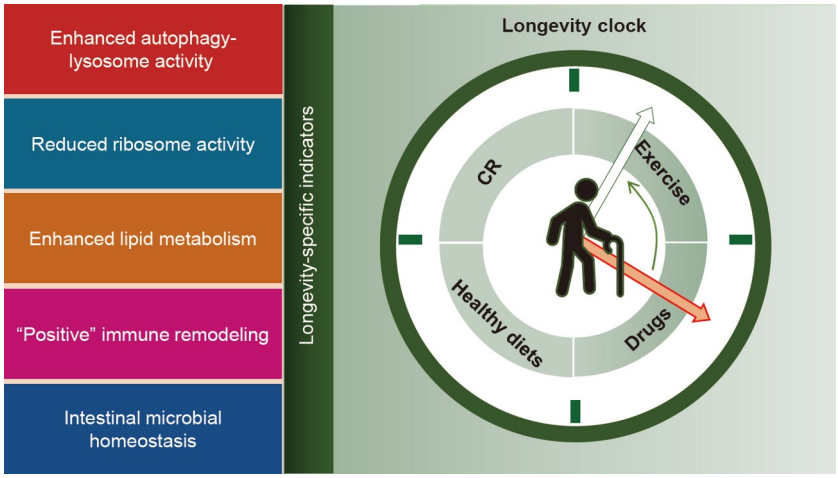

Figure 28 The clock that regulates longevity. Longevity indicators identified from longevous cohorts to guide construction of new "longevity clocks", and their application in assessing rejuvenation interventions (e.g., CR) may rewind the clock. Abbreviations: CR, caloric restriction.

sification, explaining 19.8% of the variance in lifespan) when information on disease status and lifestyles was included (Liu et al., 2021b).

Given the limited heritability (15%–35%) of longevity (Gögele et al., 2011), extensive work has attempted to characterize potential biomarkers (or indicators) of healthy human aging and longevity in long-lived cohorts at other molecular levels, including gene expression, protein expression, DNA methylation, and metabolism (Figure 28). Accumulating evidence suggests that the longevity advantage of centenarians is reflected in their younger epigenetic age (i.e., biological age) relative to their chronological age (Daunay et al., 2022). Transcriptomic evidence from long-lived individuals and functional experiments has also shown that increased autophagy-lysosome activity and decreased ribosome activity are two important indicators of healthy aging and longevity in humans (Xiao et al., 2018b; Xiao et al., 2022). Long-lived individuals also tend to exhibit a favorable lipid profile, which may be partly associated with the enhanced lipid metabolism activity found in long-lived individuals (Barzilai et al., 2003; Gonzalez-Covarrubias et al., 2013; Li et al., 2022a). A serum proteome study of centenarian families also identified several proteins associated with abundance and human survival (Sebastiani et al., 2021). In addition, progressive decline in immune function with advanced age is a major risk factor of age-related diseases (e.g., cancer and infection) in older individuals. A recent single-cell RNA sequencing study of centenarian families revealed a "positive" immune remodeling process in centenarians, marked by increased numbers and functional reinforcement of cytotoxic T cells that kill cancer cells (Dong et al., 2022; Weigelin et al., 2021). Aging is also characterized by persistent low-grade systematic inflammation. A recent study suggests that long-lived individuals however have relatively lower inflammation levels (e.g., *IL6*, *TNFα*), which likely result from the upregulation of activating transcription factor 7 (*ATF7*) (Huang et al., 2022d). In addition, several studies on the gut microbiome have revealed unique signatures in centenarians (Biagi et al., 2016; Kong et al., 2016; Sato et al., 2021). For example, some centenarians harbor abundant strains of Odoribacteraceae that can generate unique secondary bile acids and protect against enteropathogenic infection (Sato et al., 2021). Taken together, these studies suggest that longevous cohorts can facilitate the identification of longevity-specific indicators and/or biomarkers associated with healthy human aging, but non-linearly associated with age, for the construction of more efficient longevity clocks.

Rewinding the biological (or longevity) clocks by interventions

Unlike chronological age, individual biological age can be delayed, or healthspan/lifespan can be extended, by intervention strategies such as healthy lifestyle, dietary therapy, and drug therapy (Figure 28). For instance, moderate exercise and excessive sedentary behavior can markedly improve and impair physical health, respectively. Several studies have shown that people who exercise regularly have a younger biological age compared to those who are sedentary (Rea, 2017; Sellami et al., 2021). Likewise, a healthy diet is also beneficial in reducing chronic diseases and lowering biological age (Kresovich et al., 2022). Evidence has also shown that multiple longevity-promoting strategies can delay or reverse the biological clock and may serve as good indicators for assessing intervention efficacy. Among them, caloric restriction is one of the most natural and effective dietary therapies for improving health and promoting longevity (Huang et al., 2022e). Results from various studies have shown that caloric restriction can delay age-related methylation drift in diverse species (e.g., mice and rhesus monkeys), resulting in younger DNA methylation-based biological age (Maegawa et al., 2017; Petkovich et al., 2017). Dietary rapamycin, another prevalent intervention strategy, can promote longevity by inhibiting the mTOR signaling pathway and reducing biological age (Wang et al., 2017b). Similar results have been observed in interventions with other longevity drugs, such as metformin (Li et al., 2022c). These findings indicate that biological clocks can help evaluate different longevity interventions, in part by resolving issues that are time consuming and costly.

Summary and perspectives

In conclusion, extensive efforts over several decades have successfully constructed multiple types of biological clocks (e.g., epigenetic and metabolic clocks) that can be used to predict a person's health and longevity potential. Evidence also suggests that these clocks can be utilized to assess the efficacy of various longevity interventions. However, despite tremendous advances, most current biological age related biomarkers have been identified based on the principle of chronological age correlation. In other words, many biological age related biomarkers may be a consequence of aging rather than a cause, or their biological functions have not yet been elucidated, resulting in limited accuracy and sensitivity of the corresponding biological clocks as significant predictors of longevity. Therefore, novel longevity- and health-specific molecular and biological biomarkers are required, and their application to non-linear systems with deep learning methods may facilitate the construction of more efficient longevity clocks.

Aging clocks at single-cell resolution

With the development and innovation of the latest high-throughput omics technology of single-cell sequencing, a lot of studies are devoted to age-related diseases and extending a

healthy lifespan (Leng and Pawelec, 2022; Tabula Muris, 2020; Wen and Tang, 2022; Wu et al., 2023; Zhang et al., 2022a; Zhou et al., 2022). Numerous biomarkers have been proposed to understand and measure these processes. Aging clocks, estimators of chronological age, can integrate and predict various measures of biological aging utilizing multiple omics and phenotypic data across tissues, aiming to evaluate or predict the biological age and aging speed of the body at molecular resolution (Bell et al., 2019; Palmer, 2022).

In the past decade, a series of aging clocks based on epigenomics (Bocklandt et al., 2011; Hannum et al., 2013; Horvath, 2013; Levine et al., 2018; Weidner et al., 2014), transcriptomics (Fleischer et al., 2018; Holzscheck et al., 2021; Meyer and Schumacher, 2021; Peters et al., 2015), proteomics (Lehallier et al., 2019; Tanaka et al., 2020; Tanaka et al., 2018), and metabolomics (Robinson et al., 2020; van den Akker et al., 2020) data of bulk samples have been established by machine learning methods and achieved meaningful results in animal models and human cohort studies. One of these aging clocks with excellent properties is the epigenetic clock, where cytosine methylation in CpG dinucleotides (CpG methylation) changes during aging that can be detected by microarray, genome-wide, or targeted detected by sequencing (Bocklandt et al., 2011; Horvath, 2013). Despite the strong complexity and heterogeneity of aging between and within individuals, epigenetic clocks accurately predict tissue age across a broad range of human tissue types, suggesting shared cellular senescence signals across different cell types (Horvath et al., 2018). However, bulk sequencing data could not provide insights into the molecular changes that occur in specific cell types.

It is worth exploring how the phenotypic characteristics of individual or tissue aging are affected by changes in cell type composition or cell age, and how to quantitatively assess the contribution of molecular level changes in specific cell types to individual or tissue aging, which would be a challenging but promising research field (Figure 29A). Limited by the development of single-cell omics sequencing technology, the current aging clock at single-cell resolution mainly focuses on the use of single-cell epigenome and single-cell transcriptome (Figure 29B) (Lähnemann et al., 2020). A common approach is to use machine learning methods to predict the biological age of individual cells (Figure 29C). With the development of single-cell multi-omics sequencing technology, the new aging clock is expected to achieve more precise performance. It is believed that aging clocks based on single-cell omics will completely revolutionize our understanding of aging clocks in the near future (Table S21 in Supporting Information).

Aging clocks based on single-cell epigenome

Epigenetic alteration is an important hallmark of aging, especially at CpG sites. Over the past decade, several aging clocks have been developed to profile epigenetic age (Belsky et al., 2022; Horvath et al., 2018; Lin et al., 2016; McEwen et al., 2020; Shireby et al., 2020; Vidal-Bralo et al., 2016; Zhang et al., 2019b), which suggests the homogeneity of underlying cellular senescence and masks the epigenetic heterogeneity between individual cells. Epigenetic clocks have revolutionized the study of aging and a series of studies in peripheral blood or saliva hint at its broad application prospects (Bocklandt et al., 2011; Hannum et al., 2013; Lin et al., 2016; Vidal-Bralo et al., 2016; Zhang et al., 2017c). Advances in epigenome sequencing methods the enable assessment of single-cell epigenetic profiles, including single-cell reduced-representation bisulfite sequencing (scRRBS) (Guo et al., 2013), single-cell bisulfite sequencing (scBS-seq/scWGBS) (Smallwood et al., 2014), single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) (Buenrostro et al., 2015), single-cell chromatin immunoprecipitation sequencing (scChIP-seq) (Rotem et al., 2015), and single-cell extended-representation bisulfite sequencing (scXRBS) (Shareef et al., 2021) and so on. The application of these sequencing technologies in the field of aging research has greatly advanced our understanding of molecular changes in aging at an unprecedented single-cell resolution, which puts forward higher requirements and new challenges for the aging clock.

Most previous research on epigenetic clocks of bulk samples has focused on the level of DNA methylation, such as the Hannum clock (71-CpG clock) (Hannum et al., 2013), Horvath clock (353-CpG clock) (Horvath, 2013), DmAM (8-CpG clock) (Vidal-Bralo et al., 2016) and DunedinPACE (Belsky et al., 2022), and the accurate prediction and performance of the DNA methylation clock further motivate the development of more aging clocks at the single-cell level. Recently, Trapp et al. (2021) developed a novel single-cell epigenetic clock framework scAge, which provided a solution for the complex challenges of sparse and binarized methylation profiles in single cells. The scAge is the first epigenetic clock to characterize age-associated CpG methylation changes in single cells of mammals relying on bulk DNA methylation data for calibration, which recapitulates the chronological age of hepatocytes, muscle stem cells, and embryonic stem cells. In addition, two additional models, PRC2 clock (Moqri et al., 2022) and Tarkhov clock (Tarkhov et al., 2022), would be officially released soon as predictors of cell age leveraging single-cell DNA methylation data of mice, which would continue to enrich aging prediction methods at single-cell resolution. It is worth mentioning that all these methods also use bulk samples as a reference to ensure the robustness of the algorithm or calculation results. However, the solution to predict chronological age using single-cell methylation data from blood samples remains to explore, which would greatly advance their potential value as

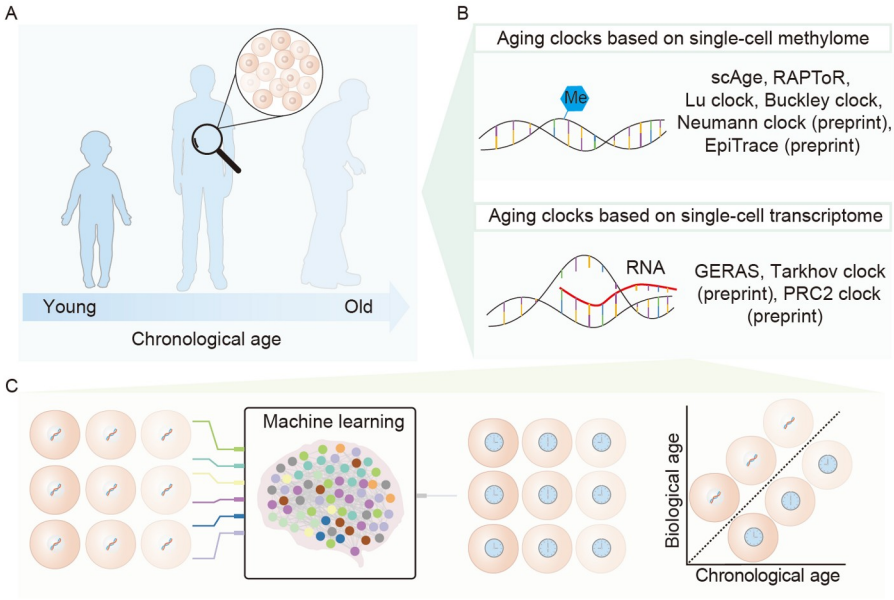

Figure 29 Aging clocks at single-cell resolution. A, Cellular senescence accompanies the whole process of individual aging. B, Current aging clocks based on single-cell omics data. C, A general flow chart for biological age prediction based on single-cell omics data.

clinically applicable biomarkers.

In the field of aging clocks, methylation clocks have been reported extensively, but the mysteries of chromatin structure, conformation, and other changes at the epigenome level during aging remain unclear. Chromatin accessibility is an important indicator for assessing the degree of aging based on the "loss of heterochromatin" theory (Liu et al., 2022a; Pal and Tyler, 2016; Wang et al., 2022a; Wu et al., 2018; Zhang et al., 2015; Zhang et al., 2020d; Zhang et al., 2022e). Some novel chromatin accessibility-based aging clocks using bulk samples remain to be developed (Rechsteiner et al., 2022). The rapid progress of scATAC-seq sequencing technology has opened up a new era for the aging clock based on single-cell big data. Recently, a promising method, EpiTrace, was actively tried on scATAC-seq data of human adult $CD34^+$ hematopoietic stem cells to determine cell age and perform lineage tracing (Rechsteiner et al., 2022). Current evidence suggests that methylation clocks hold the most promise for development and application.

Aging clocks based on single-cell transcriptome

Changes in RNA expression levels both in single cells or bulk tissues during aging have been widely reported in various common model organisms across tissues, and a large number of data resources and databases have been developed (Aging Atlas, 2021; Kang et al., 2022; Ma et al., 2020; Ma et al., 2022; Sun et al., 2023; Tabula Muris, 2020; Yan et al., 2023). The association between genes with aging makes it possible to predict the rate of aging or lifespan based on transcriptome data. There are already several aging transcriptome clocks that leverage bulk RNA-seq data to predict individual aging based on linear regression or machine learning methods (Fleischer et al., 2018; Holzscheck et al., 2021; Meyer and Schumacher, 2021; Peters et al., 2015). Single-cell resolution data could provide new perspectives on aging clocks.

Machine learning-based methods have shown robust capabilities in predicting cell age and are gaining popularity (Bulteau and Francesconi, 2022; Lu et al., 2022; Singh et al., 2018). Singh et al. (2018) established GERAS in 2018, a machine learning-based framework capable of assigning individual pancreatic cells to chronological stages based on the transcriptomes of zebrafish and human. They leverage a supervised deep learning classifier based on neural networks to classify the cell age stages which achieved an overall accuracy of 91%, while the multinomial logistic regression model only displayed an overall accuracy of 64%. In addition, Buckley et al. (2023) trained single-cell-based regression models to predict chronological age and biological age leveraging scRNA-seq data in neurogenic regions of the mice brain. Lu et al. (2022) developed a machine-learning model capable of predicting the age of individual cells in human T cell subpopulations based on their transcriptomic features, which are closely associated with their differentiation and mutation burden. All of these approaches are well-established paradigms for the study of single-cell aging clocks in different animal or cell-type models.

More aging clocks based on single-cell transcriptome data are trying to predict the biological age of cells in a wider range. Neumann et al. (2023) utilized mouse scRNA-seq data to train molecular aging clocks that distinguish between cells of young and old mice using two models: a first model trained specifically to predict the age of B cells and a second one predicting age across 70 cell types from 14 tissues. In

addition, RAPToR was developed to estimate age both during development or aging in most common animal models and humans, from bulk or single-cell expression profiles (Bulteau and Francesconi, 2022). To be noted that some studies have not been peer-reviewed and published yet, it can be predicted that these methods will help us better understand cellular senescence.

Although there is a lack of scRNA-seq data from large population cohorts, all these methods show highly predictive transcriptional features of single cells that can be used to accurately estimate cell age. Machine learning, an approach to artificial intelligence, is rapidly revolutionizing the research paradigm of aging clocks. Given the noise of the data sets and the bias of sequencing technology, one issue is to integrate large-scale single-cell data sets to evaluate the cell age and the contribution of each cell type to organisms aging across tissues.

The application of medical imaging techniques in aging clocks

The word "aging clock" vividly demonstrates the change of aging biomarkers during the process of aging. Accompanied by the revolutionary development of physical science and computer technology, abundant imaging modalities have been applied in preclinical and clinical medicine. Multiple imaging modalities, such as CT, MRI, ultrasound, optical imaging systems, single-photon emission computed tomography (SPECT) and PET, are available to provide molecular, functional and structural information of aging clock from microscale to macroscale (Tian et al., 2021). In this part, we will discuss the application of medical imaging techniques in the aging clock (Table S22 in Supporting Information), which would shed light on the purpose of clinical translation of the aging clock in managing and treating aging-induced diseases, such as neurodegenerative diseases and cardiovascular diseases.

PET/CT and PET/MRI

Medical imaging could be divided into structural, functional and molecular modalities. Functional and molecular imaging modalities provide functional and molecular alterations occurred at the early phase or stage of diseases. Hybrid PET/CT and PET/MRI imaging have been regarded as the vital modality in the clinical practice to detect early abnormalities underlying diseases (Zhang et al., 2020c). The core part of PET/CT and PET/MRI is PET, which is based on specific recognition of targets that existed in the human body. Due to the characteristics of PET, PET/CT and PET/MRI have the potential in revealing the alterations of aging biomarkers in the aging clock, which could achieve observation from DNA damages to phenotypes of organs (Table S23 in Supporting Information) (Jack et al., 2017).

For DNA damages, *in vivo* activity of PARP could be visualized by using ^{18}F-FluorThanatrace (^{18}F-FTT) or ^{18}F-PARPi PET/CT imaging, which could indicate the responsiveness to PARP inhibitor treatment in patients with cancer (Pantel et al., 2022; Schöder et al., 2020). Besides, γH2AX (a phosphorylated DNA damage repair protein) activity could be visualized by using ^{89}Zr-anti-γH2AX-TAT radioimmunoconjugates to indicate the repairment of DNA double-strand breaks (Knight et al., 2017). Meanwhile, HDACs involved in histone modification could also be observed by using radionuclides. ^{18}F-suberoylanilide hydroxamic acid (^{18}F-SAHA) achieved *in vivo* evaluation of HDACs by labeling SAHA (a broad spectrum HDAC inhibitor), while failed to demonstrate HDAC expression in the brain (Hendricks et al., 2011). Recently, ^{11}C-Martinostat successfully revealed that reduced HDAC I level in the brain of patients with AD was associated with AD pathophysiology, which compensates for the application of HDAC radiotracers (Pascoal et al., 2022). Telomeres, as a tandem repetitive sequence that protected the ends of the chromosome from progressive degradation, are closely associated with aging. Telomerase reverse transcriptase (TERT), a main part of telomerase, could be noninvasively reported by hyperpolarized [U-13C, U-2H]-glucose metabolism (Viswanath et al., 2021).

As for phenotypes of organs affected by the aging clock, PET/CT and PET/MRI have their unique superiority (dynamic, real-time and noninvasive) to investigate these alterations in the whole body. Aβ peptide and tau protein are admitted pathological alterations in the brain of patients with AD (Busche and Hyman, 2020). ^{11}C-Pittsburgh compound B (^{11}C-PiB) is one of the most widely applied radiotracers in the clinic for Aβ plaques visualization (Hatashita and Wakebe, 2020). Apart from ^{11}C-labelled ligands, three ^{18}F-labelled amyloid PET ligands (^{18}F-florbetapir, ^{18}F-flutemetamol and ^{18}F-florbetaben) are also approved by USA and applied in the clinic. For tau PET imaging, ^{18}F-AV1451 (^{18}F-flortaucipir) is the most widely used tau-specific PET radiotracer, and its distribution is in accordance with Braak staging of tau pathology (Maass et al., 2018). Advancing age induced oxidative stress and inflammatory factors accumulation has been linked with cardiovascular aging, which usually develops cardiovascular diseases in the elderly (López-Otín et al., 2023). ^{18}F-tetraphenylphosphonium (^{18}F-TPP^{+}) was used to indicate the mitochondrial membrane potential in both animals and patients showing the potential applications in myocardial imaging (Pelletier-Galarneau et al., 2021). Combined with fluorescent material, ^{18}F-5MEF (a dual-modal probe) was synthesized and achieved mitochondria-targeting myocardial dual-modal imaging (Zheng et al., 2022). Besides, ^{18}F-sodium fluoride (^{18}F-NaF) showed promising use in characterizing culprit atherosclerotic plaques in the carotid circulation (Kaczynski et al., 2022).

However, it is worth noting that PET/CT and PET/MRI just play part of their roles in investigating the aging clock as integrated CT and MRI make part use. Thus, future studies are required to make full use of each component of PET/CT and PET/MRI for a better understanding of variations of the aging clock.

MRI

MRI is a medical imaging technique that is extensively used in clinical practice to capture anatomic and functional information of the body. Its numerous scanning sequences could offer different information about the tissues and organs. Compared with PET, MRI has better contrast of soft tissues, especially the brain and abdomen. Therefore, the application of MRI in the aging clock mainly focuses on brain aging.

The intuitive change of brain aging is the loss of brain volume. T1-weighted structural MRI could be applied in evaluating brain volume and revealed that the brain volume declined about 7% of their volume between the 20s and 60s (Hedman et al., 2012). While T2-weighted structural MRI images showed that the T2 signal increased in most brain regions as a result of increased water content (Kumar et al., 2012). Diffusion imaging is a well-established MRI technique for investigating the directionality of water self-diffusion. A recent study indicated the white-matter microstructural organization during aging processes by using diffusion MRI and showed that age-related brain alterations began earlier in males than females (Toschi et al., 2020). Another important MRI scanning method without contrast agents is the BOLD technique, which linked the change of cerebral oxidative metabolism with neuronal activity. By using BOLD fMRI scanning, the shape of hemodynamic response function (HRF) was found different between young and old individuals, and elevated time-to-peak and decreased peak amplitude were discovered in old individuals (West et al., 2019).

Another important component of MRI technique is enhanced scanning based on fMRI techniques. The basic fMRI technique is based on a widely used contrast agent that is gadolinium-DTPA (Gd-DTPA). With the administration of Gd-DTPA, dynamic susceptibility-contrast perfusion imaging could reflect the real-time cerebral blood flow (Barker et al., 2013). Arterial spin labeling (ASL) techniques depend on endogenous contrast produced by blood water. Several studies have demonstrated the decrease of parenchymal CBF in old individuals (Zhang et al., 2017b). Meanwhile, different brain regions showed diverse patterns in the aged population. Superior temporal and orbitofrontal regions performed decreased perfusion, while caudate, posterior cingulate and amygdala showed increased perfusion (Lee et al., 2009). In recent decades, resting-state fMRI was used to indicate the functional connectivity in the brain. Gonneaud et al. (2021) predicted chronological age across the lifespan by using resting-state fMRI data and assessed the possibility of individual developed AD. Though MRI technique has been extensively used in clinic practice, it mainly indirectly reflects the alterations of the brain induced by the aging clock. More specific contrast agents are required to enhance the ability of MRI to image aging biomarkers.

Ultrasound

Medical ultrasound is a medical imaging technique by using sound waves with frequencies over 20,000 Hz. Since its appearance in the 1940s, medical ultrasound has been widely accepted in the clinical practice as it is flexible, convenient and economic. Medical ultrasound has various imaging mode, including amplitude mode, brightness mode, motion mode, doppler sonography and harmonic imaging. Based on these imaging modes, most of organs in the body, such as vasculature, heart, skin and kidneys, could be evaluated by medical ultrasound.

Vascular assessment is an essential content of ultrasound daily examination. Atherosclerosis is one of the obvious phenotypes of vascular aging. Ultrasound imaging was proven to have the ability of vascular assessment (Johri et al., 2016). Three-dimensional vascular ultrasound (3DVUS) revealed and quantified higher plaque burden in males with advancing age, and plaque burden was closely related with cardiovascular risk factors (López-Melgar et al., 2017). Meanwhile, a Northern Manhattan study indicated that markers of carotid atherosclerosis (such as carotid intima-media thickness) provided by ultrasound were associated with cognitive status (Gardener et al., 2017). Contrast-enhanced ultrasound achieved *in vivo* visualizing of plaque neovascularization which is a biomarker of carotid plaque vulnerability (Camps-Renom et al., 2020). Besides, ultrasound realized molecular imaging of atherosclerosis by using nanoparticles. Punjabi et al. (2019) developed a microbubble targeted to human VCAM-1 and successfully characterized the expression of VCAM-1 in the human plaques.

Ultrasound could also assess the function of the heart during aging from multiple perspectives. The function of the heart is decreased associated with advancing age. Regular cardiac ultrasound images could provide precise information for characterizing the diastolic function of the left ventricle (LV) (Omar et al., 2017). Echocardiography is a kind of medical imaging for the heart, which is dependent on standard ultrasound or Doppler ultrasound. By using echocardiography, aging was revealed to be associated with decreased left atrial (LA) reservoir function and delayed P-wave dispersion and total atrial conduction time (Abou et al., 2017). Besides, echocardiography indicated that global longitudinal strain was an independent and incremental prognostic factor for long-term risk of cardiovascular morbidity and mortality (Biering-Sørensen et al., 2017).

Photoacoustic imaging and near-infrared (NIR) fluorescence imaging

Photoacoustic imaging (PAI) is a recently developed medical imaging with the characteristics of real-time, noninvasiveness and nonionizing radiation. Based on the heat induced by non-ionizing laser pulses, transient thermoelastic expansion could be produced and lead to wideband ultrasonic emission which could be translated into images by ultrasonic transducers (Attia et al., 2019). Due to its image-forming principle, PAI owns the benefits of optical resolution and acoustic depth of penetration. Generally, PAI systems could be divided into several groups, such as PA microscopy (PAM), PA endoscopy (PAE) and PA computed tomography (PACT) (Liu et al., 2016). Based on these unparalleled advantages, PAI demonstrates a wide range of preclinical and clinical applications in aging clock, mainly focused on superficial organs. Quantitative evaluation of skin aging was achieved according to the signals from both sectioned and nonsectioned porcine skin by using PA microscopy (Murata et al., 2017). Besides, PAI system has also been extensively used in breast imaging. Breast cancer is a heterogeneous age-associated malignancy, and about 80% of breast cancers happened in females over age 50 (Benz, 2008). A PAI system called PA the Twente Photoacoustic Mammoscope succeed to detect about 97% of lesions in patients with breast cancer (Heijblom et al., 2016). Multispectral optoacoustic tomography (MSOT) was applied in visualizing pilosebaceous units which are the hair follicle structure (Ford et al., 2016). Mesoscopic and microscopic configurations of the PAI system are also applicable to demonstrate microvascular networks (Taruttis et al., 2016).

NIR fluorescence imaging is a type of medical imaging technique based on the optical signal emitted by fluorophores or endogenous molecules with the excitation of light (Frangioni, 2003). According to the emission wavelengths, NIR fluorescence imaging can be classified into fluorescence imaging within the first NIR window (NIR-I) around 150–900 nm and fluorescence imaging within the second NIR window (NIR-II) near 900–1,700 nm (Welsher et al., 2011). In the past decade, more attention has been paid to the NIR-II fluorescence imaging due to its deeper penetration. With the aid of NIR-II fluorescence molecule, NIR-II fluorescence imaging achieved dynamic vascular imaging. Li et al. (2020a) reported an organic NIR-II molecule (LZ-1105) with a long blood half-life and used it for real-time monitoring for dynamic vascular processes, such as thrombolysis in carotid artery. Similarly, another NIR-II nanoparticle based on aggregation-induced emission (AIE) strategy was conducted for multiscale vasculature visualization in animal models (Li et al., 2021d). Additionally, brain is no longer the forbidden area of NIR-II fluorescence imaging. By using neutrophils (NEs) as the carrier, 2TT-oC6B succeed in penetrating the blood-brain barrier and visualizing deeply located brain inflammation (Liu et al., 2020). Though rapidly increased number of NIR-II nanoparticles were developed in recent years, few of them were approved in the clinic. More effort is required to boost this medical imaging technique into clinical practice.

Summary and perspectives

Medical imaging techniques play an irreplaceable role in monitoring the aging process and provide a powerful tool of dynamic, real-time and noninvasive visualization of aging biomarkers. While each imaging technique has its own limitations, the combined use of different imaging methods is necessarily required for acquiring full-scale information of the aging clock. Meanwhile, with the development of basic techniques, much more imaging techniques and the reconstructed algorithm will be established to reveal the correlation between aging biomarkers and aging-induced diseases *in vivo*.

AI application in aging clocks

Advances in artificial intelligence

Artificial intelligence, also known as AI, refers to the field of computer science and engineering that is dedicated to the development of intelligent systems. These systems are characterized by their ability to make decisions, judgments, and predictions without the need for explicit programming. AI has demonstrated remarkable potential in a range of applications, including natural language processing, computer vision, and protein structure prediction. For example, more than 98.5% of the three-dimensional structures of human proteins can be predicted by artificial intelligence (Jumper et al., 2021).

AI has its origins in statistical methods and has evolved to encompass a diverse set of machine learning-based algorithms. These algorithms are designed to perform tasks such as classification, regression, clustering, and decomposition. Since 2013, deep structured learning, also called DL systems have surpassed human performance in multiple applications. In healthcare, DL systems outperformed human dermatologists, ophthalmologists, and radiologists in various tasks. DL also demonstrated significant improvement over conventional ML methods in biomedical data analysis (Aliper et al., 2016; Mamoshina et al., 2016). DL comprises a set of methods that rely on deep architectures with cascades of multiple layers, and include architectures such as DNNs, generative adversarial networks (GANs), deep reinforcement learning (RL), and others.

One of the most recent and impactful developments within AI is deep learning, which is characterized by the use of DNNs. DNNs are architectures that stack multiple neural network structures in order to form a “deep” network with hidden layers, which improves the prediction performance. With the increased computational power and the availability

of large public datasets, DL architectures have evolved to include thousands of layers and have been applied to a wide range of biological systems and drug development.

Furthermore, another example of these DL methods is Generative Adversarial Networks (GANs), which are powerful generative models that can produce new data points with a similar distribution to that of real data. GANs are composed of two models, a generator and a discriminator, which work together to produce synthetic data points that are indistinguishable from real data. GANs have already been applied in various fields, including making predictions of compound properties or for molecular structure generation (Kadurin et al., 2017a; Kadurin et al., 2017b; Putin et al., 2018a; Putin et al., 2018b).

RL is a subset of machine learning that is concerned with the design of goal-oriented algorithms that can learn to optimize a complex objective or maximize a particular dimension over many steps (Kulkarni, 2017). One of the defining characteristics of RL algorithms is that they operate in an environment where the relationship between actions and outcomes is not immediately apparent, and the effects of actions may not be fully realized until several time steps have passed. To address this challenge, RL algorithms aim to establish a correlation between immediate actions and the delayed returns they produce. Therefore, reinforcement learning is usually applied to pathway design, such as compound reverse synthesis analysis and metabolite biosynthesis prediction (Segler et al., 2018).

AI facilitated development of aging clocks

Aging is a complex and multi-factorial process that is affected by genetic, epigenetic and environmental factors at the molecular, cellular, organ and whole body levels (Khan et al., 2017). Molecular and cellular mechanisms of aging have been studied extensively, which has led to the identification of numerous "hallmarks of aging": telomere attrition, epigenetic dysregulation, genome instability, shifts in gene expression patterns and metabolic profiles (López-Otín et al., 2013). Longitudinal studies of aging have collected a plethora of "aging biomarkers", which are used to assess "biological age" and potentially predict healthspan and lifespan for an individual (Galkin et al., 2020). Quantifying aging at the individual level by biomarkers led to the development of "aging clocks", which lead to a deeper understanding of underlying mechanisms during aging, and thus develop preventive and therapeutic restorative interventions that can increase lifespan and healthspan by reversing the biological clocks back to the young, healthy state.

Current aging clocks are mainly based on statistical models of a series of biological features. These features include clinical indicators (Jylhävä et al., 2017; Tzemah-Shahar et al., 2022), instrumental parameters (di Giuseppe et al., 2012; Russoniello et al., 2013), and molecular genetic measures (Hannum et al., 2013; Zhang et al., 2014). The methods commonly used are based on univariate or multivariate regression methods (Gialluisi et al., 2019), such as principle component analysis (PCA) (Nakamura and Miyao, 2007), multilayer perceptron (MLP) (Bae et al., 2008), and the Klemera and Doubal method (KDM) (Klemera and Doubal, 2006).

Although these classical methods perform well in predicting adverse aging outcomes, they have limitations in processing multidimensional data, especially when the shape of the distribution is not suited for parametric methods (Cao et al., 2021a), and recognizing the actual interactions between the biomarkers and outcomes (Jin et al., 2020), as some significant biomarkers were proved to be nonlinear (Klemera and Doubal, 2006). While recently, the increasing availability of large-scale molecular biological data from high-throughput experiments, in parallel with technological advancements in machine learning and bioinformatics, have greatly accelerated the discovery of biomarkers and fueled the use of computational modeling to unravel complex biological phenomena by discovering multivariate relationships (Jordan and Mitchell, 2015). Since then, numerous age clocks based on DL models have been developed and have shown considerable accuracy and efficiency in age prediction (Bobrov et al., 2018; Putin et al., 2016). DL-based aging clocks may also differ through training and validation protocols. Moreover, the concept of "aging clock" expanded to further levels of biological data, using medical data include not only images (Bobrov et al., 2018), but a wide range of genomic, epigenetic, transcriptomic, proteomic, and metabolic features (Fleischer et al., 2018; Hannum et al., 2013; Hertel et al., 2016; Holly et al., 2013; Horvath, 2013; Mamoshina et al., 2018; Peters et al., 2015; Tanaka et al., 2018).

Summary and perspectives

AI enables the analysis of cross-sectional and longitudinal data related to large human populations and facilitates the development of aging biomarkers which offer a tool to assess health, quantify the effect of interventions, and produce personalized medical reports. AI allows for enhanced precision in creating panels of actionable biomarkers, enables rapid assessment, and facilitates preventive measures (Zhu et al., 2022a). Future research using DL is still evolving to achieve better performance. On one hand, aging clocks will continue to evolve as new biomarkers and integrate multiple biomarkers. Also, increasing the sample size for training by aggregating public or private datasets could further improve the model's performance. On the other hand, new DL modeling architectures (with careful modeling of parameters), could be designed and explored further to get great promise in age estimation.

For pharmaceutical companies, multimodal aging clocks enable the integration of multiple data types and provide

deeper insights into biological data management. Deep aging clocks are excellent tools for a pharmaceutical company to evaluate which type of data is affected by a drug or intervention, leading to a clearer understanding of which data are most important in a clinical trial. Aging clocks can also help to evaluate the quality of the data as well as their impact both on the prediction accuracy and on the importance of specific features. Another interesting challenge and potential extension of this work are to study how the aging clock can be used as a tool for general health monitoring, including early-onset disease identification. For instance, an inflammatory aging clock (iAge) based on deep learning made early diagnosis of inflammatory conditions and immune system decline and further identified CXCL9 as a key contributor to iAge (Sayed et al., 2021).

Although DL models seem promising applications in integrating and identifying meaningful patterns from medical profiles, experts fear that, due to the design architecture, DL models appear to be "black boxes", without showing details of inner working to reach from raw images for disease predictions (Kelly et al., 2019). Nevertheless, efforts have been made in improving the interpretability of DL in healthcare (Richards et al., 2019). It is expected that by combining modeling approaches with medical expertise, more age-related pathways and targets can be revealed, which may further boost target identification and drug discovery (Figure 30). Furthermore, many computational approaches are being developed using various kinds of techniques in the computational drug repurposing, a highly active field of pharmacology (Lamb et al., 2006; Zhu et al., 2021b).

Composite clocks

As demonstrated in the previous sections, aging clocks based on different omics data have been proposed in the past decade. These findings suggest that biological age is indeed measurable, although it sometimes remains unclear exactly how these measured variables relate to the aging-associated decline in biological function. Moreover, aging is a complex process that coordinately influences nearly all tissues and organs at multiple physiological levels, thus it is urgent to surpass aging clocks that only evaluate limited aspects of aging and constitute composite markers of aging that reflect hostile characterization of aging (Figure 31).

Nowadays, only limited studies have tried to build a composite clock to evaluate biological aging. Recently, a study conducted a longitudinal study of 106 healthy individuals aged 29–75 years, and obtained multi-omics measurements, covering transcripts, proteins, metabolites, cytokines, microbes, and clinical laboratory values for these individuals (Ahadi et al., 2020). Based on these measurements, the authors defined different aging patterns across individuals, termed "ageotypes", and highlighted the heterogeneity of the aging pace between individuals (Ahadi et al., 2020). This study divided the aging trajectories into different classes with omics datasets, implying the potential to establish an integrative aging clock. In addition, another study collected data on immune characteristics in different modalities, including cell subset phenotyping, functional responses of the cell to cytokine stimulations, and whole-blood gene expression from peripheral blood samples of 135 healthy individuals (Alpert et al., 2019). By integrating these data, the immune aging (IMM-AGE) score was defined, which was shown to have better performance in predicting mortality in older adults than the epigenetic clock. This study mainly focuses on quantifying immune aging, but not the hostile characterization of aging.

A pioneering study computed a composite index by sum-

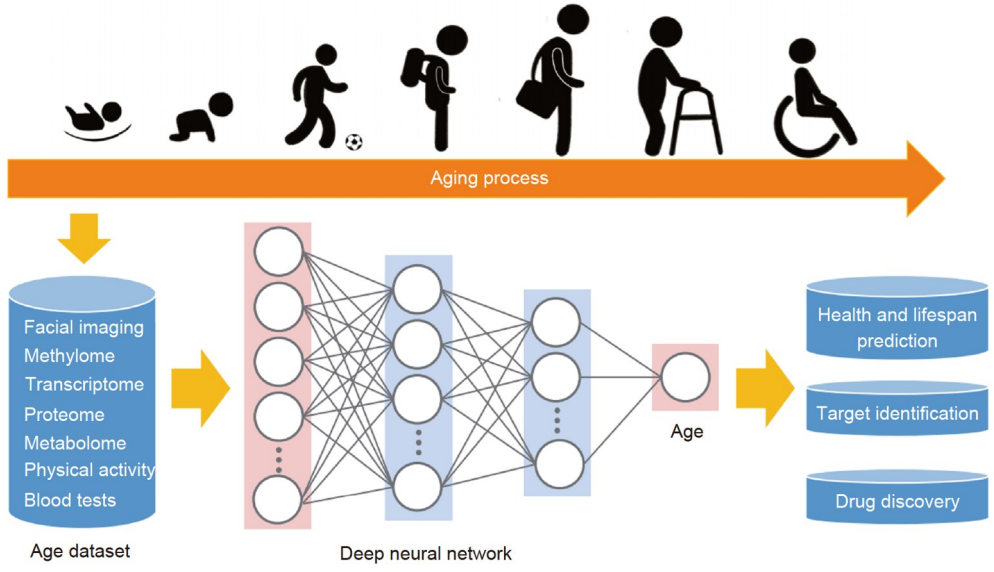

Figure 30 AI application in aging clocks. A generalized conceptual framework for applying artificial intelligence to aging clocks.

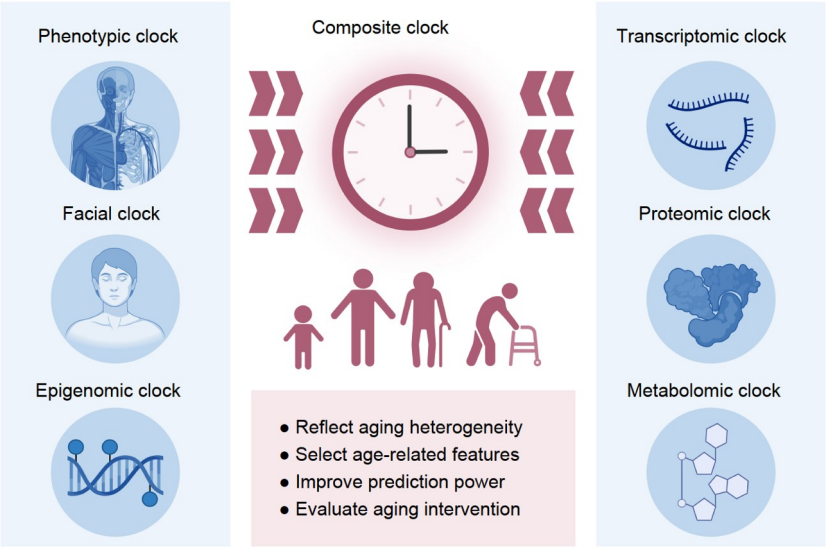

Figure 31 Composite aging clocks. A conceptual diagram showing the components of the composite clock and its advantages in evaluation of aging process.

ming five scaled biological aging indicators and found that the composite index displayed a higher correlation with health determinants than the other clocks (Jansen et al., 2021). The study measured telomere length, profiled whole-blood DNA methylation and transcriptome, serum proteome, and plasma metabolome from a single cohort, and compared their ability in predicting chronological age. Among these features, DNA methylation has the best performance in predicting age, followed by the proteomics and metabolomics data, and the telomere clock showed the worst performance. A more recent work collected biomedical information from 4,066 individuals from 20 to 45 years old, identified age-dependent changes in multi-omics variants, and established clocks indicating the biological age of different tissues/organs, such as cardiovascular age, renal age, liver age, as well as sex hormone age and gut microbiome age (Nie et al., 2022a).

Collectively, an aging clock composed of information from multiple data modalities might help to identify the key age-related features shared in different omics layers and provide a more comprehensive view of aging. Further analysis of these valuable omics datasets to establish a composite aging clock might elucidate whether incorporating more data modalities could improve prediction power. Moreover, experimental validations on these features can facilitate finding their causal effects on aging and the development of aging intervention strategies.

Ethical and social implications regarding the research of aging biomarkers

As mentioned in previous chapters, the field of aging biomarker research is rapidly evolving and has led to several breakthroughs. The advancement has demonstrated its potential to play an essential role in the diagnosis, treatment, or prevention of age-related diseases. However, aging biomarker research and its clinical translation, which often involve human beings and animals as subjects, also present a range of potential ethical, legal, and social risks. These issues should receive considerable attention from design to execution in every study and in the field of aging research.

In this chapter, we carefully explore the ethical, legal, and social risks associated with aging biomarker research and its clinical translation, and summarize them into four categories: (i) Potential scientific considerations, including difficult clinical efficacy and safety verification and limited predictive value. (ii) Informed consent issues, involving fully informed consent, informed consent capacity, and family informed consent. (iii) Disclosure of assessment or diagnostic results, as a highly complex process but lacks disclosure standards. (iv) Other specific ethical and social concerns, involving personal privacy, health insurance, and patient selection. We believe that these issues should be addressed in every study, and an ethical framework for aging biomarker research should be established to provide guidance to researchers, physicians, policy makers, and consumers to advance and safeguard the long-term development of the field.

Potential scientific considerations

The complexity and heterogeneity of aging make the study of aging biomarkers extremely complicated and expensive, far beyond the capacity of a single researcher. In this context, the clinical validity of aging biomarkers is often questioned. On

the one hand, environmental differences between preclinical studies and clinical trials of aging biomarkers (Porteri et al., 2017), such as differences in motivation, risk tolerance and psychological performance of participants/subjects, make it possible that their results may differ (Ketchum et al., 2022), and their clinical effectiveness needs further demonstration. On the other hand, the clinical application requires evidence of clinical efficacy and safety of aging biomarkers and relevant criteria. However, the time-consuming nature of data collection and the lack of collection criteria have greatly increased the difficulty of discovering and validating aging biomarkers, which makes it much harder to establish criteria for the effectiveness and safety of aging marker applications (OECD, 2011).

Currently, existing studies on aging biomarkers target a relatively homogeneous population. Larger and more diverse cohort studies are urgently needed to obtain more generalized safety and prognostic evidence. Moreover, the lack of ongoing risk follow-up has led to limited data on the associated risks, making it more difficult to analyze their efficacy and safety (Ketchum et al., 2022). The lack of effective corresponding treatments or precise prognostic assessments of aging biomarkers in the clinical setting also makes it more difficult to verify their clinical effectiveness (Bunnik et al., 2018). In addition, the predictive nature of aging biomarkers studies and the uncertainty of the associated results detract somewhat from their predictive value. Based on these potentially biased test results, it is difficult for clinicians to make a definitive diagnosis. For example, in amyloid-positive individuals, it is difficult to use aging biomarkers to determine whether and when they develop symptoms associated with AD (Vanderschaeghe, 2018).

The challenges of informed consent

The above-mentioned scientific uncertainty and incompleteness make it a great challenge to seek the informed consent of research participants/subjects. Particularly, how to fully inform participants/subjects of the potential benefits, risks and uncertainty of aging biomarkers testing in a comprehensive and understandable way is one of the challenges that researchers/clinicians have to face when conducting relevant research. Meanwhile, the process of informed consent is also challenged by the ability of research participants/subjects to give informed consent. For example, for those whose underlying pathology or physiological changes that may never result in symptoms, they could better understand the potential benefits and risks of biomarkers of aging and make more appropriate decisions (Walter and Covinsky, 2001). By contrast, for those with mild cognitive impairment, such as those with AD, their ability to understand the risks is relatively limited, and obtaining their consent and safeguarding their best interests would be more difficult (Milne et al., 2018). Furthermore, there is some controversy about whether informed consent should take into account family involvement (Porteri and Frisoni, 2014).

Disclosure of assessment or diagnostic results

Aging biomarkers research and its clinical trials also face the question of whether relevant results should be disclosed to research participants/subjects (Molinuevo et al., 2016). To some extent, the assessment of aging biomarkers or the disclosure of diagnostic results is two-sided. On the one hand, it may provide participants/subjects with opportunities for early management of age-related diseases or life planning as a way to enhance their life quality (Rostamzadeh et al., 2021). On the other hand, the limited options for aging interventions make it possible for relevant disclosures to cause specific psychosocial distress that affects the life quality of participants/subjects and alters their social environment (Paulsen et al., 2013). For example, for those at higher risk for assessment or diagnosis of biomarkers of aging, they often fear stigmatization, discrimination, disruption of family relationships or life plans (Gaille et al., 2020; Vanderschaeghe et al., 2018), and the possibility of suffering from ongoing anxiety, high stress, depression and even suicidal tendencies (Draper et al., 2010). In this sense, the potential benefits of aging biomarkers research and its clinical trials are closely linked to the attitudes, beliefs, expectations, life quality, family support, and social environment of the research participants/subjects as individuals. Considered in conjunction with the heterogeneity of researchers/clinicians, the disclosure of relevant risks becomes a complex process of ongoing communication between participants/subjects and researchers/clinicians (Rostamzadeh et al., 2021).

As can be seen, the disclosure of aging biomarkers assessment or diagnostic results has become a complex and challenging task that requires specific training and the ability to convey uncertainty (Molinuevo et al., 2016). Should researchers/clinicians inform participants/subjects of the results of the aging biomarkers assessment or diagnosis? If necessary, what should and should not be told, whether relevant results should be disclosed or shared with family members, and whether different disclosure mechanisms should be adopted depending on the differences between basic research and clinical trials? All of these questions face great difficulties due to individual differences in participants/subjects and differences between preclinical studies and clinical trials (Molinuevo et al., 2016).

More importantly, despite attempts to standardize the disclosure or consultation of aging biomarkers assessments or diagnostic results, consistent standards or systems of disclosure and consultation guidance have not been established (Alpinar-Sencan and Schicktanz, 2020; Karlawish,

2011; Roberts et al., 2013), which has also left the practice of disclosure or consultation in an unregulated or even confusing state. In practice, there have been some instances of clinicians being dishonest or withholding diagnostic information from patients, which may lead to a loss of trust, thereby affecting not only the physician-patient relationship but also the patient's family relationship (Cornett and Hall, 2008).

Other specific ethical and social concerns

Aging biomarkers research and its clinical trials may also have significant implications for research participants/subjects at the individual or social level. At the individual level, the disclosure of aging biomarkers assessment or diagnosis results and the associated data information involve the protection of personal privacy. Considering the potential stigmatization of relevant results, breaches of data information due to inadequate protection may have a negative impact on the employment and insurance of research participants/subjects (Cornett and Hall, 2008). Allowing disclosure of information about aging biomarkers to insurance companies may also make it more difficult or costly for those whose aging biomarkers indicate a shorter or more painful life span, and could even widen the gap between them and healthy individuals (Davis, 2010). At the societal level, the future clinical application of aging biomarkers may make the fair and equitable distribution of diagnostic resources or insurance coverage problematic. For example, whether aging biomarkers tests are primarily used for therapeutic or predictive needs (Rostamzadeh et al., 2021), whether they are targeted to patients or the general population, and whether the associated costs are self-imposed or covered by health insurance, pose challenges to the current health care system. This affects not only equitable access to aging biomarkers testing, but also insurance coverage for other diseases in other groups. In the long run, these issues could undermine the principle of solidarity in social security and health care systems, change the future direction of national health care systems and social security systems, and even create new health care equality issues (Gaille et al., 2020).

Not surprisingly, aging biomarkers research and its clinical trials involve the recruitment of research participants/subjects, but the recruitment criteria are controversial. For example, should the study and its clinical trials recruit young people in a healthy state, should research include those who pass the optimal treatment window (Molinuevo et al., 2016), or should research target only healthy older adults? Issues such as these are difficult to solve. In fact, current aging biomarkers studies and their clinical trials often have different recruitment sources (Ketchum et al., 2022), but there are differences in target populations, such as those with a family history of age-related diseases versus the general population (Gooblar et al., 2015), people of color versus whites (Wikler et al., 2013), and disadvantaged groups. It is equally difficult to consider the principle of diverse recruitment to ensure fair and equitable recruitment to aging biomarkers research and its clinical trials. In addition, aging biomarkers research may infringe on the welfare of experimental animals through their use. These issues also need to be addressed.

Summary and perspectives

In sum, to obtain high scientific standards for early warning and aging interventions, measurable, sensitive, reliable, and operable biomarkers based on data and scientific evidence must be determined. These studies are developing rapidly. Aging biomarker research has broad health, ethical, societal, and regulatory implications while advancing aging research as a whole. To realize the potential benefits of aging research, it has become necessary to establish an ethical framework for aging marker research to help guide researchers, physicians, policy makers, and consumers.

Epilogue

Aging biomarkers are critical to answer the three major questions in the field of aging: how old are we? Why do we get old? And how can we age slower? In this comprehensive review, we provided an encyclopedia summary of aging biomarkers covering a hierarchy of dimensions at cellular, organ, organismal and populational aging levels, along with associated ethical and social implications. We hope this review serves as a resource for readers in academia, industry and medical practice, broadening our understanding of not only what biomarkers can be used to monitor aging, but also how to use them to assess novel therapies to slow, modify or even reverse aging. As such, we can accelerate the journey of basic science discoveries in the aging field from bench to bedside.

A broad spectrum of aging biomarkers has been developed via diverse data types and modeling techniques. With these rich resources, it becomes more important to know when to use which set of biomarkers. Throughout this review, we stick to the three criteria for the selection of aging biomarkers: specific, systemic and serviceable. Thus, we do not tend to suggest a single best biomarker; rather, we provide a reliable collection of biomarkers from multiple dimensions for the prediction of the biological age and certain disease risks of a specific organ, which are practical for translation into clinical practice. They are organized according to the 6 pillars of classification: physiological characteristics, imaging traits, histologic features, cellular alteration, molecular changes, and secretory factors.

Owing to the successive release of new aging studies, especially those based on large cohorts, for example, the new initiative of the "1000 people aging research project", and the ever-expanding power of machine learning approaches, aging biomarkers and models of application are quickly evolving. Moreover, aging biomarkers in different species of animals may not be consistent or even contradictory under certain circumstances. Thus, translation into clinical practice requires the development of biomarkers applicable or specific for the assessment of aging and early warning of age-related diseases. Therefore, we tend to update this manual for aging biomarkers and their usage in the years to come to reflect our most up-to-date understanding of aging. The identification of specific, sensitive, and serviceable biomarkers of aging is the premise and basis for achieving aging interventions through various strategies (Cai et al., 2022c; Campisi et al., 2019; Chaib et al., 2022; Di Micco et al., 2021; Gasek et al., 2021; Longo and Anderson, 2022; Rando and Jones, 2021; Sun et al., 2022b). Although there is still much to learn about aging biomarkers, it is foreseeable that as the theory and application of aging biomarkers gradually advance, our understanding of the laws of aging and the prevention and treatment of aging-related diseases will usher into a new era.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *Due to space constraints, it is unavoidable that certain important works on aging biomarkers are not cited, and we offer our sincere apologies. We would like to express our sincerest gratitude to the members of the expert panel of the Major Program on Organ Aging and Degeneration. We thank all the members of the Aging Biomarker Consortium for their valuable input and suggestions. This work was supported by the National Natural Science Foundation of China (31730036, 31871380, 31871382, 31930055, 31930058, 32000500, 32022034, 32030033, 32070730, 32130046, 3217050247, 32150005, 32200595, 32222024, 81730019, 81730022, 81830014, 81921006, 81925005, 81970426, 81971301, 81971312, 82030041, 82061160495, 82070805, 82071595, 82090020, 82100841, 82120108009, 82122024, 82125002, 82125011, 82125012, 82130045, 82171284, 82173061, 82173398, 82225007, 82225015, 82225017, 82225018, 82230047, 82230088, 82271600, 91949106, 91949201, 92049116, 92049302, 92049304, 92149303, 92149306, 92157202, 92168201, 92169102, 92249301, 92268201), the National Key Research and Development Program of China (2018YFA0800700, 2018YFC2000100, 2018YFC2000102, 2018YFC2002003, 2019YFA0110900, 2019YFA0801703, 2019YFA0801903, 2019YFA0802202, 2019YFA0904800, 2020YFA0113400, 2020YFA0803401, 2020YFA0804000, 2020YFC2002900, 2020YFC2008000, 2020YFE0202200, 2021YFA0804900, 2021YFA1100103, 2021YFA1100900, 2021YFE0114200, 2021ZD0202400, 2022YFA0806001, 2022YFA0806002, 2022YFA0806600, 2022YFA1103200, 2022YFA1103601, 2022YFA1103701, 2022YFA1103800, 2022YFA1103801, 2022YFA1104100, 2022YFA1104904, 2022YFA1303000, 2022YFC2009900, 2022YFC2502401, 2022YFC3602400, 2022YFE0118000, 2022ZD0213200), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA16030302, XDB39000000, XDB39030600), the Youth Innovation Promotion Association of Chinese Academy of Sciences (2020085, 2021080), CAS Project for Young Scientists in Basic Research (YSBR-076), the Program of the Beijing Natural Science Foundation (JQ20031), Clinical Research Operating Fund of Central High level hospitals (2022-PUMCH-E-001), CAMS Innovation Fund for Medical Sciences (CIFMS) (2022-I2M-1-004), Talent Program of the Chinese Academy of Medical Science (2022-RC310-10), Research Funds from Health@InnoHK Program launched by Innovation Technology Commission of the Hong Kong Special Administrative Region, Guangdong Basic and Applied Basic Research Foundation (2020B1515020044), Guangzhou Planned Project of Science and Technology (202002020039), the Major Technology Innovation of Hubei Province (2019ACA141), the Science and Technology Major Project of Hunan Provincial Science and Technology Department (2021SK1010), Shanghai Municipal Science and Technology Major Project (2017SHZDZX01), the Natural Science Foundation of Sichuan Province (2023NSFSC0003), Yunnan Fundamental Research Project (202201AS070080) and the State Key Laboratory of Membrane Biology.*

References

Abdallah, P., Luciano, P., Runge, K.W., Lisby, M., Géli, V., Gilson, E., and Teixeira, M.T. (2009). A two-step model for senescence triggered by a single critically short telomere. Nat Cell Biol 11, 988–993.

Abou, R., Leung, M., Tonsbeek, A.M., Podlesnikar, T., Maan, A.C., Schalij, M.J., Ajmone Marsan, N., Delgado, V., and Bax, J.J. (2017). Effect of aging on left atrial compliance and electromechanical properties in subjects without structural heart disease. Am J Cardiol 120, 140–147.

Acer, E., Kaya Erdoğan, H., Kocatürk, E., Saracoğlu, Z.N., Alataş, Ö., and Bilgin, M. (2020). Evaluation of oxidative stress and psychoemotional status in premature hair graying. J Cosmet Dermatol 19, 3403–3407.

Acosta-Rodríguez, V.A., Rijo-Ferreira, F., Green, C.B., and Takahashi, J.S. (2021). Importance of circadian timing for aging and longevity. Nat Commun 12, 2862.

Acosta, J.C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J. P., Athineos, D., Kang, T.W., Lasitschka, F., Andrulis, M., et al. (2013). A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 15, 978–990.

Acosta, J.C., O'Loghlen, A., Banito, A., Guijarro, M.V., Augert, A., Raguz, S., Fumagalli, M., Da Costa, M., Brown, C., Popov, N., et al. (2008). Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell 133, 1006–1018.

Adamus, J., Aho, S., Meldrum, H., Bosko, C., and Lee, J.M. (2014). p16INK4A influences the aging phenotype in the living skin equivalent. J Invest Dermatol 134, 1131–1133.

Addison, O., Drummond, M.J., Lastayo, P.C., Dibble, L.E., Wende, A.R., McClain, D.A., and Marcus, R.L. (2014). Intramuscular fat and inflammation differ in older adults: the impact of frailty and inactivity. J Nutr Health Aging 18, 532–538.

Adelman, E.R., Huang, H.T., Roisman, A., Olsson, A., Colaprico, A., Qin, T., Lindsley, R.C., Bejar, R., Salomonis, N., Grimes, H.L., et al. (2019). Aging human hematopoietic stem cells manifest profound epigenetic reprogramming of enhancers that may predispose to leukemia. Cancer Discov 9, 1080–1101.

Agarwal, A., Barbăroşie, C., Ambar, R., and Finelli, R. (2020). The impact of single- and double-strand DNA breaks in human spermatozoa on assisted reproduction. Int J Mol Sci 21, 3882.

Aggarwal, B.B. (2003). Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 3, 745–756.

Agger, K., Cloos, P.A.C., Rudkjær, L., Williams, K., Andersen, G., Christensen, J., and Helin, K. (2009). The H3K27me3 demethylase JMJD3 contributes to the activation of the *INK4A-ARF* locus in response to oncogene- and stress-induced senescence. Genes Dev 23, 1171–1176.

Agherbi, H., Gaussmann-Wenger, A., Verthuy, C., Chasson, L., Serrano, M., and Djabali, M. (2009). Polycomb mediated epigenetic silencing and replication timing at the INK4a/ARF locus during senescence. PLoS ONE 4, e5622.

Aging Atlas, C. (2021). Aging Atlas: a multi-omics database for aging biology. Nucleic Acids Res 49, D825–D830.
Agrawal, A., and Gupta, S. (2011). Impact of aging on dendritic cell functions in humans. Ageing Res Rev 10, 336–345.
Agrawal, A., Tay, J., Ton, S., Agrawal, S., and Gupta, S. (2009). Increased reactivity of dendritic cells from aged subjects to self-antigen, the human DNA. J Immunol 182, 1138–1145.
Aguayo-Mazzucato, C., Andle, J., Lee, T.B. Jr., Midha, A., Talemal, L., Chipashvili, V., Hollister-Lock, J., van Deursen, J., Weir, G., and Bonner-Weir, S. (2019). Acceleration of β cell aging determines diabetes and senolysis improves disease outcomes. Cell Metab 30, 129–142.e4.
Ahadi, S., Zhou, W., Schüssler-Fiorenza Rose, S.M., Sailani, M.R., Contrepois, K., Avina, M., Ashland, M., Brunet, A., and Snyder, M. (2020). Personal aging markers and ageotypes revealed by deep longitudinal profiling. Nat Med 26, 83–90.
Ahlenius, H., Visan, V., Kokaia, M., Lindvall, O., and Kokaia, Z. (2009). Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. J Neurosci 29, 4408–4419.
Ahmad, H., Jahn, N., and Jaiswal, S. (2023). Clonal hematopoiesis and its impact on human health. Annu Rev Med 74, 249–260.
Ahmed, B., and Si, H. (2021). The aging of adipocytes increases expression of pro-inflammatory cytokines chronologically. Metabolites 11, 292.
Ahmed, N.S., Foote, J.B., and Singh, K.K. (2022). Impaired mitochondria promote aging-associated sebaceous gland dysfunction and pathology. Am J Pathol 192, 1546–1558.
Ahmed, T.A., Ahmed, S.M., El-Gammal, Z., Shouman, S., Ahmed, A., Mansour, R., and El-Badri, N. (2020). Oocyte Aging: The Role of Cellular and Environmental Factors and Impact on Female Fertility. In: Turksen, K., ed. Cell Biology and Translational Medicine, Volume 8. Advances in Experimental Medicine and Biology. Cham: Springer. 109–123.
Aitken, R.J., De Iuliis, G.N., and Nixon, B. (2020). The sins of our forefathers: paternal impacts on *de novo* mutation rate and development. Annu Rev Genet 54, 1–24.
Akbarian, S., Beeri, M.S., and Haroutunian, V. (2013). Epigenetic determinants of healthy and diseased brain aging and cognition. JAMA Neurol 70, 711–718.
Åkerström, B., Lögdberg, L., Berggård, T., Osmark, P., and Lindqvist, A. (2000). α1-Microglobulin: a yellow-brown lipocalin. Biochim Biophys Acta 1482, 172–184.
Akimoto, Y., Hart, G.W., Wells, L., Vosseller, K., Yamamoto, K., Munetomo, E., Ohara-Imaizumi, M., Nishiwaki, C., Nagamatsu, S., Hirano, H., et al. (2007). Elevation of the post-translational modification of proteins by O-linked N-acetylglucosamine leads to deterioration of the glucose-stimulated insulin secretion in the pancreas of diabetic Goto-Kakizaki rats. Glycobiology 17, 127–140.
Akune, T., Ohba, S., Kamekura, S., Yamaguchi, M., Chung, U., Kubota, N., Terauchi, Y., Harada, Y., Azuma, Y., Nakamura, K., et al. (2004). PPAR γ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. J Clin Invest 113, 846–855.
Al-Khalaf, H.H., and Aboussekhra, A. (2013). $p16^{INK4A}$ positively regulates $p21^{WAF1}$ expression by suppressing AUF1-dependent mRNA decay. PLoS ONE 8, e70133.
Al-Lamki, R.S., and Mayadas, T.N. (2015). TNF receptors: signaling pathways and contribution to renal dysfunction. Kidney Int 87, 281–296.
Al Bitar, , and Gali-Muhtasib, (2019). The role of the cyclin dependent kinase inhibitor $p21^{cip1/waf1}$ in targeting cancer: molecular mechanisms and novel therapeutics. Cancers 11, 1475.
Albano, D., Messina, C., Vitale, J., and Sconfienza, L.M. (2020). Imaging of sarcopenia: old evidence and new insights. Eur Radiol 30, 2199–2208.
Albrethsen, J., Frederiksen, H., Andersson, A.M., Anand-Ivell, R., Nordkap, L., Bang, A.K., Jørgensen, N., and Juul, A. (2018). Development and validation of a mass spectrometry-based assay for quantification of insulin-like factor 3 in human serum. Clin Chem Lab Med 56, 1913–1920.
Alder, J.K., Barkauskas, C.E., Limjunyawong, N., Stanley, S.E., Kembou, F., Tuder, R.M., Hogan, B.L.M., Mitzner, W., and Armanios, M. (2015). Telomere dysfunction causes alveolar stem cell failure. Proc Natl Acad Sci USA 112, 5099–5104.
Alex, L., Rajan, M., Xavier, B., Jacob, P., Rani, K., and Lakshmi, G. (2015). Microscopic study of human spleen in different age groups. Int J Res Med Sci 3, 1701–1706.
Alexeyev, M.F. (2009). Is there more to aging than mitochondrial DNA and reactive oxygen species? FEBS J 276, 5768–5787.
Alimbetov, D., Davis, T., Brook, A.J.C., Cox, L.S., Faragher, R.G.A., Nurgozhin, T., Zhumadilov, Z., and Kipling, D. (2016). Suppression of the senescence-associated secretory phenotype (SASP) in human fibroblasts using small molecule inhibitors of p38 MAP kinase and MK2. Biogerontology 17, 305–315.
Alimonti, A., Carracedo, A., Clohessy, J.G., Trotman, L.C., Nardella, C., Egia, A., Salmena, L., Sampieri, K., Haveman, W.J., Brogi, E., et al. (2010a). Subtle variations in Pten dose determine cancer susceptibility. Nat Genet 42, 454–458.
Alimonti, A., Nardella, C., Chen, Z., Clohessy, J.G., Carracedo, A., Trotman, L.C., Cheng, K., Varmeh, S., Kozma, S.C., Thomas, G., et al. (2010b). A novel type of cellular senescence that can be enhanced in mouse models and human tumor xenografts to suppress prostate tumorigenesis. J Clin Invest 120, 681–693.
Aliper, A., Plis, S., Artemov, A., Ulloa, A., Mamoshina, P., and Zhavoronkov, A. (2016). Deep learning applications for predicting pharmacological properties of drugs and drug repurposing using transcriptomic data. Mol Pharm 13, 2524–2530.
Alizadeh Pahlavani, H. (2022). Exercise therapy for people with sarcopenic obesity: myokines and adipokines as effective actors. Front Endocrinol 13, 811751.
Almada, A.E., and Wagers, A.J. (2016). Molecular circuitry of stem cell fate in skeletal muscle regeneration, ageing and disease. Nat Rev Mol Cell Biol 17, 267–279.
Almeida, M., Han, L., Martin-Millan, M., O'Brien, C.A., and Manolagas, S.C. (2007). Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting β-catenin from T cell factor- to forkhead box O-mediated transcription. J Biol Chem 282, 27298–27305.
Almeida, M., Laurent, M.R., Dubois, V., Claessens, F., O'Brien, C.A., Bouillon, R., Vanderschueren, D., and Manolagas, S.C. (2017). Estrogens and androgens in skeletal physiology and pathophysiology. Physiol Rev 97, 135–187.
Alpert, A., Pickman, Y., Leipold, M., Rosenberg-Hasson, Y., Ji, X., Gaujoux, R., Rabani, H., Starosvetsky, E., Kveler, K., Schaffert, S., et al. (2019). A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med 25, 487–495.
Alpinar-Sencan, Z., and Schicktanz, S. (2020). Addressing ethical challenges of disclosure in dementia prediction: Limitations of current guidelines and suggestions to proceed. BMC Med Ethics 21, 33.
Alvarez-Garcia, O., Matsuzaki, T., Olmer, M., Masuda, K., and Lotz, M.K. (2017). Age-related reduction in the expression of FOXO transcription factors and correlations with intervertebral disc degeneration. J Orthop Res 35, 2682–2691.
Amador, A., Steger, R.W., Bartke, A., Johns, A., Siler-khodr, T.M., Parker, C.R. Jr., and Shepherd, A.M. (1985). Testicular LH receptors during aging in Fisher 344 rats. J Androl 6, 61–64.
Amargant, F., Manuel, S.L., Tu, Q., Parkes, W.S., Rivas, F., Zhou, L.T., Rowley, J.E., Villanueva, C.E., Hornick, J.E., Shekhawat, G.S., et al. (2020). Ovarian stiffness increases with age in the mammalian ovary and depends on collagen and hyaluronan matrices. Aging Cell 19, e13259.
Ambale-Venkatesh, B., Yang, X., Wu, C.O., Liu, K., Hundley, W.G., McClelland, R., Gomes, A.S., Folsom, A.R., Shea, S., Guallar, E., et al. (2017). Cardiovascular event prediction by machine learning: the multi-ethnic study of atherosclerosis. Circ Res 121, 1092–1101.
Ambrosi, T.H., Goodnough, L.H., Steininger, H.M., Hoover, M.Y., Kim, E., Koepke, L.S., Marecic, O., Zhao, L., Seita, J., Bishop, J.A., et al. (2020). Geriatric fragility fractures are associated with a human skeletal

stem cell defect. Aging Cell 19, e13164.
Ambrosi, T.H., Longaker, M.T., and Chan, C.K.F. (2019). A revised perspective of skeletal stem cell biology. Front Cell Dev Biol 7, 189.
Ambrosi, T.H., Marecic, O., McArdle, A., Sinha, R., Gulati, G.S., Tong, X., Wang, Y., Steininger, H.M., Hoover, M.Y., Koepke, L.S., et al. (2021). Aged skeletal stem cells generate an inflammatory degenerative niche. Nature 597, 256–262.
Ambrosi, T.H., Scialdone, A., Graja, A., Gohlke, S., Jank, A.M., Bocian, C., Woelk, L., Fan, H., Logan, D.W., Schürmann, A., et al. (2017). Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration. Cell Stem Cell 20, 771–784.e6.
Amer, H., Lieske, J.C., Rule, A.D., Kremers, W.K., Larson, T.S., Palacios, C.R.F., Stegall, M.D., and Cosio, F.G. (2013). Urine high and low molecular weight proteins one-year post-kidney transplant: relationship to histology and graft survival. Am J Transplant 13, 676–684.
Amorim, J.A., Coppotelli, G., Rolo, A.P., Palmeira, C.M., Ross, J.M., and Sinclair, D.A. (2022). Mitochondrial and metabolic dysfunction in ageing and age-related diseases. Nat Rev Endocrinol 18, 243–258.
An, R., Wilms, E., Masclee, A.A.M., Smidt, H., Zoetendal, E.G., and Jonkers, D. (2018). Age-dependent changes in GI physiology and microbiota: time to reconsider? Gut 67, 2213–2222.
Anand, B.S., Vij, J.C., Mac, H.S., Chowdhury, V., and Kumar, A. (1989). Effect of aging on the pancreatic ducts: a study based on endoscopic retrograde pancreatography. Gastrointest Endosc 35, 210–213.
Anantharaju, A., Feller, A., and Chedid, A. (2002). Aging liver. Gerontology 48, 343–353.
Ando, H., Ushijima, K., Kumazaki, M., Takamura, T., Yokota, N., Saito, T., Irie, S., Kaneko, S., and Fujimura, A. (2010). Influence of age on clock gene expression in peripheral blood cells of healthy women. J Gerontol A Biol Sci Med Sci 65A, 9–13.
Andrews, C., Nettle, D., Larriva, M., Gillespie, R., Reichert, S., Brilot, B. O., Bedford, T., Monaghan, P., Spencer, K.A., and Bateson, M. (2017). A marker of biological age explains individual variation in the strength of the adult stress response. R Soc Open Sci 4, 171208.
Anerillas, C., Abdelmohsen, K., and Gorospe, M. (2020). Regulation of senescence traits by MAPKs. Geroscience 42, 397–408.
Angelidis, I., Simon, L.M., Fernandez, I.E., Strunz, M., Mayr, C.H., Greiffo, F.R., Tsitsiridis, G., Ansari, M., Graf, E., Strom, T.M., et al. (2019). An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. Nat Commun 10, 963.
Anifandis, G., Samara, M., Simopoulou, M., Messini, C.I., Chatzimeletiou, K., Thodou, E., Daponte, A., and Georgiou, I. (2021). Insights into the role of telomeres in human embryological parameters. Opinions regarding IVF. J Dev Biol 9, 49.
Antonopoulos, A.S., Siasos, G., Oikonomou, E., Gouliopoulos, N., Konsola, T., Tsigkou, V., Moschos, M., Tentolouris, N., Kassi, E., Paschou, S.A., et al. (2021). Arterial stiffness and microvascular disease in type 2 diabetes. Eur J Clin Invest 51, e13380.
Apostolova, L.G., Thompson, P.M., Rogers, S.A., Dinov, I.D., Zoumalan, C., Steiner, C.A., Siu, E., Green, A.E., Small, G.W., Toga, A.W., et al. (2010). Surface feature-guided mapping of cerebral metabolic changes in cognitively normal and mildly impaired elderly. Mol Imag Biol 12, 218–224.
Arai, T., and Takubo, K. (2007). Clinicopathological and molecular characteristics of gastric and colorectal carcinomas in the elderly. Pathol Int 57, 303–314.
Arai, Y., Kamide, K., and Hirose, N. (2019). Adipokines and aging: findings from centenarians and the very old. Front Endocrinol 10, 142.
Aravinthan, A.D., and Alexander, G.J.M. (2016). Senescence in chronic liver disease: Is the future in aging? J Hepatol 65, 825–834.
Arck, P.C., Overall, R., Spatz, K., Liezman, C., Handjiski, B., Klapp, B.F., Birch-Machin, M.A., Peters, E.M.J., Arck, P.C., Overall, R., et al. (2006). Towards a "free radical theory of graying": melanocyte apoptosis in the aging human hair follicle is an indicator of oxidative stress induced tissue damage. FASEB J 20, 1567–1569.
Arda, H.E., Li, L., Tsai, J., Torre, E.A., Rosli, Y., Peiris, H., Spitale, R.C., Dai, C., Gu, X., Qu, K., et al. (2016). Age-dependent pancreatic gene regulation reveals mechanisms governing human β cell function. Cell Metab 23, 909–920.
Arnetz, B.B., Kallner, A., and Theorell, T. (1982). The influence of aging on hemoglobin A1c (HbA1c). J Gerontol 37, 648–650.
Arnold, L., Henry, A., Poron, F.., Baba-Amer, Y., van Rooijen, N., Plonquet, A., Gherardi, R.K., and Chazaud, B... (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 204, 1057–1069.
Arosio, B., Ostan, R., Mari, D., Damanti, S., Ronchetti, F., Arcudi, S., Scurti, M., Franceschi, C., and Monti, D. (2017). Cognitive status in the oldest old and centenarians: a condition crucial for quality of life methodologically difficult to assess. Mech Ageing Dev 165, 185–194.
Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., Deroos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504, 451–455.
Asadi Shahmirzadi, A., Edgar, D., Liao, C.Y., Hsu, Y.M., Lucanic, M., Asadi Shahmirzadi, A., Wiley, C.D., Gan, G., Kim, D.E., Kasler, H.G., et al. (2020). Alpha-ketoglutarate, an endogenous metabolite, extends lifespan and compresses morbidity in aging mice. Cell Metab 32, 447–456.e6.
Ashapkin, V.V., Kutueva, L.I., and Vanyushin, B.F. (2020). The Effects of Parabiosis on Aging and Age-Related Diseases. In: Guest, P., ed. Reviews on New Drug Targets in Age-Related Disorders. Advances in Experimental Medicine and Biology. Cham: Springer. 107–122.
Astle, M.V., Hannan, K.M., Ng, P.Y., Lee, R.S., George, A.J., Hsu, A.K., Haupt, Y., Hannan, R.D., and Pearson, R.B. (2012). AKT induces senescence in human cells via mTORC1 and p53 in the absence of DNA damage: implications for targeting mTOR during malignancy. Oncogene 31, 1949–1962.
Attema, J.L., Pronk, C.J.H., Norddahl, G.L., Nygren, J.M., and Bryder, D. (2009). Hematopoietic stem cell ageing is uncoupled from $p16^{INK4A}$-mediated senescence. Oncogene 28, 2238–2243.
Attia, A.B.E., Balasundaram, G., Moothanchery, M., Dinish, U.S., Bi, R., Ntziachristos, V., and Olivo, M. (2019). A review of clinical photoacoustic imaging: current and future trends. Photoacoustics 16, 100144.
Atzmon, G., Schechter, C., Greiner, W., Davidson, D., Rennert, G., and Barzilai, N. (2004). Clinical phenotype of families with longevity. J Am Geriatr Soc 52, 274–277.
Aubert, G., Baerlocher, G.M., Vulto, I., Poon, S.S., and Lansdorp, P.M. (2012). Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. PLoS Genet 8, e1002696.
Augereau, A., T'kint de Roodenbeke, C., Simonet, T., Bauwens, S., Horard, B., Callanan, M., Leroux, D., Jallades, L., Salles, G., Gilson, E., et al. (2011). Telomeric damage in early stage of chronic lymphocytic leukemia correlates with shelterin dysregulation. Blood 118, 1316–1322.
Auro, K., Joensuu, A., Fischer, K., Kettunen, J., Salo, P., Mattsson, H., Niironen, M., Kaprio, J., Eriksson, J.G., Lehtimäki, T., et al. (2014). A metabolic view on menopause and ageing. Nat Commun 5, 4708.
Avrahami, D., Li, C., Zhang, J., Schug, J., Avrahami, R., Rao, S., Stadler, M.B., Burger, L., Schübeler, D., Glaser, B., et al. (2015). Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. Cell Metab 22, 619–632.
Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgeois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A., et al. (2017). Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell 169, 132–147.e16.
Baasch, S., Giansanti, P., Kolter, J., Riedl, A., Forde, A.J., Runge, S., Zenke, S., Elling, R., Halenius, A., Brabletz, S., et al. (2021). Cytomegalovirus subverts macrophage identity. Cell 184, 3774–3793.e25.
Babbar, M., Basu, S., Yang, B., Croteau, D.L., and Bohr, V.A. (2020). Mitophagy and DNA damage signaling in human aging. Mech Ageing

Dev 186, 111207.
Babcock, M.C., DuBose, L.E., Witten, T.L., Brubaker, A., Stauffer, B.L., Hildreth, K.L., and Moreau, K.L. (2021). Assessment of macrovascular and microvascular function in aging males. J Appl Physiol 130, 96–103.
Bacos, K., Gillberg, L., Volkov, P., Olsson, A.H., Hansen, T., Pedersen, O., Gjesing, A.P., Eiberg, H., Tuomi, T., Almgren, P., et al. (2016). Blood-based biomarkers of age-associated epigenetic changes in human islets associate with insulin secretion and diabetes. Nat Commun 7, 11089.
Bae, C.Y., Kang, Y.G., Kim, S., Cho, C., Kang, H.C., Yu, B.Y., Lee, S.W., Cho, K.H., Lee, D.C., Lee, K., et al. (2008). Development of models for predicting biological age (BA) with physical, biochemical, and hormonal parameters. Arch Gerontol Geriatr 47, 253–265.
Baerlocher, G.M., Vulto, I., de Jong, G., and Lansdorp, P.M. (2006). Flow cytometry and FISH to measure the average length of telomeres (flow FISH). Nat Protoc 1, 2365–2376.
Bailey, K.L., Kharbanda, K.K., Katafiasz, D.M., Sisson, J.H., and Wyatt, T. A. (2018). Oxidative stress associated with aging activates protein kinase Cε, leading to cilia slowing. Am J Physiol Lung Cell Mol Physiol 315, L882–L890.
Baines, H.L., Stewart, J.B., Stamp, C., Zupanic, A., Kirkwood, T.B.L., Larsson, N.G., Turnbull, D.M., and Greaves, L.C. (2014). Similar patterns of clonally expanded somatic mtDNA mutations in the colon of heterozygous mtDNA mutator mice and ageing humans. Mech Ageing Dev 139, 22–30.
Baird, D.M., Rowson, J., Wynford-Thomas, D., and Kipling, D. (2003). Extensive allelic variation and ultrashort telomeres in senescent human cells. Nat Genet 33, 203–207.
Baird, G.S., Nelson, S.K., Keeney, T.R., Stewart, A., Williams, S., Kraemer, S., Peskind, E.R., and Montine, T.J. (2012). Age-dependent changes in the cerebrospinal fluid proteome by slow off-rate modified aptamer array. Am J Pathol 180, 446–456.
Bakala, H., Delaval, E., Hamelin, M., Bismuth, J., Borot-Laloi, C., Corman, B., and Friguet, B. (2003). Changes in rat liver mitochondria with aging. Eur J Biochem 270, 2295–2302.
Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., A. Saltness, R., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., et al. (2016). Naturally occurring $p16^{Ink4a}$-positive cells shorten healthy lifespan. Nature 530, 184–189.
Baker, D.J., Wijshake, T., Tchkonia, T., Lebrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., and van Deursen, J.M. (2011). Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236.
Baker, N., Wade, S., Triolo, M., Girgis, J., Chwastek, D., Larrigan, S., Feige, P., Fujita, R., Crist, C., Rudnicki, M.A., et al. (2022). The mitochondrial protein OPA1 regulates the quiescent state of adult muscle stem cells. Cell Stem Cell 29, 1315–1332.e9.
Bakun, M., Senatorski, G., Rubel, T., Lukasik, A., Zielenkiewicz, P., Dadlez, M., and Paczek, L. (2014). Urine proteomes of healthy aging humans reveal extracellular matrix (ECM) alterations and immune system dysfunction. Age 36, 299–311.
Baldensperger, T., Eggen, M., Kappen, J., Winterhalter, P.R., Pfirrmann, T., and Glomb, M.A. (2020). Comprehensive analysis of posttranslational protein modifications in aging of subcellular compartments. Sci Rep 10, 7596.
Ballian, N., and Brunicardi, F.C. (2007). Islet vasculature as a regulator of endocrine pancreas function. World J Surg 31, 705–714.
Baman, J.R., and Eskandari, M.K. (2022). What is an abdominal aortic aneurysm? JAMA 328, 2280.
Bando, H., Zhang, C., Takada, Y., Yamasaki, R., and Saito, S. (1991). Impaired secretion of growth hormone-releasing hormone, growth hormone and IGF-I in elderly men. Acta Endocrinol 124, 31–36.
Banerjee, M., Mehr, R., Belelovsky, A., Spencer, J., and Dunn-Walters, D. K. (2002). Age- and tissue-specific differences in human germinal center B cell selection revealed by analysis of IgV_H gene hypermutation and lineage trees. Eur J Immunol 32, 1947–1957.
Banani, S.F., Lee, H.O., Hyman, A.A., and Rosen, M.K. (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 18, 285–298.
Banani, S.F., Rice, A.M., Peeples, W.B., Lin, Y., Jain, S., Parker, R., and Rosen, M.K. (2016). Compositional control of phase-separated cellular bodies. Cell 166, 651–663.
Baraibar, M.A., Hyzewicz, J., Rogowska-Wrzesinska, A., Bulteau, A.L., Prip-Buus, C., Butler-Browne, G., and Friguet, B. (2016). Impaired energy metabolism of senescent muscle satellite cells is associated with oxidative modifications of glycolytic enzymes. Aging 8, 3375–3389.
Bárcena, C., López-Otín, C., and Kroemer, G. (2019). Methionine restriction for improving progeria: another autophagy-inducing anti-aging strategy? Autophagy 15, 558–559.
Bárcena, C., Quirós, P.M., Durand, S., Mayoral, P., Rodríguez, F., Caravia, X.M., Mariño, G., Garabaya, C., Fernández-García, M.T., Kroemer, G., et al. (2018). Methionine restriction extends lifespan in progeroid mice and alters lipid and bile acid metabolism. Cell Rep 24, 2392–2403.
Barker, C.J., Li, L., Köhler, M., and Berggren, P.O. (2015). β-Cell Ca^{2+} dynamics and function are compromised in aging. Adv Biol Regul 57, 112–119.
Barker, P.B., Golay, X., and Zaharchuk, G. (2013). Clinical Perfusion MRI: Techniques and Applications. Cambridge: Cambridge University Press.
Barnaure, I., Montandon, M.L., Rodriguez, C., Herrmann, F., Lövblad, K. O., Giannakopoulos, P., and Haller, S. (2017). Clinicoradiologic correlations of cerebral microbleeds in advanced age. Am J Neuroradiol 38, 39–45.
Barnes, C.J., Covington, B.W., and Lee, M. (1999). Effect of aging and dietary restriction on rat testicular germ cell apoptosis. J Gerontol A Biol Sci Med Sci 54, B199–B204.
Barnum, K.J., and O'Connell, M.J. (2014). Cell cycle regulation by checkpoints. In: Noguchi, E., and Gadaleta, M., eds. Cell Cycle Control. Methods in Molecular Biology. New York, NY: Humana Press. 29–40.
Barradas, M., Anderton, E., Acosta, J.C., Li, S.D., Banito, A., Rodriguez-Niedenführ, M., Maertens, G., Banck, M., Zhou, M.M., Walsh, M.J., et al. (2009). Histone demethylase JMJD3 contributes to epigenetic control of *INK4a/ARF* by oncogenic RAS. Genes Dev 23, 1177–1182.
Barritt, J.A., Cohen, J., and Brenner, C.A. (2000). Mitochondrial DNA point mutation in human oocytes is associated with maternal age. Reprod Biomed Online 1, 96–100.
Barruet, E., Garcia, S.M., Striedinger, K., Wu, J., Lee, S., Byrnes, L., Wong, A., Xuefeng, S., Tamaki, S., Brack, A.S., et al. (2020). Functionally heterogeneous human satellite cells identified by single cell RNA sequencing. eLife 9, e51576.
Bartek, J., Falck, J., and Lukas, J. (2001). Chk2 kinase—a busy messenger. Nat Rev Mol Cell Biol 2, 877–886.
Barthel, F.P., Wei, W., Tang, M., Martinez-Ledesma, E., Hu, X., Amin, S. B., Akdemir, K.C., Seth, S., Song, X., Wang, Q., et al. (2017). Systematic analysis of telomere length and somatic alterations in 31 cancer types. Nat Genet 49, 349–357.
Barzilai, N., Atzmon, G., Schechter, C., Schaefer, E.J., Cupples, A.L., Lipton, R., Cheng, S., and Shuldiner, A.R. (2003). Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA 290, 2030–2040.
Barzily-Rokni, M., Friedman, N., Ron-Bigger, S., Isaac, S., Michlin, D., and Eden, A. (2011). Synergism between DNA methylation and macroH2A1 occupancy in epigenetic silencing of the tumor suppressor gene p16(CDKN2A). Nucleic Acids Res 39, 1326–1335.
Basatemur, G.L., Jørgensen, H.F., Clarke, M.C.H., Bennett, M.R., and Mallat, Z. (2019). Vascular smooth muscle cells in atherosclerosis. Nat Rev Cardiol 16, 727–744.
Basisty, N., Kale, A., Jeon, O.H., Kuehnemann, C., Payne, T., Rao, C., Holtz, A., Shah, S., Sharma, V., Ferrucci, L., et al. (2020). A proteomic atlas of senescence-associated secretomes for aging biomarker development. PLoS Biol 18, e3000599.
Bass, J., and Lazar, M.A. (2016). Circadian time signatures of fitness and disease. Science 354, 994–999.
Basso, A., Piantanelli, L., Rossolini, G., and Roth, G.S. (1998). Reduced DNA synthesis in primary cultures of hepatocytes from old mice is restored by thymus grafts. J Gerontol A Biol Sci Med Sci 53A, B111–B116.
Basu, R., Breda, E., Oberg, A.L., Powell, C.C., Dalla Man, C., Basu, A.,

Vittone, J.L., Klee, G.G., Arora, P., Jensen, M.D., et al. (2003). Mechanisms of the age-associated deterioration in glucose tolerance. Diabetes 52, 1738–1748.
Bathke, J., Konzer, A., Remes, B., McIntosh, M., and Klug, G. (2019). Comparative analyses of the variation of the transcriptome and proteome of *Rhodobacter sphaeroides* throughout growth. BMC Genomics 20, 358.
Battaglia, D.E., Goodwin, P., Klein, N.A., and Soules, M.R. (1996). Fertilization and early embryology: influence of maternal age on meiotic spindle assembly oocytes from naturally cycling women. Hum Reprod 11, 2217–2222.
Bauer, M., Caviezel, S., Teynor, A., Erbel, R., Mahabadi, A.A., and Schmidt-Trucksäss, A. (2012). Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. Swiss Med Wkly 142, w13705.
Beaman, C., Kozii, K., Hilal, S., Liu, M., Spagnolo-Allende, A.J., Polanco-Serra, G., Chen, C., Cheng, C.Y., Zambrano, D., Arikan, B., et al. (2022). Cerebral microbleeds, cerebral amyloid angiopathy, and their relationships to quantitative markers of neurodegeneration. Neurology 98, e1605–e1616.
Beauséjour, C.M., Krtolica, A., Galimi, F., Narita, M., Lowe, S.W., Yaswen, P., and Campisi, J. (2003). Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J 22, 4212–4222.
Becker, J.S., Nicetto, D., and Zaret, K.S. (2016). H3K9me3-dependent heterochromatin: barrier to cell fate changes. Trends Genet 32, 29–41.
Becklund, B.R., Purton, J.F., Ramsey, C., Favre, S., Vogt, T.K., Martin, C. E., Spasova, D.S., Sarkisyan, G., LeRoy, E., Tan, J.T., et al. (2016). The aged lymphoid tissue environment fails to support naïve T cell homeostasis. Sci Rep 6, 30842.
Bedogni, G., Miglioli, L., Masutti, F., Tiribelli, C., Marchesini, G., and Bellentani, S. (2005). Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 42, 44–52.
Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A., and Rossi, D.J. (2013). Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell Stem Cell 12, 413–425.
Beerman, I., Maloney, W.J., Weissmann, I.L., and Rossi, D.J. (2010). Stem cells and the aging hematopoietic system. Curr Opin Immunol 22, 500–506.
Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L., and Rossi, D.J. (2014). Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. Cell Stem Cell 15, 37–50.
Begovatz, P., Koliaki, C., Weber, K., Strassburger, K., Nowotny, B., Nowotny, P., Müssig, K., Bunke, J., Pacini, G., Szendrödi, J., et al. (2015). Pancreatic adipose tissue infiltration, parenchymal steatosis and beta cell function in humans. Diabetologia 58, 1646–1655.
Begueria, R., Garcia, D., Obradors, A., Poisot, F., Vassena, R., and Vernaeve, V. (2014). Paternal age and assisted reproductive outcomes in ICSI donor oocytes: is there an effect of older fathers? Hum Reprod 29, 2114–2122.
Behringer, V., Deschner, T., Murtagh, R., Stevens, J.M.G., and Hohmann, G. (2014). Age-related changes in thyroid hormone levels of bonobos and chimpanzees indicate heterochrony in development. J Hum Evol 66, 83–88.
Bell, C.G., Lowe, R., Adams, P.D., Baccarelli, A.A., Beck, S., Bell, J.T., Christensen, B.C., Gladyshev, V.N., Heijmans, B.T., Horvath, S., et al. (2019). DNA methylation aging clocks: challenges and recommendations. Genome Biol 20, 249.
Bell, J.H., Herrera, A.H., Li, Y., and Walcheck, B. (2007). Role of ADAM17 in the ectodomain shedding of TNF-α and its receptors by neutrophils and macrophages. J Leukoc Biol 82, 173–176.
Belsky, D.W., Caspi, A., Corcoran, D.L., Sugden, K., Poulton, R., Arseneault, L., Baccarelli, A., Chamarti, K., Gao, X., Hannon, E., et al. (2022). DunedinPACE, a DNA methylation biomarker of the pace of aging. eLife 11, e73420.
Belyavsky, A., Petinati, N., and Drize, N. (2021). Hematopoiesis during ontogenesis, adult life, and aging. Int J Mol Sci 22, 9231.
Ben-Saadon, R., Fajerman, I., Ziv, T., Hellman, U., Schwartz, A.L., and Ciechanover, A. (2004). The tumor suppressor protein $p16^{INK4a}$ and the human papillomavirus oncoprotein-58 E7 are naturally occurring lysine-less proteins that are degraded by the ubiquitin system. J Biol Chem 279, 41414–41421.
Ben-Zvi, A., Miller, E.A., and Morimoto, R.I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. Proc Natl Acad Sci USA 106, 14914–14919.
Bendix, L., Horn, P.B., Jensen, U.B., Rubelj, I., and Kolvraa, S. (2010). The load of short telomeres, estimated by a new method, Universal STELA, correlates with number of senescent cells. Aging Cell 9, 383–397.
Bengal, E., Perdiguero, E., Serrano, A.L., and Muñoz-Cánoves, P. (2017). Rejuvenating stem cells to restore muscle regeneration in aging. F1000Res 6, 76.
Bennett, A.E., Wahner, H.W., Riggs, B.L., and Hintz, R.L. (1984). Insulin-like growth factors I and II: aging and bone density in women. J Clin Endocrinol Metab 59, 701–704.
Bennett, I.J., and Madden, D.J. (2014). Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience 276, 187–205.
Bentov, Y., Yavorska, T., Esfandiari, N., Jurisicova, A., and Casper, R.F. (2011). The contribution of mitochondrial function to reproductive aging. J Assist Reprod Genet 28, 773–783.
Benz, C.C. (2008). Impact of aging on the biology of breast cancer. Crit Rev Oncol Hematol 66, 65–74.
Berdyshev, G.D., Korotaev, G.K., Boiarskikh, G.V., and Vaniushin, B.F. (1967). Nucleotide composition of DNA and RNA from somatic tissues of humpback and its changes during spawning (in Russian). Biokhimiia 32, 988–993.
Bernard, C., Dilharreguy, B., Helmer, C., Chanraud, S., Amieva, H., Dartigues, J.F., Allard, M., and Catheline, G. (2015). PCC characteristics at rest in 10-year memory decliners. Neurobiol Aging 36, 2812–2820.
Bernasochi, G.B., Boon, W.C., Curl, C.L., Varma, U., Pepe, S., Tare, M., Parry, L.J., Dimitriadis, E., Harrap, S.B., Nalliah, C.J., et al. (2017). Pericardial adipose and aromatase: A new translational target for aging, obesity and arrhythmogenesis? J Mol Cell Cardiol 111, 96–101.
Bernet, J.D., Doles, J.D., Hall, J.K., Kelly Tanaka, K., Carter, T.A., and Olwin, B.B. (2014). p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. Nat Med 20, 265–271.
Bernitz, J.M., Kim, H.S., MacArthur, B., Sieburg, H., and Moore, K. (2016). Hematopoietic stem cells count and remember self-renewal divisions. Cell 167, 1296–1309.e10.
Berron, D., van Westen, D., Ossenkoppele, R., Strandberg, O., and Hansson, O. (2020). Medial temporal lobe connectivity and its associations with cognition in early Alzheimer's disease. Brain 143, 1233–1248.
Berron, D., Vogel, J.W., Insel, P.S., Pereira, J.B., Xie, L., Wisse, L.E.M., Yushkevich, P.A., Palmqvist, S., Mattsson-Carlgren, N., Stomrud, E., et al. (2021). Early stages of tau pathology and its associations with functional connectivity, atrophy and memory. Brain 144, 2771–2783.
Berry, D.C., Jiang, Y., Arpke, R.W., Close, E.L., Uchida, A., Reading, D., Berglund, E.D., Kyba, M., and Graff, J.M. (2017). Cellular aging contributes to failure of cold-induced beige adipocyte formation in old mice and humans. Cell Metab 25, 166–181.
Bertrand, M.J.M., Milutinovic, S., Dickson, K.M., Ho, W.C., Boudreault, A., Durkin, J., Gillard, J.W., Jaquith, J.B., Morris, S.J., and Barker, P.A. (2008). cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 30, 689–700.
Bhasin, S., Valderrábano, R.J., and Gagliano-Jucá, T. (2000). Age-Related Changes in the Male Reproductive System. In: Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., de Herder, W.W., Dhatariya, K., Dungan, K., Hershman, J.M., Hofland, J., Kalra, S., et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.
Bhawan, J., Andersen, W., Lee, J., Labadie, R., and Solares, G. (1995).

Photoaging versus intrinsic aging: a morphologic assessment of facial skin. J Cutan Pathol 22, 154–159.
Bhushan, M., Cumberbatch, M., Dearman, R.J., Andrew, S.M., Kimber, I., and Griffiths, C.E.M. (2002). Tumour necrosis factor-α-induced migration of human Langerhans cells: the influence of ageing. Br J Dermatol 146, 32–40.
Bi, S., Liu, Z., Wu, Z., Wang, Z., Liu, X., Wang, S., Ren, J., Yao, Y., Zhang, W., Song, M., et al. (2020). SIRT7 antagonizes human stem cell aging as a heterochromatin stabilizer. Protein Cell 11, 483–504.
Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., Consolandi, C., Quercia, S., Scurti, M., Monti, D., et al. (2016). Gut microbiota and extreme longevity. Curr Biol 26, 1480–1485.
Biering-Sørensen, T., Biering-Sørensen, S.R., Olsen, F.J., Sengeløv, M., Jørgensen, P.G., Mogelvang, R., Shah, A.M., and Jensen, J.S. (2017). Global longitudinal strain by echocardiography predicts long-term risk of cardiovascular morbidity and mortality in a low-risk general population. Circ Cardiovasc Imag 10, e005521.
Biermann, M., and Reya, T. (2022). Hematopoietic stem cells and regeneration. Cold Spring Harb Perspect Biol 14, a040774.
Bilbao, A., Varesio, E., Luban, J., Strambio-De-Castillia, C., Hopfgartner, G., Müller, M., and Lisacek, F. (2015). Processing strategies and software solutions for data-independent acquisition in mass spectrometry. Proteomics 15, 964–980.
Biran, A., Zada, L., Abou Karam, P., Vadai, E., Roitman, L., Ovadya, Y., Porat, Z., and Krizhanovsky, V. (2017). Quantitative identification of senescent cells in aging and disease. Aging Cell 16, 661–671.
Birch Petersen, K., Hvidman, H.W., Forman, J.L., Pinborg, A., Larsen, E. C., Macklon, K.T., Sylvest, R., and Andersen, A.N. (2015). Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan. Hum Reprod 30, 2364–2375.
Biroccio, A., Cherfils-Vicini, J., Augereau, A., Pinte, S., Bauwens, S., Ye, J., Simonet, T., Horard, B., Jamet, K., Cervera, L., et al. (2013). TRF2 inhibits a cell-extrinsic pathway through which natural killer cells eliminate cancer cells. Nat Cell Biol 15, 818–828.
Bishop, L.A., Richter, K.S., Patounakis, G., Andriani, L., Moon, K., and Devine, K. (2017). Diminished ovarian reserve as measured by means of baseline follicle-stimulating hormone and antral follicle count is not associated with pregnancy loss in younger *in vitro* fertilization patients. Fertil Steril 108, 980–987.
Bisio, A., Latorre, E., Andreotti, V., Bressac-de Paillerets, B., Harland, M., Scarra, G.B., Ghiorzo, P., Spitale, R.C., Provenzani, A., and Inga, A. (2015). The 5′-untranslated region of $p16^{INK4a}$ melanoma tumor suppressor acts as a cellular IRES, controlling mRNA translation under hypoxia through YBX1 binding. Oncotarget 6, 39980–39994.
Bjornson, C.R.R., Cheung, T.H., Liu, L., Tripathi, P.V., Steeper, K.M., and Rando, T.A. (2012). Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells 30, 232–242.
Blackburn, E.H., Epel, E.S., and Lin, J. (2015). Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science 350, 1193–1198.
Blair, M.J., Jones, J.D., Woessner, A.E., and Quinn, K.P. (2020). Skin structure-function relationships and the wound healing response to intrinsic aging. Adv Wound Care 9, 127–143.
Blank, C.U., Haining, W.N., Held, W., Hogan, P.G., Kallies, A., Lugli, E., Lynn, R.C., Philip, M., Rao, A., Restifo, N.P., et al. (2019). Defining 'T cell exhaustion'. Nat Rev Immunol 19, 665–674.
Blebea, J.S., Houseni, M., Torigian, D.A., Fan, C., Mavi, A., Zhuge, Y., Iwanaga, T., Mishra, S., Udupa, J., Zhuang, J., et al. (2007). Structural and functional imaging of normal bone marrow and evaluation of its age-related changes. Semin Nucl Med 37, 185–194.
Blinkouskaya, Y., Caçoilo, A., Gollamudi, T., Jalalian, S., and Weickenmeier, J. (2021). Brain aging mechanisms with mechanical manifestations. Mech Ageing Dev 200, 111575.
Blinkouskaya, Y., and Weickenmeier, J. (2021). Brain shape changes associated with cerebral atrophy in healthy aging and Alzheimer's disease. Front Mech Eng 7.
Blokzijl, F., de Ligt, J., Jager, M., Sasselli, V., Roerink, S., Sasaki, N., Huch, M., Boymans, S., Kuijk, E., Prins, P., et al. (2016). Tissue-specific mutation accumulation in human adult stem cells during life. Nature 538, 260–264.
Bloomer, S.A., and Moyer, E.D. (2021). Hepatic macrophage accumulation with aging: cause for concern? Am J Physiol Gastrointest Liver Physiol 320, G496–G505.
Blume-Peytavi, U., Kottner, J., Sterry, W., Hodin, M.W., Griffiths, T.W., Watson, R.E.B., Hay, R.J., and Griffiths, C.E.M. (2016). Age-associated skin conditions and diseases: current perspectives and future options. Gerontologist 56, S230–S242.
Bobrov, E., Georgievskaya, A., Kiselev, K., Sevastopolsky, A., Zhavoronkov, A., Gurov, S., Rudakov, K., del Pilar Bonilla Tobar, M., Jaspers, S., and Clemann, S. (2018). PhotoAgeClock: deep learning algorithms for development of non-invasive visual biomarkers of aging. Aging 10, 3249–3259.
Bocklandt, S., Lin, W., Sehl, M.E., Sánchez, F.J., Sinsheimer, J.S., Horvath, S., and Vilain, E. (2011). Epigenetic predictor of age. PLoS ONE 6, e14821.
Boerrigter, M.E.T.I., Dollé, M.E.T., Martus, H.J., Gossen, J.A., and Vijg, J. (1995). Plasmid-based transgenic mouse model for studying *in vivo* mutations. Nature 377, 657–659.
Bogeska, R., Mikecin, A.M., Kaschutnig, P., Fawaz, M., Büchler-Schäff, M., Le, D., Ganuza, M., Vollmer, A., Paffenholz, S.V., Asada, N., et al. (2022). Inflammatory exposure drives long-lived impairment of hematopoietic stem cell self-renewal activity and accelerated aging. Cell Stem Cell 29, 1273–1284.e8.
Bohl, J., Steinmetz, H., and Störkel, S. (1991). Age-related accumulation of congophilic fibrillar inclusions in endocrine cells. Vichows Archiv Pathol Anat 419, 51–58.
Boisvert, M.M., Erikson, G.A., Shokhirev, M.N., and Allen, N.J. (2018). The aging astrocyte transcriptome from multiple regions of the mouse brain. Cell Rep 22, 269–285.
Bollati, V., Schwartz, J., Wright, R., Litonjua, A., Tarantini, L., Suh, H., Sparrow, D., Vokonas, P., and Baccarelli, A. (2009). Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech Ageing Dev 130, 234–239.
Bolignano, D., Mattace-Raso, F., Sijbrands, E.J.G., and Zoccali, C. (2014). The aging kidney revisited: a systematic review. Ageing Res Rev 14, 65–80.
Bonaconsa, M., Malpeli, G., Montaruli, A., Carandente, F., Grassi-Zucconi, G., and Bentivoglio, M. (2014). Differential modulation of clock gene expression in the suprachiasmatic nucleus, liver and heart of aged mice. Exp Gerontol 55, 70–79.
Bond, J., Jones, C., Haughton, M., DeMicco, C., Kipling, D., and Wynford-Thomas, D. (2004). Direct evidence from siRNA-directed "knock down" that $p16^{INK4a}$ is required for human fibroblast senescence and for limiting ras-induced epithelial cell proliferation. Exp Cell Res 292, 151–156.
Bonev, B., and Cavalli, G. (2016). Organization and function of the 3D genome. Nat Rev Genet 17, 661–678.
Bonte, S., Vandemaele, P., Verleden, S., Audenaert, K., Deblaere, K., Goethals, I., and Van Holen, R. (2017). Healthy brain ageing assessed with 18F-FDG PET and age-dependent recovery factors after partial volume effect correction. Eur J Nucl Med Mol Imag 44, 838–849.
Boonen, S., Aerssens, J., Dequeker, J., Nicholson, P., Cheng, X.G., Lowet, G., Verbeke, G., and Bouillon, R. (1997). Age-associated decline in human femoral neck cortical and trabecular content of insulin-like growth factor I: potential implications for age-related (type II) osteoporotic fracture occurrence. Calcif Tissue Int 61, 173–178.
Borgdorff, V., Lleonart, M.E., Bishop, C.L., Fessart, D., Bergin, A.H., Overhoff, M.G., and Beach, D.H. (2010). Multiple microRNAs rescue from Ras-induced senescence by inhibiting p21Waf1/Cip1. Oncogene 29, 2262–2271.
Borghesan, M., Fafián-Labora, J., Eleftheriadou, O., Carpintero-Fernández, P., Paez-Ribes, M., Vizcay-Barrena, G., Swisa, A., Kolodkin-Gal, D., Ximénez-Embún, P., Lowe, R., et al. (2019). Small extracellular vesicles are key regulators of non-cell autonomous intercellular communication in senescence via the interferon protein IFITM3. Cell Rep 27, 3956–3971.e6.

Borghesan, M., Hoogaars, W.M.H., Varela-Eirin, M., Talma, N., and Demaria, M. (2020). A senescence-centric view of aging: implications for longevity and disease. Trends Cell Biol 30, 777–791.
Boroni, M., Zonari, A., Reis de Oliveira, C., Alkatib, K., Ochoa Cruz, E.A., Brace, L.E., and Lott de Carvalho, J. (2020). Highly accurate skin-specific methylome analysis algorithm as a platform to screen and validate therapeutics for healthy aging. Clin Epigenet 12, 105.
Bosco, N., and Noti, M. (2021). The aging gut microbiome and its impact on host immunity. Genes Immun 22, 289–303.
Bosset, S., Bonnet-Duquennoy, M., Barre, P., Chalon, A., Kurfurst, R., Bonte, F., Schnebert, S., Le Varlet, B., and Nicolas, J.F. (2003). Photoageing shows histological features of chronic skin inflammation without clinical and molecular abnormalities. Br J Dermatol 149, 826–835.
Bossone, E., and Eagle, K.A. (2021). Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes. Nat Rev Cardiol 18, 331–348.
Boström, P., Wu, J., Jedrychowski, M.P., Korde, A., Ye, L., Lo, J.C., Rasbach, K.A., Boström, E.A., Choi, J.H., Long, J.Z., et al. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481, 463–468.
Bota, D.A., Van Remmen, H., and Davies, K.J.A. (2002). Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. FEBS Lett 532, 103–106.
Boutin, R.D., Houston, D.K., Chaudhari, A.S., Willis, M.H., Fausett, C.L., and Lenchik, L. (2022). Imaging of sarcopenia. Radiol Clin North Am 60, 575–582.
Bowdish, D.M.E. (2019). The aging lung. Chest 155, 391–400.
Boyle, W.J., Simonet, W.S., and Lacey, D.L. (2003). Osteoclast differentiation and activation. Nature 423, 337–342.
Brack, A.S., Conboy, M.J., Roy, S., Lee, M., Kuo, C.J., Keller, C., and Rando, T.A. (2007). Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 317, 807–810.
Bracken, A.P., Kleine-Kohlbrecher, D., Dietrich, N., Pasini, D., Gargiulo, G., Beekman, C., Theilgaard-Mönch, K., Minucci, S., Porse, B.T., Marine, J.C., et al. (2007). The Polycomb group proteins bind throughout the *INK4A-ARF* locus and are disassociated in senescent cells. Genes Dev 21, 525–530.
Branchet, M.C., Boisnic, S., Frances, C., and Robert, A.M. (1990). Skin thickness changes in normal aging skin. Gerontology 36, 28–35.
Brandhorst, S., Choi, I.Y., Wei, M., Cheng, C.W., Sedrakyan, S., Navarrete, G., Dubeau, L., Yap, L.P., Park, R., Vinciguerra, M., et al. (2015). A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. Cell Metab 22, 86–99.
Brandt, T., Mourier, A., Tain, L.S., Partridge, L., Larsson, N.G., and Kühlbrandt, W. (2017). Changes of mitochondrial ultrastructure and function during ageing in mice and *Drosophila*. eLife 6, e24662.
Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324, 1729–1732.
Braverman, I.M., and Fonferko, E. (1982). Studies in cutaneous aging: I. The elastic fiber network. J Invest Dermatol 78, 434–443.
Brenner, D.J., and Hall, E.J. (2007). Computed tomography—an increasing source of radiation exposure. N Engl J Med 357, 2277–2284.
Brinkmann, V., and Zychlinsky, A. (2007). Beneficial suicide: why neutrophils die to make NETs. Nat Rev Microbiol 5, 577–582.
Brody, H. (1955). Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. J Comp Neurol 102, 511–556.
Broekmans, F.J., Soules, M.R., and Fauser, B.C. (2009). Ovarian aging: mechanisms and clinical consequences. Endocrine Rev 30, 465–493.
Broer, S.L., Broekmans, F.J.M., Laven, J.S.E., and Fauser, B.C.J.M. (2014). Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 20, 688–701.
Brown, D.D., and Gurdon, J.B. (1964). Absence of ribosomal RNA synthesis in the anucleolate mutant of *Xenopus laevis*. Proc Natl Acad Sci USA 51, 139–146.
Brown, J.P., Wei, W., and Sedivy, J.M. (1997). Bypass of senescence after disruption of $p21^{CIP1/WAF1}$ gene in normal diploid human fibroblasts. Science 277, 831–834.
Brown, W.R., and Thore, C.R. (2011). Review: cerebral microvascular pathology in ageing and neurodegeneration. Neuropathol Appl Neurobiol 37, 56–74.
Brubaker, A.L., Rendon, J.L., Ramirez, L., Choudhry, M.A., and Kovacs, E.J. (2013). Reduced neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection with advanced age. J Immunol 190, 1746–1757.
Brugnolo, A., Morbelli, S., Arnaldi, D., De Carli, F., Accardo, J., Bossert, I., Dessi, B., Famà, F., Ferrara, M., Girtler, N., et al. (2014). Metabolic correlates of Rey auditory verbal learning test in elderly subjects with memory complaints. J Alzheimer Dis 39, 103–113.
Brunet, A., Goodell, M.A., and Rando, T.A. (2023). Ageing and rejuvenation of tissue stem cells and their niches. Nat Rev Mol Cell Biol 24, 45–62.
Bucaciuc Mracica, T., Anghel, A., Ion, C.F., Moraru, C.V., Tacutu, R., and Lazar, G.A. (2020). MetaboAge DB: a repository of known ageing-related changes in the human metabolome. Biogerontology 21, 763–771.
Bucci, M., Chiotis, K., and Nordberg, A. (2021). Alzheimer's disease profiled by fluid and imaging markers: tau PET best predicts cognitive decline. Mol Psychiatry 26, 5888–5898.
Buchwalter, A., and Hetzer, M.W. (2017). Nucleolar expansion and elevated protein translation in premature aging. Nat Commun 8, 328.
Buckley, M.T., Sun, E.D., George, B.M., Liu, L., Schaum, N., Xu, L., Reyes, J.M., Goodell, M.A., Weissman, I.L., Wyss-Coray, T., et al. (2023). Cell-type-specific aging clocks to quantify aging and rejuvenation in neurogenic regions of the brain. Nat Aging 3, 121–137.
Buenrostro, J.D., Wu, B., Litzenburger, U.M., Ruff, D., Gonzales, M.L., Snyder, M.P., Chang, H.Y., and Greenleaf, W.J. (2015). Single-cell chromatin accessibility reveals principles of regulatory variation. Nature 523, 486–490.
Buffa, S., Borzì, D., Chiarelli, R., Crapanzano, F., Lena, A.M., Nania, M., Candi, E., Triolo, F., Ruvolo, G., Melino, G., et al. (2019). Biomarkers for vascular ageing in aorta tissues and blood samples. Exp Gerontol 128, 110741.
Bulteau, R., and Francesconi, M. (2022). Real age prediction from the transcriptome with RAPToR. Nat Methods 19, 969–975.
Bunger, M.K., Wilsbacher, L.D., Moran, S.M., Clendenin, C., Radcliffe, L. A., Hogenesch, J.B., Simon, M.C., Takahashi, J.S., and Bradfield, C.A. (2000). Mop3 is an essential component of the master circadian pacemaker in mammals. Cell 103, 1009–1017.
Bunnik, E.M., Richard, E., Milne, R., and Schermer, M.H.N. (2018). On the personal utility of Alzheimer's disease-related biomarker testing in the research context. J Med Ethics 44, 830–834.
Burd, C.E., Sorrentino, J.A., Clark, K.S., Darr, D.B., Krishnamurthy, J., Deal, A.M., Bardeesy, N., Castrillon, D.H., Beach, D.H., and Sharpless, N.E. (2013). Monitoring tumorigenesis and senescence *in vivo* with a $p16^{INK4a}$-luciferase model. Cell 152, 340–351.
Burge, R., Dawson-Hughes, B., Solomon, D.H., Wong, J.B., King, A., and Tosteson, A. (2007). Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. J Bone Miner Res 22, 465–475.
Burkewitz, K., Morantte, I., Weir, H.J.M., Yeo, R., Zhang, Y., Huynh, F.K., Ilkayeva, O.R., Hirschey, M.D., Grant, A.R., and Mair, W.B. (2015). Neuronal CRTC-1 governs systemic mitochondrial metabolism and lifespan via a catecholamine signal. Cell 160, 842–855.
Burns, D.M., D'Ambrogio, A., Nottrott, S., and Richter, J.D. (2011). CPEB and two poly(A) polymerases control miR-122 stability and p53 mRNA translation. Nature 473, 105–108.
Burtner, C.R., and Kennedy, B.K. (2010). Progeria syndromes and ageing: what is the connection? Nat Rev Mol Cell Biol 11, 567–578.
Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J.L., Cerletti, M., Jang, Y., Sefik, E., Tan, T.G., Wagers, A.J., Benoist, C., et al. (2013). A special population of regulatory T cells potentiates muscle repair. Cell 155, 1282–1295.

Buscarlet, M., Provost, S., Zada, Y.F., Barhdadi, A., Bourgoin, V., Lépine, G., Mollica, L., Szuber, N., Dubé, M.P., and Busque, L. (2017). DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. Blood 130, 753–762.

Busche, M.A., and Hyman, B.T. (2020). Synergy between amyloid-β and tau in Alzheimer's disease. Nat Neurosci 23, 1183–1193.

Busnelli, A., Navarra, A., and Levi-Setti, P.E. (2021). Qualitative and quantitative ovarian and peripheral blood mitochondrial DNA (mtDNA) alterations: mechanisms and implications for female fertility. Antioxidants 10, 55.

Busuttil, R.A., Dollé, M., Campisi, J., and Vijga, J. (2004). Genomic instability, aging, and cellular senescence. Ann N Y Acad Sci 1019, 245–255.

Butterfield, D.A., and Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci 20, 148–160.

Caballero, M.Á.A., Song, Z., Rubinski, A., Duering, M., Dichgans, M., Park, D.C., and Ewers, M. (2020). Age-dependent amyloid deposition is associated with white matter alterations in cognitively normal adults during the adult life span. Alzheimers Dement 16, 651–661.

Caglar, V., Songur, A., Yagmurca, M., Acar, M., Toktas, M., and Gonul, Y. (2012). Age-related volumetric changes in pancreas: a stereological study on computed tomography. Surg Radiol Anat 34, 935–941.

Cai, J., Guo, X., Wang, K., Zhang, Y., Zhang, D., Zhang, X., and Wang, X. (2022a). Automatic quantitative evaluation of normal pancreas based on deep learning in a Chinese adult population. Abdom Radiol 47, 1082–1090.

Cai, K., Wang, F., Lu, J.Q., Shen, A.N., Zhao, S.M., Zang, W.D., Gui, Y.H., and Zhao, J.Y. (2022b). Nicotinamide mononucleotide alleviates cardiomyopathy phenotypes caused by short-chain enoyl-CoA hydratase 1 deficiency. JACC Basic Transl Sci 7, 348–362.

Cai, Y., Ji, Z., Wang, S., Zhang, W., Qu, J., Belmonte, J.C.I., and Liu, G.H. (2022c). Genetic enhancement: an avenue to combat aging-related diseases. Life Med 1, 307–318.

Cai, Y., Song, W., Li, J., Jing, Y., Liang, C., Zhang, L., Zhang, X., Zhang, W., Liu, B., An, Y., et al. (2022d). The landscape of aging. Sci China Life Sci 65, 2354–2454.

Caiado, F., Kovtonyuk, L.V., Gonullu, N.G., Fullin, J., Boettcher, S., and Manz, M.G. (2023). Aging drives *Tet2*+/− clonal hematopoiesis via IL-1 signaling. Blood 141, 886–903.

Caiado, F., Pietras, E.M., and Manz, M.G. (2021). Inflammation as a regulator of hematopoietic stem cell function in disease, aging, and clonal selection. J Exp Med 218.

Cakala-Jakimowicz, M., Kolodziej-Wojnar, P., and Puzianowska-Kuznicka, M. (2021). Aging-related cellular, structural and functional changes in the lymph nodes: a significant component of immunosenescence? An overview. Cells 10, 3148.

Calleja-Conde, J., Echeverry-Alzate, V., Bühler, K.M., Durán-González, P., Morales-García, J., Segovia-Rodríguez, L., Rodríguez de Fonseca, F., Giné, E., and López-Moreno, J. (2021). The immune system through the lens of alcohol intake and gut microbiota. Int J Mol Sci 22, 7485.

Calvani, R., Marini, F., Cesari, M., Tosato, M., Anker, S.D., von Haehling, S., Miller, R.R., Bernabei, R., Landi, F., and Marzetti, E. (2015). Biomarkers for physical frailty and sarcopenia: state of the science and future developments. J Cachexia Sarcopenia Muscle 6, 278–286.

Camell, C.D., Yousefzadeh, M.J., Zhu, Y., Prata, L.G.P.L., Huggins, M.A., Pierson, M., Zhang, L., O'Kelly, R.D., Pirtskhalava, T., Xun, P., et al. (2021). Senolytics reduce coronavirus-related mortality in old mice. Science 373, eabe4832.

Campbell, R.T., Jasilek, A., Mischak, H., Nkuipou-Kenfack, E., Latosinska, A., Welsh, P.I., Jackson, C.E., Cannon, J., McConnachie, A., Delles, C., et al. (2020). The novel urinary proteomic classifier HF1 has similar diagnostic and prognostic utility to BNP in heart failure. ESC Heart Fail 7, 1595–1604.

Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120, 513–522.

Campisi, J., Kapahi, P., Lithgow, G.J., Melov, S., Newman, J.C., and Verdin, E. (2019). From discoveries in ageing research to therapeutics for healthy ageing. Nature 571, 183–192.

Campos, C., López, N., Pera, A., Gordillo, J.J., Hassouneh, F., Tarazona, R., and Solana, R. (2015). Expression of NKp30, NKp46 and DNAM-1 activating receptors on resting and IL-2 activated NK cells from healthy donors according to CMV-serostatus and age. Biogerontology 16, 671–683.

Campos, C., Pera, A., Sanchez-Correa, B., Alonso, C., Lopez-Fernandez, I., Morgado, S., Tarazona, R., and Solana, R. (2014). Effect of age and CMV on NK cell subpopulations. Exp Gerontol 54, 130–137.

Camps-Renom, P., Prats-Sánchez, L., Casoni, F., González-de-Echávarri, J. M., Marrero-González, P., Castrillón, I., Marín, R., Jiménez-Xarrié, E., Delgado-Mederos, R., Martínez-Domeño, A., et al. (2020). Plaque neovascularization detected with contrast-enhanced ultrasound predicts ischaemic stroke recurrence in patients with carotid atherosclerosis. Eur J Neurol 27, 809–816.

Cao, J.J., Wronski, T.J., Iwaniec, U., Phleger, L., Kurimoto, P., Boudignon, B., and Halloran, B.P. (2005). Aging increases stromal/osteoblastic cell-induced osteoclastogenesis and alters the osteoclast precursor pool in the mouse. J Bone Miner Res 20, 1659–1668.

Cao, Q., Wang, W., Williams, J.B., Yang, F., Wang, Z.J., and Yan, Z. (2020). Targeting histone K4 trimethylation for treatment of cognitive and synaptic deficits in mouse models of Alzheimer's disease. Sci Adv 6, eabc8096.

Cao, X., Yang, G., Jin, X., He, L., Li, X., Zheng, Z., Liu, Z., and Wu, C. (2021a). A machine learning-based aging measure among middle-aged and older Chinese adults: The China Health and Retirement Longitudinal Study. Front Med 8, 698851.

Cao, Y., Zhou, K., Diao, W., Long, X., Tian, F., Su, M., and Jia, Z. (2021b). Age-related changes of standardized uptake values in the blood pool and liver: a decade-long retrospective study of the outcomes of 2,526 subjects. Quant Imag Med Surg 11, 95–106.

Cao, Z., Lis, R., Ginsberg, M., Chavez, D., Shido, K., Rabbany, S.Y., Fong, G.H., Sakmar, T.P., Rafii, S., and Ding, B.S. (2016). Targeting of the pulmonary capillary vascular niche promotes lung alveolar repair and ameliorates fibrosis. Nat Med 22, 154–162.

Capalbo, A., Bono, S., Spizzichino, L., Biricik, A., Baldi, M., Colamaria, S., Ubaldi, F.M., Rienzi, L., and Fiorentino, F. (2013). Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. Hum Reprod 28, 509–518.

Carlson, M.E., Hsu, M., and Conboy, I.M. (2008). Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 454, 528–532.

Carnio, S., LoVerso, F., Baraibar, M.A., Longa, E., Khan, M.M., Maffei, M., Reischl, M., Canepari, M., Loefler, S., Kern, H., et al. (2014). Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. Cell Rep 8, 1509–1521.

Carrasco, E., Gómez de las Heras, M.M., Gabandé-Rodríguez, E., Desdín-Micó, G., Aranda, J.F., and Mittelbrunn, M. (2022). The role of T cells in age-related diseases. Nat Rev Immunol 22, 97–111.

Johnston, C.D., Siegler, E.L., Rice, M.C., Derry, H.M., Hootman, K.C., Zhu, Y.S., Burchett, C.O., Gupta, S.K., Choi, M.E., and Glesby, M.J. (2021). Urine cell-free mitochondrial DNA as a marker of weight loss and body composition in older adults with HIV. J Acquir Immune Defic Syndr 88, 229–233.

Carrier, J., Monk, T.H., Buysse, D.J., and Kupfer, D.J. (1997). Sleep and morningness-eveningness in the 'middle' years of life (20-59y). J Sleep Res 6, 230–237.

Carter, E.A., Bonab, A.A., Paul, K., Yerxa, J., Tompkins, R.G., and Fischman, A.J. (2011). Association of heat production with ^{18}F-FDG accumulation in murine brown adipose tissue after stress. J Nucl Med 52, 1616–1620.

Castanheira, F.V.S., and Kubes, P. (2019). Neutrophils and NETs in modulating acute and chronic inflammation. Blood 133, 2178–2185.

Castellano, J.M., Mosher, K.I., Abbey, R.J., McBride, A.A., James, M.L., Berdnik, D., Shen, J.C., Zou, B., Xie, X.S., Tingle, M., et al. (2017).

Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature 544, 488–492.
Catalán, J., Autio, K., Kuosma, E., and Norppa, H. (1998). Age-dependent inclusion of sex chromosomes in lymphocyte micronuclei of man. Am J Hum Genet 63, 1464–1472.
Cawthon, R.M. (2002). Telomere measurement by quantitative PCR. Nucleic Acids Res 30, 47e–47.
Cayetanot, F., Bentivoglio, M., and Aujard, F. (2005). Arginine-vasopressin and vasointestinal polypeptide rhythms in the suprachiasmatic nucleus of the mouse lemur reveal aging-related alterations of circadian pacemaker neurons in a non-human primate. Eur J Neurosci 22, 902–910.
Cedar, H., and Bergman, Y. (2012). Programming of DNA methylation patterns. Ann Rev Biochem 81, 97–117.
Cedars, M.I. (2022). Evaluation of female fertility—AMH and ovarian reserve testing. J Clin Endocrinol Metab 107, 1510–1519.
Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K., and Deanfield, J.E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340, 1111–1115.
Chai, Q., Onder, L., Scandella, E., Gil-Cruz, C., Perez-Shibayama, C., Cupovic, J., Danuser, R., Sparwasser, T., Luther, S.A., Thiel, V., et al. (2013). Maturation of lymph node fibroblastic reticular cells from myofibroblastic precursors is critical for antiviral immunity. Immunity 38, 1013–1024.
Chaib, S., Tchkonia, T., and Kirkland, J.L. (2022). Cellular senescence and senolytics: the path to the clinic. Nat Med 28, 1556–1568.
Chakkalakal, J.V., Jones, K.M., Basson, M.A., and Brack, A.S. (2012). The aged niche disrupts muscle stem cell quiescence. Nature 490, 355–360.
Chakrabarti, S., Munshi, S., Banerjee, K., Thakurta, I.G., Sinha, M., and Bagh, M.B. (2011). Mitochondrial dysfunction during brain aging: role of oxidative stress and modulation by antioxidant supplementation. Aging Dis 2, 242–256.
Chakravarti, D., LaBella, K.A., and DePinho, R.A. (2021). Telomeres: history, health, and hallmarks of aging. Cell 184, 306–322.
Chaleckis, R., Murakami, I., Takada, J., Kondoh, H., and Yanagida, M. (2016). Individual variability in human blood metabolites identifies age-related differences. Proc Natl Acad Sci USA 113, 4252–4259.
Chambers, E.S., and Akbar, A.N. (2020). Can blocking inflammation enhance immunity during aging? J Allergy Clin Immunol 145, 1323–1331.
Chambers, S.M., Shaw, C.A., Gatza, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A. (2007). Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol 5, e201.
Chan, C.C.W., Liu, V.W.S., Lau, E.Y.L., Yeung, W.S.B., Ng, E.H.Y., and Ho, P.C. (2005). Mitochondrial DNA content and 4977 bp deletion in unfertilized oocytes. Mol Hum Reprod 11, 843–846.
Chan, C.K.F., Gulati, G.S., Sinha, R., Tompkins, J.V., Lopez, M., Carter, A. C., Ransom, R.C., Reinisch, A., Wearda, T., Murphy, M., et al. (2018). Identification of the human skeletal stem cell. Cell 175, 43–56.e21.
Chan, K.T., Blake, S., Zhu, H., Kang, J., Trigos, A.S., Madhamshettiwar, P. B., Diesch, J., Paavolainen, L., Horvath, P., Hannan, R.D., et al. (2020). A functional genetic screen defines the AKT-induced senescence signaling network. Cell Death Differ 27, 725–741.
Chan, M.Y., Park, D.C., Savalia, N.K., Petersen, S.E., and Wig, G.S. (2014). Decreased segregation of brain systems across the healthy adult lifespan. Proc Natl Acad Sci USA 111, E4997–E5006.
Chandra, A., and Rajawat, J. (2021). Skeletal aging and osteoporosis: mechanisms and therapeutics. Int J Mol Sci 22, 3553.
Chandra, T., Ewels, P.A., Schoenfelder, S., Furlan-Magaril, M., Wingett, S. W., Kirschner, K., Thuret, J.Y., Andrews, S., Fraser, P., and Reik, W. (2015). Global reorganization of the nuclear landscape in senescent cells. Cell Rep 10, 471–483.
Chang, A.M., and Halter, J.B. (2003). Aging and insulin secretion. Am J Physiol Endocrinol Metab 284, E7–E12.
Chang, A.M., Smith, M.J., Galecki, A.T., Bloem, C.J., and Halter, J.B. (2006). Impaired β-cell function in human aging: response to nicotinic acid-induced insulin resistance. J Clin Endocrinol Metab 91, 3303–3309.
Chang, J., Wang, Y., Shao, L., Laberge, R.M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N.E., Ding, S., Feng, W., et al. (2016). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med 22, 78–83.
Chang, J.S., Kim, T.H., Nguyen, T.T., Park, K.S., Kim, N., and Kong, I.D. (2017). Circulating irisin levels as a predictive biomarker for sarcopenia: A cross-sectional community-based study. Geriatr Gerontol Int 17, 2266–2273.
Charalambous, C., Webster, A., and Schuh, M. (2023). Aneuploidy in mammalian oocytes and the impact of maternal ageing. Nat Rev Mol Cell Biol 24, 27–44.
Chatterjee, S., Daenthanasanmak, A., Chakraborty, P., Wyatt, M.W., Dhar, P., Selvam, S.P., Fu, J., Zhang, J., Nguyen, H., Kang, I., et al. (2018). CD38-NAD$^+$axis regulates immunotherapeutic anti-tumor T cell response. Cell Metab 27, 85–100.e8.
Chaurasia, B., Ying, L., Talbot, C.L., Maschek, J.A., Cox, J., Schuchman, E.H., Hirabayashi, Y., Holland, W.L., and Summers, S.A. (2021). Ceramides are necessary and sufficient for diet-induced impairment of thermogenic adipocytes. Mol Metab 45, 101145.
Chazaud, B. (2020). Inflammation and skeletal muscle regeneration: leave it to the macrophages! Trends Immunol 41, 481–492.
Chee, C.A., Roozendaal, B., Swaab, D.F., Goudsmit, E., and Mirmiran, M. (1988). Vasoactive intestinal polypeptide neuron changes in the senile rat suprachiasmatic nucleus. Neurobiol Aging 9, 307–312.
Chen, C.Y., Logan, R.W., Ma, T., Lewis, D.A., Tseng, G.C., Sibille, E., and McClung, C.A. (2016a). Effects of aging on circadian patterns of gene expression in the human prefrontal cortex. Proc Natl Acad Sci USA 113, 206–211.
Chen, H., Luo, L., Liu, J., and Zirkin, B.R. (2007a). Cyclooxygenases in rat Leydig cells: effects of luteinizing hormone and aging. Endocrinology 148, 735–742.
Chen, J., Lippo, L., Labella, R., Tan, S.L., Marsden, B.D., Dustin, M.L., Ramasamy, S.K., and Kusumbe, A.P. (2021a). Decreased blood vessel density and endothelial cell subset dynamics during ageing of the endocrine system. EMBO J 40.
Chen, J.J., Rosas, H.D., and Salat, D.H. (2011). Age-associated reductions in cerebral blood flow are independent from regional atrophy. NeuroImage 55, 468–478.
Chen, K.Y., Cypess, A.M., Laughlin, M.R., Haft, C.R., Hu, H.H., Bredella, M.A., Enerbäck, S., Kinahan, P.E., Lichtenbelt, W.M., Lin, F.I., et al. (2016b). Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0): recommendations for standardized FDG-PET/CT experiments in humans. Cell Metab 24, 210–222.
Chen, L.Y., Liu, D., and Songyang, Z. (2007b). Telomere maintenance through spatial control of telomeric proteins. Mol Cell Biol 27, 5898–5909.
Chen, L., and Feany, M.B. (2005). α-Synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. Nat Neurosci 8, 657–663.
Chen, L., Liao, F., Wu, J., Wang, Z., Jiang, Z., Zhang, C., Luo, P., Ma, L., Gong, Q., Wang, Y., et al. (2021b). Acceleration of ageing via disturbing mTOR-regulated proteostasis by a new ageing-associated gene PC4. Aging Cell 20, e13370.
Chen, L.L., and Carmichael, G.G. (2009). Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA. Mol Cell 35, 467–478.
Chen, M., Bergman, R.N., Pacini, G., and Porte, D. Jr. (1985). Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased β-cell function. J Clin Endocrinol Metab 60, 13–20.
Chen, M.K., Mecca, A.P., Naganawa, M., Finnema, S.J., Toyonaga, T., Lin, S., Najafzadeh, S., Ropchan, J., Lu, Y., McDonald, J.W., et al. (2018a). Assessing synaptic density in Alzheimer disease with synaptic vesicle glycoprotein 2A positron emission tomographic imaging. JAMA Neurol 75, 1215–1224.
Chen, S., Bohrer, L.R., Rai, A.N., Pan, Y., Gan, L., Zhou, X., Bagchi, A., Simon, J.A., and Huang, H. (2010). Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2. Nat Cell

Biol 12, 1108–1114.
Chen, T.P., Lai, M., Lin, W.Y., Huang, K.C., and Yang, K.C. (2020a). Metabolic profiles and fibrosis of nonalcoholic fatty liver disease in the elderly: a community-based study. J Gastroenterol Hepatol 35, 1636–1643.
Chen, W., Liu, N., Zhang, H., Zhang, H., Qiao, J., Jia, W., Zhu, S., Mao, Z., and Kang, J. (2017). Sirt6 promotes DNA end joining in iPSCs derived from old mice. Cell Rep 18, 2880–2892.
Chen, W., Qian, W., Wu, G., Chen, W., Xian, B., Chen, X., Cao, Y., Green, C.D., Zhao, F., Tang, K., et al. (2015). Three-dimensional human facial morphologies as robust aging markers. Cell Res 25, 574–587.
Chen, X., Zhang, X., Chen, F., Larson, C.S., Wang, L.J., and Kaufman, D. B. (2009). Comparative study of regenerative potential of β cells from young and aged donor mice using a novel islet transplantation model. Transplantation 88, 496–503.
Chen, Y., Chen, J., Sun, X., Yu, J., Qian, Z., Wu, L., Xu, X., Wan, X., Jiang, Y., Zhang, J., et al. (2020b). The SIRT6 activator MDL-800 improves genomic stability and pluripotency of old murine-derived iPS cells. Aging Cell 19, e13185.
Chen, Y., Cui, Z., Chen, Z., Jiang, Y., and Mao, Z. (2022). IDDoR: A novel reporter mouse system for simultaneous and quantitative *in vivo* analysis of both DNA double-strand break repair pathways. Protein Cell doi: 10.1093/procel/pwac001.
Chen, Y., Geng, A., Zhang, W., Qian, Z., Wan, X., Jiang, Y., and Mao, Z. (2020c). Fight to the bitter end: DNA repair and aging. Ageing Res Rev 64, 101154.
Chen, Y., Klein, S.L., Garibaldi, B.T., Li, H., Wu, C., Osevala, N.M., Li, T., Margolick, J.B., Pawelec, G., and Leng, S.X. (2021c). Aging in COVID-19: vulnerability, immunity and intervention. Ageing Res Rev 65, 101205.
Chen, Y., Liu, S., and Leng, S.X. (2019a). Chronic low-grade inflammatory phenotype (CLIP) and senescent immune dysregulation. Clin Ther 41, 400–409.
Chen, Y., Pu, Q., Ma, Y., Zhang, H., Ye, T., Zhao, C., Huang, X., Ren, Y., Qiao, L., Liu, H.M., et al. (2021d). Aging reprograms the hematopoietic-vascular niche to impede regeneration and promote fibrosis. Cell Metab 33, 395–410.e4.
Chen, Z., Amro, E.M., Becker, F., Hölzer, M., Rasa, S.M.M., Njeru, S.N., Han, B., Di Sanzo, S., Chen, Y., Tang, D., et al. (2019b). Cohesin-mediated NF-κB signaling limits hematopoietic stem cell self-renewal in aging and inflammation. J Exp Med 216, 152–175.
Chen, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A., Niki, M., Koutcher, J.A., Scher, H.I., Ludwig, T., Gerald, W., et al. (2005). Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 436, 725–730.
Chen, Z., Yoo, S.H., and Takahashi, J.S. (2018b). Development and therapeutic potential of small-molecule modulators of circadian systems. Annu Rev Pharmacol Toxicol 58, 231–252.
Cheng, C.W., Adams, G.B., Perin, L., Wei, M., Zhou, X., Lam, B.S., Da Sacco, S., Mirisola, M., Quinn, D.I., Dorff, T.B., et al. (2014). Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell 14, 810–823.
Cherfils-Vicini, J., Iltis, C., Cervera, L., Pisano, S., Croce, O., Sadouni, N., Győrffy, B., Collet, R., Renault, V.M., Rey-Millet, M., et al. (2019). Cancer cells induce immune escape via glycocalyx changes controlled by the telomeric protein TRF2. EMBO J 38.
Cheung, I., Shulha, H.P., Jiang, Y., Matevossian, A., Wang, J., Weng, Z., and Akbarian, S. (2010). Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proc Natl Acad Sci USA 107, 8824–8829.
Chhetri, J.K., Xue, Q.L., Ma, L., Chan, P., and Varadhan, R. (2021). Intrinsic capacity as a determinant of physical resilience in older adults. J Nutr Health Aging 25, 1006–1011.
Chianca, V., Albano, D., Messina, C., Gitto, S., Ruffo, G., Guarino, S., Del Grande, F., and Sconfienza, L.M. (2022). Sarcopenia: imaging assessment and clinical application. Abdom Radiol 47, 3205–3216.
Chianese, C., Brilli, S., and Krausz, C. (2014). Genomic changes in spermatozoa of the aging male. In: Baldi, E., and Muratori, M., eds. Genetic Damage in Human Spermatozoa. Advances in Experimental Medicine and Biology. New York, NY: Springer. 13–26.
Chiang, H.M., Chen, H.C., Chiu, H.H., Chen, C.W., Wang, S.M., and Wen, K.C. (2013). *Neonauclea reticulata* (Havil.) Merr stimulates skin regeneration after UVB exposure via ROS scavenging and modulation of the MAPK/MMPs/collagen pathway. Evid Based Complement Alternat Med 2013, 1–9.
Chiang, T., Duncan, F.E., Schindler, K., Schultz, R.M., and Lampson, M.A. (2010). Evidence that weakened centromere cohesion is a leading cause of age-related aneuploidy in oocytes. Curr Biol 20, 1522–1528.
Chien, Y., Scuoppo, C., Wang, X., Fang, X., Balgley, B., Bolden, J.E., Premsrirut, P., Luo, W., Chicas, A., Lee, C.S., et al. (2011). Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. Genes Dev 25, 2125–2136.
Childs, B.G., Baker, D.J., Wijshake, T., Conover, C.A., Campisi, J., and van Deursen, J.M. (2016). Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 354, 472–477.
Childs, B.G., Durik, M., Baker, D.J., and van Deursen, J.M. (2015). Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med 21, 1424–1435.
Chin, D.W.L., Yoshizato, T., Virding Culleton, S., Grasso, F., Barbachowska, M., Ogawa, S., Jacobsen, S.E.W., and Woll, P.S. (2022). Aged healthy mice acquire clonal hematopoiesis mutations. Blood 139, 629–634.
Chin, R.M., Fu, X., Pai, M.Y., Vergnes, L., Hwang, H., Deng, G., Diep, S., Lomenick, B., Meli, V.S., Monsalve, G.C., et al. (2014). The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature 510, 397–401.
Chini, C.C.S., Peclat, T.R., Warner, G.M., Kashyap, S., Espindola-Netto, J. M., de Oliveira, G.C., Gomez, L.S., Hogan, K.A., Tarragó, M.G., Puranik, A.S., et al. (2020). CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD^+ and NMN levels. Nat Metab 2, 1284–1304.
Chini, C.C.S., Zeidler, J.D., Kashyap, S., Warner, G., and Chini, E.N. (2021). Evolving concepts in NAD^+ metabolism. Cell Metab 33, 1076–1087.
Chio, I.I.C., Sasaki, M., Ghazarian, D., Moreno, J., Done, S., Ueda, T., Inoue, S., Chang, Y.L., Chen, N.J., and Mak, T.W. (2012). TRADD contributes to tumour suppression by regulating ULF-dependent p19Arf ubiquitylation. Nat Cell Biol 14, 625–633.
Cho, H., Choi, J.Y., Hwang, M.S., Kim, Y.J., Lee, H.M., Lee, H.S., Lee, J. H., Ryu, Y.H., Lee, M.S., and Lyoo, C.H. (2016). *In vivo* cortical spreading pattern of tau and amyloid in the Alzheimer disease spectrum. Ann Neurol 80, 247–258.
Cho, H.J., Lee, J., Yoon, S.R., Lee, H.G., and Jung, H. (2020). Regulation of hematopoietic stem cell fate and malignancy. Int J Mol Sci 21, 4780.
Cho, S., Jung, S.E., Hong, S.R., Lee, E.H., Lee, J.H., Lee, S.D., and Lee, H. Y. (2017). Independent validation of DNA-based approaches for age prediction in blood. Forensic Sci Int Genet 29, 250–256.
Cho, S.J., and Stout-Delgado, H.W. (2020). Aging and lung disease. Annu Rev Physiol 82, 433–459.
Choi, J., Park, J.E., Tsagkogeorga, G., Yanagita, M., Koo, B.K., Han, N., and Lee, J.H. (2020). Inflammatory signals induce AT2 Cell-derived damage-associated transient progenitors that mediate alveolar regeneration. Cell Stem Cell 27, 366–382.e7.
Chondronasiou, D., Gill, D., Mosteiro, L., Urdinguio, R.G., Berenguer-Llergo, A., Aguilera, M., Durand, S., Aprahamian, F., Nirmalathasan, N., Abad, M., et al. (2022). Multi-omic rejuvenation of naturally aged tissues by a single cycle of transient reprogramming. Aging Cell 21.
Chong, Y.H., Pankhurst, M.W., and McLennan, I.S. (2017). The testicular hormones AMH, InhB, INSL3, and testosterone can be independently deficient in older men. J Gerontol A Biol Sci Med Sci 72, 548–553.
Chotiyarnwong, P., and McCloskey, E.V. (2020). Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. Nat Rev Endocrinol 16, 437–447.
Choudhury, S., Huang, A.Y., Kim, J., Zhou, Z., Morillo, K., Maury, E.A., Tsai, J.W., Miller, M.B., Lodato, M.A., Araten, S., et al. (2022).

Somatic mutations in single human cardiomyocytes reveal age-associated DNA damage and widespread oxidative genotoxicity. Nat Aging 2, 714–725.
Chouliaras, L., L.A. van den Hove, D., Kenis, G., Keitel, S., R. Hof, P., van Os, J., W.M. Steinbusch, H., Schmitz, C., and P.F. Rutten, B. (2012). Age-related increase in levels of 5-hydroxymethylcytosine in mouse hippocampus is prevented by caloric restriction. Curr Alzheimer Res 9, 536–544.
Chow, F.Y., Nikolic-Paterson, D.J., Ozols, E., Atkins, R.C., Rollin, B.J., and Tesch, G.H. (2006). Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. Kidney Int 69, 73–80.
Christensen, B.C., Houseman, E.A., Marsit, C.J., Zheng, S., Wrensch, M. R., Wiemels, J.L., Nelson, H.H., Karagas, M.R., Padbury, J.F., Bueno, R., et al. (2009). Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet 5, e1000602.
Christopikou, D., Tsorva, E., Economou, K., Shelley, P., Davies, S., Mastrominas, M., and Handyside, A.H. (2013). Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. Hum Reprod 28, 1426–1434.
Christov, C., Chretien, F., Abou-Khalil, R., Bassez, G., Vallet, G., Authier, F.J., Bassaglia, Y., Shinin, V., Tajbakhsh, S., Chazaud, B., et al. (2007). Muscle satellite cells and endothelial cells: close neighbors and privileged partners. Mol Biol Cell 18, 1397–1409.
Chung, P.L., Zhou, S., Eslami, B., Shen, L., LeBoff, M.S., and Glowacki, J. (2014). Effect of age on regulation of human osteoclast differentiation. J Cell Biochem 115, 1412–1419.
Claesson, M.J., Jeffery, I.B., Conde, S., Power, S.E., O'Connor, E.M., Cusack, S., Harris, H.M.B., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., et al. (2012). Gut microbiota composition correlates with diet and health in the elderly. Nature 488, 178–184.
Clarke, B.L., Ebeling, P.R., Jones, J.D., Wahner, H.W., O'Fallon, W.M., Riggs, B.L., and Fitzpatrick, L.A. (1996). Changes in quantitative bone histomorphometry in aging healthy men. J Clin Endocrinol Metab 81, 2264–2270.
Clarke, L.E., Liddelow, S.A., Chakraborty, C., Münch, A.E., Heiman, M., and Barres, B.A. (2018). Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci USA 115, E1896–E1905.
Coca, S.G., Nadkarni, G.N., Huang, Y., Moledina, D.G., Rao, V., Zhang, J., Ferket, B., Crowley, S.T., Fried, L.F., and Parikh, C.R. (2017). Plasma biomarkers and kidney function decline in early and established diabetic kidney disease. J Am Soc Nephrol 28, 2786–2793.
Coffey, S., Roberts-Thomson, R., Brown, A., Carapetis, J., Chen, M., Enriquez-Sarano, M., Zühlke, L., and Prendergast, B.D. (2021). Global epidemiology of valvular heart disease. Nat Rev Cardiol 18, 853–864.
Cogger, V.C., Svistounov, D., Warren, A., Zykova, S., Melvin, R.G., Solon-Biet, S.M., O'Reilly, J.N., McMahon, A.C., Ballard, J.W.O., De Cabo, R., et al. (2014). Liver aging and pseudocapillarization in a Werner syndrome mouse model. J Gerontol A Biol Sci Med Sci 69, 1076–1086.
Cogger, V.C., Warren, A., Fraser, R., Ngu, M., McLean, A.J., and Le Couteur, D.G. (2003). Hepatic sinusoidal pseudocapillarization with aging in the non-human primate. Exp Gerontol 38, 1101–1107.
Cohen, P., and Kajimura, S. (2021). The cellular and functional complexity of thermogenic fat. Nat Rev Mol Cell Biol 22, 393–409.
Cohen, S., Janicki-Deverts, D., Turner, R.B., Casselbrant, M.L., Li-Korotky, H.S., Epel, E.S., and Doyle, W.J. (2013). Association between telomere length and experimentally induced upper respiratory viral infection in healthy adults. JAMA 309, 699–705.
Cole, J.H., Poudel, R.P.K., Tsagkrasoulis, D., Caan, M.W.A., Steves, C., Spector, T.D., and Montana, G. (2017). Predicting brain age with deep learning from raw imaging data results in a reliable and heritable biomarker. NeuroImage 163, 115–124.
Conboy, I.M., Conboy, M.J., Smythe, G.M., and Rando, T.A. (2003). Notch-mediated restoration of regenerative potential to aged muscle. Science 302, 1575–1577.
Conley, K.E., Jubrias, S.A., and Esselman, P.C. (2000). Oxidative capacity and ageing in human muscle. J Physiol 526, 203–210.
Connor, K.M., Hsu, Y., Aggarwal, P.K., Capone, S., Colombo, A.R., and Ramsingh, G. (2018). Understanding metabolic changes in aging bone marrow. Exp Hematol Oncol 7, 13.
Conover, C.A., and Bale, L.K. (2022). Senescence induces proteolytically-active PAPP-A secretion and association with extracellular vesicles in human pre-adipocytes. Exp Gerontol 172, 112070.
Cooper, E.B., Watowich, M.M., Beeby, N., Whalen, C., Montague, M.J., Brent, L.J.N., Snyder-Mackler, N., and Higham, J.P. (2022). Concentrations of urinary neopterin, but not suPAR, positively correlate with age in rhesus macaques. Front Ecol Evol 10.
Coordt, M.C., Ruhe, R.C., and McDonald, R.B. (1995). Aging and insulin secretion. Exp Biol Med 209, 213–222.
Copley, S.J. (2016). Morphology of the aging lung on computed tomography. J Thoracic Imag 31, 140–150.
Coppé, J.P., Rodier, F., Patil, C.K., Freund, A., Desprez, P.Y., and Campisi, J. (2011). Tumor suppressor and aging biomarker $p16^{INK4a}$ induces cellular senescence without the associated inflammatory secretory phenotype. J Biol Chem 286, 36396–36403.
Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., and Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6, 2853–2868.
Corberand, J., Ngyen, F., Laharrague, P., Fontanilles, A.M., Gleyzes, B., Gyrard, E., and Senegas, C. (1981). Polymorphonuclear functions and aging in humans. J Am Geriatr Soc 29, 391–397.
Cornett, P., and Hall, J. (2008). Issues in disclosing a diagnosis of dementia. Arch Clin Neuropsychol 23, 251–256.
Cornwell, G.G., and Westermark, P. (1980). Senile amyloidosis: a protean manifestation of the aging process. J Clin Pathol 33, 1146–1152.
Corpet, A., Kleijwegt, C., Roubille, S., Juillard, F., Jacquet, K., Texier, P., and Lomonte, P. (2020). PML nuclear bodies and chromatin dynamics: catch me if you can! Nucleic Acids Res 48, 11890–11912.
Corral-Debrinski, M., Horton, T., Lott, M.T., Shoffner, J.M., Flint Beal, M., and Wallace, D.C. (1992). Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nat Genet 2, 324–329.
Correa-de-Araujo, R., Harris-Love, M.O., Miljkovic, I., Fragala, M.S., Anthony, B.W., and Manini, T.M. (2017). The need for standardized assessment of muscle quality in skeletal muscle function deficit and other aging-related muscle dysfunctions: a symposium report. Front Physiol 8, 87.
Correia-Melo, C., Hewitt, G., and Passos, J.F. (2014). Telomeres, oxidative stress and inflammatory factors: partners in cellular senescence? Longev Healthspan 3, 1.
Correia-Melo, C., Kamrad, S., Tengölics, R., Messner, C.B., Trebulle, P., Townsend, S.J., Jayasree Varma, S., Freiwald, A., Heineike, B.M., Campbell, K., et al. (2023). Cell-cell metabolite exchange creates a pro-survival metabolic environment that extends lifespan. Cell 186, 63–79. e21.
Cortopassi, G.A., and Arnheim, N. (1990). Detection of a specific mitochondrial DNA deletion in tissues of older humans. Nucl Acids Res 18, 6927–6933.
Coschigano, K.T., Clemmons, D., Bellush, L.L., and Kopchick, J.J. (2000). Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 141, 2608–2613.
Costa-Mattioli, M., and Walter, P. (2020). The integrated stress response: From mechanism to disease. Science 368.
Coughlan, M.T., Yap, F.Y.T., Tong, D.C.K., Andrikopoulos, S., Gasser, A., Thallas-Bonke, V., Webster, D.E., Miyazaki, J., Kay, T.W., Slattery, R. M., et al. (2011). Advanced glycation end products are direct modulators of β-cell function. Diabetes 60, 2523–2532.
Courtney, J., and Steinbach, J.H. (1981). Age changes in neuromuscular junction morphology and acetylcholine receptor distribution on rat skeletal muscle fibres. J Physiol 320, 435–447.
Couser, W.G. (2017). Primary membranous nephropathy. Clin J Am Soc Nephrol 12, 983–997.

Covarrubias, A.J., Kale, A., Perrone, R., Lopez-Dominguez, J.A., Pisco, A. O., Kasler, H.G., Schmidt, M.S., Heckenbach, I., Kwok, R., Wiley, C. D., et al. (2020). Senescent cells promote tissue NAD^+ decline during ageing via the activation of $CD38^+$ macrophages. Nat Metab 2, 1265–1283.

Covarrubias, A.J., Perrone, R., Grozio, A., and Verdin, E. (2021). NAD^+ metabolism and its roles in cellular processes during ageing. Nat Rev Mol Cell Biol 22, 119–141.

Cree, L.M., Patel, S.K., Pyle, A., Lynn, S., Turnbull, D.M., Chinnery, P.F., and Walker, M. (2008). Age-related decline in mitochondrial DNA copy number in isolated human pancreatic islets. Diabetologia 51, 1440–1443.

Criscione, S.W., De Cecco, M., Siranosian, B., Zhang, Y., Kreiling, J.A., Sedivy, J.M., and Neretti, N. (2016a). Reorganization of chromosome architecture in replicative cellular senescence. Sci Adv 2, e1500882.

Criscione, S.W., Teo, Y.V., and Neretti, N. (2016b). The chromatin landscape of cellular senescence. Trends Genet 32, 751–761.

Crook, H., Raza, S., Nowell, J., Young, M., and Edison, P. (2021). Long covid—mechanisms, risk factors, and management. BMJ 374, n1648.

Crotti, A., and Ransohoff, R.M. (2016). Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. Immunity 44, 505–515.

Crow, J.F. (2000). The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet 1, 40–47.

Cruz-Jentoft, A.J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A.A., et al. (2019). Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48, 16–31.

Cruz-Jentoft, A.J., and Sayer, A.A. (2019). Sarcopenia. Lancet 393, 2636–2646.

Cruz, C., Della Rosa, M., Krueger, C., Gao, Q., Horkai, D., King, M., Field, L., and Houseley, J. (2018). Tri-methylation of histone H3 lysine 4 facilitates gene expression in ageing cells. eLife 7, e34081.

Csapo, R., Malis, V., Sinha, U., Du, J., and Sinha, S. (2014). Age-associated differences in triceps surae muscle composition and strength—an MRI-based cross-sectional comparison of contractile, adipose and connective tissue. BMC Musculoskelet Disord 15, 209.

Csiszar, A., Sosnowska, D., Wang, M., Lakatta, E.G., Sonntag, W.E., and Ungvari, Z. (2012). Age-associated proinflammatory secretory phenotype in vascular smooth muscle cells from the non-human primate *Macaca mulatta*: reversal by resveratrol treatment. J Gerontol A Biol Sci Med Sci 67, 811–820.

Cunnane, S.C., Trushina, E., Morland, C., Prigione, A., Casadesus, G., Andrews, Z.B., Beal, M.F., Bergersen, L.H., Brinton, R.D., de la Monte, S., et al. (2020). Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov 19, 609–633.

Curley, M., Milne, L., Smith, S., J∅rgensen, A., Frederiksen, H., Hadoke, P., Potter, P., and Smith, L.B. (2019). A young testicular microenvironment protects Leydig cells against age-related dysfunction in a mouse model of premature aging. FASEB J 33, 978–995.

Curtis, A.B., Karki, R., Hattoum, A., and Sharma, U.C. (2018). Arrhythmias in patients ≥80 years of age: pathophysiology, management, and outcomes. J Am Coll Cardiol 71, 2041–2057.

Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F.C., Palmer, E.L., Tseng, Y.H., Doria, A., et al. (2009). Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360, 1509–1517.

Czekalla, C., Schönborn, K.H., Lademann, J., and Meinke, M.C. (2019). Noninvasive determination of epidermal and stratum corneum thickness *in vivo* using two-photon microscopy and optical coherence tomography: impact of body area, age, and gender. Skin Pharmacol Physiol 32, 142–150.

d'Adda di Fagagna, F. (2008). Living on a break: cellular senescence as a DNA-damage response. Nat Rev Cancer 8, 512–522.

d'Adda di Fagagna, F.A., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., von Zglinicki, T., Saretzki, G., Carter, N.P., and Jackson, S.P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. Nature 426, 194–198.

d'Alessandro, M., Cameli, P., Refini, R.M., Bergantini, L., Alonzi, V., Lanzarone, N., Bennett, D., Rana, G.D., Montagnani, F., Scolletta, S., et al. (2020). Serum KL-6 concentrations as a novel biomarker of severe COVID-19. J Med Virol 92, 2216–2220.

Dai, D.F., Chen, T., Johnson, S.C., Szeto, H., and Rabinovitch, P.S. (2012). Cardiac aging: from molecular mechanisms to significance in human health and disease. Antioxid Redox Signal 16, 1492–1526.

Dai, D.F., Karunadharma, P.P., Chiao, Y.A., Basisty, N., Crispin, D., Hsieh, E.J., Chen, T., Gu, H., Djukovic, D., Raftery, D., et al. (2014). Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. Aging Cell 13, 529–539.

Dakouane, M., Bicchieray, L., Bergere, M., Albert, M., Vialard, F., and Selva, J. (2005). A histomorphometric and cytogenetic study of testis from men 29–102 years old. Fertil Steril 83, 923–928.

Daneault, A., Prawitt, J., Fabien Soulé, V., Coxam, V., and Wittrant, Y. (2017). Biological effect of hydrolyzed collagen on bone metabolism. Crit Rev Food Sci Nutr 57, 1922–1937.

Dang, W., Steffen, K.K., Perry, R., Dorsey, J.A., Johnson, F.B., Shilatifard, A., Kaeberlein, M., Kennedy, B.K., and Berger, S.L. (2009). Histone H4 lysine 16 acetylation regulates cellular lifespan. Nature 459, 802–807.

Daniali, L., Benetos, A., Susser, E., Kark, J.D., Labat, C., Kimura, M., Desai, K.K., Granick, M., and Aviv, A. (2013). Telomeres shorten at equivalent rates in somatic tissues of adults. Nat Commun 4, 1597.

Danka Mohammed, C.P., Park, J.S., Nam, H.G., and Kim, K. (2017). MicroRNAs in brain aging. Mech Ageing Dev 168, 3–9.

Darcy, J., Fang, Y., McFadden, S., Lynes, M.D., Leiria, L.O., Dreyfuss, J. M., Bussburg, V., Tolstikov, V., Greenwood, B., Narain, N.R., et al. (2020). Integrated metabolomics reveals altered lipid metabolism in adipose tissue in a model of extreme longevity. Geroscience 42, 1527–1546.

Das, A., and Destouni, A. (2023). Novel insights into reproductive ageing and menopause from genomics. Hum Reprod 38, 195–203.

Das, A., Huang, G.X., Bonkowski, M.S., Longchamp, A., Li, C., Schultz, M.B., Kim, L.J., Osborne, B., Joshi, S., Lu, Y., et al. (2018). Impairment of an endothelial NAD^+-H2S signaling network is a reversible cause of vascular aging. Cell 173, 74–89.e20.

Dasgupta, N., and Adams, P.D. (2023). Is aging a "Retro"spective event? Cell 186, 233–235.

Daum, B., Walter, A., Horst, A., Osiewacz, H.D., and Kühlbrandt, W. (2013). Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria. Proc Natl Acad Sci USA 110, 15301–15306.

Daunay, A., Hardy, L.M., Bouyacoub, Y., Sahbatou, M., Touvier, M., Blanché, H., Deleuze, J.F., and How-Kit, A. (2022). Centenarians consistently present a younger epigenetic age than their chronological age with four epigenetic clocks based on a small number of CpG sites. Aging 14, 7718–7733.

David, J.P., Ghozali, F., Fallet-Bianco, C., Wattez, A., Delaine, S., Boniface, B., Di Menza, C., and Delacourte, A. (1997). Glial reaction in the hippocampal formation is highly correlated with aging in human brain. Neurosci Lett 235, 53–56.

Davis, J.K. (2010). Justice, insurance, and biomarkers of aging. Exp Gerontol 45, 814–818.

De Cecco, M., Ito, T., Petrashen, A.P., Elias, A.E., Skvir, N.J., Criscione, S. W., Caligiana, A., Brocculi, G., Adney, E.M., Boeke, J.D., et al. (2019). L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature 566, 73–78.

de Groot, E., Hovingh, G.K., Wiegman, A., Duriez, P., Smit, A.J., Fruchart, J.C., and Kastelein, J.J.P. (2004). Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation 109, III33.

de Haan, G., and Lazare, S.S. (2018). Aging of hematopoietic stem cells. Blood 131, 479–487.

de Lange, T. (2005). Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 19, 2100–2110.

de Laval, B., Maurizio, J., Kandalla, P.K., Brisou, G., Simonnet, L., Huber, C., Gimenez, G., Matcovitch-Natan, O., Reinhardt, S., David, E., et al.

(2020). C/EBPβ-dependent epigenetic memory induces trained immunity in hematopoietic stem cells. Cell Stem Cell 26, 657–674.e8.
de Lemos, J.A., Ayers, C.R., Levine, B.D., deFilippi, C.R., Wang, T.J., Hundley, W.G., Berry, J.D., Seliger, S.L., McGuire, D.K., Ouyang, P., et al. (2017). Multimodality strategy for cardiovascular risk assessment: performance in 2 population-based cohorts. Circulation 135, 2119–2132.
de Lemos, J.A., Drazner, M.H., Omland, T., Ayers, C.R., Khera, A., Rohatgi, A., Hashim, I., Berry, J.D., Das, S.R., Morrow, D.A., et al. (2010). Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA 304, 2503–2512.
De Luca, M., Mandala, M., and Rose, G. (2021). Towards an understanding of the mechanoreciprocity process in adipocytes and its perturbation with aging. Mech Ageing Dev 197, 111522.
De Majo, F., Martens, L., Hegenbarth, J.C., Rühle, F., Hamczyk, M.R., Nevado, R.M., Andrés, V., Hilbold, E., Bär, C., Thum, T., et al. (2021). Genomic instability in the naturally and prematurely aged myocardium. Proc Natl Acad Sci USA 118, e2022974118.
De Micheli, A.J., Spector, J.A., Elemento, O., and Cosgrove, B.D. (2020). A reference single-cell transcriptomic atlas of human skeletal muscle tissue reveals bifurcated muscle stem cell populations. Skelet Muscle 10, 19.
De Miguel, Z., Khoury, N., Betley, M.J., Lehallier, B., Willoughby, D., Olsson, N., Yang, A.C., Hahn, O., Lu, N., Vest, R.T., et al. (2021). Exercise plasma boosts memory and dampens brain inflammation via clusterin. Nature 600, 494–499.
De Vis, J.B., Peng, S.L., Chen, X., Li, Y., Liu, P., Sur, S., Rodrigue, K.M., Park, D.C., and Lu, H. (2018). Arterial-spin-labeling (ASL) perfusion MRI predicts cognitive function in elderly individuals: A 4-year longitudinal study. J Magn Reson Imag 48, 449–458.
Debacq-Chainiaux, F., Boilan, E., Dedessus Le Moutier, J., Weemaels, G., and Toussaint, O. (2010). p38(MAPK) in the senescence of human and murine fibroblasts. In: Tavernarakis, N., ed. Protein Metabolism and Homeostasis in Aging. Advances in Experimental Medicine and Biology. Boston: Springer. 126–137.
DeBruyne, J.P., Weaver, D.R., and Reppert, S.M. (2007). CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. Nat Neurosci 10, 543–545.
DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., Beiser, A., D'Agostino, R., and Wolf, P.A. (2005). Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. Neurobiol Aging 26, 491–510.
Deczkowska, A., Weiner, A., and Amit, I. (2020). The physiology, pathology, and potential therapeutic applications of the TREM2 signaling pathway. Cell 181, 1207–1217.
Deelen, J., Kettunen, J., Fischer, K., van der Spek, A., Trompet, S., Kastenmüller, G., Boyd, A., Zierer, J., van den Akker, E.B., Ala-Korpela, M., et al. (2019). A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. Nat Commun 10, 3346.
Deenadayal Mettler, A., Govindarajan, M., Srinivas, S., Mithraprabhu, S., Evenson, D., and Mahendran, T. (2020). Male age is associated with sperm DNA/chromatin integrity. Aging Male 23, 822–829.
Degens, H. (2010). The role of systemic inflammation in age-related muscle weakness and wasting. Scand J Med Sci Sports 20, 28–38.
DeJong, E.N., Surette, M.G., and Bowdish, D.M.E. (2020). The gut microbiota and unhealthy aging: disentangling cause from consequence. Cell Host Microbe 28, 180–189.
Dekaban, A.S. (1978). Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. Ann Neurol 4, 345–356.
DellaCroce, J.T., and Vitale, A.T. (2008). Hypertension and the eye. Curr Opin Ophthalmol 19, 493–498.
Delles, C., Schiffer, E., von zur Muhlen, C., Peter, K., Rossing, P., Parving, H.H., Dymott, J.A., Neisius, U., Zimmerli, L.U., Snell-Bergeon, J.K., et al. (2010). Urinary proteomic diagnosis of coronary artery disease: identification and clinical validation in 623 individuals. J Hypertension 28, 2316–2322.
Delmonico, M.J., Harris, T.B., Visser, M., Park, S.W., Conroy, M.B., Velasquez-Mieyer, P., Boudreau, R., Manini, T.M., Nevitt, M., Newman, A.B., et al. (2009). Longitudinal study of muscle strength, quality, and adipose tissue infiltration. Am J Clin Nutr 90, 1579–1585.
Demanelis, K., Jasmine, F., Chen, L.S., Chernoff, M., Tong, L., Delgado, D., Zhang, C., Shinkle, J., Sabarinathan, M., Lin, H., et al. (2020). Determinants of telomere length across human tissues. Science 369.
Demaria, M., Ohtani, N., Youssef, S.A., Rodier, F., Toussaint, W., Mitchell, J.R., Laberge, R.M., Vijg, J., Van Steeg, H., Dollé, M.E.T., et al. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 31, 722–733.
Denchi, E.L., and de Lange, T. (2007). Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature 448, 1068–1071.
Deng, L., Ren, R., Liu, Z., Song, M., Li, J., Wu, Z., Ren, X., Fu, L., Li, W., Zhang, W., et al. (2019). Stabilizing heterochromatin by DGCR8 alleviates senescence and osteoarthritis. Nat Commun 10, 3329.
Deng, P., Yuan, Q., Cheng, Y., Li, J., Liu, Z., Liu, Y., Li, Y., Su, T., Wang, J., Salvo, M.E., et al. (2021). Loss of KDM4B exacerbates bone-fat imbalance and mesenchymal stromal cell exhaustion in skeletal aging. Cell Stem Cell 28, 1057–1073.e7.
Deng, X., Su, R., Weng, H., Huang, H., Li, Z., and Chen, J. (2018). RNA *N*6-methyladenosine modification in cancers: current status and perspectives. Cell Res 28, 507–517.
Denic, A., Glassock, R.J., and Rule, A.D. (2016). Structural and functional changes with the aging kidney. Adv Chronic Kidney Dis 23, 19–28.
Denic, A., Lieske, J.C., Chakkera, H.A., Poggio, E.D., Alexander, M.P., Singh, P., Kremers, W.K., Lerman, L.O., and Rule, A.D. (2017). The substantial loss of nephrons in healthy human kidneys with aging. J Am Soc Nephrol 28, 313–320.
DeNino, W.F., Tchernof, A., Dionne, I.J., Toth, M.J., Ades, P.A., Sites, C. K., and Poehlman, E.T. (2001). Contribution of abdominal adiposity to age-related differences in insulin sensitivity and plasma lipids in healthy nonobese women. Diabetes Care 24, 925–932.
Denkinger, M.D., Leins, H., Schirmbeck, R., Florian, M.C., and Geiger, H. (2015). HSC aging and senescent immune remodeling. Trends Immunol 36, 815–824.
Dennison, E.M., Sayer, A.A., and Cooper, C. (2017). Epidemiology of sarcopenia and insight into possible therapeutic targets. Nat Rev Rheumatol 13, 340–347.
DePinho, R.A. (2000). The age of cancer. Nature 408, 248–254.
Desai, N., Sabanegh, E. Jr., Kim, T., and Agarwal, A. (2010). Free radical theory of aging: implications in male infertility. Urology 75, 14–19.
Desai, T.J., Brownfield, D.G., and Krasnow, M.A. (2014). Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507, 190–194.
Deschênes-Simard, X., Gaumont-Leclerc, M.F., Bourdeau, V., Lessard, F., Moiseeva, O., Forest, V., Igelmann, S., Mallette, F.A., Saba-El-Leil, M. K., Meloche, S., et al. (2013). Tumor suppressor activity of the ERK/MAPK pathway by promoting selective protein degradation. Genes Dev 27, 900–915.
Desgraz, R., Bonal, C., and Herrera, P.L. (2011). β-cell regeneration: the pancreatic intrinsic faculty. Trends Endocrinol Metab 22, 34–43.
Detlefsen, S., Sipos, B., Feyerabend, B., and Klöppel, G. (2005). Pancreatic fibrosis associated with age and ductal papillary hyperplasia. Virchows Arch 447, 800–805.
di Giuseppe, R., Arcari, A., Serafini, M., Di Castelnuovo, A., Zito, F., De Curtis, A., Sieri, S., Krogh, V., Pellegrini, N., Schünemann, H.J., et al. (2012). Total dietary antioxidant capacity and lung function in an Italian population: a favorable role in premenopausal/never smoker women. Eur J Clin Nutr 66, 61–68.
Di Micco, R., Krizhanovsky, V., Baker, D., and d'Adda di Fagagna, F. (2021). Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol 22, 75–95.
Di Narzo, A.F., Telesco, S.E., Brodmerkel, C., Argmann, C., Peters, L.A., Li, K., Kidd, B., Dudley, J., Cho, J., Schadt, E.E., et al. (2017). High-throughput characterization of blood serum proteomics of IBD patients

with respect to aging and genetic factors. PLoS Genet 13, e1006565.
Diao, Z., Ji, Q., Wu, Z., Zhang, W., Cai, Y., Wang, Z., Hu, J., Liu, Z., Wang, Q., Bi, S., et al. (2021). SIRT3 consolidates heterochromatin and counteracts senescence. Nucleic Acids Res 49, 4203–4219.
Dias, H.C., Cordeiro, C., Pereira, J., Pinto, C., Real, F.C., Cunha, E., and Manco, L. (2020). DNA methylation age estimation in blood samples of living and deceased individuals using a multiplex SNaPshot assay. Forensic Sci Int 311, 110267.
Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 72, 517–549.
Dickstein, D.L., Kabaso, D., Rocher, A.B., Luebke, J.I., Wearne, S.L., and Hof, P.R. (2007). Changes in the structural complexity of the aged brain. Aging Cell 6, 275–284.
Dietrich, N., Bracken, A.P., Trinh, E., Schjerling, C.K., Koseki, H., Rappsilber, J., Helin, K., and Hansen, K.H. (2007). Bypass of senescence by the polycomb group protein CBX8 through direct binding to the INK4A-ARF locus. EMBO J 26, 1637–1648.
Dikic, I. (2017). Proteasomal and autophagic degradation systems. Annu Rev Biochem 86, 193–224.
Dilberger, B., Baumanns, S., Schmitt, F., Schmiedl, T., Hardt, M., Wenzel, U., and Eckert, G.P. (2019). Mitochondrial oxidative stress impairs energy metabolism and reduces stress resistance and longevity of *C. elegans*. Oxid Med Cell Longev 2019, 1–14.
Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., et al. (1995). A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. Proc Natl Acad Sci USA 92, 9363–9367.
Ding, J., Ji, J., Rabow, Z., Shen, T., Folz, J., Brydges, C.R., Fan, S., Lu, X., Mehta, S., Showalter, M.R., et al. (2021a). A metabolome atlas of the aging mouse brain. Nat Commun 12, 6021.
Ding, J., Sidore, C., Butler, T.J., Wing, M.K., Qian, Y., Meirelles, O., Busonero, F., Tsoi, L.C., Maschio, A., Angius, A., et al. (2015). Assessing mitochondrial DNA variation and copy number in lymphocytes of ~2,000 sardinians using tailored sequencing analysis tools. PLoS Genet 11, e1005306.
Ding, J., Sigurðsson, S., Jónsson, P.V., Eiriksdottir, G., Meirelles, O., Kjartansson, O., Lopez, O.L., van Buchem, M.A., Gudnason, V., and Launer, L.J. (2017). Space and location of cerebral microbleeds, cognitive decline, and dementia in the community. Neurology 88, 2089–2097.
Ding, X., Kakanj, P., Leptin, M., and Eming, S.A. (2021b). Regulation of the wound healing response during aging. J Invest Dermatol 141, 1063–1070.
Ding, Y.N., Wang, H.Y., Chen, H.Z., and Liu, D.P. (2022). Targeting senescent cells for vascular aging and related diseases. J Mol Cell Cardiol 162, 43–52.
Diniz, D.G., Foro, C.A.R., Rego, C.M.D., Gloria, D.A., De Oliveira, F.R. R., Paes, J.M.P., De Sousa, A.A., Tokuhashi, T.P., Trindade, L.S., Turiel, M.C.P., et al. (2010). Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. Eur J Neurosci 32, 509–519.
Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380.
Diz, A.P., Martínez-fernández, M., and Rolán-alvarez, E. (2012). Proteomics in evolutionary ecology: linking the genotype with the phenotype. Mol Ecol 21, 1060–1080.
Dodds, R.M., Syddall, H.E., Cooper, R., Benzeval, M., Deary, I.J., Dennison, E.M., Der, G., Gale, C.R., Inskip, H.M., Jagger, C., et al. (2014). Grip strength across the life course: normative data from twelve British studies. PLoS ONE 9, e113637.
Doles, J., Storer, M., Cozzuto, L., Roma, G., and Keyes, W.M. (2012). Age-associated inflammation inhibits epidermal stem cell function. Genes Dev 26, 2144–2153.
Dollé, M.E.T., Busuttil, R.A., Garcia, A.M., Wijnhoven, S., van Drunen, E., Niedernhofer, L.J., van der Horst, G., Hoeijmakers, J.H.J., van Steeg, H., and Vijg, J. (2006). Increased genomic instability is not a prerequisite for shortened lifespan in DNA repair deficient mice. Mutat Res 596, 22–35.
Dollé, M.E.T., Giese, H., Hopkins, C.L., Martus, H.J., Hausdorff, J.M., and Vijg, J. (1997). Rapid accumulation of genome rearrangements in liver but not in brain of old mice. Nat Genet 17, 431–434.
Dollé, M.E.T., Snyder, W.K., Gossen, J.A., Lohman, P.H.M., and Vijg, J. (2000). Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. Proc Natl Acad Sci USA 97, 8403–8408.
Dominice, J., Levasseur, C., Larno, S., Ronot, X., and Adolphe, M. (1986). Age-related changes in rabbit articular chondrocytes. Mech Ageing Dev 37, 231–240.
Dong, C., Miao, Y.R., Zhao, R., Yang, M., Guo, A.Y., Xue, Z.H., Li, T., Zhang, Q., Bao, Y., Shen, C., et al. (2022). Single-cell transcriptomics reveals longevity immune remodeling features shared by centenarians and their offspring. Adv Sci 9, 2204849.
Dong, X., Tong, F., Qian, C., Zhang, R., Dong, J., Wu, G., and Hu, Y. (2015). NEMO modulates radiation-induced endothelial senescence of human umbilical veins through NF-κB signal pathway. Radiat Res 183, 82–93.
Dos Santos, M., Backer, S., Saintpierre, B., Izac, B., Andrieu, M., Letourneur, F., Relaix, F., Sotiropoulos, A., and Maire, P. (2020). Single-nucleus RNA-seq and FISH identify coordinated transcriptional activity in mammalian myofibers. Nat Commun 11, 5102.
Dossin, O. (2011). Laboratory tests for diagnosis of gastrointestinal and pancreatic diseases. Top Companion Anim Med 26, 86–97.
Dou, Z., Ghosh, K., Vizioli, M.G., Zhu, J., Sen, P., Wangensteen, K.J., Simithy, J., Lan, Y., Lin, Y., Zhou, Z., et al. (2017). Cytoplasmic chromatin triggers inflammation in senescence and cancer. Nature 550, 402–406.
Draceni, Y., and Pechmann, S. (2019). Pervasive convergent evolution and extreme phenotypes define chaperone requirements of protein homeostasis. Proc Natl Acad Sci USA 116, 20009–20014.
Draper, B., Peisah, C., Snowdon, J., and Brodaty, H. (2010). Early dementia diagnosis and the risk of suicide and euthanasia. Alzheimers Dement 6, 75–82.
Dries, R., Zhu, Q., Dong, R., Eng, C.H.L., Li, H., Liu, K., Fu, Y., Zhao, T., Sarkar, A., Bao, F., et al. (2021). Giotto: a toolbox for integrative analysis and visualization of spatial expression data. Genome Biol 22, 78.
Driscoll, I., Davatzikos, C., An, Y., Wu, X., Shen, D., Kraut, M., and Resnick, S.M. (2009). Longitudinal pattern of regional brain volume change differentiates normal aging from MCI. Neurology 72, 1906–1913.
Du, S., Ling, H., Guo, Z., Cao, Q., and Song, C. (2021). Roles of exosomal miRNA in vascular aging. Pharmacol Res 165, 105278.
Duffy, D.M., Ko, C.M., Jo, M., Brannstrom, M., and Curry, T.E. (2019). Ovulation: parallels with inflammatory processes. Endocrine Rev 40, 369–416.
Duggal, N.A., Niemiro, G., Harridge, S.D.R., Simpson, R.J., and Lord, J.M. (2019). Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? Nat Rev Immunol 19, 563–572.
Dumanski, J.P., Lambert, J.C., Rasi, C., Giedraitis, V., Davies, H., Grenier-Boley, B., Lindgren, C.M., Campion, D., Dufouil, C., Pasquier, F., et al. (2016). Mosaic loss of chromosome Y in blood is associated with Alzheimer disease. Am J Hum Genet 98, 1208–1219.
Duncan, F.E., Hornick, J.E., Lampson, M.A., Schultz, R.M., Shea, L.D., and Woodruff, T.K. (2012). Chromosome cohesion decreases in human eggs with advanced maternal age. Aging Cell 11, 1121–1124.
Duran-Ortiz, S., List, E.O., Ikeno, Y., Young, J., Basu, R., Bell, S., McHugh, T., Funk, K., Mathes, S., Qian, Y., et al. (2021). Growth hormone receptor gene disruption in mature-adult mice improves male insulin sensitivity and extends female lifespan. Aging Cell 20, e13506.
Durham, A.L., Speer, M.Y., Scatena, M., Giachelli, C.M., and Shanahan, C. M. (2018). Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res 114, 590–600.

Dykstra, B., and de Haan, G. (2008). Hematopoietic stem cell aging and self-renewal. Cell Tissue Res 331, 91–101.
Dykstra, B., Olthof, S., Schreuder, J., Ritsema, M., and de Haan, G. (2011). Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med 208, 2691–2703.
Eastell, R., and Szulc, P. (2017). Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol 5, 908–923.
Eckel-Mahan, K., Ribas Latre, A., and Kolonin, M.G. (2020). Adipose stromal cell expansion and exhaustion: mechanisms and consequences. Cells 9, 863.
Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A., et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med 22, 1428–1438.
Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonacci, L., et al. (2009). Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11, 1305–1314.
Eissa, S., Matboli, M., Aboushahba, R., Bekhet, M.M., and Soliman, Y. (2016). Urinary exosomal microRNA panel unravels novel biomarkers for diagnosis of type 2 diabetic kidney disease. J Diabetes Complications 30, 1585–1592.
el-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. Cell 75, 817–825.
El-Domyati, M., Attia, S., Saleh, F., Brown, D., Birk, D.E., Gasparro, F., Ahmad, H., and Uitto, J. (2002). Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. Exp Dermatol 11, 398–405.
El-Far, A.H., Lebda, M.A., Noreldin, A.E., Atta, M.S., Elewa, Y.H.A., Elfeky, M., and Mousa, S.A. (2020). Quercetin attenuates pancreatic and renal D-galactose-induced aging-related oxidative alterations in rats. Int J Mol Sci 21, 4348.
El-Naseery, N.I., Mousa, H.S.E., Noreldin, A.E., El-Far, A.H., and Elewa, Y.H.A. (2020). Aging-associated immunosenescence via alterations in splenic immune cell populations in rat. Life Sci 241, 117168.
Elderkin, S., Maertens, G.N., Endoh, M., Mallery, D.L., Morrice, N., Koseki, H., Peters, G., Brockdorff, N., and Hiom, K. (2007). A phosphorylated form of Mel-18 targets the Ring1B histone H2A ubiquitin ligase to chromatin. Mol Cell 28, 107–120.
Elderman, M., Sovran, B., Hugenholtz, F., Graversen, K., Huijskes, M., Houtsma, E., Belzer, C., Boekschoten, M., de Vos, P., Dekker, J., et al. (2017). The effect of age on the intestinal mucus thickness, microbiota composition and immunity in relation to sex in mice. PLoS ONE 12, e0184274.
Elhassan, Y.S., Kluckova, K., Fletcher, R.S., Schmidt, M.S., Garten, A., Doig, C.L., Cartwright, D.M., Oakey, L., Burley, C.V., Jenkinson, N., et al. (2019). Nicotinamide riboside augments the aged human skeletal muscle NAD^+ metabolome and induces transcriptomic and anti-inflammatory signatures. Cell Rep 28, 1717–1728.e6.
Emechebe, U., Nelson, J.W., Alkayed, N.J., Kaul, S., Adey, A.C., and Barnes, A.P. (2021). Age-dependent transcriptional alterations in cardiac endothelial cells. Physiol Genomics 53, 295–308.
Engberg, H., Oksuzyan, A., Jeune, B., Vaupel, J.W., and Christensen, K. (2009). Centenarians—a useful model for healthy aging? A 29-year follow-up of hospitalizations among 40,000 Danes born in 1905. Aging Cell 8, 270–276.
Enge, M., Arda, H.E., Mignardi, M., Beausang, J., Bottino, R., Kim, S.K., and Quake, S.R. (2017). Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. Cell 171, 321–330.e14.
Enomoto, M., Adachi, H., Fukami, A., Furuki, K., Satoh, A., Otsuka, M., Kumagae, S., Nanjo, Y., Shigetoh, Y., and Imaizumi, T. (2008). Serum dehydroepiandrosterone sulfate levels predict longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). J Am Geriatr Soc 56, 994–998.
Enright, P.L., Kronmal, R.A., Manolio, T.A., Schenker, M.B., and Hyatt, R. E. (1994). Respiratory muscle strength in the elderly. Correlates and reference values. Cardiovascular Health Study Research Group. Am J Respir Crit Care Med 149, 430–438.
Ensor, R.E., Fleg, J.L., Kim, Y.C., De Leon, E.F., and Goldman, S.M. (1983). Longitudinal chest X-ray changes in normal men. J Gerontol 38, 307–314.
Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., and Weiss, S. (2004). Aging Results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J Neurosci 24, 8354–8365.
Epel, E.S., and Prather, A.A. (2018). Stress, telomeres, and psychopathology: toward a deeper understanding of a triad of early aging. Annu Rev Clin Psychol 14, 371–397.
Eppig, J.J. (1991). Intercommunication between mammalian oocytes and companion somatic cells. BioEssays 13, 569–574.
Eppley, S.E., Mansberger, S.L., Ramanathan, S., and Lowry, E.A. (2019). Characteristics associated with adherence to annual dilated eye examinations among US patients with diagnosed diabetes. Ophthalmology 126, 1492–1499.
Eriksson, M., Brown, W.T., Gordon, L.B., Glynn, M.W., Singer, J., Scott, L., Erdos, M.R., Robbins, C.M., Moses, T.Y., Berglund, P., et al. (2003). Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423, 293–298.
Erten-Lyons, D., Dodge, H.H., Woltjer, R., Silbert, L.C., Howieson, D.B., Kramer, P., and Kaye, J.A. (2013). Neuropathologic basis of age-associated brain atrophy. JAMA Neurol 70, 616–622.
Espinoza, S., and Walston, J.D. (2005). Frailty in older adults: insights and interventions. Cleve Clin J Med 72, 1105–1112.
Esposito, I., and Häberle, L. (2022). Lobulocentric atrophy. In: Esposito, I., and Karamitopoulou-Diamantis, E., eds. Pathology of the Pancreas. Cham: Springer International Publishing. 116–118.
Evenson, D.P., Djira, G., Kasperson, K., and Christianson, J. (2020). Relationships between the age of 25,445 men attending infertility clinics and sperm chromatin structure assay (SCSA®) defined sperm DNA and chromatin integrity. Fertil Steril 114, 311–320.
Fabre, M.A., de Almeida, J.G., Fiorillo, E., Mitchell, E., Damaskou, A., Rak, J., Orrù, V., Marongiu, M., Chapman, M.S., Vijayabaskar, M.S., et al. (2022). The longitudinal dynamics and natural history of clonal haematopoiesis. Nature 606, 335–342.
Fadista, J., Vikman, P., Laakso, E.O., Mollet, I.G., Esguerra, J.L., Taneera, J., Storm, P., Osmark, P., Ladenvall, C., Prasad, R.B., et al. (2014). Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism. Proc Natl Acad Sci USA 111, 13924–13929.
Faget, D.V., Ren, Q., and Stewart, S.A. (2019). Unmasking senescence: context-dependent effects of SASP in cancer. Nat Rev Cancer 19, 439–453.
Fahy, G.M., Brooke, R.T., Watson, J.P., Good, Z., Vasanawala, S.S., Maecker, H., Leipold, M.D., Lin, D.T.S., Kobor, M.S., and Horvath, S. (2019). Reversal of epigenetic aging and immunosenescent trends in humans. Aging Cell 18, e13028.
Faitg, J., Leduc-Gaudet, J.P., Reynaud, O., Ferland, G., Gaudreau, P., and Gouspillou, G. (2019). Effects of aging and caloric restriction on fiber type composition, mitochondrial morphology and dynamics in rat oxidative and glycolytic muscles. Front Physiol 10, 420.
Fakhry, M., Roszkowska, M., Briolay, A., Bougault, C., Guignandon, A., Diaz-Hernandez, J.I., Diaz-Hernandez, M., Pikula, S., Buchet, R., Hamade, E., et al. (2017). TNAP stimulates vascular smooth muscle cell trans-differentiation into chondrocytes through calcium deposition and BMP-2 activation: possible implication in atherosclerotic plaque stability. Biochim Biophys Acta 1863, 643–653.
Fan, A., Wu, G., Wang, J., Lu, L., Wang, J., Wei, H., Sun, Y., Xu, Y., Mo, C., Zhang, X., et al. (2023). Inhibition of fibroblast activation protein ameliorates cartilage matrix degradation and osteoarthritis progression. Bone Res 11, 3.
Fan, J.G., Zhu, J., Li, X.J., Chen, L., Lu, Y.S., Li, L., Dai, F., Li, F., and Chen, S.Y. (2005). Fatty liver and the metabolic syndrome among Shanghai adults. J Gastroenterol Hepatol 20, 1825–1832.

Fang, E.F., Hou, Y., Palikaras, K., Adriaanse, B.A., Kerr, J.S., Yang, B., Lautrup, S., Hasan-Olive, M.M., Caponio, D., Dan, X., et al. (2019). Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci 22, 401–412.
Fang, E.F., Scheibye-Knudsen, M., Brace, L.E., Kassahun, H., SenGupta, T., Nilsen, H., Mitchell, J.R., Croteau, D.L., and Bohr, V.A. (2014). Defective mitophagy in XPA via PARP-1 hyperactivation and NAD^+/SIRT1 reduction. Cell 157, 882–896.
Farage, M.A., Miller, K.W., Elsner, P., and Maibach, H.I. (2013). Characteristics of the aging skin. Adv Wound Care 2, 5–10.
Farley, K.I., Surovtseva, Y., Merkel, J., and Baserga, S.J. (2015). Determinants of mammalian nucleolar architecture. Chromosoma 124, 323–331.
Fasshauer, M., and Blüher, M. (2015). Adipokines in health and disease. Trends Pharmacol Sci 36, 461–470.
Fayet, G., Jansson, M., Sternberg, D., Moslemi, A.R., Blondy, P., Lombès, A., Fardeau, M., and Oldfors, A. (2002). Ageing muscle: clonal expansions of mitochondrial DNA point mutations and deletions cause focal impairment of mitochondrial function. Neuromuscul Disord 12, 484–493.
Fealy, C.E., Grevendonk, L., Hoeks, J., and Hesselink, M.K.C. (2021). Skeletal muscle mitochondrial network dynamics in metabolic disorders and aging. Trends Mol Med 27, 1033–1044.
Fedorova, M., Bollineni, R.C., and Hoffmann, R. (2014). Protein carbonylation as a major hallmark of oxidative damage: update of analytical strategies. Mass Spec Rev 33, 79–97.
Feibusch, J.M., and Holt, P.R. (1982). Impaired absorptive capacity for carbohydrate in the aging human. Digest Dis Sci 27, 1095–1100.
Fenech, M., Holland, N., Kirsch-Volders, M., Knudsen, L.E., Wagner, K. H., Stopper, H., Knasmueller, S., Bolognesi, C., El-Zein, R., and Bonassi, S. (2020). Micronuclei and disease—Report of HUMN project workshop at Rennes 2019 EEMGS conference. Mutat Res Genet Toxicol Environ Mutagen 850-851, 503133.
Feng, H., Jiang, B., Xing, W., Sun, J., Greenblatt, M.B., and Zou, W. (2022). Skeletal Stem Cells: Origins, definitions, and functions in bone development and disease. Life Med 1, 276–293.
Feng, Y., Lv, L.L., Wu, W.J., Li, Z.L., Chen, J., Ni, H.F., Zhou, L.T., Tang, T.T., Wang, F.M., Wang, B., et al. (2018). Urinary exosomes and exosomal CCL2 mRNA as biomarkers of active histologic injury in IgA nephropathy. Am J Pathol 188, 2542–2552.
Fernandez-Flores, A., Saeb-Lima, M., and Cassarino, D.S. (2019). Histopathology of aging of the hair follicle. J Cutan Pathol 46, 508–519.
Fernandez-Rebollo, E., Franzen, J., Goetzke, R., Hollmann, J., Ostrowska, A., Oliverio, M., Sieben, T., Rath, B., Kornfeld, J.W., and Wagner, W. (2020). Senescence-associated metabolomic phenotype in primary and iPSC-derived mesenchymal stromal cells. Stem Cell Rep 14, 201–209.
Ferreira-Gonzalez, S., Rodrigo-Torres, D., Gadd, V.L., and Forbes, S.J. (2021). Cellular senescence in liver disease and regeneration. Semin Liver Dis 41, 050–066.
Ferreira, J.A., de Oliveira, P., and Pinto, L. (2020). Aging effect on iontophoretic transdermal drug delivery. SIAM J Appl Math 80, 1882–1907.
Field, A.E., Robertson, N.A., Wang, T., Havas, A., Ideker, T., and Adams, P.D. (2018). DNA methylation clocks in aging: categories, causes, and consequences. Mol Cell 71, 882–895.
Fikry, M.E. (1968). Exocrine pancreatic functions in the aged. J Am Geriatr Soc 16, 463–467.
Finkel, T. (2015). The metabolic regulation of aging. Nat Med 21, 1416–1423.
Fischer, K., Kettunen, J., Wurtz, P., Haller, T., Havulinna, A.S., Kangas, A. J., Soininen, P., Esko, T., Tammesoo, M.L., Magi, R., et al. (2014). Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med 11, e1001606.
Fisher, G.J., Kang, S., Varani, J., Bata-Csorgo, Z., Wan, Y., Datta, S., and Voorhees, J.J. (2002). Mechanisms of photoaging and chronological skin aging. Arch Dermatol 138, 1462–1470.
Fitsiou, E., Pulido, T., Campisi, J., Alimirah, F., and Demaria, M. (2021). Cellular senescence and the senescence-associated secretory phenotype as drivers of skin photoaging. J Invest Dermatol 141, 1119–1126.
Fitzner, B., Müller, S., Walther, M., Fischer, M., Engelmann, R., Müller-Hilke, B., Pützer, B.M., Kreutzer, M., Nizze, H., and Jaster, R. (2012). Senescence determines the fate of activated rat pancreatic stellate cells. J Cell Mol Med 16, 2620–2630.
Fivenson, E.M., Lautrup, S., Sun, N., Scheibye-Knudsen, M., Stevnsner, T., Nilsen, H., Bohr, V.A., and Fang, E.F. (2017). Mitophagy in neurodegeneration and aging. Neurochem Int 109, 202–209.
Fjell, A.M., and Walhovd, K.B. (2010). Structural brain changes in aging: courses, causes and cognitive consequences. Rev Neuroscis 21, 187–221.
Fjell, A.M., Westlye, L.T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Dale, A.M., and Walhovd, K.B. (2014). Accelerating cortical thinning: unique to dementia or universal in aging? Cereb Cortex 24, 919–934.
Fleischer, J.G., Schulte, R., Tsai, H.H., Tyagi, S., Ibarra, A., Shokhirev, M. N., Huang, L., Hetzer, M.W., and Navlakha, S. (2018). Predicting age from the transcriptome of human dermal fibroblasts. Genome Biol 19, 221.
Flohr Svendsen, A., Yang, D., Kim, K.M., Lazare, S., Skinder, N., Zwart, E., Mura-Meszaros, A., Ausema, A., von Eyss, B., de Haan, G., et al. (2021). A comprehensive transcriptome signature of murine hematopoietic stem cell aging. Blood 138, 439–451.
Floreani, A. (2007). Liver diseases in the elderly: an update. Dig Dis 25, 138–143.
Florian, M.C., Klose, M., Sacma, M., Jablanovic, J., Knudson, L., Nattamai, K.J., Marka, G., Vollmer, A., Soller, K., Sakk, V., et al. (2018). Aging alters the epigenetic asymmetry of HSC division. PLoS Biol 16, e2003389.
Flynn, J.M., O'Leary, M.N., Zambataro, C.A., Academia, E.C., Presley, M. P., Garrett, B.J., Zykovich, A., Mooney, S.D., Strong, R., Rosen, C.J., et al. (2013). Late-life rapamycin treatment reverses age-related heart dysfunction. Aging Cell 12, 851–862.
Foley, K.G., Pritchard, M.T., and Duncan, F.E. (2021). Macrophage-derived multinucleated giant cells: hallmarks of the aging ovary. Reproduction 161, V5–V9.
Ford, S.J., Bigliardi, P.L., Sardella, T.C.P., Urich, A., Burton, N.C., Kacprowicz, M., Bigliardi, M., Olivo, M., and Razansky, D. (2016). Structural and functional analysis of intact hair follicles and pilosebaceous units by volumetric multispectral optoacoustic tomography. J Invest Dermatol 136, 753–761.
Forsberg, L.A., Rasi, C., Malmqvist, N., Davies, H., Pasupulati, S., Pakalapati, G., Sandgren, J., de Ståhl, T.D., Zaghlool, A., Giedraitis, V., et al. (2014). Mosaic loss of chromosome Y in peripheral blood is associated with shorter survival and higher risk of cancer. Nat Genet 46, 624–628.
Forsberg, L.A., Rasi, C., Razzaghian, H.R., Pakalapati, G., Waite, L., Thilbeault, K.S., Ronowicz, A., Wineinger, N.E., Tiwari, H.K., Boomsma, D., et al. (2012). Age-related somatic structural changes in the nuclear genome of human blood cells. Am J Hum Genet 90, 217–228.
Fox, A.H., Lam, Y.W., Leung, A.K.L., Lyon, C.E., Andersen, J., Mann, M., and Lamond, A.I. (2002). Paraspeckles. Curr Biol 12, 13–25.
Fox, N.C., and Schott, J.M. (2004). Imaging cerebral atrophy: normal ageing to Alzheimer's disease. Lancet 363, 392–394.
Fraga, C.G., Shigenaga, M.K., Park, J.W., Degan, P., and Ames, B.N. (1990). Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. Proc Natl Acad Sci USA 87, 4533–4537.
Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., and De Benedictis, G. (2000). Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908, 244–254.
Franceschi, C., and Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 69, S4–S9.
Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., and Santoro, A.

(2018). Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14, 576–590.
Franco, A.C., Aveleira, C., and Cavadas, C. (2022). Skin senescence: mechanisms and impact on whole-body aging. Trends Mol Med 28, 97–109.
Frangioni, J. (2003). *In vivo* near-infrared fluorescence imaging. Curr Opin Chem Biol 7, 626–634.
Franzago, M., Pilenzi, L., Di Rado, S., Vitacolonna, E., and Stuppia, L. (2022). The epigenetic aging, obesity, and lifestyle. Front Cell Dev Biol 10, 985274.
Frasca, D., and Blomberg, B.B. (2016). Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 17, 7–19.
Frasca, D., Diaz, A., Romero, M., Garcia, D., and Blomberg, B.B. (2020). B cell immunosenescence. Annu Rev Cell Dev Biol 36, 551–574.
Freeman, E.W., Sammel, M.D., Lin, H., and Gracia, C.R. (2012). Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab 97, 1673–1680.
Freund, A., Orjalo, A.V., Desprez, P.Y., and Campisi, J. (2010). Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 16, 238–246.
Freund, A., Patil, C.K., and Campisi, J. (2011). p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 30, 1536–1548.
Frey, M.R., and Matera, A.G. (2001). RNA-mediated interaction of Cajal bodies and U2 snRNA genes. J Cell Biol 154, 499–510.
Fried, L.P., Tangen, C.M., Walston, J., Newman, A.B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W.J., Burke, G., et al. (2001). Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56, M146–M157.
Frisch, B.J., Hoffman, C.M., Latchney, S.E., LaMere, M.W., Myers, J., Ashton, J., Li, A.J., Saunders Ii, J., Palis, J., Perkins, A.S., et al. (2019). Aged marrow macrophages expand platelet-biased hematopoietic stem cells via interleukin-1B. JCI Insight 4.
Frisoni, G.B., Boccardi, M., Barkhof, F., Blennow, K., Cappa, S., Chiotis, K., Démonet, J.F., Garibotto, V., Giannakopoulos, P., Gietl, A., et al. (2017). Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. Lancet Neurol 16, 661–676.
Fritsche, A., Madaus, A., Stefan, N., Tschritter, O., Maerker, E., Teigeler, A., Haring, H., and Stumvoll, M. (2002). Relationships among age, proinsulin conversion, and β-cell function in nondiabetic humans. Diabetes 51, S234–S239.
Frohlich, J., and Vinciguerra, M. (2020). Candidate rejuvenating factor GDF11 and tissue fibrosis: friend or foe? Geroscience 42, 1475–1498.
Frontera, W.R., Hughes, V.A., Fielding, R.A., Fiatarone, M.A., Evans, W.J., and Roubenoff, R. (2000). Aging of skeletal muscle: a 12-yr longitudinal study. J Appl Physiol 88, 1321–1326.
Frottin, F., Schueder, F., Tiwary, S., Gupta, R., Körner, R., Schlichthaerle, T., Cox, J., Jungmann, R., Hartl, F.U., and Hipp, M.S. (2019). The nucleolus functions as a phase-separated protein quality control compartment. Science 365, 342–347.
Fu, L., Xu, X., Ren, R., Wu, J., Zhang, W., Yang, J., Ren, X., Wang, S., Zhao, Y., Sun, L., et al. (2016). Modeling xeroderma pigmentosum associated neurological pathologies with patients-derived iPSCs. Protein Cell 7, 210–221.
Fu, W., Hu, W., Yi, Y.S., Hettinghouse, A., Sun, G., Bi, Y., He, W., Zhang, L., Gao, G., Liu, J., et al. (2021). TNFR2/14-3-3ε signaling complex instructs macrophage plasticity in inflammation and autoimmunity. J Clin Invest 131.
Fujino, T., Asada, S., Goyama, S., and Kitamura, T. (2022). Mechanisms involved in hematopoietic stem cell aging. Cell Mol Life Sci 79, 473.
Funk, M.C., Zhou, J., and Boutros, M. (2020). Ageing, metabolism and the intestine. EMBO Rep 21, e50047.
Furrer, K., Rickenbacher, A., Tian, Y., Jochum, W., Bittermann, A.G., Käch, A., Humar, B., Graf, R., Moritz, W., and Clavien, P.A. (2011). Serotonin reverts age-related capillarization and failure of regeneration in the liver through a VEGF-dependent pathway. Proc Natl Acad Sci USA 108, 2945–2950.
Gaille, M., Araneda, M., Dubost, C., Guillermain, C., Kaakai, S., Ricadat, E., Todd, N., and Rera, M. (2020). Ethical and social implications of approaching death prediction in humans—when the biology of ageing meets existential issues. BMC Med Ethics 21, 64.
Galganski, L., Urbanek, M.O., and Krzyzosiak, W.J. (2017). Nuclear speckles: molecular organization, biological function and role in disease. Nucleic Acids Res 45, 10350–10368.
Galkin, F., Mamoshina, P., Aliper, A., de Magalhães, J.P., Gladyshev, V.N., and Zhavoronkov, A. (2020). Biohorology and biomarkers of aging: Current state-of-the-art, challenges and opportunities. Ageing Res Rev 60, 101050.
Gallagher, E.J., and LeRoith, D. (2011). Is growth hormone resistance/IGF-1 reduction good for you? Cell Metab 13, 355–356.
Galvan, V., and Jin, K. (2007). Neurogenesis in the aging brain. CIA 2, 605–610.
Gamble, K.L., Berry, R., Frank, S.J., and Young, M.E. (2014). Circadian clock control of endocrine factors. Nat Rev Endocrinol 10, 466–475.
Gan, Q., Huang, J., Zhou, R., Niu, J., Zhu, X., Wang, J., Zhang, Z., and Tong, T. (2008). PPARγ accelerates cellular senescence by inducing p16INK4α expression in human diploid fibroblasts. J Cell Sci 121, 2235–2245.
Ganz, P., Heidecker, B., Hveem, K., Jonasson, C., Kato, S., Segal, M.R., Sterling, D.G., and Williams, S.A. (2016). Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. JAMA 315, 2532–2541.
García-Prat, L., Martínez-Vicente, M., Perdiguero, E., Ortet, L., Rodríguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A.L., et al. (2016). Autophagy maintains stemness by preventing senescence. Nature 529, 37–42.
García-Prat, L., Perdiguero, E., Alonso-Martín, S., Dell'Orso, S., Ravichandran, S., Brooks, S.R., Juan, A.H., Campanario, S., Jiang, K., Hong, X., et al. (2020). FoxO maintains a genuine muscle stem-cell quiescent state until geriatric age. Nat Cell Biol 22, 1307–1318.
Gardener, H., Caunca, M.R., Dong, C., Cheung, Y.K., Elkind, M.S.V., Sacco, R.L., Rundek, T., and Wright, C.B. (2017). Ultrasound markers of carotid atherosclerosis and cognition. Stroke 48, 1855–1861.
Gardner, S.E., Humphry, M., Bennett, M.R., and Clarke, M.C.H. (2015). Senescent vascular smooth muscle cells drive inflammation through an interleukin-1α-dependent senescence-associated secretory phenotype. Arterioscler Thromb Vasc Biol 35, 1963–1974.
Garrett-Bakelman, F.E., Darshi, M., Green, S.J., Gur, R.C., Lin, L., Macias, B.R., McKenna, M.J., Meydan, C., Mishra, T., Nasrini, J., et al. (2019). The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. Science 364.
Gasek, N.S., Kuchel, G.A., Kirkland, J.L., and Xu, M. (2021). Strategies for targeting senescent cells in human disease. Nat Aging 1, 870–879.
Gate, D., Saligrama, N., Leventhal, O., Yang, A.C., Unger, M.S., Middeldorp, J., Chen, K., Lehallier, B., Channappa, D., De Los Santos, M.B., et al. (2020). Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. Nature 577, 399–404.
Gate, D., Tapp, E., Leventhal, O., Shahid, M., Nonninger, T.J., Yang, A.C., Strempfl, K., Unger, M.S., Fehlmann, T., Oh, H., et al. (2021). $CD4^+$ T cells contribute to neurodegeneration in Lewy body dementia. Science 374, 868–874.
Ge, Y., Miao, Y., Gur-Cohen, S., Gomez, N., Yang, H., Nikolova, M., Polak, L., Hu, Y., Verma, A., Elemento, O., et al. (2020). The aging skin microenvironment dictates stem cell behavior. Proc Natl Acad Sci USA 117, 5339–5350.
Geiger, H., de Haan, G., and Florian, M.C. (2013). The ageing haematopoietic stem cell compartment. Nat Rev Immunol 13, 376–389.
Gekakis, N., Staknis, D., Nguyen, H.B., Davis, F.C., Wilsbacher, L.D., King, D.P., Takahashi, J.S., and Weitz, C.J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. Science 280, 1564–1569.
Gekas, C., and Graf, T. (2013). CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age. Blood 121, 4463–4472.
Geng, H., Chen, H., Wang, H., and Wang, L. (2021). The Histone Mod-

ifications of Neuronal Plasticity. Neural Plast 2021, 6690523.
Geng, L., Liu, Z., Zhang, W., Li, W., Wu, Z., Wang, W., Ren, R., Su, Y., Wang, P., Sun, L., et al. (2019). Chemical screen identifies a geroprotective role of quercetin in premature aging. Protein Cell 10, 417–435.
Geng, L., Zhang, B., Liu, H., Wang, S., Cai, Y., Yang, K., Zou, Z., Jiang, X., Liu, Z., Li, W., et al. (2022). A comparative study of metformin and nicotinamide riboside in alleviating tissue aging in rats. Life Med doi: 10.1093/lifemedi/lnac045.
Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., et al. (2014). Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 371, 2477–2487.
Gensler, H.L., and Bernstein, H. (1981). DNA damage as the primary cause of aging. Q Rev Biol 56, 279–303.
Gerlach, B., Cordier, S.M., Schmukle, A.C., Emmerich, C.H., Rieser, E., Haas, T.L., Webb, A.I., Rickard, J.A., Anderton, H., Wong, W.W.L., et al. (2011). Linear ubiquitination prevents inflammation and regulates immune signalling. Nature 471, 591–596.
Gesualdo, L., Paolo, S.D., Calabró, A., Milani, S., Maiorano, E., Ranieri, E., Pannarale, G., and Schena, F.P. (1996). Expression of epidermal growth factor and its receptor in normal and diseased human kidney: an immunohistochemical and in situ hybridization study. Kidney Int 49, 656–665.
Ghaben, A.L., and Scherer, P.E. (2019). Adipogenesis and metabolic health. Nat Rev Mol Cell Biol 20, 242–258.
Ghilain, C., Gilson, E., and Giraud-Panis, M.J. (2021). Multifunctionality of the telomere-capping shelterin complex explained by variations in its protein composition. Cells 10, 1753.
Ghosh, D.K., Pande, S., Kumar, J., Yesodharan, D., Nampoothiri, S., Radhakrishnan, P., Reddy, C.G., Ranjan, A., and Girisha, K.M. (2022a). The^{E262K} mutation in Lamin A links nuclear proteostasis imbalance to laminopathy-associated premature aging. Aging Cell 21, e13688.
Ghosh, T.S., Shanahan, F., and O'Toole, P.W. (2022b). The gut microbiome as a modulator of healthy ageing. Nat Rev Gastroenterol Hepatol 19, 565–584.
Gialluisi, A., Di Castelnuovo, A., Donati, M.B., de Gaetano, G., and Iacoviello, L. (2019). Machine learning approaches for the estimation of biological aging: the road ahead for population studies. Front Med 6, 146.
Giangreco, A., Goldie, S.J., Failla, V., Saintigny, G., and Watt, F.M. (2010). Human skin aging is associated with reduced expression of the stem cell markers β1 integrin and MCSP. J Invest Dermatol 130, 604–608.
Giangreco, A., Qin, M., Pintar, J.E., and Watt, F.M. (2008). Epidermal stem cells are retained *in vivo* throughout skin aging. Aging Cell 7, 250–259.
Gil, J., Bernard, D., Martínez, D., and Beach, D. (2004). Polycomb CBX7 has a unifying role in cellular lifespan. Nat Cell Biol 6, 67–72.
Gil, J., and Peters, G. (2006). Regulation of the *INK4b-ARF-INK4a* tumour suppressor locus: all for one or one for all. Nat Rev Mol Cell Biol 7, 667–677.
Gilchrist, R.B., Lane, M., and Thompson, J.G. (2008). Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update 14, 159–177.
Gillis, N.K., Ball, M., Zhang, Q., Ma, Z., Zhao, Y.L., Yoder, S.J., Balasis, M.E., Mesa, T.E., Sallman, D.A., Lancet, J.E., et al. (2017). Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol 18, 112–121.
Gilson, E., and Géli, V. (2007). How telomeres are replicated. Nat Rev Mol Cell Biol 8, 825–838.
Giorgi, C., Marchi, S., Simoes, I.C.M., Ren, Z., Morciano, G., Perrone, M., Patalas-Krawczyk, P., Borchard, S., Jedrak, P., Pierzynowska, K., et al. (2018). Mitochondria and reactive oxygen species in aging and age-related diseases. Int Rev Cell Mol Biol 340, 209–344.
Gire, V., and Dulić, V. (2015). Senescence from G2 arrest, revisited. Cell Cycle 14, 297–304.
Gire, V., Roux, P., Wynford-Thomas, D., Brondello, J.M., and Dulic, V. (2004). DNA damage checkpoint kinase Chk2 triggers replicative senescence. EMBO J 23, 2554–2563.
Glaser, J., and Stienecker, K. (2000). Pancreas and aging: a study using ultrasonography. Gerontology 46, 93–96.
Glück, S., Guey, B., Gulen, M.F., Wolter, K., Kang, T.W., Schmacke, N.A., Bridgeman, A., Rehwinkel, J., Zender, L., and Ablasser, A. (2017). Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. Nat Cell Biol 19, 1061–1070.
Gögele, M., Pattaro, C., Fuchsberger, C., Minelli, C., Pramstaller, P.P., and Wjst, M. (2011). Heritability analysis of life span in a semi-isolated population followed across four centuries reveals the presence of pleiotropy between life span and reproduction. J Gerontol A Biol Sci Med Sci 66A, 26–37.
Gohlke, S., Zagoriy, V., Cuadros Inostroza, A., Méret, M., Mancini, C., Japtok, L., Schumacher, F., Kuhlow, D., Graja, A., Stephanowitz, H., et al. (2019). Identification of functional lipid metabolism biomarkers of brown adipose tissue aging. Mol Metab 24, 1–17.
Goldberg, L.R. (2021). Extracellular vesicles and hematopoietic stem cell aging. Arterioscler Thromb Vasc Biol 41.
Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A. E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., et al. (2004). Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci USA 101, 8963–8968.
Gomatou, G., Trontzas, I., Ioannou, S., Drizou, M., Syrigos, N., and Kotteas, E. (2021). Mechanisms of resistance to cyclin-dependent kinase 4/6 inhibitors. Mol Biol Rep 48, 915–925.
Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J.H., Petersen, R.C., Parisi, J.E., and Hyman, B.T. (1997). Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 41, 17–24.
Gómez-Santos, B., Saenz de Urturi, D., Nuñez-García, M., Gonzalez-Romero, F., Buque, X., Aurrekoetxea, I., Gutiérrez de Juan, V., Gonzalez-Rellan, M.J., García-Monzón, C., González-Rodríguez, Á., et al. (2020). Liver osteopontin is required to prevent the progression of age-related nonalcoholic fatty liver disease. Aging Cell 19, e13183.
Gong, H., Pang, J., Han, Y., Dai, Y., Dai, D., Cai, J., and Zhang, T.M. (2014). Age-dependent tissue expression patterns of Sirt1 in senescence-accelerated mice. Mol Med Rep 10, 3296–3302.
Gong, H., Qian, H., Ertl, R., Astle, C.M., Wang, G.G., Harrison, D.E., and Xu, X. (2015). Histone modifications change with age, dietary restriction and rapamycin treatment in mouse brain. Oncotarget 6, 15882–15890.
Gonneaud, J., Baria, A.T., Pichet Binette, A., Gordon, B.A., Chhatwal, J.P., Cruchaga, C., Jucker, M., Levin, J., Salloway, S., Farlow, M., et al. (2021). Accelerated functional brain aging in pre-clinical familial Alzheimer's disease. Nat Commun 12, 5346.
Gonzalez-Covarrubias, V., Beekman, M., Uh, H.W., Dane, A., Troost, J., Paliukhovich, I., Kloet, F.M., Houwing-Duistermaat, J., Vreeken, R.J., Hankemeier, T., et al. (2013). Lipidomics of familial longevity. Aging Cell 12, 426–434.
Gonzalez-Teuber, V., Albert-Gasco, H., Auyeung, V.C., Papa, F.R., Mallucci, G.R., and Hetz, C. (2019). Small molecules to improve ER proteostasis in disease. Trends Pharmacol Sci 40, 684–695.
Gooblar, J., Roe, C.M., Selsor, N.J., Gabel, M.J., and Morris, J.C. (2015). Attitudes of research participants and the general public regarding disclosure of Alzheimer disease research results. JAMA Neurol 72, 1484–1490.
Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N.A., Friston, K.J., and Frackowiak, R.S.J. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage 14, 21–36.
Goodpaster, B.H., Kelley, D.E., Thaete, F.L., He, J., and Ross, R. (2000). Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol 89, 104–110.
Gopcevic, K.R., Gkaliagkousi, E., Nemcsik, J., Acet, Ö., Bernal-Lopez, M. R., Bruno, R.M., Climie, R.E., Fountoulakis, N., Fraenkel, E., Lazaridis, A., et al. (2021). Pathophysiology of circulating biomarkers and relationship with vascular aging: a review of the literature from

VascAgeNet Group on circulating biomarkers, European Cooperation in science and technology action 18216. Front Physiol 12, 789690.
Gorczyca, J., Litwin, J.A., Pityński, K., and Miodoński, A.J. (2010). Vascular system of human fetal pancreas demonstrated by corrosion casting and scanning electron microscopy. Anat Sci Int 85, 235–240.
Gorgoulis, V., Adams, P.D., Alimonti, A., Bennett, D.C., Bischof, O., Bishop, C., Campisi, J., Collado, M., Evangelou, K., Ferbeyre, G., et al. (2019). Cellular senescence: defining a path forward. Cell 179, 813–827.
Gorki, A.D., Symmank, D., Zahalka, S., Lakovits, K., Hladik, A., Langer, B., Maurer, B., Sexl, V., Kain, R., and Knapp, S. (2022). Murine *ex vivo* cultured alveolar macrophages provide a novel tool to study tissue-resident macrophage behavior and function. Am J Respir Cell Mol Biol 66, 64–75.
Gosden, R.G., Richardson, D.W., Brown, N., and Davidson, D.W. (1982). Structure and gametogenic potential of seminiferous tubules in ageing mice. Reproduction 64, 127–133.
Goto, S., Nakamura, A., Radak, Z., Nakamoto, H., Takahashi, R., Yasuda, K., Sakurai, Y., and Ishii, N. (1999). Carbonylated proteins in aging and exercise: immunoblot approaches. Mech Ageing Dev 107, 245–253.
Gou, M., Li, J., Yi, L., Li, H., Ye, X., Wang, H., Liu, L., Sun, B., Zhang, S., Zhu, Z., et al. (2022). Reprogramming of ovarian aging epigenome by resveratrol. PNAS Nexus 2, pgac310.
Gougeon, A., Ecochard, R., and Thalabard, J.C. (1994). Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. Biol Reprod 50, 653–663.
Grajauskas, L.A., Siu, W., Medvedev, G., Guo, H., D'Arcy, R.C.N., and Song, X. (2019). MRI-based evaluation of structural degeneration in the ageing brain: Pathophysiology and assessment. Ageing Res Rev 49, 67–82.
Grassmann, F., Mengelkamp, J., Brandl, C., Harsch, S., Zimmermann, M. E., Linkohr, B., Peters, A., Heid, I.M., Palm, C., and Weber, B.H.F. (2018). A deep learning algorithm for prediction of age-related eye disease study severity scale for age-related macular degeneration from color fundus photography. Ophthalmology 125, 1410–1420.
Gravitz, L. (2018). Skin. Nature 563, S83.
Greaves, L.C., Elson, J.L., Nooteboom, M., Grady, J.P., Taylor, G.A., Taylor, R.W., Mathers, J.C., Kirkwood, T.B., and Turnbull, D.M. (2012). Comparison of mitochondrial mutation spectra in ageing human colonic epithelium and disease: absence of evidence for purifying selection in somatic mitochondrial DNA point mutations. PLoS Genet 8, e1003082.
Greaves, L.C., Preston, S.L., Tadrous, P.J., Taylor, R.W., Barron, M.J., Oukrif, D., Leedham, S.J., Deheragoda, M., Sasieni, P., Novelli, M.R., et al. (2006). Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. Proc Natl Acad Sci USA 103, 714–719.
Grigoryan, A., Guidi, N., Senger, K., Liehr, T., Soller, K., Marka, G., Vollmer, A., Markaki, Y., Leonhardt, H., Buske, C., et al. (2018). LaminA/C regulates epigenetic and chromatin architecture changes upon aging of hematopoietic stem cells. Genome Biol 19, 189.
Groarke, E.M., and Young, N.S. (2019). Aging and hematopoiesis. Clin Geriatr Med 35, 285–293.
Grootaert, M.O.J., and Bennett, M.R. (2022). Sirtuins in atherosclerosis: guardians of healthspan and therapeutic targets. Nat Rev Cardiol 19, 668–683.
Grover, A., Sanjuan-Pla, A., Thongjuea, S., Carrelha, J., Giustacchini, A., Gambardella, A., Macaulay, I., Mancini, E., Luis, T.C., Mead, A., et al. (2016). Single-cell RNA sequencing reveals molecular and functional platelet bias of aged haematopoietic stem cells. Nat Commun 7, 11075.
Guerrero, A., De Strooper, B., and Arancibia-Cárcamo, I.L. (2021). Cellular senescence at the crossroads of inflammation and Alzheimer's disease. Trends Neuroscis 44, 714–727.
Guevara, E.E., Lawler, R.R., Staes, N., White, C.M., Sherwood, C.C., Ely, J.J., Hopkins, W.D., and Bradley, B.J. (2020). Age-associated epigenetic change in chimpanzees and humans. Phil Trans R Soc B 375, 20190616.
Guillermo, A.R.R., Chocian, K., Gavriilidis, G., Vandamme, J., Salcini, A. E., Mellor, J., and Woollard, A. (2021). H3K27 modifiers regulate lifespan in *C. elegans* in a context-dependent manner. BMC Biol 19, 59.
Gumbiner, B., Polonsky, K.S., Beltz, W.F., Wallace, P., Brechtel, G., and Fink, R.I. (1989). Effects of aging on insulin secretion. Diabetes 38, 1549–1556.
Gumucio, J.P., and Mendias, C.L. (2013). Atrogin-1, MuRF-1, and sarcopenia. Endocrine 43, 12–21.
Guntur, A.R., and Rosen, C.J. (2012). Bone as an endocrine organ. Endocrine Pract 18, 758–762.
Guo, G.E., Ma, L.W., Jiang, B., Yi, J., Tong, T.J., and Wang, W.G. (2010). Hydrogen peroxide induces $p16^{INK4a}$ through an AUF1-dependent manner. J Cell Biochem 109, 1000–1005.
Guo, H., Zhu, P., Wu, X., Li, X., Wen, L., and Tang, F. (2013). Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. Genome Res 23, 2126–2135.
Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., and Li, J. (2022). Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Sig Transduct Target Ther 7, 391.
Guo, M., Yao, J., Li, J., Zhang, J., Wang, D., Zuo, H., Zhang, Y., Xu, B., Zhong, Y., Shen, F., et al. (2023). Irisin ameliorates age-associated sarcopenia and metabolic dysfunction. J Cachexia Sarcopenia Muscle 14, 391–405.
Guo, Q., Chen, X., Chen, J., Zheng, G., Xie, C., Wu, H., Miao, Z., Lin, Y., Wang, X., Gao, W., et al. (2021). STING promotes senescence, apoptosis, and extracellular matrix degradation in osteoarthritis via the NF-κB signaling pathway. Cell Death Dis 12, 13.
Gupta, D.K., Khandker, N., Stacy, K., Tatsuoka, C.M., and Preston, D.C. (2017). Utility of combining a simulation-based method with a lecture-based method for fundoscopy training in neurology residency. JAMA Neurol 74, 1223–1227.
Gupta, R., and Kumar, N.A. (2018). Morphology and aging of the human adult pancreas: an electron microscopic study. Acta Med Iran 56, 106–112.
Gupta, R., Sahu, M., Tripathi, R., Ambasta, R.K., and Kumar, P. (2022). Protein *S*-sulfhydration: unraveling the prospective of hydrogen sulfide in the brain, vasculature and neurological manifestations. Ageing Res Rev 76, 101579.
Gupta, R.K., Arany, Z., Seale, P., Mepani, R.J., Ye, L., Conroe, H.M., Roby, Y.A., Kulaga, H., Reed, R.R., and Spiegelman, B.M. (2010). Transcriptional control of preadipocyte determination by Zfp423. Nature 464, 619–623.
Gupta, S. (2014). Role of dendritic cells in innate and adaptive immune response in human aging. Exp Gerontol 54, 47–52.
Gutiérrez, O.M., Shlipak, M.G., Katz, R., Waikar, S.S., Greenberg, J.H., Schrauben, S.J., Coca, S., Parikh, C.R., Vasan, R.S., Feldman, H.I., et al. (2022). Associations of plasma biomarkers of inflammation, fibrosis, and kidney tubular injury with progression of diabetic kidney disease: a cohort study. Am J Kidney Dis 79, 849–857.e1.
Guttenbach, M., Schakowski, R., and Schmid, M. (1994). Aneuploidy and ageing: sex chromosome exclusion into micronuclei. Hum Genet 94, 295–298.
Gyr, K., Beglinger, C., and Stalder, G.A. (1985). Interaction of the endo- and exocrine pancreas (in German). Schweiz Med Wochenschr 115, 1299–1306.
Habes, M., Sotiras, A., Erus, G., Toledo, J.B., Janowitz, D., Wolk, D.A., Shou, H., Bryan, N.R., Doshi, J., Völzke, H., et al. (2018). White matter lesions: spatial heterogeneity, links to risk factors, cognition, genetics, and atrophy. Neurology 91, e964–e975.
Habib, N., McCabe, C., Medina, S., Varshavsky, M., Kitsberg, D., Dvir-Szternfeld, R., Green, G., Dionne, D., Nguyen, L., Marshall, J.L., et al. (2020). Disease-associated astrocytes in Alzheimer's disease and aging. Nat Neurosci 23, 701–706.
Habibe, J.J., Clemente-Olivo, M.P., Scheithauer, T.P.M., Rampanelli, E., Herrema, H., Vos, M., Mieremet, A., Nieuwdorp, M., van Raalte, D.H., Eringa, E.C., et al. (2022). Glucose-mediated insulin secretion is improved in FHL2-deficient mice and elevated FHL2 expression in

humans is associated with type 2 diabetes. Diabetologia 65, 1721–1733.
Haden, S.T., Glowacki, J., Hurwitz, S., Rosen, C., and LeBoff, M.S. (2000). Effects of age on serum dehydroepiandrosterone sulfate, IGF-I, and IL-6 levels in women. Calcif Tissue Int 66, 414–418.
Hagen, M., and Derudder, E. (2020). Inflammation and the alteration of B-cell physiology in aging. Gerontology 66, 105–113.
Hagen, T.M., Yowe, D.L., Bartholomew, J.C., Wehr, C.M., Do, K.L., Park, J.Y., and Ames, B.N. (1997). Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. Proc Natl Acad Sci USA 94, 3064–3069.
Hager, K., Machein, U., Krieger, S., Platt, D., Seefried, G., and Bauer, J. (1994). Interleukin-6 and selected plasma proteins in healthy persons of different ages. Neurobiol Aging 15, 771–772.
Haider, S., and Pal, R. (2013). Integrated analysis of transcriptomic and proteomic data. CG 14, 91–110.
Hainaut, M., and Clarke, H.J. (2021). Germ cells of the mammalian female: A limited or renewable resource? Biol Reprod 105, 774–788.
Haithcock, E., Dayani, Y., Neufeld, E., Zahand, A.J., Feinstein, N., Mattout, A., Gruenbaum, Y., and Liu, J. (2005). Age-related changes of nuclear architecture in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 102, 16690–16695.
Haji, M., Tanaka, S., Nishi, Y., Yanase, T., Takayanagi, R., Hasegawa, Y., Sasamoto, S., and Nawata, H. (1994). Sertoli cell function declines earlier than Leydig cell function in aging Japanese men. Maturitas 18, 143–153.
Haller, S., Vernooij, M.W., Kuijer, J.P.A., Larsson, E.M., Jäger, H.R., and Barkhof, F. (2018). Cerebral microbleeds: imaging and clinical significance. Radiology 287, 11–28.
Halloran, P.F., Melk, A., and Barth, C. (1999). Rethinking chronic allograft nephropathy: the concept of accelerated senescence. J Am Soc Nephrol 10, 167–181.
Halter, J.B. (2010). Carbohydrate metabolism. In: Terjung, R., ed. Comprehensive Physiology. Hoboken: John Wiley & Sons, Ltd. 119–145.
Hämäläinen, R.H., Landoni, J.C., Ahlqvist, K.J., Goffart, S., Ryytty, S., Rahman, M.O., Brilhante, V., Icay, K., Hautaniemi, S., Wang, L., et al. (2019). Defects in mtDNA replication challenge nuclear genome stability through nucleotide depletion and provide a unifying mechanism for mouse progerias. Nat Metab 1, 958–965.
Hamatani, T., Falco, G., Carter, M.G., Akutsu, H., Stagg, C.A., Sharov, A. A., Dudekula, D.B., VanBuren, V., and Ko, M.S.H. (2004). Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet 13, 2263–2278.
Hamburg, N.M., Palmisano, J., Larson, M.G., Sullivan, L.M., Lehman, B. T., Vasan, R.S., Levy, D., Mitchell, G.F., Vita, J.A., and Benjamin, E.J. (2011). Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. Hypertension 57, 390–396.
Hamczyk, M.R., Nevado, R.M., Barettino, A., Fuster, V., and Andrés, V. (2020). Biological versus chronological aging: JACC focus seminar. J Am Coll Cardiol 75, 919–930.
Hampton, T. (2011). Studies probe role of telomere length in predicting, modulating cancer risk. JAMA 305, 2278–2279.
Han, H., Tan, J., Wang, R., Wan, H., He, Y., Yan, X., Guo, J., Gao, Q., Li, J., Shang, S., et al. (2020a). PINK1 phosphorylates Drp1^{S616} to regulate mitophagy-independent mitochondrial dynamics. EMBO Rep 21, e48686.
Han, L., Long, Q., Li, S., Xu, Q., Zhang, B., Dou, X., Qian, M., Jiramongkol, Y., Guo, J., Cao, L., et al. (2020b). Senescent stromal cells promote cancer resistance through SIRT1 loss-potentiated overproduction of small extracellular vesicles. Cancer Res 80, 3383–3398.
Han, X., Chen, H., Gong, H., Tang, X., Huang, N., Xu, W., Tai, H., Zhang, G., Zhao, T., Gong, C., et al. (2020c). Autolysosomal degradation of cytosolic chromatin fragments antagonizes oxidative stress-induced senescence. J Biol Chem 295, 4451–4463.
Han, W.K., Bailly, V., Abichandani, R., Thadhani, R., and Bonventre, J.V. (2002). Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 62, 237–244.
Hando, J.C., Nath, J., and Tucker, J.D. (1994). Sex chromosomes, micronuclei and aging in women. Chromosoma 103, 186–192.
Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S.V., Klotzle, B., Bibikova, M., Fan, J.B., Gao, Y., et al. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell 49, 359–367.
Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio, D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., et al. (2019). Association of amyloid and Tau with cognition in preclinical Alzheimer disease: a longitudinal study. JAMA Neurol 76, 915–924.
Hansen, K. (2013). Predicting reproductive age with biomarkers of ovarian reserve—how (and what) are we measuring? Semin Reprod Med 31, 416–426.
Hansen, K.R., Hodnett, G.M., Knowlton, N., and Craig, L.T.B. (2011). Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 95, 170–175.
Harley, C.B., Futcher, A.B., and Greider, C.W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.
Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J Gerontol 11, 298–300.
Harman, D. (1972). The biologic clock: the mitochondria? J Am Geriatr Soc 20, 145–147.
Harms, M., and Seale, P. (2013). Brown and beige fat: development, function and therapeutic potential. Nat Med 19, 1252–1263.
Harrison, D.E. (1983). Long-term erythropoietic repopulating ability of old, young, and fetal stem cells. J Exp Med 157, 1496–1504.
Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., et al. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.
Harrison, T.M., La Joie, R., Maass, A., Baker, S.L., Swinnerton, K., Fenton, L., Mellinger, T.J., Edwards, L., Pham, J., Miller, B.L., et al. (2019). Longitudinal tau accumulation and atrophy in aging and alzheimer disease. Ann Neurol 85, 229–240.
Hartl, F.U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332.
Hartleben, B., Gödel, M., Meyer-Schwesinger, C., Liu, S., Ulrich, T., Köbler, S., Wiech, T., Grahammer, F., Arnold, S.J., Lindenmeyer, M.T., et al. (2010). Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. J Clin Invest 120, 1084–1096.
Harvey, A., Montezano, A.C., Lopes, R.A., Rios, F., and Touyz, R.M. (2016). Vascular fibrosis in aging and hypertension: molecular mechanisms and clinical implications. Can J Cardiol 32, 659–668.
Hastier, P., Buckley, M.J.M., Dumas, R., Kuhdorf, H., Staccini, P., Demarquay, J.F., Caroli-Bosc, F.X., and Delmont, J.P. (1998). A study of the effect of age on pancreatic duct morphology. Gastrointest Endosc 48, 53–57.
Hastings, M.H., Maywood, E.S., and Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. Nat Rev Neurosci 19, 453–469.
Hatashita, S., and Wakebe, D. (2020). Longitudinal assessment of amyloid-beta deposition in initially amyloid-negative individuals with [11C]-PIB PET imaging. Alzheimers Dement 16, e039595.
Haug, H., Kühl, S., Mecke, E., Sass, N.L., and Wasner, K. (1984). The significance of morphometric procedures in the investigation of age changes in cytoarchitectonic structures of human brain. J Hirnforsch 25, 353–374.
Haycock, P.C., Heydon, E.E., Kaptoge, S., Butterworth, A.S., Thompson, A., and Willeit, P. (2014). Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. BMJ 349, g4227.
Hayden, M.S., and Ghosh, S. (2012). NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 26, 203–234.
Haydont, V., Bernard, B.A., and Fortunel, N.O. (2019). Age-related evolutions of the dermis: clinical signs, fibroblast and extracellular

matrix dynamics. Mech Ageing Dev 177, 150–156.
Hayek, S.S., Sever, S., Ko, Y.A., Trachtman, H., Awad, M., Wadhwani, S., Altintas, M.M., Wei, C., Hotton, A.L., French, A.L., et al. (2015). Soluble urokinase receptor and chronic kidney disease. N Engl J Med 373, 1916–1925.
He, P., Lim, K., Sun, D., Pett, J.P., Jeng, Q., Polanski, K., Dong, Z., Bolt, L., Richardson, L., Mamanova, L., et al. (2022). A human fetal lung cell atlas uncovers proximal-distal gradients of differentiation and key regulators of epithelial fates. Cell 185, 4841–4860.e25.
He, S., Fu, T., Yu, Y., Liang, Q., Li, L., Liu, J., Zhang, X., Zhou, Q., Guo, Q., Xu, D., et al. (2021). IRE1α regulates skeletal muscle regeneration through myostatin mRNA decay. J Clin Invest 131.
He, T., Quan, T., Shao, Y., Voorhees, J.J., and Fisher, G.J. (2014a). Oxidative exposure impairs TGF-β pathway via reduction of type II receptor and SMAD3 in human skin fibroblasts. Age 36, 9623.
He, Y.H., Lu, X., Wu, H., Cai, W.W., Yang, L.Q., Xu, L.Y., Sun, H.P., and Kong, Q.P. (2014b). Mitochondrial DNA content contributes to healthy aging in Chinese: a study from nonagenarians and centenarians. Neurobiol Aging 35, 1779.e1–1779.e4.
Hedman, A.M., van Haren, N.E.M., Schnack, H.G., Kahn, R.S., and Hulshoff Pol, H.E. (2012). Human brain changes across the life span: a review of 56 longitudinal magnetic resonance imaging studies. Hum Brain Mapp 33, 1987–2002.
Heffernan, K.S., Patvardhan, E.A., Kapur, N.K., Karas, R.H., and Kuvin, J. T. (2012). Peripheral augmentation index as a biomarker of vascular aging: an invasive hemodynamics approach. Eur J Appl Physiol 112, 2871–2879.
Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B., and Monaghan, P. (2012). Telomere length in early life predicts lifespan. Proc Natl Acad Sci USA 109, 1743–1748.
Heijblom, M., Piras, D., van den Engh, F.M., van der Schaaf, M., Klaase, J. M., Steenbergen, W., and Manohar, S. (2016). The state of the art in breast imaging using the Twente Photoacoustic Mammoscope: results from 31 measurements on malignancies. Eur Radiol 26, 3874–3887.
Heinze, I., Bens, M., Calzia, E., Holtze, S., Dakhovnik, O., Sahm, A., Kirkpatrick, J.M., Szafranski, K., Romanov, N., Sama, S.N., et al. (2018). Species comparison of liver proteomes reveals links to naked mole-rat longevity and human aging. BMC Biol 16, 82.
Helman, A., Klochendler, A., Azazmeh, N., Gabai, Y., Horwitz, E., Anzi, S., Swisa, A., Condiotti, R., Granit, R.Z., Nevo, Y., et al. (2016). $p16^{Ink4a}$-induced senescence of pancreatic beta cells enhances insulin secretion. Nat Med 22, 412–420.
Hendricks, J.A., Keliher, E.J., Marinelli, B., Reiner, T., Weissleder, R., and Mazitschek, R. (2011). *In vivo* PET imaging of histone deacetylases by ^{18}F-suberoylanilide hydroxamic acid (^{18}F-SAHA). J Med Chem 54, 5576–5582.
Hepler, C., Shan, B., Zhang, Q., Henry, G.H., Shao, M., Vishvanath, L., Ghaben, A.L., Mobley, A.B., Strand, D., Hon, G.C., et al. (2018). Identification of functionally distinct fibro-inflammatory and adipogenic stromal subpopulations in visceral adipose tissue of adult mice. eLife 7.
Herbig, U., Jobling, W.A., Chen, B.P.C., Chen, D.J., and Sedivy, J.M. (2004). Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and $p21^{CIP1}$, but not $p16^{INK4a}$. Mol Cell 14, 501–513.
Herbst, A., Pak, J.W., McKenzie, D., Bua, E., Bassiouni, M., and Aiken, J. M. (2007). Accumulation of mitochondrial DNA deletion mutations in aged muscle fibers: evidence for a causal role in muscle fiber loss. J Gerontol A Biol Sci Med Sci 62, 235–245.
Herdy, J.R., Traxler, L., Agarwal, R.K., Karbacher, L., Schlachetzki, J.C. M., Boehnke, L., Zangwill, D., Galasko, D., Glass, C.K., Mertens, J., et al. (2022). Increased post-mitotic senescence in aged human neurons is a pathological feature of Alzheimer's disease. Cell Stem Cell 29, 1637–1652.e6.
Heredia, J.E., Mukundan, L., Chen, F.M., Mueller, A.A., Deo, R.C., Locksley, R.M., Rando, T.A., and Chawla, A. (2013). Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. Cell 153, 376–388.
Heringa, S.M., Reijmer, Y.D., Leemans, A., Koek, H.L., Kappelle, L.J., and Biessels, G.J. (2014). Multiple microbleeds are related to cerebral network disruptions in patients with early Alzheimer's disease. J Alzheimer Dis 38, 211–221.
Hernandez-Segura, A., de Jong, T.V., Melov, S., Guryev, V., Campisi, J., and Demaria, M. (2017). Unmasking transcriptional heterogeneity in senescent cells. Curr Biol 27, 2652–2660.e4.
Hernandez-Segura, A., Nehme, J., and Demaria, M. (2018). Hallmarks of cellular senescence. Trends Cell Biol 28, 436–453.
Hernlund, E., Svedbom, A., Ivergård, M., Compston, J., Cooper, C., Stenmark, J., McCloskey, E.V., Jönsson, B., and Kanis, J.A. (2013). Osteoporosis in the European Union: medical management, epidemiology and economic burden. Arch Osteoporos 8, 136.
Herranz, N., Gallage, S., Mellone, M., Wuestefeld, T., Klotz, S., Hanley, C. J., Raguz, S., Acosta, J.C., Innes, A.J., Banito, A., et al. (2015). mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol 17, 1205–1217.
Hertel, J., Friedrich, N., Wittfeld, K., Pietzner, M., Budde, K., Van der Auwera, S., Lohmann, T., Teumer, A., Völzke, H., Nauck, M., et al. (2016). Measuring biological age via metabonomics: the metabolic age score. J Proteome Res 15, 400–410.
Herzig, K.H., Purhonen, A.K., Räsänen, K.M., Idziak, J., Juvonen, P., Phillps, R., and Walkowiak, J. (2011). Fecal pancreatic elastase-1 levels in older individuals without known gastrointestinal diseases or diabetes mellitus. BMC Geriatr 11, 4.
Hidalgo, A., Libby, P., Soehnlein, O., Aramburu, I.V., Papayannopoulos, V., and Silvestre-Roig, C. (2022). Neutrophil extracellular traps: from physiology to pathology. Cardiovasc Res 118, 2737–2753.
Hide, D., Warren, A., Fernández-Iglesias, A., Maeso-Díaz, R., Peralta, C., Le Couteur, D.G., Bosch, J., Cogger, V.C., and Gracia-Sancho, J. (2020). Ischemia/reperfusion injury in the aged liver: the importance of the sinusoidal endothelium in developing therapeutic strategies for the elderly. J Gerontol A Biol Sci Med Sci 75, 268–277.
Higgins-Chen, A.T., Thrush, K.L., and Levine, M.E. (2021). Aging biomarkers and the brain. Semin Cell Dev Biol 116, 180–193.
Hilmer, S.N., Cogger, V.C., and Couteur, D.G.L. (2007). Basal activity of Kupffer cells increases with old age. J Gerontol A Biol Sci Med Sci 62, 973–978.
Hilvo, M., Meikle, P.J., Pedersen, E.R., Tell, G.S., Dhar, I., Brenner, H., Schöttker, B., Lääperi, M., Kauhanen, D., Koistinen, K.M., et al. (2020). Development and validation of a ceramide- and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. Eur Heart J 41, 371–380.
Hinge, A., He, J., Bartram, J., Javier, J., Xu, J., Fjellman, E., Sesaki, H., Li, T., Yu, J., Wunderlich, M., et al. (2020). Asymmetrically segregated mitochondria provide cellular memory of hematopoietic stem cell replicative history and drive HSC attrition. Cell Stem Cell 26, 420–430. e6.
Hipp, M.S., Kasturi, P., and Hartl, F.U. (2019). The proteostasis network and its decline in ageing. Nat Rev Mol Cell Biol 20, 421–435.
Hirose, T., Ninomiya, K., Nakagawa, S., and Yamazaki, T. (2022). A guide to membraneless organelles and their various roles in gene regulation. Nat Rev Mol Cell Biol doi: 10.1038/s41580-022-00558-8.
Hitt, R., Young-Xu, Y., Silver, M., and Perls, T. (1999). Centenarians: the older you get, the healthier you have been. Lancet 354, 652.
Ho, K.C., Roessmann, U., Straumfjord, J.V., and Monroe, G. (1980). Analysis of brain weight. I. Adult brain weight in relation to sex, race, and age. Arch Pathol Lab Med 104, 635–639.
Ho, Y.H., del Toro, R., Rivera-Torres, J., Rak, J., Korn, C., García-García, A., Macías, D., González-Gómez, C., del Monte, A., Wittner, M., et al. (2019). Remodeling of bone marrow hematopoietic stem cell niches promotes myeloid cell expansion during premature or physiological aging. Cell Stem Cell 25, 407–418.e6.
Hodgin, J.B., Bitzer, M., Wickman, L., Afshinnia, F., Wang, S.Q., O'Connor, C., Yang, Y., Meadowbrooke, C., Chowdhury, M., Kikuchi, M., et al. (2015). Glomerular aging and focal global glomerulosclerosis: a podometric perspective. J Am Soc Nephrol 26, 3162–3178.

Hoffmann, A., and Baltimore, D. (2006). Circuitry of nuclear factor kappaB signaling. Immunol Rev 210, 171–186.
Hofman, M.A., and Swaab, D.F. (1994). Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res 651, 134–142.
Hohman, L.S., and Osborne, L.C. (2022). A gut-centric view of aging: Do intestinal epithelial cells contribute to age-associated microbiota changes, inflammaging, and immunosenescence? Aging Cell 21.
Höhn, A., Weber, D., Jung, T., Ott, C., Hugo, M., Kochlik, B., Kehm, R., König, J., Grune, T., and Castro, J.P. (2017). Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biol 11, 482–501.
Holly, A.C., Melzer, D., Pilling, L.C., Henley, W., Hernandez, D.G., Singleton, A.B., Bandinelli, S., Guralnik, J.M., Ferrucci, L., and Harries, L.W. (2013). Towards a gene expression biomarker set for human biological age. Aging Cell 12, 324–326.
Holtmann, M., and Neurath, M. (2004). Differential TNF-signaling in chronic inflammatory disorders. Curr Mol Med 4, 439–444.
Holzscheck, N., Falckenhayn, C., Söhle, J., Kristof, B., Siegner, R., Werner, A., Schössow, J., Jürgens, C., Völzke, H., Wenck, H., et al. (2021). Modeling transcriptomic age using knowledge-primed artificial neural networks. NPJ Aging Mech Dis 7, 15.
Homma, S., Hirose, N., Ishida, H., Ishii, T., and Araki, G. (2001). Carotid plaque and intima-media thickness assessed by b-mode ultrasonography in subjects ranging from young adults to centenarians. Stroke 32, 830–835.
Hommos, M.S., Glassock, R.J., and Rule, A.D. (2017). Structural and functional changes in human kidneys with healthy aging. J Am Soc Nephrol 28, 2838–2844.
Honoré, L.H. (1978). Ageing changes in the human testis: a light-microscopic study. Gerontology 24, 58–65.
Hood, S., and Amir, S. (2017). The aging clock: circadian rhythms and later life. J Clin Invest 127, 437–446.
Hormaechea-Agulla, D., Matatall, K.A., Le, D.T., Kain, B., Long, X., Kus, P., Jaksik, R., Challen, G.A., Kimmel, M., and King, K.Y. (2021). Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFNγ signaling. Cell Stem Cell 28, 1428–1442.e6.
Horne, J.A., and Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 4, 97–110.
Horný, L., Roubalová, L., Kronek, J., Chlup, H., Adámek, T., Blanková, A., Petřivý, Z., Suchý, T., and Tichý, P. (2022). Correlation between age, location, orientation, loading velocity and delamination strength in the human aorta. J Mech Behav Biomed Mater 133, 105340.
Horvath, S. (2013). DNA methylation age of human tissues and cell types. Genome Biol 14, R115.
Horvath, S. (2015). Erratum to: DNA methylation age of human tissues and cell types. Genome Biol 16, 96.
Horvath, S., Haghani, A., Macoretta, N., Ablaeva, J., Zoller, J.A., Li, C.Z., Zhang, J., Takasugi, M., Zhao, Y., Rydkina, E., et al. (2022). DNA methylation clocks tick in naked mole rats but queens age more slowly than nonbreeders. Nat Aging 2, 46–59.
Horvath, S., Erhart, W., Brosch, M., Ammerpohl, O., von Schönfels, W., Ahrens, M., Heits, N., Bell, J.T., Tsai, P.C., Spector, T.D., et al. (2014). Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci USA 111, 15538–15543.
Horvath, S., Langfelder, P., Kwak, S., Aaronson, J., Rosinski, J., Vogt, T.F., Eszes, M., Faull, R.L.M., Curtis, M.A., Waldvogel, H.J., et al. (2016). Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. Aging 8, 1485–1512.
Horvath, S., Lu, A.T., Cohen, H., and Raj, K. (2019). Rapamycin retards epigenetic ageing of keratinocytes independently of its effects on replicative senescence, proliferation and differentiation. Aging 11, 3238–3249.
Horvath, S., Oshima, J., Martin, G.M., Lu, A.T., Quach, A., Cohen, H., Felton, S., Matsuyama, M., Lowe, D., Kabacik, S., et al. (2018). Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. Aging 10, 1758–1775.
Horvath, S., and Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet 19, 371–384.
Hou, X., Wei, Z., Zouboulis, C.C., and Ju, Q. (2022). Aging in the sebaceous gland. Front Cell Dev Biol 10, 909694.
Houtkooper, R.H., Argmann, C., Houten, S.M., Cantó, C., Jeninga, E.H., Andreux, P.A., Thomas, C., Doenlen, R., Schoonjans, K., and Auwerx, J. (2011). The metabolic footprint of aging in mice. Sci Rep 1, 134.
Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497, 451–457.
Hsu, H., Shu, H.B., Pan, M.G., and Goeddel, D.V. (1996). TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell 84, 299–308.
Hu, H., Ji, Q., Song, M., Ren, J., Liu, Z., Wang, Z., Liu, X., Yan, K., Hu, J., Jing, Y., et al. (2020). ZKSCAN3 counteracts cellular senescence by stabilizing heterochromatin. Nucleic Acids Res 48, 6001–6018.
Hu, L., Mauro, T.M., Dang, E., Man, G., Zhang, J., Lee, D., Wang, G., Feingold, K.R., Elias, P.M., and Man, M.Q. (2017). Epidermal dysfunction leads to an age-associated increase in levels of serum inflammatory cytokines. J Invest Dermatol 137, 1277–1285.
Hu, S.C.S., Lin, C.L., and Yu, H.S. (2019a). Dermoscopic assessment of xerosis severity, pigmentation pattern and vascular morphology in subjects with physiological aging and photoaging. Eur J Dermatol 29, 274–280.
Hu, W., Chan, C.S., Wu, R., Zhang, C., Sun, Y., Song, J.S., Tang, L.H., Levine, A.J., and Feng, Z. (2010). Negative regulation of tumor suppressor p53 by microRNA miR-504. Mol Cell 38, 689–699.
Hu, W.T., Howell, J.C., Ozturk, T., Gangishetti, U., Kollhoff, A.L., Hatcher-Martin, J.M., Anderson, A.M., and Tyor, W.R. (2019b). CSF cytokines in aging, multiple sclerosis, and dementia. Front Immunol 10, 480.
Hu, X., Ono, M., Chimge, N.O., Chosa, K., Nguyen, C., Melendez, E., Lou, C.H., Lim, P., Termini, J., Lai, K.K.Y., et al. (2021). Differential Kat3 usage orchestrates the integration of cellular metabolism with differentiation. Cancers 13, 5884.
Hua, Y., Zhang, Y., Ceylan-Isik, A.F., Wold, L.E., Nunn, J.M., and Ren, J. (2011). Chronic Akt activation accentuates aging-induced cardiac hypertrophy and myocardial contractile dysfunction: role of autophagy. Basic Res Cardiol 106, 1173–1191.
Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020a). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506.
Huang, D., Zuo, Y., Zhang, C., Sun, G., Jing, Y., Lei, J., Ma, S., Sun, S., Lu, H., Zhang, X., et al. (2022a). A single-nucleus transcriptomic atlas of primate testicular aging reveals exhaustion of the spermatogonial stem cell reservoir and loss of Sertoli cell homeostasis. Protein Cell doi: 10.1093/procel/pwac057.
Huang, H., Weng, H., and Chen, J. (2020b). The biogenesis and precise control of RNA m^6A methylation. Trends Genet 36, 44–52.
Huang, S., Wang, J., Fan, D.Y., Luo, T., Li, Y., Tu, Y.F., Shen, Y.Y., Zeng, G.H., Chen, D.W., Wang, Y.R., et al. (2022b). The association of serum uric acid with cognitive impairment and ATN biomarkers. Front Aging Neurosci 14, 943380.
Huang, W., Hickson, L.T.J., Eirin, A., Kirkland, J.L., and Lerman, L.O. (2022c). Cellular senescence: the good, the bad and the unknown. Nat Rev Nephrol 18, 611–627.
Huang, W., Tan, D., Wang, X., Han, S., Tan, J., Zhao, Y., Lu, J., and Huang, B. (2006). Histone deacetylase 3 represses p15INK4b and p21WAF1/cip1 transcription by interacting with Sp1. Biochem Biophys Res Commun 339, 165–171.
Huang, Y., Ge, M.X., Li, Y.H., Li, J.L., Yu, Q., Xiao, F.H., Ao, H.S., Yang, L.Q., Li, J., He, Y., et al. (2022d). Longevity-associated transcription factor *ATF7* promotes healthspan by suppressing cellular senescence and systematic inflammation. Aging Dis doi: 10.14336/AD.2022.1217.
Huang, Y., Liu, D., Wei, X., Huang, C., Li, C., and Zhang, H. (2022e). Time-restricted eating on weight loss: implications from the TREATY study. Life Med 1, 58–60.
Huang, Y., Wu, J., Li, R., Wang, P., Han, L., Zhang, Z., and Tong, T. (2011).

B-MYB delays cell aging by repressing $p16^{INK4\alpha}$ transcription. Cell Mol Life Sci 68, 893–901.
Huang, Y.H., Chen, C.W., Sundaramurthy, V., Słabicki, M., Hao, D., Watson, C.J., Tovy, A., Reyes, J.M., Dakhova, O., Crovetti, B.R., et al. (2022f). Systematic profiling of *DNMT3A* variants reveals protein instability mediated by the DCAF8 E3 ubiquitin ligase adaptor. Cancer Discov 12, 220–235.
Huber, A.K., Giles, D.A., Segal, B.M., and Irani, D.N. (2018). An emerging role for eotaxins in neurodegenerative disease. Clin Immunol 189, 29–33.
Huff, W.X., Kwon, J.H., Henriquez, M., Fetcko, K., and Dey, M. (2019). The evolving role of $CD8^+CD28^-$ immunosenescent T cells in cancer immunology. Int J Mol Sci 20, 2810.
Hughes, M.C., Bredoux, C., Salas, F., Lombard, D., Strutton, G.M., Fourtanier, A., and Green, A.C. (2011). Comparison of histological measures of skin photoaging. Dermatology 223, 140–151.
Hughes, V.A., Roubenoff, R., Wood, M., Frontera, W.R., Evans, W.J., and Fiatarone Singh, M.A. (2004). Anthropometric assessment of 10-y changes in body composition in the elderly. Am J Clin Nutr 80, 475–482.
Huh, J.Y., Mougios, V., Kabasakalis, A., Fatouros, I., Siopi, A., Douroudos, I.I., Filippaios, A., Panagiotou, G., Park, K.H., and Mantzoros, C.S. (2014). Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. J Clin Endocrinol Metab 99, E2154–E2161.
Huhtaniemi, I.T., Tajar, A., Lee, D.M., O'Neill, T.W., Finn, J.D., Bartfai, G., Boonen, S., Casanueva, F.F., Giwercman, A., Han, T.S., et al. (2012). Comparison of serum testosterone and estradiol measurements in 3174 European men using platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men. Eur J Endocrinol 166, 983–991.
Hume, S., Dianov, G.L., and Ramadan, K. (2020). A unified model for the G1/S cell cycle transition. Nucleic Acids Res 48, 12483–12501.
Humphrey, J.D., Harrison, D.G., Figueroa, C.A., Lacolley, P., and Laurent, S. (2016). Central artery stiffness in hypertension and aging: a problem with cause and consequence. Circ Res 118, 379–381.
Hunt, P.A., and Hassold, T.J. (2008). Human female meiosis: what makes a good egg go bad? Trends Genet 24, 86–93.
Hwang, S.M., Im, K., Chang, Y.H., Park, H.S., Kim, J.A., Kim, S.M., and Lee, D.S. (2018). Are clonal cells circulating in the peripheral blood of myelodysplastic syndrome?: Quantitative comparison between bone marrow and peripheral blood by targeted gene sequencing and fluorescence in situ hybridization. Leuk Res 71, 92–94.
Hwangbo, N., Zhang, X., Raftery, D., Gu, H., Hu, S.C., Montine, T.J., Quinn, J.F., Chung, K.A., Hiller, A.L., Wang, D., et al. (2022). A metabolomic aging clock using human cerebrospinal fluid. J Gerontol A Biol Sci Med Sci 77, 744–754.
Iakova, P., Awad, S.S., and Timchenko, N.A. (2003). Aging reduces proliferative capacities of liver by switching pathways of C/EBPα growth arrest. Cell 113, 495–506.
Iarovaia, O.V., Minina, E.P., Sheval, E.V., Onichtchouk, D., Dokudovskaya, S., Razin, S.V., and Vassetzky, Y.S. (2019). Nucleolus: a central hub for nuclear functions. Trends Cell Biol 29, 647–659.
Ichihara, I., Kawamura, H., and Pelliniemi, L.J. (1993). Ultrastructure and morphometry of testicular Leydig cells and the interstitial components correlated with testosterone in aging rats. Cell Tissue Res 271, 241–255.
Idda, M.L., McClusky, W.G., Lodde, V., Munk, R., Abdelmohsen, K., Rossi, M., and Gorospe, M. (2020). Survey of senescent cell markers with age in human tissues. Aging 12, 4052–4066.
Igarashi, M., Miura, M., Williams, E., Jaksch, F., Kadowaki, T., Yamauchi, T., and Guarente, L. (2019). NAD^+ supplementation rejuvenates aged gut adult stem cells. Aging Cell 18, e12935.
Ihara, K., Skupien, J., Kobayashi, H., Md Dom, Z.I., Wilson, J.M., O'Neil, K., Badger, H.S., Bowsman, L.M., Satake, E., Breyer, M.D., et al. (2020). Profibrotic circulating proteins and risk of early progressive renal decline in patients with type 2 diabetes with and without albuminuria. Diabetes Care 43, 2760–2767.
Ihm, S.H., Moon, H.J., Kang, J.G., Park, C.Y., Oh, K.W., Jeong, I.K., Oh, Y.S., and Park, S.W. (2007). Effect of aging on insulin secretory function and expression of beta cell function-related genes of islets. Diabetes Res Clin Pract 77, S150–S154.
Ikeda, F., Deribe, Y.L., Skånland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S.J.L., Goswami, P., Nagy, V., Terzic, J., et al. (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF-κB activity and apoptosis. Nature 471, 637–641.
Ikeda, T. (1995). Age-related reduction in bone matrix protein mRNA expression in rat bone tissues: application of histomorphometry to *in situ* hybridization. Bone 16, 17–23.
Inaba, Y., Chen, J.A., and Bergmann, S.R. (2012). Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. Atherosclerosis 220, 128–133.
Iram, T., Kern, F., Kaur, A., Myneni, S., Morningstar, A.R., Shin, H., Garcia, M.A., Yerra, L., Palovics, R., Yang, A.C., et al. (2022). Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17. Nature 605, 509–515.
Irvine, K.M., Skoien, R., Bokil, N.J., Melino, M., Thomas, G.P., Loo, D., Gabrielli, B., Hill, M.M., Sweet, M.J., Clouston, A.D., et al. (2014). Senescent human hepatocytes express a unique secretory phenotype and promote macrophage migration. World J Gastroenterol 20, 17851–17862.
Irving, A., and Harvey, J. (2021). Regulation of hippocampal synaptic function by the metabolic hormone leptin: Implications for health and disease. Prog Lipid Res 82, 101098.
Irwin, D.J., Cohen, T.J., Grossman, M., Arnold, S.E., Xie, S.X., Lee, V.M. Y., and Trojanowski, J.Q. (2012). Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. Brain 135, 807–818.
Isaev, N.K., Stelmashook, E.V., and Genrikhs, E.E. (2019). Neurogenesis and brain aging. Rev Neuroscis 30, 573–580.
Ishigaki, Y., Oka, Y., and Katagiri, H. (2009). Circulating oxidized LDL: a biomarker and a pathogenic factor. Curr Opin Lipidol 20, 363–369.
Ishov, A.M., Sotnikov, A.G., Negorev, D., Vladimirova, O.V., Neff, N., Kamitani, T., Yeh, E.T.H., Strauss Iii, J.F., and Maul, G.G. (1999). PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. J Cell Biol 147, 221–234.
Ito, Y., Hoare, M., and Narita, M. (2017). Spatial and temporal control of senescence. Trends Cell Biol 27, 820–832.
Ito, Y., Sørensen, K.K., Bethea, N.W., Svistounov, D., McCuskey, M.K., Smedsrød, B.H., and McCuskey, R.S. (2007). Age-related changes in the hepatic microcirculation in mice. Exp Gerontol 42, 789–797.
Itokawa, N., Oshima, M., Koide, S., Takayama, N., Kuribayashi, W., Nakajima-Takagi, Y., Aoyama, K., Yamazaki, S., Yamaguchi, K., Furukawa, Y., et al. (2022). Epigenetic traits inscribed in chromatin accessibility in aged hematopoietic stem cells. Nat Commun 13, 2691.
Ivanov, A., Pawlikowski, J., Manoharan, I., van Tuyn, J., Nelson, D.M., Rai, T.S., Shah, P.P., Hewitt, G., Korolchuk, V.I., Passos, J.F., et al. (2013). Lysosome-mediated processing of chromatin in senescence. J Cell Biol 202, 129–143.
Ivell, R., and Anand-Ivell, R. (2009). Biology of insulin-like factor 3 in human reproduction. Hum Reprod Update 15, 463–476.
Ivell, R., Heng, K., Severn, K., Antonio, L., Bartfai, G., Casanueva, F.F., Huhtaniemi, I.T., Giwercman, A., Maggi, M., O'Connor, D.B., et al. (2022). The Leydig cell biomarker INSL3 as a predictor of age-related morbidity: Findings from the EMAS cohort. Front Endocrinol 13, 1016107.
Ivell, R., Wade, J.D., and Anand-Ivell, R. (2013). INSL3 as a biomarker of Leydig cell functionality. Biol Reprod 88, 147.
Iwawaki, T., Akai, R., Kohno, K., and Miura, M. (2004). A transgenic mouse model for monitoring endoplasmic reticulum stress. Cell Struct Funct 29, 95.
Ix, J.H., Katz, R., Bansal, N., Foster, M., Weiner, D.E., Tracy, R., Jotwani, V., Hughes-Austin, J., McKay, D., Gabbai, F., et al. (2017). Urine fibrosis markers and risk of allograft failure in kidney transplant recipients: a case-cohort ancillary study of the FAVORIT trial. Am J

Kidney Dis 69, 410–419.
Ix, J.H., and Shlipak, M.G. (2021). The promise of tubule biomarkers in kidney disease: a review. Am J Kidney Dis 78, 719–727.
Jacinto, T.A., Meireles, G.S., Dias, A.T., Aires, R., Porto, M.L., Gava, A.L., Vasquez, E.C., Pereira, T.M.C., Campagnaro, B.P., and Meyrelles, S.S. (2018). Increased ROS production and DNA damage in monocytes are biomarkers of aging and atherosclerosis. Biol Res 51, 33.
Jack, C.R. Jr., Barrio, J.R., and Kepe, V. (2013). Cerebral amyloid PET imaging in Alzheimer's disease. Acta Neuropathol 126, 643–657.
Jack, C.R. Jr., Wiste, H.J., Weigand, S.D., Therneau, T.M., Lowe, V.J., Knopman, D.S., Gunter, J.L., Senjem, M.L., Jones, D.T., Kantarci, K., et al. (2017). Defining imaging biomarker cut points for brain aging and Alzheimer's disease. Alzheimers Dement 13, 205–216.
Jacobs, K.B., Yeager, M., Zhou, W., Wacholder, S., Wang, Z., Rodriguez-Santiago, B., Hutchinson, A., Deng, X., Liu, C., Horner, M.J., et al. (2012). Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet 44, 651–658.
Jacome Burbano, M.S., and Gilson, E. (2020). Long-lived post-mitotic cell aging: is a telomere clock at play? Mech Ageing Dev 189, 111256.
Jaenisch, R., and Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33, 245–254.
JafariNasabian, P., Inglis, J.E., Reilly, W., Kelly, O.J., and Ilich, J.Z. (2017). Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake. J Endocrinol 234, R37–R51.
Jaiswal, S. (2020). Clonal hematopoiesis and non-hematologic disorders. Blood 136, 1606–1614.
Jaiswal, S., and Ebert, B.L. (2019). Clonal hematopoiesis in human aging and disease. Science 366, eaan4673.
Jaiswal, S., and Libby, P. (2020). Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. Nat Rev Cardiol 17, 137–144.
Jakovljevic, D.G. (2018). Physical activity and cardiovascular aging: Physiological and molecular insights. Exp Gerontol 109, 67–74.
James, M.T., Hemmelgarn, B.R., Wiebe, N., Pannu, N., Manns, B.J., Klarenbach, S.W., and Tonelli, M. (2010). Glomerular filtration rate, proteinuria, and the incidence and consequences of acute kidney injury: a cohort study. Lancet 376, 2096–2103.
Jan, M., Ebert, B.L., and Jaiswal, S. (2017). Clonal hematopoiesis. Semin Hematol 54, 43–50.
Jansen, P.L.M. (2002). Liver disease in the elderly. Best Pract Res Clin Gastroenterol 16, 149–158.
Jansen, R., Han, L.K., Verhoeven, J.E., Aberg, K.A., van den Oord, E.C., Milaneschi, Y., and Penninx, B.W. (2021). An integrative study of five biological clocks in somatic and mental health. eLife 10, e59479.
Janssen, J., and Papavassiliou, I. (2014). Effect of aging and diffuse chronic pancreatitis on pancreas elasticity evaluated using semiquantitative EUS elastography. Ultraschall Med 35, 253–258.
Jasper, H. (2020). Intestinal stem cell aging: origins and interventions. Annu Rev Physiol 82, 203–226.
Jenkins, N.C., Liu, T., Cassidy, P., Leachman, S.A., Boucher, K.M., Goodson, A.G., Samadashwily, G., and Grossman, D. (2011). The $p16^{INK4A}$ tumor suppressor regulates cellular oxidative stress. Oncogene 30, 265–274.
Jennette, J.C., and Nachman, P.H. (2017). ANCA glomerulonephritis and vasculitis. Clin J Am Soc Nephrol 12, 1680–1691.
Jentzsch, V., Davis, J., and Djamgoz, M. (2020). Pancreatic Cancer (PDAC): introduction of evidence-based complementary measures into integrative clinical management. Cancers 12, 3096.
Jeon, H.J., Kim, Y.S., Kim, J.G., Heo, K., Pyo, J.H., Yamaguchi, M., Park, J.S., and Yoo, M.A. (2018). Effect of heterochromatin stability on intestinal stem cell aging in *Drosophila*. Mech Ageing Dev 173, 50–60.
Jessberger, R. (2010). Deterioration without replenishment—the misery of oocyte cohesin. Genes Dev 24, 2587–2591.
Jiang, H., Zhu, W.J., Li, J., Chen, Q.J., Liang, W.B., and Gu, Y.Q. (2014a). Quantitative histological analysis and ultrastructure of the aging human testis. Int Urol Nephrol 46, 879–885.
Jiang, M.H., Cai, B., Tuo, Y., Wang, J., Zang, Z.J., Tu, X., Gao, Y., Su, Z., Li, W., Li, G., et al. (2014b). Characterization of Nestin-positive stem Leydig cells as a potential source for the treatment of testicular Leydig cell dysfunction. Cell Res 24, 1466–1485.
Jiang, T., Wei, F., and Xie, K. (2022). Clinical significance of pancreatic ductal metaplasia. J Pathol 257, 125–139.
Jiang, X., Yan, J., Sheng, Y., Sun, M., Cui, L., and Chen, Z.J. (2018). Low anti-Müllerian hormone concentration is associated with increased risk of embryonic aneuploidy in women of advanced age. Reprod Biomed Online 37, 178–183.
Jiang, Z., Wang, Y., Li, Z., Dai, Z., He, Y., Chu, K., Gu, J., Ji, Y.X., Sun, N., Yang, F., et al. (2021). The m^6A mRNA demethylase FTO in granulosa cells retards FOS-dependent ovarian aging. Cell Death Dis 12, 744.
Jin, X., Wang, K., Wang, L., Liu, W., Zhang, C., Qiu, Y., Liu, W., Zhang, H., Zhang, D., Yang, Z., et al. (2022). RAB7 activity is required for the regulation of mitophagy in oocyte meiosis and oocyte quality control during ovarian aging. Autophagy 18, 643–660.
Jin, X., Xiong, S., Ju, S.Y., Zeng, Y., Yan, L.L., and Yao, Y. (2020). Serum 25-hydroxyvitamin D, albumin, and mortality among Chinese older adults: a population-based longitudinal study. J Clin Endocrinol Metab 105, 2762–2770.
Jing, Y., Shaheen, E., Drake, R.R., Chen, N., Gravenstein, S., and Deng, Y. (2009). Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. Hum Immunol 70, 777–784.
Jing, Y., Zuo, Y., Yu, Y., Sun, L., Yu, Z., Ma, S., Zhao, Q., Sun, G., Hu, H., Li, J., et al. (2022). Single-nucleus profiling unveils a geroprotective role of the FOXO3 in primate skeletal muscle aging. Protein Cell doi: 10.1093/procel/pwac061.
Joe, A.W.B., Yi, L., Natarajan, A., Le Grand, F., So, L., Wang, J., Rudnicki, M.A., and Rossi, F.M.V. (2010). Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 12, 153–163.
Johnson, A.A., Shokhirev, M.N., and Lehallier, B. (2021). The protein inputs of an ultra-predictive aging clock represent viable anti-aging drug targets. Ageing Res Rev 70, 101404.
Johnson, A.A., Shokhirev, M.N., Wyss-Coray, T., and Lehallier, B. (2020). Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. Ageing Res Rev 60, 101070.
Johnson, J.L. (2007). Matrix metalloproteinases: influence on smooth muscle cells and atherosclerotic plaque stability. Expert Rev Cardiovasc Ther 5, 265–282.
Johnson, K.M., Owen, K., and Witte, P.L. (2002). Aging and developmental transitions in the B cell lineage. Int Immunol 14, 1313–1323.
Johnson, L. (1989). Evaluation of the human testis and its age-related dysfunction. Prog Clin Biol Res 302, 35–60.
Johnson, L., Nguyen, H.B., Petty, C.S., and Neaves, W.B. (1987). Quantification of human spermatogenesis: germ cell degeneration during spermatocytogenesis and meiosis in testes from younger and older adult men. Biol Reprod 37, 739–747.
Johnson, L., Petty, C.S., and Neaves, W.B. (1984a). Influence of age on sperm production and testicular weights in men. Reproduction 70, 211–218.
Johnson, L., Zane, R.S., Petty, C.S., and Neaves, W.B. (1984b). Quantification of the human sertoli cell population: its distribution, relation to germ cell numbers, and age-related decline. Biol Reprod 31, 785–795.
Johnson, L.C., Parker, K., Aguirre, B.F., Nemkov, T.G., D'Alessandro, A., Johnson, S.A., Seals, D.R., and Martens, C.R. (2019). The plasma metabolome as a predictor of biological aging in humans. Geroscience 41, 895–906.
Johnson, S.L., Dunleavy, J., Gemmell, N.J., and Nakagawa, S. (2015). Consistent age-dependent declines in human semen quality: a systematic review and meta-analysis. Ageing Res Rev 19, 22–33.
Johri, A.M., Calnan, C.M., Matangi, M.F., MacHaalany, J., and Hétu, M.F. (2016). Focused vascular ultrasound for the assessment of

atherosclerosis: a proof-of-concept study. J Am Soc Echocard 29, 842–849.
Jones, C.I. (2016). Platelet function and ageing. Mamm Genome 27, 358–366.
Jones, M., Osawa, G., Regal, J.A., Weinberg, D.N., Taggart, J., Kocak, H., Friedman, A., Ferguson, D.O., Keegan, C.E., and Maillard, I. (2014). Hematopoietic stem cells are acutely sensitive to Acd shelterin gene inactivation. J Clin Invest 124, 353–366.
Jones, R.A., Harrison, C., Eaton, S.L., Llavero Hurtado, M., Graham, L.C., Alkhammash, L., Oladiran, O.A., Gale, A., Lamont, D.J., Simpson, H., et al. (2017). Cellular and molecular anatomy of the human neuromuscular junction. Cell Rep 21, 2348–2356.
Jordan, M.I., and Mitchell, T.M. (2015). Machine learning: Trends, perspectives, and prospects. Science 349, 255–260.
Jordan, S., Tung, N., Casanova-Acebes, M., Chang, C., Cantoni, C., Zhang, D., Wirtz, T.H., Naik, S., Rose, S.A., Brocker, C.N., et al. (2019). Dietary intake regulates the circulating inflammatory monocyte pool. Cell 178, 1102–1114.e17.
Josephson, A.M., Bradaschia-Correa, V., Lee, S., Leclerc, K., Patel, K.S., Muinos Lopez, E., Litwa, H.P., Neibart, S.S., Kadiyala, M., Wong, M. Z., et al. (2019). Age-related inflammation triggers skeletal stem/progenitor cell dysfunction. Proc Natl Acad Sci USA 116, 6995–7004.
Ju, W., Nair, V., Smith, S., Zhu, L., Shedden, K., Song, P.X.K., Mariani, L. H., Eichinger, F.H., Berthier, C.C., Randolph, A., et al. (2015). Tissue transcriptome-driven identification of epidermal growth factor as a chronic kidney disease biomarker. Sci Transl Med 7.
Ju, Y.J., Lee, K.H., Park, J.E., Yi, Y.S., Yun, M.Y., Ham, Y.H., Kim, T.J., Choi, H.M., Han, G.J., Lee, J.H., et al. (2006). Decreased expression of DNA repair proteins Ku70 and Mre11 is associated with aging and may contribute to the cellular senescence. Exp Mol Med 38, 686–693.
Ju, Z., Jiang, H., Jaworski, M., Rathinam, C., Gompf, A., Klein, C., Trumpp, A., and Rudolph, K.L. (2007). Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med 13, 742–747.
Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589.
Jung, K.H., Stephens, K.A., Yochim, K.M., Riphagen, J.M., Kim, C.M., Buckner, R.L., and Salat, D.H. (2021). Heterogeneity of cerebral white matter lesions and clinical correlates in older adults. Stroke 52, 620–630.
Jung, M., and Pfeifer, G.P. (2015). Aging and DNA methylation. BMC Biol 13, 7.
Jung, S.E., Lim, S.M., Hong, S.R., Lee, E.H., Shin, K.J., and Lee, H.Y. (2019a). DNA methylation of the *ELOVL2*, *FHL2*, *KLF14*, *C1orf132/MIR29B2C*, and *TRIM59* genes for age prediction from blood, saliva, and buccal swab samples. Forensic Sci Int Genet 38, 1–8.
Jung, S.H., Hwang, H.J., Kang, D., Park, H.A., Lee, H.C., Jeong, D., Lee, K., Park, H.J., Ko, Y.G., and Lee, J.S. (2019b). mTOR kinase leads to PTEN-loss-induced cellular senescence by phosphorylating p53. Oncogene 38, 1639–1650.
Jung, Y.S., Qian, Y., and Chen, X. (2010). Examination of the expanding pathways for the regulation of p21 expression and activity. Cell Signal 22, 1003–1012.
Junnila, R.K., List, E.O., Berryman, D.E., Murrey, J.W., and Kopchick, J.J. (2013). The GH/IGF-1 axis in ageing and longevity. Nat Rev Endocrinol 9, 366–376.
Jurivich, D.A., Manocha, G.D., Trivedi, R., Lizakowski, M., Rakoczy, S., and Brown-Borg, H. (2020). Multifactorial attenuation of the murine heat shock response with age. J Gerontol A Biol Sci Med Sci 75, 1846–1852.
Justice, J.N., Ferrucci, L., Newman, A.B., Aroda, V.R., Bahnson, J.L., Divers, J., Espeland, M.A., Marcovina, S., Pollak, M.N., Kritchevsky, S.B., et al. (2018). A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. Geroscience 40, 419–436.
Jylhävä, J., Pedersen, N.L., and Hägg, S. (2017). Biological age predictors. Ebiomedicine 21, 29–36.
Kabacik, S., Lowe, D., Fransen, L., Leonard, M., Ang, S.L., Whiteman, C., Corsi, S., Cohen, H., Felton, S., Bali, R., et al. (2022). The relationship between epigenetic age and the hallmarks of aging in human cells. Nat Aging 2, 484–493.
Kaczynski, J., Sellers, S., Seidman, M.A., Syed, M., Dennis, M., Mcnaught, G., Jansen, M., Semple, S.I., Alcaide-Corral, C., Tavares, A.A.S., et al. (2022). ^{18}F-NaF PET/MRI for detection of carotid atheroma in acute neurovascular syndrome. Radiology 305, 137–148.
Kadurin, A., Aliper, A., Kazennov, A., Mamoshina, P., Vanhaelen, Q., Khrabrov, K., and Zhavoronkov, A. (2017a). The cornucopia of meaningful leads: applying deep adversarial autoencoders for new molecule development in oncology. Oncotarget 8, 10883–10890.
Kadurin, A., Nikolenko, S., Khrabrov, K., Aliper, A., and Zhavoronkov, A. (2017b). druGAN: an advanced generative adversarial autoencoder model for *de novo* generation of new molecules with desired molecular properties *in silico*. Mol Pharm 14, 3098–3104.
Kahn, S.E., Larson, V.G., Beard, J.C., Cain, K.C., Fellingham, G.W., Schwartz, R.S., Veith, R.C., Stratton, J.R., Cerqueira, M.D., and Abrass, I.B. (1990). Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol Endocrinol Metab 258, E937–E943.
Kajiya, K., Kim, Y.K., Kinemura, Y., Kishimoto, J., and Chung, J.H. (2011). Structural alterations of the cutaneous vasculature in aged and in photoaged human skin *in vivo*. J Dermatol Sci 61, 206–208.
Kale, A., Sharma, A., Stolzing, A., Desprez, P.Y., and Campisi, J. (2020). Role of immune cells in the removal of deleterious senescent cells. Immun Ageing 17, 16.
Kalinkovich, A., and Livshits, G. (2017). Sarcopenic obesity or obese sarcopenia: a cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. Ageing Res Rev 35, 200–221.
Kalwat, M.A., Scheuner, D., Rodrigues-dos-Santos, K., Eizirik, D.L., and Cobb, M.H. (2021). The pancreatic beta-cell response to secretory demands and adaption to stress. Endocrinology 162.
Kanaki, T., Makrantonaki, E., and Zouboulis, C.C. (2016). Biomarkers of skin aging. Rev Endocr Metab Disord 17, 433–442.
Kandhaya-Pillai, R., Miro-Mur, F., Alijotas-Reig, J., Tchkonia, T., Kirkland, J.L., and Schwartz, S. (2017). TNFα-senescence initiates a STAT-dependent positive feedback loop, leading to a sustained interferon signature, DNA damage, and cytokine secretion. Aging 9, 2411–2435.
Kaneko, S., Li, G., Son, J., Xu, C.F., Margueron, R., Neubert, T.A., and Reinberg, D. (2010). Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. Genes Dev 24, 2615–2620.
Kang, H.T., Park, J.T., Choi, K., Kim, Y., Choi, H.J.C., Jung, C.W., Lee, Y. S., and Park, S.C. (2017). Chemical screening identifies ATM as a target for alleviating senescence. Nat Chem Biol 13, 616–623.
Kang, H.Y., Lee, J.W., Papaccio, F., Bellei, B., and Picardo, M. (2021). Alterations of the pigmentation system in the aging process. Pigment Cell Melanoma Res 34, 800–813.
Kang, W., Jin, T., Zhang, T., Ma, S., Yan, H., Liu, Z., Ji, Z., Cai, Y., Wang, S., Song, M., et al. (2022). Regeneration Roadmap: database resources for regenerative biology. Nucleic Acids Res 50, D1085–D1090.
Kania, D.M., Binkley, N., Checovich, M., Havighurst, T., Schilling, M., and Ershler, W.B. (1995). Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. J Am Geriatr Soc 43, 236–239.
Kanis, J.A. (2002). Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359, 1929–1936.
Kantarci, K., Schwarz, C.G., Reid, R.I., Przybelski, S.A., Lesnick, T.G., Zuk, S.M., Senjem, M.L., Gunter, J.L., Lowe, V., Machulda, M.M., et al. (2014). White matter integrity determined with diffusion tensor imaging in older adults without dementia: influence of amyloid load and neurodegeneration. JAMA Neurol 71, 1547–1554.
Kar, S.P., Quiros, P.M., Gu, M., Jiang, T., Mitchell, J., Langdon, R., Iyer, V., Barcena, C., Vijayabaskar, M.S., Fabre, M.A., et al. (2022). Genome-

wide analyses of 200,453 individuals yield new insights into the causes and consequences of clonal hematopoiesis. Nat Genet 54, 1155–1166.
Karlawish, J. (2011). Addressing the ethical, policy, and social challenges of preclinical Alzheimer disease. Neurology 77, 1487–1493.
Kasapoğlu, I., and Seli, E. (2020). Mitochondrial dysfunction and ovarian aging. Endocrinology 161.
Kass, E.M., Lim, P.X., Helgadottir, H.R., Moynahan, M.E., and Jasin, M. (2016). Robust homology-directed repair within mouse mammary tissue is not specifically affected by *Brca2* mutation. Nat Commun 7, 13241.
Kasselimi, E., Pefani, D.E., Taraviras, S., and Lygerou, Z. (2022). Ribosomal DNA and the nucleolus at the heart of aging. Trends Biochem Sci 47, 328–341.
Kastenhuber, E.R., and Lowe, S.W. (2017). Putting p53 in context. Cell 170, 1062–1078.
Katsimpardi, L., Litterman, N.K., Schein, P.A., Miller, C.M., Loffredo, F. S., Wojtkiewicz, G.R., Chen, J.W., Lee, R.T., Wagers, A.J., and Rubin, L.L. (2014). Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 344, 630–634.
Kaufman, J.M., Lapauw, B., Mahmoud, A., T'Sjoen, G., and Huhtaniemi, I. T. (2019). Aging and the male reproductive system. Endocrine Rev 40, 906–972.
Kaul, Z., Cesare, A.J., Huschtscha, L.I., Neumann, A.A., and Reddel, R.R. (2011). Five dysfunctional telomeres predict onset of senescence in human cells. EMBO Rep 13, 52–59.
Kauppila, T.E.S., Kauppila, J.H.K., and Larsson, N.G. (2017). Mammalian mitochondria and aging: an update. Cell Metab 25, 57–71.
Kaushik, S., and Cuervo, A.M. (2015). Proteostasis and aging. Nat Med 21, 1406–1415.
Kavcic, V., Ni, H., Zhu, T., Zhong, J., and Duffy, C.J. (2008). White matter integrity linked to functional impairments in aging and early Alzheimer's disease. Alzheimers Dement 4, 381–389.
Kawaguchi, T., Machida, S., Kurumizaka, H., Tagami, H., and Nakayama, J. (2017). Phosphorylation of CBX2 controls its nucleosome-binding specificity. J Biochem 162, 343–355.
Ke, H., Tang, S., Guo, T., Hou, D., Jiao, X., Li, S., Luo, W., Xu, B., Zhao, S., Li, G., et al. (2023). Landscape of pathogenic mutations in premature ovarian insufficiency. Nat Med 29, 483–492.
Kearney, P., Boniface, J.J., Price, N.D., and Hood, L. (2018). The building blocks of successful translation of proteomics to the clinic. Curr Opin Biotechnol 51, 123–129.
Kedlian, V.R., Wang, Y., Liu, T., Chen, X., Bolt, L., Shen, Z., Fasouli, E.S., Prigmore, E., Kleshchevnikov, V., Li, T., et al. (2022). Human skeletal muscle ageing atlas. bioRxiv 2022.05.24.493094.
Keefe, D., Kumar, M., and Kalmbach, K. (2015). Oocyte competency is the key to embryo potential. Fertil Steril 103, 317–322.
Keefe, D., Liu, L., Wang, W., and Silva, C. (2003). Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. Reprod Biomed Online 7, 24–29.
Keefe, D.L., Marquard, K., and Liu, L. (2006). The telomere theory of reproductive senescence in women. Curr Opin Obstet GynEcol 18, 280–285.
Kefaloyianni, E., Gaitanaki, C., and Beis, I. (2006). ERK1/2 and p38-MAPK signalling pathways, through MSK1, are involved in NF-κB transactivation during oxidative stress in skeletal myoblasts. Cell Signal 18, 2238–2251.
Kehm, R., König, J., Nowotny, K., Jung, T., Deubel, S., Gohlke, S., Schulz, T.J., and Höhn, A. (2018). Age-related oxidative changes in pancreatic islets are predominantly located in the vascular system. Redox Biol 15, 387–393.
Kelly, C.J., Karthikesalingam, A., Suleyman, M., Corrado, G., and King, D. (2019). Key challenges for delivering clinical impact with artificial intelligence. BMC Med 17, 195.
Kempfer, R., and Pombo, A. (2020). Methods for mapping 3D chromosome architecture. Nat Rev Genet 21, 207–226.
Kendall, M.D., Johnson, H.R., and Singh, J. (1980). The weight of the human thymus gland at necropsy. J Anat 131(Pt 3), 483–497.
Kennaway, D.J., Lushington, K., Dawson, D., Lack, L., Heuvel, C., and Rogers, N. (1999). Urinary 6-sulfatoxymelatonin excretion and aging: new results and a critical review of the literature. J Pineal Res 27, 210–220.
Kennedy, B.K., Gotta, M., Sinclair, D.A., Mills, K., McNabb, D.S., Murthy, M., Pak, S.M., Laroche, T., Gasser, S.M., and Guarente, L. (1997). Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. Cell 89, 381–391.
Kenney, W.L., Wolf, S.T., Dillon, G.A., Berry, C.W., and Alexander, L.M. (2021). Temperature regulation during exercise in the heat: Insights for the aging athlete. J Sci Med Sport 24, 739–746.
Keramida, G., and Peters, A.M. (2020). FDG PET/CT of the non-malignant liver in an increasingly obese world population. Clin Physiol Funct Imag 40, 304–319.
Kessler, M.D., Damask, A., O'Keeffe, S., Banerjee, N., Li, D., Watanabe, K., Marketta, A., Van Meter, M., Semrau, S., Horowitz, J., et al. (2022). Common and rare variant associations with clonal haematopoiesis phenotypes. Nature 612, 301–309.
Ketchum, F.B., Chin, N.A., Grill, J., Gleason, C.E., Erickson, C., Clark, L. R., Paulsen, J.S., and Kind, A.J.H. (2022). Moving beyond disclosure: stages of care in preclinical Alzheimer's disease biomarker testing. Alzheimers Dement 18, 1969–1979.
Keyes, B.E., Liu, S., Asare, A., Naik, S., Levorse, J., Polak, L., Lu, C.P., Nikolova, M., Pasolli, H.A., and Fuchs, E. (2016). Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. Cell 167, 1323–1338.e14.
Keyes, B.E., Segal, J.P., Heller, E., Lien, W.H., Chang, C.Y., Guo, X., Oristian, D.S., Zheng, D., and Fuchs, E. (2013). *Nfatc1* orchestrates aging in hair follicle stem cells. Proc Natl Acad Sci USA 110, E4950–4959.
Khan, S., Sclabas, G., and Reid-Lombardo, K.M. (2010). Population-based epidemiology, risk factors and screening of intraductal papillary mucinous neoplasm patients. World J Gastrointest Surg 2, 314–318.
Khan, S.S., Singer, B.D., and Vaughan, D.E. (2017). Molecular and physiological manifestations and measurement of aging in humans. Aging Cell 16, 624–633.
Kida, Y., Tchao, B.N., and Yamaguchi, I. (2014). Peritubular capillary rarefaction: a new therapeutic target in chronic kidney disease. Pediatr Nephrol 29, 333–342.
Kiebzak, G.M. (1991). Age-related bone changes. Exp Gerontol 26, 171–187.
Kikuchi, W., Ichihara, K., Mori, K., and Shimizu, Y. (2021). Biological sources of variations of tartrate-resistant acid phosphatase 5b in a healthy Japanese population. Ann Clin Biochem 58, 358–367.
Kim-Ha, J., and Kim, Y.J. (2016). Age-related epigenetic regulation in the brain and its role in neuronal diseases. BMB Rep 49, 671–680.
Kim, B.J., Lee, S.H., and Koh, J.M. (2020a). Clinical insights into the kynurenine pathway in age-related diseases. Exp Gerontol 130, 110793.
Kim, H., Park, S., Jeong, I.G., Song, S.H., Jeong, Y., Kim, C.S., and Lee, K. H. (2021). Noninvasive precision screening of prostate cancer by urinary multimarker sensor and artificial intelligence analysis. ACS Nano 15, 4054–4065.
Kim, H., Yoo, J.E., Cho, J.Y., Oh, B.K., Yoon, Y.S., Han, H.S., Lee, H.S., Jang, J.J., Jeong, S.H., Kim, J.W., et al. (2013). Telomere length, TERT and shelterin complex proteins in hepatocellular carcinomas expressing "stemness"-related markers. J Hepatol 59, 746–752.
Kim, M., Franke, V., Brandt, B., Lowenstein, E.D., Schöwel, V., Spuler, S., Akalin, A., and Birchmeier, C. (2020b). Single-nucleus transcriptomics reveals functional compartmentalization in syncytial skeletal muscle cells. Nat Commun 11, 6375.
Kim, M., Moon, H.B., and Spangrude, G.J. (2003). Major age-related changes of mouse hematopoietic stem/progenitor cells. Ann N Y Acad Sci 996, 195–208.
Kim, S., Myers, L., Wyckoff, J., Cherry, K.E., and Jazwinski, S.M. (2017). The frailty index outperforms DNA methylation age and its derivatives as an indicator of biological age. Geroscience 39, 83–92.
Kim, W.Y., and Sharpless, N.E. (2006). The regulation of INK4/ARF in cancer and aging. Cell 127, 265–275.
Kimmel, D.B., Vennin, S., Desyatova, A., Turner, J.A., Akhter, M.P., Lappe, J.M., and Recker, R.R. (2022). Bone architecture, bone material

properties, and bone turnover in non-osteoporotic post-menopausal women with fragility fracture. Osteoporos Int 33, 1125–1136.
Kimura, M., Itoh, N., Takagi, S., Sasao, T., Takahashi, A., Masumori, N., and Tsukamoto, T. (2003). Balance of apoptosis and proliferation of germ cells related to spermatogenesis in aged men. J Androl 24, 185–191.
Kinn, P.M., Holdren, G.O., Westermeyer, B.A., Abuissa, M., Fischer, C.L., Fairley, J.A., Brogden, K.A., and Brogden, N.K. (2015). Age-dependent variation in cytokines, chemokines and biologic analytes rinsed from the surface of healthy human skin. Sci Rep 5, 10472.
Kitajima, K., Nakamoto, Y., Senda, M., Onishi, Y., Okizuka, H., and Sugimura, K. (2007). Normal uptake of 18F-FDG in the testis: an assessment by PET/CT. Ann Nucl Med 21, 405–410.
Kitani, K. (1992). Liver and aging. Gastroenterol Jpn 27, 276–285.
Kleefeldt, F., Bömmel, H., Broede, B., Thomsen, M., Pfeiffer, V., Wörsdörfer, P., Karnati, S., Wagner, N., Rueckschloss, U., and Ergün, S. (2019). Aging-related carcinoembryonic antigen-related cell adhesion molecule 1 signaling promotes vascular dysfunction. Aging Cell 18, e13025.
Klemera, P., and Doubal, S. (2006). A new approach to the concept and computation of biological age. Mech Ageing Dev 127, 240–248.
Klöppel, G., Detlefsen, S., and Feyerabend, B. (2003). Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. Virchows Arch 445, 1–8.
Knez, J., Winckelmans, E., Plusquin, M., Thijs, L., Cauwenberghs, N., Gu, Y., Staessen, J.A., Nawrot, T.S., and Kuznetsova, T. (2016). Correlates of peripheral blood mitochondrial DNA content in a general population. Am J Epidemiol 183, 138–146.
Knight, J.C., Mosley, M.J., Bravo, L.C., Kersemans, V., Allen, P.D., Mukherjee, S., O'Neill, E., and Cornelissen, B. (2017). 89Zr-anti-γH2AX-TAT but not ^{18}F-FDG allows early monitoring of response to chemotherapy in a mouse model of pancreatic ductal adenocarcinoma. Clin Cancer Res 23, 6498–6504.
Kobayashi, T. (2011). Regulation of ribosomal RNA gene copy number and its role in modulating genome integrity and evolutionary adaptability in yeast. Cell Mol Life Sci 68, 1395–1403.
Koch, C.M., Joussen, S., Schellenberg, A., Lin, Q., Zenke, M., and Wagner, W. (2012). Monitoring of cellular senescence by DNA-methylation at specific CpG sites. Aging Cell 11, 366–369.
Koch, C.M., and Wagner, W. (2011). Epigenetic-aging-signature to determine age in different tissues. Aging 3, 1018–1027.
Kockelkoren, R., Vos, A., Van Hecke, W., Vink, A., Bleys, R.L.A.W., Verdoorn, D., Mali, W.P.T.M., Hendrikse, J., Koek, H.L., de Jong, P.A., et al. (2017). Computed tomographic distinction of intimal and medial calcification in the intracranial internal carotid artery. PLoS ONE 12, e0168360.
Koehler, C.L., Perkins, G.A., Ellisman, M.H., and Jones, D.L. (2017). Pink1 and Parkin regulate *Drosophila* intestinal stem cell proliferation during stress and aging. J Cell Biol 216, 2315–2327.
Koguchi-Yoshioka, H., Hoffer, E., Cheuk, S., Matsumura, Y., Vo, S., Kjellman, P., Grema, L., Ishitsuka, Y., Nakamura, Y., Okiyama, N., et al. (2021). Skin T cells maintain their diversity and functionality in the elderly. Commun Biol 4, 13.
Koh, J.M., Khang, Y.H., Jung, C.H., Bae, S., Kim, D.J., Chung, Y.E., and Kim, G.S. (2005). Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporos Int 16, 1263–1271.
Kohl, E., Steinbauer, J., Landthaler, M., and Szeimies, R.M. (2011). Skin ageing. J Eur Acad Dermatol Venereol 25, 873–884.
Kohut, M.L., Senchina, D.S., Madden, K.S., Martin, A.E., Felten, D.L., and Moynihan, J.A. (2004). Age effects on macrophage function vary by tissue site, nature of stimulant, and exercise behavior. Exp Gerontol 39, 1347–1360.
Kokoszka, J.E., Coskun, P., Esposito, L.A., and Wallace, D.C. (2001). Increased mitochondrial oxidative stress in the Sod2(+/−) mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis. Proc Natl Acad Sci USA 98, 2278–2283.
Kolker, D.E., Fukuyama, H., Huang, D.S., Takahashi, J.S., Horton, T.H., and Turek, F.W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. J Biol Rhythms 18, 159–169.
Kolker, D.E., Vitaterna, M.H., Fruechte, E.M., Takahashi, J.S., and Turek, F.W. (2004). Effects of age on circadian rhythms are similar in wild-type and heterozygous Clock mutant mice. Neurobiol Aging 25, 517–523.
Komutrattananont, P., Mahakkanukrauh, P., and Das, S. (2019). Morphology of the human aorta and age-related changes: anatomical facts. Anat Cell Biol 52, 109–114.
Kong, F., Hua, Y., Zeng, B., Ning, R., Li, Y., and Zhao, J. (2016). Gut microbiota signatures of longevity. Curr Biol 26, R832–R833.
Kong, M., Guo, L., Xu, W., He, C., Jia, X., Zhao, Z., and Gu, Z. (2022). Aging-associated accumulation of mitochondrial DNA mutations in tumor origin. Life Med 1, 149–167.
Konieczny, J., and Arranz, L. (2018). Updates on old and weary haematopoiesis. Int J Mol Sci 19, 2567.
Kotake, Y., Cao, R., Viatour, P., Sage, J., Zhang, Y., and Xiong, Y. (2007). pRB family proteins are required for H3K27 trimethylation and Polycomb repression complexes binding to and silencing p16^{INK4a} tumor suppressor gene. Genes Dev 21, 49–54.
Kotake, Y., Nakagawa, T., Kitagawa, K., Suzuki, S., Liu, N., Kitagawa, M., and Xiong, Y. (2011). Long non-coding RNA *ANRIL* is required for the PRC2 recruitment to and silencing of *p15*^{INK4B} tumor suppressor gene. Oncogene 30, 1956–1962.
Kotake, Y., Ozawa, Y., Harada, M., Kitagawa, K., Niida, H., Morita, Y., Tanaka, K., Suda, T., and Kitagawa, M. (2013). YB1 binds to and represses the *p16* tumor suppressor gene. Genes Cells 18, 999–1006.
Kotake, Y., Zeng, Y., and Xiong, Y. (2009). DDB1-CUL4 and MLL1 mediate oncogene-induced p16^{INK4a} activation. Cancer Res 69, 1809–1814.
Kovtonyuk, L.V., Caiado, F., Garcia-Martin, S., Manz, E.M., Helbling, P., Takizawa, H., Boettcher, S., Al-Shahrour, F., Nombela-Arrieta, C., Slack, E., et al. (2022). IL-1 mediates microbiome-induced inflammaging of hematopoietic stem cells in mice. Blood 139, 44–58.
Krabbe, K.S., Pedersen, M., and Bruunsgaard, H. (2004). Inflammatory mediators in the elderly. Exp Gerontol 39, 687–699.
Kragstrup, T.W., Kjaer, M., and Mackey, A.L. (2011). Structural, biochemical, cellular, and functional changes in skeletal muscle extracellular matrix with aging. Scand J Med Sci Sports 21, 749–757.
Kramer, H., Pickhardt, P.J., Kliewer, M.A., Hernando, D., Chen, G.H., Zagzebski, J.A., and Reeder, S.B. (2017). Accuracy of liver fat quantification with advanced CT, MRI, and ultrasound techniques: prospective comparison with MR spectroscopy. Am J Roentgenol 208, 92–100.
Kresovich, J.K., Park, Y.M.M., Keller, J.A., Sandler, D.P., and Taylor, J.A. (2022). Healthy eating patterns and epigenetic measures of biological age. Am J Clin Nutr 115, 171–179.
Krishnaraj, R. (1997). Senescence and cytokines modulate the NK cell expression. Mech Ageing Dev 96, 89–101.
Krištić, J., Vučković, F., Menni, C., Klarić, L., Keser, T., Beceheli, I., Pučić-Baković, M., Novokmet, M., Mangino, M., Thaqi, K., et al. (2014). Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci 69, 779–789.
Krizhanovsky, V., Yon, M., Dickins, R.A., Hearn, S., Simon, J., Miething, C., Yee, H., Zender, L., and Lowe, S.W. (2008). Senescence of activated stellate cells limits liver fibrosis. Cell 134, 657–667.
Krtolica, A., Parrinello, S., Lockett, S., Desprez, P.Y., and Campisi, J. (2001). Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci USA 98, 12072–12077.
Krus, U., King, B.C., Nagaraj, V., Gandasi, N.R., Sjölander, J., Buda, P., Garcia-Vaz, E., Gomez, M.F., Ottosson-Laakso, E., Storm, P., et al. (2014). The complement inhibitor CD59 regulates insulin secretion by modulating exocytotic events. Cell Metab 19, 883–890.
Kruse, J.P., and Gu, W. (2009). Modes of p53 regulation. Cell 137, 609–622.

Kubben, N., Zhang, W., Wang, L., Voss, T.C., Yang, J., Qu, J., Liu, G.H., and Misteli, T. (2016). Repression of the antioxidant NRF2 pathway in premature aging. Cell 165, 1361–1374.
Kudryavtsev, B.N., Kudryavtseva, M.V., Sakuta, G.A., and Stein, G.I. (1993). Human hepatocyte polyploidization kinetics in the course of life cycle. Virchows Arch B Cell Pathol Incl Mol Pathol 64, 387–393.
Kuh, D., Cooper, R., Sattar, N., Welsh, P., Hardy, R., and Ben-Shlomo, Y. (2019). Systemic inflammation and cardio-renal organ damage biomarkers in middle age are associated with physical capability up to 9 years later. Circulation 139, 1988–1999.
Kuilman, T., Michaloglou, C., Mooi, W.J., and Peeper, D.S. (2010). The essence of senescence. Genes Dev 24, 2463–2479.
Kuilman, T., Michaloglou, C., Vredeveld, L.C.W., Douma, S., van Doorn, R., Desmet, C.J., Aarden, L.A., Mooi, W.J., and Peeper, D.S. (2008). Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell 133, 1019–1031.
Kuilman, T., and Peeper, D.S. (2009). Senescence-messaging secretome: SMS-ing cellular stress. Nat Rev Cancer 9, 81–94.
Kujoth, G.C., Hiona, A., Pugh, T.D., Someya, S., Panzer, K., Wohlgemuth, S.E., Hofer, T., Seo, A.Y., Sullivan, R., Jobling, W.A., et al. (2005). Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309, 481–484.
Kuk, J.L., Saunders, T.J., Davidson, L.E., and Ross, R. (2009). Age-related changes in total and regional fat distribution. Ageing Res Rev 8, 339–348.
Kulkarni, P. (2017). Reinforcement and Deep Reinforcement Machine Learning. In: Reverse Hypothesis Machine Learning. Intelligent Systems Reference Library. Cham: Springer. 59–83.
Kumar, P.P., Emechebe, U., Smith, R., Franklin, S., Moore, B., Yandell, M., Lessnick, S.L., and Moon, A.M. (2014). Coordinated control of senescence by lncRNA and a novel T-box3 co-repressor complex. eLife 3, e02805.
Kumar, R., Delshad, S., Woo, M.A., Macey, P.M., and Harper, R.M. (2012). Age-related regional brain T2-relaxation changes in healthy adults. J Magn Reson Imag 35, 300–308.
Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., and Reppert, S.M. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.
Kunieda, T., Minamino, T., Nishi, J., Tateno, K., Oyama, T., Katsuno, T., Miyauchi, H., Orimo, M., Okada, S., Takamura, M., et al. (2006). Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. Circulation 114, 953–960.
Kupesic, S., and Kurjak, A. (2002). Predictors of IVF outcome by three-dimensional ultrasound. Hum Reprod 17, 950–955.
Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E., et al. (1997). Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390, 45–51.
Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., McGuinness, O.P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., et al. (2005). Suppression of aging in mice by the hormone Klotho. Science 309, 1829–1833.
Kurz, D.J., Decary, S., Hong, Y., Trivier, E., Akhmedov, A., and Erusalimsky, J.D. (2004). Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J Cell Sci 117, 2417–2426.
Kuswanto, W., Burzyn, D., Panduro, M., Wang, K.K., Jang, Y.C., Wagers, A.J., Benoist, C., and Mathis, D. (2016). Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. Immunity 44, 355–367.
Kuvin, J.T., Patel, A.R., Sliney, K.A., Pandian, N.G., Sheffy, J., Schnall, R. P., Karas, R.H., and Udelson, J.E. (2003). Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J 146, 168–174.
Kwon, J.H., Moon, K.M., and Min, K.W. (2020). Exercise-induced myokines can explain the importance of physical activity in the elderly: an overview. Healthcare 8, 378.
Labbadia, J., and Morimoto, R.I. (2015). The biology of proteostasis in aging and disease. Annu Rev Biochem 84, 435–464.
Lacey, D.L., Timms, E., Tan, H.L., Kelley, M.J., Dunstan, C.R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., et al. (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93, 165–176.
Lacolley, P., Regnault, V., and Avolio, A.P. (2018). Smooth muscle cell and arterial aging: basic and clinical aspects. Cardiovasc Res 114, 513–528.
Lacolley, P., Regnault, V., Segers, P., and Laurent, S. (2017). Vascular smooth muscle cells and arterial stiffening: relevance in development, aging, and disease. Physiol Rev 97, 1555–1617.
Lafarga, V., Cuadrado, A., Lopez de Silanes, I., Bengoechea, R., Fernandez-Capetillo, O., and Nebreda, A.R. (2009). p38 mitogen-activated protein kinase- and HuR-dependent stabilization of $p21^{Cip1}$ mRNA mediates the G_1/S checkpoint. Mol Cell Biol 29, 4341–4351.
Lafontaine, D.L.J., Riback, J.A., Bascetin, R., and Brangwynne, C.P. (2021). The nucleolus as a multiphase liquid condensate. Nat Rev Mol Cell Biol 22, 165–182.
Lähnemann, D., Köster, J., Szczurek, E., McCarthy, D.J., Hicks, S.C., Robinson, M.D., Vallejos, C.A., Campbell, K.R., Beerenwinkel, N., Mahfouz, A., et al. (2020). Eleven grand challenges in single-cell data science. Genome Biol 21, 31.
Lai, T.P., Zhang, N., Noh, J., Mender, I., Tedone, E., Huang, E., Wright, W. E., Danuser, G., and Shay, J.W. (2017). A method for measuring the distribution of the shortest telomeres in cells and tissues. Nat Commun 8, 1356.
Lajoie, I., Nugent, S., Debacker, C., Dyson, K., Tancredi, F.B., Badhwar, A. P., Belleville, S., Deschaintre, Y., Bellec, P., Doyon, J., et al. (2017). Application of calibrated fMRI in Alzheimer's disease. Neuroimage Clin 15, 348–358.
Lal, A., Kim, H.H., Abdelmohsen, K., Kuwano, Y., Pullmann, R., Srikantan, S., Subrahmanyam, R., Martindale, J.L., Yang, X., Ahmed, F., et al. (2008). $p16^{INK4a}$ translation suppressed by miR-24. PLoS ONE 3, e1864.
Lallemand-Breitenbach, V., and de Thé, H. (2018). PML nuclear bodies: from architecture to function. Curr Opin Cell Biol 52, 154–161.
Lamb, J., Crawford, E.D., Peck, D., Modell, J.W., Blat, I.C., Wrobel, M.J., Lerner, J., Brunet, J.P., Subramanian, A., Ross, K.N., et al. (2006). The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 313, 1929–1935.
Lamballais, S., Vinke, E.J., Vernooij, M.W., Ikram, M.A., and Muetzel, R. L. (2020). Cortical gyrification in relation to age and cognition in older adults. NeuroImage 212, 116637.
Lambin, P., Leijenaar, R.T.H., Deist, T.M., Peerlings, J., de Jong, E.E.C., van Timmeren, J., Sanduleanu, S., Larue, R.T.H.M., Even, A.J.G., Jochems, A., et al. (2017). Radiomics: the bridge between medical imaging and personalized medicine. Nat Rev Clin Oncol 14, 749–762.
Lan, Y.Y., Heather, J.M., Eisenhaure, T., Garris, C.S., Lieb, D., Raychowdhury, R., and Hacohen, N. (2019). Extranuclear DNA accumulates in aged cells and contributes to senescence and inflammation. Aging Cell 18, e12901.
Lang, T., Streeper, T., Cawthon, P., Baldwin, K., Taaffe, D.R., and Harris, T.B. (2010). Sarcopenia: etiology, clinical consequences, intervention, and assessment. Osteoporos Int 21, 543–559.
Lansdorp, P.M., Verwoerd, N.P., van de Rijke, F.M., Dragowska, V., Little, M.T., Dirks, R.W., Raap, A.K., and Tanke, H.J. (1996). Heterogeneity in telomere length of human chromosomes. Hum Mol Genet 5, 685–691.
Lanzer, P., Boehm, M., Sorribas, V., Thiriet, M., Janzen, J., Zeller, T., St Hilaire, C., and Shanahan, C. (2014). Medial vascular calcification revisited: review and perspectives. Eur Heart J 35, 1515–1525.
Larbi, A., Franceschi, C., Mazzatti, D., Solana, R., Wikby, A., and Pawelec, G. (2008). Aging of the immune system as a prognostic factor for human longevity. Physiology 23, 64–74.
Larsson, L., Degens, H., Li, M., Salviati, L., Lee, Y., Thompson, W., Kirkland, J.L., and Sandri, M. (2019). Sarcopenia: aging-related loss of muscle mass and function. Physiol Rev 99, 427–511.

Larsson, L., Sjödin, B., and Karlsson, J. (1978). Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22-65 years. Acta Physiol Scand 103, 31–39.
Larsson, N.G. (2010). Somatic mitochondrial DNA mutations in mammalian aging. Annu Rev Biochem 79, 683–706.
Lasry, A., and Ben-Neriah, Y. (2015). Senescence-associated inflammatory responses: aging and cancer perspectives. Trends Immunol 36, 217–228.
Lass, A. (2004). Assessment of ovarian reserve: Is there still a role for ovarian biopsy in the light of new data? Hum Reprod 19, 467–469.
Latenstein, A.E.J., van der Geest, L.G.M., Bonsing, B.A., Groot Koerkamp, B., Haj Mohammad, N., de Hingh, I.H.J.T., de Meijer, V.E., Molenaar, I. Q., van Santvoort, H.C., van Tienhoven, G., et al. (2020). Nationwide trends in incidence, treatment and survival of pancreatic ductal adenocarcinoma. Eur J Cancer 125, 83–93.
Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P., and Benetos, A. (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 37, 1236–1241.
Laurentino, S., Cremers, J.F., Horsthemke, B., Tüttelmann, F., Czeloth, K., Zitzmann, M., Pohl, E., Rahmann, S., Schröder, C., Berres, S., et al. (2020). A germ cell-specific ageing pattern in otherwise healthy men. Aging Cell 19, e13242.
Laurie, C.C., Laurie, C.A., Rice, K., Doheny, K.F., Zelnick, L.R., McHugh, C.P., Ling, H., Hetrick, K.N., Pugh, E.W., Amos, C., et al. (2012). Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet 44, 642–650.
Lavker, R.M. (1979). Structural alterations in exposed and unexposed aged skin. J Invest Dermatol 73, 59–66.
Lavker, R.M., Zheng, P., and Dong, G. (1986). Morphology of aged skin. Dermatol Clin 4, 379–389.
Lavker, R.M., Zheng, P., and Dong, G. (1987). Aged skin: a study by light, transmission electron, and scanning electron microscopy. J Invest Dermatol 88, 44s–51s.
Law, J., Morris, D.E., Izzi-Engbeaya, C., Salem, V., Coello, C., Robinson, L., Jayasinghe, M., Scott, R., Gunn, R., Rabiner, E., et al. (2018). Thermal imaging is a noninvasive alternative to PET/CT for measurement of brown adipose tissue activity in humans. J Nucl Med 59, 516–522.
Lazarus, N.R., Lord, J.M., and Harridge, S.D.R. (2019). The relationships and interactions between age, exercise and physiological function. J Physiol 597, 1299–1309.
Lazorthes, S., Vallot, C., Briois, S., Aguirrebengoa, M., Thuret, J.Y., Laurent, G.S., Rougeulle, C., Kapranov, P., Mann, C., Trouche, D., et al. (2015). A vlincRNA participates in senescence maintenance by relieving H2AZ-mediated repression at the *INK4* locus. Nat Commun 6, 5971.
Walter, L.C., and Covinsky, K.E. (2001). Cancer screening in elderly patients: a framework for individualized decision making. JAMA 285, 2750–2756.
Le Couteur, D.G., Cogger, V.C., Markus, A.M., Harvey, P.J., Yin, Z.L., Ansselin, A.D., and McLean, A.J. (2001). Pseudocapillarization and associated energy limitation in the aged rat liver. Hepatology 33, 537–543.
Le Goallec, A., Diai, S., Collin, S., Prost, J.B., Vincent, T., and Patel, C.J. (2022). Using deep learning to predict abdominal age from liver and pancreas magnetic resonance images. Nat Commun 13, 1979.
Le, R., Huang, Y., Zhang, Y., Wang, H., Lin, J., Dong, Y., Li, Z., Guo, M., Kou, X., Zhao, Y., et al. (2021). Dcaf11 activates Zscan4-mediated alternative telomere lengthening in early embryos and embryonic stem cells. Cell Stem Cell 28, 732–747.e9.
Lebel, M., and Monnat, R.J. (2018). Werner syndrome (*WRN*) gene variants and their association with altered function and age-associated diseases. Ageing Res Rev 41, 82–97.
Lechner, J., Malloth, N.A., Jennings, P., Heckl, D., Pfaller, W., and Seppi, T. (2007). Opposing roles of EGF in IFN-α-induced epithelial barrier destabilization and tissue repair. Am J Physiol Cell Physiol 293, C1843–C1850.
Lecka-Czernik, B., Rosen, C.J., and Kawai, M. (2010). Skeletal aging and the adipocyte program: New insights from an “old” molecule. Cell Cycle 9, 3648–3654.
Lee, C., Lopez, O.L., Becker, J.T., Raji, C., Dai, W., Kuller, L.H., and Gach, H.M. (2009). Imaging cerebral blood flow in the cognitively normal aging brain with arterial spin labeling: implications for imaging of neurodegenerative disease. J Neuroimag 19, 344–352.
Lee, C.K., Klopp, R.G., Weindruch, R., and Prolla, T.A. (1999). Gene expression profile of aging and its retardation by caloric restriction. Science 285, 1390–1393.
Lee, G., Kim, Y.Y., Jang, H., Han, J.S., Nahmgoong, H., Park, Y.J., Han, S. M., Cho, C., Lim, S., Noh, J.R., et al. (2022a). SREBP1c-PARP1 axis tunes anti-senescence activity of adipocytes and ameliorates metabolic imbalance in obesity. Cell Metab 34, 702–718.e5.
Lee, J.H., and Lee, J. (2022). Endoplasmic reticulum (ER) stress and its role in pancreatic β-cell dysfunction and senescence in type 2 diabetes. Int J Mol Sci 23, 4843.
Lee, J.J., Lee, J.H., Ko, Y.G., Hong, S.I., and Lee, J.S. (2010). Prevention of premature senescence requires JNK regulation of Bcl-2 and reactive oxygen species. Oncogene 29, 561–575.
Lee, L.E., Chandrasekar, B., Yu, P., and Ma, L. (2022b). Quantification of myocardial fibrosis using noninvasive T_2-mapping magnetic resonance imaging: Preclinical models of aging and pressure overload. NMR Biomed 35, e4641.
Lee, M.Y., Huang, C.H., Kuo, C.J., Lin, C.L.S., Lai, W.T., and Chiou, S.H. (2015). Clinical proteomics identifies urinary CD14 as a potential biomarker for diagnosis of stable coronary artery disease. PLoS ONE 10, e0117169.
Lee, W.J., Brown, J.A., Kim, H.R., La Joie, R., Cho, H., Lyoo, C.H., Rabinovici, G.D., Seong, J.K., and Seeley, W.W. (2022c). Regional Aβ-tau interactions promote onset and acceleration of Alzheimer’s disease tau spreading. Neuron 110, 1932–1943.e5.
Lefebvre, S., Bürglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80, 155–165.
Lehallier, B., Gate, D., Schaum, N., Nanasi, T., Lee, S.E., Yousef, H., Moran Losada, P., Berdnik, D., Keller, A., Verghese, J., et al. (2019). Undulating changes in human plasma proteome profiles across the lifespan. Nat Med 25, 1843–1850.
Lehallier, B., Shokhirev, M.N., Wyss-Coray, T., and Johnson, A.A. (2020). Data mining of human plasma proteins generates a multitude of highly predictive aging clocks that reflect different aspects of aging. Aging Cell 19, e13256.
Lemaitre, H., Goldman, A.L., Sambataro, F., Verchinski, B.A., Meyer-Lindenberg, A., Weinberger, D.R., and Mattay, V.S. (2012). Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiol Aging 33, 617.e1–617.e9.
Lenchik, L., and Boutin, R. (2018). Sarcopenia: beyond muscle atrophy and into the new frontiers of opportunistic imaging, precision medicine, and machine learning. Semin Musculoskelet Radiol 22, 307–322.
Leng, S.X., and Pawelec, G. (2022). Single-cell immune atlas for human aging and frailty. Life Med 1, 67–70.
Lenoir, O., Tharaux, P.L., and Huber, T.B. (2016). Autophagy in kidney disease and aging: lessons from rodent models. Kidney Int 90, 950–964.
Leon, M.L.A., and Zuckerman, S.H. (2005). Gamma interferon: a central mediator in atherosclerosis. Inflamm res 54, 395–411.
Lerner, C., and Harrison, D.E. (1990). 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation. Exp Hematol 18, 114–118.
Levine, M.E., Lu, A.T., Quach, A., Chen, B.H., Assimes, T.L., Bandinelli, S., Hou, L., Baccarelli, A.A., Stewart, J.D., Li, Y., et al. (2018). An epigenetic biomarker of aging for lifespan and healthspan. Aging 10, 573–591.
Lewinska, A., Adamczyk-Grochala, J., Deregowska, A., and Wnuk, M. (2017). Sulforaphane-induced cell cycle arrest and senescence are accompanied by DNA hypomethylation and changes in microRNA

profile in breast cancer cells. Theranostics 7, 3461–3477.
Lexell, J. (1995). Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci 50A, 11–16.
Lexell, J., Taylor, C.C., and Sjöström, M. (1988). What is the cause of the ageing atrophy? J Neurol Sci 84, 275–294.
Li, B., Zhao, M., Feng, L., Dou, C., Ding, S., Zhou, G., Lu, L., Zhang, H., Chen, F., Li, X., et al. (2020a). Organic NIR-II molecule with long blood half-life for *in vivo* dynamic vascular imaging. Nat Commun 11, 3102.
Li, C.J., Xiao, Y., Sun, Y.C., He, W.Z., Liu, L., Huang, M., He, C., Huang, M., Chen, K.X., Hou, J., et al. (2021a). Senescent immune cells release grancalcin to promote skeletal aging. Cell Metab 33, 1957–1973.e6.
Li, C.W., Yu, K., Shyh-Chang, N., Li, G.X., Jiang, L.J., Yu, S.L., Xu, L.Y., Liu, R.J., Guo, Z.J., Xie, H.Y., et al. (2019a). Circulating factors associated with sarcopenia during ageing and after intensive lifestyle intervention. J Cachexia Sarcopenia Muscle 10, 586–600.
Li, G., Petkova, T.D., Laritsky, E., Kessler, N., Baker, M.S., Zhu, S., and Waterland, R.A. (2019b). Early postnatal overnutrition accelerates aging-associated epigenetic drift in pancreatic islets. Environmental Epigenetics 5, dvz015.
Li, G., Tang, X., Zhang, S., Jin, M., Wang, M., Deng, Z., Liu, Z., Qian, M., Shi, W., Wang, Z., et al. (2020b). ^{SIRT} 7 activates quiescent hair follicle stem cells to ensure hair growth in mice. EMBO J 39.
Li, G.H., Han, F., Xiao, F.H., Gu, K.S., Shen, Q., Xu, W., Li, W.X., Wang, Y.L., Liang, B., Huang, J.F., et al. (2022a). System-level metabolic modeling facilitates unveiling metabolic signature in exceptional longevity. Aging Cell 21, e13595.
Li, H., Chen, Q., Li, C., Zhong, R., Zhao, Y., Zhang, Q., Tong, W., Zhu, D., and Zhang, Y. (2019c). Muscle-secreted granulocyte colony-stimulating factor functions as metabolic niche factor ameliorating loss of muscle stem cells in aged mice. EMBO J 38.
Li, H., and Satinoff, E. (1998). Fetal tissue containing the suprachiasmatic nucleus restores multiple circadian rhythms in old rats. Am J Physiol Regul Integr Comp Physiol 275, R1735–R1744.
Li, H., Wang, C., He, T., Zhao, T., Chen, Y., Shen, Y., Zhang, X., and Wang, L. (2019d). Mitochondrial transfer from bone marrow mesenchymal stem cells to motor neurons in spinal cord injury rats via gap junction. Theranostics 9, 2017–2035.
Li, J., Cui, J., and Tian, Y. (2022b). Neuron-periphery mitochondrial stress communication in aging and diseases. Life Med 1, 168–178.
Li, J., Poi, M.J., and Tsai, M.D. (2011a). Regulatory mechanisms of tumor suppressor $P16^{INK4A}$ and their relevance to cancer. Biochemistry 50, 5566–5582.
Li, J., Xie, Y., Yuan, F., Song, B., and Tang, C. (2011b). Noninvasive quantification of pancreatic fat in healthy male population using chemical shift magnetic resonance imaging: effect of aging on pancreatic fat content. Pancreas 40, 295–299.
Li, J., Zheng, Y., Yan, P., Song, M., Wang, S., Sun, L., Liu, Z., Ma, S., Izpisua Belmonte, J.C., Chan, P., et al. (2021b). A single-cell transcriptomic atlas of primate pancreatic islet aging. Natl Sci Rev 8, nwaa127.
Li, L., Xiong, W.C., and Mei, L. (2018a). Neuromuscular junction formation, aging, and disorders. Annu Rev Physiol 80, 159–188.
Li, M., Bao, L., Zhu, P., and Wang, S. (2022c). Effect of metformin on the epigenetic age of peripheral blood in patients with diabetes mellitus. Front Genet 13, 955835.
Li, N., Chen, H., and Wang, J. (2022d). DNA damage and repair in the hematopoietic system. Acta Biochim Biophys Sin 54, 847–857.
Li, N., Zhang, S., Xiong, F., Eizirik, D.L., and Wang, C.Y. (2020c). SUMOylation, a multifaceted regulatory mechanism in the pancreatic beta cells. Semin Cell Dev Biol 103, 51–58.
Li, P., Gan, Y., Xu, Y., Song, L., Wang, L., Ouyang, B., Zhang, C., and Zhou, Q. (2017a). The inflammatory cytokine TNF-α promotes the premature senescence of rat nucleus pulposus cells via the PI3K/Akt signaling pathway. Sci Rep 7, 42938.
Li, Q., Geng, X.D., Zheng, W., Tang, J., Xu, B., and Shi, Q.H. (2012). Current understanding of ovarian aging. Sci China Life Sci 55, 659–669.
Li, Q., Li, X., Tang, H., Jiang, B., Dou, Y., Gorospe, M., and Wang, W. (2017b). NSUN2-mediated m5C methylation and METTL3/METTL14-mediated m6A methylation cooperatively enhance p21 translation. J Cell Biochem 118, 2587–2598.
Li, Q., Zhang, Y., Fu, J., Han, L., Xue, L., Lv, C., Wang, P., Li, G., and Tong, T. (2013). FOXA1 mediates $p16^{INK4a}$ activation during cellular senescence. EMBO J 32, 858–873.
Li, W., and Liu, M. (2011). Distribution of 5-hydroxymethylcytosine in different human tissues. J Nucleic Acids 2011, 1–5.
Li, W., Qin, S., Li, F., and Wang, L. (2021c). MAD-UNet: A deep U-shaped network combined with an attention mechanism for pancreas segmentation in CT images. Med Phys 48, 329–341.
Li, W., Zou, Z., Cai, Y., Yang, K., Wang, S., Liu, Z., Geng, L., Chu, Q., Ji, Z., Chan, P., et al. (2022e). Low-dose chloroquine treatment extends the lifespan of aged rats. Protein Cell 13, 454–461.
Li, X., Feng, L., Zhang, C., Wang, J., Wang, S., and Hu, L. (2022f). Insulin-like growth factor binding proteins 7 prevents dental pulp-derived mesenchymal stem cell senescence via metabolic downregulation of p21. Sci China Life Sci 65, 2218–2232.
Li, X., Li, J., Zhu, D., Zhang, N., Hao, X., Zhang, W., Zhang, Q., Liu, Y., Wu, X., and Tian, Y. (2022g). Protein disulfide isomerase PDI-6 regulates Wnt secretion to coordinate inter-tissue UPRmt activation and lifespan extension in *C. elegans*. Cell Rep 39, 110931.
Li, X., Sun, X., and Carmeliet, P. (2019e). Hallmarks of endothelial cell metabolism in health and disease. Cell Metab 30, 414–433.
Li, X., Wang, J., Wang, L.Y., Gao, Y., Feng, G., Li, G., Zou, J., Yu, M., Li, Y.F., Liu, C., et al. (2022h). Lipid metabolism dysfunction induced by age-dependent DNA methylation accelerates aging. Sig Transduct Target Ther 7, 162.
Li, Y., Hu, D., Sheng, Z., Min, T., Zha, M., Ni, J.S., Zheng, H., and Li, K. (2021d). Self-assembled AIEgen nanoparticles for multiscale NIR-II vascular imaging. Biomaterials 264, 120365.
Li, Y., Lee, Y., and Thompson, W.J. (2011c). Changes in aging mouse neuromuscular junctions are explained by degeneration and regeneration of muscle fiber segments at the synapse. J Neurosci 31, 14910–14919.
Li, Y., Li, R.Q., Ou, S.B., Zhang, N.F., Ren, L., Wei, L.N., Zhang, Q.X., and Yang, D.Z. (2014). Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. Reprod Biol Endocrinol 12, 81.
Li, Y., Ma, Y., Song, L., Yu, L., Zhang, L., Zhang, Y., Xing, Y., Yin, Y., and Ma, H. (2018b). SIRT3 deficiency exacerbates p53/Parkin-mediated mitophagy inhibition and promotes mitochondrial dysfunction: Implication for aged hearts. Int J Mol Med 41, 3517–3526.
Li, Y., Wang, D., Ping, X., Zhang, Y., Zhang, T., Wang, L., Jin, L., Zhao, W., Guo, M., Shen, F., et al. (2022i). Local hyperthermia therapy induces browning of white fat and treats obesity. Cell 185, 949–966.e19.
Li, Y., Yu, H., Chen, C., Li, S., Zhang, Z., Xu, H., Zhu, F., Liu, J., Spencer, P.S., Dai, Z., et al. (2020d). Proteomic profile of mouse brain aging contributions to mitochondrial dysfunction, DNA Oxidative damage, loss of neurotrophic factor, and synaptic and ribosomal proteins. Oxid Med Cell Longev 2020, 1–21.
Li, Y., Zhang, W., Chang, L., Han, Y., Sun, L., Gong, X., Tang, H., Liu, Z., Deng, H., Ye, Y., et al. (2016a). Vitamin C alleviates aging defects in a stem cell model for Werner syndrome. Protein Cell 7, 478–488.
Li, Y., Zhang, Y., Wang, L., Wang, P., Xue, Y., Li, X., Qiao, X., Zhang, X., Xu, T., Liu, G., et al. (2017c). Autophagy impairment mediated by *S*-nitrosation of ATG4B leads to neurotoxicity in response to hyperglycemia. Autophagy 13, 1145–1160.
Li, Z., Jiao, Y., Fan, E.K., Scott, M.J., Li, Y., Li, S., Billiar, T.R., Wilson, M.A., Shi, X., and Fan, J. (2017d). Aging-impaired filamentous actin polymerization signaling reduces alveolar macrophage phagocytosis of bacteria. J Immunol 199, 3176–3186.
Li, Z., Zhang, W., Chen, Y., Guo, W., Zhang, J., Tang, H., Xu, Z., Zhang, H., Tao, Y., Wang, F., et al. (2016b). Impaired DNA double-strand break repair contributes to the age-associated rise of genomic instability in humans. Cell Death Differ 23, 1765–1777.

Liang, C., Ke, Q., Liu, Z., Ren, J., Zhang, W., Hu, J., Wang, Z., Chen, H., Xia, K., Lai, X., et al. (2022). BMAL1 moonlighting as a gatekeeper for LINE1 repression and cellular senescence in primates. Nucleic Acids Res 50, 3323–3347.
Liang, C., Liu, Z., Song, M., Li, W., Wu, Z., Wang, Z., Wang, Q., Wang, S., Yan, K., Sun, L., et al. (2021). Stabilization of heterochromatin by CLOCK promotes stem cell rejuvenation and cartilage regeneration. Cell Res 31, 187–205.
Liao, E.Y., and Cao, X. (2013). Xiangya Metabolic Osteology (in Chinese). Beijing: Science Press.
Liao, M., Chen, R., Yang, Y., He, H., Xu, L., Jiang, Y., Guo, Z., He, W., Jiang, H., and Wang, J. (2022). Aging-elevated inflammation promotes DNMT3A R878H-driven clonal hematopoiesis. Acta Pharm Sin B 12, 678–691.
Libby, P., Buring, J.E., Badimon, L., Hansson, G.K., Deanfield, J., Bittencourt, M.S., Tokgözoğlu, L., and Lewis, E.F. (2019). Atherosclerosis. Nat Rev Dis Primers 5, 56.
Liberale, L., Kraler, S., Camici, G.G., and Lüscher, T.F. (2020). Ageing and longevity genes in cardiovascular diseases. Basic Clin Pharmacol Toxicol 127, 120–131.
Licastro, F., Candore, G., Lio, D., Porcellini, E., Colonna-Romano, G., Franceschi, C., and Caruso, C. (2005). Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immun Ageing 2, 8.
Lieb, W., Beiser, A.S., Vasan, R.S., Tan, Z.S., Au, R., Harris, T.B., Roubenoff, R., Auerbach, S., DeCarli, C., Wolf, P.A., et al. (2009). Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. JAMA 302, 2565–2572.
Lim, H.K., Nebes, R., Snitz, B., Cohen, A., Mathis, C., Price, J., Weissfeld, L., Klunk, W., and Aizenstein, H.J. (2014). Regional amyloid burden and intrinsic connectivity networks in cognitively normal elderly subjects. Brain 137, 3327–3338.
Lim, J., and Luderer, U. (2011). Oxidative damage increases and antioxidant gene expression decreases with aging in the mouse ovary. Biol Reprod 84, 775–782.
Lim, M., Park, L., Shin, G., Hong, H., Kang, I., and Park, Y. (2008). Induction of apoptosis of β cells of the pancreas by advanced glycation end-products, important mediators of chronic complications of diabetes mellitus. Ann N Y Acad Sci 1150, 311–315.
Lin, D., Wang, Z., Li, H., Zhang, H., Deng, L., Ren, H., Sun, S., Zheng, F., Zhou, J., and Wang, M. (2023). Automated measurement of pancreatic fat deposition on Dixon MRI using nnU-Net. Magn Reson Imag 57, 296–307.
Lin, K., Ma, H., Sarnari, R., Li, D., Lloyd-Jones, D.M., Markl, M., and Carr, J.C. (2021). Cardiac MRI reveals late diastolic changes in left ventricular relaxation patterns during healthy aging. Magn Reson Imag 53, 766–774.
Lin, Q., Weidner, C.I., Costa, I.G., Marioni, R.E., Ferreira, M.R.P., Deary, I. J., and Wagner, W. (2016). DNA methylation levels at individual age-associated CpG sites can be indicative for life expectancy. Aging 8, 394–401.
Lin, Z., Sur, S., Soldan, A., Pettigrew, C., Miller, M., Oishi, K., Bilgel, M., Moghekar, A., Pillai, J.J., Albert, M., et al. (2019). Brain oxygen extraction by using MRI in older individuals: relationship to apolipoprotein E genotype and amyloid burden. Radiology 292, 140–148.
Linehan, E., Dombrowski, Y., Snoddy, R., Fallon, P.G., Kissenpfennig, A., and Fitzgerald, D.C. (2014). Aging impairs peritoneal but not bone marrow-derived macrophage phagocytosis. Aging Cell 13, 699–708.
Linnane, A.W., Ozawa, T., Marzuki, S., and Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 333, 642–645.
Lintzeri, D.A., Karimian, N., Blume-Peytavi, U., and Kottner, J. (2022). Epidermal thickness in healthy humans: a systematic review and meta-analysis. Acad Dermatol Venereol 36, 1191–1200.
Lipska, K.J., Krumholz, H., Soones, T., and Lee, S.J. (2016). Polypharmacy in the aging patient: a review of glycemic control in older adults with type 2 diabetes. JAMA 315, 1034–1045.
Liu, A., Guo, E., Yang, J., Yang, Y., Liu, S., Jiang, X., Hu, Q., Dirsch, O., Dahmen, U., Zhang, C., et al. (2018a). Young plasma reverses age-dependent alterations in hepatic function through the restoration of autophagy. Aging Cell 17, e12708.
Liu, B., Qu, J., Zhang, W., Izpisua Belmonte, J.C., and Liu, G.H. (2022a). A stem cell aging framework, from mechanisms to interventions. Cell Rep 41, 111451.
Liu, B., Wang, J., Chan, K.M., Tjia, W.M., Deng, W., Guan, X., Huang, J., Li, K.M., Chau, P.Y., Chen, D.J., et al. (2005). Genomic instability in laminopathy-based premature aging. Nat Med 11, 780–785.
Liu, C., Meng, M., Xu, B., Xu, Y., Li, G., Cao, Y., Wang, D., Qiu, J., Yu, J., Xu, L., et al. (2023a). Fibroblast growth factor 6 promotes adipocyte progenitor cell proliferation for adipose tissue homeostasis. Diabetes doi: 10.2337/db22-0585.
Liu, G.Y., and Sabatini, D.M. (2020). mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21, 183–203.
Liu, H., Ho, P.W.L., Leung, C.T., Pang, S.Y.Y., Chang, E.E.S., Choi, Z.Y. K., Kung, M.H.W., Ramsden, D.B., and Ho, S.L. (2021a). Aberrant mitochondrial morphology and function associated with impaired mitophagy and DNM1L-MAPK/ERK signaling are found in aged mutant Parkinsonian LRRK2^{R1441G} mice. Autophagy 17, 3196–3220.
Liu, H., Yang, Y., Xia, Y., Zhu, W., Leak, R.K., Wei, Z., Wang, J., and Hu, X. (2017a). Aging of cerebral white matter. Ageing Res Rev 34, 64–76.
Liu, L., Charville, G.W., Cheung, T.H., Yoo, B., Santos, P.J., Schroeder, M., and Rando, T.A. (2018b). Impaired Notch signaling leads to a decrease in p53 activity and mitotic catastrophe in aged muscle stem cells. Cell Stem Cell 23, 544–556.e4.
Liu, L., Cheung, T.H., Charville, G.W., Hurgo, B.M.C., Leavitt, T., Shih, J., Brunet, A., and Rando, T.A. (2013). Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. Cell Rep 4, 189–204.
Liu, L., and Keefe, D.L. (2008). Defective cohesin is associated with age-dependent misaligned chromosomes in oocytes. Reprod Biomed Online 16, 103–112.
Liu, L., and Rando, T.A. (2011). Manifestations and mechanisms of stem cell aging. J Cell Biol 193, 257–266.
Liu, N., Matsumura, H., Kato, T., Ichinose, S., Takada, A., Namiki, T., Asakawa, K., Morinaga, H., Mohri, Y., De Arcangelis, A., et al. (2019). Stem cell competition orchestrates skin homeostasis and ageing. Nature 568, 344–350.
Liu, Q., Fischer, U., Wang, F., and Dreyfuss, G. (1997). The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 90, 1013–1021.
Liu, S., Chen, C., Li, Y., Zhang, H., Liu, J., Wang, R., Wong, S.T.H., Lam, J.W.Y., Ding, D., and Tang, B.Z. (2020). Constitutional isomerization enables bright NIR-II AIEgen for brain-inflammation imaging. Adv Funct Mater 30, 1908125.
Liu, S., Hartleben, B., Kretz, O., Wiech, T., Igarashi, P., Mizushima, N., Walz, G., and Huber, T.B. (2012a). Autophagy plays a critical role in kidney tubule maintenance, aging and ischemia-reperfusion injury. Autophagy 8, 826–837.
Liu, T., Zhang, L., Joo, D., and Sun, S.C. (2017b). NF-κB signaling in inflammation. Sig Transduct Target Ther 2, 17023.
Liu, X., Liu, Z., Wu, Z., Ren, J., Fan, Y., Sun, L., Cao, G., Niu, Y., Zhang, B., Ji, Q., et al. (2023b). Resurrection of endogenous retroviruses during aging reinforces senescence. Cell 186, 287–304.e26.
Liu, X., Song, Z., Li, Y., Yao, Y., Fang, M., Bai, C., An, P., Chen, H., Chen, Z., Tang, B., et al. (2021b). Integrated genetic analyses revealed novel human longevity loci and reduced risks of multiple diseases in a cohort study of 15,651 Chinese individuals. Aging Cell 20, e13323.
Liu, Y., Han, M., Li, X., Wang, H., Ma, M., Zhang, S., Guo, Y., Wang, S., Wang, Y., Duan, N., et al. (2017c). Age-related changes in the mitochondria of human mural granulosa cells. Hum Reprod 32, 2465–2473.
Liu, Y., Liu, F., Yu, H., Zhao, X., Sashida, G., Deblasio, A., Harr, M., She, Q.B., Chen, Z., Lin, H.K., et al. (2012b). Akt phosphorylates the transcriptional repressor Bmi1 to block its effects on the tumor-

suppressing *Ink4a-Arf* locus. Sci Signal 5.
Liu, Y., Nie, L., and Chen, X. (2016). Photoacoustic molecular imaging: from multiscale biomedical applications towards early-stage theranostics. Trends Biotechnol 34, 420–433.
Liu, Z., Belmonte, J.C.I., Zhang, W., Qu, J., and Liu, G.H. (2022b). Deciphering aging at three-dimensional genomic resolution. Cell Insight 1, 100034.
Liu, Z., Ji, Q., Ren, J., Yan, P., Wu, Z., Wang, S., Sun, L., Wang, Z., Li, J., Sun, G., et al. (2022c). Large-scale chromatin reorganization reactivates placenta-specific genes that drive cellular aging. Dev Cell 57, 1347–1368.e12.
Liu, Z., Li, W., Geng, L., Sun, L., Wang, Q., Yu, Y., Yan, P., Liang, C., Ren, J., Song, M., et al. (2022d). Cross-species metabolomic analysis identifies uridine as a potent regeneration promoting factor. Cell Discov 8, 6.
Llarena, N., and Hine, C. (2021). Reproductive longevity and aging: geroscience approaches to maintain long-term ovarian fitness. J Gerontol A Biol Sci Med Sci 76, 1551–1560.
Lob, M., and Hugonnaud, C. (1978). Danger of pneumoconiosis due to hard metals and berilosis in the dental technicians who work with metallic prosthesis (in Italian). Dent Press 14, 21–26.
Lock-Andersen, J., Therkildsen, P., Olivarius, F.F., Gniadecka, M., Dahlstrem, K., Poulsen, T., and Wulf, H.C. (1997). Epidermal thickness, skin pigmentation and constitutive photosensitivity. Photodermatol Photoimmunol Photomed 13, 153–158.
Loeb, L.A., Wallace, D.C., and Martin, G.M. (2005). The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. Proc Natl Acad Sci USA 102, 18769–18770.
Löhr, J.M., Panic, N., Vujasinovic, M., and Verbeke, C.S. (2018). The ageing pancreas: a systematic review of the evidence and analysis of the consequences. J Intern Med 283, 446–460.
Long, C., Liu, H., Zhan, W., Chen, L., Yu, Z., Tian, S., Xiang, Y., Chen, S., and Tian, X.L. (2022). Chronological attenuation of NPRA/PKG/AMPK signaling promotes vascular aging and elevates blood pressure. Aging Cell 21.
Long, H., Wang, Y., Wang, L., Lu, Y., Nie, Y., Cai, Y., Liu, Z., Jia, M., Lyu, Q., Kuang, Y., et al. (2018). Age-related nomograms of serum anti-Mullerian hormone levels in female monkeys: Comparison of rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) monkeys. Gen Comp Endocrinol 269, 171–176.
Longnecker, D.S. (2014). Anatomy and histology of the pancreas. Pancreaped Exocrine Pancreas Knowledge Base doi:10.3998/PANC.2014.3.
Longo, V.D., and Anderson, R.M. (2022). Nutrition, longevity and disease: from molecular mechanisms to interventions. Cell 185, 1455–1470.
López-Melgar, B., Fernández-Friera, L., Oliva, B., García-Ruiz, J.M., Peñalvo, J.L., Gómez-Talavera, S., Sánchez-González, J., Mendiguren, J.M., Ibáñez, B., Fernández-Ortiz, A., et al. (2017). Subclinical atherosclerosis burden by 3D ultrasound in mid-life. J Am Coll Cardiol 70, 301–313.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell 153, 1194–1217.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2023). Hallmarks of aging: An expanding universe. Cell 186, 243–278.
López-Otín, C., Galluzzi, L., Freije, J.M.P., Madeo, F., and Kroemer, G. (2016). Metabolic control of longevity. Cell 166, 802–821.
Lorenz, M.W., Markus, H.S., Bots, M.L., Rosvall, M., and Sitzer, M. (2007). Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 115, 459–467.
Lorenz, M.W., Price, J.F., Robertson, C., Bots, M.L., Polak, J.F., Poppert, H., Kavousi, M., Dörr, M., Stensland, E., Ducimetiere, P., et al. (2015). Carotid intima-media thickness progression and risk of vascular events in people with diabetes: results from the PROG-IMT collaboration. Diabetes Care 38, 1921–1929.
Lorenz, G., Schmalenberg, M., Kemmner, S., Haller, B., Steubl, D., Pham, D., Schreiegg, A., Bachmann, Q., Schmidt, A., Haderer, S., et al. (2018). Mortality prediction in stable hemodialysis patients is refined by YKL-40, a 40-kDa glycoprotein associated with inflammation. Kidney Int 93, 221–230.
Lototska, L., Yue, J.X., Li, J., Giraud-Panis, M.J., Songyang, Z., Royle, N. J., Liti, G., Ye, J., Gilson, E., and Mendez-Bermudez, A. (2020). Human RAP1 specifically protects telomeres of senescent cells from DNA damage. EMBO Rep 21, e49076.
Louveau, A., Smirnov, I., Keyes, T.J., Eccles, J.D., Rouhani, S.J., Peske, J. D., Derecki, N.C., Castle, D., Mandell, J.W., Lee, K.S., et al. (2016). Corrigendum: Structural and functional features of central nervous system lymphatic vessels. Nature 533, 278.
Lovell, C.R., Smolenski, K.A., Duance, V.C., Light, N.D., Young, S., and Dyson, M. (1987). Type I and III collagen content and fibre distribution in normal human skin during ageing. Br J Dermatol 117, 419–428.
Lovshin, J.A., Bjornstad, P., Lovblom, L.E., Bai, J.W., Lytvyn, Y., Boulet, G., Farooqi, M.A., Santiago, S., Orszag, A., Scarr, D., et al. (2018). Atherosclerosis and microvascular complications: results from the Canadian study of longevity in type 1 diabetes. Diabetes Care 41, 2570–2578.
Lowery, J.W., and Rosen, V. (2018). The BMP pathway and its inhibitors in the skeleton. Physiol Rev 98, 2431–2452.
Lu, A.T., Fei, Z., Haghani, A., Robeck, T.R., Zoller, J.A., Li, C.Z., Zhang, J., Ablaeva, J., Adams, D.M., Almunia, J., et al. (2021). Universal DNA methylation age across mammalian tissues. bioRxiv 2021.2001.2018.426733.
Lu, A.T., Quach, A., Wilson, J.G., Reiner, A.P., Aviv, A., Raj, K., Hou, L., Baccarelli, A.A., Li, Y., Stewart, J.D., et al. (2019). DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging 11, 303–327.
Lu, J., Ahmad, R., Nguyen, T., Cifello, J., Hemani, H., Li, J., Chen, J., Li, S., Wang, J., Achour, A., et al. (2022). Heterogeneity and transcriptome changes of human $CD8^+$ T cells across nine decades of life. Nat Commun 13, 5128.
Lu, Y., Brommer, B., Tian, X., Krishnan, A., Meer, M., Wang, C., Vera, D. L., Zeng, Q., Yu, D., Bonkowski, M.S., et al. (2020). Reprogramming to recover youthful epigenetic information and restore vision. Nature 588, 124–129.
Lu, Y., Ma, W., Li, Z., Lu, J., and Wang, X. (2017). The interplay between p16 serine phosphorylation and arginine methylation determines its function in modulating cellular apoptosis and senescence. Sci Rep 7, 41390.
Lue, L.F., Pai, M.C., Chen, T.F., Hu, C.J., Huang, L.K., Lin, W.C., Wu, C. C., Jeng, J.S., Blennow, K., Sabbagh, M.N., et al. (2019). Age-dependent relationship between plasma Aβ40 and Aβ42 and total Tau levels in cognitively normal subjects. Front Aging Neurosci 11, 222.
Luis, N.M., Morey, L., Mejetta, S., Pascual, G., Janich, P., Kuebler, B., Roma, G., Nascimento, E., Frye, M., Di Croce, L., et al. (2011). Regulation of human epidermal stem cell proliferation and senescence requires polycomb- dependent and -independent functions of Cbx4. Cell Stem Cell 9, 233–246.
Lukjanenko, L., Jung, M.J., Hegde, N., Perruisseau-Carrier, C., Migliavacca, E., Rozo, M., Karaz, S., Jacot, G., Schmidt, M., Li, L., et al. (2016). Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice. Nat Med 22, 897–905.
Lukjanenko, L., Karaz, S., Stuelsatz, P., Gurriaran-Rodriguez, U., Michaud, J., Dammone, G., Sizzano, F., Mashinchian, O., Ancel, S., Migliavacca, E., et al. (2019). Aging disrupts muscle stem cell function by impairing matricellular WISP1 secretion from fibro-adipogenic progenitors. Cell Stem Cell 24, 433–446.e7.
Lumeng, C.N., Liu, J., Geletka, L., Delaney, C., Delproposto, J., Desai, A., Oatmen, K., Martinez-Santibanez, G., Julius, A., Garg, S., et al. (2011). Aging is associated with an increase in T cells and inflammatory macrophages in visceral adipose tissue. J Immunol 187, 6208–6216.
Luo, H., Liu, W., Zhang, Y., Yang, Y., Jiang, X., Wu, S., and Shao, L. (2021). METTL3-mediated m6A modification regulates cell cycle progression of dental pulp stem cells. Stem Cell Res Ther 12, 159.
Luo, M., Yan, D., Liang, X., Huang, Y., Luo, P., Yang, Z., Zhang, Y., Xu, T., Gao, S., Zhang, L., et al. (2022a). Association between plasma

fibulin-1 and brachial-ankle pulse wave velocity in arterial stiffness. Front Cardiovasc Med 9, 837490.
Luo, O.J., Lei, W., Zhu, G., Ren, Z., Xu, Y., Xiao, C., Zhang, H., Cai, J., Luo, Z., Gao, L., et al. (2022b). Multidimensional single-cell analysis of human peripheral blood reveals characteristic features of the immune system landscape in aging and frailty. Nat Aging 2, 348–364.
Luo, Y., Viswanathan, R., Hande, M.P., Loh, A.H.P., and Cheow, L.F. (2020). Massively parallel single-molecule telomere length measurement with digital real-time PCR. Sci Adv 6, eabb7944.
Lüth, S., Teyssen, S., Forssmann, K., Kölbel, C., Krummenauer, F., and Singer, M. (2001). Fecal elastase-1 determination: 'gold standard' of indirect pancreatic function tests? Scand J Gastroenterol 36, 1092–1099.
Lv, L., Wang, J., Gao, B., Wu, L., Wang, F., Cui, Z., He, K., Zhang, L., Chen, M., and Zhao, M.H. (2018). Serum uromodulin and progression of kidney disease in patients with chronic kidney disease. J Transl Med 16, 316.
Lynch, M.A. (2010). Age-related neuroinflammatory changes negatively impact on neuronal function. Front Aging Neurosci 1, 6.
Lynch, M.D., and Watt, F.M. (2018). Fibroblast heterogeneity: implications for human disease. J Clin Invest 128, 26–35.
Lyon, A.S., Peeples, W.B., and Rosen, M.K. (2021). A framework for understanding the functions of biomolecular condensates across scales. Nat Rev Mol Cell Biol 22, 215–235.
Ma, S., Sun, S., Geng, L., Song, M., Wang, W., Ye, Y., Ji, Q., Zou, Z., Wang, S., He, X., et al. (2020). Caloric restriction reprograms the single-cell transcriptional landscape of rattus norvegicus aging. Cell 180, 984–1001.e22.
Ma, S., Sun, S., Li, J., Fan, Y., Qu, J., Sun, L., Wang, S., Zhang, Y., Yang, S., Liu, Z., et al. (2021). Single-cell transcriptomic atlas of primate cardiopulmonary aging. Cell Res 31, 415–432.
Ma, S., Wang, S., Ye, Y., Ren, J., Chen, R., Li, W., Li, J., Zhao, L., Zhao, Q., Sun, G., et al. (2022). Heterochronic parabiosis induces stem cell revitalization and systemic rejuvenation across aged tissues. Cell Stem Cell 29, 990–1005.e10.
Ma, X., Xu, L., Gavrilova, O., and Mueller, E. (2014). Role of forkhead box protein A3 in age-associated metabolic decline. Proc Natl Acad Sci USA 111, 14289–14294.
Ma, X., Xu, L., and Mueller, E. (2016). Forkhead box A3 mediates glucocorticoid receptor function in adipose tissue. Proc Natl Acad Sci USA 113, 3377–3382.
Maass, A., Lockhart, S.N., Harrison, T.M., Bell, R.K., Mellinger, T., Swinnerton, K., Baker, S.L., Rabinovici, G.D., and Jagust, W.J. (2018). Entorhinal Tau pathology, episodic memory decline, and neurodegeneration in aging. J Neurosci 38, 530–543.
Mabbott, N.A. (2015). A breakdown in communication? Understanding the effects of aging on the human small intestine epithelium. Clin Sci 129, 529–531.
Machiela, M.J., Zhou, W., Karlins, E., Sampson, J.N., Freedman, N.D., Yang, Q., Hicks, B., Dagnall, C., Hautman, C., Jacobs, K.B., et al. (2016). Female chromosome X mosaicism is age-related and preferentially affects the inactivated X chromosome. Nat Commun 7, 11843.
Madan, C.R. (2021). Age-related decrements in cortical gyrification: Evidence from an accelerated longitudinal dataset. Eur J Neurosci 53, 1661–1671.
Madole, M.B., Iyer, C.M., Madivalar, M.T., Wadde, S.K., and Howale, D.S. (2016). Evaluation of biochemical markers serum amylase and serum lipase for the assessment of pancreatic exocrine function in diabetes mellitus. J Clin Diagn Res 10, BC01–BC04.
Maegawa, S., Lu, Y., Tahara, T., Lee, J.T., Madzo, J., Liang, S., Jelinek, J., Colman, R.J., and Issa, J.P.J. (2017). Caloric restriction delays age-related methylation drift. Nat Commun 8, 539.
Maertens, G.N., El Messaoudi-Aubert, S., Elderkin, S., Hiom, K., and Peters, G. (2010). Ubiquitin-specific proteases 7 and 11 modulate Polycomb regulation of the INK4a tumour suppressor. EMBO J 29, 2553–2565.
Maertens, G.N., El Messaoudi-Aubert, S., Racek, T., Stock, J.K., Nicholls, J., Rodriguez-Niedenführ, M., Gil, J., and Peters, G. (2009). Several distinct polycomb complexes regulate and co-localize on the INK4a tumor suppressor locus. PloS ONE 4, e6380.
Maeso-Díaz, R., and Gracia-Sancho, J. (2020). Aging and chronic liver disease. Semin Liver Dis 40, 373–384.
Maeso-Díaz, R., Ortega-Ribera, M., Fernández-Iglesias, A., Hide, D., Muñoz, L., Hessheimer, A.J., Vila, S., Francés, R., Fondevila, C., Albillos, A., et al. (2018). Effects of aging on liver microcirculatory function and sinusoidal phenotype. Aging Cell 17, e12829.
Mahdi, F., Shariat-Madar, Z., Todd Iii, R.F., Figueroa, C.D., and Schmaier, A.H. (2001). Expression and colocalization of cytokeratin 1 and urokinase plasminogen activator receptor on endothelial cells. Blood 97, 2342–2350.
Maheshwari, A., Fowler, P., and Bhattacharya, S. (2006). Assessment of ovarian reserve—should we perform tests of ovarian reserve routinely? Hum Reprod 21, 2729–2735.
Mähl, P., Lutz, Y., Puvion, E., and Fuchs, J.P. (1989). Rapid effect of heat shock on two heterogeneous nuclear ribonucleoprotein-associated antigens in HeLa cells. J Cell Biol 109, 1921–1935.
Mahmoud, A.M., Goemaere, S., El-Garem, Y., Van Pottelbergh, I., Comhaire, F.H., and Kaufman, J.M. (2003). Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. J Clin Endocrinol Metab 88, 179–184.
Mahrouf-Yorgov, M., de l'Hortet, A.C., Cosson, C., Slama, A., Abdoun, E., Guidotti, J.E., Fromenty, B., Mitchell, C., and Gilgenkrantz, H. (2011). Increased susceptibility to liver fibrosis with age is correlated with an altered inflammatory response. Rejuvenation Res 14, 353–363.
Mahtal, N., Lenoir, O., Tinel, C., Anglicheau, D., and Tharaux, P.L. (2022). MicroRNAs in kidney injury and disease. Nat Rev Nephrol 18, 643–662.
Maison, C., and Almouzni, G. (2004). HP1 and the dynamics of heterochromatin maintenance. Nat Rev Mol Cell Biol 5, 296–305.
Mäkelä, J.A., Koskenniemi, J.J., Virtanen, H.E., and Toppari, J. (2019). Testis development. Endocrine Rev 40, 857–905.
Malhotra, R., Katz, R., Jotwani, V., Ambrosius, W.T., Raphael, K.L., Haley, W., Rastogi, A., Cheung, A.K., Freedman, B.I., Punzi, H., et al. (2020). Urine markers of kidney tubule cell injury and kidney function decline in SPRINT trial participants with CKD. Clin J Am Soc Nephrol 15, 349–358.
Mamalis, A., Ho, D., and Jagdeo, J. (2015). Optical coherence tomography imaging of normal, chronologically aged, photoaged and photodamaged skin: a systematic review. Dermatol Surg 41, 993–1005.
Mamoshina, P., Vieira, A., Putin, E., and Zhavoronkov, A. (2016). Applications of deep learning in biomedicine. Mol Pharm 13, 1445–1454.
Mamoshina, P., Volosnikova, M., Ozerov, I.V., Putin, E., Skibina, E., Cortese, F., and Zhavoronkov, A. (2018). Machine learning on human muscle transcriptomic data for biomarker discovery and tissue-specific drug target identification. Front Genet 9, 242.
Manczak, M., and Reddy, P.H. (2012). Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage. Hum Mol Genet 21, 2538–2547.
Mann, C.J., Perdiguero, E., Kharraz, Y., Aguilar, S., Pessina, P., Serrano, A. L., and Muñoz-Cánoves, P. (2011). Aberrant repair and fibrosis development in skeletal muscle. Skelet Muscle 1, 21.
Mann, M., Mehta, A., de Boer, C.G., Kowalczyk, M.S., Lee, K., Haldeman, P., Rogel, N., Knecht, A.R., Farouq, D., Regev, A., et al. (2018). Heterogeneous responses of hematopoietic stem cells to inflammatory stimuli are altered with age. Cell Rep 25, 2992–3005.e5.
Mao, Q., Fan, L., Wang, X., Lin, X., Cao, Y., Zheng, C., Zhang, Y., Zhang, H., Garcia-Milian, R., Kang, L., et al. (2019). Transcriptome-wide piRNA profiling in human brains for aging genetic factors. Jacobs J Genet 4, 14.
Marín-Aguilar, F., Lechuga-Vieco, A.V., Alcocer-Gómez, E., Castejón-Vega, B., Lucas, J., Garrido, C., Peralta-Garcia, A., Pérez-Pulido, A.J., Varela-López, A., Quiles, J.L., et al. (2020). NLRP3 inflammasome suppression improves longevity and prevents cardiac aging in male

mice. Aging Cell 19, e13050.
Marioni, R.E., Shah, S., McRae, A.F., Chen, B.H., Colicino, E., Harris, S. E., Gibson, J., Henders, A.K., Redmond, P., Cox, S.R., et al. (2015). DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol 16, 25.
Markousis-Mavrogenis, G., Tromp, J., Ouwerkerk, W., Devalaraja, M., Anker, S.D., Cleland, J.G., Dickstein, K., Filippatos, G.S., Harst, P., Lang, C.C., et al. (2019). The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. Eur J Heart Fail 21, 965–973.
Maroni, L., Pinto, C., Giordano, D.M., Saccomanno, S., Banales, J.M., Spallacci, D., Albertini, M.C., Orlando, F., Provinciali, M., Milkiewicz, M., et al. (2019). Aging-related expression of Twinfilin-1 regulates cholangiocyte biological response to injury. Hepatology 70, 883–898.
Marsh, E., Gonzalez, D.G., Lathrop, E.A., Boucher, J., and Greco, V. (2018). Positional stability and membrane occupancy define skin fibroblast homeostasis *in vivo*. Cell 175, 1620–1633.e13.
Martin, J.E., and Sheaff, M.T. (2007). Renal ageing. J Pathol 211, 198–205.
Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., Wedge, D.C., Fullam, A., Alexandrov, L.B., Tubio, J.M., et al. (2015). High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348, 880–886.
Marttila, S., Chatsirisupachai, K., Palmer, D., and de Magalhães, J.P. (2020). Ageing-associated changes in the expression of lncRNAs in human tissues reflect a transcriptional modulation in ageing pathways. Mech Ageing Dev 185, 111177.
Masiero, E., Agatea, L., Mammucari, C., Blaauw, B., Loro, E., Komatsu, M., Metzger, D., Reggiani, C., Schiaffino, S., and Sandri, M. (2009). Autophagy is required to maintain muscle mass. Cell Metab 10, 507–515.
Masters, A.R., Hall, A., Bartley, J.M., Keilich, S.R., Lorenzo, E.C., Jellison, E.R., Puddington, L., and Haynes, L. (2019). Assessment of Lymph Node Stromal Cells as an Underlying Factor in Age-Related Immune Impairment. J Gerontol A Biol Sci Med Sci 74, 1734–1743.
Masters, C.L., Bateman, R., Blennow, K., Rowe, C.C., Sperling, R.A., and Cummings, J.L. (2015). Alzheimer's disease. Nat Rev Dis Primers 1, 15056.
Matsuda, Y. (2019). Age-related morphological changes in the pancreas and their association with pancreatic carcinogenesis. Pathol Int 69, 450–462.
Matsuda, Y., Furukawa, T., Yachida, S., Nishimura, M., Seki, A., Nonaka, K., Aida, J., Takubo, K., Ishiwata, T., Kimura, W., et al. (2017). The prevalence and clinicopathological characteristics of high-grade pancreatic intraepithelial neoplasia: autopsy study evaluating the entire pancreatic parenchyma. Pancreas 46, 658–664.
Matsuda, Y., Ishiwata, T., Izumiyama-Shimomura, N., Hamayasu, H., Fujiwara, M., Tomita, K., Hiraishi, N., Nakamura, K., Ishikawa, N., Aida, J., et al. (2015). Gradual telomere shortening and increasing chromosomal instability among PanIN grades and normal ductal epithelia with and without cancer in the pancreas. PLoS ONE 10, e0117575.
Matsumoto, S., Harada, H., Tanaka, J., Ochi, K., Ishibashi, T., Takeda, M., Yoshida, M., Miyake, H., and Kimura, I. (1989). Aging and exocrine pancreatic function (in Japanese). Jap J Geriatr 26, 146–152.
Matsumura, H., Mohri, Y., Binh, N.T., Morinaga, H., Fukuda, M., Ito, M., Kurata, S., Hoeijmakers, J., and Nishimura, E.K. (2016). Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. Science 351.
Matsuzawa, Y., Kwon, T.G., Lennon, R.J., Lerman, L.O., and Lerman, A. (2015). Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. J Am Heart Assoc 4.
Mattson, M.P., and Arumugam, T.V. (2018). Hallmarks of brain aging: adaptive and pathological modification by metabolic states. Cell Metab 27, 1176–1199.
Matzkin, M.E., Calandra, R.S., Rossi, S.P., Bartke, A., and Frungieri, M.B. (2021). Hallmarks of testicular aging: the challenge of anti-inflammatory and antioxidant therapies using natural and/or pharmacological compounds to improve the physiopathological status of the aged male gonad. Cells 10, 3114.
May-Panloup, P., Boucret, L., Chao de la Barca, J.M., Desquiret-Dumas, V., Ferré-L'Hotellier, V., Morinière, C., Descamps, P., Procaccio, V., and Reynier, P. (2016). Ovarian ageing: the role of mitochondria in oocytes and follicles. Hum Reprod Update 22, 725–743.
Mazziotti, G., Lania, A.G., and Canalis, E. (2022). Skeletal disorders associated with the growth hormone-insulin-like growth factor 1 axis. Nat Rev Endocrinol 18, 353–365.
McClelland, R.L., Chung, H., Detrano, R., Post, W., and Kronmal, R.A. (2006). Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 113, 30–37.
McConnell, B.B., Starborg, M., Brookes, S., and Peters, G. (1998). Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts. Curr Biol 8, 351–354.
McCord, R.P., Nazario-Toole, A., Zhang, H., Chines, P.S., Zhan, Y., Erdos, M.R., Collins, F.S., Dekker, J., and Cao, K. (2013). Correlated alterations in genome organization, histone methylation, and DNA-lamin A/C interactions in Hutchinson-Gilford progeria syndrome. Genome Res 23, 260–269.
McCrory, C., Fiorito, G., Hernandez, B., Polidoro, S., O'Halloran, A.M., Hever, A., Ni Cheallaigh, C., Lu, A.T., Horvath, S., Vineis, P., et al. (2021). GrimAge outperforms other epigenetic clocks in the prediction of age-related clinical phenotypes and all-cause mortality. J Gerontol A Biol Sci Med Sci 76, 741–749.
McEwen, L.M., O'Donnell, K.J., McGill, M.G., Edgar, R.D., Jones, M.J., MacIsaac, J.L., Lin, D.T.S., Ramadori, K., Morin, A., Gladish, N., et al. (2020). The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. Proc Natl Acad Sci USA 117, 23329–23335.
McGreevy, C., Horan, J., Jones, D., Biswas, K., O'Meara, Y.M., and Mulkerrin, E.C. (2008). A study of tubular potassium secretory capacity in older patients with hyperkalaemia. J Nutr Health Aging 12, 152–155.
McGregor, R.A., Cameron-Smith, D., and Poppitt, S.D. (2014). It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. Longev Healthspan 3, 9.
McLean, A.J., Cogger, V.C., Chong, G.C., Warren, A., Markus, A.M., Dahlstrom, J.E., and Le Couteur, D.G. (2003). Age-related pseudocapillarization of the human liver. J Pathol 200, 112–117.
McQuattie-Pimentel, A.C., Ren, Z., Joshi, N., Watanabe, S., Stoeger, T., Chi, M., Lu, Z., Sichizya, L., Aillon, R.P., Chen, C.I., et al. (2021). The lung microenvironment shapes a dysfunctional response of alveolar macrophages in aging. J Clin Invest 131.
Meer, M.V., Podolskiy, D.I., Tyshkovskiy, A., and Gladyshev, V.N. (2018). A whole lifespan mouse multi-tissue DNA methylation clock. eLife 7, e40675.
Mehdizadeh, M., Aguilar, M., Thorin, E., Ferbeyre, G., and Nattel, S. (2022). The role of cellular senescence in cardiac disease: basic biology and clinical relevance. Nat Rev Cardiol 19, 250–264.
Meier, J.M., Alavi, A., Iruvuri, S., Alzeair, S., Parker, R., Houseni, M., Hernandez-Pampaloni, M., Mong, A., and Torigian, D.A. (2007). Assessment of age-related changes in abdominal organ structure and function with computed tomography and positron emission tomography. Semin Nucl Med 37, 154–172.
Meier, U.T. (2017). RNA modification in Cajal bodies. RNA Biol 14, 693–700.
Mejia-Ramirez, E., and Florian, M.C. (2020). Understanding intrinsic hematopoietic stem cell aging. Haematologica 105, 22–37.
Mencke, R., Olauson, H., and Hillebrands, J.L. (2017). Effects of Klotho on fibrosis and cancer: a renal focus on mechanisms and therapeutic strategies. Adv Drug Deliv Rev 121, 85–100.
Mender, I., Zhang, A., Ren, Z., Han, C., Deng, Y., Siteni, S., Li, H., Zhu, J., Vemula, A., Shay, J.W., et al. (2020). Telomere stress potentiates STING-dependent anti-tumor immunity. Cancer Cell 38, 400–411.e6.
Mendez-Bermudez, A., Giraud-Panis, M.J., Ye, J., and Gilson, E. (2020). Heterochromatin replication goes hand in hand with telomere

protection. Nat Struct Mol Biol 27, 313–318.
Meng, D., Pan, X., Gokulnath, P., Vulugundam, G., and Xiao, J. (2023). Abdominal aortic aneurysm formation: a yap's view. J Cardiovasc Trans Res 16, 63–64.
Meng, J., Lv, Z., Qiao, X., Li, X., Li, Y., Zhang, Y., and Chen, C. (2017). The decay of redox-stress response capacity is a substantive characteristic of aging: revising the redox theory of aging. Redox Biol 11, 365–374.
Meng, J., Lv, Z., Wang, Y., and Chen, C. (2022). Identification of the redox-stress signaling threshold (RST): Increased RST helps to delay aging in *C. elegans*. Free Radic Biol Med 178, 54–58.
Meng, J., Lv, Z., Zhang, Y., Wang, Y., Qiao, X., Sun, C., Chen, Y., Guo, M., Han, W., Ye, A., et al. (2021). Precision redox: the key for antioxidant pharmacology. Antioxid Redox Signal 34, 1069–1082.
Mengel-From, J., Thinggaard, M., Dalgård, C., Kyvik, K.O., Christensen, K., and Christiansen, L. (2014). Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. Hum Genet 133, 1149–1159.
Merritt, E.K., Stec, M.J., Thalacker-Mercer, A., Windham, S.T., Cross, J. M., Shelley, D.P., Craig Tuggle, S., Kosek, D.J., Kim, J., and Bamman, M.M. (2013). Heightened muscle inflammation susceptibility may impair regenerative capacity in aging humans. J Appl Physiol 115, 937–948.
Meyer, D.H., and Schumacher, B. (2021). BiT age: a transcriptome-based aging clock near the theoretical limit of accuracy. Aging Cell 20, e13320.
Meyer, J.G., Softic, S., Basisty, N., Rardin, M.J., Verdin, E., Gibson, B.W., Ilkayeva, O., Newgard, C.B., Kahn, C.R., and Schilling, B. (2018). Temporal dynamics of liver mitochondrial protein acetylation and succinylation and metabolites due to high fat diet and/or excess glucose or fructose. PLoS ONE 13, e0208973.
Meyer, J.H., Cervenka, S., Kim, M.J., Kreisl, W.C., Henter, I.D., and Innis, R.B. (2020). Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. Lancet Psychiatry 7, 1064–1074.
Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., Cesari, M., and Nourhashemi, F. (2013). Proinflammatory cytokines, aging, and age-related diseases. J Am Med Dir Assoc 14, 877–882.
Micheau, O., and Tschopp, J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114, 181–190.
Middleton, E.A., He, X.Y., Denorme, F., Campbell, R.A., Ng, D., Salvatore, S.P., Mostyka, M., Baxter-Stoltzfus, A., Borczuk, A.C., Loda, M., et al. (2020). Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 136, 1169–1179.
Miething, A. (1993). Multinucleated spermatocytes in the aging human testis: formation, morphology, and degenerative fate. Andrologia 25, 317–323.
Migliavacca, E., Tay, S.K.H., Patel, H.P., Sonntag, T., Civiletto, G., McFarlane, C., Forrester, T., Barton, S.J., Leow, M.K., Antoun, E., et al. (2019). Mitochondrial oxidative capacity and NAD^+ biosynthesis are reduced in human sarcopenia across ethnicities. Nat Commun 10, 5808.
Mihaylova, M.M., Cheng, C.W., Cao, A.Q., Tripathi, S., Mana, M.D., Bauer-Rowe, K.E., Abu-Remaileh, M., Clavain, L., Erdemir, A., Lewis, C.A., et al. (2018). Fasting activates fatty acid oxidation to enhance intestinal stem cell function during homeostasis and aging. Cell Stem Cell 22, 769–778.e4.
Mikwar, M., MacFarlane, A.J., and Marchetti, F. (2020). Mechanisms of oocyte aneuploidy associated with advanced maternal age. Mutat Res Rev Mutat Res 785, 108320.
Milanovic, M., Fan, D.N.Y., Belenki, D., Däbritz, J.H.M., Zhao, Z., Yu, Y., Dörr, J.R., Dimitrova, L., Lenze, D., Monteiro Barbosa, I.A., et al. (2018). Senescence-associated reprogramming promotes cancer stemness. Nature 553, 96–100.
Mills, K.F., Yoshida, S., Stein, L.R., Grozio, A., Kubota, S., Sasaki, Y., Redpath, P., Migaud, M.E., Apte, R.S., Uchida, K., et al. (2016). Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab 24, 795–806.
Milne, R., Diaz, A., Badger, S., Bunnik, E., Fauria, K., and Wells, K. (2018). At, with and beyond risk: expectations of living with the possibility of future dementia. Sociol Health Illn 40, 969–987.
Milosevic-Djordjevic, O., Grujicic, D., Novakovic, T., Arsenijevic, S., and Marinkovic, D. (2002). Micronuclei and ageing in a sample of Yugoslavian population (in Russian). Genetika 38, 264–267.
Mimran, A., Ribstein, J., and Jover, B. (1992). Aging and sodium homeostasis. Kidney Int Suppl 37, S107–S113.
Min, K.W., Zealy, R.W., Davila, S., Fomin, M., Cummings, J.C., Makowsky, D., Mcdowell, C.H., Thigpen, H., Hafner, M., Kwon, S. H., et al. (2018). Profiling of m^6A RNA modifications identified an age-associated regulation of *AGO2* mRNA stability. Aging Cell 17, e12753.
Minamino, T., and Komuro, I. (2007). Vascular cell senescence: contribution to atherosclerosis. Circ Res 100, 15–26.
Miquel, J., Economos, A.C., Fleming, J., and Johnson, J.E. Jr. (1980). Mitochondrial role in cell aging. Exp Gerontol 15, 575–591.
Misaka, T., Kimishima, Y., Yokokawa, T., Ikeda, K., and Takeishi, Y. (2023). Clonal hematopoiesis and cardiovascular diseases: role of JAK2V617F. J Cardiol 81, 3–9.
Mishra, A., Brown, A.L., Yao, X., Yang, S., Park, S.J., Liu, C., Dagur, P.K., McCoy, J.P., Keeran, K.J., Nugent, G.Z., et al. (2015a). Dendritic cells induce Th2-mediated airway inflammatory responses to house dust mite via DNA-dependent protein kinase. Nat Commun 6, 6224.
Mishra, P., Varuzhanyan, G., Pham, A.H., and Chan, D.C. (2015b). Mitochondrial dynamics is a distinguishing feature of skeletal muscle fiber types and regulates organellar compartmentalization. Cell Metab 22, 1033–1044.
Mishra, S., and Kass, D.A. (2021). Cellular and molecular pathobiology of heart failure with preserved ejection fraction. Nat Rev Cardiol 18, 400–423.
Mistry, K., van der Steen, B., Clifford, T., van Holthoon, F., Kleinnijenhuis, A., Prawitt, J., Labus, M., Vanhoecke, B., Lovat, P.E., and McConnell, A. (2021). Potentiating cutaneous wound healing in young and aged skin with nutraceutical collagen peptides. Clin Exp Derm 46, 109–117.
Mitchell, E., Spencer Chapman, M., Williams, N., Dawson, K.J., Mende, N., Calderbank, E.F., Jung, H., Mitchell, T., Coorens, T.H.H., Spencer, D.H., et al. (2022). Clonal dynamics of haematopoiesis across the human lifespan. Nature 606, 343–350.
Mitchell, G.F., Hwang, S.J., Vasan, R.S., Larson, M.G., Pencina, M.J., Hamburg, N.M., Vita, J.A., Levy, D., and Benjamin, E.J. (2010). Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation 121, 505–511.
Mittelbrunn, M., and Kroemer, G. (2021). Hallmarks of T cell aging. Nat Immunol 22, 687–698.
Miyachi, Y., Tsuchiya, K., Komiya, C., Shiba, K., Shimazu, N., Yamaguchi, S., Deushi, M., Osaka, M., Inoue, K., Sato, Y., et al. (2017). Roles for cell-cell adhesion and contact in obesity-induced hepatic myeloid cell accumulation and glucose intolerance. Cell Rep 18, 2766–2779.
Miyamoto-Mikami, E., Sato, K., Kurihara, T., Hasegawa, N., Fujie, S., Fujita, S., Sanada, K., Hamaoka, T., Tabata, I., and Iemitsu, M. (2015). Endurance training-induced increase in circulating irisin levels is associated with reduction of abdominal visceral fat in middle-aged and older adults. PLoS ONE 10, e0120354.
Miyauchi, H., Minamino, T., Tateno, K., Kunieda, T., Toko, H., and Komuro, I. (2004). Akt negatively regulates the *in vitro* lifespan of human endothelial cells via a p53/p21-dependent pathway. EMBO J 23, 212–220.
Mizukami, H., Takahashi, K., Inaba, W., Osonoi, S., Kamata, K., Tsuboi, K., and Yagihashi, S. (2014). Age-associated changes of islet endocrine cells and the effects of body mass index in Japanese. J Diabetes Invest 5, 38–47.
Moaddel, R., Ubaida-Mohien, C., Tanaka, T., Lyashkov, A., Basisty, N., Schilling, B., Semba, R.D., Franceschi, C., Gorospe, M., and Ferrucci, L. (2021). Proteomics in aging research: a roadmap to clinical, translational research. Aging Cell 20, e13325.
Mocchegiani, E., Giacconi, R., Cipriano, C., and Malavolta, M. (2009). NK and NKT cells in aging and longevity: role of zinc and metallothioneins. J Clin Immunol 29, 416–425.
Mogilenko, D.A., Shchukina, I., and Artyomov, M.N. (2022). Immune

ageing at single-cell resolution. Nat Rev Immunol 22, 484–498.
Mogilenko, D.A., Shpynov, O., Andhey, P.S., Arthur, L., Swain, A., Esaulova, E., Brioschi, S., Shchukina, I., Kerndl, M., Bambouskova, M., et al. (2021). Comprehensive profiling of an aging immune system reveals clonal GZMK$^+$ CD8$^+$ T cells as conserved hallmark of inflammaging. Immunity 54, 99–115.e12.
Mohamad, M., Mitchell, S.J., Wu, L.E., White, M.Y., Cordwell, S.J., Mach, J., Solon-Biet, S.M., Boyer, D., Nines, D., Das, A., et al. (2016). Ultrastructure of the liver microcirculation influences hepatic and systemic insulin activity and provides a mechanism for age-related insulin resistance. Aging Cell 15, 706–715.
Mohammed, S., Thadathil, N., Selvarani, R., Nicklas, E.H., Wang, D., Miller, B.F., Richardson, A., and Deepa, S.S. (2021). Necroptosis contributes to chronic inflammation and fibrosis in aging liver. Aging Cell 20, e13512.
Mohawk, J.A., Green, C.B., and Takahashi, J.S. (2012). Central and peripheral circadian clocks in mammals. Annu Rev Neurosci 35, 445–462.
Moiseeva, V., Cisneros, A., Sica, V., Deryagin, O., Lai, Y., Jung, S., Andrés, E., An, J., Segalés, J., Ortet, L., et al. (2023). Senescence atlas reveals an aged-like inflamed niche that blunts muscle regeneration. Nature 613, 169–178.
Molinuevo, J.L., Cami, J., Carné, X., Carrillo, M.C., Georges, J., Isaac, M. B., Khachaturian, Z., Kim, S.Y.H., Morris, J.C., Pasquier, F., et al. (2016). Ethical challenges in preclinical Alzheimer's disease observational studies and trials: Results of the Barcelona summit. Alzheimers Dement 12, 614–622.
Molka, B., Gwladys, B., Dorian, B., Lucie, M., Mustapha, B., Rosalie, C., Brigitte, G., Hafida, K.C., and Moncef, B. (2022). Follicular fluid growth factors and interleukin profiling as potential predictors of IVF outcomes. Front Physiol 13, 859790.
Moll, L., Roitenberg, N., Bejerano-Sagie, M., Boocholez, H., Carvalhal Marques, F., Volovik, Y., Elami, T., Siddiqui, A.A., Grushko, D., Biram, A., et al. (2018). The insulin/IGF signaling cascade modulates SUMOylation to regulate aging and proteostasis in *Caenorhabditis elegans*. eLife 7, e38635.
Mongelli, A., Barbi, V., Gottardi Zamperla, M., Atlante, S., Forleo, L., Nesta, M., Massetti, M., Pontecorvi, A., Nanni, S., Farsetti, A., et al. (2021). Evidence for biological age acceleration and telomere shortening in COVID-19 survivors. Int J Mol Sci 22, 6151.
Mongi-Bragato, B., Grondona, E., Sosa, L.V., Zlocowski, N., Venier, A.C., Torres, A.I., Latini, A., Leal, R.B., Gutiérrez, S., and De Paul, A.L. (2020). Pivotal role of NF-κB in cellular senescence of experimental pituitary tumours. J Endocrinol 245, 179–191.
Monk, T.H., Buysse, D.J., Carrier, J., and Kupfer, D.J. (2000). Inducing jet-lag in older people: directional asymmetry. J Sleep Res 9, 101–116.
Monk, T.H., Buysse, D.J., Reynolds Iii, C.F., and Kupfer, D.J. (1993). Inducing jet lag in older people: adjusting to a 6-hour phase advance in routine. Exp Gerontol 28, 119–133.
Montagna, W., and Carlisle, K. (1990). Structural changes in ageing skin. Br J Dermatol 122, 61–70.
Mook-Kanamori, D.O., El-Din, S.M.M., Takiddin, A.H., Al-Homsi, H., Al-Mahmoud, K.A.S., Al-Obaidli, A., Zirie, M.A., Rowe, J., Yousri, N.A., Karoly, E.D., et al. (2017). 1,5-Anhydroglucitol in saliva is a non-invasive marker of short-term glycemic control (vol 99, pg E479, 2014). J Clin Endocrinol Metab 102, 3867–3867.
Mooradian, A.D., and Chehade, J.M. (2000). Serum leptin response to endogenous hyperinsulinemia in aging rats. Mech Ageing Dev 115, 101–106.
Moore, L.D., Le, T., and Fan, G. (2013). DNA methylation and its basic function. Neuropsychopharmacol 38, 23–38.
Moqri, M., Cipriano, A., Nachun, D., Murty, T., de Sena Brandine, G., Rasouli, S., Tarkhov, A., Aberg, K.A., van den Oord, E., Zhou, W., et al. (2022). PRC2 clock: a universal epigenetic biomarker of aging and rejuvenation. bioRxiv 2022.2006.2003.494609.
Morgan, M., Thorburn, J., Pandolfi, P.P., and Thorburn, A. (2002). Nuclear and cytoplasmic shuttling of TRADD induces apoptosis via different mechanisms. J Cell Biol 157, 975–984.
Morgan, R.G., Walker, A.E., Trott, D.W., Machin, D.R., Henson, G.D., Reihl, K.D., Cawthon, R.M., Denchi, E.L., Liu, Y., Bloom, S.I., et al. (2019). Induced Trf2 deletion leads to aging vascular phenotype in mice associated with arterial telomere uncapping, senescence signaling, and oxidative stress. J Mol Cell Cardiol 127, 74–82.
Morin, O., Vallières, M., Jochems, A., Woodruff, H.C., Valdes, G., Braunstein, S.E., Wildberger, J.E., Villanueva-Meyer, J.E., Kearney, V., Yom, S.S., et al. (2018). A deep look into the future of quantitative imaging in oncology: a statement of working principles and proposal for change. Int J Radiat Oncol Biol Phys 102, 1074–1082.
Morrison, S.J., Wandycz, A.M., Akashi, K., Globerson, A., and Weissman, I.L. (1996). The aging of hematopoietic stem cells. Nat Med 2, 1011–1016.
Mosteiro, L., Pantoja, C., Alcazar, N., Marión, R.M., Chondronasiou, D., Rovira, M., Fernandez-Marcos, P.J., Muñoz-Martin, M., Blanco-Aparicio, C., Pastor, J., et al. (2016). Tissue damage and senescence provide critical signals for cellular reprogramming *in vivo*. Science 354.
Mtintsilana, A., Micklesfield, L.K., Chorell, E., Olsson, T., and Goedecke, J.H. (2019). Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study. Nutr Diabetes 9, 12.
Mukherjee, A.B., and Thomas, S. (1997). A longitudinal study of human age-related chromosomal analysis in skin fibroblasts. Exp Cell Res 235, 161–169.
Mularoni, V., Esposito, V., Di Persio, S., Vicini, E., Spadetta, G., Berloco, P., Fanelli, F., Mezzullo, M., Pagotto, U., Pelusi, C., et al. (2020). Age-related changes in human Leydig cell status. Hum Reprod 35, 2663–2676.
Muller, D.C., Elahi, D., Tobin, J.D., and Andres, R. (1996). Insulin response during the oral glucose tolerance test: the role of age, sex, body fat and the pattern of fat distribution. Aging Clin Exp Res 8, 13–21.
Muñoz-Espín, D., Cañamero, M., Maraver, A., Gómez-López, G., Contreras, J., Murillo-Cuesta, S., Rodríguez-Baeza, A., Varela-Nieto, I., Ruberte, J., Collado, M., et al. (2013). Programmed cell senescence during mammalian embryonic development. Cell 155, 1104–1118.
Muñoz-Espín, D., and Serrano, M. (2014). Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 15, 482–496.
Murakami, G. (2004). Histologic heterogeneity and intranodal shunt flow in lymph nodes from elderly subjects: a cadaveric study. Ann Surg Oncol 11, 279S–284S.
Murakami, R., Saisho, Y., Watanabe, Y., Inaishi, J., Tsuchiya, T., Kou, K., Sato, S., Kitago, M., Kitagawa, Y., Yamada, T., et al. (2017). Pancreas fat and β cell mass in humans with and without diabetes: an analysis in the Japanese population. J Clin Endocrinol Metab 102, 3251–3260.
Murao, N., Yokoi, N., Takahashi, H., Hayami, T., Minami, Y., and Seino, S. (2022). Increased glycolysis affects β-cell function and identity in aging and diabetes. Mol Metab 55, 101414.
Murata, Y., Namita, T., Kondo, K., Yamakawa, M., and Shiina, T. (2017). Quantitative evaluation of skin aging with photoacoustic microscopy. In: 2017 IEEE International Ultrasonics Symposium (IUS). Washington.
Murgia, M., Toniolo, L., Nagaraj, N., Ciciliot, S., Vindigni, V., Schiaffino, S., Reggiani, C., and Mann, M. (2017). Single muscle fiber proteomics reveals fiber-type-specific features of human muscle aging. Cell Rep 19, 2396–2409.
Nacarelli, T., Lau, L., Fukumoto, T., Zundell, J., Fatkhutdinov, N., Wu, S., Aird, K.M., Iwasaki, O., Kossenkov, A.V., Schultz, D., et al. (2019). NAD$^+$ metabolism governs the proinflammatory senescence-associated secretome. Nat Cell Biol 21, 397–407.
Nagata, T. (2012). RNA synthesis in the pancreatic acinar cells of aging mice as revealed by electron microscopic radioautography. CRP 5, 5–14.
Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D.W., Kushugulova, A., Marotta, F., and Yadav, H. (2018). Gut microbiome and aging: physiological and mechanistic insights. Nutr Healthy Aging 4, 267–285.
Nakamura, E., and Miyao, K. (2007). A method for identifying biomarkers

of aging and constructing an index of biological age in humans. J Gerontol A Biol Sci Med Sci 62, 1096–1105.
Nakamura, T.J., Nakamura, W., Tokuda, I.T., Ishikawa, T., Kudo, T., Colwell, C.S., and Block, G.D. (2015). Age-related changes in the circadian system unmasked by constant conditions. eNeuro 2, ENEURO.0064-15.2015.
Nakamura, T.J., Nakamura, W., Yamazaki, S., Kudo, T., Cutler, T., Colwell, C.S., and Block, G.D. (2011). Age-related decline in circadian output. J Neurosci 31, 10201–10205.
Nakano-Kurimoto, R., Ikeda, K., Uraoka, M., Nakagawa, Y., Yutaka, K., Koide, M., Takahashi, T., Matoba, S., Yamada, H., Okigaki, M., et al. (2009). Replicative senescence of vascular smooth muscle cells enhances the calcification through initiating the osteoblastic transition. Am J Physiol Heart Circ Physiol 297, H1673–H1684.
Nakayama, H., Nishida, K., and Otsu, K. (2016). Macromolecular degradation systems and cardiovascular aging. Circ Res 118, 1577–1592.
Nalapareddy, K., Hassan, A., Sampson, L.L., Zheng, Y., and Geiger, H. (2021). Suppression of elevated Cdc42 activity promotes the regenerative potential of aged intestinal stem cells. iScience 24, 102362.
Nalapareddy, K., Nattamai, K.J., Kumar, R.S., Karns, R., Wikenheiser-Brokamp, K.A., Sampson, L.L., Mahe, M.M., Sundaram, N., Yacyshyn, M.B., Yacyshyn, B., et al. (2017). Canonical Wnt signaling ameliorates aging of intestinal stem cells. Cell Rep 18, 2608–2621.
Nalapareddy, K., Zheng, Y., and Geiger, H. (2022). Aging of intestinal stem cells. Stem Cell Rep 17, 734–740.
Nandi, N., and Krämer, H. (2018). Cdk5-mediated Acn/Acinus phosphorylation regulates basal autophagy independently of metabolic stress. Autophagy 14, 1271–1272.
Naqvi, T.Z., and Lee, M.S. (2014). Carotid intima-media thickness and plaque in cardiovascular risk assessment. JACC Cardiovasc Imag 7, 1025–1038.
Naranjo, J.D., Dziki, J.L., and Badylak, S.F. (2017). Regenerative medicine approaches for age-related muscle loss and sarcopenia: a mini-review. Gerontology 63, 580–589.
Narita, M., Narita, M., Krizhanovsky, V., Nuñez, S., Chicas, A., Hearn, S. A., Myers, M.P., and Lowe, S.W. (2006). A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126, 503–514.
Narita, M., Nuñez, S., Heard, E., Narita, M., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., and Lowe, S.W. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113, 703–716.
Navarro, A., and Boveris, A. (2004). Rat brain and liver mitochondria develop oxidative stress and lose enzymatic activities on aging. Am J Physiol Regul Integr Comp Physiol 287, R1244–R1249.
Navik, U., Sheth, V.G., Khurana, A., Jawalekar, S.S., Allawadhi, P., Gaddam, R.R., Bhatti, J.S., and Tikoo, K. (2021). Methionine as a double-edged sword in health and disease: current perspective and future challenges. Ageing Res Rev 72, 101500.
Navot, D., Bergh, R.A., Williams, M.A., Garrisi, G.J., Guzman, I., Sandler, B., and Grunfeld, L. (1991). Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 337, 1375–1377.
Naylor, K., Li, G., Vallejo, A.N., Lee, W.W., Koetz, K., Bryl, E., Witkowski, J., Fulbright, J., Weyand, C.M., and Goronzy, J.J. (2005). The influence of age on T cell generation and TCR diversity. J Immunol 174, 7446–7452.
Neaves, W.B., Johnson, L., and Petty, C.S. (1985). Age-related change in numbers of other interstitial cells in testes of adult men: evidence bearing on the fate of Leydig cells lost with increasing age. Biol Reprod 33, 259–269.
Neaves, W.B., Johnson, L., Porter, J.C., Parker, C.R. Jr., and Petty, C.S. (1984). Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 59, 756–763.
Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 293, E444–E452.
Nestle, F.O., Di Meglio, P., Qin, J.Z., and Nickoloff, B.J. (2009). Skin immune sentinels in health and disease. Nat Rev Immunol 9, 679–691.
Neumann, J.F., Leote, A.C., Liersch, M., and Beyer, A. (2023). Predicting murine age across tissues and cell types using single cell transcriptome data. bioRxiv 2022.2010.2019.512922.
Nguyen, H.P., Lin, F., Yi, D., Xie, Y., Dinh, J., Xue, P., and Sul, H.S. (2021). Aging-dependent regulatory cells emerge in subcutaneous fat to inhibit adipogenesis. Dev Cell 56, 1437–1451.e3.
Nichols, N.R., Day, J.R., Laping, N.J., Johnson, S.A., and Finch, C.E. (1993). GFAP mRNA increases with age in rat and human brain. Neurobiol Aging 14, 421–429.
Nie, C., Li, Y., Li, R., Yan, Y., Zhang, D., Li, T., Li, Z., Sun, Y., Zhen, H., Ding, J., et al. (2022a). Distinct biological ages of organs and systems identified from a multi-omics study. Cell Rep 38, 110459.
Nie, X., Munyoki, S.K., Sukhwani, M., Schmid, N., Missel, A., Emery, B. R., DonorConnect, B.R., Stukenborg, J.B., Mayerhofer, A., Orwig, K. E., et al. (2022b). Single-cell analysis of human testis aging and correlation with elevated body mass index. Dev Cell 57, 1160–1176.e5.
Niemann, J., Johne, C., Schröder, S., Koch, F., Ibrahim, S.M., Schultz, J., Tiedge, M., and Baltrusch, S. (2017). An mtDNA mutation accelerates liver aging by interfering with the ROS response and mitochondrial life cycle. Free Radic Biol Med 102, 174–187.
NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, (2001). Osteoporosis prevention, diagnosis, and therapy. JAMA 285, 785–795.
Nishimura, E.K., Granter, S.R., and Fisher, D.E. (2005). Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science 307, 720–724.
Nishimura, K., Kumazawa, T., Kuroda, T., Katagiri, N., Tsuchiya, M., Goto, N., Furumai, R., Murayama, A., Yanagisawa, J., and Kimura, K. (2015). Perturbation of ribosome biogenesis drives cells into senescence through 5S RNP-mediated p53 activation. Cell Rep 10, 1310–1323.
Nistal, M., Codesal, J., and Paniagua, R. (1986). Multinucleate spermatids in aging human testes. Arch Androl 16, 125–129.
Nistal, M., Codesal, J., Paniagua, R., and Santamaria, L. (1987). Decrease in the number of human Ap and Ad spermatogonia and in the Ap/Ad ratio with advancing age. New data on the spermatogonial stem cell. J Androl 8, 64–68.
Nitta, K., Okada, K., Yanai, M., and Takahashi, S. (2013). Aging and chronic kidney disease. Kidney Blood Press Res 38, 109–120.
Noguchi, G.M., and Huising, M.O. (2019). Integrating the inputs that shape pancreatic islet hormone release. Nat Metab 1, 1189–1201.
Nogueira, V., Park, Y., Chen, C.C., Xu, P.Z., Chen, M.L., Tonic, I., Unterman, T., and Hay, N. (2008). Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. Cancer Cell 14, 458–470.
Noh, J.H., Kim, K.M., Pandey, P.R., Noren Hooten, N., Munk, R., Kundu, G., De, S., Martindale, J.L., Yang, X., Evans, M.K., et al. (2019). Loss of RNA-binding protein GRSF1 activates mTOR to elicit a proinflammatory transcriptional program. Nucleic Acids Res 47, 2472–2486.
Norvik, J.V., Harskamp, L.R., Nair, V., Shedden, K., Solbu, M.D., Eriksen, B.O., Kretzler, M., Gansevoort, R.T., Ju, W., and Melsom, T. (2021). Urinary excretion of epidermal growth factor and rapid loss of kidney function. Nephrol Dial Transplant 36, 1882–1892.
Noureddin, M., Yates, K.P., Vaughn, I.A., Neuschwander-Tetri, B.A., Sanyal, A.J., McCullough, A., Merriman, R., Hameed, B., Doo, E., Kleiner, D.E., et al. (2013). Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. Hepatology 58, 1644–1654.
Ntostis, P., Iles, D., Kokkali, G., Vaxevanoglou, T., Kanavakis, E., Pantou, A., Huntriss, J., Pantos, K., and Picton, H.M. (2021). The impact of maternal age on gene expression during the GV to MII transition in euploid human oocytes. Hum Reprod 37, 80–92.
Nunes, A.D.C., Weigl, M., Schneider, A., Noureddine, S., Yu, L., Lahde, C., Saccon, T.D., Mitra, K., Beltran, E., Grillari, J., et al. (2022). miR-146a-5p modulates cellular senescence and apoptosis in visceral

adipose tissue of long-lived Ames dwarf mice and in cultured pre-adipocytes. Geroscience 44, 503–518.
O'Connor, J.P.B., Aboagye, E.O., Adams, J.E., Aerts, H.J.W.L., Barrington, S.F., Beer, A.J., Boellaard, R., Bohndiek, S.E., Brady, M., Brown, G., et al. (2017). Imaging biomarker roadmap for cancer studies. Nat Rev Clin Oncol 14, 169–186.
O'Driscoll, M., Ribeiro Dos Santos, G., Wang, L., Cummings, D.A.T., Azman, A.S., Paireau, J., Fontanet, A., Cauchemez, S., and Salje, H. (2021). Age-specific mortality and immunity patterns of SARS-CoV-2. Nature 590, 140–145.
O'Driscoll, S.W., Saris, D.B., Ito, Y., and Fitzimmons, J.S. (2001). The chondrogenic potential of periosteum decreases with age. J Orthop Res 19, 95–103.
O'Sullivan, E.D., Hughes, J., and Ferenbach, D.A. (2017). Renal aging: causes and consequences. J Am Soc Nephrol 28, 407–420.
O'Sullivan, R.J., Kubicek, S., Schreiber, S.L., and Karlseder, J. (2010). Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. Nat Struct Mol Biol 17, 1218–1225.
O'Toole, P.W., and Jeffery, I.B. (2015). Gut microbiota and aging. Science 350, 1214–1215.
Oates, P.S., and Morgan, R.G.H. (1986). Changes in pancreatic acinar cell nuclear number and DNA content during aging in the rat. Am J Anat 177, 547–554.
Obas, V., and Vasan, R.S. (2018). The aging heart. Clin Sci 132, 1367–1382.
Ocampo, A., Reddy, P., Martinez-Redondo, P., Platero-Luengo, A., Hatanaka, F., Hishida, T., Li, M., Lam, D., Kurita, M., Beyret, E., et al. (2016). *In vivo* amelioration of age-associated hallmarks by partial reprogramming. Cell 167, 1719–1733.e12.
Odom, D.T., Zizlsperger, N., Gordon, D.B., Bell, G.W., Rinaldi, N.J., Murray, H.L., Volkert, T.L., Schreiber, J.., Rolfe, P.A., Gifford, D.K., et al. (2004). Control of pancreas and liver gene expression by HNF transcription factors. Science 303, 1378–1381.
OECD. (2011). Policy Issues for the Development and Use of Biomarkers in Health. Paris: OECD.
Ogata, Y., Yamada, T., Hasegawa, S., Sanada, A., Iwata, Y., Arima, M., Nakata, S., Sugiura, K., and Akamatsu, H. (2021). SASP-induced macrophage dysfunction may contribute to accelerated senescent fibroblast accumulation in the dermis. Exp Dermatol 30, 84–91.
Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C.L., Lahat, A., Day, C.P., Burt, A., Palmer, A., Anstee, Q.M., et al. (2017). Cellular senescence drives age-dependent hepatic steatosis. Nat Commun 8, 15691.
Ohsaki, Y., Kawai, T., Yoshikawa, Y., Cheng, J., Jokitalo, E., and Fujimoto, T. (2016). PML isoform II plays a critical role in nuclear lipid droplet formation. J Cell Biol 212, 29–38.
Ohsugi, M., Adachi, K., Horai, R., Kakuta, S., Sudo, K., Kotaki, H., Tokai-Nishizumi, N., Sagara, H., Iwakura, Y., and Yamamoto, T. (2008). Kid-mediated chromosome compaction ensures proper nuclear envelope formation. Cell 132, 771–782.
Ohtani, N., Zebedee, Z., Huot, T.J.G., Stinson, J.A., Sugimoto, M., Ohashi, Y., Sharrocks, A.D., Peters, G., and Hara, E. (2001). Opposing effects of Ets and Id proteins on $p16^{INK4a}$ expression during cellular senescence. Nature 409, 1067–1070.
Oldham, J.M. (2021). Interstitial Lung Abnormalities and Aging Biomarkers: A Mediation. Am J Respir Crit Care Med 203, 1058–1060.
Olsen, F.J., Johansen, N.D., Skaarup, K.G., Lassen, M.C.H., Ravnkilde, K., Schnohr, P., Jensen, G.B., Marott, J.L., Søgaard, P., Møgelvang, R., et al. (2021). Changes in left atrial structure and function over a decade in the general population. Eur Heart J Cardiovasc Imag 23, 124–136.
Omar, A.M.S., Narula, S., Abdel Rahman, M.A., Pedrizzetti, G., Raslan, H., Rifaie, O., Narula, J., and Sengupta, P.P. (2017). Precision phenotyping in heart failure and pattern clustering of ultrasound data for the assessment of diastolic dysfunction. JACC Cardiovasc Imag 10, 1291–1303.
Onishi, M., Yamano, K., Sato, M., Matsuda, N., and Okamoto, K. (2021). Molecular mechanisms and physiological functions of mitophagy. EMBO J 40, e104705.
Ono, T., Ikehata, H., Nakamura, S., Saito, Y., Hosoi, Y., Takai, Y., Yamada, S., Onodera, J., and Yamamoto, K. (2000). Age-associated increase of spontaneous mutant frequency and molecular nature of mutation in newborn and old lacZ-transgenic mouse. Mutat Res Fundamental Mol Mech Mutagenesis 447, 165–177.
Ono, T., Miyamura, Y., Ikehata, H., Yamanaka, H., Kurishita, A., Yamamoto, K., Suzuki, T., Nohmi, T., Hayashi, M., and Sofuni, T. (1995). Spontaneous mutant frequency of lacZ gene in spleen of transgenic mouse increases with age. Mutat Res DNAging 338, 183–188.
Orjalo, A.V., Bhaumik, D., Gengler, B.K., Scott, G.K., and Campisi, J. (2009). Cell surface-bound IL-1α is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci USA 106, 17031–17036.
Orta, T., and Günebakan, S. (2012). The effect of aging on micronuclei frequency and proliferation in human peripheral blood lymphocytes. Ind J Hum Genet 18, 95–100.
Ortega-Martínez, M., Rodríguez-Flores, L.E., Ancer-Arellano, A., Cerda-Flores, R.M., de-la-Garza-González, C., Ancer-Rodríguez, J., and Jaramillo-Rangel, G. (2016). Analysis of cell turnover in the bronchiolar epithelium through the normal aging process. Lung 194, 581–587.
Ou, M.Y., Zhang, H., Tan, P.C., Zhou, S.B., and Li, Q.F. (2022). Adipose tissue aging: mechanisms and therapeutic implications. Cell Death Dis 13, 300.
Özcan, S., Alessio, N., Acar, M.B., Mert, E., Omerli, F., Peluso, G., and Galderisi, U. (2016). Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging 8, 1316–1329.
Ozkurede, U., and Miller, R.A. (2019). Improved mitochondrial stress response in long-lived Snell dwarf mice. Aging Cell 18, e13030.
Pagel, P.S., Tawil, J.N., Boettcher, B.T., Izquierdo, D.A., Lazicki, T.J., Crystal, G.J., and Freed, J.K. (2021). Heart failure with preserved ejection fraction: a comprehensive review and update of diagnosis, pathophysiology, treatment, and perioperative implications. J Cardiothorac Vasc Anesth 35, 1839–1859.
Pal, S., and Tyler, J.K. (2016). Epigenetics and aging. Sci Adv 2, e1600584.
Palikaras, K., Lionaki, E., and Tavernarakis, N. (2015). Coupling mitogenesis and mitophagy for longevity. Autophagy 11, 1428–1430.
Palla, A.R., Hilgendorf, K.I., Yang, A.V., Kerr, J.P., Hinken, A.C., Demeter, J., Kraft, P., Mooney, N.A., Yucel, N., Burns, D.M., et al. (2022). Primary cilia on muscle stem cells are critical to maintain regenerative capacity and are lost during aging. Nat Commun 13, 1439.
Palla, A.R., Ravichandran, M., Wang, Y.X., Alexandrova, L., Yang, A.V., Kraft, P., Holbrook, C.A., Schürch, C.M., Ho, A.T.V., and Blau, H.M. (2021). Inhibition of prostaglandin-degrading enzyme 15-PGDH rejuvenates aged muscle mass and strength. Science 371.
Palmer, A.K., Xu, M., Zhu, Y., Pirtskhalava, T., Weivoda, M.M., Hachfeld, C.M., Prata, L.G., Dijk, T.H., Verkade, E., Casaclang-Verzosa, G., et al. (2019). Targeting senescent cells alleviates obesity-induced metabolic dysfunction. Aging Cell 18, e12950.
Palmer, R.D. (2022). Aging clocks & mortality timers, methylation, glycomic, telomeric and more. A window to measuring biological age. Aging Med 5, 120–125.
Palmeri, M., Misiano, G., Malaguarnera, M., Forte, G.I., Vaccarino, L., Milano, S., Scola, L., Caruso, C., Motta, M., Maugeri, D., et al. (2012). Cytokine serum profile in a group of Sicilian nonagenarians. J Immunoassay Immunochem 33, 82–90.
Pálovics, R., Keller, A., Schaum, N., Tan, W., Fehlmann, T., Borja, M., Kern, F., Bonanno, L., Calcuttawala, K., Webber, J., et al. (2022). Molecular hallmarks of heterochronic parabiosis at single-cell resolution. Nature 603, 309–314.
Pan, K., Chen, Y., Roth, M., Wang, W., Wang, S., Yee, A.S., and Zhang, X. (2013). HBP1-mediated transcriptional regulation of DNA methyltransferase 1 and its impact on cell senescence. Mol Cell Biol 33, 887–903.
Panagopoulos, A., and Altmeyer, M. (2021). The hammer and the dance of cell cycle control. Trends Biochem Sci 46, 301–314.

Panda, A., Qian, F., Mohanty, S., van Duin, D., Newman, F.K., Zhang, L., Chen, S., Towle, V., Belshe, R.B., Fikrig, E., et al. (2010). Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. J Immunol 184, 2518–2527.
Panda, S., Hogenesch, J.B., and Kay, S.A. (2002). Circadian rhythms from flies to human. Nature 417, 329–335.
Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D. J., Schrier, S.L., and Weissman, I.L. (2011). Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci USA 108, 20012–20017.
Paniagua, R., Amat, P., Nistal, M., and Martin, A. (1985). Ultrastructural changes in Sertoli cells in ageing humans. Int J Androl 8, 295–312.
Paniagua, R., Nistal, M., Amat, P., Rodriguez, M.C., and Martin, A. (1987). Seminiferous tubule involution in elderly men. Biol Reprod 36, 939–947.
Paniagua, R., Nistal, M., Sáez, F.J., and Fraile, B. (1991). Ultrastructure of the aging human testis. J Elec Microsc Tech 19, 241–260.
Pannérec, A., Springer, M., Migliavacca, E., Ireland, A., Piasecki, M., Karaz, S., Jacot, G., Métairon, S., Danenberg, E., Raymond, F., et al. (2016). A robust neuromuscular system protects rat and human skeletal muscle from sarcopenia. Aging 8, 712–728.
Pantel, A.R., Gitto, S.B., Makvandi, M., Kim, H., Medvedv, S., Weeks, J. K., Torigian, D.A., Hsieh, C.J., Ferman, B., Latif, N.A., et al. (2022). [18F]FluorThanatrace ([18F]FTT) PET imaging of PARP-inhibitor drug-target engagement as a biomarker of response in ovarian cancer, a pilot study. Clin Cancer Res doi: 10.1158/1078-0432.CCR-22-1602.
Panwar, P., Lamour, G., Mackenzie, N.C.W., Yang, H., Ko, F., Li, H., and Brömme, D. (2015). Changes in structural-mechanical properties and degradability of collagen during aging-associated modifications. J Biol Chem 290, 23291–23306.
Panyard, D.J., Yu, B., and Snyder, M.P. (2022). The metabolomics of human aging: Advances, challenges, and opportunities. Sci Adv 8, eadd6155.
Papandreou, M.E., Konstantinidis, G., and Tavernarakis, N. (2022). Nucleophagy delays aging and preserves germline immortality. Nat Aging 3, 34–46.
Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 18, 134–147.
Park, J.H., Seo, S.W., Kim, C., Kim, G.H., Noh, H.J., Kim, S.T., Kwak, K. C., Yoon, U., Lee, J.M., Lee, J.W., et al. (2013). Pathogenesis of cerebral microbleeds: *in vivo* imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment. Ann Neurol 73, 584–593.
Park, J.S., Na, H.J., Pyo, J.H., Jeon, H.J., Kim, Y.S., and Yoo, M.A. (2015). Requirement of ATR for maintenance of intestinal stem cells in aging *Drosophila*. Aging 7, 307–318.
Park, J.Y., Cho, M.O., Leonard, S., Calder, B., Mian, I.S., Kim, W.H., Wijnhoven, S., van Steeg, H., Mitchell, J., van der Horst, G.T.J., et al. (2008). Homeostatic imbalance between apoptosis and cell renewal in the liver of premature aging Xpd mice. PLoS ONE 3, e2346.
Park, M.J., Ahn, J.W., Kim, K.H., Bang, J., Kim, S.C., Jeong, J.Y., Choi, Y. E., Kim, C.W., and Joo, B.S. (2020). Prediction of ovarian aging using ovarian expression of BMP15, GDF9, and C-KIT. Exp Biol Med 245, 711–719.
Park, S.J., Gavrilova, O., Brown, A.L., Soto, J.E., Bremner, S., Kim, J., Xu, X., Yang, S., Um, J.H., Koch, L.G., et al. (2017). DNA-PK promotes the mitochondrial, metabolic, and physical decline that occurs during aging. Cell Metab 25, 1135–1146.e7.
Parkhitko, A.A., Jouandin, P., Mohr, S.E., and Perrimon, N. (2019). Methionine metabolism and methyltransferases in the regulation of aging and lifespan extension across species. Aging Cell 18, e13034.
Parkinson, L.G., Toro, A., Zhao, H., Brown, K., Tebbutt, S.J., and Granville, D.J. (2015). Granzyme B mediates both direct and indirect cleavage of extracellular matrix in skin after chronic low-dose ultraviolet light irradiation. Aging Cell 14, 67–77.
Parrish, A.R. (2017). The impact of aging on epithelial barriers. Tissue Barriers 5, e1343172.
Partridge, L., Deelen, J., and Slagboom, P.E. (2018). Facing up to the global challenges of ageing. Nature 561, 45–56.
Paschalis, E.P., Fratzl, P., Gamsjaeger, S., Hassler, N., Brozek, W., Eriksen, E.F., Rauch, F., Glorieux, F.H., Shane, E., Dempster, D., et al. (2016). Aging versus postmenopausal osteoporosis: bone composition and maturation kinetics at actively-forming trabecular surfaces of female subjects aged 1 to 84 years. J Bone Miner Res 31, 347–357.
Pascoal, T.A., Benedet, A.L., Tudorascu, D.L., Therriault, J., Mathotaarachchi, S., Savard, M., Lussier, F.Z., Tissot, C., Chamoun, M., Kang, M.S., et al. (2021). Longitudinal 18F-MK-6240 tau tangles accumulation follows Braak stages. Brain 144, 3517–3528.
Pascoal, T.A., Chamoun, M., Lax, E., Wey, H.Y., Shin, M., Ng, K.P., Kang, M.S., Mathotaarachchi, S., Benedet, A.L., Therriault, J., et al. (2022). [11C]Martinostat PET analysis reveals reduced HDAC I availability in Alzheimer's disease. Nat Commun 13, 4171.
Passegué, E., and Wagner, E.F. (2000). JunB suppresses cell proliferation by transcriptional activation of $p16^{INK4a}$ expression. EMBO J 19, 2969–2979.
Patke, A., Young, M.W., and Axelrod, S. (2020). Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol 21, 67–84.
Paul, C., and Robaire, B. (2013). Impaired function of the blood-testis barrier during aging is preceded by a decline in cell adhesion proteins and GTPases. PLoS ONE 8, e84354.
Paulsen, J.S., Nance, M., Kim, J.I., Carlozzi, N.E., Panegyres, P.K., Erwin, C., Goh, A., McCusker, E., and Williams, J.K. (2013). A review of quality of life after predictive testing for and earlier identification of neurodegenerative diseases. Prog Neurobiol 110, 2–28.
Payne, B.A.I., and Chinnery, P.F. (2015). Mitochondrial dysfunction in aging: much progress but many unresolved questions. Biochim Biophys Acta 1847, 1347–1353.
Peake, J., Gatta, P.D., and Cameron-Smith, D. (2010). Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. Am J Physiol Regul Integr Comp Physiol 298, R1485–R1495.
Pearce, E.E., Alsaggaf, R., Katta, S., Dagnall, C., Aubert, G., Hicks, B.D., Spellman, S.R., Savage, S.A., Horvath, S., and Gadalla, S.M. (2022). Telomere length and epigenetic clocks as markers of cellular aging: a comparative study. Geroscience 44, 1861–1869.
Pearson, M., Carbone, R., Sebastiani, C., Cioce, M., Fagioli, M., Saito, S., Higashimoto, Y., Appella, E., Minucci, S., Pandolfi, P.P., et al. (2000). PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 406, 207–210.
Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R.C., Cota, P., Wittnam, J.L., Gogol-Doering, A., Opitz, L., et al. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 328, 753–756.
Pelletier-Galarneau, M., Petibon, Y., Ma, C., Han, P., Kim, S.J.W., Detmer, F.J., Yokell, D., Guehl, N., Normandin, M., El Fakhri, G., et al. (2021). *In vivo* quantitative mapping of human mitochondrial cardiac membrane potential: a feasibility study. Eur J Nucl Med Mol Imag 48, 414–420.
Peng, H., Hu, B., Xie, L.Q., Su, T., Li, C.J., Liu, Y., Yang, M., Xiao, Y., Feng, X., Zhou, R., et al. (2022). A mechanosensitive lipolytic factor in the bone marrow promotes osteogenesis and lymphopoiesis. Cell Metab 34, 1168–1182.e6.
Peng, S.L., Dumas, J.A., Park, D.C., Liu, P., Filbey, F.M., McAdams, C.J., Pinkham, A.E., Adinoff, B., Zhang, R., and Lu, H. (2014). Age-related increase of resting metabolic rate in the human brain. NeuroImage 98, 176–183.
Peng, X., Zhang, C., Bao, J.P., Zhu, L., Shi, R., Xie, Z.Y., Wang, F., Wang, K., and Wu, X. (2020). A20 of nucleus pulposus cells plays a self-protection role via the nuclear factor-kappa B pathway in the inflammatory microenvironment. Bone Joint Res 9, 225–235.
Pentinmikko, N., Iqbal, S., Mana, M., Andersson, S., Cognetta Iii, A.B., Suciu, R.M., Roper, J., Luopajärvi, K., Markelin, E., Gopalakrishnan, S., et al. (2019). Notum produced by Paneth cells attenuates regeneration of aged intestinal epithelium. Nature 571, 398–402.
Perez, K., Ciotlos, S., McGirr, J., Limbad, C., Doi, R., Nederveen, J.P.,

Nilsson, M.I., Winer, D.A., Evans, W., Tarnopolsky, M., et al. (2022). Single nuclei profiling identifies cell specific markers of skeletal muscle aging, frailty, and senescence. Aging 14, 9393–9422.
Perheentupa, A., and Huhtaniemi, I. (2009). Aging of the human ovary and testis. Mol Cell Endocrinol 299, 2–13.
Pertynska-Marczewska, M., and Diamanti-Kandarakis, E. (2017). Aging ovary and the role for advanced glycation end products. Menopause 24, 345–351.
Peters, A. (1993). The absence of significant neuronal loss from cerebral cortex with age. Neurobiol Aging 14, 657–658.
Peters, A. (2009). The effects of normal aging on myelinated nerve fibers in monkey central nervous system. Front Neuroanat 3, 11.
Peters, A., Verderosa, A., and Sethares, C. (2008). The neuroglial population in the primary visual cortex of the aging rhesus monkey. Glia 56, 1151–1161.
Peters, A.E., Mihalas, B.P., Bromfield, E.G., Roman, S.D., Nixon, B., and Sutherland, J.M. (2020). Autophagy in female fertility: a role in oxidative stress and aging. Antioxid Redox Signal 32, 550–568.
Peters, M.J., Joehanes, R., Pilling, L.C., Schurmann, C., Conneely, K.N., Powell, J., Reinmaa, E., Sutphin, G.L., Zhernakova, A., Schramm, K., et al. (2015). The transcriptional landscape of age in human peripheral blood. Nat Commun 6, 8570.
Peters, R. (2006). Ageing and the brain. Postgrad Med J 82, 84–88.
Petersen, K.F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D. L., DiPietro, L., Cline, G.W., and Shulman, G.I. (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300, 1140–1142.
Petkovich, D.A., Podolskiy, D.I., Lobanov, A.V., Lee, S.G., Miller, R.A., and Gladyshev, V.N. (2017). Using DNA methylation profiling to evaluate biological age and longevity interventions. Cell Metab 25, 954–960.e6.
Petrany, M.J., Swoboda, C.O., Sun, C., Chetal, K., Chen, X., Weirauch, M. T., Salomonis, N., and Millay, D.P. (2020). Single-nucleus RNA-seq identifies transcriptional heterogeneity in multinucleated skeletal myofibers. Nat Commun 11, 6374.
Pfefferbaum, A., Mathalon, D.H., Sullivan, E.V., Rawles, J.M., Zipursky, R.B., and Lim, K.O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. Arch Neurol 51, 874–887.
Pham, T.X., Lee, J., Guan, J., Caporarello, N., Meridew, J.A., Jones, D.L., Tan, Q., Huang, S.K., Tschumperlin, D.J., and Ligresti, G. (2022). Transcriptional analysis of lung fibroblasts identifies PIM1 signaling as a driver of aging-associated persistent fibrosis. JCI Insight 7.
Phan, T.G., Grigorova, I., Okada, T., and Cyster, J.G. (2007). Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. Nat Immunol 8, 992–1000.
Pibiri, M. (2018). Liver regeneration in aged mice: new insights. Aging 10, 1801–1824.
Picca, A., Mankowski, R.T., Burman, J.L., Donisi, L., Kim, J.S., Marzetti, E., and Leeuwenburgh, C. (2018). Mitochondrial quality control mechanisms as molecular targets in cardiac ageing. Nat Rev Cardiol 15, 543–554.
Piciucchi, M., Capurso, G., Archibugi, L., Delle Fave, M.M., Capasso, M., and Delle Fave, G. (2015). Exocrine pancreatic insufficiency in diabetic patients: prevalence, mechanisms, and treatment. Int J Endocrinol 2015, 1–7.
Piehl, N., van Olst, L., Ramakrishnan, A., Teregulova, V., Simonton, B., Zhang, Z., Tapp, E., Channappa, D., Oh, H., Losada, P.M., et al. (2022). Cerebrospinal fluid immune dysregulation during healthy brain aging and cognitive impairment. Cell 185, 5028–5039.e13.
Piera-Velazquez, S., and Jimenez, S.A. (2019). Endothelial to mesenchymal transition: role in physiology and in the pathogenesis of human diseases. Physiol Rev 99, 1281–1324.
Pignolo, R.J., Law, S.F., and Chandra, A. (2021). Bone aging, cellular senescence, and osteoporosis. JBMR Plus 5, e10488.
Pikó, L., Hougham, A.J., and Bulpitt, K.J. (1988). Studies of sequence heterogeneity of mitochondrial DNA from rat and mouse tissues: evidence for an increased frequency of deletions/additions with aging. Mech Ageing Dev 43, 279–293.
Pino, V., Sanz, A., Valdés, N., Crosby, J., and Mackenna, A. (2020). The effects of aging on semen parameters and sperm DNA fragmentation. JBRA Assist Reprod 24, 82–86.
Pinti, M., Appay, V., Campisi, J., Frasca, D., Fülöp, T., Sauce, D., Larbi, A., Weinberger, B., and Cossarizza, A. (2016). Aging of the immune system: Focus on inflammation and vaccination. Eur J Immunol 46, 2286–2301.
Poels, M..M.F., Vernooij, M.W., Ikram, M.A., Hofman, A., Krestin, G.P., van der Lugt, A., and Breteler, M.M.B. (2010). Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. Stroke 41, S103–106.
Polanowski, A.M., Robbins, J., Chandler, D., and Jarman, S.N. (2014). Epigenetic estimation of age in humpback whales. Mol Ecol Resour 14, 976–987.
Ponti, F., Santoro, A., Mercatelli, D., Gasperini, C., Conte, M., Martucci, M., Sangiorgi, L., Franceschi, C., and Bazzocchi, A. (2019). Aging and imaging assessment of body composition: from fat to facts. Front Endocrinol 10, 861.
Popper, H. (1986). Aging and the liver. Prog Liver Dis 8, 659–683.
Porpiglia, E., Mai, T., Kraft, P., Holbrook, C.A., de Morree, A., Gonzalez, V.D., Hilgendorf, K.I., Frésard, L., Trejo, A., Bhimaraju, S., et al. (2022). Elevated CD47 is a hallmark of dysfunctional aged muscle stem cells that can be targeted to augment regeneration. Cell Stem Cell 29, 1653–1668.e8.
Porteri, C., Albanese, E., Scerri, C., Carrillo, M.C., Snyder, H.M., Martensson, B., Baker, M., Giacobini, E., Boccardi, M., Winblad, B., et al. (2017). The biomarker-based diagnosis of Alzheimer's disease. 1—ethical and societal issues. Neurobiol Aging 52, 132–140.
Porteri, C., and Frisoni, G.B. (2014). Biomarker-based diagnosis of mild cognitive impairment due to Alzheimer's disease: how and what to tell. A kickstart to an ethical discussion. Front Aging Neurosci 6, 41.
Potabattula, R., Zacchini, F., Ptak, G.E., Dittrich, M., Müller, T., El Hajj, N., Hahn, T., Drummer, C., Behr, R., Lucas-Hahn, A., et al. (2020). Increasing methylation of sperm rDNA and other repetitive elements in the aging male mammalian germline. Aging Cell 19, e13181.
Poulter, N.R., Prabhakaran, D., and Caulfield, M. (2015). Hypertension. Lancet 386, 801–812.
Pour, P.M., Sayed, S., and Sayed, G. (1982). Hyperplastic, preneoplastic and neoplastic lesions found in 83 human pancreases. Am J Clin Pathol 77, 137–152.
Practice, C.o.G. (2015). Committee opinion no. 618: Ovarian reserve testing. Obstet Gynecol 125, 268–273.
Pramodkumar, T.A., Jayashri, R., Gokulakrishnan, K., Velmurugan, K., Pradeepa, R., Anjana, R.M., and Mohan, V. (2016). Relationship of glycemic control markers—1,5 anhydroglucitol, fructosamine, and glycated hemoglobin among Asian Indians with different degrees of glucose intolerance. Ind J Endocr Metab 20, 690–695.
Prasanna, P.G., Citrin, D.E., Hildesheim, J., Ahmed, M.M., Venkatachalam, S., Riscuta, G., Xi, D., Zheng, G., Deursen, J., Goronzy, J., et al. (2021). Therapy-induced senescence: opportunities to improve anticancer therapy. JNCI J Natl Cancer Inst 113, 1285–1298.
Price, A.J., Manjegowda, M.C., Kain, J., Anandh, S., and Bochkis, I.M. (2020). Hdac3, Setdb1, and Kap1 mark H3K9me3/H3K14ac bivalent regions in young and aged liver. Aging Cell 19, e13092.
Price, F.D., von Maltzahn, J., Bentzinger, C.F., Dumont, N.A., Yin, H., Chang, N.C., Wilson, D.H., Frenette, J., and Rudnicki, M.A. (2014). Inhibition of JAK-STAT signaling stimulates adult satellite cell function. Nat Med 20, 1174–1181.
Pu, M., Wang, M., Wang, W., Velayudhan, S.S., and Lee, S.S. (2018). Unique patterns of trimethylation of histone H3 lysine 4 are prone to changes during aging in *Caenorhabditis elegans* somatic cells. PLoS Genet 14, e1007466.
Puleston, D.J., Buck, M.D., Klein Geltink, R.I., Kyle, R.L., Caputa, G., O'Sullivan, D., Cameron, A.M., Castoldi, A., Musa, Y., Kabat, A.M., et al. (2019). Polyamines and eIF5A hypusination modulate mitochondrial respiration and macrophage activation. Cell Metab 30, 352–363.e8.
Punjabi, M., Xu, L., Ochoa-Espinosa, A., Kosareva, A., Wolff, T., Murtaja,

A., Broisat, A., Devoogdt, N., and Kaufmann, B.A. (2019). Ultrasound molecular imaging of atherosclerosis with nanobodies. Arterioscler Thromb Vasc Biol 39, 2520–2530.
Puthumana, J., Thiessen-Philbrook, H., Xu, L., Coca, S.G., Garg, A.X., Himmelfarb, J., Bhatraju, P.K., Ikizler, T.A., Siew, E.D., Ware, L.B., et al. (2021). Biomarkers of inflammation and repair in kidney disease progression. J Clin Invest 131.
Putin, E., Asadulaev, A., Ivanenkov, Y., Aladinskiy, V., Sanchez-Lengeling, B., Aspuru-Guzik, A., and Zhavoronkov, A. (2018a). Reinforced adversarial neural computer for *de novo* molecular design. J Chem Inf Model 58, 1194–1204.
Putin, E., Asadulaev, A., Vanhaelen, Q., Ivanenkov, Y., Aladinskaya, A.V., Aliper, A., and Zhavoronkov, A. (2018b). Adversarial threshold neural computer for molecular *de novo* design. Mol Pharm 15, 4386–4397.
Putin, E., Mamoshina, P., Aliper, A., Korzinkin, M., Moskalev, A., Kolosov, A., Ostrovskiy, A., Cantor, C., Vijg, J., and Zhavoronkov, A. (2016). Deep biomarkers of human aging: application of deep neural networks to biomarker development. Aging 8, 1021–1033.
Puvvula, P.K., Desetty, R.D., Pineau, P., Marchio, A., Moon, A., Dejean, A., and Bischof, O. (2014). Long noncoding RNA PANDA and scaffold-attachment-factor SAFA control senescence entry and exit. Nat Commun 5, 5323.
Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., Skalska, A., Jonas, M., Franek, E., and Mossakowska, M. (2016). Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun Ageing 13, 21.
Pyrkov, T.V., Avchaciov, K., Tarkhov, A.E., Menshikov, L.I., Gudkov, A.V., and Fedichev, P.O. (2021). Longitudinal analysis of blood markers reveals progressive loss of resilience and predicts human lifespan limit. Nat Commun 12, 2765.
Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.Y., Olshen, R.A., Weyand, C.M., Boyd, S.D., and Goronzy, J.J. (2014). Diversity and clonal selection in the human T-cell repertoire. Proc Natl Acad Sci USA 111, 13139–13144.
Qiao, J., Wang, Z.B., Feng, H.L., Miao, Y.L., Wang, Q., Yu, Y., Wei, Y.C., Yan, J., Wang, W.H., Shen, W., et al. (2014). The root of reduced fertility in aged women and possible therapentic options: current status and future perspects. Mol Aspects Med 38, 54–85.
Qiao, X., Zhang, Y., Ye, A., Zhang, Y., Xie, T., Lv, Z., Shi, C., Wu, D., Chu, B., Wu, X., et al. (2022). ER reductive stress caused by Ero1α *S*-nitrosation accelerates senescence. Free Radic Biol Med 180, 165–178.
Qin, Z., Balimunkwe, R.M., and Quan, T. (2017). Age-related reduction of dermal fibroblast size upregulates multiple matrix metalloproteinases as observed in aged human skin *in vivo*. Br J Dermatol 177, 1337–1348.
Qiu, J., Papatsenko, D., Niu, X., Schaniel, C., and Moore, K. (2014). Divisional history and hematopoietic stem cell function during homeostasis. Stem Cell Rep 2, 473–490.
Quan, T., Wang, F., Shao, Y., Rittié, L., Xia, W., Orringer, J.S., Voorhees, J. J., and Fisher, G.J. (2013). Enhancing structural support of the dermal microenvironment activates fibroblasts, endothelial cells, and keratinocytes in aged human skin *in vivo*. J Invest Dermatol 133, 658–667.
Quarles, L.D. (2012). Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism. Nat Rev Endocrinol 8, 276–286.
Quirk, J.D., Sukstanskii, A.L., Woods, J.C., Lutey, B.A., Conradi, M.S., Gierada, D.S., Yusen, R.D., Castro, M., and Yablonskiy, D.A. (2016). Experimental evidence of age-related adaptive changes in human acinar airways. J Appl Physiol 120, 159–165.
Raaijmakers, M.H.G.P. (2019). Aging of the hematopoietic stem cell niche: an unnerving matter. Cell Stem Cell 25, 301–303.
Raggi, P., Gongora, M.C., Gopal, A., Callister, T.Q., Budoff, M., and Shaw, L.J. (2008). Coronary artery calcium to predict all-cause mortality in elderly men and women. J Am Coll Cardiol 52, 17–23.
Ragonnaud, E., and Biragyn, A. (2021). Gut microbiota as the key controllers of "healthy" aging of elderly people. Immun Ageing 18, 2.
Raguso, C.A., Kyle, U., Kossovsky, M.P., Roynette, C., Paoloni-Giacobino, A., Hans, D., Genton, L., and Pichard, C. (2006). A 3-year longitudinal study on body composition changes in the elderly: role of physical exercise. Clin Nutr 25, 573–580.
Rahier, J., Guiot, Y., Goebbels, R.M., Sempoux, C., and Henquin, J.C. (2008). Pancreatic β-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 10, 32–42.
Rahmati, M., Nalesso, G., Mobasheri, A., and Mozafari, M. (2017). Aging and osteoarthritis: central role of the extracellular matrix. Ageing Res Rev 40, 20–30.
Rai, N., Venugopalan, G., Pradhan, R., Ambastha, A., Upadhyay, A.D., Dwivedi, S., Dey, A.B., and Dey, S. (2018). Exploration of novel anti-oxidant protein sestrin in frailty syndrome in elderly. Aging Dis 9, 220–227.
Rai, R., Chen, Y., Lei, M., and Chang, S. (2016). TRF2-RAP1 is required to protect telomeres from engaging in homologous recombination-mediated deletions and fusions. Nat Commun 7, 10881.
Rai, R., Ghosh, A.K., Eren, M., Mackie, A.R., Levine, D.C., Kim, S.Y., Cedernaes, J., Ramirez, V., Procissi, D., Smith, L.H., et al. (2017). Downregulation of the apelinergic axis accelerates aging, whereas its systemic restoration improves the mammalian healthspan. Cell Rep 21, 1471–1480.
Rajan, E., Clain, J.E., Levy, M.J., Norton, I.D., Wang, K.K., Wiersema, M. J., Vazquez-Sequeiros, E., Nelson, B.J., Jondal, M.L., Kendall, R.K., et al. (2005). Age-related changes in the pancreas identified by EUS: a prospective evaluation. Gastrointest Endosc 61, 401–406.
Rakyan, V.K., Down, T.A., Maslau, S., Andrew, T., Yang, T.P., Beyan, H., Whittaker, P., McCann, O.T., Finer, S., Valdes, A.M., et al. (2010). Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20, 434–439.
Ramage, L.E., Akyol, M., Fletcher, A.M., Forsythe, J., Nixon, M., Carter, R.N., van Beek, E.J.R., Morton, N.M., Walker, B.R., and Stimson, R.H. (2016). Glucocorticoids acutely increase brown adipose tissue activity in humans, revealing species-specific differences in UCP-1 regulation. Cell Metab 24, 130–141.
Ramezani Tehrani, F., Mansournia, M.A., Solaymani-Dodaran, M., Minooee, S., and Azizi, F. (2017). Serum variations of anti-mullerian hormone and total testosterone with aging in healthy adult Iranian men: A population-based study. PLoS ONE 12, e0179634.
Ramirez, T., Li, Y.M., Yin, S., Xu, M.J., Feng, D., Zhou, Z., Zang, M., Mukhopadhyay, P., Varga, Z.V., Pacher, P., et al. (2017). Aging aggravates alcoholic liver injury and fibrosis in mice by downregulating sirtuin 1 expression. J Hepatol 66, 601–609.
Randles, M.J., Lausecker, F., Kong, Q., Suleiman, H., Reid, G., Kolatsi-Joannou, M., Davenport, B., Tian, P., Falcone, S., Potter, P., et al. (2021). Identification of an altered matrix signature in kidney aging and disease. J Am Soc Nephrol 32, 1713–1732.
Rando, T.A., and Jones, D.L. (2021). Regeneration, rejuvenation, and replacement: turning back the clock on tissue aging. Cold Spring Harb Perspect Biol 13, a040907.
Rappold, P.M., Cui, M., Grima, J.C., Fan, R.Z., de Mesy-Bentley, K.L., Chen, L., Zhuang, X., Bowers, W.J., and Tieu, K. (2014). Drp1 inhibition attenuates neurotoxicity and dopamine release deficits *in vivo*. Nat Commun 5, 5244.
Razavi, P., Li, B.T., Brown, D.N., Jung, B., Hubbell, E., Shen, R., Abida, W., Juluru, K., De Bruijn, I., Hou, C., et al. (2019). High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med 25, 1928–1937.
Rea, I.M. (2017). Towards ageing well: use it or lose it: exercise, epigenetics and cognition. Biogerontology 18, 679–691.
Rechsteiner, C., Morandini, F., Perez, K., Praz, V., López-García, G., Hinte, L., von Meyenn, F., and Ocampo, A. (2022). Development of a novel aging clock based on chromatin accessibility. bioRxiv 2022.2008.2011.502778.
Redwood, A.B., Perkins, S.M., Vanderwaal, R.P., Feng, Z., Biehl, K.J., Gonzalez-Suarez, I., Morgado-Palacin, L., Shi, W., Sage, J., Roti-Roti, J.L., et al. (2011). A dual role for A-type lamins in DNA double-strand break repair. Cell Cycle 10, 2549–2560.
Reeder, S.B., Cruite, I., Hamilton, G., and Sirlin, C.B. (2011). Quantitative assessment of liver fat with magnetic resonance imaging and

spectroscopy. J Magn Reson Imag 34, 729–749.
Reers, C., Erbel, S., Esposito, I., Schmied, B., Büchler, M.W., Nawroth, P. P., and Ritzel, R.A. (2009). Impaired islet turnover in human donor pancreata with aging. Eur J Endocrinol 160, 185–191.
Regadera, J., Nistal, M., and Paniagua, R. (1985). Testis, epididymis, and spermatic cord in elderly men. Correlation of angiographic and histologic studies with systemic arteriosclerosis. Arch Pathol Lab Med 109, 663–667.
Reick, M., Garcia, J.A., Dudley, C., and McKnight, S.L. (2001). NPAS2: An analog of clock operative in the mammalian forebrain. Science 293, 506–509.
Reiter, R.J., Richardson, B.A., Johnson, L.Y., Ferguson, B.N., and Dinh, D. T. (1980). Pineal melatonin rhythm: reduction in aging Syrian hamsters. Science 210, 1372–1373.
Reiter, R.J., Tan, D., Kim, S.J., Manchester, L.C., Qi, W., Garcia, J.J., Cabrera, J.C., El-Sokkary, G., and Rouvier-Garay, V. (1999). Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. Mech Ageing Dev 110, 157–173.
Ren, R., Deng, L., Xue, Y., Suzuki, K., Zhang, W., Yu, Y., Wu, J., Sun, L., Gong, X., Luan, H., et al. (2017). Visualization of aging-associated chromatin alterations with an engineered TALE system. Cell Res 27, 483–504.
Ren, X., Hu, B., Song, M., Ding, Z., Dang, Y., Liu, Z., Zhang, W., Ji, Q., Ren, R., Ding, J., et al. (2019). Maintenance of nucleolar homeostasis by CBX4 alleviates senescence and osteoarthritis. Cell Rep 26, 3643–3656.e7.
Resnick, S.M., Goldszal, A.F., Davatzikos, C., Golski, S., Kraut, M.A., Metter, E.J., Bryan, R.N., and Zonderman, A.B. (2000). One-year age changes in MRI brain volumes in older adults. Cereb Cortex 10, 464–472.
Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., and Davatzikos, C. (2003). Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. J Neurosci 23, 3295–3301.
Revelli, A., Piane, L.D., Casano, S., Molinari, E., Massobrio, M., and Rinaudo, P. (2009). Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol 7, 40.
Reyes, J., Chen, J.Y., Stewart-Ornstein, J., Karhohs, K.W., Mock, C.S., and Lahav, G. (2018). Fluctuations in p53 signaling allow escape from cell-cycle arrest. Mol Cell 71, 581–591.e5.
Ribeiro, J.D., Morey, L., Mas, A., Gutierrez, A., Luis, N.M., Mejetta, S., Richly, H., Benitah, S.A., Keyes, W.M., and Di Croce, L. (2013). ZRF1 controls oncogene-induced senescence through the INK4-ARF locus. Oncogene 32, 2161–2168.
Richards, B.A., Lillicrap, T.P., Beaudoin, P., Bengio, Y., Bogacz, R., Christensen, A., Clopath, C., Costa, R.P., de Berker, A., Ganguli, S., et al. (2019). A deep learning framework for neuroscience. Nat Neurosci 22, 1761–1770.
Riddle, D.R., Sonntag, W.E., and Lichtenwalner, R.J. (2003). Microvascular plasticity in aging. Ageing Res Rev 2, 149–168.
Rinnerthaler, M., Duschl, J., Steinbacher, P., Salzmann, M., Bischof, J., Schuller, M., Wimmer, H., Peer, T., Bauer, J.W., and Richter, K. (2013). Age-related changes in the composition of the cornified envelope in human skin. Exp Dermatol 22, 329–335.
Risques, R.A., and Kennedy, S.R. (2018). Aging and the rise of somatic cancer-associated mutations in normal tissues. PLoS Genet 14, e1007108.
Rist, M.J., Roth, A., Frommherz, L., Weinert, C.H., Kruger, R., Merz, B., Bunzel, D., Mack, C., Egert, B., Bub, A., et al. (2017). Metabolite patterns predicting sex and age in participants of the Karlsruhe Metabolomics and Nutrition (KarMeN) study. PloS ONE 12, e0183228.
Rivero-Segura, N.A., Bello-Chavolla, O.Y., Barrera-Vázquez, O.S., Gutierrez-Robledo, L.M., and Gomez-Verjan, J.C. (2020). Promising biomarkers of human aging: In search of a multi-omics panel to understand the aging process from a multidimensional perspective. Ageing Res Rev 64, 101164.
Rizza, S., Cardaci, S., Montagna, C., Di Giacomo, G., De Zio, D., Bordi, M., Maiani, E., Campello, S., Borreca, A., Puca, A.A., et al. (2018). *S*-nitrosylation drives cell senescence and aging in mammals by controlling mitochondrial dynamics and mitophagy. Proc Natl Acad Sci USA 115, E3388–E3397.
Roberts, J.S., Dunn, L.B., and Rabinovici, G.D. (2013). Amyloid imaging, risk disclosure and Alzheimer's disease: ethical and practical issues. Neurodegener Dis Manag 3, 219–229.
Robin, J.D., Jacome Burbano, M.S., Peng, H., Croce, O., Thomas, J.L., Laberthonniere, C., Renault, V., Lototska, L., Pousse, M., Tessier, F., et al. (2020). Mitochondrial function in skeletal myofibers is controlled by a TRF2-SIRT3 axis over lifetime. Aging Cell 19, e13097.
Robinson, O., Chadeau Hyam, M., Karaman, I., Climaco Pinto, R., Ala-Korpela, M., Handakas, E., Fiorito, G., Gao, H., Heard, A., Jarvelin, M. R., et al. (2020). Determinants of accelerated metabolomic and epigenetic aging in a UK cohort. Aging Cell 19, e13149.
Rodier, F., and Campisi, J. (2011). Four faces of cellular senescence. J Cell Biol 192, 547–556.
Rodier, F., Coppé, J.P., Patil, C.K., Hoeijmakers, W.A.M., Muñoz, D.P., Raza, S.R., Freund, A., Campeau, E., Davalos, A.R., and Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 11, 973–979.
Rodier, F., Muñoz, D.P., Teachenor, R., Chu, V., Le, O., Bhaumik, D., Coppé, J.P., Campeau, E., Beauséjour, C.M., Kim, S.H., et al. (2011). DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. J Cell Sci 124, 68–81.
Rodríguez, J.J., Yeh, C.Y., Terzieva, S., Olabarria, M., Kulijewicz-Nawrot, M., and Verkhratsky, A. (2014). Complex and region-specific changes in astroglial markers in the aging brain. Neurobiol Aging 35, 15–23.
Rodriguez, K.A., Valentine, J.M., Kramer, D.A., Gelfond, J.A., Kristan, D. M., Nevo, E., and Buffenstein, R. (2016). Determinants of rodent longevity in the chaperone-protein degradation network. Cell Stress Chaperones 21, 453–466.
Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., and Merrow, M. (2007). Epidemiology of the human circadian clock. Sleep Med Rev 11, 429–438.
Roger, L., Tomas, F., and Gire, V. (2021). Mechanisms and regulation of cellular senescence. Int J Mol Sci 22, 13173.
Roman, M.A., Rossiter, H.B., and Casaburi, R. (2016). Exercise, ageing and the lung. Eur Respir J 48, 1471–1486.
Romero, J.R., Preis, S.R., Beiser, A., DeCarli, C., Viswanathan, A., Martinez-Ramirez, S., Kase, C.S., Wolf, P.A., and Seshadri, S. (2014). Risk factors, stroke prevention treatments, and prevalence of cerebral microbleeds in the Framingham Heart Study. Stroke 45, 1492–1494.
Rong, Y.D., Bian, A.L., Hu, H.Y., Ma, Y., and Zhou, X.Z. (2018). Study on relationship between elderly sarcopenia and inflammatory cytokine IL-6, anti-inflammatory cytokine IL-10. BMC Geriatr 18, 308.
Roozendaal, B., van Gool, W.A., Swaab, D.F., Hoogendijk, J.E., and Mirmiran, M. (1987). Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. Brain Res 409, 259–264.
Roseman, D.A., Hwang, S.J., Oyama-Manabe, N., Chuang, M.L., O'Donnell, C.J., Manning, W.J., and Fox, C.S. (2017). Clinical associations of total kidney volume: the Framingham Heart Study. Nephrol Dial Transplant 32, 1344–1350.
Rosenberg, I.H. (1997). Sarcopenia: origins and clinical relevance. J Nutr 127, S990–S991.
Rosiak-Gill, A., Gill, K., Jakubik, J., Fraczek, M., Patorski, L., Gaczarzewicz, D., Kurzawa, R., Kurpisz, M., and Piasecka, M. (2019). Age-related changes in human sperm DNA integrity. Aging 11, 5399–5411.
Ross, J.M., Stewart, J.B., Hagström, E., Brené, S., Mourier, A., Coppotelli, G., Freyer, C., Lagouge, M., Hoffer, B.J., Olson, L., et al. (2013). Germline mitochondrial DNA mutations aggravate ageing and can impair brain development. Nature 501, 412–415.
Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L. (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447, 725–729.
Rossi, F.E., Lira, F.S., Silva, B.S.A., Freire, A.P.C.F., Ramos, E.M.C., and Gobbo, L.A. (2019). Influence of skeletal muscle mass and fat mass on

the metabolic and inflammatory profile in sarcopenic and non-sarcopenic overfat elderly. Aging Clin Exp Res 31, 629–635.
Rossi, L., Nicoletti, M.C., Carmosino, M., Mastrofrancesco, L., Di Franco, A., Indrio, F., Lella, R., Laviola, L., Giorgino, F., Svelto, M., et al. (2017). Urinary excretion of kidney aquaporins as possible diagnostic biomarker of diabetic nephropathy. J Diabetes Res 2017, 1–13.
Rostamzadeh, A., Schwegler, C., Gil-Navarro, S., Rosende-Roca, M., Romotzky, V., Ortega, G., Canabate, P., Moreno, M., Schmitz-Luhn, B., Boada, M., et al. (2021). Biomarker-based risk prediction of Alzheimer's disease dementia in mild cognitive impairment: psychosocial, ethical, and legal aspects. J Alzheimer Dis 80, 601–617.
Rotem, A., Ram, O., Shoresh, N., Sperling, R.A., Goren, A., Weitz, D.A., and Bernstein, B.E. (2015). Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state. Nat Biotechnol 33, 1165–1172.
Rothe, J., Gehr, G., Loetscher, H., and Lesslauer, W. (1992). Tumor necrosis factor receptors—structure and function. Immunol Res 11, 81–90.
Rottenberg, H., and Wu, S. (1997). Mitochondrial dysfunction in lymphocytes from old mice: enhanced activation of the permeability transition. Biochem Biophys Res Commun 240, 68–74.
Rovillain, E., Mansfield, L., Caetano, C., Alvarez-Fernandez, M., Caballero, O.L., Medema, R.H., Hummerich, H., and Jat, P.S. (2011). Activation of nuclear factor-kappa B signalling promotes cellular senescence. Oncogene 30, 2356–2366.
Rowe, J.W., Shock, N.W., and DeFronzo, R.A. (1976). The influence of age on the renal response to water deprivation in man. Nephron 17, 270–278.
Roy, A.L., Sierra, F., Howcroft, K., Singer, D.S., Sharpless, N., Hodes, R.J., Wilder, E.L., and Anderson, J.M. (2020). A blueprint for characterizing senescence. Cell 183, 1143–1146.
Rozo, M., Li, L., and Fan, C.M. (2016). Targeting β1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice. Nat Med 22, 889–896.
Rube, C.E., Fricke, A., Widmann, T.A., Furst, T., Madry, H., Pfreundschuh, M., and Rube, C. (2011). Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. PLoS ONE 6, e17487.
Rubenstein, A.B., Smith, G.R., Raue, U., Begue, G., Minchev, K., Ruf-Zamojski, F., Nair, V.D., Wang, X., Zhou, L., Zaslavsky, E., et al. (2020). Single-cell transcriptional profiles in human skeletal muscle. Sci Rep 10, 229.
Rufini, A., Tucci, P., Celardo, I., and Melino, G. (2013). Senescence and aging: the critical roles of p53. Oncogene 32, 5129–5143.
Rule, A.D., Amer, H., Cornell, L.D., Taler, S.J., Cosio, F.G., Kremers, W. K., Textor, S.C., and Stegall, M.D. (2010). The association between age and nephrosclerosis on renal biopsy among healthy adults. Ann Intern Med 152, 561–567.
Russoniello, C.V., Zhirnov, Y.N., Pougatchev, V.I., and Gribkov, E.N. (2013). Heart rate variability and biological age: implications for health and gaming. Cyberpsychol Behav Soc Netw 16, 302–308.
Ruth, K.S., Day, F.R., Hussain, J., Martínez-Marchal, A., Aiken, C.E., Azad, A., Thompson, D.J., Knoblochova, L., Abe, H., Tarry-Adkins, J. L., et al. (2021). Genetic insights into biological mechanisms governing human ovarian ageing. Nature 596, 393–397.
Rutledge, J., Oh, H., and Wyss-Coray, T. (2022). Measuring biological age using omics data. Nat Rev Genet 23, 715–727.
Ryan, A.S., and Elahi, D. (1998). Loss of bone mineral density in women athletes during aging. Calcif Tissue Int 63, 287–292.
Ryan, N.A., Zwetsloot, K.A., Westerkamp, L.M., Hickner, R.C., Pofahl, W. E., and Gavin, T.P. (2006). Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. J Appl Physiol 100, 178–185.
Sabari, B.R., Dall'Agnese, A., and Young, R.A. (2020). Biomolecular condensates in the nucleus. Trends Biochem Sci 45, 961–977.
Sabio, G., and Davis, R.J. (2014). TNF and MAP kinase signalling pathways. Semin Immunol 26, 237–245.
Sacco, A.J., Granatosky, M.C., Laird, M.F., and Milich, K.M. (2021). Validation of a method for quantifying urinary C-peptide in platyrrhine monkeys. Gen Comp Endocrinol 300, 113644.
Saeed, A., Nambi, V., Sun, W., Virani, S.S., Taffet, G.E., Deswal, A., Selvin, E., Matsushita, K., Wagenknecht, L.E., Hoogeveen, R., et al. (2018). Short-term global cardiovascular disease risk prediction in older adults. J Am Coll Cardiol 71, 2527–2536.
Sage, A.P., Tsiantoulas, D., Binder, C.J., and Mallat, Z. (2019). The role of B cells in atherosclerosis. Nat Rev Cardiol 16, 180–196.
Sahin, E., Colla, S., Liesa, M., Moslehi, J., Müller, F.L., Guo, M., Cooper, M., Kotton, D., Fabian, A.J., Walkey, C., et al. (2011). Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 470, 359–365.
Saisho, Y., Butler, A.E., Manesso, E., Elashoff, D., Rizza, R.A., and Butler, P.C. (2013). β-cell mass and turnover in humans: effects of obesity and aging. Diabetes Care 36, 111–117.
Sakalihasan, N., Limet, R., and Defawe, O.D. (2005). Abdominal aortic aneurysm. Lancet 365, 1577–1589.
Sakamoto, J., Miura, T., Shimamoto, K., and Horio, Y. (2004). Predominant expression of Sir2α, an NAD-dependent histone deacetylase, in the embryonic mouse heart and brain. FEBS Lett 556, 281–286.
Sakers, A., De Siqueira, M.K., Seale, P., and Villanueva, C.J. (2022). Adipose-tissue plasticity in health and disease. Cell 185, 419–446.
Sala, A.J., and Morimoto, R.I. (2022). Protecting the future: balancing proteostasis for reproduction. Trends Cell Biol 32, 202–215.
Salahudeen, A.A., Choi, S.S., Rustagi, A., Zhu, J., van Unen, V., de la O, S. M., Flynn, R.A., Margalef-Català, M., Santos, A.J.M., Ju, J., et al. (2020). Progenitor identification and SARS-CoV-2 infection in human distal lung organoids. Nature 588, 670–675.
Salama, R., Sadaie, M., Hoare, M., and Narita, M. (2014). Cellular senescence and its effector programs. Genes Dev 28, 99–114.
Salameh, Y., Bejaoui, Y., and El Hajj, N. (2020). DNA methylation biomarkers in aging and age-related diseases. Front Genet 11, 171.
Salat, D.H., Tuch, D.S., Greve, D.N., van der Kouwe, A.J.W., Hevelone, N. D., Zaleta, A.K., Rosen, B.R., Fischl, B., Corkin, S., Rosas, H.D., et al. (2005). Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiol Aging 26, 1215–1227.
Salminen, A. (2020). Activation of immunosuppressive network in the aging process. Ageing Res Rev 57, 100998.
Salminen, A. (2022). Role of indoleamine 2,3-dioxygenase 1 (IDO1) and kynurenine pathway in the regulation of the aging process. Ageing Res Rev 75, 101573.
Salminen, A., Kaarniranta, K., and Kauppinen, A. (2012a). Inflammaging: disturbed interplay between autophagy and inflammasomes. Aging 4, 166–175.
Salminen, A., Kaarniranta, K., and Kauppinen, A. (2016). Age-related changes in AMPK activation: role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. Ageing Res Rev 28, 15–26.
Salminen, A., Kaarniranta, K., and Kauppinen, A. (2022). Photoaging: UV radiation-induced inflammation and immunosuppression accelerate the aging process in the skin. Inflamm Res 71, 817–831.
Salminen, A., Kauppinen, A., and Kaarniranta, K. (2012b). Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal 24, 835–845.
Salonia, A., Rastrelli, G., Hackett, G., Seminara, S.B., Huhtaniemi, I.T., Rey, R.A., Hellstrom, W.J.G., Palmert, M.R., Corona, G., Dohle, G.R., et al. (2019). Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers 5, 38.
Sanchez-Vazquez, R., Guío-Carrión, A., Zapatero-Gaviria, A., Martínez, P., and Blasco, M.A. (2021). Shorter telomere lengths in patients with severe COVID-19 disease. Aging 13, 1–15.
Sanchez, J.S., Becker, J.A., Jacobs, H.I.L., Hanseeuw, B.J., Jiang, S., Schultz, A.P., Properzi, M.J., Katz, S.R., Beiser, A., Satizabal, C.L., et al. (2021). The cortical origin and initial spread of medial temporal tauopathy in Alzheimer's disease assessed with positron emission tomography. Sci Transl Med 13.
Sanders, J.L., Putman, R.K., Dupuis, J., Xu, H., Murabito, J.M., Araki, T., Nishino, M., Benjamin, E.J., Levy, D., Ramachandran, V.S., et al. (2021). The association of aging biomarkers, interstitial lung

abnormalities, and mortality. Am J Respir Crit Care Med 203, 1149–1157.
Sandovici, I., Smith, N.H., Nitert, M.D., Ackers-Johnson, M., Uribe-Lewis, S., Ito, Y., Jones, R.H., Marquez, V.E., Cairns, W., Tadayyon, M., et al. (2011). Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the *Hnf4a* gene in rat pancreatic islets. Proc Natl Acad Sci USA 108, 5449–5454.
Sangiorgi, G., Rumberger, J.A., Severson, A., Edwards, W.D., Gregoire, J., Fitzpatrick, L.A., and Schwartz, R.S. (1998). Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol 31, 126–133.
Sano, S., Horitani, K., Ogawa, H., Halvardson, J., Chavkin, N.W., Wang, Y., Sano, M., Mattisson, J., Hata, A., Danielsson, M., et al. (2022). Hematopoietic loss of Y chromosome leads to cardiac fibrosis and heart failure mortality. Science 377, 292–297.
Santiago, J., Silva, J.V., Alves, M.G., Oliveira, P.F., and Fardilha, M. (2019). Testicular aging: an overview of ultrastructural, cellular, and molecular alterations. J Gerontol A Biol Sci Med Sci 74, 860–871.
Santos, A.L., and Lindner, A.B. (2017). Protein posttranslational modifications: roles in aging and age-related disease. Oxid Med Cell Longev 2017, 1–19.
Sasano, N., and Ichijo, S. (1969). Vascular patterns of the human testis with special reference to its senile changes. Tohoku J Exp Med 99, 269–280.
Sasazaki, Y., Seedhom, B.B., and Shore, R. (2008). Morphology of the bovine chondrocyte and of its cytoskeleton in isolation and in situ: are chondrocytes ubiquitously paired through the entire layer of articular cartilage? Rheumatology 47, 1641–1646.
Sastre, J., Pallardó, F.V., Plá, R., Pellín, A., Juan, G., O'Connor, J.E., Estrela, J.M., Miquel, J., and Viña, J. (1996). Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology 24, 1199–1205.
Sati, S., Bonev, B., Szabo, Q., Jost, D., Bensadoun, P., Serra, F., Loubiere, V., Papadopoulos, G.L., Rivera-Mulia, J.C., Fritsch, L., et al. (2020). 4D genome rewiring during oncogene-induced and replicative senescence. Mol Cell 78, 522–538.e9.
Sato, I., Morita, I., Kaji, K., Ikeda, M., Nagao, M., and Murota, S. (1993). Reduction of nitric oxide producing activity associated with *in vitro* aging in cultured human umbilical vein endothelial cell. Biochem Biophys Res Commun 195, 1070–1076.
Sato, S., Solanas, G., Peixoto, F.O., Bee, L., Symeonidi, A., Schmidt, M.S., Brenner, C., Masri, S., Benitah, S.A., and Sassone-Corsi, P. (2017). Circadian reprogramming in the liver identifies metabolic pathways of aging. Cell 170, 664–677.e11.
Sato, T., Ito, K., Tamada, T., Sone, T., Noda, Y., Higaki, A., Kanki, A., Tanimoto, D., and Higashi, H. (2012). Age-related changes in normal adult pancreas: MR imaging evaluation. Eur J Radiol 81, 2093–2098.
Sato, Y., Atarashi, K., Plichta, D.R., Arai, Y., Sasajima, S., Kearney, S.M., Suda, W., Takeshita, K., Sasaki, T., Okamoto, S., et al. (2021). Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. Nature 599, 458–464.
Sato, Y., and Yanagita, M. (2019). Immunology of the ageing kidney. Nat Rev Nephrol 15, 625–640.
Satyanarayana, A., and Kaldis, P. (2009). Mammalian cell-cycle regulation: several Cdks, numerous cyclins and diverse compensatory mechanisms. Oncogene 28, 2925–2939.
Saunders, J.T., Nambi, V., de Lemos, J.A., Chambless, L.E., Virani, S.S., Boerwinkle, E., Hoogeveen, R.C., Liu, X., Astor, B.C., Mosley, T.H., et al. (2011). Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation 123, 1367–1376.
Sayed, N., Huang, Y., Nguyen, K., Krejciova-Rajaniemi, Z., Grawe, A.P., Gao, T., Tibshirani, R., Hastie, T., Alpert, A., Cui, L., et al. (2021). An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging. Nat Aging 1, 598–615.
Sayer, A.A., and Cruz-Jentoft, A. (2022). Sarcopenia definition, diagnosis and treatment: consensus is growing. Age Ageing 51.
Scaffidi, P., and Misteli, T. (2006). Lamin A-dependent nuclear defects in human aging. Science 312, 1059–1063.
Schaap, L.A., Pluijm, S.M.F., Deeg, D.J.H., and Visser, M. (2006). Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 119, 526.e9–526.e17.
Schaum, N., Lehallier, B., Hahn, O., Pálovics, R., Hosseinzadeh, S., Lee, S. E., Sit, R., Lee, D.P., Losada, P.M., Zardeneta, M.E., et al. (2020). Ageing hallmarks exhibit organ-specific temporal signatures. Nature 583, 596–602.
Scheffer, G.J., Broekmans, F.J.M., Dorland, M., Habbema, J.D.F., Looman, C.W.N., and te Velde, E.R. (1999). Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. Fertil Steril 72, 845–851.
Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C.E., Cummings, J., and van der Flier, W.M. (2021). Alzheimer's disease. Lancet 397, 1577–1590.
Scherer, M., and Stamminger, T. (2016). Emerging role of PML nuclear bodies in innate immune signaling. J Virol 90, 5850–5854.
Schipper, B.M., Marra, K.G., Zhang, W., Donnenberg, A.D., and Rubin, J. P. (2008). Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg 60, 538–544.
Schmidt, I.M., Hall, I.E., Kale, S., Lee, S., He, C.H., Lee, Y., Chupp, G.L., Moeckel, G.W., Lee, C.G., Elias, J.A., et al. (2013). Chitinase-like protein Brp-39/YKL-40 modulates the renal response to ischemic injury and predicts delayed allograft function. J Am Soc Nephrol 24, 309–319.
Schmitt, R., and Melk, A. (2017). Molecular mechanisms of renal aging. Kidney Int 92, 569–579.
Schmucker, D.L., Woodhouse, K.W., Wang, R.K., Wynne, H., James, O.F., McManus, M., and Kremers, P. (1990). Effects of age and gender on *in vitro* properties of human liver microsomal monooxygenases. Clin Pharmacol Ther 48, 365–374.
Schneider, J.L., Rowe, J.H., Garcia-de-Alba, C., Kim, C.F., Sharpe, A.H., and Haigis, M.C. (2021). The aging lung: physiology, disease, and immunity. Cell 184, 1990–2019.
Schneider, J.L., Villarroya, J., Diaz-Carretero, A., Patel, B., Urbanska, A. M., Thi, M.M., Villarroya, F., Santambrogio, L., and Cuervo, A.M. (2015). Loss of hepatic chaperone-mediated autophagy accelerates proteostasis failure in aging. Aging Cell 14, 249–264.
Schöder, H., França, P.D.D.S., Nakajima, R., Burnazi, E., Roberts, S., Brand, C., Grkovski, M., Mauguen, A., Dunphy, M.P., Ghossein, R.A., et al. (2020). Safety and feasibility of PARP1/2 imaging with 18F-PARPi in patients with head and neck cancer. Clin Cancer Res 26, 3110–3116.
Schrauben, S.J., Shou, H., Zhang, X., Anderson, A.H., Bonventre, J.V., Chen, J., Coca, S., Furth, S.L., Greenberg, J.H., Gutierrez, O.M., et al. (2021). Association of multiple plasma biomarker concentrations with progression of prevalent diabetic kidney disease: findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. J Am Soc Nephrol 32, 115–126.
Schüler, S.C., Kirkpatrick, J.M., Schmidt, M., Santinha, D., Koch, P., Di Sanzo, S., Cirri, E., Hemberg, M., Ori, A., and von Maltzahn, J. (2021). Extensive remodeling of the extracellular matrix during aging contributes to age-dependent impairments of muscle stem cell functionality. Cell Rep 35, 109223.
Schuliga, M., and Bartlett, N. (2019). Modeling the impact of low-dose particulate matter on lung health. Am J Physiol Lung Cell Mol Physiol 317, L550–L553.
Schuliga, M., Read, J., and Knight, D.A. (2021). Ageing mechanisms that contribute to tissue remodeling in lung disease. Ageing Res Rev 70, 101405.
Schultz, M.B., Kane, A.E., Mitchell, S.J., MacArthur, M.R., Warner, E., Vogel, D.S., Mitchell, J.R., Howlett, S.E., Bonkowski, M.S., and Sinclair, D.A. (2020). Age and life expectancy clocks based on machine learning analysis of mouse frailty. Nat Commun 11, 4618.
Schupp, J.C., Adams, T.S., Cosme, C. Jr., Raredon, M.S.B., Yuan, Y., Omote, N., Poli, S., Chioccioli, M., Rose, K.A., Manning, E.P., et al. (2021). Integrated single-cell atlas of endothelial cells of the human

lung. Circulation 144, 286–302.
Schwalie, P.C., Dong, H., Zachara, M., Russeil, J., Alpern, D., Akchiche, N., Caprara, C., Sun, W., Schlaudraff, K.U., Soldati, G., et al. (2018). A stromal cell population that inhibits adipogenesis in mammalian fat depots. Nature 559, 103–108.
Schwarz, A.J., Yu, P., Miller, B.B., Shcherbinin, S., Dickson, J., Navitsky, M., Joshi, A.D., Devous Sr, M.D., and Mintun, M.S. (2016). Regional profiles of the candidate tau PET ligand ^{18}F-AV-1451 recapitulate key features of Braak histopathological stages. Brain 139, 1539–1550.
Screaton, G., and Xu, X.N. (2000). T cell life and death signalling via TNF-receptor family members. Curr Opin Immunol 12, 316–322.
Sebastiani, P., Federico, A., Morris, M., Gurinovich, A., Tanaka, T., Chandler, K.B., Andersen, S.L., Denis, G., Costello, C.E., Ferrucci, L., et al. (2021). Protein signatures of centenarians and their offspring suggest centenarians age slower than other humans. Aging Cell 20, e13290.
Sebastiani, P., Gurinovich, A., Nygaard, M., Sasaki, T., Sweigart, B., Bae, H., Andersen, S.L., Villa, F., Atzmon, G., Christensen, K., et al. (2019). *APOE* alleles and extreme human longevity. J Gerontol A Biol Sci Med Sci 74, 44–51.
Sebo, Z.L., Rendina-Ruedy, E., Ables, G.P., Lindskog, D.M., Rodeheffer, M.S., Fazeli, P.K., and Horowitz, M.C. (2019). Bone marrow adiposity: basic and clinical implications. Endocrine Rev 40, 1187–1206.
Secomandi, L., Borghesan, M., Velarde, M., and Demaria, M. (2022). The role of cellular senescence in female reproductive aging and the potential for senotherapeutic interventions. Hum Reprod Update 28, 172–189.
Sedelnikova, O.A., Horikawa, I., Redon, C., Nakamura, A., Zimonjic, D.B., Popescu, N.C., and Bonner, W.M. (2008). Delayed kinetics of DNA double-strand break processing in normal and pathological aging. Aging Cell 7, 89–100.
Sedelnikova, O.A., Horikawa, I., Zimonjic, D.B., Popescu, N.C., Bonner, W.M., and Barrett, J.C. (2004). Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat Cell Biol 6, 168–170.
Segler, M.H.S., Preuss, M., and Waller, M.P. (2018). Planning chemical syntheses with deep neural networks and symbolic AI. Nature 555, 604–610.
Segurado-Miravalles, G., Jiménez-Gómez, N., Moreno-Arrones, O., Alarcón-Salazar, I., Alegre-Sánchez, A., Saceda-Corralo, D., Jaén-Olasolo, P., and González-Rodríguez, S. (2018). Assessment of the effect of 3% diclofenac sodium on photodamaged skin by means of reflectance confocal microscopy. Acta Derm Venerol 98, 963–969.
Seibyl, J.P. (2022). α-Synuclein PET and parkinson disease therapeutic trials: ever the twain shall meet? J Nucl Med 63, 1463–1466.
Sellami, M., Bragazzi, N., Prince, M.S., Denham, J., and Elrayess, M. (2021). Regular, intense exercise training as a healthy aging lifestyle strategy: preventing DNA damage, telomere shortening and adverse DNA methylation changes over a lifetime. Front Genet 12, 652497.
Sellayah, D., and Sikder, D. (2014). Orexin restores aging-related brown adipose tissue dysfunction in male mice. Endocrinology 155, 485–501.
Sellix, M.T., Evans, J.A., Leise, T.L., Castanon-Cervantes, O., Hill, D.J.D., DeLisser, P., Block, G.D., Menaker, M., and Davidson, A.J. (2012). Aging differentially affects the re-entrainment response of central and peripheral circadian oscillators. J Neurosci 32, 16193–16202.
Selvaratnam, J., Paul, C., and Robaire, B. (2015). Male rat germ cells display age-dependent and cell-specific susceptibility in response to oxidative stress challenges. Biol Reprod 93, 72.
Sendama, W. (2020). The effect of ageing on the resolution of inflammation. Ageing Res Rev 57, 101000.
Sene, A., Khan, A.A., Cox, D., Nakamura, R.E.I., Santeford, A., Kim, B. M., Sidhu, R., Onken, M.D., Harbour, J.W., Hagbi-Levi, S., et al. (2013). Impaired cholesterol efflux in senescent macrophages promotes age-related macular degeneration. Cell Metab 17, 549–561.
Sengupta, U., and Kayed, R. (2022). Amyloid β, Tau, and α-synuclein aggregates in the pathogenesis, prognosis, and therapeutics for neurodegenerative diseases. Prog Neurobiol 214, 102270.
Seo, Y., Ishizu, T., Ieda, M., and Ohte, N. (2020). Elderly Japanese standard data of echocardiography; from J-LONG study. J Echocardiogr 18, 175–182.
Shahnazari, M., Dwyer, D., Chu, V., Asuncion, F., Stolina, M., Ominsky, M., Kostenuik, P., and Halloran, B. (2012). Bone turnover markers in peripheral blood and marrow plasma reflect trabecular bone loss but not endocortical expansion in aging mice. Bone 50, 628–637.
Shan, H., Geng, L., Jiang, X., Song, M., Wang, J., Liu, Z., Zhuo, X., Wu, Z., Hu, J., Ji, Z., et al. (2022). Large-scale chemical screen identifies Gallic acid as a geroprotector for human stem cells. Protein Cell 13, 532–539.
Shareef, S.J., Bevill, S.M., Raman, A.T., Aryee, M.J., van Galen, P., Hovestadt, V., and Bernstein, B.E. (2021). Extended-representation bisulfite sequencing of gene regulatory elements in multiplexed samples and single cells. Nat Biotechnol 39, 1086–1094.
Sharma, G., and Goodwin, J. (2006). Effect of aging on respiratory system physiology and immunology. Clin Interv Aging 1, 253–260.
Sharma, R., Agarwal, A., Rohra, V.K., Assidi, M., Abu-Elmagd, M., and Turki, R.F. (2015). Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. Reprod Biol Endocrinol 13, 35.
Sharpless, N.E., and Sherr, C.J. (2015). Forging a signature of *in vivo* senescence. Nat Rev Cancer 15, 397–408.
Shaw, A.C., Joshi, S., Greenwood, H., Panda, A., and Lord, J.M. (2010). Aging of the innate immune system. Curr Opin Immunol 22, 507–513.
Shay, J., Pereira-Smith, O.M., and Wright, W.E. (1991). A role for both RB and p53 in the regulation of human cellular senescence. Exp Cell Res 196, 33–39.
Sheedfar, F., Biase, S.D., Koonen, D., and Vinciguerra, M. (2013). Liver diseases and aging: friends or foes? Aging Cell 12, 950–954.
Shelton, D.N., Chang, E., Whittier, P.S., Choi, D., and Funk, W.D. (1999). Microarray analysis of replicative senescence. Curr Biol 9, 939–945.
Shen, B., Tasdogan, A., Ubellacker, J.M., Zhang, J., Nosyreva, E.D., Du, L., Murphy, M.M., Hu, S., Yi, Y., Kara, N., et al. (2021). A mechanosensitive peri-arteriolar niche for osteogenesis and lymphopoiesis. Nature 591, 438–444.
Shen, E.Z., Song, C.Q., Lin, Y., Zhang, W.H., Su, P.F., Liu, W.Y., Zhang, P., Xu, J., Lin, N., Zhan, C., et al. (2014). Mitoflash frequency in early adulthood predicts lifespan in *Caenorhabditis elegans*. Nature 508, 128–132.
Shen, T.H., Lin, H.K., Scaglioni, P.P., Yung, T.M., and Pandolfi, P.P. (2006). The mechanisms of PML-nuclear body formation. Mol Cell 24, 331–339.
Shen, W., Wan, X., Hou, J., Liu, Z., Mao, G., Xu, X., Yu, C., Zhu, X., and Ju, Z. (2022). Peroxisome proliferator-activated receptor γ coactivator 1α maintains NAD^+ bioavailability protecting against steatohepatitis. Life Med 1, 207–220.
Sheng, Y., Chen, Y.J., Qian, Z.M., Zheng, J., and Liu, Y. (2020). Cyclophosphamide induces a significant increase in iron content in the liver and spleen of mice. Hum Exp Toxicol 39, 973–983.
Sherwani, S.I., Khan, H.A., Ekhzaimy, A., Masood, A., and Sakharkar, M. K. (2016). Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomark Insights 11, BMI.S38440.
Shi, M., Xu, B., Wang, X., Aoyama, K., Michie, S.A., and Takeuchi, T. (2004). Oxidative damages in chronic inflammation of a mouse autoimmune disease model. Immunol Lett 95, 233–236.
Shi, R., and Babu, S. (2021). Modern approaches and innovations in the diagnosis and treatment of peripheral vascular diseases. Front Biosci 13, 173–180.
Shi, Y., and Carroll, K.S. (2020). Activity-based sensing for site-specific proteomic analysis of cysteine oxidation. Acc Chem Res 53, 20–31.
Shi, Z., Geng, Y., Liu, J., Zhang, H., Zhou, L., Lin, Q., Yu, J., Zhang, K., Liu, J., Gao, X., et al. (2018). Single-cell transcriptomics reveals gene signatures and alterations associated with aging in distinct neural stem/progenitor cell subpopulations. Protein Cell 9, 351–364.
Shimi, T., Butin-Israeli, V., Adam, S.A., Hamanaka, R.B., Goldman, A.E., Lucas, C.A., Shumaker, D.K., Kosak, S.T., Chandel, N.S., and Goldman, R.D. (2011). The role of nuclear lamin B1 in cell proliferation and senescence. Genes Dev 25, 2579–2593.

Shimobayashi, S.F., Ronceray, P., Sanders, D.W., Haataja, M.P., and Brangwynne, C.P. (2021). Nucleation landscape of biomolecular condensates. Nature 599, 503–506.
Shin, C.S., Moon, B.S., Park, K.S., Kim, S.Y., Park, S.J., Chung, M.H., and Lee, H.K. (2001). Serum 8-hydroxy-guanine levels are increased in diabetic patients. Diabetes Care 24, 733–737.
Shin, J.W., Kwon, S.H., Choi, J.Y., Na, J.I., Huh, C.H., Choi, H.R., and Park, K.C. (2019). Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci 20, 2126.
Shin, J., Yang, J., Lee, J.C., and Baek, K.H. (2013). Depletion of ERK2 but not ERK1 abrogates oncogenic Ras-induced senescence. Cell Signal 25, 2540–2547.
Shin, Y., and Brangwynne, C.P. (2017). Liquid phase condensation in cell physiology and disease. Science 357.
Shireby, G.L., Davies, J.P., Francis, P.T., Burrage, J., Walker, E.M., Neilson, G.W.A., Dahir, A., Thomas, A.J., Love, S., Smith, R.G., et al. (2020). Recalibrating the epigenetic clock: implications for assessing biological age in the human cortex. Brain 143, 3763–3775.
Shirendeb, U.P., Calkins, M.J., Manczak, M., Anekonda, V., Dufour, B., McBride, J.L., Mao, P., and Reddy, P.H. (2012). Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. Hum Mol Genet 21, 406–420.
Shook, B.A., Lennington, J.B., Acabchuk, R.L., Halling, M., Sun, Y., Peters, J., Wu, Q., Mahajan, A., Fellows, D.W., and Conover, J.C. (2014). Ventriculomegaly associated with ependymal gliosis and declines in barrier integrity in the aging human and mouse brain. Aging Cell 13, 340–350.
Shrestha, S., Erikson, G., Lyon, J., Spigelman, A.F., Bautista, A., Manning Fox, J.E., dos Santos, C., Shokhirev, M., Cartailler, J.P., Hetzer, M.W., et al. (2022). Aging compromises human islet beta cell function and identity by decreasing transcription factor activity and inducing ER stress. Sci Adv 8, eabo3932.
Shvedunova, M., and Akhtar, A. (2022). Modulation of cellular processes by histone and non-histone protein acetylation. Nat Rev Mol Cell Biol 23, 329–349.
Sidlow, R., Lin, A.E., Gupta, D., Bolton, K.L., Steensma, D.P., Levine, R. L., Ebert, B.L., and Libby, P. (2020). The clinical challenge of clonal hematopoiesis, a newly recognized cardiovascular risk factor. JAMA Cardiol 5, 958–961.
Siervo, M., Lara, J., Celis-Morales, C., Vacca, M., Oggioni, C., Battezzati, A., Leone, A., Tagliabue, A., Spadafranca, A., and Bertoli, S. (2016). Age-related changes in basal substrate oxidation and visceral adiposity and their association with metabolic syndrome. Eur J Nutr 55, 1755–1767.
Sies, H., Belousov, V.V., Chandel, N.S., Davies, M.J., Jones, D.P., Mann, G. E., Murphy, M.P., Yamamoto, M., and Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat Rev Mol Cell Biol 23, 499–515.
Sikora, E., Bielak-Zmijewska, A., Dudkowska, M., Krzystyniak, A., Mosieniak, G., Wesierska, M., and Wlodarczyk, J. (2021). Cellular senescence in brain aging. Front Aging Neurosci 13, 646924.
Simcox, J., Geoghegan, G., Maschek, J.A., Bensard, C.L., Pasquali, M., Miao, R., Lee, S., Jiang, L., Huck, I., Kershaw, E.E., et al. (2017). Global analysis of plasma lipids identifies liver-derived acylcarnitines as a fuel source for brown fat thermogenesis. Cell Metab 26, 509–522. e6.
Simon, M., Van Meter, M., Ablaeva, J., Ke, Z., Gonzalez, R.S., Taguchi, T., De Cecco, M., Leonova, K.I., Kogan, V., Helfand, S.L., et al. (2019). LINE1 derepression in aged wild-type and SIRT6-deficient mice drives inflammation. Cell Metab 29, 871–885.e5.
Sinclair, D.A., and Guarente, L. (1997). Extrachromosomal rDNA circles—a cause of aging in yeast. Cell 91, 1033–1042.
Singh, L., Brennan, T.A., Russell, E., Kim, J.H., Chen, Q., Brad Johnson, F., and Pignolo, R.J. (2016). Aging alters bone-fat reciprocity by shifting *in vivo* mesenchymal precursor cell fate towards an adipogenic lineage. Bone 85, 29–36.
Singh, P., Coskun, Z.Z., Goode, C., Dean, A., Thompson-Snipes, L.A., and Darlington, G. (2008). Lymphoid neogenesis and immune infiltration in aged liver. Hepatology 47, 1680–1690.
Singh, P., Marcu, K.B., Goldring, M.B., and Otero, M. (2019). Phenotypic instability of chondrocytes in osteoarthritis: on a path to hypertrophy. Ann N Y Acad Sci 1442, 17–34.
Singh, S.P., Janjuha, S., Chaudhuri, S., Reinhardt, S., Kränkel, A., Dietz, S., Eugster, A., Bilgin, H., Korkmaz, S., Zararsız, G., et al. (2018). Machine learning based classification of cells into chronological stages using single-cell transcriptomics. Sci Rep 8, 17156.
Sivam, S., Phillips, C.L., Trenell, M.I., Yee, B.J., Liu, P.Y., Wong, K.K., and Grunstein, R.R. (2012). Effects of 8 weeks of continuous positive airway pressure on abdominal adiposity in obstructive sleep apnoea. Eur Respir J 40, 913–918.
Skaug, E.A., Aspenes, S.T., Oldervoll, L., Mørkedal, B., Vatten, L., Wisløff, U., and Ellingsen, Ø. (2013). Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. Eur J Prev Cardiol 20, 531–540.
Skloot, G.S. (2017). The effects of aging on lung structure and function. Clin Geriatr Med 33, 447–457.
Smallwood, S.A., Lee, H.J., Angermueller, C., Krueger, F., Saadeh, H., Peat, J., Andrews, S.R., Stegle, O., Reik, W., and Kelsey, G. (2014). Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. Nat Methods 11, 817–820.
Smith, K.P., Carter, K.C., Johnson, C.V., and Lawrence, J.B. (1995). U2 and U1 snRNA gene loci associate with coiled bodies. J Cell Biochem 59, 473–485.
Smith, L.K., He, Y., Park, J.S., Bieri, G., Snethlage, C.E., Lin, K., Gontier, G., Wabl, R., Plambeck, K.E., Udeochu, J., et al. (2015). β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. Nat Med 21, 932–937.
Smith, Z.D., and Meissner, A. (2013). DNA methylation: roles in mammalian development. Nat Rev Genet 14, 204–220.
Snyder, P.J. (2022). Symptoms of late-onset hypogonadism in men. Endocrinol Metab Clin North Am 51, 755–760.
Sohal, R.S. (1970). Mitochondrial changes in the heart of *Drosophila repleta*, wollaston with age. Exp Gerontol 5, 213–214.
Solimena, M., Schulte, A.M., Marselli, L., Ehehalt, F., Richter, D., Kleeberg, M., Mziaut, H., Knoch, K.P., Parnis, J., Bugliani, M., et al. (2018). Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. Diabetologia 61, 641–657.
Solvang, S.H., Hodge, A., Watne, L.O., Cabral-Marques, O., Nordrehaug, J.E., Giles, G.G., Dugue, P.A., Nygard, O., Ueland, P.M., McCann, A., et al. (2022). Kynurenine pathway metabolites in the blood and cerebrospinal fluid are associated with human aging. Oxid Med Cell Longev 2022, 5019752.
Sonel, A., Sasseen, B.M., Fineberg, N., Bang, N., and Wilensky, R.L. (2000). Prospective study correlating fibrinopeptide A, troponin I, myoglobin, and myosin light chain levels with early and late ischemic events in consecutive patients presenting to the emergency department with chest pain. Circulation 102, 1107–1113.
Song, A., Lusk, J.B., Roh, K.M., Jackson, K.J., Scherr, K.A., McNabb, R. P., Chatterjee, R., and Kuo, A.N. (2022a). Practice patterns of fundoscopic examination for diabetic retinopathy screening in primary care. JAMA Netw Open 5, e2218753.
Song, J., Ni, Q., Sun, J., Xie, J., Liu, J., Ning, G., Wang, W., and Wang, Q. (2022b). Aging impairs adaptive unfolded protein response and drives beta cell dedifferentiation in humans. J Clin Endocrinol Metab 107, 3231–3241.
Song, L. (2017). Calcium and bone metabolism indices. Adv Clin Chem 82, 1–46.
Song, M., Franco, A., Fleischer, J.A., Zhang, L., and Dorn Ii, G.W. (2017). Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence. Cell Metab 26, 872–883.e5.
Song, S., Lam, E.W.F., Tchkonia, T., Kirkland, J.L., and Sun, Y. (2020a). Senescent cells: emerging targets for human aging and age-related diseases. Trends Biochem Sci 45, 578–592.
Song, S., Tchkonia, T., Jiang, J., Kirkland, J.L., and Sun, Y. (2020b).

Targeting senescent cells for a healthier aging: challenges and opportunities. Adv Sci 7, 2002611.
Soreq, L., Rose, J., Soreq, E., Hardy, J., Trabzuni, D., Cookson, M.R., Smith, C., Ryten, M., Patani, R., and Ule, J. (2017). Major shifts in glial regional identity are a transcriptional hallmark of human brain aging. Cell Rep 18, 557–570.
Sousa-Victor, P., García-Prat, L., and Muñoz-Cánoves, P. (2022). Control of satellite cell function in muscle regeneration and its disruption in ageing. Nat Rev Mol Cell Biol 23, 204–226.
Sousa-Victor, P., Gutarra, S., García-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jardí, M., Ballestar, E., González, S., Serrano, A. L., et al. (2014). Geriatric muscle stem cells switch reversible quiescence into senescence. Nature 506, 316–321.
Sousa-Victor, P., and Muñoz-Cánoves, P. (2016). Regenerative decline of stem cells in sarcopenia. Mol Aspects Med 50, 109–117.
Sousa-Victor, P., Neves, J., Cedron-Craft, W., Ventura, P.B., Liao, C.Y., Riley, R.R., Soifer, I., van Bruggen, N., Kolumam, G.A., Villeda, S.A., et al. (2019). MANF regulates metabolic and immune homeostasis in ageing and protects against liver damage. Nat Metab 1, 276–290.
Sperling, R.A., LaViolette, P.S., O'Keefe, K., O'Brien, J., Rentz, D.M., Pihlajamaki, M., Marshall, G., Hyman, B.T., Selkoe, D.J., Hedden, T., et al. (2009). Amyloid deposition is associated with impaired default network function in older persons without dementia. Neuron 63, 178–188.
Srivastava, A., Schmidt, I.M., Palsson, R., Weins, A., Bonventre, J.V., Sabbisetti, V., Stillman, I.E., Rennke, H.G., and Waikar, S.S. (2021). The associations of plasma biomarkers of inflammation with histopathologic lesions, kidney disease progression, and mortality—The Boston Kidney Biopsy Cohort Study. Kidney Int Rep 6, 685–694.
Stahl, E.C., Haschak, M.J., Popovic, B., and Brown, B.N. (2018). Macrophages in the aging liver and age-related liver disease. Front Immunol 9, 2795.
Stam, H., Hrachovina, V., Stijnen, T., and Versprille, A. (1994). Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 76, 2356–2363.
Staunton, L., Zweyer, M., Swandulla, D., and Ohlendieck, K. (2012). Mass spectrometry-based proteomic analysis of middle-aged vs. aged vastus lateralis reveals increased levels of carbonic anhydrase isoform 3 in senescent human skeletal muscle. Int J Mol Med 30, 723–733.
Stearns-Reider, K.M., D'Amore, A., Beezhold, K., Rothrauff, B., Cavalli, L., Wagner, W.R., Vorp, D.A., Tsamis, A., Shinde, S., Zhang, C., et al. (2017). Aging of the skeletal muscle extracellular matrix drives a stem cell fibrogenic conversion. Aging Cell 16, 518–528.
Steer, M.L., Waxman, I., and Freedman, S. (1995). Chronic pancreatitis. N Engl J Med 332, 1482–1490.
Stefanska, A., Eng, D., Kaverina, N., Duffield, J.S., Pippin, J.W., Rabinovitch, P., and Shankland, S.J. (2015). Interstitial pericytes decrease in aged mouse kidneys. Aging 7, 370–382.
Stein, G.H., Drullinger, L.F., Soulard, A., and Dulic, V. (1999). Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. Mol Cell Biol 19, 2109–2117.
Stensen, M.H., Tanbo, T., Storeng, R., and Fedorcsak, P. (2014). Advanced glycation end products and their receptor contribute to ovarian ageing. Hum Reprod 29, 125–134.
Stervbo, U., Meier, S., Mälzer, J.N., Baron, U., Bozzetti, C., Jürchott, K., Nienen, M., Olek, S., Rachwalik, D., Schulz, A.R., et al. (2015). Effects of aging on human leukocytes (part I): immunophenotyping of innate immune cells. Age 37, 92.
Steuerwald, N.M., Bermúdez, M.G., Wells, D., Munné, S., and Cohen, J. (2007). Maternal age-related differential global expression profiles observed in human oocytes. Reprod Biomed Online 14, 700–708.
Stevens, B.R., Goel, R., Seungbum, K., Richards, E.M., Holbert, R.C., Pepine, C.J., and Raizada, M.K. (2018). Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut 67, 1555.2–1557.
Stolk, L., Perry, J.R.B., Chasman, D.I., He, C., Mangino, M., Sulem, P., Barbalic, M., Broer, L., Byrne, E.M., Ernst, F., et al. (2012). Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet 44, 260–268.
Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M.C., Di Giacomo, V., Yosef, R., Pilpel, N., Krizhanovsky, V., Sharpe, J., et al. (2013). Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell 155, 1119–1130.
Storsve, A.B., Fjell, A.M., Tamnes, C.K., Westlye, L.T., Overbye, K., Aasland, H.W., and Walhovd, K.B. (2014). Differential longitudinal changes in cortical thickness, surface area and volume across the adult life span: regions of accelerating and decelerating change. J Neurosci 34, 8488–8498.
Stotland, A., and Gottlieb, R.A. (2016). α-MHC MitoTimer mouse: *In vivo* mitochondrial turnover model reveals remarkable mitochondrial heterogeneity in the heart. J Mol Cell Cardiol 90, 53–58.
Stout-Delgado, H.W., Du, W., Shirali, A.C., Booth, C.J., and Goldstein, D. R. (2009). Aging promotes neutrophil-induced mortality by augmenting IL-17 production during viral infection. Cell Host Microbe 6, 446–456.
Støylen, A., Mølmen, H.E., and Dalen, H. (2020). Over all variability of mitral annular plane peak systolic velocity and peak global longitudinal strain rate in relation to age, body size, and sex: The HUNT Study. Echocardiography 37, 578–585.
Strangfeld, A., Schäfer, M., Gianfrancesco, M.A., Lawson-Tovey, S., Liew, J.W., Ljung, L., Mateus, E.F., Richez, C., Santos, M.J., Schmajuk, G., et al. (2021). Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. Ann Rheum Dis 80, 930–942.
Streit, W.J. (2006). Microglial senescence: does the brain's immune system have an expiration date? Trends Neuroscis 29, 506–510.
Streit, W.J., Sammons, N.W., Kuhns, A.J., and Sparks, D.L. (2004). Dystrophic microglia in the aging human brain. Glia 45, 208–212.
Streit, W.J., and Xue, Q.S. (2010). The brain's aging immune system. Aging Dis 1, 254–261.
Stuart, B.D., Lee, J.S., Kozlitina, J., Noth, I., Devine, M.S., Glazer, C.S., Torres, F., Kaza, V., Girod, C.E., Jones, K.D., et al. (2014). Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. Lancet Respir Med 2, 557–565.
Stuart, G.R., Oda, Y., de Boer, J.G., and Glickman, B.W. (2000). Mutation frequency and specificity with age in liver, bladder and brain of lacI transgenic mice. Genetics 154, 1291–1300.
Stubbs, T.M., Bonder, M.J., Stark, A.K., Krueger, F., von Meyenn, F., Stegle, O., and Reik, W. (2017). Multi-tissue DNA methylation age predictor in mouse. Genome Biol 18, 68.
Su, Y., Misumi, Y., Ueda, M., Shono, M., Tasaki, M., Guo, J., Jono, H., Obayashi, K., Senokuchi, T., Yamagata, K., et al. (2012). The occurrence of islet amyloid polypeptide amyloidosis in Japanese subjects. Pancreas 41, 971–973.
Su, Y., Wang, T., Wu, N., Li, D., Fan, X., Xu, Z., Mishra, S.K., and Yang, M. (2019). Alpha-ketoglutarate extends *Drosophila* lifespan by inhibiting mTOR and activating AMPK. Aging 11, 4183–4197.
Sugrue, M.M., Wang, Y., Rideout, H.J., Chalmers-Redman, R.M.E., and Tatton, W.G. (1999). Reduced mitochondrial membrane potential and altered responsiveness of a mitochondrial membrane megachannel in p53-induced senescence. Biochem Biophys Res Commun 261, 123–130.
Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., Wang, H., Le, T., Faull, K.F., Chen, R., et al. (2014). Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell 14, 673–688.
Sun, J., Wang, M., Zhong, Y., Ma, X., Sun, S., Xu, C., Peng, L., Li, G., Zhang, L., Liu, Z., et al. (2022a). A Glb1-2A-mCherry reporter monitors systemic aging and predicts lifespan in middle-aged mice. Nat Commun 13, 7028.
Sun, N., Youle, R.J., and Finkel, T. (2016). The mitochondrial basis of aging. Mol Cell 61, 654–666.
Sun, S., Ma, S., Cai, Y., Wang, S., Ren, J., Yang, Y., Ping, J., Wang, X.,

Zhang, Y., Yan, H., et al. (2023). A single-cell transcriptomic atlas of exercise-induced anti-inflammatory and geroprotective effects across the body. Innovation 4, 100380.
Sun, S., Qin, W., Tang, X., Meng, Y., Hu, W., Zhang, S., Qian, M., Liu, Z., Cao, X., Pang, Q., et al. (2020a). Vascular endothelium-targeted *Sirt7* gene therapy rejuvenates blood vessels and extends life span in a Hutchinson-Gilford progeria model. Sci Adv 6, eaay5556.
Sun, X., Shi, B., Zheng, H., Min, L., Yang, J., Li, X., Liao, X., Huang, W., Zhang, M., Xu, S., et al. (2018a). Senescence-associated secretory factors induced by cisplatin in melanoma cells promote non-senescent melanoma cell growth through activation of the ERK1/2-RSK1 pathway. Cell Death Dis 9, 260.
Sun, X., Zhang, Y., Hu, Y., An, J., Li, L., Wang, Y., and Zhang, X. (2021). Decreased expression of m^6A demethylase FTO in ovarian aging. Arch Gynecol Obstet 303, 1363–1369.
Sun, Y., Coppé, J.P., and Lam, E.W.F. (2018b). Cellular senescence: the sought or the unwanted? Trends Mol Med 24, 871–885.
Sun, Y., Li, Q., and Kirkland, J.L. (2022b). Targeting senescent cells for a healthier longevity: the roadmap for an era of global aging. Life Med 1, 103–119.
Sun, Y., Liang, L., Dong, M., Li, C., Liu, Z., and Gao, H. (2019). Cofilin 2 in serum as a novel biomarker for Alzheimer's disease in Han Chinese. Front Aging Neurosci 11, 214.
Sun, Y., Lu, F., Yu, X., Wang, B., Chen, J., Lu, F., Peng, S., Sun, X., Yu, M., Chen, H., et al. (2020b). Exogenous H_2S promoted USP8 sulfhydration to regulate mitophagy in the hearts of db/db mice. Aging Dis 11, 269–285.
Sundaresan, V., Griffanti, L., Kindalova, P., Alfaro-Almagro, F., Zamboni, G., Rothwell, P.M., Nichols, T.E., and Jenkinson, M. (2019). Modelling the distribution of white matter hyperintensities due to ageing on MRI images using Bayesian inference. NeuroImage 185, 434–445.
Sunderland, P., Augustyniak, J., Lenart, J., Bużańska, L., Carlessi, L., Delia, D., and Sikora, E. (2020). ATM-deficient neural precursors develop senescence phenotype with disturbances in autophagy. Mech Ageing Dev 190, 111296.
Suzan, V., Yavuzer, H., Bag Soytas, R., Bektan Kanat, B., Arman, P., Emiroglu Gedik, T., Unal, D., Atar, O., Bolayirli, I.M., and Doventas, A. (2021). The relationship between primary sarcopenia and SARC-F, serum MMP9, TIMP1 levels, and MMP9/TIMP1 ratio in the geriatric patients. Eur Geriatr Med 12, 1229–1235.
Suzuki, T., Aoki, K., Shimokobe, K., Omiya, S., Funayama, C., Takahashi, T., and Kato, M. (2022). Age-related morphological and functional changes in the small intestine of senescence-accelerated mouse. Exp Gerontol 163, 111795.
Svennerholm, L., Boström, K., and Jungbjer, B. (1997). Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. Acta Neuropathol 94, 345–352.
Svoboda, P., Ko, S.H., Cho, B.L., Yoo, S.H., Choi, S.W., Ye, S.K., Kasai, H., and Chung, M.H. (2008). Neopterin, a marker of immune response, and 8-hydroxy-2′-deoxyguanosine, a marker of oxidative stress, correlate at high age as determined by automated simultaneous high-performance liquid chromatography analysis of human urine. Anal Biochem 383, 236–242.
Swaab, D.F., Fliers, E., and Partiman, T.S. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 342, 37–44.
Swanlund, J.M., Kregel, K.C., and Oberley, T.D. (2008). Autophagy following heat stress: the role of aging and protein nitration. Autophagy 4, 936–939.
Swatek, K.N., and Komander, D. (2016). Ubiquitin modifications. Cell Res 26, 399–422.
Symonds, M.E., Henderson, K., Elvidge, L., Bosman, C., Sharkey, D., Perkins, A.C., and Budge, H. (2012). Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. J Pediatr 161, 892–898.
Szabo, Q., Bantignies, F., and Cavalli, G. (2019). Principles of genome folding into topologically associating domains. Sci Adv 5, eaaw1668.
Szakal, A.K., Aydar, Y., Balogh, P., and Tew, J.G. (2002). Molecular interactions of FDCs with B cells in aging. Semin Immunol 14, 267–274.
Tabibian, J.H., O'Hara, S.P., Splinter, P.L., Trussoni, C.E., and LaRusso, N. F. (2014). Cholangiocyte senescence by way of N-ras activation is a characteristic of primary sclerosing cholangitis. Hepatology 59, 2263–2275.
Tabibzadeh, S. (2021). CircadiOmic medicine and aging. Ageing Res Rev 71, 101424.
Tabula Muris, C. (2020). A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. Nature 583, 590–595.
Tajima, K., Ikeda, K., Chang, H.Y., Chang, C.H., Yoneshiro, T., Oguri, Y., Jun, H., Wu, J., Ishihama, Y., and Kajimura, S. (2019). Mitochondrial lipoylation integrates age-associated decline in brown fat thermogenesis. Nat Metab 1, 886–898.
Takada, Y., Yaman-Deveci, R., Shirakawa, T., Sharif, J., Tomizawa, S., Miura, F., Ito, T., Ono, M., Nakajima, K., Koseki, Y., et al. (2021). Maintenance DNA methylation in pre-meiotic germ cells regulates meiotic prophase by facilitating homologous chromosome pairing. Development 148.
Takahashi, H., Okamura, D., Starr, M.E., Saito, H., and Mark Evers, B. (2012). Age-dependent reduction of the PI3K regulatory subunit p85α suppresses pancreatic acinar cell proliferation. Aging Cell 11, 305–314.
Takahashi, H., Yoshimatsu, G., and Faustman, D.L. (2022). The roles of TNFR2 signaling in cancer cells and the tumor microenvironment and the potency of TNFR2 targeted therapy. Cells 11, 1952.
Takahashi, J.S. (2017). Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet 18, 164–179.
Takahashi, J.S., Hong, H.K., Ko, C.H., and McDearmon, E.L. (2008). The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet 9, 764–775.
Takahashi, M., Hoshino, H., and Kushida, K. (1999). Measurements of urinary nonisomerized form of type I collagen degradation products (α-CTx) in aging, menopause, and osteoporosis with fractures. Clin Chim Acta 279, 69–76.
Takai, K.K., Kibe, T., Donigian, J.R., Frescas, D., and de Lange, T. (2011). Telomere protection by TPP1/POT1 requires tethering to TIN2. Mol Cell 44, 647–659.
Takasugi, M., Okada, R., Takahashi, A., Virya Chen, D., Watanabe, S., and Hara, E. (2017). Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. Nat Commun 8, 15729.
Taki, Y., Kinomura, S., Sato, K., Goto, R., Kawashima, R., and Fukuda, H. (2011). A longitudinal study of gray matter volume decline with age and modifying factors. Neurobiol Aging 32, 907–915.
Talchai, C., Xuan, S., Lin, H.V., Sussel, L., and Accili, D. (2012). Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 150, 1223–1234.
Talens, R.P., Christensen, K., Putter, H., Willemsen, G., Christiansen, L., Kremer, D., Suchiman, H.E.D., Slagboom, P.E., Boomsma, D.I., and Heijmans, B.T. (2012). Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694–703.
Tallam, H., Elton, D.C., Lee, S., Wakim, P., Pickhardt, P.J., and Summers, R.M. (2022). Fully automated abdominal CT biomarkers for type 2 diabetes using deep learning. Radiology 304, 85–95.
Tam, Z.Y., Ng, S.P., Tan, L.Q., Lin, C.H., Rothenbacher, D., Klenk, J., Boehm, B.O., Kiat, K.G.K., Suwanchaikasem, P., Tipthara, P., et al. (2017). Metabolite profiling in identifying metabolic biomarkers in older people with late-onset type 2 diabetes mellitus. Sci Rep 7, 4392.
Tamura, Y., Takubo, K., Aida, J., Araki, A., and Ito, H. (2016). Telomere attrition and diabetes mellitus. Geriatr Gerontol Int 16, 66–74.
Tan, R.J., and Liu, Y. (2012). Matrix metalloproteinases in kidney homeostasis and diseases. Am J Physiol Renal Physiol 302, F1351–F1361.
Tanaka, T., Basisty, N., Fantoni, G., Candia, J., Moore, A.Z., Biancotto, A., Schilling, B., Bandinelli, S., and Ferrucci, L. (2020). Plasma proteomic

biomarker signature of age predicts health and life span. eLife 9, e61073.
Tanaka, T., Biancotto, A., Moaddel, R., Moore, A.Z., Gonzalez-Freire, M., Aon, M.A., Candia, J., Zhang, P., Cheung, F., Fantoni, G., et al. (2018). Plasma proteomic signature of age in healthy humans. Aging Cell 17, e12799.
Taneera, J., Lang, S., Sharma, A., Fadista, J., Zhou, Y., Ahlqvist, E., Jonsson, A., Lyssenko, V., Vikman, P., Hansson, O., et al. (2012). A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab 16, 122–134.
Taneike, M., Yamaguchi, O., Nakai, A., Hikoso, S., Takeda, T., Mizote, I., Oka, T., Tamai, T., Oyabu, J., Murakawa, T., et al. (2010). Inhibition of autophagy in the heart induces age-related cardiomyopathy. Autophagy 6, 600–606.
Tanemura, K., Kurohmaru, M., kuramoto, K., Matsumotot, M., and Hayashi, Y. (1994). Age-related changes in cytoskeletal components of the BDF1, mouse sertoli cell. Tissue Cell 26, 447–455.
Tang, D., Tao, S., Chen, Z., Koliesnik, I.O., Calmes, P.G., Hoerr, V., Han, B., Gebert, N., Zörnig, M., Löffler, B., et al. (2016). Dietary restriction improves repopulation but impairs lymphoid differentiation capacity of hematopoietic stem cells in early aging. J Exp Med 213, 535–553.
Tang, X., Chen, X.F., Sun, X., Xu, P., Zhao, X., Tong, Y., Wang, X.M., Yang, K., Zhu, Y.T., Hao, D.L., et al. (2021). Short-chain enoyl-CoA hydratase mediates histone crotonylation and contributes to cardiac homeostasis. Circulation 143, 1066–1069.
Tang, X., Chen, X.F., Wang, N.Y., Wang, X.M., Liang, S.T., Zheng, W., Lu, Y.B., Zhao, X., Hao, D.L., Zhang, Z.Q., et al. (2017). SIRT2 acts as a cardioprotective deacetylase in pathological cardiac hypertrophy. Circulation 136, 2051–2067.
Tang, X., Li, P.H., and Chen, H.Z. (2020). Cardiomyocyte senescence and cellular communications within myocardial microenvironments. Front Endocrinol 11, 280.
Tao, X., Landis, J.N., Krisher, R.L., Duncan, F.E., Silva, E., Lonczak, A., Scott Iii, R.T., Zhan, Y., Chu, T., Scott, R.T. Jr., et al. (2017). Mitochondrial DNA content is associated with ploidy status, maternal age, and oocyte maturation methods in mouse blastocysts. J Assist Reprod Genet 34, 1587–1594.
Tarkhov, A.E., Lindstrom-Vautrin, T., Zhang, S., Ying, K., Moqri, M., Zhang, B., and Gladyshev, V.N. (2022). Nature of epigenetic aging from a single-cell perspective. bioRxiv 2022.2009.2026.509592.
Tarumi, T., Khan, M.A., Liu, J., Tseng, B.M., Parker, R., Riley, J., Tinajero, C., and Zhang, R. (2014). Cerebral hemodynamics in normal aging: central artery stiffness, wave reflection, and pressure pulsatility. J Cereb Blood Flow Metab 34, 971–978.
Taruttis, A., Timmermans, A.C., Wouters, P.C., Kacprowicz, M., van Dam, G.M., and Ntziachristos, V. (2016). Optoacoustic imaging of human vasculature: feasibility by using a handheld probe. Radiology 281, 256–263.
Tasdemir, N., Banito, A., Roe, J.S., Alonso-Curbelo, D., Camiolo, M., Tschaharganeh, D.F., Huang, C.H., Aksoy, O., Bolden, J.E., Chen, C.C., et al. (2016). BRD4 connects enhancer remodeling to senescence immune surveillance. Cancer Discov 6, 612–629.
Tatomer, D.C., Terzo, E., Curry, K.P., Salzler, H., Sabath, I., Zapotoczny, G., McKay, D.J., Dominski, Z., Marzluff, W.F., and Duronio, R.J. (2016). Concentrating pre-mRNA processing factors in the histone locus body facilitates efficient histone mRNA biogenesis. J Cell Biol 213, 557–570.
Tatone, C., and Amicarelli, F. (2013). The aging ovary—the poor granulosa cells. Fertil Steril 99, 12–17.
Tatone, C., Amicarelli, F., Carbone, M.C., Monteleone, P., Caserta, D., Marci, R., Artini, P.G., Piomboni, P., and Focarelli, R. (2008). Cellular and molecular aspects of ovarian follicle ageing. Hum Reprod Update 14, 131–142.
Tatone, C., Heizenrieder, T., Di Emidio, G., Treffon, P., Amicarelli, F., Seidel, T., and Eichenlaub-Ritter, U. (2011). Evidence that carbonyl stress by methylglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial integrity in mammalian oocytes and contributes to oocyte ageing. Hum Reprod 26, 1843–1859.
Tauchi, H., Sato, T., and Ito, Y. (1994). Morphological aspects of aging liver: half a century of progress in Japan. Arch Gerontol Geriatr 19, 135–144.
Tawo, R., Pokrzywa, W., Kevei, É., Akyuz, M.E., Balaji, V., Adrian, S., Höhfeld, J., and Hoppe, T. (2017). The ubiquitin ligase CHIP integrates proteostasis and aging by regulation of insulin receptor turnover. Cell 169, 470–482.e13.
Taylor, R.W., Barron, M.J., Borthwick, G.M., Gospel, A., Chinnery, P.F., Samuels, D.C., Taylor, G.A., Plusa, S.M., Needham, S.J., Greaves, L.C., et al. (2003). Mitochondrial DNA mutations in human colonic crypt stem cells. J Clin Invest 112, 1351–1360.
Tchkonia, T., Morbeck, D.E., Von Zglinicki, T., Van Deursen, J., Lustgarten, J., Scrable, H., Khosla, S., Jensen, M.D., and Kirkland, J. L. (2010). Fat tissue, aging, and cellular senescence. Aging Cell 9, 667–684.
Tchkonia, T., Palmer, A.K., and Kirkland, J.L. (2021). New horizons: novel approaches to enhance healthspan through targeting cellular senescence and related aging mechanisms. J Clin Endocrinol Metab 106, e1481–e1487.
Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J., and Kirkland, J.L. (2013). Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 123, 966–972.
te Velde, E.R., and Pearson, E.R. (2002). The variability of female reproductive ageing. Hum Reprod Update 8, 141–154.
Tehrani, F.R., Firouzi, F., and Behboudi-Gandevani, S. (2022). Investigating the clinical utility of the anti-mullerian hormone testing for the prediction of age at menopause and assessment of functional ovarian reserve: a practical approach and recent updates. Aging Dis 13, 458–467.
Teissier, T., Boulanger, E., and Deramecourt, V. (2020). Normal ageing of the brain: Histological and biological aspects. Rev Neurol 176, 649–660.
Textor, J., Mandl, J.N., and de Boer, R.J. (2016). The reticular cell network: a robust backbone for immune responses. PLoS Biol 14, e2000827.
Tezze, C., Romanello, V., Desbats, M.A., Fadini, G.P., Albiero, M., Favaro, G., Ciciliot, S., Soriano, M.E., Morbidoni, V., Cerqua, C., et al. (2017). Age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. Cell Metab 25, 1374–1389.e6.
Tham, C.Y., Poon, L.F., Yan, T.D., Koh, J.Y.P., Ramlee, M.K., Teoh, V.S.I., Zhang, S., Cai, Y., Hong, Z., Lee, G.S., et al. (2023). High-throughput telomere length measurement at nucleotide resolution using the PacBio high fidelity sequencing platform. Nat Commun 14, 281.
Thijssen, D.H.J., Bruno, R.M., van Mil, A.C.C.M., Holder, S.M., Faita, F., Greyling, A., Zock, P.L., Taddei, S., Deanfield, J.E., Luscher, T., et al. (2019). Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J 40, 2534–2547.
Thompson, M.J., vonHoldt, B., Horvath, S., and Pellegrini, M. (2017). An epigenetic aging clock for dogs and wolves. Aging 9, 1055–1068.
Thuault, S. (2023). Endogenous retroviruses and aging. Nat Aging 3, 145.
Thurman, J.M. (2015). Complement in kidney disease: core curriculum 2015. Am J Kidney Dis 65, 156–168.
Thuringer, J.M., and Katzberg, A.A. (1959). The effect of age on mitosis in the human epidermis. J Invest Dermatol 33, 35–39.
Tian, M., He, X., Jin, C., He, X., Wu, S., Zhou, R., Zhang, X., Zhang, K., Gu, W., Wang, J., et al. (2021). Transpathology: molecular imaging-based pathology. Eur J Nucl Med Mol Imag 48, 2338–2350.
Tian, X.L., and Li, Y. (2014). Endothelial cell senescence and age-related vascular diseases. J Genet Genomics 41, 485–495.
Tian, Y., Wang, S., Jiao, F., Kong, Q., Liu, C., and Wu, Y. (2019). Telomere length: a potential biomarker for the risk and prognosis of stroke. Front Neurol 10, 624.
Tieland, M., Trouwborst, I., and Clark, B.C. (2018). Skeletal muscle performance and ageing. J Cachexia Sarcopenia Muscle 9, 3–19.
Tierney, M.T., Aydogdu, T., Sala, D., Malecova, B., Gatto, S., Puri, P.L., Latella, L., and Sacco, A. (2014). STAT3 signaling controls satellite cell expansion and skeletal muscle repair. Nat Med 20, 1182–1186.

Tierney, M.T., Stec, M.J., Rulands, S., Simons, B.D., and Sacco, A. (2018). Muscle stem cells exhibit distinct clonal dynamics in response to tissue repair and homeostatic aging. Cell Stem Cell 22, 119–127.e3.
Tietz, N.W., Shuey, D.F., and Wekstein, D.R. (1992). Laboratory values in fit aging individuals—sexagenarians through centenarians. Clin Chem 38, 1167–1185.
Tiku, V., Jain, C., Raz, Y., Nakamura, S., Heestand, B., Liu, W., Späth, M., Suchiman, H.E.D., Müller, R.U., Slagboom, P.E., et al. (2017). Small nucleoli are a cellular hallmark of longevity. Nat Commun 8, 16083.
Tilg, H., and Moschen, A.R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6, 772–783.
Tintignac, L.A., Brenner, H.R., and Rüegg, M.A. (2015). Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting. Physiol Rev 95, 809–852.
Tiscornia, O.M., Cresta, M.A., de Lehmann, E.S., Celener, D., and Dreiling, D.A. (1986). Effects of sex and age on pancreatic secretion. Int J Pancreatol 1, 95–118.
Tokida, R., Uehara, M., Nakano, M., Suzuki, T., Sakai, N., Ikegami, S., Takahashi, J., Nakamura, Y., and Kato, H. (2021). Reference values for bone metabolism in a Japanese cohort survey randomly sampled from a basic elderly resident registry. Sci Rep 11, 7822.
Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S.I., Tanaka, K., Nakano, H., and Iwai, K. (2011). SHARPIN is a component of the NF-κB-activating linear ubiquitin chain assembly complex. Nature 471, 633–636.
Toledo, C., Andrade, D.C., Díaz, H.S., Inestrosa, N.C., and Del Rio, R. (2019). Neurocognitive disorders in heart failure: novel pathophysiological mechanisms underpinning memory loss and learning impairment. Mol Neurobiol 56, 8035–8051.
Tolep, K., Higgins, N., Muza, S., Criner, G., and Kelsen, S.G. (1995). Comparison of diaphragm strength between healthy adult elderly and young men. Am J Respir Crit Care Med 152, 677–682.
Toner, J.P., and Seifer, D.B. (2013). Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril 99, 1825–1830.
Toppari, J. (2021). Insulin-like factor 3 emerges from the shadow of testosterone as a leydig cell biomarker. J Clin Endocrinol Metab 106, e370–e371.
Torres, D.D., Rossini, M., Manno, C., Mattace-Raso, F., D'Altri, C., Ranieri, E., Pontrelli, P., Grandaliano, G., Gesualdo, L., and Schena, F. P. (2008). The ratio of epidermal growth factor to monocyte chemotactic peptide-1 in the urine predicts renal prognosis in IgA nephropathy. Kidney Int 73, 327–333.
Toschi, N., Gisbert, R.A., Passamonti, L., Canals, S., and De Santis, S. (2020). Multishell diffusion imaging reveals sex-specific trajectories of early white matter degeneration in normal aging. Neurobiol Aging 86, 191–200.
Tosh, D., and Slack, J.M.W. (2002). How cells change their phenotype. Nat Rev Mol Cell Biol 3, 187–194.
Tost, J. (2010). DNA methylation: an introduction to the biology and the disease-associated changes of a promising biomarker. Mol Biotechnol 44, 71–81.
Touitou, Y., Fevre, M., Lagoguey, M., Carayon, A., Bogdan, A., Reinberg, A., Beck, H., Cesselin, F., and Touitou, C. (1981). Age- and mental health-related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man. J Endocrinol 91, 467–475.
Townsend, R.R., Wilkinson, I.B., Schiffrin, E.L., Avolio, A.P., Chirinos, J. A., Cockcroft, J.R., Heffernan, K.S., Lakatta, E.G., McEniery, C.M., Mitchell, G.F., et al. (2015). Recommendations for improving and standardizing vascular research on arterial stiffness: a scientific statement from the American Heart Association. Hypertension 66, 698–722.
Townsley, D.M., Dumitriu, B., Liu, D., Biancotto, A., Weinstein, B., Chen, C., Hardy, N., Mihalek, A.D., Lingala, S., Kim, Y.J., et al. (2016). Danazol treatment for telomere diseases. N Engl J Med 374, 1922–1931.
Toyoda, M., Nakamura, M., Luo, Y., and Morohashi, M. (2001). Ultrastructural characterization of microvasculature in photoaging. J Dermatol Sci 27, 32–41.
Tracy, E., Rowe, G., and LeBlanc, A.J. (2020). Cardiac tissue remodeling in healthy aging: the road to pathology. Am J Physiol Cell Physiol 319, C166–C182.
Trapp, A., Kerepesi, C., and Gladyshev, V.N. (2021). Profiling epigenetic age in single cells. Nat Aging 1, 1189–1201.
Travaglini, K.J., Nabhan, A.N., Penland, L., Sinha, R., Gillich, A., Sit, R. V., Chang, S., Conley, S.D., Mori, Y., Seita, J., et al. (2020). A molecular cell atlas of the human lung from single-cell RNA sequencing. Nature 587, 619–625.
Treff, N.R., Su, J., Taylor, D., and Scott, R.T., Jr. (2011). Telomere DNA deficiency is associated with development of human embryonic aneuploidy. PLoS Genet 7, e1002161.
Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A. T., Bruder, C.E., Bohlooly-Y, M., Gidlöf, S., Oldfors, A., Wibom, R., et al. (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417–423.
Trompette, A., Gollwitzer, E.S., Pattaroni, C., Lopez-Mejia, I.C., Riva, E., Pernot, J., Ubags, N., Fajas, L., Nicod, L.P., and Marsland, B.J. (2018). Dietary fiber confers protection against flu by shaping $Ly6c^{-}$ patrolling monocyte hematopoiesis and $CD8^{+}$ T cell metabolism. Immunity 48, 992–1005.e8.
Trowbridge, J.J., and Starczynowski, D.T. (2021). Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J Exp Med 218.
Tsurumi, A., and Li, W. (2012). Global heterochromatin loss: a unifying theory of aging? Epigenetics 7, 680–688.
Tu, W., Ni, D., Yang, H., Zhao, F., Yang, C., Zhao, X., Guo, Z., Yu, K., Wang, J., Hu, Z., et al. (2022). Deciphering the dynamics of the ovarian reserve in cynomolgus monkey through a quantitative morphometric study. Sci Bull 67, 1854–1859.
Turner, V.M., and Mabbott, N.A. (2017a). Influence of ageing on the microarchitecture of the spleen and lymph nodes. Biogerontology 18, 723–738.
Turner, V.M., and Mabbott, N.A. (2017b). Structural and functional changes to lymph nodes in ageing mice. Immunology 151, 239–247.
Tzemah-Shahar, R., Hochner, H., Iktilat, K., and Agmon, M. (2022). What can we learn from physical capacity about biological age? A systematic review. Ageing Res Rev 77, 101609.
Uddin, M.N., Nishio, N., Ito, S., Suzuki, H., and Isobe, K. (2012). Autophagic activity in thymus and liver during aging. Age 34, 75–85.
Uesugi, N., Shimazu, Y., Kikuchi, K., and Nagata, M. (2016). Age-related renal microvascular changes: evaluation by three-dimensional digital imaging of the human renal microcirculation using virtual microscopy. Int J Mol Sci 17, 1831.
Uezumi, A., Fukada, S., Yamamoto, N., Takeda, S., and Tsuchida, K. (2010). Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 12, 143–152.
Uezumi, A., Ito, T., Morikawa, D., Shimizu, N., Yoneda, T., Segawa, M., Yamaguchi, M., Ogawa, R., Matev, M.M., Miyagoe-Suzuki, Y., et al. (2011). Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. J Cell Sci 124, 3654–3664.
Uitto, J. (2008). The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. J Drugs Dermatol 7, s12–s16.
Ulrich, N.D., and Marsh, E.E. (2019). Ovarian reserve testing: a review of the options, their applications, and their limitations. Clin Obstet Gynecol 62, 228–237.
Umehara, T., Winstanley, Y.E., Andreas, E., Morimoto, A., Williams, E.J., Smith, K.M., Carroll, J., Febbraio, M.A., Shimada, M., Russell, D.L., et al. (2022). Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. Sci Adv 8, eabn4564.
Umemoto, T., Hashimoto, M., Matsumura, T., Nakamura-Ishizu, A., and Suda, T. (2018). $Ca2^{+}$-mitochondria axis drives cell division in hematopoietic stem cells. J Exp Med 215, 2097–2113.

Ungvari, Z., Tarantini, S., Kiss, T., Wren, J.D., Giles, C.B., Griffin, C.T., Murfee, W.L., Pacher, P., and Csiszar, A. (2018). Endothelial dysfunction and angiogenesis impairment in the ageing vasculature. Nat Rev Cardiol 15, 555–565.

Urbanek, T., Krasinski, Z., Suminska-Jasinska, K., Baum, E., Borej-Nowicka, G., Begier-Krasinska, B., and Breborowicz, A. (2016). Sulodexide reduces the inflammatory reaction and senescence of endothelial cells in conditions involving chronic venous disease. Int Angiol 35, 140–147.

Uryga, A.K., Grootaert, M.O.J., Garrido, A.M., Oc, S., Foote, K., Chappell, J., Finigan, A., Rossiello, F., d'Adda di Fagagna, F., Aravani, D., et al. (2021). Telomere damage promotes vascular smooth muscle cell senescence and immune cell recruitment after vessel injury. Commun Biol 4, 611.

Vaidya, A., Mao, Z.Y., Tian, X., Spencer, B., Seluanov, A., and Gorbunova, V. (2014). Knock-in reporter mice demonstrate that DNA repair by non-homologous end joining declines with age. PLoS Genet 10, e1004511.

Vaiserman, A., and Krasnienkov, D. (2020). Telomere length as a marker of biological age: state-of-the-art, open issues, and future perspectives. Front Genet 11, 630186.

van Beek, A.A., Van den Bossche, J., Mastroberardino, P.G., de Winther, M.P.J., and Leenen, P.J.M. (2019). Metabolic alterations in aging macrophages: ingredients for inflammaging? Trends Immunol 40, 113–127.

Van Bortel, L.M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J.K., De Backer, T., Filipovsky, J., Huybrechts, S., Mattace-Raso, F.U.S., Protogerou, A.D., et al. (2012). Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertension 30, 445–448.

van den Akker, E.B., Trompet, S., Barkey Wolf, J.J.H., Beekman, M., Suchiman, H.E.D., Deelen, J., Asselbergs, F.W., Boersma, E., Cats, D., Elders, P.M., et al. (2020). Metabolic age based on the BBMRI-NL ^{1}H-NMR metabolomics repository as biomarker of age-related disease. Circ Genom Precis Med 13, 541–547.

van der Goot, A.T., and Nollen, E.A.A. (2013). Tryptophan metabolism: entering the field of aging and age-related pathologies. Trends Mol Med 19, 336–344.

van der Horst, G.T.J.V.D., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S.I., Takao, M., Wit, J.D., Verkerk, A., Eker, A.P.M., Leenen, D. V., et al. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature 398, 627–630.

van Deursen, J.M. (2014). The role of senescent cells in ageing. Nature 509, 439–446.

van Leeuwen, N., Beekman, M., Deelen, J., van den Akker, E.B., de Craen, A.J.M., Slagboom, P.E., and 't Hart, L.M. (2014). Low mitochondrial DNA content associates with familial longevity: the Leiden Longevity Study. Age 36, 9629.

van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M.A.F.L., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., and Teule, G.J.J. (2009). Cold-activated brown adipose tissue in healthy men. N Engl J Med 360, 1500–1508.

Vandenabeele, P., Declercq, W., Beyaert, R., and Fiers, W. (1995). Two tumour necrosis factor receptors: structure and function. Trends Cell Biol 5, 392–399.

Vanderschaeghe, G. (2018). Ethical challenges in the disclosure of biomarker-based information in the early stage of Alzheimer's disease. Dissertation for Doctoral Degree. Leuven: University of Leuven.

Vanderschaeghe, G., Dierickx, K., and Vandenberghe, R. (2018). Review of the ethical issues of a biomarker-based diagnoses in the early stage of Alzheimer's disease. Bioeth Inq 15, 219–230.

VanDussen, K.L., Carulli, A.J., Keeley, T.M., Patel, S.R., Puthoff, B.J., Magness, S.T., Tran, I.T., Maillard, I., Siebel, C., Kolterud, Å., et al. (2012). Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. Development 139, 488–497.

Vanyushin, B.F., Nemirovsky, L.E., Klimenko, V.V., Vasiliev, V.K., and Belozersky, A.N. (1973). The 5-methylcytosine in DNA of rats. Gerontology 19, 138–152.

Varela-Eirin, M., Loureiro, J., Fonseca, E., Corrochano, S., Caeiro, J.R., Collado, M., and Mayan, M.D. (2018). Cartilage regeneration and ageing: Targeting cellular plasticity in osteoarthritis. Ageing Res Rev 42, 56–71.

Varela, A., and Jolette, J. (2018). Bone toolbox: biomarkers, imaging tools, biomechanics, and histomorphometry. Toxicol Pathol 46, 511–529.

Vasa, M., Breitschopf, K., Zeiher, A.M., and Dimmeler, S. (2000). Nitric oxide activates telomerase and delays endothelial cell senescence. Circ Res 87, 540–542.

Vaz Fragoso, C.A., and Lee, P.J. (2012). The aging lung. J Gerontol A Biol Sci Med Sci 67A, 233–235.

Velicescu, M., Weisenberger, D.J., Gonzales, F.A., Tsai, Y.C., Nguyen, C. T., and Jones, P.A. (2002). Cell division is required for de novo methylation of CpG islands in bladder cancer cells. Cancer Res 62, 2378–2384.

Velickovic, K., Wayne, D., Leija, H.A.L., Bloor, I., Morris, D.E., Law, J., Budge, H., Sacks, H., Symonds, M.E., and Sottile, V. (2019). Caffeine exposure induces browning features in adipose tissue *in vitro* and *in vivo*. Sci Rep 9, 9104.

Vellas, B., Balas, D., Moreau, J., Bouisson, M., Senegas-Balas, F., Guidet, M., and Ribet, A. (1988). Exocrine pancreatic secretion in the elderly. Int J Pancreatol 3, 497–502.

Vemuri, P., Lesnick, T.G., Knopman, D.S., Przybelski, S.A., Reid, R.I., Mielke, M.M., Graff-Radford, J., Lowe, V.J., Machulda, M.M., Petersen, R.C., et al. (2019). Amyloid, vascular, and resilience pathways associated with cognitive aging. Ann Neurol 86, 866–877.

Venkataram, S., Dunn, B., Li, Y., Agarwala, A., Chang, J., Ebel, E.R., Geiler-Samerotte, K., Hérissant, L., Blundell, J.R., Levy, S.F., et al. (2016). Development of a comprehensive genotype-to-fitness map of adaptation-driving mutations in yeast. Cell 166, 1585–1596.e22.

Venturelli, S., Berger, A., Weiland, T., Essmann, F., Waibel, M., Nuebling, T., Häcker, S., Schenk, M., Schulze-Osthoff, K., Salih, H.R., et al. (2013). Differential induction of apoptosis and senescence by the DNA methyltransferase inhibitors 5-azacytidine and 5-aza-2′-deoxycytidine in solid tumor cells. Mol Cancer Ther 12, 2226–2236.

Verbeken, E.K., Cauberghs, M., Mertens, I., Clement, J., Lauweryns, J.M., and Van de Woestijne, K.P. (1992). The senile lung. Chest 101, 800–809.

Verdijk, L.B., Koopman, R., Schaart, G., Meijer, K., Savelberg, H.H.C.M., and van Loon, L.J.C. (2007). Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. Am J Physiol Endocrinol Metab 292, E151–E157.

Vergilio, M.M., Vasques, L.I., and Leonardi, G.R. (2021). Characterization of skin aging through high-frequency ultrasound imaging as a technique for evaluating the effectiveness of anti-aging products and procedures: a review. Skin Res Technol 27, 966–973.

Verma, M., Asakura, Y., Murakonda, B.S.R., Pengo, T., Latroche, C., Chazaud, B., McLoon, L.K., and Asakura, A. (2018). Muscle satellite cell cross-talk with a vascular niche maintains quiescence via VEGF and Notch signaling. Cell Stem Cell 23, 530–543.e9.

Verma, S., Tachtatzis, P., Penrhyn-Lowe, S., Scarpini, C., Jurk, D., Von Zglinicki, T., Coleman, N., and Alexander, G.J.M. (2012). Sustained telomere length in hepatocytes and cholangiocytes with increasing age in normal liver. Hepatology 56, 1510–1520.

Vermeulen, A., Verdonck, L., and Kaufman, J.M. (1999). A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84, 3666–3672.

Vermeulen, L., De Wilde, G., Van Damme, P., Vanden Berghe, W., and Haegeman, G. (2003). Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J 22, 1313–1324.

Verón, G.L., Tissera, A.D., Bello, R., Beltramone, F., Estofan, G., Molina, R.I., and Vazquez-Levin, M.H. (2018). Impact of age, clinical conditions, and lifestyle on routine semen parameters and sperm kinematics. Fertil Steril 110, 68–75.e4.

Vicentini, F.T.M.C., He, T., Shao, Y., Fonseca, M.J.V., Verri, W.A., Fisher, G.J., and Xu, Y. (2011). Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF-κB pathway. J Dermatol Sci 61, 162–168.

Victorelli, S., Lagnado, A., Halim, J., Moore, W., Talbot, D., Barrett, K.,

Chapman, J., Birch, J., Ogrodnik, M., Meves, A., et al. (2019). Senescent human melanocytes drive skin ageing via paracrine telomere dysfunction. EMBO J 38.
Vidal-Bralo, L., Lopez-Golan, Y., and Gonzalez, A. (2016). Simplified assay for epigenetic age estimation in whole blood of adults. Front Genet 7, 126.
Vijayakumar, A., Novosyadlyy, R., Wu, Y.J., Yakar, S., and LeRoith, D. (2010). Biological effects of growth hormone on carbohydrate and lipid metabolism. Growth Hormone IGF Res 20, 1–7.
Villeda, S.A., Luo, J., Mosher, K.I., Zou, B., Britschgi, M., Bieri, G., Stan, T.M., Fainberg, N., Ding, Z., Eggel, A., et al. (2011). The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477, 90–94.
Villeponteau, B. (1997). The heterochromatin loss model of aging. Exp Gerontol 32, 383–394.
Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradère, J.P., Le Gonidec, S., Dortignac, A., Geoffre, N., Pereira, O., Karaz, S., et al. (2018). The exerkine apelin reverses age-associated sarcopenia. Nat Med 24, 1360–1371.
Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N.J., Enerbäck, S., et al. (2009). Functional brown adipose tissue in healthy adults. N Engl J Med 360, 1518–1525.
Visser, J.A., de Jong, F.H., Laven, J.S.E., and Themmen, A.P.N. (2006). Anti-Mullerian hormone: a new marker for ovarian function. Reproduction 131, 1–9.
Viswanath, P., Batsios, G., Ayyappan, V., Taglang, C., Gillespie, A.M., Larson, P.E.Z., Luchman, H.A., Costello, J.F., Pieper, R.O., and Ronen, S.M. (2021). Metabolic imaging detects elevated glucose flux through the pentose phosphate pathway associated with TERT expression in low-grade gliomas. Neuro Oncol 23, 1509–1522.
Vizioli, M.G., Liu, T., Miller, K.N., Robertson, N.A., Gilroy, K., Lagnado, A.B., Perez-Garcia, A., Kiourtis, C., Dasgupta, N., Lei, X., et al. (2020). Mitochondria-to-nucleus retrograde signaling drives formation of cytoplasmic chromatin and inflammation in senescence. Genes Dev 34, 428–445.
Vogel, J.W., Varga Doležalová, M., La Joie, R., Marks, S.M., Schwimmer, H.D., Landau, S.M., and Jagust, W.J. (2017). Subjective cognitive decline and β-amyloid burden predict cognitive change in healthy elderly. Neurology 89, 2002–2009.
Vollmar, B., Pradarutti, S., Richter, S., and Menger, M.D. (2002). *In vivo* quantification of ageing changes in the rat liver from early juvenile to senescent life. Liver 22, 330–341.
Volpato, S., Bianchi, L., Cherubini, A., Landi, F., Maggio, M., Savino, E., Bandinelli, S., Ceda, G.P., Guralnik, J.M., Zuliani, G., et al. (2014). Prevalence and clinical correlates of sarcopenia in community-dwelling older people: application of the EWGSOP definition and diagnostic algorithm. J Gerontol A Biol Sci Med Sci 69, 438–446.
von Zglinicki, T., Saretzki, G., Ladhoff, J., Fagagna, F., and Jackson, S.P. (2005). Human cell senescence as a DNA damage response. Mech Ageing Dev 126, 111–117.
Voncken, J.W., Niessen, H., Neufeld, B., Rennefahrt, U., Dahlmans, V., Kubben, N., Holzer, B., Ludwig, S., and Rapp, U.R. (2005). MAPKAP kinase 3pK phosphorylates and regulates chromatin association of the polycomb group protein Bmi1. J Biol Chem 280, 5178–5187.
Vu, R., Jin, S., Sun, P., Haensel, D., Nguyen, Q.H., Dragan, M., Kessenbrock, K., Nie, Q., and Dai, X. (2022). Wound healing in aged skin exhibits systems-level alterations in cellular composition and cell-cell communication. Cell Rep 40, 111155.
Vyas, C.M., Ogata, S., Reynolds, C.F., Mischoulon, D., Chang, G., Cook, N.R., Manson, J.A.E., Crous-Bou, M., De Vivo, I., and Okereke, O.I. (2020). Lifestyle and behavioral factors and mitochondrial DNA copy number in a diverse cohort of mid-life and older adults. PLoS ONE 15, e0237235.
Waaijer, M.E.C., Parish, W.E., Strongitharm, B.H., van Heemst, D., Slagboom, P.E., de Craen, A.J.M., Sedivy, J.M., Westendorp, R.G.J., Gunn, D.A., and Maier, A.B. (2012). The number of $p16^{INK4a}$ positive cells in human skin reflects biological age. Aging Cell 11, 722–725.
Waddington, C.H. (2012). The epigenotype. Int J Epidemiol 41, 10–13.
Wade, M., Wang, Y.V., and Wahl, G.M. (2010). The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol 20, 299–309.
Wagner, K.D., Ying, Y., Leong, W., Jiang, J., Hu, X., Chen, Y., Michiels, J. F., Lu, Y., Gilson, E., Wagner, N., et al. (2017). The differential spatiotemporal expression pattern of shelterin genes throughout lifespan. Aging 9, 1219–1232.
Wakabayashi, H., Nishiyama, Y., Ushiyama, T., Maeba, T., and Maeta, H. (2002). Evaluation of the effect of age on functioning hepatocyte mass and liver blood flow using liver scintigraphy in preoperative estimations for surgical patients: comparison with CT volumetry. J Surg Res 106, 246–253.
Waldera Lupa, D.M., Kalfalah, F., Safferling, K., Boukamp, P., Poschmann, G., Volpi, E., Götz-Rösch, C., Bernerd, F., Haag, L., Huebenthal, U., et al. (2015). Characterization of skin aging-associated secreted proteins (SAASP) produced by dermal fibroblasts isolated from intrinsically aged human skin. J Invest Dermatol 135, 1954–1968.
Walter, C.A., Intano, G.W., McCarrey, J.R., McMahan, C.A., and Walter, R. B. (1998). Mutation frequency declines during spermatogenesis in young mice but increases in old mice. Proc Natl Acad Sci USA 95, 10015–10019.
Walters, M.N.I. (1966). Adipose atrophy of the exocrine pancreas. J Pathol 92, 547–557.
Walther, D.M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., Mann, M., et al. (2015). Widespread proteome remodeling and aggregation in aging *C. elegans*. Cell 161, 919–932.
Wan, J., Wu, X., Chen, H., Xia, X., Song, X., Chen, S., Lu, X., Jin, J., Su, Q., Cai, D., et al. (2020). Aging-induced aberrant RAGE/PPARα axis promotes hepatic steatosis via dysfunctional mitochondrial β oxidation. Aging Cell 19, e13238.
Wan, L., Xu, K., Wei, Y., Zhang, J., Han, T., Fry, C., Zhang, Z., Wang, Y.V., Huang, L., Yuan, M., et al. (2018). Phosphorylation of EZH2 by AMPK suppresses PRC2 methyltransferase activity and oncogenic function. Mol Cell 69, 279–291.e5.
Wang, B., Zhang, L., Dai, T., Qin, Z., Lu, H., Zhang, L., and Zhou, F. (2021a). Liquid-liquid phase separation in human health and diseases. Sig Transduct Target Ther 6, 290.
Wang, C., Tang, H., Geng, A., Dai, B., Zhang, H., Sun, X., Chen, Y., Qiao, Z., Zhu, H., Yang, J., et al. (2020a). Rational combination therapy for hepatocellular carcinoma with PARP1 and DNA-PK inhibitors. Proc Natl Acad Sci USA 117, 26356–26365.
Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., and Von Zglinicki, T. (2009). DNA damage response and cellular senescence in tissues of aging mice. Aging Cell 8, 311–323.
Wang, G.L., Salisbury, E., Shi, X., Timchenko, L., Medrano, E.E., and Timchenko, N.A. (2008). HDAC1 cooperates with C/EBPα in the inhibition of liver proliferation in old mice. J Biol Chem 283, 26169–26178.
Wang, H., Chen, Y.E., and Eitzman, D.T. (2014a). Imaging body fat: techniques and cardiometabolic implications. Arterioscler Thromb Vasc Biol 34, 2217–2223.
Wang, H., Diao, D., Shi, Z., Zhu, X., Gao, Y., Gao, S., Liu, X., Wu, Y., Rudolph, K.L., Liu, G., et al. (2016a). SIRT6 controls hematopoietic stem cell homeostasis through epigenetic regulation of Wnt signaling. Cell Stem Cell 18, 495–507.
Wang, H., Guan, J., Lin, J., Zhang, Z., Li, S., Guo, Y., and Cai, H. (2018a). Diffusion-weighted and magnetization transfer imaging in testicular spermatogenic function evaluation: preliminary results. J Magn Reson Imag 47, 186–190.
Wang, H., Zheng, C., Lu, Y., Jiang, Q., Yin, R., Zhu, P., Zhou, M., and Liu, Z. (2017a). Urinary fibrinogen as a predictor of progression of CKD. Clin J Am Soc Nephrol 12, 1922–1929.
Wang, J., Zhu, Q., Cao, D., Peng, Q., Zhang, X., Li, C., Zhang, C., Zhou, B. O., and Yue, R. (2023). Bone marrow-derived IGF-1 orchestrates maintenance and regeneration of the adult skeleton. Proc Natl Acad Sci USA 120, e2203779120.
Wang, J. (2001). DNA damage and apoptosis. Cell Death Differ 8, 1047–

1048.
Wang, K., Liu, H., Hu, Q., Wang, L., Liu, J., Zheng, Z., Zhang, W., Ren, J., Zhu, F., and Liu, G.H. (2022a). Epigenetic regulation of aging: implications for interventions of aging and diseases. Sig Transduct Target Ther 7, 374.
Wang, K., Wang, X., Zheng, S., Niu, Y., Zheng, W., Qin, X., Li, Z., Luo, J., Jiang, W., Zhou, X., et al. (2018b). iTRAQ-based quantitative analysis of age-specific variations in salivary proteome of caries-susceptible individuals. J Transl Med 16, 293.
Wang, L., Jia, H., Lin, G., and Zheng, S. (2021b). Magnetic resonance imaging investigation of age-related morphological changes in the pancreases of 226 Chinese. Aging Med 4, 297–303.
Wang, L., Li, H., Liang, Y., Zhang, J., Li, X., Shu, N., Wang, Y.Y., and Zhang, Z. (2013a). Amnestic mild cognitive impairment: topological reorganization of the default-mode network. Radiology 268, 501–514.
Wang, L., Liu, J., Liu, H., Yazawa, M., and Zhu, F. (2022b). The secret of youth: how is systemic rejuvenation achieved at the single cell level? Life Med 1, 258–260.
Wang, L., Liu, T., Liang, R., Wang, G., Liu, Y., Zou, J., Liu, N., Zhang, B., Liu, Y., Ding, X., et al. (2020b). Mesenchymal stem cells ameliorate β cell dysfunction of human type 2 diabetic islets by reversing β cell dedifferentiation. Ebiomedicine 51, 102615.
Wang, M., and Lemos, B. (2019). Ribosomal DNA harbors an evolutionarily conserved clock of biological aging. Genome Res 29, 325–333.
Wang, M., Takagi, G., Asai, K., Resuello, R.G., Natividad, F.F., Vatner, D. E., Vatner, S.F., and Lakatta, E.G. (2003). Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. Hypertension 41, 1308–1316.
Wang, M., Wang, L., Qian, M., Tang, X., Liu, Z., Lai, Y., Ao, Y., Huang, Y., Meng, Y., Shi, L., et al. (2020c). PML2-mediated thread-like nuclear bodies mark late senescence in Hutchinson-Gilford progeria syndrome. Aging Cell 19, e13147.
Wang, M.J., Chen, F., Li, J.X., Liu, C.C., Zhang, H.B., Xia, Y., Yu, B., You, P., Xiang, D., Lu, L., et al. (2014b). Reversal of hepatocyte senescence after continuous *in vivo* cell proliferation. Hepatology 60, 349–361.
Wang, N., He, Y., Liu, S., Makarcyzk, M.J., Lei, G., Chang, A., Alexander, P.G., Hao, T., Padget, A.M., de Pedro, N., et al. (2022c). Engineering osteoarthritic cartilage model through differentiating senescent human mesenchymal stem cells for testing disease-modifying drugs. Sci China Life Sci 65, 309–327.
Wang, Q., Sawyer, I.A., Sung, M.H., Sturgill, D., Shevtsov, S.P., Pegoraro, G., Hakim, O., Baek, S., Hager, G.L., and Dundr, M. (2016b). Cajal bodies are linked to genome conformation. Nat Commun 7, 10966.
Wang, S., Hu, S., and Mao, Y. (2021c). The mechanisms of vascular aging. Aging Med 4, 153–158.
Wang, S., Su, J.H., Beliveau, B.J., Bintu, B., Moffitt, J.R., Wu, C., and Zhuang, X. (2016c). Spatial organization of chromatin domains and compartments in single chromosomes. Science 353, 598–602.
Wang, S., Sun, Q., Xiang, B., and Li, X.J. (2013b). Pancreatic islet cell autophagy during aging in rats. Clin Invest Med 36, 72.
Wang, S., Yao, X., Ma, S., Ping, Y., Fan, Y., Sun, S., He, Z., Shi, Y., Sun, L., Xiao, S., et al. (2021d). A single-cell transcriptomic landscape of the lungs of patients with COVID-19. Nat Cell Biol 23, 1314–1328.
Wang, S., Zheng, Y., Li, J., Yu, Y., Zhang, W., Song, M., Liu, Z., Min, Z., Hu, H., Jing, Y., et al. (2020d). Single-cell transcriptomic atlas of primate ovarian aging. Cell 180, 585–600.e19.
Wang, T., Tsui, B., Kreisberg, J.F., Robertson, N.A., Gross, A.M., Yu, M. K., Carter, H., Brown-Borg, H.M., Adams, P.D., and Ideker, T. (2017b). Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. Genome Biol 18, 57.
Wang, T., Zhang, M., Jiang, Z., and Seli, E. (2017c). Mitochondrial dysfunction and ovarian aging. Am J Reprod Immunol 77, e12651.
Wang, T.W., Johmura, Y., Suzuki, N., Omori, S., Migita, T., Yamaguchi, K., Hatakeyama, S., Yamazaki, S., Shimizu, E., Imoto, S., et al. (2022d). Blocking PD-L1-PD-1 improves senescence surveillance and ageing phenotypes. Nature 611, 358–364.
Wang, W., Ishibashi, J., Trefely, S., Shao, M., Cowan, A.J., Sakers, A., Lim, H.W., O'Connor, S., Doan, M.T., Cohen, P., et al. (2019). A PRDM16-driven metabolic signal from adipocytes regulates precursor cell fate. Cell Metab 30, 174–189.e5.
Wang, W., Zheng, Y., Sun, S., Li, W., Song, M., Ji, Q., Wu, Z., Liu, Z., Fan, Y., Liu, F., et al. (2021e). A genome-wide CRISPR-based screen identifies *KAT7* as a driver of cellular senescence. Sci Transl Med 13.
Wang, X., Cairns, B.R., and Guo, J. (2022e). When spermatogenesis meets human aging and elevated body mass. Life Med 1, 267–269.
Wang, X., Feng, Y., Pan, L., Wang, Y., Xu, X., Lu, J., and Huang, B. (2007). The proximal GC-rich region of $p16^{INK4a}$ gene promoter plays a role in its transcriptional regulation. Mol Cell Biochem 301, 259–266.
Wang, X., Jin, L., Mao, Y., Shi, J., Huang, R., Jiang, Y., Zhang, C., and Liang, X. (2021f). Evaluation of ovarian reserve tests and age in the prediction of poor ovarian response to controlled ovarian stimulation—a real-world data analysis of 89,002 patients. Front Endocrinol 12, 702061.
Wang, X.J., Shen, C.L., Dyson, M.T., Eimerl, S., Orly, J., Hutson, J.C., and Stocco, D.M. (2005). Cyclooxygenase-2 regulation of the age-related decline in testosterone biosynthesis. Endocrinology 146, 4202–4208.
Wang, X., Vrtiska, T.J., Avula, R.T., Walters, L.R., Chakkera, H.A., Kremers, W.K., Lerman, L.O., and Rule, A.D. (2014c). Age, kidney function, and risk factors associate differently with cortical and medullary volumes of the kidney. Kidney Int 85, 677–685.
Wang, Y., Chang, Y.J., Xu, L.P., Liu, K.Y., Liu, D.H., Zhang, X.H., Chen, H., Han, W., Chen, Y.H., Wang, F.R., et al. (2014d). Who is the best donor for a related HLA haplotype-mismatched transplant? Blood 124, 843–850.
Wang, Y., Chen, F., Ye, L., Zirkin, B., and Chen, H. (2017d). Steroidogenesis in Leydig cells: effects of aging and environmental factors. Reproduction 154, R111–R122.
Wang, Y., and Chen, L.L. (2020). Organization and function of para-speckles. Essays Biochem 64, 875–882.
Wang, Y., Deng, P., Liu, Y., Wu, Y., Chen, Y., Guo, Y., Zhang, S., Zheng, X., Zhou, L., Liu, W., et al. (2020e). Alpha-ketoglutarate ameliorates age-related osteoporosis via regulating histone methylations. Nat Commun 11, 5596.
Wang, Y., Holbrook, C.A., Hamilton, J., Garoussian, J., Afshar, M., Su, S., Schürch, C.M., Lee, M. Y., Goltsev, Y., Kundaje, A., et al. (2022f). A single cell spatial temporal atlas of skeletal muscle reveals cellular neighborhoods that orchestrate regeneration and become disrupted in aging. bioRxiv 2022.06.10.494732.
Wang, Y., Osborne, M.T., Tung, B., Li, M., and Li, Y. (2018c). Imaging cardiovascular calcification. J Am Heart Assoc 7.
Wang, Y., Shen, M.F., and Chang, S. (2011). Essential roles for Pot1b in HSC self-renewal and survival. Blood 118, 6068–6077.
Wang, Y., Welc, S.S., Wehling-Henricks, M., and Tidball, J.G. (2018d). Myeloid cell-derived tumor necrosis factor-alpha promotes sarcopenia and regulates muscle cell fusion with aging muscle fibers. Aging Cell 17, e12828.
Wang, Y., Yuan, Y., Zhang, Y., Lei, C., Zhou, Y., He, J., and Sun, Z. (2017e). Serum 1,5-anhydroglucitol level as a screening tool for diabetes mellitus in a community-based population at high risk of diabetes. Acta Diabetol 54, 425–431.
Wang, Y.X.J. (2021). Gender-specific liver aging and magnetic resonance imaging. Quant Imag Med Surg 11, 2893–2904.
Wang, Z., Sun, F., and Xiong, W. (2022g). Fat shapes fate: unlock the destiny of a cell with single-cell metabolomics. Life Med 1, 261–263.
Wansleeben, C., Bowie, E., Hotten, D.F., Yu, Y.R.A., and Hogan, B.L.M. (2014). Age-related changes in the cellular composition and epithelial organization of the mouse trachea. PLoS ONE 9, e93496.
Warren, A., Bertolino, P., Cogger, V., Mclean, A., Fraser, R., and Couteur, D. (2005). Hepatic pseudocapillarization in aged mice. Exp Gerontol 40, 807–812.
Warren, A., Cogger, V.C., Fraser, R., DeLeve, L.D., McCuskey, R.S., and Le Couteur, D.G. (2011). The effects of old age on hepatic stellate cells. Curr Gerontol Geriatr Res 2011, 1–7.
Watanabe, A., Shibata, S., and Watanabe, S. (1995). Circadian rhythm of spontaneous neuronal activity in the suprachiasmatic nucleus of old

hamster *in vitro*. Brain Res 695, 237–239.
Watanabe, T., and Tanaka, Y. (1982). Age-related alterations in the size of human hepatocytes. Virchows Archiv B Cell Pathol 39, 9–20.
Watson, C.J., Papula, A.L., Poon, G.Y.P., Wong, W.H., Young, A.L., Druley, T.E., Fisher, D.S., and Blundell, J.R. (2020a). The evolutionary dynamics and fitness landscape of clonal hematopoiesis. Science 367, 1449–1454.
Watson, J.K., Sanders, P., Dunmore, R., Rosignoli, G., Julé, Y., Rawlins, E. L., Mustelin, T., May, R., Clarke, D., and Finch, D.K. (2020b). Distal lung epithelial progenitor cell function declines with age. Sci Rep 10, 10490.
Weber, M.H., and Verwiebe, R. (1992). Alpha 1-microglobulin (protein HC): features of a promising indicator of proximal tubular dysfunction. Eur J Clin Chem Clin Biochem 30, 683–691.
Webley, K., Bond, J.A., Jones, C.J., Blaydes, J.P., Craig, A., Hupp, T., and Wynford-Thomas, D. (2000). Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. Mol Cell Biol 20, 2803–2808.
Wei, C., Wang, M., and Wang, X.J. (2023). Evolutionary conservation analysis of human arachidonic acid metabolism pathway genes. Life Med doi: 10.1093/lifemedi/lnad004.
Wei, H., Xu, Y., Wang, Y., Xu, L., Mo, C., Li, L., Shen, B., Sun, Y., Cheng, P., Yang, L., et al. (2020). Identification of fibroblast activation protein as an osteogenic suppressor and anti-osteoporosis drug target. Cell Rep 33, 108252.
Wei, J., Xu, H., Davies, J.L., and Hemmings, G.P. (1992). Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci 51, 1953–1956.
Wei, Y., Chen, Y.H., Li, L.Y., Lang, J., Yeh, S.P., Shi, B., Yang, C.C., Yang, J.Y., Lin, C.Y., Lai, C.C., et al. (2011). CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. Nat Cell Biol 13, 87–94.
Weidner, C., Lin, Q., Koch, C., Eisele, L., Beier, F., Ziegler, P., Bauerschlag, D., Jöckel, K.H., Erbel, R., Mühleisen, T., et al. (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol 15, R24.
Weigelin, B., den Boer, A.T., Wagena, E., Broen, K., Dolstra, H., de Boer, R.J., Figdor, C.G., Textor, J., and Friedl, P. (2021). Cytotoxic T cells are able to efficiently eliminate cancer cells by additive cytotoxicity. Nat Commun 12, 5217.
Weir, G.C., Gaglia, J., and Bonner-Weir, S. (2020). Inadequate β-cell mass is essential for the pathogenesis of type 2 diabetes. Lancet Diabetes Endocrinol 8, 249–256.
Weir, H.J., Yao, P., Huynh, F.K., Escoubas, C.C., Goncalves, R.L., Burkewitz, K., Laboy, R., Hirschey, M.D., and Mair, W.B. (2017). Dietary restriction and AMPK increase lifespan via mitochondrial network and peroxisome remodeling. Cell Metab 26, 884–896.e5.
Well, D., Yang, H., Houseni, M., Iruvuri, S., Alzeair, S., Sansovini, M., Wintering, N., Alavi, A., and Torigian, D.A. (2007). Age-related structural and metabolic changes in the pelvic reproductive end organs. Semin Nucl Med 37, 173–184.
Welle, S., Bhatt, K., Shah, B., Needler, N., Delehanty, J.M., and Thornton, C.A. (2003). Reduced amount of mitochondrial DNA in aged human muscle. J Appl Physiol 94, 1479–1484.
Welsher, K., Sherlock, S.P., and Dai, H. (2011). Deep-tissue anatomical imaging of mice using carbon nanotube fluorophores in the second near-infrared window. Proc Natl Acad Sci USA 108, 8943–8948.
Wen, L., and Tang, F. (2022). Recent advances in single-cell sequencing technologies. Precis Clin Med 5, pbac002.
Wendt, R., Siwy, J., He, T., Latosinska, A., Wiech, T., Zipfel, P.F., Tserga, A., Vlahou, A., Rupprecht, H., Catanese, L., et al. (2021). Molecular mapping of urinary complement peptides in kidney diseases. Proteomes 9, 49.
Weng, N., Akbar, A.N., and Goronzy, J. (2009). $CD28^-$ T cells: their role in the age-associated decline of immune function. Trends Immunol 30, 306–312.
Werring, D.J., Frazer, D.W., Coward, L.J., Losseff, N.A., Watt, H., Cipolotti, L., Brown, M.M., and Jager, H.R. (2004). Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. Brain 127, 2265–2275.
Wertz, I.E., O'Rourke, K.M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S., Wu, P., Wiesmann, C., Baker, R., Boone, D.L., et al. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. Nature 430, 694–699.
Wesemann, D.R., Qin, H., Kokorina, N., and Benveniste, E.N. (2004). TRADD interacts with STAT1-α and influences interferon-γ signaling. Nat Immunol 5, 199–207.
West, K.L., Zuppichini, M.D., Turner, M.P., Sivakolundu, D.K., Zhao, Y., Abdelkarim, D., Spence, J.S., and Rypma, B. (2019). BOLD hemodynamic response function changes significantly with healthy aging. NeuroImage 188, 198–207.
West, M.J., Coleman, P.D., Flood, D.G., and Troncoso, J.C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 344, 769–772.
Westacott, M.J., Farnsworth, N.L., St. Clair, J.R., Poffenberger, G., Heintz, A., Ludin, N.W., Hart, N.J., Powers, A.C., and Benninger, R.K.P. (2017). Age-dependent decline in the coordinated $[Ca^{2+}]$ and insulin secretory dynamics in human pancreatic islets. Diabetes 66, 2436–2445.
Whitham, M., and Febbraio, M.A. (2016). The ever-expanding myokinome: discovery challenges and therapeutic implications. Nat Rev Drug Discov 15, 719–729.
WHO. (2021). WHO laboratory manual for the examination and processing of human semen. 6th ed. Geneva: World Health Organization.
Wigglesworth, K., Lee, K.B., O'Brien, M.J., Peng, J., Matzuk, M.M., and Eppig, J.J. (2013). Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. Proc Natl Acad Sci USA 110, E3723–E3729.
Wikler, E.M., Blendon, R.J., and Benson, J.M. (2013). Would you want to know? Public attitudes on early diagnostic testing for Alzheimer's disease. Alzheimers Res Ther 5, 43.
Wiktor-Brown, D.M., Hendricks, C.A., Olipitz, W., and Engelward, B.P. (2006). Age-dependent accumulation of recombinant cells in the mouse pancreas revealed by *in situ* fluorescence imaging. Proc Natl Acad Sci USA 103, 11862–11867.
Wiley, C.D., Flynn, J.M., Morrissey, C., Lebofsky, R., Shuga, J., Dong, X., Unger, M.A., Vijg, J., Melov, S., and Campisi, J. (2017). Analysis of individual cells identifies cell-to-cell variability following induction of cellular senescence. Aging Cell 16, 1043–1050.
Wiley, C.D., Liu, S., Limbad, C., Zawadzka, A.M., Beck, J., Demaria, M., Artwood, R., Alimirah, F., Lopez-Dominguez, J.A., Kuehnemann, C., et al. (2019). SILAC analysis reveals increased secretion of hemostasis-related factors by senescent cells. Cell Rep 28, 3329–3337.e5.
Wiley, C.D., Sharma, R., Davis, S.S., Lopez-Dominguez, J.A., Mitchell, K. P., Wiley, S., Alimirah, F., Kim, D.E., Payne, T., Rosko, A., et al. (2021). Oxylipin biosynthesis reinforces cellular senescence and allows detection of senolysis. Cell Metab 33, 1124–1136.e5.
Wiley, C.D., Velarde, M.C., Lecot, P., Liu, S., Sarnoski, E.A., Freund, A., Shirakawa, K., Lim, H.W., Davis, S.S., Ramanathan, A., et al. (2016). Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. Cell Metab 23, 303–314.
Wilkinson, A.C., Igarashi, K.J., and Nakauchi, H. (2020). Haematopoietic stem cell self-renewal *in vivo* and *ex vivo*. Nat Rev Genet 21, 541–554.
Wilkinson, D.J., Piasecki, M., and Atherton, P.J. (2018). The age-related loss of skeletal muscle mass and function: measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. Ageing Res Rev 47, 123–132.
Willcox, B.J., Donlon, T.A., He, Q., Chen, R., Grove, J.S., Yano, K., Masaki, K.H., Willcox, D.C., Rodriguez, B., and Curb, J.D. (2008). FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci USA 105, 13987–13992.
Williams, S.A., Kivimaki, M., Langenberg, C., Hingorani, A.D., Casas, J. P., Bouchard, C., Jonasson, C., Sarzynski, M.A., Shipley, M.J., Alexander, L., et al. (2019). Plasma protein patterns as comprehensive indicators of health. Nat Med 25, 1851–1857.
Williamson, E.J., Walker, A.J., Bhaskaran, K., Bacon, S., Bates, C.,

Morton, C.E., Curtis, H.J., Mehrkar, A., Evans, D., Inglesby, P., et al. (2020). Factors associated with COVID-19-related death using OpenSAFELY. Nature 584, 430–436.
Wilmanski, T., Diener, C., Rappaport, N., Patwardhan, S., Wiedrick, J., Lapidus, J., Earls, J.C., Zimmer, A., Glusman, G., Robinson, M., et al. (2021). Gut microbiome pattern reflects healthy ageing and predicts survival in humans. Nat Metab 3, 274–286.
Wilson, A., Laurenti, E., Oser, G., van der Wath, R.C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C.F., Eshkind, L., Bockamp, E., et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118–1129.
Wilson, V.L., and Jones, P.A. (1983). DNA methylation decreases in aging but not in immortal cells. Science 220, 1055–1057.
Wilson, V.L., Smith, R.A., Ma, S., and Cutler, R.G. (1987). Genomic 5-methyldeoxycytidine decreases with age. J Biol Chem 262, 9948–9951.
Wingo, A.P., Dammer, E.B., Breen, M.S., Logsdon, B.A., Duong, D.M., Troncosco, J.C., Thambisetty, M., Beach, T.G., Serrano, G.E., Reiman, E.M., et al. (2019). Large-scale proteomic analysis of human brain identifies proteins associated with cognitive trajectory in advanced age. Nat Commun 10, 1619.
Wishart, D.S., Feunang, Y.D., Marcu, A., Guo, A.C., Liang, K., Vázquez-Fresno, R., Sajed, T., Johnson, D., Li, C., Karu, N., et al. (2018). HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res 46, D608–D617.
Wojda, A., Zietkiewicz, E., and Witt, M. (2007). Effects of age and gender on micronucleus and chromosome nondisjunction frequencies in centenarians and younger subjects. Mutagenesis 22, 195–200.
Wolf, K.N., Wildt, D.E., Vargas, A., Marinari, P.E., Kreeger, J.S., Ottinger, M.A., and Howard, J.G. (2000). Age-dependent changes in sperm production, semen quality, and testicular volume in the black-footed ferret (*Mustela nigripes*). Biol Reprod 63, 179–187.
Wollheim, C.B. (2000). Beta-cell mitochondria in the regulation of insulin secretion: a new culprit in type II diabetes. Diabetologia 43, 265–277.
Wollheim, C.B., and Maechler, P. (2002). β-cell mitochondria and insulin secretion: messenger role of nucleotides and metabolites. Diabetes 51, S37–S42.
Wong, C.K., Smith, C.A., Sakamoto, K., Kaminski, N., Koff, J.L., and Goldstein, D.R. (2017). Aging impairs alveolar macrophage phagocytosis and increases influenza-induced mortality in mice. J Immunol 199, 1060–1068.
Wong, L.Y.R., Zheng, J., Wilhelmsen, K., Li, K., Ortiz, M.E., Schnicker, N. J., Thurman, A., Pezzulo, A.A., Szachowicz, P.J., Li, P., et al. (2022). Eicosanoid signalling blockade protects middle-aged mice from severe COVID-19. Nature 605, 146–151.
Wood, M., and Rajkovic, A. (2013). Genomic markers of ovarian reserve. Semin Reprod Med 31, 399–415.
Wosczyna, M.N., Konishi, C.T., Perez Carbajal, E.E., Wang, T.T., Walsh, R.A., Gan, Q., Wagner, M.W., and Rando, T.A. (2019). Mesenchymal stromal cells are required for regeneration and homeostatic maintenance of skeletal muscle. Cell Rep 27, 2029–2035.e5.
Wosczyna, M.N., Perez Carbajal, E.E., Wagner, M.W., Paredes, S., Konishi, C.T., Liu, L., Wang, T.T., Walsh, R.A., Gan, Q., Morrissey, C.S., et al. (2021). Targeting microRNA-mediated gene repression limits adipogenic conversion of skeletal muscle mesenchymal stromal cells. Cell Stem Cell 28, 1323–1334.e8.
Wu, F.C.W., Tajar, A., Pye, S.R., Silman, A.J., Finn, J.D., O'Neill, T.W., Bartfai, G., Casanueva, F., Forti, G., Giwercman, A., et al. (2008). Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab 93, 2737–2745.
Wu, H., Balsbaugh, J.L., Chandler, H., Georgilis, A., Zullow, H., Shabanowitz, J., Hunt, D.F., Gil, J., Peters, G., and Bernstein, E. (2013). Mitogen-activated protein kinase signaling mediates phosphorylation of polycomb ortholog Cbx7. J Biol Chem 288, 36398–36408.
Wu, H., Luo, C., Rong, Y., Yu, R., Zhao, Y., Yan, L., and Wang, H. (2023). Elucidating the dynamics of polypeptide hormones in the physiological and preeclampsic placental trophoblast cells across gestation at single-cell level. Life Med doi: 10.1093/lifemedi/lnad003.
Wu, L., Li, X.Q., Chang, D.Y., Zhang, H., Li, J.J., Wu, S.L., Zhang, L.X., Chen, M., and Zhao, M.H. (2020a). Associations of urinary epidermal growth factor and monocyte chemotactic protein-1 with kidney involvement in patients with diabetic kidney disease. Nephrol Dial Transplant 35, 291–297.
Wu, M., Junker, D., Branca, R.T., and Karampinos, D.C. (2020b). Magnetic resonance imaging techniques for brown adipose tissue detection. Front Endocrinol 11, 421.
Wu, M., Ye, H., Shao, C., Zheng, X., Li, Q., Wang, L., Zhao, M., Lu, G., Chen, B., Zhang, J., et al. (2017). Metabolomics-proteomics combined approach identifies differential metabolism-associated molecular events between senescence and apoptosis. J Proteome Res 16, 2250–2261.
Wu, S., Yu, Y., Liu, C., Zhang, X., Zhu, P., Peng, Y., Yan, X., Li, Y., Hua, P., Li, Q., et al. (2022a). Single-cell transcriptomics reveals lineage trajectory of human scalp hair follicle and informs mechanisms of hair graying. Cell Discov 8, 49.
Wu, Y., Yang, L., Su, T., Wang, C., Liu, G., and Li, X. (2010). Pathological significance of a panel of urinary biomarkers in patients with drug-induced tubulointerstitial nephritis. Clin J Am Soc Nephrol 5, 1954–1959.
Wu, Z., Shi, Y., Lu, M., Song, M., Yu, Z., Wang, J., Wang, S., Ren, J., Yang, Y.G., Liu, G.H., et al. (2020c). METTL3 counteracts premature aging via m6A-dependent stabilization of MIS12 mRNA. Nucleic Acids Res 48, 11083–11096.
Wu, Z., Wang, S., Belmonte, J.C.I., Zhang, W., Qu, J., and Liu, G.H. (2022b). Emerging role of RNA m^6A modification in aging regulation. Curr Med 1, 8.
Wu, Z., Zhang, W., Song, M., Wang, W., Wei, G., Li, W., Lei, J., Huang, Y., Sang, Y., Chan, P., et al. (2018). Differential stem cell aging kinetics in Hutchinson-Gilford progeria syndrome and Werner syndrome. Protein Cell 9, 333–350.
Wyckelsma, V.L., Levinger, I., McKenna, M.J., Formosa, L.E., Ryan, M.T., Petersen, A.C., Anderson, M.J., and Murphy, R.M. (2017). Preservation of skeletal muscle mitochondrial content in older adults: relationship between mitochondria, fibre type and high-intensity exercise training. J Physiol 595, 3345–3359.
Wynn, T.A. (2008). Cellular and molecular mechanisms of fibrosis. J Pathol 214, 199–210.
Wynn, T., and Barron, L. (2010). Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 30, 245–257.
Wynne, H.A., Cope, L.H., Mutch, E., Rawlins, M.D., Woodhouse, K.W., and James, O.F.W. (1989). The effect of age upon liver volume and apparent liver blood flow in healthy man. Hepatology 9, 297–301.
Xia, K., Chen, H., Wang, J., Feng, X., Gao, Y., Wang, Y., Deng, R., Wu, C., Luo, P., Zhang, M., et al. (2020a). Restorative functions of Autologous Stem Leydig Cell transplantation in a Testosterone-deficient non-human primate model. Theranostics 10, 8705–8720.
Xia, X., Chen, X., Wu, G., Li, F., Wang, Y., Chen, Y., Chen, M., Wang, X., Chen, W., Xian, B., et al. (2020b). Three-dimensional facial-image analysis to predict heterogeneity of the human ageing rate and the impact of lifestyle. Nat Metab 2, 946–957.
Xiao, F.H., Chen, X.Q., He, Y.H., and Kong, Q.P. (2018a). Accelerated DNA methylation changes in middle-aged men define sexual dimorphism in human lifespans. Clin Epigenet 10, 133.
Xiao, F.H., Chen, X.Q., Yu, Q., Ye, Y., Liu, Y.W., Yan, D., Yang, L.Q., Chen, G., Lin, R., Yang, L., et al. (2018b). Transcriptome evidence reveals enhanced autophagy-lysosomal function in centenarians. Genome Res 28, 1601–1610.
Xiao, F.H., Wang, H.T., and Kong, Q.P. (2019). Dynamic DNA methylation during aging: a "prophet" of age-related outcomes. Front Genet 10, 107.
Xiao, F.H., Yu, Q., Deng, Z.L., Yang, K., Ye, Y., Ge, M.X., Yan, D., Wang, H.T., Chen, X.Q., Yang, L.Q., et al. (2022). ETS1 acts as a regulator of human healthy aging via decreasing ribosomal activity. Sci Adv 8, eabf2017.
Xiao, J., Lin, H., Luo, X., Luo, X., and Wang, Z. (2011). *miR-605* joins p53 network to form a p53:*miR-605*:Mdm2 positive feedback loop in response to stress. EMBO J 30, 524–532.

Xie, M., Lu, C., Wang, J., McLellan, M.D., Johnson, K.J., Wendl, M.C., McMichael, J.F., Schmidt, H.K., Yellapantula, V., Miller, C.A., et al. (2014). Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 20, 1472–1478.
Xie, Y., Chen, D., Jiang, K., Song, L., Qian, N., Du, Y., Yang, Y., Wang, F., and Chen, T. (2022). Hair shaft miniaturization causes stem cell depletion through mechanosensory signals mediated by a Piezo1-calcium-TNF-α axis. Cell Stem Cell 29, 70–85.e6.
Xin, H., Liu, D., Wan, M., Safari, A., Kim, H., Sun, W., O'Connor, M.S., and Songyang, Z. (2007). TPP1 is a homologue of ciliate TEBP-β and interacts with POT1 to recruit telomerase. Nature 445, 559–562.
Xiong, F., and Frasch, W.D. (2021). ΩqPCR measures telomere length from single-cells in base pair units. Nucleic Acids Res 49, e120.
Xu, D., Zhao, H., Jin, M., Zhu, H., Shan, B., Geng, J., Dziedzic, S.A., Amin, P., Mifflin, L., Naito, M.G., et al. (2020). Modulating TRADD to restore cellular homeostasis and inhibit apoptosis. Nature 587, 133–138.
Xu, H., Beasley, M.D., Warren, W.D., van der Horst, G.T.J., and McKay, M.J. (2005). Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell 8, 949–961.
Xu, H., Zhang, M., Zhang, H., Alpadi, K., Wang, L., Li, R., and Qiao, J. (2021). Clinical applications of serum anti-Müllerian hormone measurements in both males and females: an update. Innovation 2, 100091.
Xu, L., Li, Y., Finkel, T., and Ma, X. (2022). Local hyperthermia therapy: a novel strategy turning white fat brown. Life Med 1, 78–80.
Xu, L., Ma, X., Verma, N.K., Wang, D., Gavrilova, O., Proia, R.L., Finkel, T., and Mueller, E. (2018a). Ablation of PPARγ in subcutaneous fat exacerbates age-associated obesity and metabolic decline. Aging Cell 17, e12721.
Xu, L.P., Wang, S.Q., Ma, Y.R., Gao, S.J., Cheng, Y.F., Zhang, Y.Y., Mo, W.J., Mo, X.D., Zhang, Y.P., Yan, C.H., et al. (2019). Who is the best haploidentical donor for acquired severe aplastic anemia? Experience from a multicenter study. J Hematol Oncol 12, 87.
Xu, M., Pirtskhalava, T., Farr, J.N., Weigand, B.M., Palmer, A.K., Weivoda, M.M., Inman, C.L., Ogrodnik, M.B., Hachfeld, C.M., Fraser, D.G., et al. (2018b). Senolytics improve physical function and increase lifespan in old age. Nat Med 24, 1246–1256.
Xu, X., Chen, X., Zhang, X., Liu, Y., Wang, Z., Wang, P., Du, Y., Qin, Y., and Chen, Z.J. (2017). Impaired telomere length and telomerase activity in peripheral blood leukocytes and granulosa cells in patients with biochemical primary ovarian insufficiency. Hum Reprod 32, 201–207.
Xu, X., Hueckstaedt, L.K., and Ren, J. (2013). Deficiency of insulin-like growth factor 1 attenuates aging-induced changes in hepatic function: role of autophagy. J Hepatol 59, 308–317.
Xu, X., Laird, N., Dockery, D.W., Schouten, J.P., Rijcken, B., and Weiss, S. T. (1995). Age, period, and cohort effects on pulmonary function in a 24-year longitudinal study. Am J Epidemiol 141, 554–566.
Xu, Z., Zhang, L., Zhang, W., Meng, D., Zhang, H., Jiang, Y., Xu, X., Van Meter, M., Seluanov, A., Gorbunova, V., et al. (2015). SIRT6 rescues the age related decline in base excision repair in a PARP1-dependent manner. Cell Cycle 14, 269–276.
Xue, Q.L. (2011). The frailty syndrome: definition and natural history. Clin Geriatric Med 27, 1–15.
Yagi, M., Kabata, M., Tanaka, A., Ukai, T., Ohta, S., Nakabayashi, K., Shimizu, M., Hata, K., Meissner, A., Yamamoto, T., et al. (2020). Identification of distinct loci for de novo DNA methylation by DNMT3A and DNMT3B during mammalian development. Nat Commun 11, 3199.
Yalcin, A., Silay, K., Balik, A.R., Avcioğlu, G., and Aydin, A.S. (2018). The relationship between plasma interleukin-15 levels and sarcopenia in outpatient older people. Aging Clin Exp Res 30, 783–790.
Yamakoshi, K., Takahashi, A., Hirota, F., Nakayama, R., Ishimaru, N., Kubo, Y., Mann, D.J., Ohmura, M., Hirao, A., Saya, H., et al. (2009). Real-time *in vivo* imaging of $p16^{Ink4a}$ reveals cross talk with p53. J Cell Biol 186, 393–407.
Yamamuro, T., Kawabata, T., Fukuhara, A., Saita, S., Nakamura, S., Takeshita, H., Fujiwara, M., Enokidani, Y., Yoshida, G., Tabata, K., et al. (2020). Age-dependent loss of adipose Rubicon promotes metabolic disorders via excess autophagy. Nat Commun 11, 4150.
Yamashita, M., and Iwama, A. (2022). Aging and clonal behavior of hematopoietic stem cells. Int J Mol Sci 23, 1948.
Yamauchi, T., and Kadowaki, T. (2013). Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. Cell Metab 17, 185–196.
Yamazaki, S., Straume, M., Tei, H., Sakaki, Y., Menaker, M., and Block, G. D. (2002). Effects of aging on central and peripheral mammalian clocks. Proc Natl Acad Sci USA 99, 10801–10806.
Yan, F., Zhao, Q., Li, Y., Zheng, Z., Kong, X., Shu, C., Liu, Y., and Shi, Y. (2022). The role of oxidative stress in ovarian aging: a review. J Ovarian Res 15, 100.
Yan, H., Wang, R., Ma, S., Huang, D., Wang, S., Ren, J., Lu, C., Chen, X., Lu, X., Zheng, Z., et al. (2023). Lineage Landscape: a comprehensive database that records lineage commitment across species. Nucleic Acids Res 51, D1061–D1066.
Yan, S., Xu, Z., Lou, F., Zhang, L., Ke, F., Bai, J., Liu, Z., Liu, J., Wang, H., Zhu, H., et al. (2015). NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. Nat Commun 6, 7652.
Yan, Y., Wang, H., Hu, M., Jiang, L., Wang, Y., Liu, P., Liang, X., Liu, J., Li, C., Lindström-Battle, A., et al. (2017). HDAC6 suppresses age-dependent ectopic fat accumulation by maintaining the proteostasis of PLIN2 in *Drosophila*. Dev Cell 43, 99–111.e5.
Yan, Z., Wang, G., and Shi, X. (2021). Advances in the Progression and Prognosis Biomarkers of Chronic Kidney Disease. Front Pharmacol 12, 785375.
Yang, D., McCrann, D.J., Nguyen, H., Hilaire, C.S., DePinho, R.A., Jones, M.R., and Ravid, K. (2007). Increased polyploidy in aortic vascular smooth muscle cells during aging is marked by cellular senescence. Aging Cell 6, 257–260.
Yang, H., Chryssikos, T., Houseni, M., Alzeair, S., Sansovini, M., Iruvuri, S., Torigian, D.A., Zhuang, H., Dadparvar, S., Basu, S., et al. (2011). The effects of aging on testicular volume and glucose metabolism: an investigation with ultrasonography and FDG-PET. Mol Imag Biol 13, 391–398.
Yang, H., Wang, H., Ren, J., Chen, Q., and Chen, Z.J. (2017). cGAS is essential for cellular senescence. Proc Natl Acad Sci USA 114, E4612–E4620.
Yang, J.H., Hayano, M., Griffin, P.T., Amorim, J.A., Bonkowski, M.S., Apostolides, J.K., Salfati, E.L., Blanchette, M., Munding, E.M., Bhakta, M., et al. (2023). Loss of epigenetic information as a cause of mammalian aging. Cell 186, 305–326.e27.
Yang, L., Lin, X., Tang, H., Fan, Y., Zeng, S., Jia, L., Li, Y., Shi, Y., He, S., Wang, H., et al. (2020). Mitochondrial DNA mutation exacerbates female reproductive aging via impairment of the NADH/NAD^+ redox. Aging Cell 19.
Yang, N., Zhang, Y., Huang, Y., Yan, J., Qian, Z., Li, H., Luo, P., Yang, Z., Luo, M., Wei, X., et al. (2022a). FGF21 at physiological concentrations regulates vascular endothelial cell function through multiple pathways. Biochim Biophys Acta 1868, 166558.
Yang, S.C., Yu, E.J., Park, J.K., Kim, T.H., Eum, J.H., Paek, S.K., Hwang, J.Y., Lyu, S.W., Kim, J.Y., Lee, W.S., et al. (2021). The ratio of mitochondrial DNA to genomic DNA copy number in cumulus cell may serve as a biomarker of embryo quality in IVF cycles. Reprod Sci 28, 2495–2502.
Yang, Y., Li, X., Zhang, T., and Xu, D. (2022b). RIP kinases and necroptosis in aging and aging-related diseases. Life Med 1, 2–20.
Yang, Y.C., Lu, F.H., Wu, J.S., and Chang, C.J. (1997). Age and sex effects on HbA1c: a study in a healthy Chinese population. Diabetes Care 20, 988–991.
Yao, R.W., Xu, G., Wang, Y., Shan, L., Luan, P.F., Wang, Y., Wu, M., Yang, L.Z., Xing, Y.H., Yang, L., et al. (2019). Nascent pre-rRNA sorting via phase separation drives the assembly of dense fibrillar components in the human nucleolus. Mol Cell 76, 767–783.e11.
Yap, K.L., Li, S., Muñoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J., and Zhou, M.M. (2010). Molecular interplay of the noncoding RNA *ANRIL* and methylated histone H3 lysine 27 by

polycomb CBX7 in transcriptional silencing of *INK4a*. Mol Cell 38, 662–674.
Yarasheski, K.E., Bhasin, S., Sinha-Hikim, I., Pak-Loduca, J., and Gonzalez-Cadavid, N.F. (2002). Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. J Nutr Health Aging 6, 343–348.
Ye, C.X., Yi, J.L., Lai, W., and Zheng, Y. (2021). Skin barrier damaging and repairing process: a new application field of dermoscopy. J Cosmet Dermatol 20, 897–905.
Ye, J., Lenain, C., Bauwens, S., Rizzo, A., Saint-Léger, A., Poulet, A., Benarroch, D., Magdinier, F., Morere, J., Amiard, S., et al. (2010). TRF2 and apollo cooperate with topoisomerase 2α to protect human telomeres from replicative damage. Cell 142, 230–242.
Ye, J., Renault, V.M., Jamet, K., and Gilson, E. (2014). Transcriptional outcome of telomere signalling. Nat Rev Genet 15, 491–503.
Yeager, T.R., Neumann, A.A., Englezou, A., Huschtscha, L.I., Noble, J.R., and Reddel, R.R. (1999). Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer Res 59, 4175–4179.
Yeh, S., Lin, C.H., Yang, Y.J., Lin, L.W., Tseng, C.W., and Yang, K.D. (2021). Higher senescence associated secretory phenotype and lower defense mediator in urinary extracellular vesicles of elders with and without Parkinson disease. Sci Rep 11, 15783.
Yen, T.C., Su, J.H., King, K.L., and Wei, Y.H. (1991). Ageing-associated 5 kb deletion in human liver mitochondrial DNA. Biochem Biophys Res Commun 178, 124–131.
Yetil, A., Anchang, B., Gouw, A.M., Adam, S.J., Zabuawala, T., Parameswaran, R., van Riggelen, J., Plevritis, S., and Felsher, D.W. (2015). p19ARF is a critical mediator of both cellular senescence and an innate immune response associated with MYC inactivation in mouse model of acute leukemia. Oncotarget 6, 3563–3577.
Yin, C., Imms, P., Cheng, M., Amgalan, A., Chowdhury, N.F., Massett, R. J., Chaudhari, N.N., Chen, X., Thompson, P.M., Bogdan, P., et al. (2023). Anatomically interpretable deep learning of brain age captures domain-specific cognitive impairment. Proc Natl Acad Sci USA 120, e2214634120.
Yin, J., Yang, L., Xie, Y., Liu, Y., Li, S., Yang, W., Xu, B., Ji, H., Ding, L., Wang, K., et al. (2018). Dkk3 dependent transcriptional regulation controls age related skeletal muscle atrophy. Nat Commun 9, 1752.
Ying, Y., Hu, X., Han, P., Mendez-Bermudez, A., Bauwens, S., Eid, R., Tan, L., Pousse, M., Giraud-Panis, M.J., Lu, Y., et al. (2022). The non-telomeric evolutionary trajectory of TRF2 in zebrafish reveals its specific roles in neurodevelopment and aging. Nucleic Acids Res 50, 2081–2095.
Ying, Z., Chen, K., Zheng, L., Wu, Y., Li, L., Wang, R., Long, Q., Yang, L., Guo, J., Yao, D., et al. (2016). Transient activation of mitoflashes modulates nanog at the early phase of somatic cell reprogramming. Cell Metab 23, 220–226.
Yokoyama, K., Fukumoto, K., Murakami, T., Harada, S., Hosono, R., Wadhwa, R., Mitsui, Y., and Ohkuma, S. (2002). Extended longevity of *Caenorhabditis elegans* by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett 516, 53–57.
Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., Miyagawa, M., Tsujisaki, M., and Saito, M. (2011). Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity 19, 1755–1760.
Yoneyama, K., Venkatesh, B.A., Bluemke, D.A., McClelland, R.L., and Lima, J.A.C. (2017). Cardiovascular magnetic resonance in an adult human population: serial observations from the multi-ethnic study of atherosclerosis. J Cardiovasc Magn Reson 19, 52.
Yoon, J., Kim, K.G., Kim, Y.J., Lim, S., Park, Y.H., Kim, D., Kang, H.T., and Lee, D.H. (2020). Distribution and characteristics of pancreatic volume using computed tomography volumetry. Healthc Inform Res 26, 321–327.
Yoshida, Y., Nakanishi, K., Daimon, M., Ishiwata, J., Sawada, N., Hirokawa, M., Kaneko, H., Nakao, T., Mizuno, Y., Morita, H., et al. (2019). Alteration of cardiac performance and serum B-type natriuretic peptide level in healthy aging. J Am Coll Cardiol 74, 1789–1800.
Yoshizawa, H., Gazes, Y., Stern, Y., Miyata, Y., and Uchiyama, S. (2014). Characterizing the normative profile of 18F-FDG PET brain imaging: sex difference, aging effect, and cognitive reserve. Psychiatry Res Neuroimag 221, 78–85.
Young, K., Eudy, E., Bell, R., Loberg, M.A., Stearns, T., Sharma, D., Velten, L., Haas, S., Filippi, M.D., and Trowbridge, J.J. (2021). Decline in IGF1 in the bone marrow microenvironment initiates hematopoietic stem cell aging. Cell Stem Cell 28, 1473–1482.e7.
Yu, B., Huo, L., Liu, Y., Deng, P., Szymanski, J., Li, J., Luo, X., Hong, C., Lin, J., and Wang, C.Y. (2018). PGC-1α controls skeletal stem cell fate and bone-fat balance in osteoporosis and skeletal aging by inducing TAZ. Cell Stem Cell 23, 193–209.e5.
Yu, B., Ma, J., Li, J., Wang, D., Wang, Z., and Wang, S. (2020a). Mitochondrial phosphatase PGAM5 modulates cellular senescence by regulating mitochondrial dynamics. Nat Commun 11, 2549.
Yu, B., Zheng, Y., Nettleton, J.A., Alexander, D., Coresh, J., and Boerwinkle, E. (2014). Serum metabolomic profiling and incident CKD among African Americans. Clin J Am Soc Nephrol 9, 1410–1417.
Yu, M., Du, H., Wang, B., Chen, J., Lu, F., Peng, S., Sun, Y., Liu, N., Sun, X., Shiyun, D., et al. (2020b). Exogenous H_2S induces Hrd1 S-sulfhydration and prevents CD36 translocation via VAMP3 ubiquitylation in diabetic hearts. Aging Dis 11, 286–300.
Yu, Y., Mutlu, A.S., Liu, H., and Wang, M.C. (2017). High-throughput screens using photo-highlighting discover BMP signaling in mitochondrial lipid oxidation. Nat Commun 8, 865.
Yu, Y., Zhang, X., Liu, F., Zhu, P., Zhang, L., Peng, Y., Yan, X., Li, Y., Hua, P., Liu, C., et al. (2021). A stress-induced miR-31-CLOCK-ERK pathway is a key driver and therapeutic target for skin aging. Nat Aging 1, 795–809.
Yu, Z., Zhai, G., Singmann, P., He, Y., Xu, T., Prehn, C., Römisch-Margl, W., Lattka, E., Gieger, C., Soranzo, N., et al. (2012). Human serum metabolic profiles are age dependent. Aging Cell 11, 960–967.
Yuan, Y., Li, J., He, Z., Fan, X., Mao, X., Yang, M., and Yang, D. (2021). tRNA-derived fragments as new hallmarks of aging and age-related diseases. Aging Dis 12, 1304–1322.
Yue, R., Shen, B., and Morrison, S.J. (2016). Clec11a/osteolectin is an osteogenic growth factor that promotes the maintenance of the adult skeleton. eLife 5.
Yura, Y., Sano, S., and Walsh, K. (2020). Clonal hematopoiesis: a new step linking inflammation to heart failure. JACC Basic Transl Sci 5, 196–207.
Yureneva, S., Averkova, V., Silachev, D., Donnikov, A., Gavisova, A., Serov, V., and Sukhikh, G. (2021). Searching for female reproductive aging and longevity biomarkers. Aging 13, 16873–16894.
Zacharski, L.R., Ornstein, D.L., Woloshin, S., and Schwartz, L.M. (2000). Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data. Am Heart J 140, 98–104.
Zamboni, M., Nori, N., Brunelli, A., and Zoico, E. (2021). How does adipose tissue contribute to inflammageing? Exp Gerontol 143, 111162.
Zbieć-Piekarska, R., Spólnicka, M., Kupiec, T., Parys-Proszek, A., Makowska, Ż., Pałeczka, A., Kucharczyk, K., Płoski, R., and Branicki, W. (2015). Development of a forensically useful age prediction method based on DNA methylation analysis. Forens Sci Int Genet 17, 173–179.
Zeng, W., Chen, Y., Zhu, Z., Gao, S., Xia, J., Chen, X., Jia, J., and Zhang, Z. (2020). Severity of white matter hyperintensities: Lesion patterns, cognition, and microstructural changes. J Cereb Blood Flow Metab 40, 2454–2463.
Zhai, C., Fang, G., Liu, W., Wu, T., Miao, L., Zhang, L., Ma, J., Zhang, Y., Zong, C., Zhang, S., et al. (2021). Robust scalable-manufactured smart fabric surfaces based on azobenzene-containing maleimide copolymers for rewritable information storage and hydrogen fluoride visual sensor. ACS Appl Mater Interfaces 13, 42024–42034.
Zhan, X., Yang, J., Mao, Z., and Yu, W. (2018). PIM1-catalyzed CBX8 phosphorylation promotes the oncogene-induced senescence of human diploid fibroblast. Biochem Biophys Res Commun 501, 779–785.
Zhang, B., Long, Q., Wu, S., Xu, Q., Song, S., Han, L., Qian, M., Ren, X., Liu, H., Jiang, J., et al. (2021a). KDM4 orchestrates epigenomic

remodeling of senescent cells and potentiates the senescence-associated secretory phenotype. Nat Aging 1, 454–472.
Zhang, C., and Cuervo, A.M. (2008). Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. Nat Med 14, 959–965.
Zhang, C., and Tao, J. (2018). Expert consensus on clinical assessment and intervention of vascular aging in China (2018). Aging Med 1, 228–237.
Zhang, C., Wang, D., Wang, J., Wang, L., Qiu, W., Kume, T., Dowell, R., and Yi, R. (2021b). Escape of hair follicle stem cells causes stem cell exhaustion during aging. Nat Aging 1, 889–903.
Zhang, C., Xie, Y., Chen, H., Lv, L., Yao, J., Zhang, M., Xia, K., Feng, X., Li, Y., Liang, X., et al. (2020a). FOXO4-DRI alleviates age-related testosterone secretion insufficiency by targeting senescent Leydig cells in aged mice. Aging 12, 1272–1284.
Zhang, C., Zhou, S., Chang, H., Zhuang, F., Shi, Y., Chang, L., Ai, W., Du, J., Liu, W., Liu, H., et al. (2021c). Metabolomic profiling identified serum metabolite biomarkers and related metabolic pathways of colorectal cancer. Dis Markers 2021, 6858809.
Zhang, D., Lv, J., Tang, R., Feng, Y., Zhao, Y., Fei, X., Chian, R., and Xie, Q. (2021d). Association of exosomal microRNAs in human ovarian follicular fluid with oocyte quality. Biochem Biophys Res Commun 534, 468–473.
Zhang, F., Qiu, H., Dong, X., Zhang, X., Wang, C., Li, X., Zhang, X., Na, J., Zhou, J., and Wang, C. (2022a). Single-cell atlas of multilineage cardiac organoids derived from human induced pluripotent stem cells. Life Med 1, 179–195.
Zhang, H., Alsaleh, G., Feltham, J., Sun, Y., Napolitano, G., Riffelmacher, T., Charles, P., Frau, L., Hublitz, P., Yu, Z., et al. (2019a). Polyamines control eIF5A hypusination, TFEB translation, and autophagy to reverse B cell senescence. Mol Cell 76, 110–125.e9.
Zhang, H., Cai, B., Geng, A., Tang, H., Zhang, W., Li, S., Jiang, Y., Tan, R., Wan, X., and Mao, Z. (2020b). Base excision repair but not DNA double-strand break repair is impaired in aged human adipose-derived stem cells. Aging Cell 19.
Zhang, H., Li, J., Ren, J., Sun, S., Ma, S., Zhang, W., Yu, Y., Cai, Y., Yan, K., Li, W., et al. (2021e). Single-nucleus transcriptomic landscape of primate hippocampal aging. Protein Cell 12, 695–716.
Zhang, H., Menzies, K.J., and Auwerx, J. (2018a). The role of mitochondria in stem cell fate and aging. Development 145.
Zhang, H., Ryu, D., Wu, Y., Gariani, K., Wang, X., Luan, P., D'Amico, D., Ropelle, E.R., Lutolf, M.P., Aebersold, R., et al. (2016). NAD^+ repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352, 1436–1443.
Zhang, H., Wang, Y., Lyu, D., Li, Y., Li, W., Wang, Q., Li, Y., Qin, Q., Wang, X., Gong, M., et al. (2021f). Cerebral blood flow in mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. Ageing Res Rev 71, 101450.
Zhang, H., Weyand, C.M., and Goronzy, J.J. (2021g). Hallmarks of the aging T-cell system. FEBS J 288, 7123–7142.
Zhang, J., Goodlett, D.R., Peskind, E.R., Quinn, J.F., Zhou, Y., Wang, Q., Pan, C., Yi, E., Eng, J., Aebersold, R.H., et al. (2005a). Quantitative proteomic analysis of age-related changes in human cerebrospinal fluid. Neurobiol Aging 26, 207–227.
Zhang, J., Pickering, C.R., Holst, C.R., Gauthier, M.L., and Tlsty, T.D. (2006). $p16^{INK4a}$ modulates p53 in primary human mammary epithelial cells. Cancer Res 66, 10325–10331.
Zhang, J., Ren, P., Wang, Y., Feng, S., Wang, C., Shen, X., Weng, C., Lang, X., Chen, Z., Jiang, H., et al. (2017a). Serum matrix metalloproteinase-7 level is associated with fibrosis and renal survival in patients with IgA nephropathy. Kidney Blood Press Res 42, 541–552.
Zhang, J., Yin, J., Heng, Y., Xie, K., Chen, A., Amit, I., Bian, X., and Xu, X. (2022b). Spatiotemporal Omics-Refining the landscape of precision medicine. Life Med 1, 84–102.
Zhang, K., Sun, Y., Wu, S., Zhou, M., Zhang, X., Zhou, R., Zhang, T., Gao, Y., Chen, T., Chen, Y., et al. (2020c). Systematic imaging in medicine: a comprehensive review. Eur J Nucl Med Mol Imag doi: 10.1007/s00259-020-05107-z.
Zhang, N., Gordon, M.L., and Goldberg, T.E. (2017b). Cerebral blood flow measured by arterial spin labeling MRI at resting state in normal aging and Alzheimer's disease. Neurosci Biobehaval Rev 72, 168–175.
Zhang, Q., Vallerga, C.L., Walker, R.M., Lin, T., Henders, A.K., Montgomery, G.W., He, J., Fan, D., Fowdar, J., Kennedy, M., et al. (2019b). Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. Genome Med 11, 54.
Zhang, R., Chen, W., and Adams, P.D. (2007). Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol 27, 2343–2358.
Zhang, R., Poustovoitov, M.V., Ye, X., Santos, H.A., Chen, W., Daganzo, S. M., Erzberger, J.P., Serebriiskii, I.G., Canutescu, A.A., Dunbrack, R.L., et al. (2005b). Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 8, 19–30.
Zhang, S., Li, H., Zheng, L., Li, H., Feng, C., and Zhang, W. (2019c). Identification of functional tRNA-derived fragments in senescence-accelerated mouse prone 8 brain. Aging 11, 10485–10498.
Zhang, S., Wu, Z., Shi, Y., Wang, S., Ren, J., Yu, Z., Huang, D., Yan, K., He, Y., Liu, X., et al. (2022c). FTO stabilizes MIS12 and counteracts senescence. Protein Cell 13, 954–960.
Zhang, W., Li, J., Suzuki, K., Qu, J., Wang, P., Zhou, J., Liu, X., Ren, R., Xu, X., Ocampo, A., et al. (2015). A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. Science 348, 1160–1163.
Zhang, W., Qu, J., Liu, G.H., and Belmonte, J.C.I. (2020d). The ageing epigenome and its rejuvenation. Nat Rev Mol Cell Biol 21, 137–150.
Zhang, W., Song, M., Qu, J., and Liu, G.H. (2018b). Epigenetic modifications in cardiovascular aging and diseases. Circ Res 123, 773–786.
Zhang, W., Zhang, S., Yan, P., Ren, J., Song, M., Li, J., Lei, J., Pan, H., Wang, S., Ma, X., et al. (2020e). A single-cell transcriptomic landscape of primate arterial aging. Nat Commun 11, 2202.
Zhang, W.B., Aleksic, S., Gao, T., Weiss, E.F., Demetriou, E., Verghese, J., Holtzer, R., Barzilai, N., and Milman, S. (2020f). Insulin-like growth factor-1 and IGF binding proteins predict all-cause mortality and morbidity in older adults. Cells 9, 1368.
Zhang, W.G., Zhu, S.Y., Bai, X.J., Zhao, D.L., Jiang, S.M., Li, J., Li, Z.X., Fu, B., Cai, G.Y., Sun, X.F., et al. (2014). Select aging biomarkers based on telomere length and chronological age to build a biological age equation. Age 36, 9639.
Zhang, X., and Gao, F. (2021). Exercise improves vascular health: Role of mitochondria. Free Radic Biol Med 177, 347–359.
Zhang, X., Habiballa, L., Aversa, Z., Ng, Y.E., Sakamoto, A.E., Englund, D.A., Pearsall, V.M., White, T.A., Robinson, M.M., Rivas, D.A., et al. (2022d). Characterization of cellular senescence in aging skeletal muscle. Nat Aging 2, 601–615.
Zhang, X., Huang, B., Zhang, L., Zhang, Y., Zhao, Y., Guo, X., Qiao, X., and Chen, C. (2012). SNObase, a database for S-nitrosation modification. Protein Cell 3, 929–933.
Zhang, Y., Amaral, M.L., Zhu, C., Grieco, S.F., Hou, X., Lin, L., Buchanan, J., Tong, L., Preissl, S., Xu, X., et al. (2022e). Single-cell epigenome analysis reveals age-associated decay of heterochromatin domains in excitatory neurons in the mouse brain. Cell Res 32, 1008–1021.
Zhang, Y., Jiang, M., Nouraie, M., Roth, M.G., Tabib, T., Winters, S., Chen, X., Sembrat, J., Chu, Y., Cardenes, N., et al. (2019d). GDF15 is an epithelial-derived biomarker of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 317, L510–L521.
Zhang, Y., Lanjuin, A., Chowdhury, S.R., Mistry, M., Silva-García, C.G., Weir, H.J., Lee, C.L., Escoubas, C.C., Tabakovic, E., and Mair, W.B. (2019e). Neuronal TORC1 modulates longevity via AMPK and cell nonautonomous regulation of mitochondrial dynamics in *C. elegans*. eLife 8, e49158.
Zhang, Y., Wilson, R., Heiss, J., Breitling, L.P., Saum, K.U., Schöttker, B., Holleczek, B., Waldenberger, M., Peters, A., and Brenner, H. (2017c). DNA methylation signatures in peripheral blood strongly predict all-cause mortality. Nat Commun 8, 14617.
Zhang, Y., Yan, J., Yang, N., Qian, Z., Nie, H., Yang, Z., Yan, D., Wei, X., Ruan, L., Huang, Y., et al. (2021h). High-level serum fibroblast growth

factor 21 concentration is closely associated with an increased risk of cardiovascular diseases: a systematic review and meta-analysis. Front Cardiovasc Med 8, 705273.
Zhang, Y., Ye, Y., Tang, X., Wang, H., Tanaka, T., Tian, R., Yang, X., Wang, L., Xiao, Y., Hu, X., et al. (2022f). CCL17 acts as a novel therapeutic target in pathological cardiac hypertrophy and heart failure. J Exp Med 219, e20200418.
Zhang, Y., Yuan, D., Li, Y., Yang, F., Hou, L., Yu, Y., Sun, C., Duan, G., Meng, C., Yan, H., et al. (2021i). Paraquat promotes acute lung injury in rats by regulating alveolar macrophage polarization through glycolysis. Ecotoxicol Environ Saf 223, 112571.
Zhang, Y., Zheng, Y., Wang, S., Fan, Y., Ye, Y., Jing, Y., Liu, Z., Yang, S., Xiong, M., Yang, K., et al. (2022g). Single-nucleus transcriptomics reveals a gatekeeper role for FOXP1 in primate cardiac aging. Protein Cell doi: 10.1093/procel/pwac038.
Zhang, Z., He, C., Gao, Y., Zhang, L., Song, Y., Zhu, T., Zhu, K., Lv, D., Wang, J., Tian, X., et al. (2021j). α-ketoglutarate delays age-related fertility decline in mammals. Aging Cell 20.
Zhang, K., Mizuma, H., Zhang, X., Takahashi, K., Jin, C., Song, F., Gao, Y., Kanayama, Y., Wu, Y., Li, Y., et al. (2021k). PET imaging of neural activity, β-amyloid, and tau in normal brain aging. Eur J Nucl Med Mol Imaging, 48, 3859–3871.
Zhang, Z., Liu, S., Zhang, B., Qiao, L., Zhang, Y., and Zhang, Y. (2020g). T cell dysfunction and exhaustion in cancer. Front Cell Dev Biol 8, 17.
Zhao, B.S., Roundtree, I.A., and He, C. (2017). Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol 18, 31–42.
Zhao, D., and Chen, S. (2022). Failures at every level: breakdown of the epigenetic machinery of aging. Life Med 1, 81–83.
Zhao, H., Ji, Q., Wu, Z., Wang, S., Ren, J., Yan, K., Wang, Z., Hu, J., Chu, Q., Hu, H., et al. (2022a). Destabilizing heterochromatin by APOE mediates senescence. Nat Aging 2, 303–316.
Zhao, J.L., Ma, C., O'Connell, R.M., Mehta, A., DiLoreto, R., Heath, J.R., and Baltimore, D. (2014). Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell 14, 445–459.
Zhao, Q., Zheng, Y., Zhao, D., Zhao, L., Geng, L., Ma, S., Cai, Y., Liu, C., Yan, Y., Izpisua Belmonte, J.C., et al. (2022b). Single-cell profiling reveals a potent role of quercetin in promoting hair regeneration. Protein Cell doi: 10.1093/procel/pwac062.
Zhao, Y., Feng, B., Lee, J., Lu, N., and Pierce, D.M. (2020a). A multi-layered model of human skin elucidates mechanisms of wrinkling in the forehead. J Mech Behav BioMed Mater 105, 103694.
Zhao, Y., Shao, Q., and Peng, G. (2020b). Exhaustion and senescence: two crucial dysfunctional states of T cells in the tumor microenvironment. Cell Mol Immunol 17, 27–35.
Zhao, Z., Zhao, C., Zhang, X.H., Zheng, F., Cai, W., Vlassara, H., and Ma, Z.A. (2009). Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. Endocrinology 150, 2569–2576.
Zhao, Z.Y., Xie, Y., Fu, Y.R., Bogdan, A., and Touitou, Y. (2002). Aging and the circadian rhythm of melatonin: a cross-sectional study of chinese subjects 30–110 yr of age. Chronobiol Int 19, 1171–1182.
Zhavoronkov, A., Kochetov, K., Diamandis, P., and Mitina, M. (2020). PsychoAge and SubjAge: development of deep markers of psychological and subjective age using artificial intelligence. Aging 12, 23548–23577.
Zheng, H., and Xie, W. (2019). The role of 3D genome organization in development and cell differentiation. Nat Rev Mol Cell Biol 20, 535–550.
Zheng, L., Wang, Z., Zhang, X., Zhou, Y., Ji, A., Lou, H., Liu, X., Chen, H., and Cheng, Z. (2022). Development of mitochondria-targeted small-molecule dyes for myocardial PET and fluorescence bimodal imaging. J Med Chem 65, 497–506.
Zheng, S.X., Vrindts, Y., Lopez, M., De Groote, D., Zangerle, P.F., Collette, J., Franchimont, N., Geenen, V., Albert, A., and Reginster, J.Y. (1997). Increase in cytokine production (IL-1β, IL-6, TNF-α but not IFN-γ, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas 26, 63–71.
Zheng, W.Q., Zhang, Y., Yao, Q., Chen, Y., Qiao, X., Wang, E.D., Chen, C., and Zhou, X.L. (2020). Nitrosative stress inhibits aminoacylation and editing activities of mitochondrial threonyl-tRNA synthetase by S-nitrosation. Nucleic Acids Res 48, 6799–6810.
Zhou, D., Tian, Y., Sun, L., Zhou, L., Xiao, L., Tan, R.J., Tian, J., Fu, H., Hou, F.F., and Liu, Y. (2017). Matrix metalloproteinase-7 is a urinary biomarker and pathogenic mediator of kidney fibrosis. J Am Soc Nephrol 28, 598–611.
Zhou, J.N., Hofman, M.A., and Swaab, D.F. (1995). VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol Aging 16, 571–576.
Zhou, J.N., and Swaab, D.F. (1999). Activation and degeneration during aging: a morphometric study of the human hypothalamus. Microsc Res Tech 44, 36–48.
Zhou, Q., Zhu, L., Zhang, D., Li, N., Li, Q., Dai, P., Mao, Y., Li, X., Ma, J., and Huang, S. (2016). Oxidative stress-related biomarkers in postmenopausal osteoporosis: a systematic review and meta-analyses. Dis Markers 2016, 1–12.
Zhou, S., Liu, L., and Lu, X. (2023). Endogenous retroviruses make aging go viral. Life Med doi: 10.1093/lifemedi/lnad001.
Zhou, T., Kiran, M., Lui, K.O., and Ding, Q. (2022). Decoding liver fibrogenesis with single-cell technologies. Life Med 1, 333–344.
Zhou, X., and McElhaney, J.E. (2011). Age-related changes in memory and effector T cells responding to influenza A/H3N2 and pandemic A/H1N1 strains in humans. Vaccine 29, 2169–2177.
Zhu, B., Gong, Y., Yan, G., Wang, D., Wang, Q., Qiao, Y., Hou, J., Liu, B., and Tang, C. (2017). Atorvastatin treatment modulates *p16* promoter methylation to regulate *p16* expression. FEBS J 284, 1868–1881.
Zhu, D., Xu, G., Ghandhi, S., and Hubbard, K. (2002). Modulation of the expression of $p16^{INK4a}$ and $p14^{ARF}$ by hnRNP A1 and A2 RNA binding proteins: implications for cellular senescence. J Cell Physiol 193, 19–25.
Zhu, H., Sun, B., Zhu, L., Zou, G., and Shen, Q. (2021a). *N*6-methyladenosine induced miR-34a-5p promotes TNF-α-induced nucleus pulposus cell senescence by targeting SIRT1. Front Cell Dev Biol 9, 642437.
Zhu, J., An, Y., Wang, X., Huang, L., Kong, W., Gao, M., Wang, J., Sun, X., Zhu, S., and Xie, Z. (2022a). The natural product rotundic acid treats both aging and obesity by inhibiting PTP1B. Life Med 1, 372–386.
Zhu, J., Wang, J., Wang, X., Gao, M., Guo, B., Gao, M., Liu, J., Yu, Y., Wang, L., Kong, W., et al. (2021b). Prediction of drug efficacy from transcriptional profiles with deep learning. Nat Biotechnol 39, 1444–1452.
Zhu, M., Kohan, E., Bradley, J., Hedrick, M., Benhaim, P., and Zuk, P. (2009). The effect of age on osteogenic, adipogenic and proliferative potential of female adipose-derived stem cells. J Tissue Eng Regen Med 3, 290–301.
Zhu, Q., Ding, L., and Yue, R. (2022b). Skeletal stem cells: a game changer of skeletal biology and regenerative medicine? Life Med 1, 294–306.
Zhu, Z., Xu, W., and Liu, L. (2022c). Ovarian aging: mechanisms and intervention strategies. Med Rev doi: 10.1515/mr-2022-0031.
Ziaei, S., Shimada, N., Kucharavy, H., and Hubbard, K. (2012). MNK1 expression increases during cellular senescence and modulates the subcellular localization of hnRNP A1. Exp Cell Res 318, 500–508.
Ziemkiewicz, N., Hilliard, G., Pullen, N.A., and Garg, K. (2021). The role of innate and adaptive immune cells in skeletal muscle regeneration. Int J Mol Sci 22, 3265.
Zimmermann, M., Kibe, T., Kabir, S., and de Lange, T. (2014). TRF1 negotiates TTAGGG repeat-associated replication problems by recruiting the BLM helicase and the TPP1/POT1 repressor of ATR signaling. Genes Dev 28, 2477–2491.
Zink, F., Stacey, S.N., Norddahl, G.L., Frigge, M.L., Magnusson, O.T., Jonsdottir, I., Thorgeirsson, T.E., Sigurdsson, A., Gudjonsson, S.A., Gudmundsson, J., et al. (2017). Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood 130, 742–752.
Zivanovic, J., Kouroussis, E., Kohl, J.B., Adhikari, B., Bursac, B., Schott-

Roux, S., Petrovic, D., Miljkovic, J.L., Thomas-Lopez, D., Jung, Y., et al. (2019). Selective persulfide detection reveals evolutionarily conserved antiaging effects of S-sulfhydration. Cell Metab 30, 1152–1170.e13.
Zlotorynski, E. (2023). Younger endogenous retroviruses make us older. Nat Rev Mol Cell Biol 24, 165.
Zoico, E., Rossi, A., Di Francesco, V., Sepe, A., Olioso, D., Pizzini, F., Fantin, F., Bosello, O., Cominacini, L., Harris, T.B., et al. (2010). Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. J Gerontol A Biol Sci Med Sci 65A, 295–299.
Zoli, M., Magalotti, D., Bianchi, G., Gueli, C., Orlandini, C., Grimaldi, M., and Marchesini, G. (1999). Total and functional hepatic blood flow decrease in parallel with ageing. Age Ageing 28, 29–33.
Zou, X., Dai, X., Mentis, A.F.A., Esteban, M.A., Liu, L., and Han, L. (2022). From monkey single-cell atlases into a broader biomedical perspective. Life Med 1, 254–257.
Zou, Y., Wang, A., Huang, L., Zhu, X., Hu, Q., Zhang, Y., Chen, X., Li, F., Wang, Q., Wang, H., et al. (2020). Illuminating NAD(+) metabolism in live cells and *in vivo* using a genetically encoded fluorescent sensor. Dev Cell 53, 240–252.e.
Zou, Z., Long, X., Zhao, Q., Zheng, Y., Song, M., Ma, S., Jing, Y., Wang, S., He, Y., Esteban, C.R., et al. (2021). A single-cell transcriptomic atlas of human skin aging. Dev Cell 56, 383–397.e8.
Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., Benhaim, P., Lorenz, H.P., and Hedrick, M.H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7, 211–228.
Zwick, R.K., Guerrero-Juarez, C.F., Horsley, V., and Plikus, M.V. (2018). Anatomical, physiological, and functional diversity of adipose tissue. Cell Metab 27, 68–83.

SUPPORTING INFORMATION

The supporting information is available online at https://doi.org/10.1007/s11427-023-2305-0. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.