Chapter

Tissue Specificity of Exosomes and Their Prospects as a Drug Delivery System

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Abstract

This chapter reviewed the various sources of exosomes and their characteristics. Exosomes, which in the context of the proposed chapter are the synonym for extracellular vesicles up to 200 nm, play a pivotal role in cell to cell communication thus leading to the involvement of exosomes in inflammation and cancer development. This brings exosomes to the forefront of promising markers of a sub-clinical stage of the disease, which makes identifying exosome's source and destination one of the main goals in exosome research. However, due to some biogenesis features and technological difficulties, which are discussed further, identification of a certain exosome's address, or its specificity for a certain tissue or cell type, becomes a non-trivial task. The chapter covers the following questions: some of the main barriers on the way of testing tissue specificity hypothesis of exosomes, exosomes from synovial fluid and CSF and their features, exosomes from mesenchymal stem cells (MSCs) of different origins, and some membrane and cargo exosomal markers for tissue specificity and the prospect of exosomes as a drug delivery approach.

Keywords: exosomes, tissue specificity, synovial fluid, cerebrospinal fluid, mesenchymal stem cells of different origins, drug delivery

1. Introduction

The term "exosome" has been known since the beginning of 1970s, when it was first used in papers published in "The Proceedings of the National Academy of Sciences (PNAS)" and dedicated to the transfer of DNA fragments between Drosophila or Neurospora cells. The term in that context had little in common with lipid bilayer vesicles, which may contain a wide variety of nucleic acids and proteins and was mainly related to DNA fragments that are not integrated into the exome and eliminated during meiosis [1]. The first use of the term "exosomes" in the extracellular vesicles context happened in 1980s, when Trams et al. in 1981 described vesicles that are produced directly by outward budding at the plasma membrane [2]. Nowadays, the ISEV (International Society for Extracellular Vesicles) consensus recommendation on nomenclature is to use "extracellular vesicle" as the "generic term for particles naturally released from the cell that is delimited by a lipid bilayer and cannot replicate" and to modify "EV" based on clear, measurable characteristics such as the cell of origin, molecular markers, size, density, function, etc [1]. In this paper we assume exosomes and extracellular vesicles (EV) as synonym terms. They are assumed to be a tool for intracellular communication and are promising biomarkers in different pathologies, including tumor growth. Another perspective trend for exosome' application is the use of the latter as a delivery system for various therapeutic targets. These microvesicles have some of the features of an ideal drug delivery system such as high biocompatibility, minimal toxicity, and tissue specificity. The latter feature may seem difficult to be objectively determined as far as the experimental design for confirmation or refutation of exosomes` tissue specificity is troublesome. This chapter proposes to discuss various aspects of the tissue specificity of exosomes, including some of the main barriers on the way of testing the tissue specificity hypothesis of exosomes, exosomes from synovial fluid and CSF and their features, exosomes from mesenchymal stem cells (MSCs) of different origin and some of membrane and cargo exosomal markers for tissue specificity the prospect of exosomes as a drug delivery approach.

2. Some of the main barriers on the way of testing the tissue specificity hypothesis of exosomes

First of all, culturing difficulties should be admitted. The difference between cell lines and even the corresponding primary cell cultures is obvious and does not allow to extrapolation of data obtained on cell lines to corresponding cells of an organism without limitations. Barriers in experiments with exosomes on the primary cell cultures lie on the surface: during obtaining the ex vivo culture of human cells successive steps should be performed, including dissection and/or disaggregation of the tissue, which may be accompanied by the formation of vesicles as the result of mechanical destruction of cells, especially in working with dense tissues, requiring preliminary mechanical grinding [3]. Another barrier, and perhaps more serious, is time limits. It is well known, that the longer primary cell cultures exist the less they reflect the ex vivo state of the corresponding tissue. Anyway, the latter reason along with others such as changes in morphology and signaling under the influence of antibiotics, culture flask, absence of tissue architectonics, etc., is not unique to exosome experiments.

Secondly, in terms of biogenesis exosomes are secreted intraluminal vesicles (ILVs), which sequester specific proteins, lipids, and cytosolic components, whilst multivesicular bodies (MVBs), and late endosomes are a subset of specialized endosomal compartments rich in intraluminal vesicles. ILVs are generated by the inward budding of endosomal membranes and MVBs get transported to plasma membrane *via* cytoskeletal and microtubule network, after that they undergo exocytosis post-fusion with the cell surface whereby the ILVs get secreted as exosomes [4]. The biology of this process does not allow us to make a firm assumption, that exosomes, even secreted by different cell types, have distinct superficial/membrane markers, identifying their specificity. Nevertheless, there is a probability of determining exosome's cell or tissue origin or tissue-specific engagement in disease by the presence of specific combinations of surface proteins and their abundance [5]. Anyway, there is always a possibility to suppose the origin of exosomes by their cargo, for example, by miRNA profile, which is discussed later, but considering all of the above identification of exosome fraction of certain miRNA profiles is a non-trivial task. Moreover, the effects of exosomes from different sources might overlap with each other and, for example, might act complementary in eliciting inflammatory reactions, e.g. as has been observed for microvesicles from atherosclerotic plaques [6, 7].

Thirdly, partly due to exosomes' size, there are technical difficulties in identifying exosomal membrane markers especially when there is a complex of these markers. Currently, opportunities for the detection of exosomes are improving due to the new emerged technologies. Imaging flow cytometry is on the edge of these technologies, remaining one of the basic instruments for phenotype description. Imaging flow cytometry overcomes obstacles in traditional flow cytometry by including a CCD camera with a 60× objective, allowing detection of vesicles with sizes below 500 nm through enhanced fluorescence with only a small number of fluorophore-labeled antibodies like two protein targets per exosome [5]. However, this area is growing rapidly, and flow cytometry kits based on beads carrying up to 37 exosomal protein markers on their surface are nowadays available. There are some alternative options for multiplex surface markers recognition on exosomes like proximity-dependent barcoding assay, converting the protein composition to DNA sequence information via bound antibody-DNA conjugates with the following decoding by NGS [5].

And, fourthly, the biological materials which are widely available for sampling are blood, urine, and saliva. Apparently, every of this biomaterial is a stock of everything from everywhere and it is extremely difficult to determine the origin of exosomes, isolated from these biomaterials. A major source of microvesicles in plasma is represented by platelets, other sources of exosomes in blood plasma are endothelial cells, smooth muscle cells, monocytes, lymphocytes, and erythrocytes [6, 8]. Berckmans René et al. in 2001 discovered, that among total exosomes, isolated from human plasma, 82% were platelet exosomes, 15% from erythrocytes, and 3% from leukocytes; in 2019 they discovered, that among total isolated plasma, 52% are platelet EVs, 29% erythrocyte Evs, 20% leucocyte Evs and a low concentration of EVs from (activated) endothelial cells (E-selectin, CD62E) can be detected [9]. The biofluids which allow assuming the specificity of the isolated exosomes are synovial fluid and cerebrospinal fluid (CSF), exosomes from these biomaterials are discussed below.

3. Exosomes from synovial fluid and CFS

The biofluid, which is available for research in a limited number of cases, is synovial fluid. With a large synovial microvesicular pore radius reaching 40 nm, it can be assumed, that the exosomes, isolated from the synovial fluid, are mostly exosomes, produced by fibroblast-like synoviocytes and by chondrocytes, which constitute the synovial membrane and joint cartilage respectively [10].

Huang et al. investigated exosomes from synovial fluids of patients with different joint diseases: gout, rheumatoid arthritis (RA), axial spondyloarthritis (axSpA), and osteoarthritis (OA). The main goal of the experiment was not to identify tissue or cell-specific markers in exosomes but to determine markers, which would primarily allow differentiating these disease states in patients. However, this is valuable research in characterizing tissue specificity of exosomes, which include samples of synovial fluid of total of 100 patients. Twenty-five proteins were found highly expressed in gout uniquely, lysozyme C and protein S100-A9 included, whose bioinformatic analysis was significantly involved in "neutrophil degranulation" and "prion diseases". Along with differentially expressed proteins, there were thirty-nine proteins highly expressed in axSpA uniquely and twenty-eight proteins in RA. In axSpA among others there were RNA-binding protein 8A and protein transport protein Sec24C included, whose bioinformatic analysis was significantly involved in “acute-phase response” and “citrate cycle”. In RA, these uniquely expressed proteins included pregnancy zone protein (PZP) and stromelysin-1, whose bioinformatic analysis was significantly involved in “serine-type endopeptidase inhibitor activity” and “complement and coagulation cascade” [11]. Apparently, these molecular events may have distinct functional consequences: exosomes isolated from synovial fibroblasts, which were cultured in conditions mimicking OA, were able to induce MMP-13 and aggrecan expression in articular chondrocytes isolated from healthy synovial joints, suggesting in vitro this would lead to tissue degeneration [12]. Moreover, Esa et al. admit in their review that exosomes produced by both synovial fibroblasts and chondrocytes under OA-like conditions upregulate the release of pro-inflammatory cytokine cascades, including MMP-13, creating a “positive-feedback loop” that drives inflammation within the joint and ultimately leads to the damage of articular cartilage and a loss of structural integrity [13]. By the way, it is interesting to note, that, unlike exosomes from MSCs of different origins, exosomes from healthy individuals or individuals with OA do not differ either in the concentration (OA: 1.18 × 1010 particles/ml, $n = 6$; non-OA: 1.59 × 1010 particles/ml, $n = 6$) or in the size (OA: 0.128 μm, $n = 6$; non-OA: 0.127 μm, $n = 6$) [14].

Another biofluid that is also available in a limited number of cases is cerebrospinal fluid. Taking into account that CSF is produced by ependymal cells and permeability of blood-brain barrier to hydrophobic molecules and small non-polar molecules, it is possible to assume exosomes, isolated from CSF, are specific for the cells, making up and supporting functioning the central nerve system, such as neurons, astrocytes, microglia, and oligodendrocytes [15]. On the other hand, it should be admitted exosomes can cross BBB in blood-brain direction, which makes them a promising approach for the target delivery of therapeutic agents [15, 16]. The ability of exosomes to cross the blood-brain barrier (BBB) in the opposite direction makes them a highly attractive source of biomarkers originating in the CNS that could be isolated from the blood [17].

Otake et al. detected 14,807 genes in CSF exosomes, of which 4580 genes were commonly detected among four individuals, including neuron-enriched genes such as TUBB3 and CAMK2A. Gene Ontology analysis and pathway analysis with these genes revealed functional enrichment of ubiquitin-proteasome pathway, oxidative stress response, and unfolded protein response in CSF from patients with amyotrophic lateral sclerosis. These pathways are related to pathomechanisms of amyotrophic lateral sclerosis [18]. Along with common exosomal protein markers, expressed on the surface of CSF exosomes and in mRNA exosomal content, there are such proteins as NCAM, L1CAM, SOD1, α-synuclein Aβ42, total tau, TDP-43, and pT181-tau [16, 18]. The presence of SOD1, which is one of the most studied causes of amyotrophic lateral sclerosis, in exosomes secreted from motor-neuron-like NSC-34 cells overexpressing human wild-type or mutant SOD1 provided the first evidence for the secretion and cell-to-cell transmission of SOD1 in the context of ALS [16]. TDP-43 is assumed to facilitate a prion-like spread of its misfolded species [19]. NCAM is a neuronal cell adhesion protein, which is involved in cell-cell and cell-matrix interactions, and L1CAM is an axonal glycoprotein that plays an important role in nervous-system development and its mutations cause neurological syndromes known as CRASH [19, 20].

4. Exosomes from mesenchymal stem cells (MSCs) of different origin

Mesenchymal stem cells (MSCs) are the subject of intense research as they are a potential therapeutic tool for several clinical applications and among others one of the most available options to study stem cells, what is one of the main reasons why exosomes from these cells seem to be mostly described and studied. Thus, exosomes from different variants of mesenchymal stem cells seem to be well described and characterized.

In 2021 González-Cubero et al. described the phenotype of adipose tissue-derived mesenchymal stem cells (ASCs-derived) exosomes: from 37 exosomal surface epitomes 31 were detected and 6 were undetected. Among the detected ones were CD3, CD4, CD19, CD8, HLA-DRDPDQ, CD56, CD105, CD2, CD1c, CD25, CD49e, ROR1, CD209, CD9, SSEA-4, HLA-ABC, CD63, CD40, CD11c, CD81, CD41b, CD86, CD326, CD133/1, CD29, CD69, CD45, CD31, CD20, CD14, while CD3+, CD45+, CD56+, HLA-ABC, and HLA-DRDPDQ were particularly strongly enhanced in samples with ASCs-derived exosomes (99.99% ±0.06%, 55.45% ±6.36%, 88.68% ±4.29%, 84.66% ±5.99%, 59.98% ±7.45%, respectively). However, CD42a, CD44, CD62P, CD142, CD146, and MCSP were undetectable [20]. Like ASCs-derived exosomes, it was shown, that exosomes from BMSC do not express CD146 and CD42a. However, CD1c, CD2, CD3, CD4, CD14, CD20, CD25, CD31, CD40, CD45, CD49e, CD56, CD69, CD133/1, and CD326 also were undetectable in exosomes from BMSC [21].

Moreover, 1 year in 2020 Wang et al. compared the exosomes, isolated from bone marrow-derived MSC (BM-MSC), umbilical cord-derived MSC (UC-MSC), and adipose tissue-derived MSCs (AT-MSC). They found that AT-MSCs produced exosomes more intensively, as far as the concentration of exosomes in the supernatant, collected for the same time period, was higher than that of BM-MSC or UC-MSC exosomes [22]. However, simultaneously in 2020 Xu et al. showed this is not a strict regularity: during the experiment, they got supernatant with the density 2.38 × 1011/mL in exosomes from BMSCs; 1.08 × 1011/mL in exosomes from ADMSCs and 1.75 × 1011/mL in exosomes from UCMSCs [23]. In the research of Wang et al., exosomes from all three different tissue sources were studied with TEM, typical cup-shaped vesicles were observed and no differences in shape among the exosomes were noted [22]. Xu et al. showed there is sometimes possibly a slight difference in the size distribution of exosomes from BMMSCs, ADMSCs, and UCMSCs: in the case of BMMSCs, exosomes were round or dish-shaped with a diameter of 40–100 nm, the average particle diameter of exosomes was 70.3 nm. Exosomes from ADMSCs were uniform in size with a diameter of 30–100 nm with the average particle diameter within 95 nm and the majority of exosomes were 72.8 nm, while the UCMSCs exosomes were round in shape with a diameter of 10–90 nm and most of the particles had diameters of about 80.6 nm [23].

A detailed proteomic analysis revealed 771, 457, and 431 proteins in exosomes from BM-MSC, AT-MSC, and UC-MSC, respectively; comparison of the three types of exosomes revealed 355 common proteins, and 341, 23, and 37 proteins unique to the exosomes from BM-MSC, AT-MSC, and UC-MSC, respectively. In terms of biological process, proteins from BM-MSC exosomes were mainly involved in granulocyte activation and regulation of cell migration, whereas proteins from AT-MSC exosomes were enriched in leukocyte activation involved in immune response and UC-MSC exosomes along with leukocyte activation proteins involved in immune response were enriched in proteins of collagen metabolic process. As for molecular function, AT-MSC exo and UC-MSC exo proteins were both significantly enriched in cell adhesion molecule binding, whereas BM-MSC exo proteins were mostly involved in protein complex binding and integrin binding. Along with protein cargo, Wang et al. examined membrane markers of isolated exosomes and identified some membrane proteins, that are differentially expressed: ATP2B1 and ATP1A1 showed high expression in AT-MSC exosomes, whereas ITGA2 and LRP1 showed low expression. LTGB3 and SLC44A1 showed low expression in UC-MSC exosomes. In contrast, ADAM9, ADAM10, CD81, CACNA2D1, NOTCH2, and HLA-A showed high expression in BM-MSC exosomes [23]. There is a strong data, exosomes of all three sources—BMMSCs, ADMSCs, and UCMSCs—show highly expressed exosomes specific markers CD63, HSP70, CD81, and CD9 [21, 23].

Exosomes from MSCs are known to express another protein, a milk fat globule- epidermal growth factor-factor VIII (MFGE8), a glycoprotein that bridges externalized phosphatidylserine (PS) on the apoptotic cell surface to alphaVbeta3 or alphaVbeta5 integrins on the phagocyte. The expression of this protein has certain functional consequences in exosomes: their administration increases macrophage uptake of apoptotic bodies in the border zone of infarction and is associated with reduced proinflammatory response, increase in neovascularization, lower infarct size, and an improvement in cardiac function [24].

Three years before that in 2017 Mead B. et al. discovered the difference in membrane proteins expression between exosomes from BMSC and fibroblasts: more CD11c+ and CD63+ exosomes were detected on the BMSC exosomes (20.3% ± 8.3%, 81.7% ± 12.3%, respectively) compared to fibroblast exosomes (7.7 ± 0.7, 49.6 ± 2.4, respectively), whereas more CD29+ and CD81+ exosomes were detected on fibroblast exosomes (32.4% ± 0.75%, 39% ± 3.3%, respectively) compared to BMSC exosomes (20.5% ± 1.9%, 15.3% ± 10.6%, respectively) [24]. On the surface of AT-MSCs exosomes along with an abundance of well-known exosomal markers CD63, CD9, and CD81, there was revealed the expression of CD105, an MSC marker, as well as CD44, CD29, CD49e, and melanoma-associated chondroitin sulfate proteoglycan (MCSP). In addition, MSC-exosomes were found to be preferentially distributed in the damaged kidneys of mice with glycerol-induced AKI compared to in the healthy kidneys of control mice [25].

Exosomes from BM-MSCs and ADSCs show similar profiles, which are positive for CD105, CD73, CD90, and CD44; negative for CD45, CD31, and CD34 [26]. Nevertheless, there is a difference in functional capabilities of exosomes derived from BM-MSCs and ADSCs, the latter has a more significant neprilysin (NEP) activity: NEP-specific enzyme activity accounted for 38.3 ± 4.5% of total enzyme activity of ADSCs exosomes while BM-MSCs showed weak or undetectable NEP enzyme activity. Katsuda et al. calculated NEP-specific activity after the subtraction of fluorescence in the presence of thiorphan and they demonstrated that all ADSCs exhibited NEP-specific enzyme activity [27]. This makes ADSCs exosomes a promising approach for Alzheimer's disease treatment. This difference between exosomes from ADSCs and BM-MSCs is also determined in protein expression: immunoblot analysis revealed that the NEP protein expression level in ADSCs was ~4-fold higher than that of BM-MSCs [26].

5. Some membrane and cargo exosomal markers for tissue specificity

As it was already mentioned above, identifying the source of a certain exosome with its membrane markers is a non-trivial task, requiring consideration of the combination of different proteins and their abundance.

Skogberg et al. in their study of exosomes from human thymic epithelial cells (TEC) revealed the typical mTEC-associated cytokeratins K5, K14, and in both cells and exosomes, while the typical cortical thymic epithelial cell (cTEC) associated cytokeratin K8 and, for example, involucrin, a marker for late-stage mTEC differentiation, and CLAUDIN-1 were only identified in cells. Amidst the markers, which were found only in exosomes and not in cells, there were classical exosomal markers such as TSG101, CD82, CD63, MFG-E8, FLOTILIN-1 and immunoproteasome subunits PSMB9 and PSMB10, while PSMB8 was found in both, cells and exosomes. Other proteins, which were identified in cells and in exosomes from these cells, were CP, ALDOC, COL6A1, LAMA2, SRI, HSPG2, TSN, AOC3, SLC34A2, and F13A1 [28].

Mathivanan et al. also revealed some markers, which are specific for colorectal cancer cells (LIM1215) and can be identified both in cells and in exosomes. Amidst others, these are A33 antigen, cadherin-17, carcinoembryonic antigen, and ephrin-B1 and -B2, cell type-specific proteins associated with the gastrointestinal tract. Comparing proteomes of LIM125-derived exosome with previously published proteomes of human urinary exosomes and mast cell-derived exosomes, they found 31 proteins to be common between all of three exosomal proteomes, whereas 79 and 96 proteins were in common between LIM1215-mast cell and LIM1215-urine data sets. The 31 common proteins include Alix, transferrin, actins (α, β, and γ), RAB5B, RAB5C, EH-domain containing 4, heat shock proteins, annexins A6 and A11, and ADP-ribosylation factor 1 among others. Moreover, they found, that LIM125-derived pure exosome proteins are enriched with tetraspanin-containing proteins (p-value 0.0001) when compared with the entire human proteome and were the first to report the presence of phospholipid scramblase 3 in exosomes [29].

Saheera et al. in their recent review admit, exosomes from cardiomyocytes are enriched with proteins, which play critical roles in cardiomyocyte growth and survival like heat shock proteins (Hsp) like Hsp20, Hsp60, and Hsp70. Furthermore, these exosomes are known to be loaded with such inflammatory factors responsible for cardiac remodeling as IL-6 and TNF-α. Among others, these exosomes include GLUT4, GLUT1, and lactate dehydrogenase, different miRNAs, namely miR-320 and miR-126 [29]. The specificity of exosomes is possible to be used in target delivery purposes: the delivery of exosomes, expressing cardiac-targeting peptide on their membrane, in H9C2 cells was 16% greater than that of exosomes, which did not express this peptide, whereas the delivery of the exosomes of these two types was not different in HEK 293 cells exosomes expressing cardiac-targeting peptide (CTP)-Lamp2b on their membrane (CTP-Exo) was generated by introducing vectors encoding CTP-Lamp2b into HEK 293 cells. Moreover, compared with exosomes without cardiac-targeting peptide on its membrane, the in vivo delivery of exosomes to the hearts of mice was increased by 15% with CTP-Exo ($P = 0.035$) [30].

In exosomes from ECC1 cells, which are the most accurate resemblence of the endometrial luminal epithelium, Greening et al. found 14 proteins, which are essential for embryo implantation. As exosomal protein cargo, there were complement decay-accelerating factor (CD55, Rsc 7.1), perlecan (HSPG2, Rsc 5.9), and EGFR (Rsc 5.1), which are highly regulated at the time of blastocyst apposition and attachment [31, 32]. In general, it should be emphasized exosomes participate not only in tissue-specific processes like blastocyst apposition and attachment but in common processes like inflammation, cancer development, and cell senescence. Saheera et al. in their review, dedicated to exosomes' role in cell aging, admit senescence-associated exosomes could transfer many molecules and could accelerate the aging process or associated pathologies in an autocrine, paracrine, and endocrine fashion. Moreover, senescence-associated exosomes can intensify the aging process by cargos transfer between cells that may be recruited to increase the exosome release observed during cellular senescence. Exosomes from older individuals were shown to have MHC-II expression on monocytes and they are taken up faster by B cells in older individuals when compared to young, and as a result, the levels of circulating exosomes could be reduced [32].

It is worth it to note in some cases exosomes may be one of the features causing graft rejection. Sharma et al. in 2018 revealed a higher expression of some proteins in exosomes isolated from patients with complications compared with patients without complications. They collected serum samples from patients who had undergone lung (n = 30), heart (n = 8), or kidney (n = 15) transplantations. Using western blot along with CD9 identified tissue-associated lung SAgs, collagen V (Col-V) and K-alpha 1 tubulin (Kα1T), heart SAgs, myosin and vimentin, and kidney SAgs, fibronectin and collagen IV (Col-IV). Lung transplant recipients diagnosed with bronchiolitis obliterans syndrome had exosomes with higher expression of Col-V (4.2 fold) and Kα1T (37.1fold) than stable. Heart recipients with coronary artery vasculopathy had a 3.9-fold increase in myosin and a 4.7-fold increase in vimentin compared with stable. Exosomes in kidney transplant recipients diagnosed with transplant glomerulopathy 2-fold increased expression of fibronectin and 2.5-fold increased in Col-IV compared with exosomes from stable patients [33]. This is not a unique case of exosomes involvement in heart pathology processes: exosomes from macrophages exposed to diabetic milieu (high glucose or db/db mice) significantly increase inflammatory and profibrogenic responses in fibroblast (in vitro) and cardiac fibrosis in mice [34].

Some of the features which are specific for exosomes of certain origins are listed in the **Table 1**.

6. Exosomes as a promising approach for drug delivery

Exosome delivery is a novel nanoscale delivery system with many advantages, such as biocompatibility, biodegradability, less toxicity, specificity to the target cells, small size, promotes plasma membrane fusion, among different cells, longer half-life,

#	Exosomes' source	Characteristics/effects of certain exosomes in vitro or in vivo	Material/experiment design	Reference
1	Synovial fluid	25 proteins, including lysozyme C and protein S100-A9, uniquely highly expressed in gout patients; 39 proteins including RNA-binding protein 8A and protein transport protein Sec24C uniquely high expressed in axSpA; pregnancy zone protein (PZP) and stromelysin-1 highly expressed in RA	Samples from 100 patients with gout, rheumatoid arthritis (RA), axial spondyloarthritis (axSpA)	[11]
2	Synovial fluid	Induced MMP-13 and aggrecan expression	Exosomes from synovial fibroblasts cultured in OA conditions were added to articular chondrocytes isolated from healthy synovial joints	[12]

#	Exosomes' source	Characteristics/effects of certain exosomes in vitro or in vivo	Material/experiment design	Reference
3	CSF	Exosomes with highly expressed NCAM, L1CAM, SOD1, α-synuclein Aβ42, total tau, TDP-43 and pT181-tau Exosomes with functional enrichment of genes of ubiquitin-proteasome pathway, oxidative stress response, and unfolded protein response in CSF from patients with amyotrophic lateral sclerosis.	CSF from patients including patients with amyoptophic lateral sclerosis	[17, 18]
4	adipose tissue-derived mesenchymal stem cells	ASC-derived exosomes were positive for 31 surface markers including CD3, CD4, CD19, CD8, HLA-DRDPDQ , CD56, CD105, CD2, CD1c, CD25, CD49e, ROR1, CD209, CD9, SSEA-4, HLA-ABC, CD63, CD40, CD11c, CD81, CD41b, CD86, CD326, CD133/1, CD29, CD69, CD45, CD31, CD20, CD14, while CD3+, CD45+, CD56+, HLA-ABC, and HLA-DRDPDQ and negative for CD42a, CD44, CD62P, CD142, CD146, and MCSP	Exosomes isolated from culture media of adipose tissue-derived mesenchymal stem cells	[21]
5	Bone Marrow-MSC, Adipose Tissue-MSC, and Umbilical Cord-MSC	A detailed proteomic analysis revealed 355 common proteins between BM-MSC, AT-MSC, and UC-MSC-derived exosomes, and 341 (out of 771 proteins detected in BM-MSC-derived exosomes), 23 (out of 457 proteins detected in AT-MSC-derived exosomes), and 37 (out of 431 proteins detected in UC-MSC-derived exosomes) proteins unique to the exosomes from BM-MSC, AT-MSC, and UC-MSC, respectively. In AT-MSC exosomes ATP2B1 and ATP1A1 showed high expression; ITGA2 and LRP1 showed low expression. In UC-MSC exosomes LTGB3 and SLC44A1 showed low expression. In BM-MSC exosomes ADAM9, ADAM10, CD81, CACNA2D1, NOTCH2, and HLA-A showed high expression	Exosomes isolated from culture media of Bone Marrow derived MSC, adipose tissue-derived MSC, and umbilical cord- derived MSCs	[22, 23]
6	BM-derived stem cells and fibroblasts	On the exosomes from BMSC there were more CD11c+ and CD63+ (20.3% ± 8.3%, 81.7% ± 12.3%, respectively) compared to fibroblast exosomes (7.7 ± 0.7, 49.6 ± 2.4, respectively), whereas on the fibroblasts derived exosomes there were more CD29+ and CD81+ (32.4% ± 0.75%, 39% ± 3.3%, respectively) compared to BMSC derived exosomes (20.5% ± 1.9%, 15.3% ± 10.6%, respectively).	Exosomes were isolated from cultured media of BM derived MSC and fibroblasts	[24]

#	Exosomes' source	Characteristics/effects of certain exosomes in vitro or in vivo	Material/experiment design	Reference
7	Exosomes from human thymic epithelial cells (TEC)	mTEC exosomes expressed the typical mTEC-associated cytokeratins K5, K14 (like TEC) and were negative for involucrin, cytokeratin K8 and CLAUDIN-1, the typical cortical thymic epithelial cell (cTEC) associated markers. mTEC exosomes were positive classical exosomal markers such as TSG101, CD82, CD63, MFG-E8, FLOTILIN-1 and immunoproteasome subunits PSMB9 and PSMB10 (cells were negative for these markers), while PSMB8 was found in both, cells and exosomes. Such markers as CP, ALDOC, COL6A1, LAMA2, SRI, HSPG2, TSN, AOC3, SLC34A2, F13A1 were identified both in cells and exosomes.	Exosomes were isolated from media of TEC	[28]
8	Exosomes from colorectal cancer cell LIM1215	Exosomes and cells were positive for A33 antigen, cadherin-17, carcinoembryonic antigen, ephrin-B1 and -B2. 31 proteins were be common between exosomal proteomes of LIM1215 cells, mast cells and exosomes from urine, whereas 79 and 96 proteins were in common between LIM1215-mast cell and LIM1215-urine data sets. The 31 common proteins include Alix, transferrin, actins (α, β, and γ), RAB5B, RAB5C, EH-domain containing 4, heat shock proteins, annexins A6 and A11, and ADP-ribosylation factor 1 among others. LIM125-derived pure exosome proteins were enriched with tetraspanin-containing proteins when compared with the entire human proteome and were the first to report the presence of phospholipid scramblase 3 in exosomes.	Exosomes were isolated from culture media of LIM1215 cells	[29]

Table 1.
Some of the surface markers and proteome particularities of the exosomes of a certain origin.

low-uptake machinery, capability to pass contents from one cell to another cell, low immunogenicity and the unique feature that they have more tendency to accumulate in the cancerous cell than normal cells [35]. Other features making exosome a promising delivery system are innate stability, the ability to cross biological barriers, and enhanced loading capability of biological molecules [36]. It should be noted, due to the preferential homing of exosomes for their source cells, cancer exosomes should not or should be used as drug carriers with particular attention because they may promote tumor invasion or epithelial-mesenchymal transition, or they may transfer tumor resistance genes to tumor cells [37].

Their superior tissue-homing capabilities have been identified such as unidirectional synaptic transfer of microRNA from T cells to antigen-presenting cells.

Moreover, depending on the integrin expression pattern of the parent cells, different mammalian tumor exosomes were shown to preferentially target healthy cells in the predicted tissue. As it was mentioned above, cancer exosomes, like exosomes from sarcoma cells, show preferential tumor homing. As for the biodistribution, exosomes accumulate primarily in the liver, lung, spleen, and gastrointestinal tract and this distribution in some cases, like with systemic exosome administration, is not specific [38]. Nevertheless, depending on the origin of exosomes, the biodistribution may be changed: dendritic-cell-derived exosomes are preferentially taken up by the spleen, melanoma-cell-derived exosomes accumulate more prominently in the liver [38]. Despite a shorter half-life compared with liposomes, reaching a maximum of 60 minutes, exosomes were superior in escaping stress-relaxing environments and had a comparatively longer circulation half-life [38, 39].

Among others, there are three relatively simple and effective options for loading cargo into exosomes: electroporation, passive transport of the target during incubation, and sonication. Electroporation is a well-known method for different genetic structures loading into cells has the same principle in exosome loading: pores are created in the exosomal membrane by applying an electric field to a suspension of exosomes facilitating the movement of cargo into the lumen of the exosomes. A very simple and nevertheless effective way of cargo loading is a simple incubation of exosomes with the cargo: curcumin was efficiently loaded into exosomes after only 5 min of incubation at 22°C [40]. Another method to load cargo into exosomes is sonication with the same basic idea as electroporation, which is making pores in the bilipid exosomal membrane and cargo loading into exosomes via these pores. An accurately chosen regimen of sonication does not cause significant changes in the structure and content of exosomal membranes [40]. Thus, the appropriate method for cargo loading should be chosen based on the exosomes concentration, preliminary procedures like method of isolation and exosome storage condition and the loading target. Other methods of loading cargo into exosomes are transfection, freeze-thaw cycles, extrusion, surfactant treatment, and hypotonic dialysis.

Exosomes, being biodegradable nanoparticles, have successfully encapsulated bioactive molecules such as curcumin, paclitaxel, neurotoxin-I, and dexamethasone. Additionally, exosomes are utilized as drug delivery vesicles for multiple disease models of cancers, diabetes, and brain diseases such as Alzheimer's, prions, and Parkinson's [41].

Due to the biocompatibility of exosomes, various routes of administration are possible such as intravenous, intraperitoneal, oral, intranasal, and intratumoral. The exosomes have been considered a transporter of biomolecules, including lipids, proteins, enzymes, transmembrane proteins, cytoskeletal proteins, and genetic material. Exosomes were shown to effectively deliver an antibody-drug conjugate (trastuzumab-emtansine) to cancer cells in HER2-positive cancer [42]. Barok et al. showed that antibody-drug-conjugated exosomes bound to HER2+ cancer cells with growth inhibition and activation of caspases-3 [42]. Another example of successful use of cancer-related exosomes in cancer treatment is loading of modification of inhibitor of apoptosis protein survivin-survivin-T34A, which is a dominant-negative mutant of survivin—into exosomes isolated from melanoma cell lines. These exosomes were shown to effectively induce apoptosis in cancer cells [43, 44]. Kooijmans et al. anchored anti-epidermal growth factor receptor nanobodies to the surfaces of exosome vesicles via glycosylphosphatidylinositol to improve the interactions between exosomes and epidermal growth factor receptor-expressing tumor cells [45].

Elucidation of the mechanisms underlying protein and RNA sorting in exosomes may have great potential for developing various therapeutic applications. Although in clinical trials exosomes are commonly used as diagnostic and/or prognostic and/or predictive markers they are more than viable candidates for targeted drug delivery innovation due to the various benefits mentioned above [41].

7. Conclusion

Working with exosomes isolated from biological fluids we do not havestrong arguments allowing us to firmly assume the tissue origin of the isolated exosomes. This is due to the biogensis features of exosomes and methodological difficulties in performing experiments to identify the tissue specificity of exosomes. All this explains a few data, allowing us to compare membrane proteins and protein and nucleic acids cargo of exosomes of a certain origin except the research on cell lines, which have a wide range of limitations in the extrapolation of obtained data to the corresponding tissue. However, exosomes remain a promising diagnostic approach for different pathologies and gain more interest as therapeutic agents delivery systems because of their biocompatibility, safety, and tissue-homing capabilities. Further research in exosomes biology would provide a big future for the application of these biomolecules for different aspects of clinical medicine.

Conflict of interest

The authors declare no conflict of interest.

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