Radiovesicolomics—new approach in medical imaging

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This review introduces extracellular vesicles (EVs) to a molecular imaging field. The idea of modern analyses based on the use of omics studies, using high-throughput methods to characterize the molecular content of a single biological system, vesicomics seems to be the new approach to collect molecular data about EV content, to find novel biomarkers or therapeutic targets. The use of various imaging techniques, including those based on radionuclides as positron emission tomography (PET) or single photon emission computed tomography (SPECT), combining molecular data on EVs, opens up the new space for radiovesicolomics—a new approach to be used in theranostics.

KEYWORDS extracellular vesicles, medical imaging, positronium imaging, PET, total-body PET, theranostics

Introduction

What is omics?

The magic word omics appeared in the 1980s as a figment of the imagination and extraordinary creativity of three scientists in the field of genetics: Dr. Thomas H. Roderick (a geneticist at the Jackson Laboratory, Bar Harbor), Dr. Frank Ruddle (Yale University) and Dr. Victor McKusick (The Johns Hopkins University) in 1986. During an international meeting in Kuska (1998) on the feasibility of mapping the entire human genome, they “assembled” a short sub-meeting to discuss starting a new genome-oriented scientific journal. The invention of a new word by T.H. Roderick was the beginning of this vocabulary scientiﬁc activity which, like a rush of life-giving water, spilled over all the specialties of molecular biology, yielding crops in the form of all kinds of omic fruits (Kuska, 1998). Following genomics, the word proteomics was first proposed much later by Marc Wilkins in 1993 to describe the variety of proteins that make up the whole organism (Yadav, 2007). A typical downstream neologism is a portmanteau derived from two words: one that describes the biological or molecular level of analysis and the suffix -omics to denote all studies conducted on a wide scale in a living organism.

The outstanding development of molecular biology techniques, such as mass spectrometry, molecular sequencing, and chromatographic techniques, caused the creation of many datasets containing quantitative and qualitative characteristics, as the results of large-scale experiments: genomics, transcriptomics, metabolomics,
miromics and lipidomics (Skotland et al., 2017; Skotland et al., 2020), as well as glycomic studies (Gerlach and Griffin, 2016) (Figure 1). These datasets are stored in repositories as open data sources and are constantly expanded and cured. Great improvements have also been observed in cytometric methods, including flow cytometry techniques, using high-resolution flow cytometry based on the scattering angle analysis and the development of imaging flow cytometry, which is a combination of quantitative flow power with high-quality analysis of large numbers of images. This allowed for the improvement of resolution of flow cytometry for spatial discrimination between nuclear and cytoplasmic fluorescence or cellular morphometry (Pierzchalski et al., 2011).

These new approaches in molecular biology have been transferred to cytometry, a laboratory technique, which development resulted in providing new parameters, complementary to fluorescence intensity, e.g. like fluorescence localization, cell shape and morphology. Translation of new characteristics derived from biochemistry and molecular biology to cellular systems, expressed as the imaging data, together with the strength of "population" statistics, opened up a new window for a novel branch of systemic study—cytomics (Valet et al., 2006). Cytomics is defined as the multimolecular cytometric analysis of cell and cell system heterogeneity by means of supervised or unsupervised data-mining algorithms. This approach allows for data extraction and effective analysis of multi-parameter data sets in order to obtain the maximum information about the molecular phenotypes of cells (Valet et al., 2006; Valet, 2022)

Another example of this emergence is the formation of the word radiomics that currently appeared in the medical literature. Despite some controversies that may result from the connotation of this word, the literature adopts radiomics as the application not only radiological examinations, but all imaging tests aimed at comprehensive diagnosis to extract a large number of quantitative features from digital images (Figure 1). The main directions and applications of radiomics to personalize the patient treatment have been established by the “Father of Radiomics” Dr Robert (Bob) J. Gillies in his fundamental article (Gillies et al., 2016).
The next step in the development of the concept of comprehensive image data analysis is the idea of using data from the whole-body (WB) images—omics (Strand et al., 2017). The concept of omics derived from the holistic Magnetic Resonance Imaging (MRI) data analysis, where information in each voxel is collected from a patient to compare between patients or analyzed over time and integrated with other omics data within a patient to visualize fat tissue distribution (Lind et al., 2019).

In our scheme, in an equal level of this creative terminology, we have placed the second term, created to draw attention to the comprehensiveness and emergence of research into new biomarkers: extracellular vesicles (EVs)—vesicolomics. Starting from this article, we would like to apply the vesicolomics as a new concept to characterize EVs and collect data to extract maximal information from standard and high-throughput methods used in EV research.

What omics can do for theranostics?

Theranostics is a portmanteau word derived from terms therapeutic (Greek therapeia) and diagnostics (Greek diagnōsis). Without an accurate diagnosis, based on laboratory or imaging tests, an appropriate treatment cannot be applied. Theranostics means the use of molecular and functional imaging to determine the location, size and type of a lesion. It can be a neoplastic lesion, but also other types of lesions, such as an enlarged thyroid gland, prostate gland, pituitary gland, inflammatory lesion and the like (Opalińska et al., 2021). If the marker for the lesion localization is a radioisotope (radioactive element), diagnostics consists in measuring the signal (radiation), and analyzing the distribution of the radiation signal in four dimensions: 3D in space and a time dimension. The simultaneous use of radioactive isotopes for diagnosis also enables radiotherapy, which may be used alone or in combination with other treatments for the individual patient (Weineisen et al., 2015; Królicki and Kunikowska, 2021). Omics is an approach to the foundation of new biomarkers that allows objective extraction and selection of new therapeutic and diagnostic targets based on the analysis of clinical and experimental (genetic, biochemical, histological and imaging) data (Gillies et al., 2016; Wrobel 2021).

The new therapeutic targets are very important for theranostics, there is limited number of theranostic radionuclides (Choinski and Lyczko, 2021; Matulewicz 2021), but the number of theranostic biomarkers and targets is endless.

Extracellular vesicles—new objects for omics

Extracellular vesicles definition and classification

EVs are nano- and micro-sized double-layered membrane entities produced by most cell types and released into biological fluids enabling cell-to-cell communication at close or distant sites (Kim et al., 2014). These nano- and microfragments of cell membranes are classified according to their formation and differences in size (diameter), into subgroups, including exosomes (Ex) with a diameter of 30–100 nm, ectosomes with a diameter of 100 nm to 1 μm (Figure 2), and apoptotic bodies (AB) with a size between 1 and 5 μm (Gurunathan et al., 2021). A single cell can release different types of EVs, resulting their heterogeneity within the EVs subtype. Currently, the recommendations of the International Society of Extracellular vesicles (ISEV), namely the 2018 MISEV guidelines, endorsed the use of the term “extracellular vesicles” (small, medium or large) instead of e.g. exosome, ectosome etc. (Théry et al., 2018).

Vesicolomics as the multimodal approach for EVs characterization

The dynamic development of cell biology and, above all, the interest of biologists and biophysicists in EVs, resulted in offering new research tools and techniques used in material sciences and their application in research at the subcellular and nanoscale levels, e.g. atomic force microscopy (Gajos et al., 2017; Stepieni et al., 2018) infrared Attenuated Total Reflectance (ATR) spectroscopy (Stepien et al., 2018; Paolini et al., 2020), dynamic light scattering (DLS) (van der Pool et al., 2013) and tunable resistive pulse sensing (van der Pol et al., 2013). Such analyses, combined with results of high-throughput techniques, produce a vast number of multiparametric (quantitative and qualitative) data from less number of examined samples. It needs systematic and multimodal analyses for integration of omics datasets and selection highly correlated biological features using different bioinformatics methods like Canonical Correlation Analysis (CCA) (Jun et al., 2018; Turek et al., 2020; Wrobel 2021) or deep/machine learning algorithms for better selection of biological interrelationships (Stahlschmidt et al., 2022).

EVs as biomarkers for liquid biopsy

The rapid expansion of molecular biology techniques, including high-throughput genetic techniques such as DNA and RNA sequencing (next generation sequencing—NGS) (Cheng et al., 2014; San Lucas et al., 2016), as well as mass spectroscopy techniques in proteomic, lipidomic, metabolomic and glycomic (Williams et al., 2018) studies, has created new perspectives for offering extracellular vesicles as biomarkers in different pathologies. Circulation EVs are present in all body fluids to offer a high level of sensitivity and specificity of the non-invasive medical procedure through the collection of body fluid samples such as blood or urine (Stepien et al., 2012; Stepieni et al., 2020). EVs were proposed as biomarkers in such diseases as diabetes (Tokarz et al., 2015, 2019; Alexandru et al., 2016; Stepieni et al., 2018; Kamińska et al., 2022; Roman et al., 2019), cardiovascular diseases including stroke (Lee et al., 1993; Lundström et al., 2020), myocardial infarction (Stepien et al., 2012; Burrello et al., 2020), atherosclerosis and
stable coronary artery disease (Chyrchel et al., 2019; Gkaliagkousi et al., 2021). The uniqueness of EVs in terms of their molecular composition has become a great promise to be explored as a new source of biomarkers in liquid biopsy, which was recognized in cancer diagnostics (Surman et al., 2018; Möller and Lobb, 2020; Stepieni et al., 2021a).

Immune system and the dynamics of EV biodistribution

The average plasma half-life of intravenously delivered EVs is reported to be between 30 and 80 min (Lai, et al., 2014; Charoenviriyakul et al., 2018) (Figure 2B). It is due to the phagocytizing activity of mononuclear phagocyte system (MPS), which is generally involved in biodistribution, organ accumulation and a half-life of EVs. Most of the intravenously delivered EVs are internalized and transported by phagocytizing cells and ultimately accumulate in the spleen, lungs, liver, and gastrointestinal tracts (Matsumoto, et al., 2017; Charoenviriyakul et al., 2018) (Figure 2C). In such targeting organs EVs have the preference to endothelial and Kupffer cells (Kooijmans et al., 2016; Németh et al., 2021). The EV half-life can be potentially increased by the reduction of cationic α-D-mannose monosaccharide or phosphatidylserine exposure (Escrivente et al., 2011; Matsumoto et al., 2017). Such modifications of...
EVs to control their uptake and biodistribution are called “eat me/do not eat me” strategy to achieve effective drug delivery: MPS saturation (eat me) to increase dendritic cells stimulation or EV uptake by cancer cells or avoid phagocytosis and increase organ targeting (do not eat me) (Escrevente et al., 2011; Choi et al., 2019; Belhadj et al., 2020).

Interestingly, the cellular origin of EVs also influence their distribution, suggesting that EVs from different cellular sources have different targeting properties. In order for EVs to perform their function, they must first bind to the target cell, and it is known that different EVs are able to preferentially bind to specific target cell types. This innate ability of EVs to bind to target cells is a feature that can be exploited to target EV drug carriers to the desired sites of action.

**Extracellular vesicles as drug delivery systems**

In addition to the outstanding and unique diagnostic applications of EVs as a liquid biopsy, the other virtually limitless potential of EVs is seen, as possible drug delivery systems (DDS) in many diseases (van Dommelen et al., 2012; Hwang et al., 2015; Kojima et al., 2018). Exosomes as nanoscale membrane vesicles with a special ability to target specific cells may serve as carriers to mediate a horizontal gene transfer. This potential has been firstly recognized as the transfer of mRNA or short, non-coding RNA (micro RNA-miRNA) in health and diseases (Baj-Krzyworzeka et al., 2006; van Dommelen et al., 2012) to set new directions for research on the biomimetic properties of EVs. Currently, loading of EVs with exogenous miRNA or pre-miRNA is a tempting strategy to achieve the antitumor effect of EVs (Ohno et al., 2013; Sutaria et al., 2017).

The most important characteristic to nominate EVs as candidates for contemporary DDS are as follows: biological stability, cell targeting, plasma protein interactions (pharmacodynamics) and controlled drug release.

**Biological stability and lifespan of extracellular vesicles**

EVs are continuously released parental cell and uptaken by target cells, which can be distant significant, thus it is impractical to evaluate the lifespan of an average vesicle, and such information is still missing. The EV stability and shelf life in biological fluids is better described. The best conditions for EV storage is freezing. The small EVs (exosomes) preserve their size and protein content at -80°C for 28 days, showing comparable biodistribution to freshly isolated ones (Wu et al., 2021). Also freezing temperatures preserved most EV particles, and 4°C and 20°C would cause significant loss of EVs (Lórińcz et al., 2014). Biological activity of exosomes is significantly increase after addition of trehalose to improve their long-term stability (Bosch et al., 2016; Charoenviriyakul et al., 2018). In contrast, dendritic cells-derived EVs are stable and can be stored frozen for at least 6 months (Alvarez-Erviti et al., 2011). Another way to enhance EV stability is a modification by polyethylene glycol (PEGylation) to achieve better blood residence and cell targeting (Kooijmans et al., 2016; Shi et al., 2019).

**Cell targeting by extracellular vesicles**

The pivotal feature of EV-based DDS is their targeting capacity, which is limited by two physiological boundaries: a vascular barrier and a target recognition. Systemic administration via intra venous injection of EVs is not effective when target cells are located distantly (organized tumor) or when delivery is to the brain. In such case the direct administration to the organ or by peritoneal injection is a method to improve cell targeting (Wiklander et al., 2015; Chen et al., 2021). The other method is to tag of EVs with a signaling peptide or ligand to improve targeting (Alvarez-Erviti et al., 2011). The generation of tagged EVs through transfection with ligand expression vectors for lactadherin or Rabies Viral Glycoprotein (RVG) peptides is a proposed protocol for a direct cell targeting (Alvarez-Erviti et al., 2011; Matsumoto et al., 2017; Kojima et al., 2018). Lamp2b, and tetraspanins could serve as a promising strategy for active targeting of cancer cells for therapeutic exosomes (Kojima et al., 2018; Wang, 2022). The other strategy is to produce tumor EVs (Tu-EVs), which have a natural housing behavior to target cells (Lara et al., 2020; Gong et al., 2021) or are used as a tumor antigens-adjuvant utilizing Tu-EVs as a tumor cell-based vaccine to target dendritic cells (DCs) (Huang et al., 2022). EVs from HEK293T cells accumulated in subcutaneous tumors, which may be exploited by EV-based anticancer therapies (Murphy et al., 2019).

"Eat me" strategy can be also used to improve cell targeting by altering the EV glycation pattern (Escrevente et al., 2011; Choi et al., 2019) (see Cell targeting by extracellular vesicles). Very promising approach to EV targeting is using EVs derived from immune cells: macrophages or dendritic cells (DCs) to target inflammatory sites and regulate the inflammatory response. This strategy is applied to deliver therapeutic agent directly to neural cells, brain tumors (Alvarez-Erviti et al., 2011; Liang et al., 2021) or affect antitumor immune responses (Fernández-Delgado et al., 2020).

**Passing the brain-blood barrier by EVs to achieve therapeutic effect in glioma**

Several types of circulating EVs interact with brain microvascular endothelial cells and modulate the integrity of the brain-blood barrier (BBB), e.g. glioma-derived EVs can pass...
the intact BBB and are detected in the peripheral blood of patients (García-Romero et al., 2017). This process is controlled by various mechanisms among them inflammation being the larger contributor. A similar mechanism can be used by DDD to target glioma cell in the brain enhanced by adoptive transcytosis to enter the central nervous system parenchyma (Krämer-Albers et al., 2022). To deliver drugs to brain tumors, peptide-modified EVs need to be generated to pass the BBB and targeted glioma. The best results are observed for a peptide targeting low-density lipoprotein receptor-related protein-1 (LRP1), which mediates the transcytosis across the BBB, such as Angiopep-2 peptide or the integrin family protein - leukocyte function associated antigen 1 (LFA-1) (Shan et al., 2022; Zhu et al., 2022). The other strategy may apply tumor derived EVs as a potential glioma vaccination due to their ability to display tumor antigens that can activate DCs, which can then activate CD8+ T cells having antitumor potential (Fernández-Delgado et al., 2020).

Systems radiomics and extracellular vesicles tracking

Radiomics can be performed with tomographic images obtained from computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET) (Gillies et al., 2016). The most sensitive imaging modality is PET (Alavi et al., 2021), allowing to detect $10^{-11}$–$10^{-12}$ M concentrations of radiolabeled agent, which is an equivalent of nanograms for an injection to a human body (Skotland et al., 2022). Single photon emission computed tomography (SPECT) is more convenient because of availability of radionuclides and detectors, nevertheless the sensitivity of this technique is one order of magnitude lower then PET (Skotland et al., 2022). In preclinical (animal) studies, the most common modalities are optical and fluorescence techniques utilizing lipophilic fluorescence dyes (Kojima et al., 2018; Wu et al., 2021). The greatest advantage of fluorescence markers is their accessibility and utility, the disadvantage is the low sensitivity falling to the concentration of
fluorescent agent in the range of $10^{-9}$ to $10^{-11}$ M and the limit for depth detection between 1 and 10 cm (Lázaro-Ibáñez et al., 2021; Skotland et al., 2022). These EV imaging approaches are used in preclinical studies and needs the translation to clinical practice (Arifin et al., 2022).

**Strategies for radiolabeling of EVs**

Since extracellular vesicles are the cell-derived structures enveloped by a lipid bilayer, which is like a typical biological membrane, the strategies for EV labeling are almost identical with those for cell labelling (Gawne et al., 2022). Consequently, EVs can be radiolabeled by surface tagging or intraluminal loading methods (Figure 2A). In the surface radiolabeling, radionuclide can be a part of a stable radiopharmaceutical (antibody or ligand) which recognize a specific antigen or receptor on a EV surface (Moriyama et al., 2015).

Alternatively, a radiopharmaceutical can be directly incorporated to a lipid membrane. In the covalent binding strategy, a useful chelator for various radioisotopes (e.g. NOTA) can be conjugated with the amine group of the membrane proteins on EVs (Royo et al., 2019; Shi et al., 2019; Jung et al., 2020; Lázaro-Ibáñez et al., 2021). In the intraluminal radiolabeling strategy, a lipophilic radiotracer can easily penetrate to the EV lumen or ionophores allow radionuclides to be transported across the lipid membrane where they can be trapped as their loose lipophilicity (Son et al., 2020; Khan et al., 2022).

**In vivo tracking of EVs**

Current studies demonstrated that radiolabeling is the most sensitive EV tracking approach for a quantitative biodistribution and pharmacokinetic study (Lázaro-Ibáñez et al., 2021). EVs radiolabeled with a bifunctional chelator as diethylenetriaminepentaacetic acid (DTPA) and the indium trivalent isotope ([111In]-DTPA) were detected in BALB/c tumor-bearing mice at a dose of $10^5$ vesicles administered i. v. (Lázaro-Ibáñez et al., 2021). With its radioactive decay half-life ($t_{1/2}$) of 2.83 days, the 111In isotope is an appropriate for the biodistribution studies that extend over several days. The alternative isotope is technetium (99mTc) with its radioactive $t_{1/2}$ of 6 h. The advantages of 99mTc radiolabeling are an easy preparation of a radiopharmaceutical before its administration and the emission of a monochromatic γ radiation (140.5 keV, 98.6%) make this radioisotope achievable for different preclinical and clinical studies. For both radionuclides, SPECT and SPECT/CT are imaging modalities in biodistribution studies (Hwang et al., 2015; Lázaro-Ibáñez et al., 2021). However, for the in vivo EV tracking, 99mTc radiolabeling appears to be inefficient due to its short decay half-life $t_{1/2}$. Using this radiotracer, the uptake of red blood cell-derived exosome-mimetic vesicles ([99mTc]-RBC-EMVs) was shown to be dose- and time-dependent reaching its maximum at 12–18 h of incubation, too long to be clinically applicable (Son et al., 2020).

The in vivo PET imaging of EVs is achievable by the gallium 68Ga ($t_{1/2} = 68$ min), cooper 64Cu ($t_{1/2} = 12.7$ h), zirconium 89Zr ($t_{1/2} = 78.4$ h) and iodine 124I ($t_{1/2} = 100$ h) isotopes, with the increasing half-life (Royo et al., 2019; Jung et al., 2020; Khan et al., 2022). The advent of high sensitivity total-body PET scanners opens possibilities for an efficient in vivo tracking of EVs in the whole human body simultaneously (Stepieri et al., 2021b; Vandenbergh et al., 2020) (Figure 2C). The long half-life of mentioned radionuclides is ideal for long term in vivo tracking of EVs, but for the practical achievability of a radionuclide decides its chemical properties and the availability of a biological ligand, which determines radiolabeling conditions (Khan and de Rosales, 2021).

**Radiovesicolomics—Applications of EVs in nuclear imaging**

Nuclear imaging, especially PET, in the extremely developing imaging modality having the most realistic perspective to be used in radiovesicolomics. The main advantage of nuclear imaging is the ability to obtain 3D whole body images (Khan and de Rosales, 2021; Lázaro-Ibáñez et al., 2021). Notably, recently a new generation of PET scanners was introduced enabling dynamic and kinetic model based imaging of all tissues and organs simultaneously (Badawi et al., 2019; Karp et al., 2020; Moskal et al., 2021a; Moskal et al., 2021b; Moskal and Stepieri, 2020; Preosil et al., 2022; Spencer et al., 2021). Another advantage of SPECT or PET imaging modalities is their very high sensitivity reaching a factor 10$^4$ with compare to MRI. This allows application of the radionuclide in a dose from very small dose 0.2–1 MBq per mouse for whole-body imaging with the use of EVs in a concentration 10$^{10}$ of particles/gram body weight (p/g) (Lázaro-Ibáñez et al., 2021; Khan et al., 2022). Depending on imaging radionuclides and the detection system, the applied dose can vary from 37 kBq ([99mTc]-biotin) (Matsumoto et al., 2017) to 5–10 MBq ([111In] DTPA) (Lázaro-Ibáñez et al., 2021), 7 MBq ([64Cu]-NOTA) (Banerjee et al., 2019), 3.7 MBq ([89Zr]Tc) (Son et al., 2020), 2 MBq ([64Cu]-NOTA) (Shi et al., 2019), and 0.2–1 µg [89Zr]PANC1 (Khan et al., 2022) to track/ image EVs using either SPECT or PET in a mouse. Radiovesicolomics may benefit also from the new multi-photon PET scanners (Moskal et al., 2020; Moskal et al., 2021a; Moskal et al., 2021b; Moskal et al., 2021c) which enable simultaneous multi-tracer imaging (Moskal and Stepieri, 2022) and hence studying simultaneously the kinetics of two different types of EVs by marking them with different isotopes.

**Radiovesicolomics in cardiovascular diseases**

Regenerative medicine is the promising perspective for use of EVs in cardiovascular Theranostics (Gašcėka et al., 2018). Recent studies have shown that EVs exhibit various regenerative properties valuable in the treatment of cardiovascular disease. EVs derived from bone marrow mesenchymal stem cells improve cardiac function and promote angiogenesis in acute myocardial infarction (AMI) (Wang...
et al., 2017). These pro-angiogenic potential arises from paracrine effectors regulated by NF-kB signaling including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) identified both in endothelial and stem cell derived EVs (Tokarz et al., 2015; Anderson et al., 2016). Another possible mechanism is epigenetic regulation via miRNA carried by EVs which may have both pro- and anti-angiogenic activity (Beijing et al., 2017; Stepien et al., 2018; Wang et al., 2017). The transfer of these bioactive molecules is possible due to the internalization of EVs by the recipient angiogenic cells (Durak-Kozica et al., 2018). In this case, radiovesicolomics proposes the use of imaging studies, with the use of radionuclide-labeled EVs. This approach will allow the precise location and distribution of EVs having an angiogenic potential in the treatment of heart failure caused by hypoxia or inflammation (Figure 3).

Radiovesicolomics in diabetes

Global diabetes mellitus prevalence in the population ages 20 to 79 reached 9.8% in 2021. Among many complications caused by diabetes, microvascular complications are the most common, typically including retinopathy, nephropathy, and neuropathy, and contribute to increased treatment costs. To lower the cost of treating diabetic complications, new and sensitive biomarkers are needed to accelerate the diagnosis process and allow for the elimination of diabetic complications at the early stage of the disease. To this end, EVs appear to be an ideal biomarker demonstrating their characteristic molecular profile, including miRNA, proteins, lipids and metabolites (Tataruch-Weinert et al., 2016; Kamińska et al., 2021; Kamińska et al., 2022) (Figure 3). There is lack of imaging studies using EVs in the course of diabetes as a carrier of radionuclides and the development of safe and efficacious delivery strategies for EVs in diabetes therapies is still the unmet need in theranostics (Li et al., 2022).

Radiovesicolomics in cancer

The concept of omics and theranostics is related to personalized treatment, which is of greatest importance in terms of neoplastic disease. What is most important in personalized medicine is the development of a treatment procedure tailored to the patient’s condition, his genetic predispositions (which are mainly related to the metabolic capacity of the drug) and the production of a drug targeted at a given type of cancer. The deep characteristic of EVs content in cancer is giving the opportunity to find new biomarkers (shedding cancer proteins, metabolites, miRNAs) related with cancer development and metastasis, or recognizing the new metabolic targets (Surman et al., 2018; Möller and Lobb, 2020; Stepien et al., 2021a). Using the example of overall glioma, EVs as the entities passing the BBB barrier, can serve as prognostic and therapeutics approaches (Garcia-Romero et al., 2017; Krämer-Albers, 2022). Radiovesicolomics will help to develop strategies for tumor EVs (Tu-EVs) labelling and use them for recognition of cancer location or monitoring cancer interaction with other cells e.g. immune cells, which are modulated by Tu-EVs.

Conclusion

This review is the first proposal of radiovesicolomic approaches in theranostics. Undoubtedly, the results of the studies gathered in this manuscript demonstrate that manufacturing, radiolabeling and administering EVs is feasible and safe, but there are still some limitations as the unknown lifespan of an average EV, the availability of detection systems and unknown mechanism of EV accumulation. The advantages of radionuclide-based imaging modalities make them a promising tool to validate the use of EVs in clinical setting, as they have a potential to characterize in vivo the pharmacokinetics and biological behavior of extracellular vesicles. Although PET offers better quantification two- or even 3-fold higher sensitivity than SPECT, the latter is still the most widely used imaging technology due to cost-effectiveness, availability and the existence of a wider range of suitable radionuclides. The strategies described here demonstrate how molecular imaging can be useful in guiding the development of biomedical applications of EVs for medical diagnosis and treatment in the ever-evolving field of nanotechnology and theranostics.

Author contributions

ES: Conceptualization, Methodology, Resources, Data Curation, Writing original draft, Review and Editing, Supervision, Funding acquisition; CR: Visualization, Editing; PM: Conceptualization, Writing, Review and Editing, Resources.

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Conflict of interest

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