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Extracellular vesicles - biogenesis and role in cancer treatment

Lea Sleiman[1], Andreea Lazăr[1], Sorina Dinescu[1,2]✉, Marieta Costache[1,2]

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independenței, 050095 Bucharest, Romania; [2]Research Institute of the University of Bucharest, Bucharest, Romania

✉Correspondence to: Sorina Dinescu, Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independentei, 050095 Bucharest, Romania E-mail: sorina.dinescu@bio.unibuc.ro

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Abstract Extracellular vesicles represent a group of cell-derived structures of different sizes encapsulating a variety of molecules. Cargos in exosomes, such as nucleic acids (RNA and DNA), proteins, and lipids serve as external stimuli for recipient cells, thus modifying the intercellular communication pathway in both homeostatic physiological and pathological conditions, including cancer. Due to the heterogeneity of these extracellular vesicles, it has been shown that these vesicles can also carry tumor-associated molecules, favoring the formation of pre-metastatic niches and cancer metastasis. Knowledge of the cellular processes that stand at the basis of their biogenesis and relation with the tumor microenvironment is essential for their potential use for clinical application. This review introduces the expanding and promising field of exosome research focusing on their biogenesis and composition and presenting different methods for their isolation and detection. In addition, this review also discusses their inter-relations within the tumor microenvironment and possible use as potential therapeutic targets.

Keywords: extracellular vesicles, exosomes, biogenesis, exosome detection, intercellular communication, cancer, metastasis, exosome-targeted therapy

Introduction

Extracellular vesicles (EVs) represent a novel mechanism of intercellular communication important for maintaining tissue homeostasis and cellular functions in multicellular organisms. EVs are small lipid membrane-bound vesicles originating from the endosome or plasma membrane and secreted from almost all kinds of cells into the extracellular space. EVs were described initially by Pan and Johnstone (1983). At first, these vesicles were considered as a part of a disposal mechanism playing the role of "waste carriers". Further research demonstrated that the release of EVs is not only important in discarding unwanted materials from cells but also in mediating cell-to-cell communication involved in both normal and pathological processes. (Frühbeis et al., 2012; Marcilla et al., 2012; Regev-Rudzki et al., 2013). For example, tumor-derived EVs have been reported to play significant roles in modifying the tumor microenvironment and promoting cancer metastasis. Moreover, recent studies showed that these vesicles are also involved in the establishment of a premetastatic niche (Peinado et al., 2012), destruction of the peritoneum (Yokoi et al., 2017) or the blood-brain barrier (BBB) (Tominaga et al., 2015), promotion of angiogenesis (Kosaka et al., 2013), formation of the heterogeneity of cancer-associated fibroblasts (Naito et al., 2019), and induction of drug resistance (Wei et al., 2017). Considering all these observations, understanding the mechanism that stands at the basis of EVs role in intercellular communication and in altering the tumor microenvironment is currently expected to be a novel therapeutic target (Kosaka et al., 2016).

Generally, all types of mammalian cells are capable of releasing and taking up EVs (Kowal et al., 2014). EVs can be isolated from normal functioning cells such as immune cells (Raposo et al., 1996), adipocytes (Thomou et al., 2017), brain resident cells (Zhang et al., 2017), or even from cancer cells. EVs isolated from cancer patients encapsulate various tumor-specific bioactive molecules such as nucleic acids (DNA, mRNA, ncRNAs in particular miRNAs), proteins, and lipids. To this date, the ExoCarta exosome database (http://www.exocarta.org) has collected 9769 proteins, 3408 mRNAs, 2838 miRNAs, and 1116 lipids that have been found in exosomes from multiple cell lines. The content may vary with respect to their mode of secretion, cell type, and cell condition whether involved in normal or pathological processes. Once released, these EVs can be internalized via membrane fusion or endocytosis thus contributing to cancer malignancy and recipient cell's function diversion. Therefore, understanding the process that stands at the basis of their capacity to communicate and exchange molecular components between cells and to act as

signaling “bio-vehicles” in both normal and pathological conditions can provide a novel therapeutic strategy for cancer treatment, serving as an important diagnostic and prognostic tool (Henderson and Azorsa 2012).

In this review, we summarize the types of EVs giving an overview of their biogenesis, nucleic acid, protein, and lipid cargos. We will also present different methods for their detection, isolation, and characterization. Moreover, we will focus on the function of exosomes in cancer development and metastasis, plus their possible therapeutic-targeting application in cancer treatment.

Biogenesis of extracellular vesicles

Extracellular vesicles can be divided into three main subgroups based on their size and origin: exosomes, microvesicles, and apoptotic bodies (ApoEVs).

Exosomes biogenesis

Exosomes represent a subset of EVs secreted by most eukaryotic cells. They are lipid-bilayered and have a size range of 40-160 nanometers (averaging 100 nanometers). The inward budding of the endosomal membrane generates early endosomes; these early endosomes give rise to late endosomes and multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs); the fusion of the plasma membrane with MVBs results in the release of exosomes into the extracellular matrix; if lysosomes fuse with these exosomes, the MVBs are degraded (Fig.1) (Yáñez-Mó et al., 2015). Exosome biogenesis and formation are controlled by the endosomal sorting complex required for transport (ESCRT) machinery, such as ESCRT-0, -I, -II, and -III, and accessory proteins (Henne et al., 2013).

Various types of specific surface markers and membrane proteins such as tetraspanins (CD9, CD63, CD81, CD82, and CD106), MVB synthesis proteins (ALG-2-interacting protein X (ALIX)), heat shock proteins (Hsp60, Hsp70, and Hsp90), proteasome component (HSC10), and tumor susceptibility gene 101 protein (TSG101)) have been reported to be associated with exosomes (Fig.1) (Bobrie et al., 2012).

Microvesicles and ApoEVs biogenesis

Microvesicles (MVs) represent another subset of EVs and have a size range of 50-1000 nanometers. In addition to their role in blood coagulation (Sims et al., 1988), studies have shown that these microvesicles can be also involved in cell-cell communication in different cell types, including cancer cells (Al-Nedawi et al., 2008). In this condition, these vesicles are denoted as oncosomes.

Apoptotic bodies (ApoEVs) having a size range of 1000-5000 nanometers house the nuclear protein histones and DNA (van der Pol et al., 2012). Both exosomes and microvesicles are secreted from normal cells, on the other hand, ApoEVs are released from apoptotic cells.

Unlike exosomes, microvesicles and apoptotic bodies are generated by the outward blebbing and fission of the plasma membrane, not from MVBs, followed by the subsequent release of vesicles into the extracellular space (Fig.1) (Tricarico et al., 2017). ARF6 and GTPase RHOA were successively reported to be involved in controlling the microvesicle release mechanism (Muralidharan-Chari et al., 2009; Li et al., 2012).

All three above-mentioned types of EVs contribute to the local and distant modulation of cell communication. Continuous attempts exist to unravel the mechanisms underlying the respective specific functions of the different types of EVs and to uncover the fate of these vesicles’ cargos in target cells. Although it has been shown that other types of EVs contribute to cancer development, this review focuses on exosome function in tumor development and metastasis.

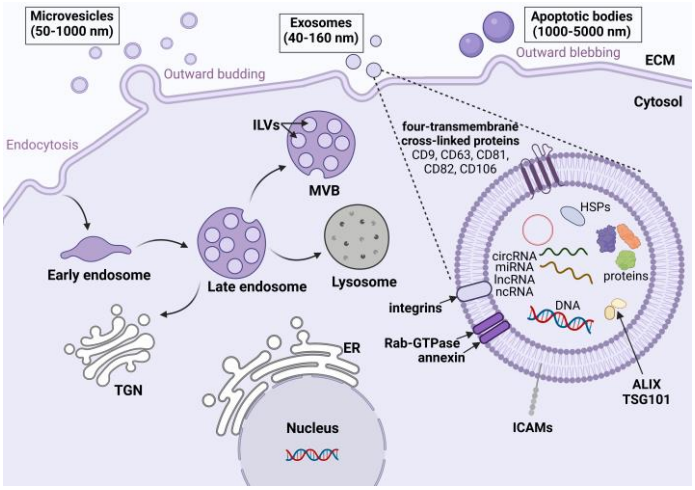

Fig. 1. Extracellular vesicles biogenesis. Exosomes originate from the inward budding of the endosomal membrane. Exosomes that originate from MVBs contain nucleic acids (DNA, miRNA, lncRNA, and circRNA), proteins (membrane transporters, HSPs, and other proteins), and lipids. Microvesicles and apoptotic bodies originate from the outward budding of the plasma membrane. MVBs: Multivesicular bodies, ILVs: Intraluminal vesicles, HSPs: Heat shock proteins, TGN: Trans-Golgi network, ER: Endoplasmic reticulum, ECM: Extracellular matrix.

Exosome composition

The nature, abundance, and mode of action of exosomes with the recipient cells are specific to cell type and influenced by the normal homeostatic or pathological state of these cells. Endocytosis, receptor-ligand interaction, or fusion with the cell membrane favors the internalization of exosomes by recipient cells. Several studies showed that different surface markers and adhesion-associated molecules exposed on the surface of exosomes determine the interaction between exosomes and recipient cells. For example, exosomes uptake through micropinocytosis by tumor cells can be facilitated by enhancing integrin CD47 expression (Kamerkar et al., 2017).

Exosomal nucleic acid content

Genetic material has been detected in exosomes including both genomic and mitochondrial DNA (Elzanowska et al., 2020). But in general, exosomes are mainly enriched with RNA (mRNAs, miRNAs, and rRNAs). Using different techniques, such as next-generation sequencing, other RNA species have been found to be present in exosomes such as tRNA fragments, long and short non-coding RNA, and piwi-interacting RNA.

Exosomal protein content

Exosomal proteins are considered to be essential elements of exosomes and present two specific characteristics known as ubiquitination and deubiquitination. Ubiquitination represents a crucial step in protein recognition by ESCRT-0, whereas deubiquitination is essential for their sorting into ILVs. The most commonly found proteins in EVs are those associated with the mechanisms responsible for biogenesis. Exosomal proteins include (i) tetraspanins such as CD9, CD63, CD81, CD82, CD106, Tspan8, and intercellular adhesion molecules (ICAMs); (ii) membrane transport and fusion-related proteins such as annexin, Rab-GTPase, and heat shock proteins (HSPs) including Hsp60, Hsp70, and Hsp90; (iii) MVBs-related proteins, such as ALIX and TSG101; and (iv) other proteins, such as integrins, actin, and myosin (Li et al., 2023).

Exosomal lipid content

In addition to proteins and RNAs, lipids can be also enriched within exosomes. Extensive research has been done on the lipid composition of EVs and it was found that exosomes share a similar lipid composition to that of their cell of origin. Lipids such as cholesterol, phosphatidylserine, ganglioside GM3, sphingomyelin, ceramide, and desaturated lipids are found to be present in exosomes (Llorente et al. 2013).

Methods to detect, isolate and characterize exosomes

Exosomes as mentioned above are associated with various membrane-bound proteins or so-called vesicle-associated proteins. The state of cancer disease can be predicted by analyzing the molecular phenotype and evaluating the physiochemical properties of exosomes and EVs. Several commercial EVs isolation kits exist, but they are not ideal for detecting and isolating exosomes due to their potential to capture soluble proteins. Thus, effective EV-associated protein biomarker platforms are required for standardized EVs collection. Various techniques have been put to use in the last decade to detect and efficiently isolate exosomes from body fluids or culture supernatants without the need for repeated ultracentrifugation. However, to this date no unique method for EVs accurate detection was established, thus EVs analysis remains challenging.

Among the currently used methods (Fig. 2) we mention the following 1) ExoTEST which represents an Immunocapture-based ELISA method that allows the detection and quantification of EVs in both human body fluids (plasma) and cell culture supernatants. In this method two antibodies are used, the first is directed against a classical exosomal housekeeping protein and allows the capture of exosomes, while the second is directed against either a potential disease marker or another exosomal housekeeping protein and allows the characterization and quantification of the captured EVs (Logozzi et al., 2020). 2) EV Array which represents a method for EVs detection and phenotyping based on protein microarray technology. On a microarray slide captured antibodies are printed, thus allowing EVs to be detected in a high-throughput manner based on their membrane proteins. For example, for the detection of exosomes captured on the EV Array and no other types of EVs a mix of antibodies against tetraspanins CD9, CD63, and CD81 can be used (Jørgensen et al., 2013). 3) ZnO-nanorods integrated (ZNI) microfluidic chip captures and quantifies plasma EVs at the micro-scale. It represents a highly sensitive and rapid analytical microfluidic chip platform. It was reported that using a cocktail of antibodies against tetraspanins CD81 and CD63 on the ZNI chip yields a great number of total EVs. Using this microfluidic chip it was shown that the total amount of EVs present in plasma was significantly different between osteosarcoma (OS) and healthy donors (Xu et al., 2021). 4) Nano-plasmonic (nPLEX) exosome technology detects exosomes in a label-free manner. It is made of periodic nanohole arrays shaped in an opaque gold (Au) film. By means of light illumination, to these nanohole arrays, strong electromagnetic fields called surface plasmons (SPs) are excited. This excitation leads to extraordinary optical transmission (EOT). The binding of exosomes to the nanohole surface (via affinity ligands) would red shift the optical transmission spectral peak since it is very sensitive and highly depends on the nanohole surface. Exosomes captured on this surface can be quantified by correlating the amount of spectral shift with the molecular mass density (Im et al., 2015). Applying this method, the targeting of surface exosomal EpCAM and CD24 allowed the differentiation between ovarian cancer patients and healthy individuals (with an accuracy of 97%). 5) Surface Plasmon Resonance imaging (SPRi) for label-free quantitative detection of tumor-derived exosomes and extracellular domains of exosomal membrane proteins. SPRi is combined with antibody microarray thus providing an easy and efficient way for exosome detection and cancer diagnosis and prognosis (Zhu et al., 2014).

Compared to targeting EV membrane-bound proteins, creating a functional EV-associated RNA biomarker platform requires a more complex approach for EV harvest. Quantitative PCR (qPCR), also called quantitative real-time PCR (qRT-PCR) necessitates the use of a typical housekeeping gene,

which in the case of EV-associated RNAs is not easy to select.

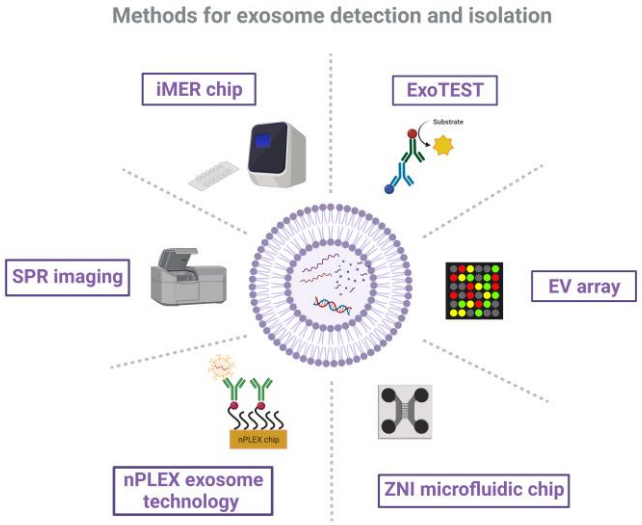

Fig. 2. Different methods to detect, isolate and identify exosomes. These techniques either target the exosomal membrane-bound proteins or the exosomal-associated miRNAs. Exosomes can be detected and efficiently isolated from body fluids or culture supernatants without the need for repeated ultracentrifugation.

Moreover, current protocols for EV-associated miRNAs detection and isolation recommend processing and compensating the tested samples from both identical input volumes and technical viability points of view, using either stable endogenous miRNAs or synthetic non-human miRNAs as internal controls. Despite the presented issue, an immune-magnetic exosome RNA (iMER) microfluidic chip (Fig. 2) was created to analyze using qPCR mRNA levels of O6-methylguanine DNA methyltransferase (MGMT) and alkylpurine-DNA-N-glycosylase (APNG) in enriched tumor exosomes obtained from blood samples of patients suffering from glioblastoma multiforme (GBM). It was shown that the levels of exosomal mRNA in these enzymes correspond with those found in parental cells and that during the treatment of seven patients, these levels change significantly making it a potential method for drug response prediction in GBM patients (Shao et al., 2015).
So far only a small number of samples have been analyzed in assessing the diagnostic capability of these developed platforms and assays. Further research is required to clearly demonstrate the usefulness of these extracellular vesicle-associated proteins and RNAs as biomarkers for cancer detection.

Relation between exosomes and tumor microenvironment

In the last decade, tumor-secreted exosomes have been gaining a growing interest as critical messengers in cancer progression and metastasis. Exosomes not only play an important role in the communication between tumor cells and surrounding normal cells within the primary tumor microenvironment but they also are believed to contribute to early steps involved in tumor progression and metastasis. Oncogenic molecules can be transferred via tumor-secreted exosomes either between cancer cells within the primary tumor or between cancer cells and stromal cells (Al-Nedawi et al., 2008). Circulating exosomes can "modify" the behavior of cells at distant sites influencing various processes such as the establishment of the pre-metastatic niche (PMN), immune response, cell growth, and survival.

Tumor-secreted exosomes and stromal cells

Several studies have demonstrated that tumor-secreted exosomes can exert complex effects on neighboring non-tumor cells. These cells take up mRNA, miRNA, lncRNA, proteins, and other molecules thus promoting primary tumor growth and mediating tumor cells-surrounding microenvironment bidirectional communication. Exosomes derived from pancreatic cancer cells and expressing CD8 recruit mRNA and protein cargo that in turn activate angiogenesis-related gene expression in endothelial cells (Nazarenko et al., 2010). Pulmonary pre-metastatic niche development is facilitated by exosomal Rab22a-NeoF1 fusion protein which also promotes osteosarcoma lung metastasis by recruiting macrophages derived from the bone marrow (Zhong et al., 2021).
Interactions between cancer cells and their surrounding tumor microenvironment via miRNA-enriched exosomes have also been reported. Elevated levels of miR-21-5p, miR-375, and let-7c-5p were detected in the urine of prostate cancer patients when compared to healthy individuals (Foj et al., 2016). Elevated levels of exosomal miR-375 and miR-1290 in the serum of advanced-stage prostate cancer patients were associated with decreased survival rates (Huang et al., 2015). Exosomal miR-934 isolated from colorectal cancer cells promotes liver metastasis of colorectal cancer by inducing M2 macrophage polarization, downregulating phosphatase and tensin homolog (PTEN) expression, and activating the PI3K/AKT signaling pathway (Zhao et al., 2020). Exosome-mediated miR-200b uptake by a new target cell can promote colorectal cancer cell proliferation upon TGF-β1 exposure (Zhang et al., 2018). Transferring miR-21, released by exosomes derived from breast cancer cells (SCP28 cells), to osteoclasts promotes pre-metastatic niche formation and breast cancer bone metastasis. Exosomal miR-21 facilitates the process of osteoclastogenesis by regulating programmed cell death protein 4 (PDCD4) levels (Yuan et al., 2021). Astrocytes-derived exosomes containing miR-19 downregulate PTEN expression in metastatic cancer cells, thus promoting brain metastasis (Zhang et al., 2015).
Despite all the results showing that exosomes are capable of mediating intercellular communication between tumor

and stromal cells, the exact specific mechanism that stands behind exosome release by stromal and uptake by cancer cells remains to be further explored.

Tumor-derived exosomes and the "seed and soil" theory

Cancer metastasis represents a complex process in which cancer cells leave their primary site of invasion and enter circulation via the lymphatic or circulatory system. Cancer cells, after surviving the harsh circulatory conditions arrive at a site situated farther from their point of origin and colonize it. In 1889, Stephen Paget proposed his "seed and soil" theory after postmortem examination of women suffering from breast cancer. He stated that metastatic tumor cells ("the seeds") will metastasize to a site where the local microenvironment is favorable ("the soil") meaning that this will allow cancer cells to survive and metastasize. Not for long, the focus has been on soluble biological factors such as chemokines, cytokines, and growth factors to validate this hypothesis and to understand how cancer cells adhere, proliferate, and circulate. However, recently, exosomes and EVs gained increasing interest due to their considerable effect on each step of the process of metastasis. The impact of exosomes on reprogramming the surrounding stromal cells in the distant sites for establishing pre-metastatic niches aligns with the "seed and soil" theory.

Exosomes derived from cancer cells are able to: (1) regulate directional cell movement and cell polarity, (2) modify the extracellular matrix (ECM) to stimulate cell proliferation, invasion, and metastasis, (3) knock down the tight junctions to establish tumor cells' intravasation, and (4) promote recipient cell's epithelial-mesenchymal transition (EMT). Highly malignant tumor cells-derived exosomes can also promote cancer cell invasion when taken up by less malignant cells. For example, tumor cell metastasis across the blood-brain barrier (BBB) takes place by means of the secretion of breast cancer cell-derived exosomes. Exosomes released from brain metastatic cancer cells transfer into the endothelial cells of the BBB miR-181c, leading to the unlocking of cell-cell junctions and therefore the destruction of the BBB to permit brain metastasis (Tominaga et al., 2015). It was reported that exosome secreted Met derived from Met-high subpopulations in melanoma cells represented a modified phenotype, resistance to v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitors, and conditioned the lung tissue to become a suitable niche for the metastatic invasion, colonization and outgrowth of melanoma cells (Adachi et al., 2016). Exosomes derived from melanoma cells can also travel to sentinel lymph nodes via the lymphatic system and condition the lymph nodes for metastasis by activating different biomolecular signals that influence vascular proliferation, extracellular matrix (ECM) modulation, and melanoma cell recruitment (Hood et al., 2011). These data support the role of exosomes in the establishment of pre-metastatic niches for disseminated tumor cells and in the progression of metastasis.

Exosomes targeting therapy

As mentioned previously, exosomes derived from cancer cells are capable of promoting tumor malignancy and progression by mediating cell-to-cell communication within the surrounding tumor microenvironment between both tumor and healthy neighboring cells. Multiple studies showed that cancer cell-derived exosomes promote cancer growth, tumor angiogenesis and coagulation, regulate the immune system, modulate the nearby parenchymal tissue, and favor therapeutic resistance which altogether support tumor outgrowth (Ciardiello et al., 2016). Therefore, one way to inhibit cancer progression is to eliminate cancer-secreted exosomes from circulation or to block their release. In the final part of this review, we will discuss the possible therapeutic strategies for tumor-derived exosomes.

The biogenesis and release of exosomes from cancer cells is a remarkable feature of tumor progression and malignancy due to the role of these nanovesicles in promoting cancer growth, tumor angiogenesis, immune suppression, and therapeutic resistance. The majority of experimental model systems target the importance of exosomes as "bio-vehicles" for the transfer and delivery of therapeutic cargos (Fig. 3A), however, fewer models aim to target their malignant activities. A novel therapeutic strategy for circulating exosome elimination was proposed in 2012. This therapeutic strategy is based on an extracorporeal hemofiltration system that uses an affinity plasmapheresis platform termed the adaptive dialysis-like affinity platform technology (Aethlon ADAPT™) system (Fig. 3B). This system is composed of plasma filtration membranes, in which the outer-capillary space is immobilized by different affinity agents such as exosome-binding antibodies and lectins, which integrate into an existing kidney dialysis system. The epidermal growth factor receptor 2 (HER-2) present on the surface of exosomes is targeted, hence their selective removal could be advantageous for cancer therapy (Marleau et al., 2012). Another strategy can be used for targeting cancer-derived exosomes. This novel strategy is based on therapeutic antibody treatment (Fig. 3B). It was reported that metastasis of breast cancer to the lungs, lymph nodes, and thoracic cavity in mouse model could be inhibited by the treatment with human-specific anti-CD9 or anti-CD63 antibodies, although no obvious effects on primary tumor growth were observed. This study suggested that exosomes derived from cancer cells and tagged with antibodies were removed by macrophages (Nishida-Aoki et al., 2017). These obtained data suggest that targeted cancer-derived exosome elimination therapy could effectively suppress EV-triggered metastasis.

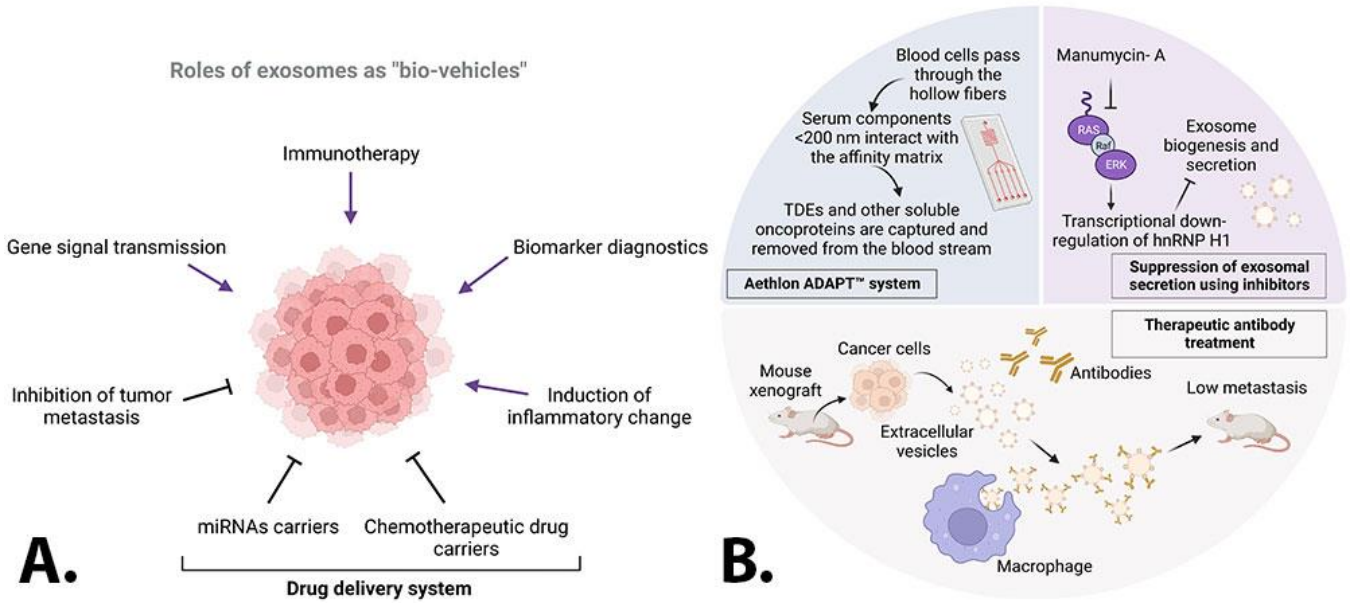

Fig. 3. Potential therapeutic strategies targeting tumor-derived exosomes (TDEs). A: Roles of exosomes as "bio-vehicles" in cancer treatment. **B:** Methods to eliminate or block tumor-derived exosomes (TDEs) biogenesis. TDEs in circulation can be eliminated using extracorporeal hemofiltration systems, such as the Aethlon ADAPTTM system, or by using therapeutic antibody treatment. TDEs release can be blocked using different inhibitors (e.g., Manumycin- A) that down-regulate specific exosomal miRNAs synthesis and secretion involved in the promotion of angiogenesis and metastasis within the tumor microenvironment.

Another approach for inhibiting exosome-mediated cancer progression is by means of blocking their secretion. The identification of the exosome secretion mechanism is crucial to establish successful therapeutic approaches. Several studies aimed to identify inhibitors of exosome biogenesis and release in various types of cancer. For example, reducing neutral sphingomyelinase 2nSMase2 activity, an enzyme that regulates the release of miRNA from exosomes and promotes angiogenesis within the tumor microenvironment as well as metastasis, blocked exosome release and transfer of miR-210-3p. Moreover, it also blocked breast cancer metastasis and tumor angiogenesis in the xenograft model (Kosaka et al., 2013). It was also shown that knocking down the activity of Ras-associated binding (RAB) proteins (RAB27A and RAB27B) inhibited exosome release without influencing the secretion of other soluble proteins. Using the High-throughput screening (HTS) technique several inhibitors for exosome secretion in human neutrophils have been reported. This inhibition is influenced by the interaction between RAB27A and JFC114 (Zhang et al., 2020).

Other inhibitors such as Manumycin- A (MA), a natural microbial metabolite, represents a potential drug to block exosome secretion and biogenesis by castration-resistant prostate cancer (CRPC) cells, however, it does not inhibit their secretion from normal prostate epithelial cells. The inhibition of Ras/Raf/ERK1/2 signaling oncogenic splicing factor hnRNP H1 and hnRNP H1 in prostate cancer cells determines the inhibitory role of MA (Fig. 3B) (Datta et al., 2017). The release of exosomes can also be reduced by modulating the tumor microenvironment, making it more alkaline (Logozzi et al., 2018). Data showed that the number of exosomes released from different cell lines including SKBR3, Me30966, and LNCaP cells, decreased successively as a function of increasing microenvironment pH.

In summary, an endless potential in EV research as therapeutic targets exist, whether inhibiting their activity or using them as "bio-vehicles" for drug transport. The clinical application of exosomes as drug delivery systems has been reported in several studies and since the research in this domain is vastly developing, using exosomes as diagnostic tools in cancer has an optimistic future. Advances in EV research may provide novel treatment strategies and may be beneficial to cancer patients.

Conclusions

It is remarkable how in the last few decades our knowledge of understanding the basic biology of EVs has expanded. Many promising findings and applications for EVs under not only physiological but also pathological conditions have been and are still being shared and published. However, the urge for common like-mindedness on the mode of isolation, detection, characterization, classification, and identification of cargos of EV subgroups is still to be set on. Future comprehensive research can focus on novel approaches and techniques to manipulate these EVs, whether by using them as novel drug delivery systems or controlling

their biogenesis, release, and content composition (for example delivery of miRNAs or proteins in order to modulate a certain pathological process response), for the purpose of developing new therapeutic approaches due to their ability to hold great potential for clinical application, especially for cancer patients.

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