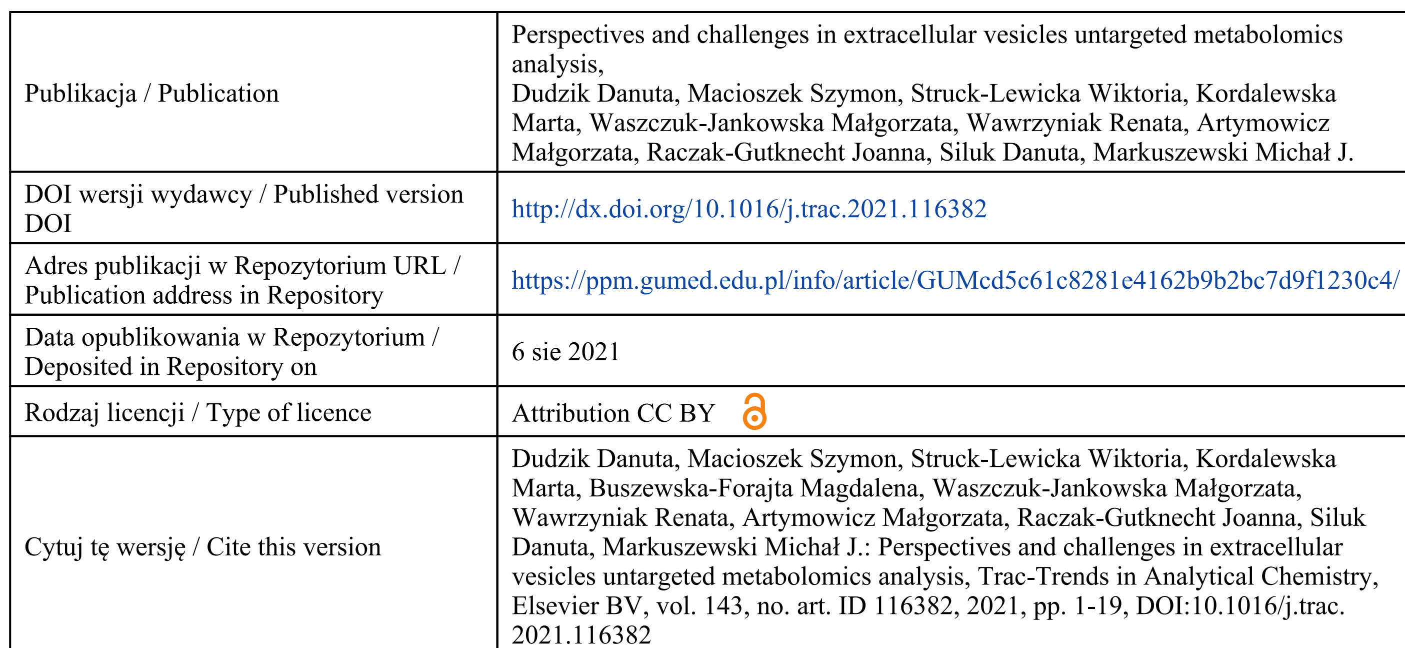
Repozytorium Gdańskiego Uniwersytetu Medycznego
Repository of Medical University of Gdańsk
https://ppm.gumed.edu.pl

Polska Platforma Medyczna
Polish Platform of Medical Research
https://ppm.edu.pl



Publikacja / Publication	Perspectives and challenges in extracellular vesicles untargeted metabolomics analysis, Dudzik Danuta, Macioszek Szymon, Struck-Lewicka Wiktoria, Kordalewska Marta, Waszczuk-Jankowska Małgorzata, Wawrzyniak Renata, Artymowicz Małgorzata, Raczak-Gutknecht Joanna, Siluk Danuta, Markuszewski Michał J.
DOI wersji wydawcy / Published version DOI	http://dx.doi.org/10.1016/j.trac.2021.116382
Adres publikacji w Repozytorium URL / Publication address in Repository	https://ppm.gumed.edu.pl/info/article/GUMcd5c61c8281e4162b9b2bc7d9f1230c4/
Data opublikowania w Repozytorium / Deposited in Repository on	6 sie 2021
Rodzaj licencji / Type of licence	Attribution CC BY
Cytuj tę wersję / Cite this version	Dudzik Danuta, Macioszek Szymon, Struck-Lewicka Wiktoria, Kordalewska Marta, Buszewska-Forajta Magdalena, Waszczuk-Jankowska Małgorzata, Wawrzyniak Renata, Artymowicz Małgorzata, Raczak-Gutknecht Joanna, Siluk Danuta, Markuszewski Michał J.: Perspectives and challenges in extracellular vesicles untargeted metabolomics analysis, Trac-Trends in Analytical Chemistry, Elsevier BV, vol. 143, no. art. ID 116382, 2021, pp. 1-19, DOI:10.1016/j.trac. 2021.116382

Trends in Analytical Chemistry 143 (2021) 116382

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Perspectives and challenges in extracellular vesicles untargeted metabolomics analysis

Danuta Dudzik [a, b, *, 1], Szymon Macioszek [a, 1], Wiktoria Struck-Lewicka [a], Marta Kordalewska [a], Magdalena Buszewska-Forajta [a, c], Małgorzata Waszczuk-Jankowska [a], Renata Wawrzyniak [a], Małgorzata Artymowicz [a], Joanna Raczak-Gutknecht [a], Danuta Siluk [a, **], Michał J. Markuszewski [a]

[a] Department of Biopharmaceutics and Pharmacodynamics, Faculty of Pharmacy, Medical University of Gdańsk, Al. J. Hallera 107, 80-416 Gdańsk, Poland
[b] Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28660 Boadilla del Monte, Madrid, Spain
[c] Institute of Veterinary Medicine, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, 87-100 Toruń, Poland

ARTICLE INFO

Article history:
Available online 5 July 2021

Keywords:
Exosomes
Microvesicles
Apoptotic bodies
Non-targeted metabolomics
Molecular profiling
Biomarkers
Mass spectrometry
Standardization
Quality control
Validation

ABSTRACT

The discovery of extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, has opened a new frontier in the study of signal transduction and understanding cell-to-cell communication. EVs play a key role regulating various biological processes such as tissue regeneration, blood coagulation, immunomodulation or carcinogenesis. Therefore, they gain much attention as potentially therapeutic and non-invasive diagnostic targets. In this aspect, a comprehensive characterization of the EVs molecular content and understanding EVs functions are especially relevant. However, routine isolation of a usually small amount of high purity EVs is challenging, and analysis requires highly sensitive advanced analytical solutions. The utility of EVs in clinical settings is still limited due to the lack of standardization in existing protocols. This review focuses on the implication of the state-of-the-art mass-spectrometry based untargeted metabolomics approach for the molecular profiling of EVs, with the emphasis on the analytical methodologies, highlighting potential challenges and limitations.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

1.1. Extracellular vesicles biology and significance

The number of scientific publications dedicated to the biology of extracellular vesicles (EVs) has increased rapidly over the past decade. Those small, spheroidal particles surrounded by a phospholipid bilayer, naturally released from various types of cells and isolated from many body fluids or cell cultures are receiving considerable attention due to their unique and potent biological function [1–5]. EVs refer to various subtypes of secreted membranous structures, classified according to their physical characteristics (size and density), biogenesis and secretory pathway, commonly described as exomeres (<50 nm), exosomes (~30–150 nm), microvesicles (~100–1000 nm) and apoptotic bodies (>1000 nm) (Fig. 1) [1–3,5,6]. Based on the current knowledge, these structures are recognized as extremely important carriers of many active molecules such as mRNA, miRNA, ecDNA, proteins, bioactive lipids, and small molecules (metabolites) [1,4,7,8]. As carriers of biological molecules, EVs have been shown to have pleiotropic physiological and pathological functions. The number of EVs increases in pathology-related conditions and their molecular cargo varies with respect to associated state or cell type [6]. EVs play a crucial role in intercellular communication by inducing interactions with target cells' receptors. Moreover, EVs can also transfer receptors to target

* Corresponding author. Department of Biopharmaceutics and Pharmacodynamics, Faculty of Pharmacy, Medical University of Gdańsk, Al. J. Hallera 107, 80-416 Gdańsk, Poland.
** Corresponding author.
E-mail addresses: danuta.dudzik@gumed.edu.pl (D. Dudzik), macioszek@gumed.edu.pl (S. Macioszek), wiktoria.struck-lewicka@gumed.edu.pl (W. Struck-Lewicka), marta.kordalewska@gumed.edu.pl (M. Kordalewska), magdalena.buszewska-forajta@gumed.edu.pl (M. Buszewska-Forajta), waska@gumed.edu.pl (M. Waszczuk-Jankowska), renata.wawrzyniak@gumed.edu.pl (R. Wawrzyniak), malgorzata.artymowicz@gumed.edu.pl (M. Artymowicz), jrgutknecht@gumed.edu.pl (J. Raczak-Gutknecht), danuta.siluk@gumed.edu.pl (D. Siluk), michal.markuszewski@gumed.edu.pl (M.J. Markuszewski).
[1] The first two authors contributed equally to this work.

https://doi.org/10.1016/j.trac.2021.116382
0165-9936/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

Abbreviations

AB	Apoptotic Bodies
ACD	Acid-Citrate-Dextrose
ACE2	Angiotensin-Converting Enzyme 2
AUC	Area Under Curve
BALF	Bronchoalveolar Lavage Fluid
BR-EVs	Bioreactor-Derived EVs
CAFs	Human Cancer-Associated Fibroblasts
capillary IC-MS	Capillary Ion Chromatography-Mass Spectrometry
CDEs	Human Cancer-Associated Fibroblasts Derived Exosomes
C-Evs	Conventional Cell Cultures EVs
CIL	Chemical Isotope Labeling
CL	Cardiolipin
CoV	Coronavirus
CRC	Colorectal Cancer
DC	Differential Centrifugation
DG	Diacylglycerols
DGU	Density Gradient Ultracentrifugation
DI-QTOF/MS	Direct-Infusion-coupled to Quadrupole-Time-of-Flight-Mass Spectrometry
DLS	Dynamic Light Scattering
DMSO	Dimethyl Sulfoxide
DVD/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
ecDNA	Extrachromosomal DNA
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELVs	Exosomes-Like Vesicles
EpCAM-positive	Epithelial Cell Adhesion Molecule-Positive
ESI	Electrospray Ionization
EVs	Extracellular Vesicles
Exo-MFA	Exosome-Mediated Metabolic Flux Analysis
GC-Q/MS	Gas Chromatography coupled to Quadrupole Mass Spectrometry
GC-QqQ/MS	Gas Chromatography coupled to Triple Quadrupole Mass Spectrometry
GC-TOF/MS	Gas Chromatography coupled to Time-of-Flight Mass Spectrometry
GP	Glycerophospholipids
HAdV	Human Adenovirus
HDL	High-Density Lipoproteins
HIV	Human Immune Deficiency Virus
HSPME GC-MS	Headspace Solid-Phase micro-Extraction/Gas Chromatography-Mass Spectrometry
Huh7	Hepatocellular Carcinoma Cells
IC-MS	Ion Chromatography with Mass Spectrometry
IDL	Intermediate-Density Lipoproteins
IFN-γ	Interferon-gamma
IS	Internal Standard
ISEV	The International Society for Extracellular Vesicles
LASSO	Least Absolute Shrinkage and Selection Operator
LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-Q-Orbitrap/MS	Liquid Chromatography coupled to Quadrupole-Orbitrap-Mass-Spectrometry
LC-QTOF/MS	Liquid Chromatography coupled to Quadrupole-Time-of-Flight Mass Spectrometry
LC-QTRAP/MS	Liquid Chromatography coupled to Quadrupole-Ion-Trap-Mass Spectrometry
LDL	Low-Density Lipoproteins
miRNA	microRNA
MPE	Malignancy Pleural Effusion
mRNA	messenger RNA
MS	Mass Spectrometry
MSCs	Human Bone Merrow-Derived Mesenchymal Stem Cells
MTBE	Methyl *tert*-butyl ether
MVB	Multivesicular Body
MVs	Microvesicles
NanoE	Nanoerythrosomes
nLC-MS	Nanoflow Liquid Chromatography coupled to Mass Spectrometry
NTA	Nanoparticle Tracking Analysis
PBS	Phosphate-Buffered Saline
PC	Phosphatidylcholines
PE	Phosphatidylethanolamines
PEG	Polyethyleneglycol
PQN	Probabilistic Quotient Normalization
QA	Quality Assurance
QC	Quality Control
RBCs	Red Blood Cells
RMs	Reference Materials
ROC	Receiver Operating Characteristic
RT	Retention Time
SD	Standard Deviation
SEC	Size-Exclusion Chromatography
SM	Sphingomyelins
SP	Sphingolipids
SPE	Solid Phase Extraction
TCA-cycle	Tricarboxylic Acid Cycle
TEM	Transmission Electron Microscopy
TIC	Total Ion Chromatogram
TPE	Tuberculosis Pleural Effusion
U87	Glioblastoma Cells
UCF	Ultracentrifugation
UHPLC-QqQ/MS	Ultra-Performance Liquid Chromatography coupled to Triple Quadrupole Mass Spectrometry
UHPLC-QTOF/MS	Ultra-Performance Liquid Chromatography coupled to Triple Quadrupole Time-of-Flight Mass Spectrometry
UHPLC-QTrap/MS	Ultra-High Performance Liquid Chromatography coupled to Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer
UHR-FTMS	Ultra High-Resolution Fourier Transform Mass Spectrometry
VLDL	Very Low-Density Lipoproteins
VOC	Volatile Organic Compounds

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

cells or even transfer cargo from one cell to another [4,9]. In that way, they can reprogram the recipient cell and thereby affect the immune system, exert cells' proliferation, cell migration and induce processes like inflammation, angiogenesis, or tumorigenesis [6,9]. Hence, EVs and particularly exosomes carry and deliver information that is essential for health. According to current knowledge, they play a significant role in diverse pathologies such as cancer [10], diabetes [11], cardiovascular [12], neurodegenerative [13], or infectious [14] diseases, among others. Recently, EVs, and in particular exosomes, are attracting much interest due to the evidence of their role in particular viral infections including human adenovirus (HAdV), human immune deficiency virus (HIV), and coronavirus (CoV) [14]. Exosomes, which are consistently produced by virus-infected cells, transfer viral components such as viral miRNAs and proteins from infected to healthy cells. Moreover, they contain virus-specific receptors such as the tetraspanin CD9 and ACE2 that make recipient cells susceptible to virus entry [15]. However, it has been postulated that exosomes may also induce immune cell responses [15], but still, the complete mechanisms of EVs action in viral infections have not been fully elucidated, and more research is warranted. The unique abilities of EVs are attracting much interest in their potential for the identification of new biomarkers and new diagnostics, and therapeutic options [16]. Currently, exosome-based so-called liquid biopsies revolutionized molecular diagnostics starting a new era of precision medicine as non-invasive tools for early disease detection and monitoring disease progression [17]. Considering also the capability of EVs to efficiently cross the blood-brain barrier and the ability to be modified or functionalized with the addition of tumour target ligands, they give new perspectives for innovative therapeutic strategies [18,19].

1.2. Challenges recognized in EVs-based research

Despite exciting findings and the growing interest, the use of EVs in clinical applications is limited due to the lack of standardization in EVs isolation and analysis, which has become one major challenge in the field [8,20,21] The use of disparate protocols for sample handling, analysis, and data normalization leads to variability and inconsistency that significantly affects downstream's analysis performance, makes inter-study comparisons difficult and might even lead to questionable conclusions [21,22]. Thereby, the International Society for Extracellular Vesicles made a great effort in order to propose consensus for EVs nomenclature, standardization of the methodologies for isolation, and EVs characterization. Bringing the challenges, recommendations given in 2018 [22] and recently released in 2020 [21,23] recognize hindrances, report on the current advancements, and provide detailed guidance for the improvement of the quality of EVs research.

Although there are different strategies currently available to characterize EVs, including Western blot, electron microscopy, Raman spectroscopy, nanoparticle tracking analysis, or flow cytometry [8,21], the cutting-edge “omics” technologies (transcriptomics, proteomics, and metabolomics) open great perspectives to decipher EVs molecular cargo [7,24–26]. Key technological advances in metabolomics and mass spectrometry technologies provide a powerful approach to expand EVs research. Untargeted, global in scope metabolomics approach is gaining more and more attraction for a deeper understanding of the EVs metabolome landscape. Therefore, the already established quality assurance (QA) and quality control (QC) recommendations [27–30] could be adapted for EVs based studies [27–30].

In this review, we summarize the current knowledge on extracellular vesicles and highlight their potential clinical applications. Furthermore, we will focus on the pre-analytical (sample collection, processing, EVs isolation, and metabolite extraction), analytical and post-analytical factors. Particularly, we aim to consider associated key issues and challenges in the aspect of untargeted metabolomics workflow. We put special emphasis on the importance of implementing robust and reproducible protocols to provide high-quality research and reliable results.

2. Pre-analytical factors impact downstream analysis

2.1. Sample quality considerations

EVs can be isolated from various body fluids, tissues, or cultured cell-conditioned media. Importantly, the selection of the source from which they are obtained as well as each step of the pre-analytical process may significantly impact the quality, amount, or EVs heterogeneity. Therefore, it is crucial to standardize all the procedures, including sample collection and EVs isolation.

More than 30 biological fluids are present in mammals, e.g., blood, saliva, urine, nasal secretions, breast milk, cerebrospinal fluid, bronchoalveolar lavage, etc. Because of its various biophysical and chemical characteristics, each must be treated individually in terms of EVs separation [22]. The composition of EVs may also vary depending on intrinsic and extrinsic factors. Therefore, it is necessary to plan all procedures and report as many pre-analytical parameters as possible (including pipetting, decanting or centrifugation, temperature, etc.) [21,31–33].

Blood is a rich source of EVs, widely applied for most studies, but it contains hard-to-remove lipoproteins and chylomicrons [21,22]. Blood plasma is preferred because additional platelet-derived EVs are released during clot formation while preparing serum [32]. However, large physical forces related to blood drawing, e.g., associated with a small-diameter needle used during venepuncture may also activate and release the platelet-derived EVs, which may cause *in vitro* haemolysis and the formation of erythrocyte microparticles. Therefore, it is recommended to use needles of 21-gauge or larger to minimize shear forces [32,33]. It is also advised to discard the first volume of collected blood, containing blood cell remnants, which could contaminate the EVs isolate [31,33].

Importantly, several plasma-derived contaminants including lipoprotein particles such as high-density lipoproteins (HDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), very low-density lipoproteins (VLDL), and chylomicrons as well as other components e.g., protein aggregates, protein-phospholipid micelles, cell and membrane fragments or intact cells overlap with extracellular vesicles (EVs) in terms of size or density and could be co-isolated in EVs fraction [23,34,35].

The choice of anticoagulant is another important factor to be considered, and it strongly depends on its properties and the downstream analysis. Among the most commonly used anticoagulants are ethylenediaminetetraacetic acid (EDTA), heparin, acid-citrate-dextrose (ACD), and sodium citrate, where the last one with the final concentration of 0.109 mol/L has been recommended by the International Society on Thrombosis and Haemostasis [32,36]. However, for instance, blood collected in heparin gave considerably higher levels of annexin V-positive microparticles than blood collected in sodium citrate. Furthermore, heparin shows typical repeating building-block polymer in MS spectra. Cations from the anticoagulant agents (e.g., lithium, sodium, potassium) contaminate samples and affect MS-based metabolomics analysis by causing ion suppression or enhancement [37–39]. Moreover, anticoagulants may also impact the efficiency of the metabolite extraction and the derivatization processes [39]. Several reviews cover this subject in great detail [31,33,37–39]. l It is important to highlight that irrespective of the sample source, and it is always mandatory to consider biofluid-specific factors when designing EVs based study [22,33].

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

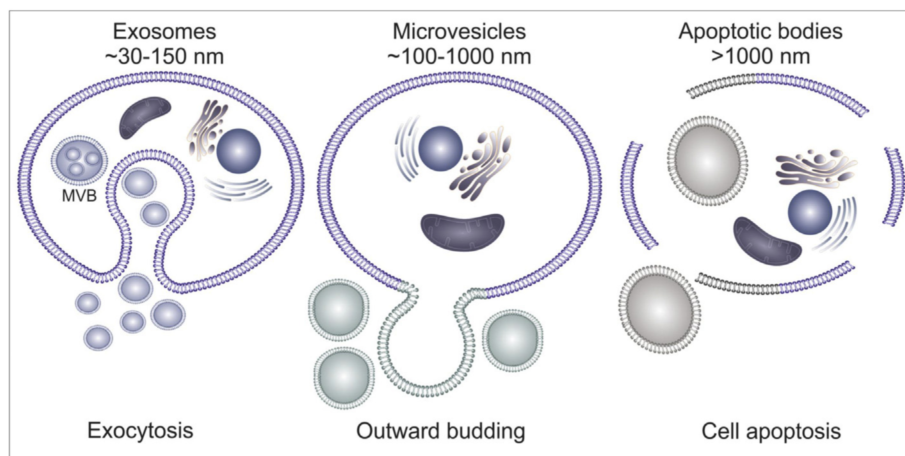


Fig. 1. Based on the size and biogenesis process, EVs can be divided into three main subpopulations: (i) exosomes, (ii) microvesicles (MVs), and (iii) apoptotic bodies (AB). Exosomes are generated by the internal budding of endosomes to produce a multivesicular body (MVB) which is released into the extracellular space after fusion with the plasma membrane. MVs are generated by direct outward plasma membrane budding. The AB is membrane blebs formed during cell apoptosis.

Special attention should be paid to EVs derived from cell culture conditioned media. Particularly, basic characterization of releasing cells and culture should be performed, as well as all harvesting conditions should be reported (e.g., seeding density, passage number, culture volume, surface coatings, gas tension, stimulations/treatments, frequency, intervals of harvesting) [22]. Importantly, cells are widely cultured *in vitro,* in a medium enriched with human, or other species serum, containing its own EVs that should be removed before adding to the cell culture [33]. An increasing number of vendors offer commercial exosome-free serum. Unfortunately, the exact steps of the depletion method are often not described, which makes it impossible to predict and control the EVs release [22]. Emphasis should be placed on quantifying the percentage of dead cells present in the culture after conditioning time since even their small number could release cell membranes, which overcame true extricated EVs. The estimated acceptable dead cell concentration should not exceed 5% [22]. Other sources of possible contaminations such as EVs originating from bacteria, e.g., *Mycoplasma* should be carefully evaluated [22,33].

Several critical remarks should be placed for EVs extraction from tissue, where it is challenging to guarantee that recovered vesicles originate from the extracellular space. It is difficult to achieve the degree of tissue disruption while minimizing cellular damage. Once cells are destroyed, intracellular components and artificially produced vesicles may co-isolate with EVs fraction [22,23].

The tissues which EVs were isolated from include brain tissue [40], visceral adipose tissue [41] or lymphoid tissues [42].

Before the analysis of urine EVs, one has to consider two critical aspects regarding sample quality: urine collection and sample storage. First or second morning urine samples can be collected as according to EV protein analysis, EVs content was similar between these two sample types [43]. Urine samples can be stored long-term, however, the storage temperature is important and should be −80°C. Immediately prior to sample preparation, urine has to be vortexed vigorously as EVs could remain attached to the plastic [43]. It should also be noted that uromodulin, the most abundant urine protein, may co-precipitate with EVs, reducing the purity of isolated vesicles [23,44].

The most important factors influencing the sample quality, which determines the accuracy of research are summarized in Fig. 2.

2.2. EVs isolation and purification

To obtain relevant results leading to coherent conclusions, EVs should be isolated in a standardized manner. As a group of heterogeneous particles of different size and content, the standardization seems to be an exceptional challenge. A multitude of isolation techniques and their modifications exist, which result in the separation of EVs subsets of various size, purity, and with different molecular cargo [45,46]. The general principles of the main isolation methods, along with recognized advantages and disadvantages are presented in Table 1.

Ultracentrifugation (UCF), considered as a gold standard, was the first technique applied for EVs isolation in 1996 [47], and still in 2016 survey results showed that 81% of EVs research is based on ultracentrifugation as an isolation technique [48]. This technique is also predominant for EVs isolation for MS-based metabolomics studies, as shown in Table 2. UCF isolates components according to their size and density through sedimentation caused by a successive rise in centrifugal forces and duration time [46]. Consequently, it is possible to remove cell debris and apoptotic bodies, then MVs, and finally exosomes pellet is formed at the highest centrifugation force. This method was applied to isolate exosomes from cell-cultured media as well as from biological matrices such as plasma, urine, saliva, breast milk, and semen. A crucial aspect related to quality assurance is the fact that isolation protocols vary substantially between research projects [45]. However, numerous parameters impact the isolation efficiency and should be considered during method development including (i) centrifugal g-force; (ii) type of rotor; (iii) sample viscosity; (iv) time of centrifugation [46].

The general UCF procedure includes several centrifugation steps at increasing acceleration and time: firstly at $400\times g$ ~10 min and $2000\times g$ ~30 min to remove cells and cell debris, then at $10\,000\times g$ ~40 min to eliminate apoptotic bodies (AB) and larger vesicles. Although the supernatant obtained at this stage contains a high concentration of exosomes, it can be contaminated by lipoproteins, MVs, and proteins. Additionally, for more efficient isolation of exosome fraction from contaminants, the last step with sedimentation at $100\,000\times g$ for at least 70 min up to 18 h is performed [46,49]. For consistency and reproducibility purposes, the same value of rotor acceleration, rotor type, and centrifugation temperature (preferably 4°C), are essential. The time should be adjusted to the specified protocols. Prolonged centrifugation co-precipitates other particles that have a negative impact on the sample purity [46,50]. Finally, pellets are washed with a large volume of phosphate-buffered saline (PBS) to separate the exosomes from lipoproteins or protein aggregates [51,52]. It can be also assumed that the majority of the reported studies usually employ single

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

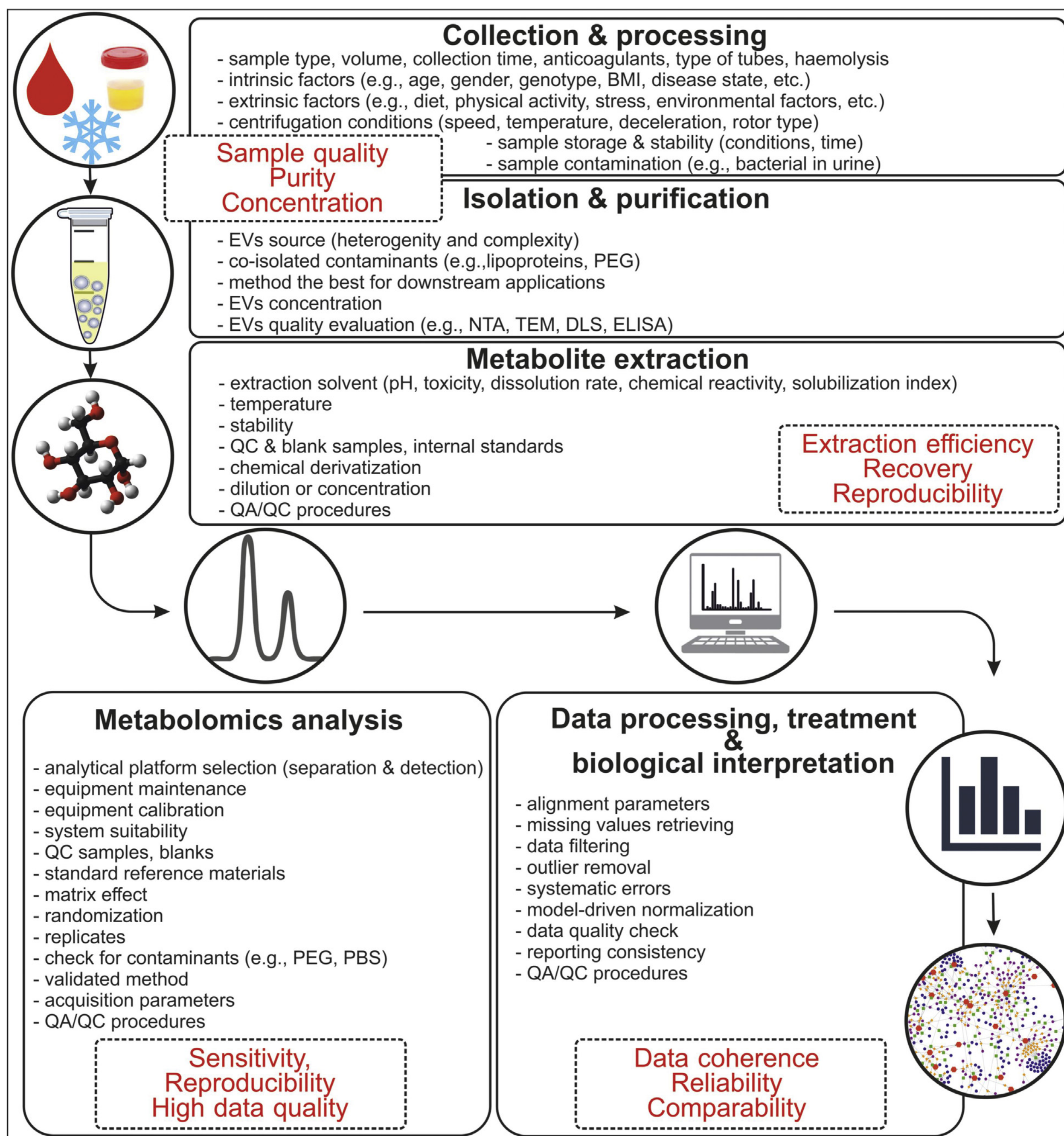


Fig. 2. Considerations on the critical factors and challenges affecting the quality of untargeted metabolomics studies together with the principal steps of the metabolomics workflow including (i) biological material collection and sample processing; (ii) EVs sample preparation – isolation and purification; (iii) metabolite extraction; (iv) metabolomics analysis; (v) data processing; (vi) data treatment; (vii) biological interpretation. BMI – Body Mass Index, PEG – Polyethyleneglycol, NTA – Nanoparticle Tracking Analysis, TEM – Transmission Electron Microscopy, DLS – Dynamic Light Scattering, ELISA – Enzyme-Linked Immunosorbent Assay, QC – Quality Control, QA – Quality Assurance, PBS – Phosphate-Buffered Saline.

[49,53,54] or double [55,56] PBS-washing, some authors applied multiple, e.g., 4× PBS-washing cycles [57]. However, apart from sample purity, washing also influences isolation yield crucially. Thus, a balance should be maintained as washing steps decrease isolation efficiency [45]. In a study by Nielsen et al. the number of EVs was compared between a washed and non-washed pellet. The introduction of a single washing step reduced the number of EVs to almost 5% of the equivalent unwashed pellet. However, the washing step also reduced significantly the co-isolated plasma proteins by more than tenfold, which is undeniably beneficial for the downstream mass spectrometry analysis [58]. Generally, it may be assumed that for metabolomics analysis, a single wash allows achieving acceptable EVs purity while ensuring detectable levels of metabolites in the analyzed extract. However, apart from sample purity, washing also influences isolation yield crucially. Thus, a balance should be maintained as too many washing steps decreases isolation efficiency [45]. The undeniable advantages of differential UCF are its reproducibility and low cost. On the other hand, the major limitation is the need to utilize specialized equipment for centrifugation, long procedure (~5–10 h) and unavoidable

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

Table 1
Summary of the EVs isolation methods.

Method	Background Principles	Advantages	Disadvantages
Ultracentrifugation (Differential Ultracentrifugation)	Separation is based on differences in size, shape, and density. Particles sediment at different centrifugal forces.	• reproducibility of the procedure • low cost of used consumables and reagents • no reagent addition • multiple samples at the same time	• lengthy methodology • labor-intensive • low yield • not suitable for low sample volumes • risk of contamination by particles of similar size • the necessity of utilization of specific costly equipment • low throughput • the potential damage of exosomes • lack of standardized procedure • efficiency is influenced by numerous parameters, limited use in clinical practice
Sucrose Density Gradient Ultracentrifugation	Combination of differential ultracentrifugation with a density gradient.	• EVs of higher purity than obtained by ultracentrifugation • easy methodology	• time-consuming • high volume sampling required • low yield • strong centrifugation force has to be put to overcome the viscous media • contamination of viruses and lipoproteins
Filtration (Ultrafiltration)	Differences in particle size - the pore size affects the isolated vesicles.	• fast and efficient • easy to perform • no special equipment required • no reagent addition	• low exosome yield • unable to separate contaminants of the same size as EVs • filters clogging • risk of vesicle deformability under forced pressure
Precipitation	Water-binding polymers lower solubility of EVs leading to their precipitation	• high yield • easy and fast procedure • low cost • commercial kits available • suitable for large volumes	• low purity (proteins and polymer) • low specificity • unable to capture subpopulations of EVs
Microfluidics	Isolation based on immunoaffinity capture or physical or mechanical properties of EVs (size, density, etc.) Novel methodologies can be based on acoustic, electrophoretic, and electromagnetic manipulations.	• low sample volumes • low cost • low consumption • high precision • easy automation and integration • fast and portable	• possible low reproducibility • possible clogging of pores and channels • lack of standardization and method • validation • low processing capacity
Size Exclusion Chromatography	Based on molecule size and the size of pores in the column gel.	• higher purity of exosome isolate • no reagent addition • intact exosomes • high yield	• low volumes required • low throughput • sample dilution
Immunoaffinity-based	Interaction between antigens on the exosome surface and immobilized antibodies.	• high purity • isolation of exosomes subpopulations • fast procedure (excluding pre-enrichment)	• usually, sample pre-enrichment is required • low yield • suitable only for low volumes • high cost
Polymer Precipitation-based Commercial Kits	Water in the solution is tied up by PEG, which induces exosome precipitation. The exosomes can be easily collected with low-speed centrifugation.	• rapid • easily scalable to large preparation volumes with high yield • does not require sophisticated equipment • capable of processing all type of samples • high efficiency	• presence of other extracellular contaminants (e.g. lipoproteins, viruses) • requires complicated clean-up steps and affects downstream analysis

contamination of the final sample by proteins and non-vesicle particles representing similar size as isolated exosomes [59]. Moreover, it is considered that centrifugation at high speed may cause exosomes fracture and aggregation with biofluid contaminants [60].

To alleviate certain drawbacks of UCF, density gradient ultracentrifugation (DGU) has been developed as an extension to the described procedure for increasing the exosome isolation efficiency [61]. It includes two alternative methods: based on sucrose or iodixanol gradient. Exosome pellets, resuspended after ultracentrifugation, are overlaid in the gradient medium with crescively decreasing density from bottom to the top (e.g., in the 30% sucrose solution with D_2O) and subsequently, undergo centrifugation at 100 000×g acceleration. As a result, isolated particles drift and stop at the point where medium density is the same as their own. DGU increases sample purity and yield. Moreover, due to the applied high-density medium, the destructive influence of high centrifugal forces on exosomes structure is minimized [62]. The main disadvantage of this method is a laborious protocol and the need for additional purification procedures to separate vesicles subpopulations from each other and non-vesicles molecules with similar density.

Other EVs isolation methods, so far commonly applied in EVs-based transcriptomics and proteomics analysis include ultrafiltration, precipitation, immunoaffinity-based, and size-exclusion chromatography (SEC) are summarized in Table 1. Briefly, ultrafiltration separates molecules by their differences in size and molecular weight. Isolating particles are sequentially passed through ultrafiltration membranes (usually cellulose) with various

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gda

Table 2
The overview of the most relevant EVs-based untargeted metabolomics studies ordered according to the year of publication.

Aim of the study	EV type and origin	Isolation method	Metabolite extraction & Analytical platform	QA/QC procedure & Normalization	Main metabolomics findings	Ref. Year
Non-invasive detection and discrimination of colorectal cancer.	CRC-derived exosomes; cell culture conditioned medium and serum	UCF	methanol-water-chloroform LC-QTOF/MS GC-Q/MS	normalization to sum area-mean centering	Identification of 37 (cell culture), 31 (serum) metabolites including organic acids, amino acids and conjugates, sugars, and 30 (cell culture) and 56 (serum) lipids with statistically significant changes. The most significant changes were observed for monomethyl phosphate, D-pinitol, Myo-inositol, xylitol (decreased) and glucose, L-pyroglutamic acid, glycolic acid (increased). Sphingolipid and glycerophospholipid metabolisms were associated with the strongest discriminative power.	[103] 2020
Characterization of EVs subpopulations composition. Assessment of metabolic differences between tuberculosis and malignancy.	large and small EVs derived from pleural effusion	UCF	methanol-MTBE-water UHPLC-QTrap/MS	NA	Metabolic profiles of EVs subpopulations overlapped with a difference from each other and considerably varied from EVs derived from pleural effusion. A panel of 4 biomarker candidates: phenylalanine, leucine, phosphatidylcholine 35:0, and sphingomyelin 44:3 showed high performance for TPE and MPE stratification.	[84] 2020
Development of colorectal cancer diagnostic model using stool microbial EVs metabolomics and metagenomics.	stool microbial EVs	centrifugation (10 000×g) 10, min, 4°C	acetonitrile-isopropanol-water GC-TOF/MS	log transformation; Pareto scaling	In stool microbial EVs, 6 amino acids, ethanolamine, phenol, 3 carboxylic acids, and 3 fatty acids were upregulated while aminoisobutyric acid and butanoic acid were downregulated as compared to healthy controls. Most metabolic markers were strongly correlated with the microbial abundance of *Firmicutes* and *Proteobacteria*.	[90] 2020
Assessment of immunosuppressive properties of cancer-derived EVs.	EVs derived from breast cancer cell line	UCF	water-methanol-chloroform LC-Q-Orbitrap/MS; capillary IC-MS	normalization according to the amount of EV protein and particle number	After destruction by lymphocyte-derived perforin, EVs secrete adenosine, which has immunosuppressive effects. Another finding suggests that IFN-γ treatment promotes intracellular tryptophan catabolism and glycolysis, but does not affect the metabolic profile in EV metabolites.	[104] 2020
Comparison of the metabolite profiles of serum-derived exosomes between head and neck cancer patients and healthy controls.	serum-derived exosomes	UCF followed by SEC	methanol GC-QqQ/MS	Total Ion Current normalization	Cancer serum-derived EVs metabolite profile is characterized by markedly upregulated 1-hexadecanol and downregulated citric acid, 4-hydroxybenzoic acid, and propylene glycol. No metabolic changes were observed in EVs as a consequence of radiotherapy.	[106] 2020

(continued on next page)

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk

Table 2 (*continued*)

Aim of the study	EV type and origin	Isolation method	Metabolite extraction & Analytical platform	QA/QC procedure & Normalization	Main metabolomics findings	Ref. Year
Identification of exosome-derived biomarkers for schizophrenia based on a large multicenter study.	serum-derived exosomes	SEC	methanol LC-QTRAP/MS	pooled QC	25 metabolites (10 upregulated and 15 downregulated) differentiated between patients with schizophrenia and healthy controls. Significant metabolites are related to glycerophospholipid metabolism and the biosynthesis of phenylalanine, tyrosine, and tryptophan. High ROC AUC value implies their potential as indicators of schizophrenia.	[65] 2020
Development of novel immunoaffinitive magnetic composites (MagG@PEI@DSP@aptamer) as a facile strategy for the capture and release of exosomes.	exosomes secreted by breast cancer cells	immunoaffinity-based MagG@PEI@DSP@aptamer	50% methanol LC-Q-Orbitrap/MS	the intensity of each ion feature was normalized using IS	The use of the developed magnetic composites provides reliable and informative results. Exosomes isolated with the novel method were characterized by significantly different metabolic profiles in the comparison between breast cancer cells and normal breast cells. The main observations include increased glutaminolysis and downregulation of aspartic acid in breast cancer.	[64] 2020
Investigation of the impact of cell growth conditions (bioreactors *vs*. conventional cell cultures) on the EVs characteristics.	EVs derived from prostate cancer cell lines	UCF	acetonitrile LC-QTOF/MS	pooled QC	The use of bioreactor increased the EVs yield >100 times compared to the conventional cell culture system. The growth conditions significantly affected the EVs metabolite profiles, e.g., bioreactor-derived EVs contained significantly less PC and PE, as well as SM.	[99] 2019
Exploring a lipid profile that can classify non-small cell lung cancer patients.	plasma-derived exosomes	UCF	acetonitrile-water-chloroform UHR-FTMS	lipid features intensities normalized using the summed intensities of lipid features	Panels of 7 and 16 lipid features selected with Random Forest and LASSO methods, respectively, distinguish lung cancer patients from healthy individuals.	[91] 2018
Proof of concept for applying a sensitive CIL nLC-MS technique in exosome metabolomics research. A comparative analysis of samples obtained from pancreatic patients before and after chemotherapy.	serum-derived exosomes	UCF	methanol-water CIL nLC-QTOF/MS	NA	The developed workflow allowed for the detection of ~1950 metabolites per sample. Significant changes were noted in alanyl-histidine, 6-dimethylaminopurine, leucyl-proline, methionine sulfoxide levels before and after chemotherapy; however, the sample size in this study was too small to draw biological conclusions.	[57] 2018
Characterization of the lipid composition of airways EVs from asthmatic and healthy subjects, with and without second-hand smoke exposure.	EVs derived from BALF	UCF	methanol-water-chloroform DI-QTOF/MS	EVs containing 1×10^9 particles; ceramide IS; lipid peaks normalized by the mean and SD value of each sample	The levels of ten lipids belonging to ceramides, sphingomyelins, and GP were significantly different between the compared groups. It was observed that second-hand smoke exposure reduces ceramide levels, which may have a protective anti-inflammatory function.	[92] 2018

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk

Development of the protocol for exosome-mediated metabolic flux analysis (Exo-MFA), to predict fluxes involved in metabolite trafficking from CAFs to cancer cells.	Human cancer-associated fibroblasts (CAFs) derived exosomes (CDEs)	Commercial kit	methanol-water-chloroform GC-Q/MS	NA	Exo-MFA algorithm integrates experimental and computational stable-isotope tracer techniques and provides insight into multicellular metabolic interactions. Exo-MFA was successfully employed to estimate the packaging fluxes in CAFs that constitute the metabolite cargo of exosomes. The study finds that lactate supply is provided by exosomes for the regulation of glycolysis pathway fluxes for 24 h, while glutamine and TCA intermediates are provided to supply up to 35% of the TCA cycle fluxes.	[102] 2017
Identification of differential content of EVs-VOCs in *in vitro* cultures of *Plasmodium falciparum* growing at two different parasitemias and study the relationship between the higher EV-VOC density and mosquito attraction.	RBCs-EVs form infected and uninfected patients	UCF	SPME extraction with the use of DVD/CAR/PDMS fiber HSPME GC-Q/MS analysis of EVs-VOCs and UCF supernatants	NA	18 VOCs were obtained from the EVs of both infected and uninfected RBCs with 1,2,3-propanetriol, diacetate (diacetin) increased in the infected EVs, regardless of the parasitemia of the culture. The UCF supernatant analysis, resulted in 56 VOCs, with pentane 2,2,4-trimethyl being present in all the UCF of uninfected erythrocytes but absent from the parasite-infected ones. Hexanal, was the only VOC present in all samples from UCF from infected erythrocytes and absent from uninfected ones, suggesting that it originates during parasite infection.	[88] 2017
Characterization of metabolites and determination of changes in metabolic profiles of EVs associated with prostate cancer. Comparison of normalization methods.	Urinary and platelet EVs from prostate cancer patients before and after prostatectomy and healthy controls	UCF	acetonitrile and water (80:20) with 1% of formic acid UHPLC-QqQ/MS 102 (urinary) and 111 (platelet) metabolites	Different normalization procedures compared: to EV volume, EV number, CD9 optical density, other metabolites, urine volume, or urine creatinine.	The study provides insights into the amounts of required material ~1×10^{10} EVs for metabolomics analysis. Normalization to EV-derived parameters or analysis of metabolite ratios would have the best potential. 2–26 fold lower levels of glucuronate, D-ribose 5-phosphate and isobutyryl-L-carnitine were observed in all pre-prostatectomy samples compared to the healthy control and post-prostatectomy samples.	* [76] 2017
Determination of metabolic content of patient-derived exosomes. Study of cancer cell metabolism in prostate and pancreatic cancers mediated CDEs.	Human cancer-associated fibroblasts (CAFs) derived exosomes (CDEs)	Commercial kit	methanol-water-chloroform GC-Q/MS	Normalization by protein concentration	CDEs contain metabolites (amino acids, lipids, TCA-cycle intermediates, lactate, acetate) that are utilized by cancer cells for central carbon metabolism, proliferation, and promote tumour growth.	[98] 2016

(*continued on next page*)

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk

Table 2 (*continued*)

Aim of the study	EV type and origin	Isolation method	Metabolite extraction & Analytical platform	QA/QC procedure & Normalization	Main metabolomics findings	Ref. Year
Comparison of protein and lipid content of exosomes, MVs.	Human cell culture (U87, Huh 7, MSCs) derived exosomes and MVs	UCF	chloroform-methanol DI-QTOF/MS	Fatty acid standards. Lipid species normalized to total protein content	Exosomes and MVs differ in lipid types. Exosomes are characterized by a higher content of glycolipids and free fatty acids, while MVs are represented by enrichment in ceramides and sphingomyelins. Lipidomes of Huh7 and MSC exosomes were similar to each other but different from U87. Huh7 and MSC exosomes contain more CL, whereas U87 exosomes contain more sphingomyelins.	[105] 2016
Description of methodology for metabolic profiling of Exosomes-Like Vesicles (ELVs).	ELVs derived from human plasma samples from patients with endometrioid adenocarcinomaELVs isolated from human pancreatic carcinoma cell culture media	UCF	water- methanol-chloroform-acetonitrile UHPLC-QTOF/MS	ELVs containing approximately 10^{11-12} particles/mL; Two IS (%CV calculated)	Plasma-derived ELVs: 29% GP/SP, 17% nucleotides and derivatives, 16% fatty acid esters/amides/alcohols, 10% peptides/peptide conjugates, 10% steroids, prenols and eicosanoids, 7% sugars and sugar conjugates, 5% cyclic alcohols, aliphatic and aromatic compounds, 3% amino acids/conjugates, 2% organic acids and derivatives, 1% others. Cell culture media derived ELVs: 56% GP/SP, 14% fatty acid esters/amides/alcohols, 7% nucleotides, and derivatives, 6% amino acids/conjugates, 5% steroids, prenols and eicosanoids, 4% organic acids and derivatives, 3% cyclic alcohols, aliphatic and aromatic compounds, 2% sugars and sugar conjugates, 2% others, 1% peptides/peptide conjugates.	[89] 2016

EV – extracellular vesicle, QA/QC – quality assurance/quality control, CRC – colorectal cancer, UCF – ultracentrifugation, LC-QTOF/MS – liquid chromatography coupled to quadrupole – time-of-flight mass spectrometry, GC-Q/MS – gas chromatography coupled to quadrupole mass spectrometry, MTBE - methyl *tert*-butyl ether, UHPLC-QTrap/MS – ultra high performance liquid chromatography coupled to hybrid triple quadrupole linear ion trap mass spectrometer, TPE – tuberculosis pleural effusion, MPE – malignancy pleural effusion, GC-TOF/MS – gas chromatography coupled to time-of-flight mass spectrometry, IC-MS – ion chromatography with mass spectrometry, IFN-γ – interferon gamma, SEC – size exclusion chromatography, GC-QqQ/MS – gas chromatography coupled to triple quadrupole mass spectrometry, ROC – Receiver Operating Characteristic, AUC – area under curve, IS – internal standards, PC – phosphatidylcholine, PE – phosphatidylethanolamine, SM – sphingomyelin, UHR-FTMS – ultra high-resolution Fourier transform mass spectrometry, LASSO - Least absolute Shrinkage and Selection Operator, CIL – chemical isotope labeling, nLC-MS – nanoflow liquid chromatography coupled to mass spectrometry, BALF - bronchoalveolar lavage fluid, DI – direct infusion, SD – standard deviation, GP – Glycerophospholipids, CAF – cancer-associated fibroblast, CDEs – CAF-derived exosomes, Exo-MFA – exosome-mediated metabolic flux analysis, TCA-tricarboxylic acid cycle, VOC – volatile organic compound, SPME – solid phase microextraction, DVD/CAR/PDMS – divinylbenzene/carboxen/polydimethylsiloxane, HSPME – headspace solid phase microextraction, RBCs – red blood cells, MV – microvesicles, U87 – glioblastoma cells, Huh7 – hepatocellular carcinoma cells, MSCs – human bone merrow-derived mesenchymal stem cells, CL – cardiolipins, ELVs – exosomes-like vesicles, CV – coefficient of variation, SP – sphingolipids, NA – data not available, * [76] this citation is referred to the wide in scope metabolite profiling. However, due to its importance, we consider it as highly relevant for the provided discussion.

molecular size and weight cut-offs [62]. In the end, the sample is filtered through a 100 nm membrane filter to separate exosomes. Isolated EVs are characterized by higher purity compared to UCF method. Nonetheless, membrane clogging by aggregates and non-vesicles contaminants vastly decrease isolation efficiency. Furthermore, it is considered that passing through nanopores induces changes in EVs morphology or their lysis [45].

EVs can also be isolated through precipitation from a solution caused by the presence of a reagent (usually a polymer) that binds water molecules and saturates the solution. A substantial disadvantage is the co-precipitation of proteins and polymer particles affecting downstream MS analysis.

Immunoaffinity techniques are based on capturing EVs by antibodies that attach to specific surface ligands. The technique is most suitable for obtaining pure exosome fraction since certain proteins such as CD63, CD9, and CD81 are characteristic for exosomes, but none of the other EVs [24]. However, the required sample pre-concentration may hamper the method's applicability [63]. Nevertheless, novel strategies based on immunoaffinity emerge, including aptamer-based immunoaffinitive magnetic composites (MagG@PEI@DSP@aptamer), developed and applied in metabolomics study by Chang et al. [64]. Such composites were able to capture a higher number of exosomes from cell culture medium than UCF, and the authors were able to detect a higher number of metabolic features.

Specific exosome fractions can be isolated using Exosome-Human EpCAM Isolation Reagent (Thermofisher, United States), which separates EpCAM-positive (epithelial cell adhesion molecule-positive) human exosome subsets from a pre-enriched medium. Magnetic beads are coated with an antibody specific for the EpCAM membrane antigen, and attached exosomes can then be magnetically separated.

A technique that has recently been used for EVs isolation from schizophrenia patients' serum samples is size-exclusion chromatography (SEC) [65]. SEC enables particle separation in a column, based on the molecule size. In this simple technique, a biofluid and porous gel filtration polymer are used as mobile and stationary phase, respectively. The porous gel allows for differential elution as bigger particles enter the big size pores only, and therefore have a shorter path to get to the end of the column and be eluted. The method is rapid, cost-effective, enables to separate EVs from soluble plasma proteins, however, it cannot differentiate between exosomes and MVs of the same size as well as SEC isolates often contain lipoproteins [46,66]. Nevertheless, SEC is considered to produce EVs isolate of higher purity than UCF and precipitation [67]. When identification of EVs subtype is needed, the additional use of the immunocapture method is advised.

Microfluidics-based techniques have been strongly developed in the last years [68]. It refers to processing small amounts of fluids through micro-channels with dimensions of tens to hundreds of micrometers. On such a small scale, fluids behave distinctly, partially because surface forces dominate volumetric forces. The most significant advantage of microfluidics is low reagent consumption and fast analysis. Due to substantial issues with standardization and validation of microfluidics methods as well as their low processing capacity, none of the currently available methods can be applied to large scale studies such as untargeted metabolomics based projects [69].

A simple and rapid EVs isolation can be achieved with commercially available kits or systems, which are based on previously described methods such as precipitation, immunoaffinity, SEC, or microfluidics [70]. Precipitation-based kits comprise the vast majority of commercial kits e.g., ExoQuick (System Biosciences, United States), Total Exosome Isolation Reagent (Invitrogen, United States), or miRCURY Exosome Isolation Kit (Exiqon, Denmark), among others. Here, normally polyethyleneglycol (PEG) is the substance responsible for water-binding and EVs precipitation. Usually, a few different kits are offered by the manufacturer appropriate for different types of biological fluids - plasma/serum, urine, cell culture media, or saliva. A huge stumbling block in applying commercial kits for EVs isolation is sample contamination with PEGs, which is particularly problematic when metabolite detection is based on mass spectrometry. Even a slight amount of polymers in an analyzed sample leads to ion suppression and interference with the ions formed by metabolites, which in general causes lower sensitivity [71]. The presence of PEGs manifests in numerous MS peaks differing by a unit of ethylene oxide (44.026 Da). Moreover, PEGs are not well separated on LC columns and elute in a wide RT window producing abundant peaks due to their easy ionization [72]. Besides, as a result of their adhesive properties, PEGs are difficult to remove from LC and MS systems (LC tubing, needle, ESI source) as well as they contaminate the chromatographic columns [73].

Another examples of EV isolation kits include ExoChip, based on a combination of microfluidics and immunoaffinity techniques. This microfluidic device functionalized with antibodies against CD63 was proved to extract EVs with intact RNA, ready for micro-RNA profiling [74]. Modified ExoChip, called new ExoChip was developed to isolate cancer-specific exosomes. As phosphatidylserine is more specific for cancer-associated EVs than CD63, the new ExoChip is conjugated with phosphatidylserine-specific protein [75]. Recently, also a commercial qEV column (Izon Science, New Zealand) was applied in an untargeted metabolomics project [65]. The qEVs are SEC-based columns that enable to obtain exosome fraction in 15 min. According to the manufacturer, the exosome isolate is significantly purer than the one obtained with UCF or precipitation-based kits.

Notwithstanding the whole range of EVs isolation techniques, there is still an urgent need to establish optimal standardized procedures. Consistent conclusions cannot be drawn as each technique captures a distinctive subset of EVs differing in size, quantity, and purity. Although ultracentrifugation is often called a ‘gold standard’ in EVs isolation and has been applied for more than 20 years, the number of parameters affecting the final isolate yield and purity make UCF difficult for standardization. Commercial kits seem to be promising in future research as they enable the isolation procedure to be standardized. Nonetheless, the aspect of sample purity first needs to be improved. Certainly, each isolation procedure should be followed by EVs characterization using nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), dynamic light scattering (DLS), or enzyme-linked immunosorbent assay (ELISA) for validation of the isolated EVs fraction. To give one example, Puhka et al. validated the quality of the EVs isolation for metabolomics study by electron microscopy and western blotting analysis [76].

2.3. The importance of sample handling and storage

Pre-analytical variables such as stability and storage procedures of EVs should be of particular concern, mainly due to their diagnostic or therapeutic potential. MISEV2018 guidance indicates that storage and retrieval conditions of both matrix and isolated EVs may affect EVs characteristics, including stability, a number of particles, aggregation, and function [22]. Therefore, in terms of stability, when planning EVs research, several factors should be taken into consideration, e.g., storage container, time and temperature, the procedure of freezing and thawing, number of freeze-thaw cycles, protective additives or storage solutions. Technical challenges regarding pre-analytical factors influencing EVs stability were recently reviewed by Ramirez et al. [77]. Current EVs storage strategies include cryopreservation, lyophilization/freeze-drying, and spray-drying, which

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

were previously thoroughly described [69,78]. Briefly, for cryopreservation one of the most critical factor, besides the controlled freezing method, remains a proper choice of cryoprotectant, ensuring EVs stability and minimizing cryodamage. Substances serving as cryoprotectants can either penetrate across cell membranes (penetrating cryoprotectants) or remain situated externally (non-penetrating cryoprotectants). Examples of the first type like glycerol, gelatin, ethylene glycol, polyvinyl alcohol, DMSO, protect from ice crystals generation during the cooling process. Substances of the second type, usually carbohydrates (e.g., sucrose, fructose, glucose) counteract cryodamage by diminishing hyperosmotic lysis of EVs. Among several protectors, trehalose is indicated as the most effective and valuable in terms of cryopreservation and reduction of EVs aggregation. Additionally, to increase the effectiveness of the process cocktails of cryoprotectants are also utilized (e.g., trehalose/sucrose; DMSO/trehalose).

Lyophilization, an extensively used technique in the preservation of peptides, proteins, or vaccines, is also recommended for assurance of EVs stability. The lyophilized biomaterial is characterized by a relatively long shelf-life and can be reconstituted by water addition. However, due to freezing and dehydration pressures generated during the freeze-drying process, and the consequent risk of EVs damage, also in this technique addition of protectants is of great importance. For this purpose, short saccharides are usually employed (e.g., sucrose, fructose, glucose), with trehalose suggested as a very effective storage cryoprotectant. Saccharides act as stability enhancers in the lyophilization technique by forming an amorphous sugar glass on the outer part of the EVs by which they increase their stability characteristics [78–80].

Spray-drying is another method employed to increase EVs stability. It is a frequently used in the production of therapeutic peptides, proteins, vaccines, with main advantages such as a single-step process and the ability to produce particles in relatively small sizes in a controllable manner [78]. Here, crucial parameters to be considered are atomization pressure and outlet temperature, which should be precisely adjusted, as unproperly optimized parameters can decrease EVs stability and contents. Spray-drying so far is less often utilized in EVs studies, yet it is listed in a US patent by Behfar, who engineered a method for exosome delivery based on EVs with a platelet-rich solution as a wound healing formulation proposal [81].

In most of the studies focused on EVs stability, the storage temperature of −80°C was indicated as the most adequate in terms of maintaining activity and contents [78,82,83]. It should be noted though, that storage conditions can be selected on the basis of EVs study purpose and ultimate application. For example, Wu et al. reported that in studies on EVs integrity and quantity, storage of EVs up to a week at 4°C could be acceptable, however, for contents and activity studies, storage at −20°C or −80°C is necessary [83].

Exceptional potential dormant in EVs phenomenon, particularly their diagnostic and therapeutic potential, impose further investigations focused on their stability during the storage. There is still a need for further studies on the stability of EVs cargo from a proteomic, transcriptomic and metabolomic perspective [77]. There is no doubt, that new recommendations, indicating standard methodologies, will ease following research and simplify comparison of results presented by different research groups [22,83].

2.4. Metabolite extraction as a critical step in metabolite profiling

Untargeted metabolomics requires an effective metabolite extraction method that guarantees all metabolites' complete and unbiased release from the EVs. However, this process is challenging due to the high chemical diversity and the wide dynamic range of metabolites that can vary by many of magnitude orders. Therefore, no single method can completely extract all metabolites and no single analytical platform is capable of their analysis. Importantly, for efficient extraction of intracellular metabolites, the EVs membrane needs to be first permeabilized, and then metabolites are extracted by organic solvent [84]. To achieve high metabolite coverage, protein removal by precipitation with cold organic solvents such as methanol, methanol/ethanol (1/1, *v/v*), or acetonitrile is the most widely used protocol for global untargeted metabolomics [85]. Protein removal preserves the LC-MS system integrity, reduces matrix effects, and significantly increases the number of detected metabolites [86]. Alternatively, organic solvents mixtures or with water addition are commonly used to increase the range of extracted metabolites. Here, combinations of e.g., a two-phase solvent system composed of a mixture of chloroform-methanol-water can be applied to recover both polar and non-polar metabolites. Nonetheless, due to chloroform's high toxicity, liquid-liquid extraction using methyl *tert*-butyl ether has become an alternative that provides comparable metabolite coverage [87].

Although solid-phase extraction (SPE) is avoided in global metabolomics analysis because of its selectivity, this method can be recommended for the determination of volatile organic compounds. Such a strategy was applied by Correa et al. in the HSPME/GC-MS analysis of red blood cell EVs from *Plasmodium falciparum* infected and uninfected individuals [88].

Consequently, the method of choice for sample preparation should be non-destructive and non-selective to cover the wide variety of metabolites present in the analyzed matrix. Moreover, the resulting extracts should be compatible with the analytical techniques used for subsequent analysis. An overview of the extraction procedures and analytical platform used for the untargeted analysis of EVs metabolome is provided in Table 2, whereas Fig. 2 highlights the most important aspects of metabolite extraction.

3. Metabolomics reveals molecular signature of extracellular vesicles

In the post-genomic era, metabolomics is increasingly gaining more and more attraction among the basic sciences. Metabolites represent the downstream products of multiple reactions between genes, transcripts, and proteins to provide a more holistic molecular perspective that intends to understand the biological complexity of the studied system. Importantly, high-quality untargeted metabolomics studies require (i) proper experimental design; (ii) thoughtful sample selection and handling; (iii) appropriate metabolite extraction; (iv) accurate data acquisition methods; (v) careful data processing and interpretation. Each step of the workflow is essential for the outcome of the study. Herein, in Fig. 2, we indicated the most crucial factors that should be considered to conduct a reliable EVs based untargeted metabolomics study.

Although a significant number of proteomics and transcriptomics studies were conducted to elucidate the role of EVs proteins and nucleic acids, still the EVs metabolome remains to be explored more extensively. Particular composition depends on the donor cell type, physiological or pathological state of the cell, and the stimuli that have led to their secretion. One of the first studies that applied the MS-based approach for evaluation of EVs metabolome composition was conducted by Altadill et al., in 2016 [89]. The applied analytical platform and non-selective extraction protocol enabled to the detection of numerous classes of lipids as well as peptides, organic acids, nucleosides, sugars, or cyclic alcohols in the EVs derived from plasma and cell culture media. In the other study, Luo et al. [57] also reported a high number of amino acids, dipeptides, purine bases, and nucleosides in serum EVs. Similarly, a wide range of metabolites was detected in stool microbial EVs [90].

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

Puhka et al. [76] detected the same above-mentioned compound classes in urine and platelet EVs, together with other predominant metabolites belonging to carnitines, vitamin B-related metabolites, and amines. Moreover, this study proved that more than 85% of EVs cargo has a cytoplasmic origin. This is a consequence of the cytosol being engulfed by EVs while budding from cell membranes. It is suggested that the rest of the EVs content is produced through enzymatic activity within EVs. In addition to common classes of metabolites, also volatile compounds were analyzed in the EVs [88] where the group of Correa detected alcohols, alkylbenzenes, phenols, aldehydes, alkanes, and benzene derivatives in the EVs of red blood cells. Since EVs are characterized by high lipid content related to their membrane structure, several studies focused only on the analysis of lipid compounds in EVs [49,91–95]. The recent review paper by Skotland et al. aims to present and compare in details advancements in the lipid composition in EVs [96]. Whereas, Peterka et al. performed comprehensive lipidomics characterization of exosomes from human plasma and provide absolute molar concentrations of lipid species from 10 lipid classes. In the following discussion, the authors refer to lipid classes as membrane lipids (PC, LPC, Cer, SM) and lipids occurring in the internal space of lipid membrane, but not incorporated in the membrane structure (TG, DG, MG) [97]. Hough et al. [92] detected sphingomyelins (SM), phosphatidylcholines (PC), ceramides, prostaglandins, and leukotrienes in EVs purified from the bronchoalveolar lavage fluid (BALF). These lipids play an essential role in cellular signalling, survival, and immune activation. According to Altadill et al. [89], 29% and 56% of total metabolome composition comprise glycerophospholipids and sphingolipids in the EVs obtained from plasma and cell culture media, respectively. In the study of Royo et al. [93] SM, PC, and phosphatidylethanolamines (PE) were also listed as the most abundant in the EVs secreted by hepatocytes. Particular interest is paid to the composition of cancer-derived EVs, produced in high amounts, which could serve as potential cancer indicators. Zhao et al. [98] found that exosomes secreted by cancer-associated fibroblasts, key components of the tumour microenvironment, promote tumour growth. Amino acids, lipids, and TCA-cycle intermediates included in their cargo are utilized by cancer cells for central carbon metabolism. In another study, breast cancer-secreted EVs' composition was compared with the content of tumour cells [94]. EVs contained more cholesterol and SM than their original cells, while PC and PE were depleted in EVs. Furthermore, unsaturated diacylglycerols (DG) were highly enriched in cancer cell-secreted EVs. The authors suggest that through protein kinase C activation, DG contributes to cell proliferation and tumour progression. It is important to note that according to the study of Palviainen et al. [99], the composition of cell-derived EVs is dependent on cell growth conditions. Bioreactor-derived EVs (BR-EVs) were compared with the EVs obtained from cells grown in conventional cell cultures (C-EVs). C-EVs were characterized by the significantly higher content of PC, PE, and SM in comparison to BR-EVs. Therefore, the cell culture conditions should be carefully planned, standardized and described in scientific reports.

With the advancement in studies on profiling EVs molecular cargo, free web-enabled tools including Vesiclepedia [7], ExoCarta [100], and EVpedia [101] help scientific communities to identify key components in the EVs datasets. Currently, Vesiclepedia contains data obtained from 1254 EVs studies, 38 146 RNA entries, 349 988 protein entries, and 639 lipid/metabolite entries [7]. The development of such resources has added another dimension in elucidating molecular mechanisms underlying EVs biological properties and their physiological functions.

3.1. Application of EVs studies to the clinical settings

The potential of EVs and especially exosomes for explanation of mechanisms underlying disease development was mostly studied in the case of cancer-related conditions including: prostate cancer [76,98,99] pancreatic cancer [57,89,98,102], colorectal cancer [90,103], breast cancer [64,104], lung cancer [91], endometroid adenocarcinoma [89], hepatocellular carcinoma [105], glioblastoma [105] or head and neck cancer [106]. Metabolomics studies were also applied to study metabolic reprogramming of tuberculosis and malignancy [84] and characterize lipid composition of airways EVs from asthmatic and healthy subjects, with and without second-hand smoke exposure [92]. In another study, Du et al. conducted a large multicenter project to identify exosome-derived biomarkers for schizophrenia [65]. Correa et al. performed the analysis of EVs-VOCs in *in vitro* cultures of *Plasmodium falciparum* growing at two different parasitemias and studied the relationship between the higher EV-VOC density and mosquito attraction [88]. The main findings of the aforementioned studies have been summarized in Table 2.

The ultimate goal of untargeted metabolomics studies, including EVs based research is to translate the results from basic science to the clinic. There is no doubt that there is a great potential in the EVs based studies, however, much work is needed to validate previous findings. That remains the challenge due to the methodological variability across the studies. Therefore, even promising the results of those studies need to be carefully interpreted.

4. Key elements of a successful metabolomics study

4.1. Quality assurance/quality control (QA/QC) procedures

Well-defined and properly applied QA/QC procedures in untargeted metabolomics studies provide proper standardization and harmonization for multi-laboratory experiments [30]. Both QA and QC are part of a quality management system that centres on fulfilling analytical quality requirements [28]. QA and QC approach constitutes a crucial point to ensure high quality and reproducibility in untargeted metabolomics. The most common types of QC refers to QC samples (i) raw (pooled intra-study); (ii) external (surrogate); (iii) commercially available; (iv) group-specific; or (v) post-extraction QC sample [27,28]. The implementation of QC results in several advantages including (i) analytical system equilibration and control; (ii) signal intensity control; (iii) system performance control; (iv) estimation of measurement precision; (v) signal drift correction and normalization. Therefore, it is highly recommended that QC is homogenous and representative in terms of studied sample composition as it assures measurement stability monitored through its interaction with the analytical platform (e.g., through matrix-specific and sample-specific ionization suppression) [28]. For integrity and stability, QC samples should undergo the same storing conditions and freeze-thaw cycles as experimental samples, if applicable. QC replicates should be analyzed through the whole analytical batch and used to calculate precision for each metabolite feature. However, the preparation of QC samples is dependent on the type of sample to be studied and the amount of biological material available. A pooled post-extraction QC samples may provide a reliable alternative for low sample volume that can possess technical difficulties for sample pool or sub-aliquot preparation. Nonetheless, this type of QC allows only to control variation derived from analytical measurements and data preprocessing but does not consider the variation related to the

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

sample preparation procedure [28,29]. In such instances, as it could be in the case of EVs, the alternative solution's choice is particularly important. Thus, the application of an artificial QC sample (e.g., the mixture of authentic chemical standards) or standard reference materials (RMs) might be evaluated in order to ensure the quality of the measurements. In such a case, it should be considered that not all metabolites from the biological samples will be present in such QC; therefore, the resulting data must be carefully interpreted. Reference materials could benefit in (i) calibration and analytical platform sensitivity quantification; (ii) assay validation between assays, instruments and laboratories; and (iii) standardized quality control reference materials. Despite great potential, the use of RMs in EVs studies is limited. Currently, only uncertified RMs are commercially available, and they can lack accuracy and lead to bias in sample characterization and measurement inaccuracy [27]. Valkonen et al. focused on the perspectives for the RM development which reflect properties equal to EVs and they evaluated the use of nanoerythrosomes (NanoE), vesicles from erythrocytes, that due to their resemblance to EV might be considered as potential RM [107]. More recently, Welsh et al. provided a detailed discussion on the RM application and highlighted the urgent need for their incorporation as a standard protocol for EVs-based research [108]. Another important action has been taken through the European Joint Research Project METVES II (Standardization of Concentration Measurements of Extracellular Vesicles for Medical Diagnoses; www.metves.eu) that was established with the overall aim to enable the standardization of concentration measurements of cell-specific EVs by developing RM and related reference measurement methods [109]. The detailed discussion on the QC approach adapted to untargeted metabolomics workflow has been reviewed extensively [27,29].

A crucial aspect of QA/QC that cannot be underestimated is the analysis of the blank samples (collection blanks, extraction blanks, solvent blanks) that can be helpful to estimate carry-over, identify contaminants and other artefacts originating from e.g. tubes, solvent impurities, sample additives, preservatives or other sources [27]. An alternative method that can limit the analytical bias and enhance data quality, especially in studies with limited sample size and sample volume is the analysis of technical replicate e.g., a triplicate of the same biological sample performed to determine method reproducibility. Such a strategy allows to calculate measurement precision and allows to minimize the false findings [27].

Regarding the most relevant untargeted metabolomics-based studies focused on EVs analysis, published during the last 5 years (Table 2), there are no sufficient details concerning QCs preparation and application that is presumably limited by available low sample size. However, a recently published study attempts to implement standard QC protocol recommended for metabolomics analysis [65]. Nevertheless, the QA/QC procedures cannot be omitted and it has to be considered as an obligatory step in any biomedical analysis.

4.2. Metabolomics data integrity

Advanced technologies and high-throughput mass spectrometry-based metabolomics platforms allow the measurements of thousands of metabolite variables generating highly complex multidimensional data. Therefore, key considerations should be taken on their integrity. Metabolomics data consequently need to undergo several processing steps, namely, (i) background noise removal; (ii) peak peaking and deconvolution; (iii) alignment; (iv) missingness imputation; (v) filtration; (vi) normalization, which is crucial for the data quality and relevant for the interpretation of the results. Several previous reviews cover in detail the aspects of metabolomics data management [110–112]. Therefore, we will only comment on the most critical step, data normalization, which is still considered as the main bottleneck in untargeted metabolomics including EVs based studies. Emphasis should be placed that raw metabolomics data are prone to the unwanted variation that might significantly enhance systematic bias and lead to spurious correlations [113,114]. Such unwanted variation can occur due to both experimental and biological factors and could be associated with the pre-analytical, analytical, and post-analytical stages of the study [113]. There is strong evidence that especially sample handling procedures can influence sample integrity and change their molecular characteristics. Indeed, it has been recognized that in EVs-based studies substantial variability is introduced due to the isolation procedure, which is challenging regarding size overlap between different EVs subtypes and their heterogeneous distribution [16,115]. Furthermore, contaminating artefacts co-isolated in EVs fractions like serum lipoproteins, protein aggregates or components of intracellular compartments that are not completely removed during sample processing can significantly bias downstream analysis and data interpretation [16,22]. Therefore, the total available EVs sample quantity and thus the concentration of metabolites can significantly vary from one sample to another [116]. Thus, currently, the results are incomparable among studies. Clearly stated, only the high quality of EVs sample can guarantee accurate and precise characterization of their cargo and examine the true concentration differences. Several factors associated with sample heterogeneity, contributing to overall variation derived from study design, through sample handling, collection, pre-processing, measurement, to the final data treatment and interpretation have been reviewed extensively [27]. Untargeted metabolomics often requires the use of multiple analytical platforms and several analytical batches. Therefore, every experiment is also subjected to a within-platform variation that can arise from e.g., inconsistencies in sample extraction and preparation, temperature changes, the mobile phase pH shift, sample degradation, the fluctuations of the ionization efficiency caused e.g., by matrix effect and can lead to the loss of instrument performance during analytical run [27]. Such lack of precision consequently impacts the quality of metabolomics data. Hence, any unwanted variation must be removed to focus on the true biological information of interest. It should be clearly stated that without proper normalization EVs metabolomics data can provide confusing or misleading results.

To address this issue, appropriate either sample-based or data-based normalization strategies can be employed. Sample-derived approaches include: (i) intrinsic (e.g., total protein content, selected metabolite); (ii) cell-based (e.g., count, volume, markers); (iii) weight-based (biofluid volume and tissue mass); (iv) reference compound (e.g., internal standard); (v) QC-based factors and others as osmolarity or specific gravity. Normalization strategies belonging to data-based procedures count on (i) statistical methods (mean, quantile, median fold change, PQN, cubic spline, etc.) (ii) analytical platform factors (e.g., TIC, the sum of peak area, etc.); [116–119]. The normalization strategies in untargeted metabolomics studies are widely discussed in the literature. Several reviews cover the basis of the normalization algorithms and their application for various biological conditions [113,116–118,120–122]. However, limited normalization methods have been applied so far for untargeted metabolomic analysis of EVs, and there is no clear recommendation of which strategy is the most appropriate [22]. As an option, characteristics of isolated EVs (e.g., particle counts, the total amount of protein, nucleic acid, or lipids) or EVs source characteristics (e.g., the initial volume of biofluid, a mass of tissue or number of cells) is taken into consideration [22]. Clearly, data normalization is a big challenge in metabolomics studies, whether targeted or untargeted.

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

Without an appropriate normalization method, both strategies can lead to erroneous data. Therefore, we want to highlight the study reported by Puhka and co-workers due to its importance [76]. This study compared alternative normalization methods: EVs based (volume, number, CD9 optical density, other metabolites) and urine-derived factors (volume, creatinine) in the targeted LC-MS/MS analysis of over 100 metabolites in the isolated EVs, original urine samples, and platelets. The study concludes that normalization to EVs volume, number, and CD9 optical density yields comparable results. Moreover, the authors provide insight into the amount of EVs sample (approximately 1×10^{10}) that is required for successful metabolite profiling [76]. Number of EVs detected in plasma ranges from 10^4 to 10^{12} mL^{-1}, in this instance, it is important to standardize the required sample concentration for EVs based studies to achieve comparable results [123]. In the other study reported by Royo et al. the metabolic signals derived from UPLC-MS analysis of the serum samples incubated with EVs released from hepatocytes were normalized by total peak area, and the observed shift was corrected for QC time [124]. Another study by Wojakowska et al. used TIC to normalized GC-MS data from the analysis of head and neck cancer and control samples, either serum or serum-derived exosomes [106]. Kim et al. in the GC-TOF-MS analysis conducted on the gut EVs from colorectal cancer patients and healthy individuals applied the total count method [90], previously described and evaluated in metagenomic data by Pereira et al. [125]. An alternative normalization method that could benefit EVs results is quantile normalization described in metabolomics studies [113] and recently applied also for transcriptomic analysis of pancreatic cancer-derived exosomes [126]. Tadokoro et al. investigated immunosuppressive metabolites within cancer cell-derived EVs and normalized data against particle number and protein amount [104]. However, particular caution should be taken as protein concentration can vary between different EVs subtypes, and in isolated EVs could be overestimated due to co-existed contamination [8]. Additionally, EVs sample storage destabilizes the protein content, therefore if applicable, it should be measured immediately after isolation [127]. As a general recommendation, this strategy is not preferred for untargeted metabolomics studies [113]. An interesting option to consider is a sequential normalization approach proposed by Gagnebin et al. based on the combination of pre-acquisition and post-acquisition sample normalization that allows for the correction of the analytical and concentration variability [119]. What makes the choice of optimal normalization strategy the most difficult is the fact that metabolomics profiles are prone to changes considering all aforementioned variables. Thus, the challenge remains to develop a data normalization method that would take into account all discussed factors, and always model-driven specific context should be considered to decrease data variability. Already published EVs based studies together with metabolomics recommendations can serve as practical benchmarks for further EVs research improvement and development of the standard operating procedures.

5. Concluding remarks

A growing number of studies provide valuable knowledge and understanding of the EVs role in both health and disease. Although EVs hold great promise as potential diagnostic or prognostic biomarkers as well as therapeutic and drug delivery agents, their use in the clinical settings first requires methodological standardization. It has been recognized that EVs based research is particularly challenging due to their complex biogenesis and vast heterogeneity in size, composition, and origin. High-throughput untargeted metabolomics provides great perspectives to decipher EVs molecular cargo. However, it is accompanied by many herein discussed challenges that must be properly addressed to get meaningful results from complex and multidimensional data. Understanding the assumptions throughout metabolomics workflow and already established guidelines would help overcome the numerous limitations of EVs-based research. The lack of global consensus and standard operating protocols, including quality assurance and quality control procedures in EVs studies leads to significant variations in the reported results and makes inter-study comparisons impossible. Hence, given the ubiquitous nature of these limitations, considerable effort should be taken to guarantee the reliability of conducted research. We believe that given considerations might be useful for the scientific community in designing high-quality EVs studies also, but not only, based on untargeted metabolomics analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A. Jurj, O. Zanoaga, C. Braicu, V. Lazar, C. Tomuleasa, A. Irimie, I. Berindan-Neagoe, A comprehensive picture of extracellular vesicles and their contents. Molecular Transfer to Cancer Cells, Cancers (Basel). 12 (2020) 298. https://doi.org/10.3390/cancers12020298.
[2] J.C. Akers, D. Gonda, R. Kim, B.S. Carter, C.C. Chen, Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies, J. Neuro Oncol. 113 (2013) 1–11. https://doi.org/10.1007/s11060-013-1084-8.
[3] C. Théry, Exosomes: secreted vesicles and intercellular communications, F1000 Biol. Rep. 3 (2011). https://doi.org/10.3410/B3-15.
[4] M.Z. Ratajczak, J. Ratajczak, Extracellular microvesicles/exosomes: discovery, disbelief, acceptance, and the future? Leukemia (2020) 1–10. https://doi.org/10.1038/s41375-020-01041-z.
[5] G. Raposo, W. Stoorvogel, Extracellular vesicles: exosomes, microvesicles, and friends, J. Cell Biol. 200 (2013) 373–383. https://doi.org/10.1083/jcb.201211138.
[6] G. Van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (2018) 213–228. https://doi.org/10.1038/nrm.2017.125.
[7] M. Pathan, P. Fonseka, S.V. Chitti, T. Kang, R. Sanwlani, J. Van Deun, A. Hendrix, S. Mathivanan, Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles, Nucleic Acids Res. 47 (2019) D516–D519. https://doi.org/10.1093/nar/gky1029.
[8] L. Doyle, M. Wang, Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis, Cells 8 (2019) 727. https://doi.org/10.3390/cells8070727.
[9] M. Tkach, C. Théry, Communication by extracellular vesicles: where we are and where we need to go, Cell 164 (2016) 1226–1232. https://doi.org/10.1016/j.cell.2016.01.043.
[10] A. Becker, B.K. Thakur, J.M. Weiss, H.S. Kim, H. Peinado, D. Lyden, Extracellular vesicles in cancer: cell-to-cell mediators of metastasis, Canc. Cell 30 (2016) 836–848. https://doi.org/10.1016/j.ccell.2016.10.009.
[11] N. Noren Hooten, M.K. Evans, Extracellular vesicles as signaling mediators in type 2 diabetes mellitus, Am. J. Physiol. Cell Physiol. 318 (2020) C1189–C1199. https://doi.org/10.1152/ajpcell.00536.2019.
[12] C.M. Boulanger, X. Loyer, P.E. Rautou, N. Amabile, Extracellular vesicles in coronary artery disease, Nat. Rev. Cardiol. 14 (2017) 259–272. https://doi.org/10.1038/nrcardio.2017.7.
[13] A.R. Gomes, N.B. Sangani, T.G. Fernandes, M.M. Diogo, L.M.G. Curfs, C.P. Reutelingsperger, Extracellular vesicles in cns developmental disorders, Int. J. Mol. Sci. 21 (2020) 1–20. https://doi.org/10.3390/ijms21249428.
[14] A.O. Ipinmoroti, Q.L. Matthews, Extracellular vesicles: roles in human viral infections, immune-diagnostic, and therapeutic applications, Pathogens 9 (2020) 1–20. https://doi.org/10.3390/pathogens9121056.
[15] M. Hassanpour, J. Rezaie, M. Nouri, Y. Panahi, The role of extracellular vesicles in COVID-19 virus infection, Infect. Genet. Evol. 85 (2020) 104422. https://doi.org/10.1016/j.meegid.2020.104422.
[16] R.E. Lane, D. Korbie, M.M. Hill, M. Trau, Extracellular vesicles as circulating cancer biomarkers: opportunities and challenges, Clin. Transl. Med. 7 (2018). https://doi.org/10.1186/s40169-018-0192-7.
[17] X. Zheng, X. Li, X. Wang, Extracellular vesicle-based liquid biopsy holds great promise for the management of ovarian cancer, Biochim. Biophys. Acta Rev. Canc 1874 (2020) 188395. https://doi.org/10.1016/j.bbcan.2020.188395.
[18] R.S. Conlan, S. Pisano, M.I. Oliveira, M. Ferrari, I. Mendes Pinto, Exosomes as reconfigurable therapeutic systems, Trends Mol. Med. 23 (2017) 636–650. https://doi.org/10.1016/j.molmed.2017.05.003.

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

[19] M. Yáñez-Mó, P.R.-M. Siljander, Z. Andreu, A. Bedina Zavec, F.E. Borràs, E.I. Buzas, K. Buzas, E. Casal, F. Cappello, J. Carvalho, E. Colás, A. Cordeiro-da Silva, S. Fais, J.M. Falcon-Perez, I.M. Ghobrial, B. Giebel, M. Gimona, M. Graner, I. Gursel, M. Gursel, N.H.H. Heegaard, A. Hendrix, P. Kierulf, K. Kokubun, M. Kosanovic, V. Kralj-Iglic, E.-M. Krämer-Albers, S. Laitinen, C. Lässer, T. Lener, E. Ligeti, A. Linē, G. Lipps, A. Llorente, J. Lötvall, M. Manček-Keber, A. Marcilla, M. Mittelbrunn, I. Nazarenko, E.N.M. Nolte'‘t Hoen, T.A. Nyman, L. O'Driscoll, M. Olivan, C. Oliveira, É. Pállinger, H.A. del Portillo, J. Reventós, M. Rigau, E. Rohde, M. Sammar, F. Sánchez-Madrid, N. Santarém, K. Schallmoser, M. Stampe Ostenfeld, W. Stoorvogel, R. Stukelj, S.G. Van der Grein, M. Helena Vasconcelos, M.H.M. Wauben, O. De Wever, Biological properties of extracellular vesicles and their physiological functions, J. Extracell. Vesicles 4 (2015) 27066. https://doi.org/10.3402/jev.v4.27066.

[20] W. Meng, C. He, Y. Hao, L. Wang, L. Li, G. Zhu, Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source, Drug Deliv. 27 (2020) 585–598. https://doi.org/10.1080/10717544.2020.1748758.

[21] S. Gandham, X. Su, J. Wood, A.L. Nocera, S.C. Alli, L. Milane, A. Zimmerman, M. Amiji, A.R. Ivanov, Technologies and standardization in research on extracellular vesicles, Trends Biotechnol. 38 (2020) 1066–1098. https://doi.org/10.1016/j.tibtech.2020.05.012.

[22] C. Théry, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D.C. Ayre, J.M. Bach, D. Bachurski, H. Baharvand, L. Balaj, S. Baldacchino, N.N. Bauer, A.A. Baxter, M. Bebawy, C. Beckham, A. Bedina Zavec, A. Benmoussa, A.C. Berardi, P. Bergese, E. Bielska, C. Blenkiron, S. Bobis-Wozowicz, E. Boilard, W. Boireau, A. Bongiovanni, F.E. Borràs, S. Bosch, C.M. Boulanger, X. Breakefield, A.M. Breglio, M. Brennan, D.R. Brigstock, A. Brisson, M.L.D. Broekman, J.F. Bromberg, P. Bryl-Górecka, S. Buch, A.H. Buck, D. Burger, S. Busatto, D. Buschmann, B. Bussolati, E.I. Buzás, J.B. Byrd, G. Camussi, D.R.F. Carter, S. Caruso, L.W. Chamley, Y.T. Chang, A.D. Chaudhuri, C. Chen, S. Chen, L. Cheng, A.R. Chin, A. Clayton, S.P. Clerici, A. Cocks, E. Cocucci, R.J. Coffey, A. Cordeiro-da-Silva, Y. Couch, F.A.W. Coumans, B. Coyle, R. Crescitelli, M.F. Criado, C. D'Souza-Schorey, S. Das, P. de Candia, E.F. De Santana, O. De Wever, H.A. del Portillo, T. Demaret, S. Deville, A. Devitt, B. Dhondt, D. Di Vizio, L.C. Dieterich, V. Dolo, A.P. Dominguez Rubio, M. Dominici, M.R. Dourado, T.A.P. Driedonks, F.V. Duarte, H.M. Duncan, R.M. Eichenberger, K. Ekström, S. El Andaloussi, C. Elie-Caille, U. Erdbrügger, J.M. Falcón-Pérez, F. Fatima, J.E. Fish, M. Flores-Bellver, A. Försönits, A. Frelet-Barrand, F. Fricke, G. Fuhrmann, S. Gabrielsson, A. Gámez-Valero, C. Gardiner, K. Gärtner, R. Gaudin, Y.S. Gho, B. Giebel, C. Gilbert, M. Gimona, I. Giusti, D.C.I. Goberdhan, A. Görgens, S.M. Gorski, D.W. Greening, J.C. Gross, A. Gualerzi, G.N. Gupta, D. Gustafson, A. Handberg, R.A. Haraszti, P. Harrison, H. Hegyesi, A. Hendrix, A.F. Hill, F.H. Hochberg, K.F. Hoffmann, B. Holder, H. Holthofer, B. Hosseinkhani, G. Hu, Y. Huang, V. Huber, S. Hunt, A.G.E. Ibrahim, T. Ikezu, J.M. Inal, M. Isin, A. Ivanova, H.K. Jackson, S. Jacobsen, S.M. Jay, M. Jayachandran, G. Jenster, L. Jiang, S.M. Johnson, J.C. Jones, A. Jong, T. Jovanovic-Talisman, S. Jung, R. Kalluri, S. ichi Kano, S. Kaur, Y. Kawamura, E.T. Keller, D. Khamari, E. Khomyakova, A. Khvorova, P. Kierulf, K.P. Kim, T. Kislinger, M. Klingeborn, D.J. Klinke, M. Kornek, M.M. Kosanović, Á.F. Kovács, E.M. Krämer-Albers, S. Krasemann, M. Krause, I.V. Kurochkin, G.D. Kusuma, S. Kuypers, S. Laitinen, S.M. Langevin, L.R. Languino, J. Lannigan, C. Lässer, L.C. Laurent, G. Lavieu, E. Lázaro-Ibáñez, S. Le Lay, M.S. Lee, Y.X.F. Lee, D.S. Lemos, M. Lenassi, A. Leszczynska, I.T.S. Li, K. Liao, S.F. Libregts, E. Ligeti, R. Lim, S.K. Lim, A. Linē, K. Linnemannstöns, A. Llorente, C.A. Lombard, M.J. Lorenowicz, Á.M. Lörincz, J. Lötvall, J. Lovett, M.C. Lowry, X. Loyer, Q. Lu, B. Lukomska, T.R. Lunavat, S.L.N. Maas, H. Malhi, A. Marcilla, J. Mariani, J. Mariscal, E.S. Martens-Uzunova, L. Martin-Jaular, M.C. Martinez, V.R. Martins, M. Mathieu, S. Mathivanan, M. Maugeri, L.K. McGinnis, M.J. McVey, D.G. Meckes, K.L. Meehan, I. Mertens, V.R. Minciacchi, A. Möller, M. Møller Jørgensen, A. Morales-Kastresana, J. Morhayim, F. Mullier, M. Muraca, L. Musante, V. Mussack, D.C. Muth, K.H. Myburgh, T. Najrana, M. Nawaz, I. Nazarenko, P. Nejsum, C. Neri, T. Neri, R. Nieuwland, L. Nimrichter, J.P. Nolan, E.N.M. Nolte-'t Hoen, N. Noren Hooten, L. O'Driscoll, T. O'Grady, A. O'Loghlen, T. Ochiya, M. Olivier, A. Ortiz, L.A. Ortiz, X. Osteikoetxea, O. Ostegaard, M. Ostrowski, J. Park, D.M. Pegtel, H. Peinado, F. Perut, M.W. Pfaffl, D.G. Phinney, B.C.H. Pieters, R.C. Pink, D.S. Pisetsky, E. Pogge von Strandmann, I. Polakovicova, I.K.H. Poon, B.H. Powell, I. Prada, L. Pulliam, P. Quesenberry, A. Radeghieri, R.L. Raffai, S. Raimondo, J. Rak, M.I. Ramirez, G. Raposo, M.S. Rayyan, N. Regev-Rudzki, F.L. Ricklefs, P.D. Robbins, D.D. Roberts, S.C. Rodrigues, E. Rohde, S. Rome, K.M.A. Rouschop, A. Rughetti, A.E. Russell, P. Saá, S. Sahoo, E. Salas-Huenuleo, C. Sánchez, J.A. Saugstad, M.J. Saul, R.M. Schiffelers, R. Schneider, T.H. Schøyen, A. Scott, E. Shahaj, S. Sharma, O. Shatnyeva, F. Shekari, G.V. Shelke, A.K. Shetty, K. Shiba, P.R.M. Siljander, A.M. Silva, A. Skowronek, O.L. Snyder, R.P. Soares, B.W. Sódar, C. Soekmadji, J. Sotillo, P.D. Stahl, W. Stoorvogel, S.L. Stott, E.F. Strasser, S. Swift, H. Tahara, M. Tewari, K. Timms, S. Tiwari, R. Tixeira, M. Tkach, W.S. Toh, R. Tomasini, A.C. Torrecilhas, J.P. Tosar, V. Toxavidis, L. Urbanelli, P. Vader, B.W.M. van Balkom, S.G. van der Grein, J. Van Deun, M.J.C. van Herwijnen, K. Van Keuren-Jensen, G. van Niel, M.E. van Royen, A.J. van Wijnen, M.H. Vasconcelos, I.J. Vechetti, T.D. Veit, L.J. Vella, É. Velot, F.J. Verweij, B. Vestad, J.L. Viñas, T. Visnovitz, K.V. Vukman, J. Wahlgren, D.C. Watson, M.H.M. Wauben, A. Weaver, J.P. Webber, V. Weber, A.M. Wehman, D.J. Weiss, J.A. Welsh, S. Wendt, A.M. Wheelock, Z. Wiener, L. Witte, J. Wolfram, A. Xagorari, P. Xander, J. Xu, X. Yan, M. Yáñez-Mó, H. Yin, Y. Yuana, V. Zappulli, J. Zarubova, V. Žėkas, J. ye Zhang, Z. Zhao, L. Zheng, A.R. Zheutlin, A.M. Zickler, P. Zimmermann, A.M. Zivkovic, D. Zocco, E.K. Zuba-Surma, Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (2018). https://doi.org/10.1080/20013078.2018.1535750.

[23] C. Soekmadji, B. Li, Y. Huang, H. Wang, T. An, C. Liu, W. Pan, J. Chen, L. Cheung, J.M. Falcon-Perez, Y.S. Gho, H.B. Holthofer, M.T.N. Le, A. Marcilla, L. O'Driscoll, F. Shekari, T.L. Shen, A.C. Torrecilhas, X. Yan, F. Yang, H. Yin, Y. Xiao, Z. Zhao, X. Zou, Q. Wang, L. Zheng, The future of Extracellular Vesicles as Theranostics—an ISEV meeting report, J. Extracell. Vesicles 9 (2020) 1809766. https://doi.org/10.1080/20013078.2020.1809766.

[24] A. Turchinovich, O. Drapkina, A. Tonevitsky, Transcriptome of extracellular vesicles: state-of-the-art, Front. Immunol. 10 (2019). https://doi.org/10.3389/fimmu.2019.00202.

[25] S. Kreimer, A.M. Belov, I. Ghiran, S.K. Murthy, D.A. Frank, A.R. Ivanov, Mass-spectrometry-based molecular characterization of extracellular vesicles: lipidomics and proteomics, J. Proteome Res. 14 (2015) 2367–2384. https://doi.org/10.1021/pr501279t.

[26] C. Williams, M. Palviainen, N.C. Reichardt, P.R.M. Siljander, J.M. Falcón-Pérez, Metabolomics applied to the study of extracellular vesicles, Metabolites 9 (2019) 1–14. https://doi.org/10.3390/metabo9110276.

[27] D. Dudzik, C. Barbas-Bernardos, A. García, C. Barbas, Quality assurance procedures for mass spectrometry untargeted metabolomics. a review, J. Pharmaceut. Biomed. Anal. 147 (2018) 149–173. https://doi.org/10.1016/j.jpba.2017.07.044.

[28] A.M. Evans, C. O'Donovan, M. Playdon, C. Beecher, R.D. Beger, J.A. Bowden, D. Broadhurst, C.B. Clish, S. Dasari, W.B. Dunn, J.L. Griffin, T. Hartung, P.C. Hsu, T. Huan, J. Jans, C.M. Jones, M. Kachman, A. Kleensang, M.R. Lewis, M.E. Monge, J.D. Mosley, E. Taylor, F. Tayyari, G. Theodoridis, F. Torta, B.K. Ubhi, D. Vuckovic, Dissemination and analysis of the quality assurance (QA) and quality control (QC) practices of LC–MS based untargeted metabolomics practitioners, Metabolomics 16 (2020). https://doi.org/10.1007/s11306-020-01728-5.

[29] D. Broadhurst, R. Goodacre, S.N. Reinke, J. Kuligowski, I.D. Wilson, M.R. Lewis, W.B. Dunn, Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies, Metabolomics 14 (2018) 72. https://doi.org/10.1007/s11306-018-1367-3.

[30] R.D. Beger, W.B. Dunn, A. Bandukwala, B. Bethan, D. Broadhurst, C.B. Clish, S. Dasari, L. Derr, A. Evans, S. Fischer, T. Flynn, T. Hartung, D. Herrington, R. Higashi, P.C. Hsu, C. Jones, M. Kachman, H. Karuso, G. Kruppa, K. Lippa, P. Maruvada, J. Mosley, I. Ntai, C. O'Donovan, M. Playdon, D. Raftery, D. Shaughnessy, A. Souza, T. Spaeder, B. Spalholz, F. Tayyari, B. Ubhi, M. Verma, T. Walk, I. Wilson, K. Witkin, D.W. Bearden, K.A. Zanetti, Towards quality assurance and quality control in untargeted metabolomics studies, Metabolomics 15 (2019). https://doi.org/10.1007/s11306-018-1460-7.

[31] Y. Yuana, R.M. Bertina, S. Osanto, Pre-analytical and analytical issues in the analysis of blood microparticles, Thromb. Haemostasis 105 (2011) 396–408. https://doi.org/10.1160/TH10-09-0595.

[32] F.A.W. Coumans, A.R. Brisson, E.I. Buzas, F. Dignat-George, E.E.E. Drees, S. El-Andaloussi, C. Emanueli, A. Gasecka, A. Hendrix, A.F. Hill, R. Lacroix, Y. Lee, T.G. Van Leeuwen, N. Mackman, I. Mäger, J.P. Nolan, E. Van Der Pol, D.M. Pegtel, S. Sahoo, P.R.M. Siljander, G. Sturk, O. De Wever, R. Nieuwland, Methodological guidelines to study extracellular vesicles, Circ. Res. 120 (2017) 1632–1648. https://doi.org/10.1161/CIRCRESAHA.117.309417.

[33] K.W. Witwer, E.I. Buzás, L.T. Bemis, A. Bora, C. Lässer, J. Lötvall, E.N. Nolte'‘t Hoen, M.G. Piper, S. Sivaraman, J. Skog, C. Théry, M.H. Wauben, F. Hochberg, Standardization of sample collection, isolation and analysis methods in extracellular vesicle research, J. Extracell. Vesicles 2 (2013) 20360. https://www.tandfonline.com/doi/full/10.3402/jev.v2i0.20360. (Accessed 8 October 2020).

[34] G. Müller, Novel tools for the study of cell type-specific exosomes and microvesicles, J. Bioanal. Biomed. 4 (2012) 46–60. https://doi.org/10.4172/1948-593X.1000063.

[35] N. Karimi, A. Cvjetkovic, S.C. Jang, R. Crescitelli, M.A. Hosseinpour Feizi, R. Nieuwland, J. Lötvall, C. Lässer, Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins, Cell. Mol. Life Sci. 75 (2018) 2873–2886. https://doi.org/10.1007/s00018-018-2773-4.

[36] R. Lacroix, C. Judicone, M. Mooberry, M. Boucekine, N.S. Key, F. Dignat-George, A. Ambrozic, N. Bailly, C. Buffat, E. Buzas, A. Charpentier, B. Chatelain, J.M.D. Dogne, A. Falanga, E. Garcia Arias-Salgado, B. Gyorgy, M. Harrison, H. Kwaan, K. Lakota, V. Latger-Cannard, F. Massin, F. Mobarrez, F. Mojca, F. Mullier, J.P. Phelan, N. Snoj, S. Sodin-Semrl, E. Stezpień, S. Susen, A.D. Terrisse, V. Tintillier, A. Vignoli, Standardization of pre-analytical variables in plasma microparticle determination: results of the international society on Thrombosis and Haemostasis SSC collaborative workshop, J. Thromb. Haemostasis 11 (2013) 1190–1193. https://doi.org/10.1111/jth.12207.

[37] P. Yin, R. Lehmann, G. Xu, Effects of pre-analytical processes on blood samples used in metabolomics studies, Anal. Bioanal. Chem. 407 (2017) 4879–4892. https://doi.org/10.1007/s00216-015-8565-x.

[38] V.V. Hernandes, C. Barbas, D. Dudzik, A review of blood sample handling and pre-processing for metabolomics studies, Electrophoresis 38 (2017) 2232–2241. https://doi.org/10.1002/elps.201700086.

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

[39] R. González-Domínguez, Á. González-Domínguez, A. Sayago, Á. Fernández-Recamales, Recommendations and best practices for standardizing the pre-analytical processing of blood and urine samples in metabolomics, Metabolites 10 (2020) 1–18. https://doi.org/10.3390/metabo10060229.

[40] L.J. Vella, B.J. Scicluna, L. Cheng, E.G. Bawden, C.L. Masters, C.S. Ang, N. Willamson, C. McLean, K.J. Barnham, A.F. Hill, A rigorous method to enrich for exosomes from brain tissue, J. Extracell. Vesicles 6 (2017). https://doi.org/10.1080/20013078.2017.1348885.

[41] Z. Bin Deng, A. Poliakov, R.W. Hardy, R. Clements, C. Liu, Y. Liu, J. Wang, X. Xiang, S. Zhang, X. Zhuang, S.V. Shah, D. Sun, S. Michalek, W.E. Grizzle, T. Garvey, J. Mobley, H.G. Zhang, Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance, Diabetes 58 (2009). https://doi.org/10.2337/db09-0216.

[42] M. Bordas, G. Genard, S. Ohl, M. Nessling, K. Richter, T. Roider, S. Dietrich, K.K. Maaß, M. Seiffert, Optimized protocol for isolation of small extracellular vesicles from human and murine lymphoid tissues, Int. J. Mol. Sci. 21 (2020). https://doi.org/10.3390/ijms21155586.

[43] H. Zhou, P.S.T. Yuen, T. Pisitkun, P.A. Gonzales, H. Yasuda, J.W. Dear, P. Gross, M.A. Knepper, R.A. Star, Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery, Kidney Int. 69 (2006). https://doi.org/10.1038/sj.ki.5000273.

[44] Z. Liu, D.M. Cauvi, E.M.A. Bernardino, B. Lara, R.E. Lizardo, D. Hawisher, S. Bickler, A. De Maio, Isolation and characterization of human urine extracellular vesicles, Cell Stress Chaperones 23 (2018). https://doi.org/10.1007/s12192-018-0902-5.

[45] J. Li, X. He, Y. Deng, C. Yang, An update on isolation methods for proteomic studies of extracellular vesicles in biofluids, Molecules 24 (2019). https://doi.org/10.3390/molecules24193516.

[46] K. Sidhom, P.O. Obi, A. Saleem, A review of exosomal isolation methods: is size exclusion chromatography the best option? Int. J. Mol. Sci. 21 (2020) 1–19. https://doi.org/10.3390/ijms21186466.

[47] G. Raposo, H.W. Nijman, W. Stoorvogel, R. Leijendekker, C.V. Harding, C.J.M. Melief, H.J. Geuze, B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (1996) 1161–1172. https://doi.org/10.1084/jem.183.3.1161.

[48] C. Gardiner, D. Di Vizio, S. Sahoo, C. Théry, K.W. Witwer, M. Wauben, A.F. Hill, Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey, J. Extracell. Vesicles 5 (2016). https://doi.org/10.3402/jev.v5.32945.

[49] J. Burrello, V. Biemmi, M. Dei Cas, M. Amongero, S. Bolis, E. Lazzarini, S. Bollini, G. Vassalli, R. Paroni, L. Barile, Sphingolipid composition of circulating extracellular vesicles after myocardial ischemia, Sci. Rep. 10 (2020). https://doi.org/10.1038/s41598-020-73411-7.

[50] W.P. Kuo, J. Shidong (Editors), Extracellular Vesicles Methods and Protocols, Humana Press, 2017. https://doi.org/10.1007/978-1-4939-7253-1.

[51] C. Théry, S. Amigorena, G. Raposo, A. Clayton, Isolation and characterization of exosomes from cell culture supernatants and biological fluids, in: Curr. Protoc. Cell Biol, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2006. https://doi.org/10.1002/0471143030.cb0322s30. Unit 3.22.

[52] M. Clos-Garcia, A. Loizaga-Iriarte, P. Zuñiga-Garcia, P. Sánchez-Mosquera, A. Rosa Cortazar, E. González, V. Torrano, C. Alonso, M. Pérez-Cormenzana, A. Ugalde-Olano, I. Lacasa-Viscasillas, A. Castro, F. Royo, M. Unda, A. Carracedo, J.M. Falcón-Pérez, Metabolic alterations in urine extracellular vesicles are associated to prostate cancer pathogenesis and progression, J. Extracell. Vesicles 7 (2018). https://doi.org/10.1080/20013078.2018.1470442.

[53] M.P. Caby, D. Lankar, C. Vincendeau-Scherrer, G. Raposo, C. Bonnerot, Exosomal-like vesicles are present in human blood plasma, Int. Immunol. 17 (2005) 879–887. https://doi.org/10.1093/intimm/dxh267.

[54] F. Cappello, M. Logozzi, C. Campanella, C.C. Bavisotto, A. Marcilla, F. Properzi, S. Fais, Exosome levels in human body fluids: a tumor marker by themselves? Eur. J. Pharmaceut. Sci. 96 (2017) 93–98. https://doi.org/10.1016/j.ejps.2016.09.010.

[55] D. Yuan, Y. Zhao, W.A. Banks, K.M. Bullock, M. Haney, E. Batrakova, A.V. Kabanov, Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain, Biomaterials 142 (2017) 1–12. https://doi.org/10.1016/j.biomaterials.2017.07.011.

[56] M.J. Haney, N.L. Klyachko, Y. Zhao, R. Gupta, E.G. Plotnikova, Z. He, T. Patel, A. Piroyan, M. Sokolsky, A.V. Kabanov, E.V. Batrakova, Exosomes as drug delivery vehicles for Parkinson's disease therapy, J. Contr. Release 207 (2015) 18–30. https://doi.org/10.1016/j.jconrel.2015.03.033.

[57] X. Luo, M. An, K.C. Cuneo, D.M. Lubman, L. Li, High-performance chemical isotope labeling liquid chromatography mass spectrometry for exosome metabolomics, Anal. Chem. 90 (2018) 8314–8319. https://doi.org/10.1021/acs.analchem.8b01726.

[58] T. Nielsen, A.F. Kristensen, S. Pedersen, G. Christiansen, S.R. Kristensen, Investigation of procoagulant activity in extracellular vesicles isolated by differential ultracentrifugation, J. Extracell. Vesicles 7 (2018). https://doi.org/10.1080/20013078.2018.1454777.

[59] N. Ludwig, T.L. Whiteside, T.E. Reichert, Challenges in exosome isolation and analysis in health and disease, Int. J. Mol. Sci. 20 (2019). https://doi.org/10.3390/ijms20194684.

[60] E.M. Veziroglu, G.I. Mias, Characterizing extracellular vesicles and their diverse RNA contents, Front. Genet. 11 (2020). https://doi.org/10.3389/fgene.2020.00700.

[61] R. Hou, Y. Li, Z. Sui, H. Yuan, K. Yang, Z. Liang, L. Zhang, Y. Zhang, Advances in exosome isolation methods and their applications in proteomic analysis of biological samples, Anal. Bioanal. Chem. 411 (2019) 5351–5361. https://doi.org/10.1007/s00216-019-01982-0.

[62] H. Bu, D. He, X. He, K. Wang, Exosomes: isolation, analysis, and applications in cancer detection and therapy, Chembiochem 20 (2019) 451–461. https://doi.org/10.1002/cbic.201800470.

[63] D. Yang, W. Zhang, H. Zhang, F. Zhang, L. Chen, L. Ma, L.M. Larcher, S. Chen, N. Liu, Q. Zhao, P.H.L. Tran, C. Chen, R.N. Veedu, T. Wang, Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics, Theranostics 10 (2020) 3684–3707. https://doi.org/10.7150/thno.41580.

[64] M. Chang, Q. Wang, W. Qin, X. Shi, G. Xu, Rational synthesis of aptamer-functionalized polyethylenimine-modified magnetic graphene oxide composites for highly efficient enrichment and comprehensive metabolomics analysis of exosomes, Anal. Chem. 92 (2020) 15497–15505. https://doi.org/10.1021/acs.analchem.0c03374.

[65] Y. Du, L. Chen, X.-S. Li, X.-L. Li, X.-D. Xu, S.-B. Tai, G.-L. Yang, Q. Tang, H. Liu, S.-H. Liu, S.-Y. Zhang, Y. Cheng, Metabolomic identification of exosome-derived biomarkers for schizophrenia: a large multicenter study, Schizophr. Bull. (2020). https://doi.org/10.1093/schbul/sbaa166.

[66] A. Askeland, A. Borup, O. Østergaard, J.V. Olsen, S.M. Lund, G. Christiansen, S.R. Kristensen, N.H.H. Heegaard, S. Pedersen, Mass-spectrometry based proteome comparison of extracellular vesicle isolation methods: comparison of ME-kit, size-exclusion chromatography, and high-speed centrifugation, Biomedicines 8 (2020). https://doi.org/10.3390/BIOMEDICINES8080246.

[67] C.N. Davis, H. Phillips, J.J. Tomes, M.T. Swain, T.J. Wilkinson, P.M. Brophy, R.M. Morphew, The importance of extracellular vesicle purification for downstream analysis: a comparison of differential centrifugation and size exclusion chromatography for helminth pathogens, PLoS Neglected Trop. Dis. 13 (2019). https://doi.org/10.1371/journal.pntd.0007191.

[68] S. Lin, Z. Yu, D. Chen, Z. Wang, J. Miao, Q. Li, D. Zhang, J. Song, D. Cui, Progress in microfluidics-based exosome separation and detection technologies for diagnostic applications, Small 16 (2020). https://doi.org/10.1002/smll.201903916.

[69] Y. Zhang, J. Bi, J. Huang, Y. Tang, S. Du, P. Li, Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications, Int. J. Nanomed. 15 (2020) 6917–6934. https://doi.org/10.2147/IJN.S264498.

[70] M. Ding, C. Wang, X. Lu, C. Zhang, Z. Zhou, X. Chen, C.Y. Zhang, K. Zen, C. Zhang, Comparison of commercial exosome isolation kits for circulating exosomal microRNA profiling, Anal. Bioanal. Chem. 410 (2018) 3805–3814. https://doi.org/10.1007/s00216-018-1052-4.

[71] M.J. Rardin, Rapid assessment of contaminants and interferences in mass spectrometry data using skyline, J. Am. Soc. Mass Spectrom. 29 (2018) 1327–1330. https://doi.org/10.1007/s13361-018-1940-z.

[72] A. Svan, M. Hedeland, T. Arvidsson, C.E. Pettersson, The differences in matrix effect between supercritical fluid chromatography and reversed phase liquid chromatography coupled to ESI/MS, Anal. Chim. Acta 1000 (2018) 163–171. https://doi.org/10.1016/j.aca.2017.10.014.

[73] C. Zhao, P.B. O'Connor, Removal of polyethylene glycols from protein samples using titanium dioxide, Anal. Biochem. 365 (2007) 283–285. https://doi.org/10.1016/j.ab.2007.03.024.

[74] S.S. Kanwar, C.J. Dunlay, D.M. Simeone, S. Nagrath, Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes, Lab Chip 14 (2014) 1891–1900. https://doi.org/10.1039/c4lc00136b.

[75] Y.T. Kang, E. Purcell, C. Palacios-Rolston, T.W. Lo, N. Ramnath, S. Jolly, S. Nagrath, Isolation and profiling of circulating tumor-associated exosomes using extracellular vesicular lipid–protein binding affinity based microfluidic device, Small 15 (2019). https://doi.org/10.1002/smll.201903600.

[76] M. Puhka, M. Takatalo, M.E. Nordberg, S. Valkonen, J. Nandania, M. Aatonen, M. Yliperttula, S. Laitinen, V. Velagapudi, T. Mirtti, O. Kallioniemi, A. Rannikko, P.R.M. Siljander, T.M. Af Hällström, Metabolomic profiling of extracellular vesicles and alternative normalization methods reveal enriched metabolites and strategies to study prostate cancer-related changes, Theranostics 7 (2017) 3824–3841. https://doi.org/10.7150/thno.19890.

[77] M.I. Ramirez, M.G. Amorim, C. Gadelha, I. Milic, J.A. Welsh, V.M. Freitas, M. Nawaz, N. Akbar, Y. Couch, L. Makin, F. Cooke, A.L. Vettore, P.X. Batista, R. Freezor, J.A. Pezuk, L. Rosa-Fernandes, A.C.O. Carreira, A. Devitt, L. Jacobs, I.T. Silva, G. Coakley, D.N. Nunes, D. Carter, G. Palmisano, E. Dias-Neto, Technical challenges of working with extracellular vesicles, Nanoscale 10 (2018) 881–906. https://doi.org/10.1039/c7nr08360b.

[78] G.D. Kusuma, M. Barabadi, J.L. Tan, D.A.V. Morton, J.E. Frith, R. Lim, To protect and to preserve: novel preservation strategies for extracellular vesicles, Front. Pharmacol. 9 (2018). https://doi.org/10.3389/fphar.2018.01199.

[79] S. Bosch, L. De Beaurepaire, M. Allard, M. Mosser, C. Heichette, D. Chrétien, D. Jegou, J.M. Bach, Trehalose prevents aggregation of exosomes and cryodamage, Sci. Rep. 6 (2016). https://doi.org/10.1038/srep36162.

[80] C. Charoenviriyakul, Y. Takahashi, M. Nishikawa, Y. Takakura, Preservation of exosomes at room temperature using lyophilization, Int. J. Pharm. 553 (2018) 1–7. https://doi.org/10.1016/j.ijpharm.2018.10.032.

[81] A. Behfar, A. Terzic, W. Cook, R. Crespo, Exosome Delivery Technology, 2016. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2016145330. (Accessed 8 February 2021).

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

[82] A. Jeyaram, S.M. Jay, Preservation and storage stability of extracellular vesicles for therapeutic applications, AAPS J. 20 (2018). https://doi.org/10.1208/s12248-017-0160-y.
[83] J.-Y. Wu, Y.-J. Li, X.-B. Hu, S. Huang, D.-X. Xiang, Preservation of small extracellular vesicles for functional analysis and therapeutic applications: a comparative evaluation of storage conditions, Drug Deliv. 28 (2021) 162–170. https://doi.org/10.1080/10717544.2020.1869866.
[84] P. Luo, K. Mao, J. Xu, F. Wu, X. Wang, S. Wang, M. Zhou, L. Duan, Q. Tan, G. Ma, G. Yang, R. Du, H. Huang, Q. Huang, Y. Li, M. Guo, Y. Jin, Metabolic characteristics of large and small extracellular vesicles from pleural effusion reveal biomarker candidates for the diagnosis of tuberculosis and malignancy, J. Extracell. Vesicles 9 (2020). https://doi.org/10.1080/20013078.2020.1790158.
[85] E.J. Want, G. O'Maille, C.A. Smith, T.R. Brandon, W. Uritboonthai, C. Qin, S.A. Trauger, G. Siuzdak, Solvent-dependent metabolite distribution, clustering, and protein extraction for serum profiling with mass spectrometry, Anal. Chem. 78 (2006) 743–752. https://doi.org/10.1021/ac051312t.
[86] S. Tulipani, R. Llorach, M. Urpi-Sarda, C. Andres-Lacueva, Comparative analysis of sample preparation methods to handle the complexity of the blood fluid metabolome: when less is more, Anal. Chem. 85 (2013) 341–348. https://doi.org/10.1021/ac302919t.
[87] J. Sostare, R. Di Guida, J. Kirwan, K. Chalal, E. Palmer, W.B. Dunn, M.R. Viant, Comparison of modified Matyash method to conventional solvent systems for polar metabolite and lipid extractions, Anal. Chim. Acta 1037 (2018) 301–315. https://doi.org/10.1016/j.aca.2018.03.019.
[88] R. Correa, L.M. Coronado, A.C. Garrido, A.A. Durant-Archibold, C. Spadafora, Volatile organic compounds associated with Plasmodium falciparum infection in vitro, Parasites Vectors (2017) 10. https://doi.org/10.1186/s13071-017-2157-x.
[89] T. Altadill, I. Campoy, L. Lanau, K. Gill, M. Rigau, A. Gil-Moreno, J. Reventos, S. Byers, E. Colas, A.K. Cheema, Enabling metabolomics based biomarker discovery studies using molecular phenotyping of exosome-like vesicles, PloS One 11 (2016). https://doi.org/10.1371/journal.pone.0151339.
[90] D.J. Kim, J. Yang, H. Seo, W.H. Lee, D. Ho Lee, S. Kym, Y.S. Park, J.G. Kim, I.J. Jang, Y.K. Kim, J.Y. Cho, Colorectal cancer diagnostic model utilizing metagenomic and metabolomic data of stool microbial extracellular vesicles, Sci. Rep. 10 (2020). https://doi.org/10.1038/s41598-020-59529-8.
[91] T.W.M. Fan, X. Zhang, C. Wang, Y. Yang, W.Y. Kang, S. Arnold, R.M. Higashi, J. Liu, A.N. Lane, Exosomal lipids for classifying early and late stage non-small cell lung cancer, Anal. Chim. Acta 1037 (2018) 256–264. https://doi.org/10.1016/j.aca.2018.02.051.
[92] K.P. Hough, L.S. Wilson, J.L. Trevor, J.G. Strenkowski, N. Maina, Y. Il Kim, M.L. Spell, Y. Wang, D. Chanda, J.R. Dager, N.S. Sharma, M. Curtiss, V.B. Antony, M.T. Dransfield, D.D. Chaplin, C. Steele, S. Barnes, S.R. Duncan, J.K. Prasain, V.J. Thannickal, J.S. Deshane, Unique lipid signatures of extracellular vesicles from the airways of asthmatics, Sci. Rep. 8 (2018) 1–16. https://doi.org/10.1038/s41598-018-28655-9.
[93] F. Royo, D. Gil-Carton, E. Gonzalez, J. Mleczko, L. Palomo, M. Perez-Cormenzana, R. Mayo, C. Alonso, J.M. Falcon-Perez, Differences in the metabolite composition and mechanical properties of extracellular vesicles secreted by hepatic cellular models, J. Extracell. Vesicles 8 (2019). https://doi.org/10.1080/20013078.2019.1575678.
[94] N. Nishida-Aoki, Y. Izumi, H. Takeda, M. Takahashi, T. Ochiya, T. Bamba, Lipidomic analysis of cells and extracellular vesicles from high-and low-metastatic triple-negative breast cancer, Metabolites 10 (2020). https://doi.org/10.3390/metabo10020067.
[95] A. Llorente, T. Skotland, T. Sylvänne, D. Kauhanen, T. Róg, A. Orłowski, I. Vattulainen, K. Ekroos, K. Sandvig, Molecular lipidomics of exosomes released by PC-3 prostate cancer cells, Biochim, Biophys. Acta - Mol. Cell Biol. Lipids. 1831 (2013) 1302–1309. https://doi.org/10.1016/j.bbalip.2013.04.011.
[96] T. Skotland, K. Sandvig, A. Llorente, Lipids in exosomes: current knowledge and the way forward, Prog. Lipid Res. 66 (2017) 30–41. https://doi.org/10.1016/j.plipres.2017.03.001.
[97] O. Peterka, R. Jirásko, M. Chocholoušková, L. Kuchař, D. Wolrab, R. Hájek, D. Vrána, O. Strouhal, B. Melichar, M. Holčapek, Lipidomic characterization of exosomes isolated from human plasma using various mass spectrometry techniques, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1865 (2020) 158634. https://doi.org/10.1016/j.bbalip.2020.158634.
[98] H. Zhao, L. Yang, J. Baddour, A. Achreja, V. Bernard, T. Moss, J.C. Marini, T. Tudawe, E.G. Seviour, F.A. San Lucas, H. Alvarez, S. Gupta, S.N. Maiti, L. Cooper, D. Peehl, P.T. Ram, A. Maitra, D. Nagrath, Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism, Elife 5 (2016). https://doi.org/10.7554/eLife.10250.001.
[99] M. Palviainen, H. Saari, O. Kärkkäinen, J. Pekkinen, S. Auriola, M. Yliperttula, M. Puhka, K. Hanhineva, P.R.M. Siljander, Metabolic signature of extracellular vesicles depends on the cell culture conditions, J. Extracell. Vesicles 8 (2019). https://doi.org/10.1080/20013078.2019.1596669.
[100] S. Keerthikumar, D. Chisanga, D. Ariyaratne, H. Al Saffar, S. Anand, K. Zhao, M. Samuel, M. Pathan, M. Jois, N. Chilamkurti, L. Gangoda, S. Mathivanan, ExoCarta: a web-based compendium of exosomal cargo, J. Mol. Biol. 428 (2016) 688–692. https://doi.org/10.1016/j.jmb.2015.09.019.
[101] D.K. Kim, J. Lee, S.R. Kim, D.S. Choi, Y.J. Yoon, J.H. Kim, G. Go, D. Nhung, K. Hong, S.C. Jang, S.H. Kim, K.S. Park, O.Y. Kim, H.T. Park, J.H. Seo, E. Aikawa, M. Baj-Krzyworzeka, B.W.M. Van Balkom, M. Belting, L. Blanc, V. Bond, A. Bongiovanni, F.E. Borràs, L. Buée, E.I. Buzás, L. Cheng, A. Clayton, E. Cocucci, C.S. Dela Cruz, D.M. Desiderio, D. Di Vizio, K. Ekström, J.M. Falcon-Perez, C. Gardiner, B. Giebel, D.W. Greening, J. Christina Gross, D. Gupta, A. Hendrix, A.F. Hill, M.M. Hill, E. Nolte-'T Hoen, D.W. Hwang, J. Inal, M.V. Jagannadham, M. Jayachandran, Y.K. Jee, M. Jørgensen, K.P. Kim, Y.K. Kim, T. Kislinger, C. Lässer, D.S. Lee, H. Lee, J. Van Leeuwen, T. Lener, M.L. Liu, J. Lötvall, A. Marcilla, S. Mathivanan, A. Möller, J. Morhayim, F. Mullier, I. Nazarenko, R. Nieuwland, D.N. Nunes, K. Pang, J. Park, T. Patel, G. Pocsfalvi, H. Del Portillo, U. Putz, M.I. Ramirez, M.L. Rodrigues, T.Y. Roh, F. Royo, S. Sahoo, R. Schiffelers, S. Sharma, P. Siljander, R.J. Simpson, C. Soekmadji, P. Stahl, A. Stensballe, E. Stepień, H. Tahara, A. Trummer, H. Valadi, L.J. Vella, S.N. Wai, K. Witwer, M. Yánez-Mó, H. Youn, R. Zeidler, Y.S. Gho, EVpedia: a community web portal for extracellular vesicles research, Bioinformatics 31 (2015) 933–939. https://doi.org/10.1093/bioinformatics/btu741.
[102] A. Achreja, H. Zhao, L. Yang, T.H. Yun, J. Marini, D. Nagrath, Exo-MFA – a 13C metabolic flux analysis framework to dissect tumor microenvironment-secreted exosome contributions towards cancer cell metabolism, Metab. Eng. 43 (2017) 156–172. https://doi.org/10.1016/j.ymben.2017.01.001.
[103] C.C. Eylem, M. Yilmaz, B. Derkus, E. Nemutlu, C.B. Camci, E. Yilmaz, M.A. Turkoglu, B. Aytac, N. Ozyurt, E. Emregul, Untargeted multi-omic analysis of colorectal cancer-specific exosomes reveals joint pathways of colorectal cancer in both clinical samples and cell culture, Canc. Lett. 469 (2020) 186–194. https://doi.org/10.1016/j.canlet.2019.10.038.
[104] H. Tadokoro, A. Hirayama, R. Kudo, M. Hasebe, Y. Yoshioka, J. Matsuzaki, Y. Yamamoto, M. Sugimoto, T. Soga, T. Ochiya, Adenosine leakage from perforin-burst extracellular vesicles inhibits perforin secretion by cytotoxic T-lymphocytes, PloS One 15 (2020) 1–21. https://doi.org/10.1371/journal.pone.0231430.
[105] R.A. Haraszti, M.C. Didiot, E. Sapp, J. Leszyk, S.A. Shaffer, H.E. Rockwell, F. Gao, N.R. Narain, M. DiFiglia, M.A. Kiebish, N. Aronin, A. Khvorova, High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources, J. Extracell. Vesicles 5 (2016). https://doi.org/10.3402/jev.v5.32570.
[106] A. Wojakowska, A. Zebrowska, A. Skowronek, T. Rutkowski, K. Polanski, P. Widlak, L. Marczak, M. Pietrowska, Metabolic profiles of whole serum and serum-derived exosomes are different in head and neck cancer patients treated by radiotherapy, J. Personalized Med. 10 (2020) 1–14. https://doi.org/10.3390/jpm10040229.
[107] S. Valkonen, E. van der Pol, A. Böing, Y. Yuana, M. Yliperttula, R. Nieuwland, S. Laitinen, P.R.M. Siljander, Biological reference materials for extracellular vesicle studies, Eur. J. Pharmaceut. Sci. 98 (2017) 4–16. https://doi.org/10.1016/j.ejps.2016.09.008.
[108] J.A. Welsh, E. van der Pol, B.A. Bettin, D.R.F. Carter, A. Hendrix, M. Lenassi, M.A. Langlois, A. Llorente, A.S. van de Nes, R. Nieuwland, V. Tang, L. Wang, K.W. Witwer, J.C. Jones, Towards defining reference materials for measuring extracellular vesicle refractive index, epitope abundance, size and concentration, J. Extracell. Vesicles 9 (2020). https://doi.org/10.1080/20013078.2020.1816641.
[109] G. Euramet, Brunswick, Publishable Summary for 18HLT01 METVES II Standardisation of Concentration Measurements of Extracellular Vesicles for Medical Diagnoses, 2019, pp. 1–4. https://www.metves.eu/.
[110] A. Alonso, S. Marsal, A. Julià, Analytical methods in untargeted metabolomics: state of the art in 2015, Front. Bioeng. Biotechnol. 3 (2015) 23. https://doi.org/10.3389/fbioe.2015.00023.
[111] P.S. Gromski, Y. Xu, K.A. Hollywood, M.L. Turner, R. Goodacre, The influence of scaling metabolomics data on model classification accuracy, Metabolomics 11 (2015) 684–695. https://doi.org/10.1007/s11306-014-0738-7.
[112] I. Martínez-Arranz, R. Mayo, M. Pérez-Cormenzana, I. Mincholé, L. Salazar, C. Alonso, J.M. Mato, Enhancing metabolomics research through data mining, J. Proteomics. 127 (2015) 275–288. https://doi.org/10.1016/j.jprot.2015.01.019.
[113] P. Cuevas-Delgado, D. Dudzik, V. Miguel, S. Lamas, C. Barbas, Data-dependent normalization strategies for untargeted metabolomics—a case study, Anal. Bioanal. Chem. 412 (2020). https://doi.org/10.1007/s00216-020-02594-9.
[114] A.M. De Livera, D.A. Dias, D. De Souza, T. Rupasinghe, D.L. Tull, U. Roessner, M.J. Mcconville, T.P. Speed, Normalizing and integrating metabolomics data, Anal. Chem. 84 (2012) 10768–10776. https://doi.org/10.1021/ac302748b.
[115] K. Boriachek, M.N. Islam, A. Möller, C. Salomon, N.T. Nguyen, M.S.A. Hossain, Y. Yamauchi, M.J.A. Shiddiky, Biological functions and current advances in isolation and detection strategies for exosome nanovesicles, Small 14 (2018). https://doi.org/10.1002/smll.201702153.
[116] Y. Wu, L. Li, Sample normalization methods in quantitative metabolomics, J. Chromatogr. A 1430 (2016) 80–95. https://doi.org/10.1016/j.chroma.2015.12.007.
[117] B.B. Misra, Data normalization strategies in metabolomics: current challenges, approaches, and tools, Eur. J. Mass Spectrom. 26 (2020) 165–174. https://doi.org/10.1177/1469066720918446.
[118] A.M. De Livera, M. Sysi-Aho, L. Jacob, J.A. Gagnon-Bartsch, S. Castillo, J.A. Simpson, T.P. Speed, Statistical methods for handling unwanted variation in metabolomics data, Anal. Chem. 87 (2015) 3606–3615. https://doi.org/10.1021/ac502439y.
[119] Y. Gagnebin, D. Tonoli, P. Lescuyer, B. Ponte, S. de Seigneux, P.-Y. Martin, J. Schappler, J. Boccard, S. Rudaz, Metabolomic analysis of urine samples by UHPLC-QTOF-MS: impact of normalization strategies, Anal. Chim. Acta 955 (2017) 27–35. https://doi.org/10.1016/j.aca.2016.12.029.
[120] B. Li, J. Tang, Q. Yang, S. Li, X. Cui, Y. Li, Y. Chen, W. Xue, X. Li, F. Zhu, NOREVA: normalization and evaluation of MS-based metabolomics data, Nucleic Acids Res. 45 (2017) W162–W170. https://doi.org/10.1093/nar/gkx449.
[121] B.A. Ejigu, D. Valkenborg, G. Baggerman, M. Vanaerschot, E. Witters, J.-C. Dujardin, T. Burzykowski, M. Berg, Evaluation of normalization methods to

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical University of Gdańsk 2024-04-25

pave the way towards large-scale LC-MS-based metabolomics profiling experiments, OMICS A J. Integr. Biol. 17 (2013) 473–485. https://doi.org/10.1089/omi.2013.0010.
[122] A.M. De Livera, G. Olshansky, J.A. Simpson, D.J. Creek, NormalizeMets: assessing, selecting and implementing statistical methods for normalizing metabolomics data, Metabolomics 14 (2018) 54. https://doi.org/10.1007/s11306-018-1347-7.
[123] E. van der Pol, F.A.W. Coumans, A.E. Grootemaat, C. Gardiner, I.L. Sargent, P. Harrison, A. Sturk, T.G. van Leeuwen, R. Nieuwland, Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing, J. Thromb. Haemostasis 12 (2014) 1182–1192. https://doi.org/10.1111/jth.12602.
[124] F. Royo, L. Moreno, J. Mleczko, L. Palomo, E. Gonzalez, D. Cabrera, A. Cogolludo, F.P. Vizcaino, S. Van-Liempd, J.M. Falcon-Perez, Hepatocyte-secreted extracellular vesicles modify blood metabolome and endothelial function by an arginase-dependent mechanism, Sci. Rep. 7 (2017). https://doi.org/10.1038/srep42798.
[125] M.B. Pereira, M. Wallroth, V. Jonsson, E. Kristiansson, Comparison of normalization methods for the analysis of metagenomic gene abundance data, BMC Genom. 19 (2018) 274. https://doi.org/10.1186/s12864-018-4637-6.
[126] C. Lau, Y. Kim, D. Chia, N. Spielmann, G. Eibl, D. Elashoff, F. Wei, Y.L. Lin, A. Moro, T. Grogan, S. Chiang, E. Feinstein, C. Schafer, J. Farrell, D.T.W. Wong, Role of pancreatic cancer-derived exosomes in salivary biomarker development, J. Biol. Chem. 288 (2013) 2688–2697. https://doi.org/10.1074/jbc.M113.452458.
[127] R. Maroto, Y. Zhao, M. Jamaluddin, V.L. Popov, H. Wang, M. Kalubowilage, Y. Zhang, J. Luisi, H. Sun, C.T. Culbertson, S.H. Bossmann, M. Motamedi, A.R. Brasier, Effects of storage temperature on airway exosome integrity for diagnostic and functional analyses, J. Extracell. Vesicles 6 (2017). https://doi.org/10.1080/20013078.2017.1359478.

Pobrano z Repozytorium Gdańskiego Uniwersytetu Medycznego / Downloaded from Repository of Medical Univeristy of Gdańsk 2024-04-25