Exosomes: Definition, Role in Tumor Development and Clinical Implications

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Abstract

Exosomes are microvesicles released by cells in both physiological and pathological situations. They are surrounded by a lipid bilayer with proteins derived from the origin cell, and contain a variety of molecules, such as nucleic acids. They represent an emerging mechanism of intercellular communication, and they play an important role in the pathogenesis of cancer, stimulating proliferation and aggressiveness of cancer cells, inducing a microenvironment favorable to tumor development and controlling immune responses. Because of the growing understanding of the potential implications of extracellular vesicles in the development of malignancies, research on exosomes, and its role as a diagnostic and therapeutic tool, constitutes nowadays a very exciting and promising field.

Keywords Cancer · Microenvironment · Metastasis · Microvesicles · Exosomes

Introduction

Traditionally, microvesicles have been considered as elements used by cells to remove waste, such as unnecessary proteins and remains of deceased cells [1]. However, they are now believed to play an additional and critical role in intercellular communication [2].

Exosomes may be present in any biological fluid such as blood, urine or cerebro-spinal fluid, and they are released by normal cells in physiological situations. Interestingly, the levels of these microvesicles have been found to be increased in pathological conditions, such as infections, autoimmune disorders or cancer, supporting the hypothesis of its role in the pathogenesis of cancer.

Microvesicles may be characterized by several criteria, including size and the structural composition.

The aim of this review is to update the information about the role of exosomes in solid tumors, as they are one of the leading and most compelling molecules, both for the diagnosis and therapy of cancer. However the role played by these molecules in the tumorigenesis and its correlation with the

status of disease is still not fully understood, and therefore more research is required. This explains that the incorporation and use of these molecules in the clinical setting cannot be foreseen in the near future and remains a major challenge.

Microvesicles: Cellular Origin and Classification

Exosomes are cell-derived vesicles ranging from 40 to 100 nm [3]. They are generated by invagination of the cell membrane (endocytosis) and the subsequent formation of intracellular vesicles, which are released toward the extracellular space in response to different signals (exocytosis) [3].

There are also several proteins involved in the exosomes synthesis, trafficking and transport, such as GTPases, Rab, p53-regulated gene product (TSAP6) or the components of the *endosomal sorting complex responsible for transport* (ESCRT-III) such as Alix [4]. Interestingly, some pathways involved in exosome shedding, as that driven by *peptidylarginine deiminase* (PAD), are being revised as new potential ways of influence on tumor biology [5].

Extracellular vesicles can be classified into three main subtypes: exosomes, ectosomes and apoptotic bodies. Due to the diversity of techniques and the difficulties in the detection and isolation of these vesicles, there is a lack of consensus regarding this classification, and different and confusing terms can be found in the literature [3]. Different attempts based on

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physical and chemical characteristics, as protein composition [6], are being optimized in order to improve and standardize the study of these particles [7, 8]. Different methods have been used to isolate exosomes (centrifugation, filtration, electrophoresis), and the most frequently used is the flow cytometry [9, 10].

Several elements can regulate the exosomes release, such as, stressful cellular conditions, pH or ionic alterations, ischemia, phosphatidylinositol 3-kinase and loose cell-to-cell adhesion [11–13]. For instance, hypoxic situations can induce the packaging and delivery of exosomes, both involved in promoting angiogenesis mechanisms [14]. These processes may be extremely relevant in tumors where angiogenesis has shown to be a core feature.

In addition to exosomes, there are other types of microvesicles. The microparticles or ectosomes are cell-derived vesicles, from 0.1 to 1 μm of diameter, that are directly released from the cell membrane by an exocytosis process. They also contain a variety of molecules, such as lipids, proteins and nucleic acids, mainly ribonucleic acid or RNA. Finally, apoptotic bodies are cell fragments arising from late stages of the apoptosis. Its size ranges from 1 to 4 μm , and they contain deoxyribonucleic acid and histones [3, 4].

Biochemical and Molecular Structure of Exosomes

Exosomes are surrounded by a lipid bilayer with characteristic and cell-specific membrane proteins. They contain different molecules, including nucleic acids, such as *messenger-RNA* (mRNA) and *microRNA* (miRNA) [4, 15]. It has been reported that different subpopulations of exosomes with different molecular compositions and functions can be produced by the same cell. This would all depend on distinct specialized or polarized areas inside the cell [16] (Fig. 1).

The exosome surface is surrounded by diverse several cell-specific proteins, such as tetraspanins, integrins, various intercellular adhesion molecules or the major histocompatibility complex, which can also be found on the exosome surface [4]. Their molecular composition varies depending on the origin cell, thus, it has been described the presence of the Her 2 oncoprotein in breast cancer-derived exosomes as well as the expression of different Endothelial Growth Factor Receptor isoforms in pancreatic adenocarcinoma and glioblastoma [16–19].

Exosomes tend to contain nucleic acids, such as mRNA and miRNA [20]. miRNAs are a type of non-coding RNA with the capability to regulate gene expression through binding the 3' *untranslated region* (UTR) of the mRNAs of the target cell and, thus, inhibiting translation process [15, 21]. The miRNA codifying-genes can either act as oncogenes or

tumor suppressor genes, and they are involved in cell proliferation and differentiation, apoptosis and intercellular adhesion. These genes are first transcribed to *primary-miRNA* (pri-miRNA) and subsequently, through different processes mediated by Drosha and Dicer enzymes, they become mature miRNA [15]. According to some authors, miRNA expression levels may have a prognostic value in cancer patients [21–23]. As epigenetics research has been growing in the recent years, non-coding RNAs (including miRNAs) focus the most interest. In addition to miRNAs, according to preview research, mRNAs located in exosomes are introduced inside the target cell through membrane-fusion and endocytosis processes, and subsequently translated, resulting in proteins that control cell functions and gene expression [24].

According to the characteristics of each exosome, they can act in the target cell by two different mechanisms: regulating intracellular signal transduction through the exosome surface proteins or via miRNAs or mRNAs [4].

Role in the Oncogenesis

It is becoming clear that cells use exosomes as an intercellular communication system, by transferring molecules such as surface proteins, lipids or genetic information; but they are also used by cells to remove unnecessary or toxic substances [24].

Normal cells have the capacity of releasing exosomes in physiological situations, collaborating in homeostasis; for instance, they have an important role in cell differentiation and they are thought to have a role in the regulation of immune response. Thus, placenta-derived exosomes have an anti-inflammatory and immunosuppressor effect mediated by Fas ligand and miRNAs from chromosome 19 cluster. They also may induce maternal immune tolerance during pregnancy [25, 26]. Furthermore, it has been suggested that exosomes can participate in the antigen-presentation process mediated by dendritic cells.

Exosomes are released into the circulation, as they are needed, in pathological situations, adjusting pathways in tumor biology. Cancer related exosomes may act as principal signals released by tumor and stromal cells, working as a multidirectional communication system (crosstalk between stromal and tumor cells) [4, 15, 27]. Interestingly, two important stem cell populations in cancer (mesenchymal stem cells and cancer stem cells) are multifaceted regulators of tumor biology and it has been shown that they could exert partially their functions by secretion of exosomes [28].

Effects in Tumor Cells

Exosomes released by cancer cells respond not only to the nearby cells that have an autocrine or paracrine effect, but also may commonly modulate distant tissues (endocrine effect).

Fig. 1 Diagram depicting the biogenesis and composition of a typical cancer-cell derived exosome

Cancer-related exosomes have the potential to induce malignant cell transformation and, they are also regarded as a regulator of tumor growth and cell progression, like triggering cell proliferation or inhibiting apoptosis. Once released, exosomes are able to travel to distant sites to generate the pre-metastatic niche, driving tumor cells to these favorable environments. For all these reasons, exosomes are also called “oncosomes”.

The coexistence in a tumor of different cell clones is well known (tumoral heterogeneity) [29, 30]. Aggressive clones are able to enhance cell growth by stimulating growth factor signaling and progression of primary neoplastic lesions in indolent cells via exosomes. *Le et al.* described how exosomes allow the transference of miR-200 s from metastatic to non metastatic tumor cells, conferring them the capability of invading distant tissues [23]. For instance, pancreatic cancer-derived exosomes can affect proliferation and apoptosis processes of other cells through Notch-pathway [31]. Gliomas often express an *epidermal growth factor receptor* (EGFR) variant, called EGFRvIII. Exosomes containing EGFRvIII can be released by gliomas, transferring an oncogenic signal to normal cells via MAPK and AKT pathways [18, 32, 33].

Because of their diverse properties, stromal cell-derived exosomes, such as fibroblasts and myofibroblasts, can also modify tumor-cells characteristics, promoting the *epithelial–mesenchymal-transition* (EMT) [34, 35]. In the brain microenvironment, astrocyte-derived exosomes mediate an intercellular transfer of PTEN-targeting microRNAs to metastatic tumor cells (miR-19). This PTEN-loss (known tumor-suppressor gene)

increases the secretion of chemokine CCL2, which recruits myeloid cells that enhance the outgrowth of brain metastatic tumor cells [36]. Platelet-derived exosomes seem to play a role in lung cancer, by getting involved in intercellular adhesion, invasion ability, angiogenesis and metastasis through several molecules such as CD41 [37, 38]. The regulation of exosome transporters is still being elucidated, but as a consequence of the export of intracellular waste products, exosomes may enhance resistance of cytotoxic agents (such as anthracyclines) [39]. *Patel et al.* found that exosomes derived from gemcitabine-treated pancreatic cancer cells could confer chemoresistance by promoting ROS detoxification and gemcitabine-metabolising enzyme downregulation [40]. Expression of target molecules on the surface membrane (e.g., *human epidermal growth factor receptor 2-HER2*–) can represent another defense mechanism against targeted therapy. This is done by decreasing the proportion of monoclonal antibodies which have an effect on neoplastic cells [41].

Effects in Stromal Cells: Microenvironment Conditioning

Due to its nature, exosomes derived from malignant and adjacent stromal cells (endothelial, hematopoietic and immune cells or fibroblasts) act on the tumor microenvironment both locally and distantly. This raises the possibility that exosomes may have an effect on the nutritional support, the tumor growth and on distant metastases.

As for glioblastoma, when exosomes expressing EGFRvIII on their surface interact with endothelial cells, they promote a

series of changes that stimulate angiogenesis; this interaction increases the amount of oxygen and nutrients in the neoplastic tissue [18, 32]. Under hypoxia conditions different cytokines (e.g., TGF- β , VEGF, IL-6, IL-8) located in the exosomes induce the formation of new blood vessels [14]. In relation to metabolic reprogramming, it has been described how breast-cancer-secreted miR-122 in the exosomes increases nutrient availability in the pre-metastatic niche by suppressing glucose uptake in niche cells (via the downregulation of the glycolytic enzyme pyruvate kinase). *In vivo* inhibition of miR-122 restores glucose uptake in distant organs, including brain and lungs, and decreases the incidence of metastasis [42]. Some tumors are usually associated with thromboembolic phenomena, which impacts on survival, and it seems that the increased tissue factor expression on the surface membrane of the exosomes located in the plasma could be related to this event [43]. These vesicles are able to modify the structure of the extracellular matrix by modifying the fibroblasts, as they may increase degradation and hyaluronic acid concentration; these changes enhance the invasion and establishment of malignant cells. These induced alterations in the stromal cells may also affect its own secretion of exosomes which, eventually, may have a bearing on the tumor cells (multidirectional communication loops are created) [4, 15, 20, 24]. In studies with melanoma cells, these structures have been shown to produce changes which facilitate the tumor growth in the regional lymph nodes [44]. One of the mechanisms proposed for this process is the recruitment of hematopoietic progenitor cells that would create a favorable environment. Different molecules involved in these mechanisms are being identified, like the tyrosine-kinase receptor MET, located on the exosomes surface membrane of tumor cells or *Toll-like receptors* (TLR); Liu et al. describe how RNAs from tumor-derived exosomes can activate lung epithelial cells TLR3 positive, inducing chemokine secretion and subsequent neutrophil recruitment. High levels of neutrophils are known to enhance expression of pro-metastatic S100 protein, supporting their role in pre-metastatic niche formation [45, 46].

Exosomes found in the tumor microenvironment seem to play a crucial role in the creation of tumor receptive microenvironments in distant tissues, also known as pre-metastatic niches. Also, as seen in the literature, exosomes may stimulate the migration of cancer cells to the pre-metastatic niches by regulating the release of chemical signals. In general, cancer cells will first spread to the nearby lymph nodes, and finally to the rest of the body. Therefore, exosomes could have a decisive role in targeted cancer therapy [4, 44, 47].

In the nineteenth century Paget described the tropism of the metastases for certain organs and tissues. Since then, the explanation of this phenomena has remained unknown [48]. In this regard, there are different lines of research focusing on the structural components of the exosomes which are considered as functionally relevant molecules. Hoshino et al. found that

exosomes organotropism was determined by the expression of different integrins (cell-adhesion proteins). In this sense, depending on the specific integrins displayed on their surface, the exosomes would be taken up by the specific target organs [49]. Several combinations of integrins have been characterized, such as $\alpha v \beta 5$ integrin, which leads exosomes to the liver, or $\alpha 6 \beta 4$, which mediates their migration to the lung [49]. In this regard, a different group of transmembrane proteins, called tetraspanins, are being investigated; some studies suggest that the complex tetraspanin-integrin could be involved in the preparation of the pre-metastatic niche, although this relationship is still unclear [50]. Once the microvesicles reach their destination, they induce relevant changes in the metastatic deposits at a cellular and microenvironmental level. The increase of S100 proteins (important in promoting inflammation and cell migration) and the activation of Src protein have been related to this event [49]. Costa-Silva et al. also pointed at the exosomes secreted by pancreatic adenocarcinoma-cells as facilitators of liver metastases by acting on Kupffer cells through the expression of macrophage migration inhibitory factor (MIF) [51]. A study performed by Zhang et al. in gastric cancer-cell lines highlighted the role of EGFR-containing exosomes derived from cancer cells in the development of a liver-like microenvironment promoting liver-specific metastasis [52].

Other components may also influence the tropism of metastases, mechanisms that deserve further investigation. Deng et al. showed that gastric cancer-derived exosomes contain metalloproteinase-2 (MMP2) and *Fas-ligand* (FasL) protein, molecules that could be involved in the development of peritoneal metastases through mesothelial barrier disruption [53].

Several studies have also shown that tumour-derived exosomal miRNAs are able to “teach” target cells and drive malignant cells to a specific location. For instance, Fang et al. described how high serum exosomal miR-1247-3p levels correlate with lung metastasis in hepatocellular carcinoma patients [54]. miR-181c seems to be involved in the destruction of the blood–brain barrier through degradation of PDPK1, representing a mechanism of brain metastasis development [55].

Effects in the Immune System

The link between cancer and immune system is an old phenomenon of complex origin. Novel immunotherapy is being considered as one of the most promising therapeutic strategies in the last recent years.

In normal conditions, immune system cells are supposed to recognize and minimize the progression of an aggression, including the cancer cells aggression. Immunosuppression produced by malignant cells allows them to escape from the immune system control and proceed with their growth and shedding. There is an increasing evidence regarding the immune

disturbance caused by the exosomes [56, 57]. These molecules seem to be able to promote an immunosuppressive phenotype by increasing the number of suppressor cells or by decreasing the number of cytotoxic T cells, *natural killer* (NK) cells and antigen-presenting cells. All these phenomena could be influenced by these microvesicles, *inter alia* [57]. For instance, *Gao et al.* found that tumor-derived exosomes were able to transfer EGFR to host macrophages, resulting in a reduced production of type I interferon and impairing host immunity [58] and *Ludwig et al.* described how exosomes of patients with an active head and neck cancer induced apoptosis of CD8⁺ T cells and an increase of regulatory T cells more frequently than exosomes of patients without an active disease [59].

There are different types of signals and routes of communication that promote this immunosuppressive microenvironment. In this sense, the exosomes express on their surface proteins such as Fas ligand, TRAIL or TNF α , which can induce the apoptosis of activated lymphocytes by binding to death receptors [57, 60] and tumour-derived exosomal miRNAs could also have a role in post-transcriptional control of immune modulatory molecules, such as HLA antigens [61]. Moreover, NK cells may interplay with malignant cell-derived exosomes leading to a reduction of the perforin secretion, the inhibition of Jak-3 protein, the decrease in cyclin D3 or the alteration of the NKG2D receptor pathway [62]. All these effects interfere with the activation and the adequate performance of these immune cells. Furthermore, exosomes may also influence the normal maturation process of antigen-presenting cells, including dendritic cells, through different cytokines [56]. Also, exosomes are able to stimulate the proliferation of both myeloid suppressor cells and regulatory T lymphocytes (via TGF- β , Stat 3 and SMAD2/3 among other pathways) [63–65]. Therefore, by promoting all these processes, exosomes enhance the creation of an immunosuppressive state.

On the other hand, exosomes may also play a role in providing tumor antigens that can stimulate the immune system and promote rejection of neoplastic spread by the organism. In recent years, the development of immunotherapies, such as immune checkpoint inhibitors, has highlighted the importance of tumor neoantigens and their relation with responses to these new therapeutic alternatives [66]. In a way, the mutational burden (and consequently neoantigens) has been shown to correlate with the efficacy of immunotherapies [66, 67]. In this sense, exosomes could be used as a predictive biomarker as well as a tool to improve response rates to new treatments (used as a platform of neoantigens).

Andre et al. outlined the immunogenic effect of tumor cell-derived exosomes, which were extracted from ascitic fluid, when they are stimulated with dendritic cells *in vitro* [68, 69] and *Plebanek et al.* showed how exosomes from nonmetastatic melanoma cell lines can stimulate an innate

immune response by activating patrolling monocytes and inhibiting metastasis [70].

Thus, exosomes were able to increase the immune cells' ability to expand the production of cytotoxic T lymphocytes active against tumor. This may be extremely relevant, given the increasing importance of immunotherapy in cancer treatment, as well as the promising potential of combined therapy with a synergistic effect.

In addition to malignant cell-derived exosomes, dendritic cell or NK cell-derived vesicles may have similar effects on the immune system. This would be done by activating pathways that are not yet fully understood, and which would involve, the major histocompatibility system proteins, certain miRNAs, NKG2D or pro-apoptotic/cytotoxic proteins, among others [71, 72].

The balance between immunosuppressive and pro-immunogenic factors is still unknown, and it could depend both on the cells of origin and the microenvironments where they are located. Nevertheless, it seems that, at least *in vivo*, exosomes could impact negatively on the immune system, and therefore they could promote the tumor growth and spread.

All this data, emphasizing the idea that exosomes may have a positive influence on the immune system against cancer, represents an encouraging and promising line of research.

Diagnostic and Therapeutic Implications

Diagnostic Implications

Theoretically, exosomes can be isolated from any body fluid, although plasma is the most used source. Once they are extracted from the organism, these vesicles are stable structures. Considering that they take part in the key processes of the tumor development, the study of these microvesicles would provide a noteworthy information about different neoplasms.

From a diagnostic perspective, blood sampling for studying the exosomes would improve our knowledge regarding the specific peculiarities of a given tumor strain [73]. In addition, this method focuses on the cellular subpopulations of greater clinical relevance, that is to say, those able to access the circulatory system.

Along with the development of “liquid biopsy” techniques (based on the study of circulating tumor cells isolated in the bloodstream), the study of these subcellular components will provide information about the molecular structure of malignant cell-clones such as, genetic mutations present in mRNA or miRNA, cellular receptors eligible for targeted therapy, or biomarkers related to the aggressiveness of malignant cells [17, 21, 22]. Therefore, these molecules, such as integrins or miRNAs, could help us to predict the neoplastic evolution, as they could give us information about the potential of

metastatic dissemination (there are integrins related to the dissemination skills), or about the preferred target organs (locations prone to be involved in the future) [49]. Recently, different studies conducted in the early-stage disease setting have shown the usefulness of exosomal components (mainly miRNAs) as potential biomarkers through which early diagnosis of tumor relapse/persistence could be improved. There are an increasing number of specific miRNA profiles suggested for this purpose, such as those for lung adenocarcinoma, lung squamous cell carcinoma or breast carcinoma [74–76].

The inclusion of exosomes analysis as a routine in cancer studies could have many advantages, like easy access to obtaining samples (without need of further aggressive techniques), the assessment of disease response to different treatments, or the gathering of molecular details with prognostic implications [15, 21, 24, 73, 77]. Analysis by *next-generation sequencing* (NGS) of plasma exosomal nucleic acids for common BRAF, KRAS, and EGFR mutations seems to have high sensitivity compared with clinical testing of tumor tissue and testing of plasma *cell-free DNA* (cfDNA); in addition exosomal cargo could better reflect the underlying cancer biology as they originate from living cells (compared to cfDNA). Furthermore, low *mutation allelic frequency* (MAF) measured in exosomal nucleic acids may become an independent prognostic factor for longer survival based on results from *in vivo* studies [78]. Exosomes may be used to monitor, by quantitative analysis or molecular characterization, the response to the different therapeutic agents, such as chemotherapy or targeted therapy. Thus, they could help us detect resistance mechanisms which would be helpful to optimize the treatment [79–81]. As mentioned above, exosomes have recently been reported to have a role in acquired chemoresistance [82, 83].

Therapeutic Implications

Exosomes may also constitute an emergent option to fight against. In theory, exosomes eager to develop tumors could be removed from the circulatory system by means of tools capable of recognizing these vesicles and reducing their effect on the neoplastic evolution [84, 85]. Those tools should be based on the differentiating characteristics of malignant cell-derived exosomes [41]. On the other hand, exosomes could represent a new way of targeted administration of antineoplastic agents, acting as vectors in order to establish a more specific therapy against malignant cells which would reduce systemic toxicities [86–89]. As an example, a work in animal models (mice) published recently shows the possibilities of treatment with engineered exosomes loaded with short interfering RNA or short hairpin RNA specific to oncogenic mutation Kras in pancreatic cancer; the use of these particles significantly increased overall survival [90].

Finally, as a consequence of the link between exosomes and the immune system, there is a feasible basis for implementing new kinds of immunotherapy against cancer based on these structures. Those exosomes which have the capacity to stimulate anti-tumor immune responses could be the mainstays of new treatments [68, 71, 72]. *In vitro* studies have shown that exosomes could induce antigen-specific T cells independently of the expression of major histocompatibility complex proteins on their surface if whole antigen is present [91]. Conversely, exosomes with immunosuppressive functions (due to their origins or microenvironments in where they are located) could make up another plausible way to new therapeutic alternatives; in this case the mechanism should be based on microvesicles inactivation [41, 84, 85].

There are ongoing studies that are evaluating all these possibilities, mainly by using tumor cells and immune cells derived-exosomes. Although we are still in a very early stage, encouraging preclinical results have already been reported.

Conclusions

Exosomes represent an emerging pathway of intercellular communication, with recognized functions in the development of neoplastic disease. Because of their attributed functions and their physicochemical properties (stable vesicles in plasma), these structures might be the key point for multiple diagnostic and therapeutic techniques with promising results in early studies. This research area is currently at an early stage of its development with virtually untapped potential.

Compliance with Ethical Standards

The manuscript does not contain clinical studies or patient data.

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