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Review Article

Tumor exosomes: a double-edged sword in cancer therapy

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Abstract

Tumor cells produce and secrete more nucleic acids, proteins and lipids than normal cells. These molecules are transported in the blood or around the cells in membrane-encapsulated exosomes. Tumor-derived or tumor-associated exosomes (usually 30–100 nm in diameter) contain abundant biological contents resembling those of the parent cells along with signaling messengers for intercellular communication involved in the pathogenesis, development, progression, and metastasis of cancer. As these exosomes can be detected and isolated from various body fluids, they have become attractive new biomarkers for the diagnosis and prognosis of cancer. Furthermore, tumor exosomes have also attracted increasing attention due to their potential as novel therapeutic strategies for the treatment of cancers. On the one hand, the lipid bilayer membrane-encapsulated vesicles are promising carriers of drugs and other therapeutic materials targeting specific cancer cells. On the other hand, tumor exosomes are important mediators for modulation of the microenvironment that orchestrates events critical to the growth and metastasis of cancer cells as well as chemoresistance. Here, we summarize the advances in our understanding of tumor-associated or tumor-derived exosomes in recent years, and discuss their roles in cancer development, progression, invasion, and metastasis of cancers and, more importantly, their potential in strategies for precision therapy of various cancers as well as important caveats.

Keywords: exosomes; tumor; metastasis; chemotherapy; precision medicine

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Introduction

Tumor cells, similar to normal cells, are factories that constantly produce and export various biological molecules. These molecules are released from the cells and transported in the blood or around the cells in unique nano-sized lipid bilayer membrane vesicles[1]. A group of these membrane vesicles with a diameter of approximately 40 nanometer (nm) were initially identified from two types of cancer cells with electron microscopy by Trams *et al* in 1981[2]. Later, Johnstone *et al* reported vesicle externalization from multivesicular bodies using immunoelectron microscopy during reticulocyte maturation, and then, they defined the exosomes with a diameter of 30–100 nm as microvesicles[3]. At this very early stage, exosomes were erroneously identified as cellular garbage until the emergence of dendritic cell (DC)-derived exosomes that contributed to the inhibition of cancer cell growth by increasing the proliferation of cytotoxic T lymphocytes[4]. Indeed, these pioneer studies promoted the application of exosomes not only in cancer research but also in the general field of cell biology, physiology, and pharmacology. In 2013, the Nobel Prize in Physiology or Medicine was awarded to three scientists "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"[5]. Randy SCHEKMAN discovered a set of genes that were required for vesicle traffic[6]. James ROTHMAN identified protein machinery that allows vesicles to fuse with their targets to permit vesicle transfer[7]. Thomas SÜDHOF revealed how signals instruct the cell to organize microvesicle exosomes and deliver them to the right place at the right time[8]. Indeed, later studies consistently found that disturbances in the exquisitely precise control system for the transport and delivery of microvesicles/exosomes have deleterious effects and contribute to many pathological conditions, such as immunological disorders[9, 10] and cancers[11, 12]. More details about the general physiology, pathology, functionality, and roles in cancer metastasis and therapy of these molecules can be found in several recent excellent review articles[13-17]. To date, tumor-associated or tumor-derived exosomes have been shown to be extensively involved in the intercellular substance exchange and signal

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communication between the tumor and the host and play crucial roles in the pathogenesis, development, invasion, and metastasis of cancer cells; more importantly, their potential as novel therapeutic strategies for precision therapy of various cancers has been demonstrated. In this review, we summarize the advances in our understanding of tumor exosomes in recent years and discuss their roles in the development, invasion, and metastasis of cancer cells, and more importantly, the potential and limitations for the development of tumor exosome-based therapeutic strategies for cancer treatment.

Characteristics of tumor-derived and tumor-associated exosomes

Tumor cells continuously secrete membrane vesicles to the extracellular environment. These tumor-derived exosomes represent novel vehicles for cell-cell communication networks and new components of a supportive microenvironment for localization, proliferation, and survival of tumor cells[2, 18-21]. Trams *et al* first used the term "exosomes" to describe membrane-enclosed structures of variable sizes budding from the cell membranes of C-6 glioma or N-18 neuroblastoma cells[2]. Exosomes are now often used to describe small extracellular membrane vesicles with a diameter of 30–150 nm generated via an endocytic pathway. Exosomes released by malignant tumor cells contain specific repertoires of molecules, including proteins, lipids, DNA molecules, miRNAs, mRNAs and non-coding RNAs, which are important for cancer cell communication with the environment. In the ExoCarta database, 286 studies focused on exosomes were found together with the contents in the exosomes, which included 41860 proteins, 7540 RNA molecules and 1116 lipids[22-24]. Specific sorting mechanisms have been proposed for controlling the sorting and loading of selected molecules into exosomes[25].

In addition to cancer cells, multiple other cell types, including adult stem cells, stromal cells, and cancer stem cells, are known to communicate with each other through their exosomes within the tumor microenvironment[26-32]. Accumulating evidence has demonstrated that these tumor-associated exosomes mediate the interactions among different types of cells within the tumor microenvironment, thereby modulating tumor development, progression, and metastasis[29-32]. These studies have also provided new insight into how these cells interact with each other to promote resistance to anti-cancer drug therapy and contribute to tumor recurrence through exosome signaling[33-37]. While the detailed cellular and molecular mechanisms for the interaction between cancer cells and their surrounding cells, and thereby tumor progression and metastasis, remain to be further elucidated, current evidence has demonstrated that extracellular microvesicles, especially exosomes, are transferred among cancer cells and their surrounding cancer stem cells, adult stem cells and stromal cells, providing another layer of complexity in cancer cell biology associated with the tumor microenvironment and tumor progression.

Although there are differences in the chemical composition and biological functions of exosomes in various tissues and cells, the process of exosome formation is similar. Initially, primary endosomes are formed after entrapment of the cytoplasmic membrane, and then, the endosomes may fuse with the lysosomes, which trigger the formation of vesicles in the lumen after releasing the cellular contents into the endosomes. Then, these primary endosomes are shifted to secondary endosomes and are called multivesicular bodies[38]. Next, the multivesicular bodies fuse with a specific part of the cellular membrane. The multivesicular bodies fuse with the lysosomes, followed by fusing with the cellular membrane and secretion of vesicles through exocytosis via regulation of the GTPase and NsF coupling protein receptor. These substances were reported to be detected in blood, lymph fluid, urine, saliva, breast milk and ascites[39].

Upon uptake of exosomes by the target cells, the exosome content may affect the biological behaviors and induce phenotypic changes in target recipient cells by several mechanisms. Exosome miRNAs, for example, can silence mRNA targets and influence cellular functions[40-42]. Exosomes released by acute myeloid leukemia cells affected the proliferation and migration of bone marrow stromal cells[43], and multiple myeloma exosomes enhanced angiogenesis[44].

Tumor exosomes in invasion and metastasis of cancer cells

Not all cancer cells, or at least cancer cells in the initial early stages, show an invasive capacity. Invasion gradually progresses during the pathogenesis and development of most cancer cells. The biological and genetic alterations of the daughter cells of cancer cells may be transferred through exosomes after multiple rounds of division and proliferation, which then result in the sharing of biological features among cancer cells. Metastasis is a complex multistep process that involves cancer cell invasion, survival in blood vessels, and attachment to and colonization of distant host organs. A variety of molecular and functional mediators in cell interactions are involved in cancer metastasis. Tumor exosomes are now known to influence almost every step of the signaling cascade of cancer cell metastasis and thus can be targeted as a therapeutic approach. Al-Nedawi *et al* studied the effects of intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from glioma cells with EGFR mutations[45]. Abundant expression of anti-apoptotic genes was observed in the recipient cells, together with increased non-adherent growth of cancer cells and invasion. Similarly, transfer of DKO-1 (mutant KRAS allele only) to DKs-8 (wild-type KRAS allele only) resulted in enhanced three-dimensional growth of the wild-type KRAS-expressing non-transformed cells as exosomes from mutant KRAS cells contained many tumor-promoting proteins, including KRAS, EGFR, SRC family kinases and integrins[46]. These findings indicated that exosomes may serve as an important signal transfer vehicle and a vector for the delivery of genetic information[47]. Le *et al* indicated that in murine cancer and human xenograft models, extracellular vesicles from highly metastatic breast cancer cells promoted metastasis of otherwise weakly metastatic cells either nearby or at distant

sites and induced these cells to colonize distant tissues in a miR-200-dependent manner[48]. These results demonstrated that uptake of extracellular vesicles contributed to the transfer of metastatic capability in breast cancer cells.

It has been well established that organs of future cancer metastasis are not passive receivers of circulating cancer cells. The tumor microenvironment is closely related to cancer metastasis. Before metastasis, cancer cells may modulate the microenvironment of the target organs through exosomes to facilitate the metastasis and growth of cancer cells[49]. The metastasis of cancer cells to the specific target organ is highly reliant on the expression of integrins contained in the tumor exosomes[50]. Upon entering the recipient cells, exosomes may promote conditions that are suitable for the microenvironment formation before metastasis by activating Src phosphorylation and increasing S100 expression. Zhang *et al* reported that exosomal microRNA could induce microenvironmental PTEN loss in brain tissues, which then resulted in astrocytoma metastasis[51].

Increasing evidence indicates that cancer cell-derived exosomes cause complex effects on stromal cells, vascular endothelial cells (VECs), and fibroblasts, which then promote the pathogenesis and development of cancer. Alterations in vascular permeability are indicators of microenvironmental formation before metastasis[52]. Exosomes with abundant miR-105 from highly metastatic breast cancer cells could inhibit ZO-1 protein expression in endotheliocytes, which then altered the vascular permeability and increased the susceptibility to cancer. This mechanism was attributed to the hepatic metastasis of breast cancer[53]. Pancreatic cancer exosomes containing integrin β5 and αv could be specifically recognized by Kupffer cells in the hepatic tissues. After phagocytosis of Kupffer cells, exosome-released macrophage migration inhibitory factor (MIF) upregulated the secretion of TGFβ and contributed to the generation of fibronectin by Kupffer cells in the liver. This fibrotic microenvironment could enhance the recruitment of bone marrow-derived macrophages, which finally induced the formation of the microenvironment before hepatic metastasis, as well as the possibility of liver metastasis. Otherwise, strategies that inhibit MIF expression could attenuate the microenvironment formation in the liver before metastasis and thus limit hepatic metastasis of cancer cells[54, 55].

In 2016, Greening *et al* reported that exosomes from malignant mesothelioma were involved in the reconstitution and regeneration of blood vessels by enhancing the migration of VECs and fibroblasts[56]. Similarly, Cui *et al* reported that exosomes from pulmonary adenocarcinoma may be involved in the angiogenesis of cancer cells by upregulating Ephrin α3 expression in stromal cells in a miR-210-dependent manner[57]. Moreover, proteins from tumor-derived exosomes, such as Rab3D[58], TGF-β1[59], and LMP1[60], were reported to promote epithelial-mesenchymal transition (EMT), which then increased the oncogenicity and invasion of cancer cells. In addition to the effects on proliferation of endothelial cells and mesenchymal remodeling, tumor-derived exosomes can also directly regulate the distal lymph node microenvironment, which provided suitable conditions for lymph node metastasis and melanoma growth[61]. Furthermore, exosomes from stromal cells were reported to affect the biological behaviors of cancer cells. For example, Luga *et al* revealed that upon trafficking of CD81-positive fibroblast exosomes in breast cancer cells, the fibroblast WNT-11 autocrine protein contributed to the invasion and metastasis of cancer by activating the Wnt-PCP signaling pathway[62].

Exosomes involved in the degradation of extracellular matrix (ECM)

ECM is an important tissue barrier for cancer metastasis. Alterations and remodeling of ECM affect the pace of cancer cell invasion. Exosomes from human prostate cancer have varying microRNA contents, such as miR-100-5p, miR-21-5p, and miR-139-5p, which could trigger the expression of matrix metalloproteinases (MMPs), such as MMP2, MMP9 and MMP13. These exosomes are involved in the degradation of ECM and release of growth factors, which finally trigger the invasion and metastasis of cancer cells[63]. Additionally, tumor exosomes can mediate the direct transmission of MMP13 to recipient cells, which then lead to the degradation of ECM and metastasis of cancer cells[64]. Moreover, McCready *et al* showed that invasive carcinoma exosome-released Hsp90 protein could promote cellular migration by activating fibrinogenase[65].

Potential of exosomes in cancer therapy

To date, cancer therapy has primarily relied on surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy. However, the prognosis remains poor due to the recurrence and metastasis of cancer. Based on the roles of exosomes in the invasion and metastasis of cancer cells, tumor exosomes may be promising new therapeutic candidates for the treatment of cancer and may improve the prognosis and outcomes of cancer therapy.

Tumor-derived exosomes or tumor-related exosomes are considered to be closely related to the pathogenesis and microenvironmental formation of cancer, as the number of exosomes in cancer cells is higher than that of the normal cells[66]. Therefore, inhibition of the synthesis, release and absolute circulating level of exosomes may serve as an effective treatment regimen for cancer. The formation and secretion of exosomes may be modulated through several approaches. For example, sphingomyelinase 2 could facilitate the synthesis of tumor-derived exosomes. Intravenous injection of an inhibitor of sphingomyelinase 2, GW4869, effectively inhibited the secretion of exosomes in tumor-bearing mice and decreased the metastatic rate of cancer cells[67]. Chalmin *et al* reported that 5-*N,N*-dimethylamiloride could decrease the secretion of exosomes from cancer cells by blocking the H^+/Na^+ and Na^+/Ca^{2+} channels, which subsequently delayed the growth of cancer cells[68]. In contrast, these phenomena were not observed in PC3 cells[69], which indicated that these inhibitory effects might be selective to specific cell types. A few conserved protein families are closely related to the secretion of exosomes,

among which Rab27a and Rab27b have been regarded as important mediators of these biological processes[70]. Bobrie *et al* showed that silencing of Rab27a and Rab27b by RNA interference may alter the cancer microenvironment and delay the growth and metastasis of cancer cells[71]. Nevertheless, the release of exosomes was similar in CD9- and MFGE8-positive cells, which indicates that the release of exosomes was partially independent of Rab27a. Similarly, not all regulatory factors could effectively interfere with the release of exosomes. Although the expression of integrin facilitates the migration of cancer cells to specific organs, Mason *et al* reported confounding results of a phase III trial using cilengitide, an inhibitor of integrin αvβ3 and αvβ5, for treating glioblastoma[72]. Interestingly, in a recent study, utilization of a hemofiltration system (*eg*, the Aethlon ADAPT™ system) to capture the exosomes in the circulating system contributed to the decrease of exosome secretion in the circulation, which provided an potential strategy to inhibit the growth and metastasis of cancer cells[73].

The unique lipid bilayer structure of the exosome protects it from degradation and RNase damage. Compared with conventional liposome preparations, exosomes showed comparatively lower toxicity and satisfactory tissue tolerance[74]. Moreover, the integrin expression spectrum contained in these molecules is abundant, which can mediate the migration of exosomes into target organs[49]. These advantages have attracted increasing interest in developing exosomes as an excellent vector *in vivo* for the effective delivery of agents for cancer treatment. Exosomes can be used as specific vectors for the intracellular transmission of selective DNAs, miRNAs, non-coding RNAs, and proteins[75]. For example, an exosome-based delivery platform for adriamycin[76] or paclitaxel[77] was developed for targeted cancer therapy and shown to have minimal immunogenicity and toxicity. Compared with conventional drug delivery systems, the exosome-based adriamycin delivery system showed higher drug availability with lower toxicity, especially toxicity to the heart. A previous study showed that the let-7 miRNA could inhibit the PI3K signaling pathway in cancer cells, which shares the same target PI3K signaling pathway as that for gefitinib for lung cancer[78]. The overlap of targets for let-7 miRNA and gefitinib may induce sensitization of these two agents. Given that anti-miR-135 contributed to the sensitivity of lung cancer cells to taxanes, we speculated that delivery of specific miRNAs into specific cells using exosomes, which then increase the sensitivity of cancer cells to chemotherapy, could be a promising approach[79]. According to previous results, the resistance of pulmonary adenocarcinoma to paclitaxel and cisplatin was related to the loss of PTEN on chromosome[24]. Therefore, delivery of miR-181a through exosomes into pulmonary adenocarcinoma may increase the sensitivity of the cancer cells to paclitaxel and platinum-based chemotherapy[80].

Another advantage for the use of exosomes as a therapeutic vector is their small size. Exosomes can easily pass through various biological barriers, including the blood-brain barrier[81].

Extensive studies have been carried out to investigate the roles of exosomes in immunological reactions after Zitvogel *et al* reported that exosomes are involved in immunological reactions in 1998[4]. Exosomes are now considered a new hot topic in research on vaccine development in cancer treatment. In a previous study, it was reported that after stimulation of cancer-related antigens, DCs could produce exosomes containing specific cancer antigens[82]. The tumor-derived exosomes and DC-derived exosomes could migrate into specific lymph nodes and then activate $CD4^+$ and $CD8^+$ T cells to trigger anti-tumor immunological reactions. To date, there are indeed some promising reports on the development of cancer vaccines for treating lung cancer. For example, in a clinical trial, TG4010 could extend the survival duration of patients with non-small cell lung cancer (NSCLC) at stages IIIb and IV[83]. Meanwhile, the EGF vaccination was reported to be effective for the treatment of NSCLC patients[84]. Additionally, in a recent phase II clinical trial focused on the treatment of NSCLC patients, generation of exosomes by DCs in the presence of tumor-related antigens could extend the progression-free survival of advanced cancer patients with low expression of NKp30[85].

Tumor exosomes were reported to facilitate the apoptosis of cancer cells. Cancer cells could deliver survivin into the lung cancer cells through exosomes, which then facilitated the apoptosis of recipient cells by inhibiting cell growth. Survivin-D53A, a dominant-negative mutant survivin, could promote the apoptosis of pulmonary adenocarcinoma[86]. Some components in vegetal cell-derived exosomes also showed anticancer effects. For example, the combination of curcumin and triptolide could increase the apoptosis of ovarian cancer cells[87].

Caveats and limitations of exosomes for precision therapy of various cancers

Currently, clinical application of exosomes in cancer therapy is still not possible due to the following major caveats and limitations: i) specific markers for the identification of extracellular vesicles are still lacking; ii) there is currently no standard for the isolation and purification of specific tumor exosomes[88]; iii) more effective methods are needed to obtain homogenous exosomes; iv) regulatory control of exosome contents and secretion is still poorly understood. Some exosomes could also promote growth and invasion as well as metastasis of cancer cells. For example, several studies confirmed that exosomes could induce complete metastasis of cancer cells[50, 54]. Hoshino *et al* reported that unconventional secretion of exosome vesicles from multivesicular endosomes (MVE) occurs across a broad set of systems and is increased in cancer, where it promotes aggressive behavior. Analysis of cancer cells identified specialized invasive actin structures called invadopodia as specific and critical docking and secretion sites for CD63- and Rab27a-positive MVE[50]. Inhibition of invadopodia formation reduced exosome secretion into conditioned media. Addition of purified exosomes or inhibition of exosome biogenesis or secretion significantly affected multiple invadopodia life cycle steps, including invadopodia formation, stabilization, and exocytosis of proteinases. Therefore, exosome cargoes may

play a key role in promoting invasive activity and providing in situ signaling feedback. Exosome secretion also controlled cellular invasion through a 3-dimensional matrix. The synergistic interaction between exosome secretion and invadopodia biogenesis strongly suggests that regulation of tumor exosome secretion may play a fundamental role in promoting cancer cell invasiveness[50]. Pancreatic ductal adenocarcinomas (PDACs) are highly metastatic and have a poor prognosis due to delayed detection. PDAC-derived exosomes were reported to induce liver pre-metastatic niche formation in naive mice and consequently increase liver metastatic burden. Uptake of PDAC-derived exosomes by Kupffer cells caused TGFβ secretion and upregulated fibronectin production by hepatic stellate cells. This fibrotic microenvironment enhanced recruitment of bone marrow-derived macrophages. MIF was substantially higher in PDAC-derived exosomes from stage I PDAC patients who later developed liver metastasis than exosomes from patients whose pancreatic tumors did not progress. When MIF expression in the PDAC-derived exosomes was blocked, liver pre-metastatic niche formation and metastasis were also prevented. These findings suggest that MIF in tumor exosomes may prime the liver for metastasis and may be a prognostic marker for the development of PDAC liver metastasis[54]. Leal *et al* reported that tumor-derived exosomes and neutrophils may act cooperatively in cancer-associated thrombosis because the thrombosis in the cancer-bearing mice was faster than that of the normal controls[89].

Understanding the underlying and complex mechanisms of exosome-mediated immunosuppression is important for preventing the immune escape of tumor cells and for identifying novel treatments for cancer. DCs and lymphocytes in the tumor microenvironment may modulate immune responses; however, tumor-derived exosomes may mediate the death receptor pathway to induce $CD8^+$ T lymphocyte apoptosis. In addition, these molecules could mediate T lymphocyte imbalance by regulating the proliferation of T lymphocytes and inhibiting the proliferation of effector T lymphocytes, which then inhibited the immune function of the tumor microenvironment[90, 91]. As a means of cellular communication, tumor-derived exosomes can inhibit the immune function of the receptor cells or trigger the distribution of immunologic receptors and ligands of the receptor cells with a similar pattern to that of oncocytes. Thereafter, they may interfere with immune therapies via sequestration of therapeutic antibodies or elimination of vaccine-induced or adoptively transferred immune effector cells[92]. In a recent study, tumor-derived exosomes were shown to activate the transfer of epidermal growth factor receptor to macrophages in the hosts, inhibit innate immunity and induce immunocompromise[93].

Therefore, in contrast to exosomes from other sources used to deliver therapeutic agents, tumor exosomes may be a double-edged sword when used as a therapeutic tool for cancer treatment. Full elucidation of the formation, secretion, and networking function of tumor exosomes is urgently needed for the realization of this attractive and promising strategy for cancer therapy. More extensive clinical or animal studies with large sample sizes are needed in the future to investigate the features and safety of tumor exosomes serving as either a target or a vector for genes and/or drugs.

Conclusion and perspectives

In the future, we will need to broaden our understanding of the biological features and biochemical characteristics as well as the functional roles of tumor exosomes in the pathogenesis and development of cancer. Only when the specificity, efficacy, functionality, toxicity, and safety of exosomes have been fully elucidated can tumor exosomes serve as promising candidates for anti-cancer therapy and be developed as new strategies for precision therapy of various cancers.

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