Exosomes: potent regulators of tumor malignancy and potential bio-tools in clinical application

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Abstract

Multiple lines of evidence indicate that exosomes, as efficient messengers in cell-to-cell communication, play pleiotropic roles in regulating tumor malignancy. The cargos (proteins, mRNAs, and miRNAs) carried by exosomes can be functionally delivered between different types of cells and even transferred to distant locations, influencing the biological activities of tumor and non-tumor cells and promoting tumor growth, invasion, metastasis, angiogenesis, and drug resistance. Tumor-associated exosomes have been identified in biological (plasma, urine, saliva) and pathological (malignant effusions, pleural effusions, ascites) fluids from cancer patients. The contents of exosomes may vary depending on tumor types and status. Detection of exosomes in biofluids of cancer patients may represent a promising strategy to gain pathogenic information and to select specific biomarkers for the diagnosis and prognosis of cancer. Utilization of exosomes as delivery vehicles for siRNAs and therapeutic drugs brings out new concepts such as biomimetics in cancer treatment. In this review, we will mainly discuss emerging roles of exosomes in tumor invasion, metastasis, angiogenesis, and drug resistance and potential clinical application of exosomes as biomarkers and therapeutic tools.

Key words: exosomes; angiogenesis; metastasis; drug resistance; biomarkers; delivery vehicles

1. Introduction

Tumor formation and metastasis are regulated by multiple molecular mechanisms. Interplay of tumor cells with stromal cells in tumor surroundings and distant organs critically influences tumor progression. Tumor and stromal cells communicate with each other in two canonical ways: the direct cell-to-cell contact that activates signaling pathways (such as MEK/ERK, PI3K/Akt, Notch, Wnt/ β -catenin) and the indirect “talk” mediated by soluble factors, including cytokines (such as IL-6, TNF- α , IL-8, TGF- β , VEGF), chemokines (CCL-18, CCL-12), and MMPs, which are involved in the establishment of tumor microenvironment favoring tumor growth, invasion, and metastasis. However, how primary tumors communicate with distant organs during the process of metastasis is not fully understood.

Exosomes, nano-sized microvesicles (30-100 nm in diameter), are formed by inward budding of late endosomal membrane and segregating the cargos (lipids, proteins, and nucleic acids) within the membrane-covered vesicles. After fusion with the plasma membrane, exosomes are secreted into extracellular space and migrate far away from initial positions and even across physiological barriers, such as blood-brain barrier.

Mounting evidence indicates that exosomes play pleiotropic roles in regulating tumor progression, invasion, neovascularization, and metastasis by transferring functional effectors to various types of cells within tumor microenvironment and even to distant locations. Tumor cells actively shed exosomes into their surroundings. These extracellular microvesicles act as efficient messengers in the intercellular communication between tumor and stromal cells (such as fibroblasts, inflammatory cells, and endothelial cells), promoting inflammatory cell recruitment, angiogenesis, and extracellular matrix (ECM) degradation. Malignant information carried by

tumor-derived exosomes can be conveyed to other tumor cells. Drug-resistant tumor cells may spread resistance capacity to sensitive ones by releasing exosomes. Tumor-derived exosomes can travel to and modulate lymph nodes and metastatic target organs, which is critical for the establishment of pre-metastatic niches facilitating tumor cell adherence and growth. Stromal cells also produce exosomes that contribute to tumor cell migration, invasion, and dissemination.

Exosomes contain distinct subsets of proteins and miRNAs, which are either known or novel disease-specific biomarkers. Detection of the exosomes in biofluids or analysis of the protein and miRNA expression profiles in exosomes may provide useful information for tumor diagnosis and prognosis. The finding that the cargos shuttled by exosomes are functional has become a major breakthrough in this field [1]. Multiple molecules are supposed to be selectively assembled into the exosomes and delivered to target cells, thereby influencing the biological activities of target cells. Attempts are being made to create functional exosome mimetics for delivery of pharmaceutical proteins and nucleic acids in cancer treatments.

In this review, we will focus on the recent information about emerging roles of exosomes in tumor invasion, metastasis, angiogenesis, and drug-resistance and potential clinical application of exosomes as biomarkers and therapeutic tools.

2. Contents of tumor-derived exosomes

Under both physiological and pathological conditions, exosomes are released by a variety of cells into nearly all biological fluids. Tumor-associated exosomes have been identified in the plasma, urine, pleural effusions, and ascites of patients with carcinomas of lung, prostate, ovarian, bladder, and glioblastoma. Exosomes share certain common characteristics and express distinct protein markers, such as

endosomal sorting complex required for transport (ESCRT) components (Alix, HRS, Tsg101), tetraspanins (CD63, CD81, CD82, CD9), and GTPase family proteins (Rab27, Rab11, Rab35). Exosomes also contain cytoplasmic proteins (tubulin, actin, actin-binding proteins, annexins), signal transduction proteins (protein kinases, heterotrimeric G-proteins), MHC-I and -II molecules, and heat-shock proteins (HSP70, HSP90). Additionally, the molecular characterization of exosomes reveals the presence of miRNAs, which exert critical roles in various processes by regulating target genes. The majority of circulating miRNAs is concentrated in exosomes. A general database of exosomal proteins and RNAs called Exocarta can be found at www.exocarta.org and the numbers of candidates in this database are continuously increasing [2].

2.1. Proteins carried by exosomes

Functionality of exosomes under physiological and pathological conditions relies mostly on what they carry. The protein molecules associated with all biological activities of exosomes, including biogenesis, secretion, and cell-to-cell communication, may be approximately classified into two groups: commonly shared and unique proteins. The commonly shared proteins are an evolutionary-conserved set of proteins, which usually participate in the biological processes shared by the majority of exosomes from various origins, such as components of ESCRT for exosome biogenesis, GTPase family proteins for intracellular trafficking and fusion, and tetraspanins for protein sorting and target cell selection. Unique proteins are frequently expressed in the exosomes from certain types and states of tumors. The tumor-associated exosomal proteins play important roles in regulating tumor

proliferation, metastasis, and progression and may have potential diagnostic and prognostic values.

Ras-associated binding (Rab) GTPases, members of Ras superfamily of small GTPases, are important membrane trafficking organizers and exert crucial roles in intracellular trafficking, fusion, and secretion of exosomes. Rab11, which is associated with post-Golgi membrane, is involved in regulating multivesicular body (MVB) docking and fusion in a calcium-dependent way in K562 erythroleukemia cells [3, 4]. Rab27a and Rab27b participate in the regulation of membrane trafficking and exosome secretion pathway in a variety of cells [5]. The size of MVBs was enlarged upon Rab27a silencing. When Rab27b was knocked down, MVBs accumulated around perinuclear region [5]. It has been suggested that the Rab27 subfamily regulates various exocytotic pathways by using multiple organelle-specific effector proteins [6]. Rab35, which localizes to plasma membrane and internal vesicles and tubules, is known to regulate endosome secretion, MVB docking/tethering to the plasma membrane, and cargo recycling. Activation of Rab35 by TBC1 domain family member 10A-C, which functioned as a GTPase-activating protein of Rab35, was required in regulating exosome secretion. The inhibition of Rab35 function caused intracellular accumulation of endosomal vesicles and impaired exosome secretion in oligodendroglia [7]. Patrick et al. found that the DENN (differentially expressed in neoplastic versus normal cells) domain-containing protein connecdenn, a component of clathrin-coated vesicle, had intrinsic guanine nucleotide exchange factor activity specific for Rab35 and was a key regulator of Rab35 activity through its DENN domain. Loss of Rab35 activity caused an enlargement of early endosomes and inhibits cargo-selective recycling pathways [8].

The exosomal Rabs also play indispensable roles in tumor progression. It was

reported that Rab11 promoted the invasive capability of human breast cancer cell line MDA-231 by mediating the trafficking the integrin $\alpha 6\beta 4$ [9]. Rab27 GTPases and their effector proteins are suggested to be critical determinants for invasive growth and metastasis of cancer cells. Suppression of exosome secretion by stably knockdown of Rab27a reduced primary tumor growth and lung metastasis [10]. Overexpression of Rab27b enhanced the invasive capacities and stimulated metabolic reprogramming from oxidative phosphorylation towards aerobic glycolysis in breast cancer cells [11]. Clinical studies indicated that the elevated expression of Rab27b was correlated with lymph node metastasis and the secretory Rab27b was an independent risk factor for survival of breast cancer patients [12].

Tetraspanins, generally characterized as exosomal markers, are a large superfamily of four-transmembrane proteins, including CD63, CD9, CD81, CD82, and tetraspanin-1, -6, -8, -14, -15. They are enriched in membrane microdomains and interact with integrins, participating in a variety of cellular processes. However, up to now, most functions of tetraspanins in exosomes are unclear. Several studies have suggested that the protein assembly of exosomes and the selective recruitment of cargo are regulated to a large extent by tetraspanins and the exosomal tetraspanin web also contributes to target cell selection. Sorting of MHC class II molecules into dendritic cell-derived exosomes was dependent on its incorporation into CD9-containing lipid membranes [13]. Tetraspanins also exhibit diverse properties indicating their physiological importance in target cell adhesion, motility, and activation. For example, Tetraspanin-8 was associated with selective recruitment of CD106 and CD49d into exosomes, contributing to the exosome-endothelial cell adhesion. Uptake of Tspan8-49d complex-containing exosomes by endothelial cells was accompanied by enhanced endothelial cell proliferation, migration, sprouting, and

maturation of endothelial cell progenitors [14]. CD82 and CD9 synergistically reduced cellular pool of β -catenin via exosome export. The exosomal discharge of β -catenin may modulate the activity of Wnt/ β -catenin pathway [15].

The findings in the recent studies implicate that exosomal tetraspanins are involved in tumor growth, migration, and progression. For example, the level of CD63 was high in the exosomes derived from the plasma of melanoma patients [16]. CD151 and Tspan8 are considered as metastasis-promoting tetraspanins. Knockdown of CD151 or Tspan8 reduced the metastatic potential of rat pancreatic adenocarcinoma cells. However, the knockdown cells regained metastatic capacity when rats were pretreated with the wild-type exosomes, which could influence host matrix remodeling through exosomal tetraspanin-integrin and tetraspanin-protease associations, stimulate stromal cell activation by upregulation of cytokines, cytokine receptors, and proteases, and promote EMT gene expression in rat pancreatic adenocarcinoma cells [17]. Fibroblast-derived CD81-deficient exosomes also reduced breast cancer metastasis [18]. Exosomal Tspan8 was proposed as a biomarker for migrating pancreatic cancer initiating cells [19]. However, the signaling that specifically controls exosome biogenesis and the underlying mechanisms, by which these commonly shared proteins regulate tumor progression, remain elusive.

Unlike the commonly shared proteins, the unique exosomal markers are not always present in all the exosomes. Molecules packaged in exosomes vary depending on different tumor types and their activation status. During the last few years remarkable efforts have been made to identify the protein profiles of the exosomes derived from various cancers, including bladder, prostate, kidney, breast, ovarian, head and neck, and brain cancers by proteomic analysis. So far the majority of proteomic analysis has focused on the exosomes derived from cancer cell lines. However, analysis of the

exosomes derived from cancer patients are more valuable and the exosomal profiles may mostly reflect the basic characteristics of patient status, response to therapy, and tumor progression. Thus we will mainly discuss exosomal proteins identified from clinical materials.

It has been reported that the concentration of exosomes in peripheral venous blood is frequently higher in the patients with metastatic uveal melanoma and colorectal cancer than in healthy controls [20, 21]. High levels of exosomes were correlated with poorly differentiated tumors and shorter overall survival [21]. The exosomes in the serum of ovarian cancer patients contained high level of CD24 and EpCAM [22]. CD24 and EpCAM were also found in the exosomes from ovarian carcinoma cell lines, suggesting their importance in ovarian cancer [23]. In another study, claudin-4 was detected in the exosomes from the plasma of ovarian cancer patients. The ovarian tumors with high level of CA125, the most clinically useful ovarian cancer biomarker, tended to express high levels of claudin-4, implicating the potential value of the exosomal claudin-4 in the diagnosis of ovarian cancer [24]. The exosomes collected from the sera of patients with glioblastoma multiforme (GBM) expressed TGF- β and brain tumor-specific oncogenic antigen epidermal growth factor receptor (EGFR) ν III, both of which were undetectable in the exosomes from normal sera [25]. It was also found that circulating exosomes of melanoma patients expressed high levels of melanoma-specific tyrosinase-related protein-2 (TYRP2) and MET oncoprotein, which played key roles in tumorigenesis and chemoresistance [26]. A series of hypoxia-regulated proteins, such as MMP-9, caveolin-1, pentraxin 3, IL-8, platelet-derived growth factor AA/AB, CD26, and plasminogen activator inhibitor 1, were increased in the exosomes from the plasma of GBM patients and these exosomes were able to stimulate proliferation and survival of endothelial cells, reflecting the

hypoxic status of tumors and hypoxia-induced angiogenesis [27].

Exosomes can be readily isolated from other physiological and pathological fluids, such as urine, ascites, and pleural effusions. As delivery vehicles to remove unwanted proteins, exosomes are the predominant form of microvesicles in urine. Prostate cancer patients had higher levels of urinary exosomes than healthy men. In the exosomes isolated from the urine of prostate cancer patients two known prostate cancer biomarkers PCA-3 and TMPRSS2-ERG were detectable [28]. Prostate specific membrane antigens PSA and PSMA also existed in urinary exosomes from prostate cancer patients but not healthy men [29]. Proteomic analysis of the urinary exosomes from patients with bladder cancers showed that eight proteins, most of which participate in key signaling transduction mechanisms regulating cell growth, differentiation, and survival, were overexpressed. Among them, five proteins are linked to the EGFR pathway, including NRas (its mutations have been found in urothelial bladder cancer), EPS8L1, EPS8L2 (substrates for EGFR), Mucin 4 (a ligand of Her2), and EH domain-containing protein 4 (EHD4) [30]. In addition, the tumor-derived exosomes could be detected in isolated hepatic perfusion of uveal melanoma patients with liver metastasis [20].

2.2. Exosomal miRNAs

Noncoding regulatory miRNAs, which control gene expression by inducing mRNA degradation and inhibiting translation, play an important role in the regulation of cell differentiation, proliferation, and survival [31-33]. MiRNAs can be actively secreted and delivered to other cells via exosomes, which protect them from degradation. It has been demonstrated that exosomal miRNAs are transferred into subcellular sites in recipient cells and induce a miRNA-mediated repression of target genes, indicating

that exogenous miRNAs can function effectively in recipient cells at new locations [1, 34, 35]. The presence of miRNAs in exosomes has been reported in the plasma and urine samples of cancer patients. MiRNA-carrying exosomes were also isolated and characterized from saliva [36].

It was found that the level of miR-195 was increased in the plasma exosomes from breast cancer patients and decreased after tumor resection [37]. The level of circulating exosomal miR-21 was significantly higher in the patients with esophageal squamous cancer than benign diseases and positively correlated with the progression of esophageal squamous cancer [38, 39]. In ovarian cancer patients the exosomal miR-200c and miR-214 (previously demonstrated as diagnostic) were high and their levels appeared to be correlated with the stages of ovarian cancer [40]. In the patients with hepatocellular carcinoma, decreased level of serum exosomal miR-718 was related to large tumor diameter and tumor recurrence after liver transplantation [41].

Tumor-derived exosomes frequently express an array of miRNAs. In many patients with different types of cancers, such as prostate, ovarian, and lung cancers, a comprehensive set of exosomal miRNAs, rather than a single miRNA, was altered. It was reported that the profiles of exosomal miRNAs in ovarian cancer patients were significantly distinct from those observed in benign diseases [40]. In the serum exosomes derived from the patients with pancreatic cancer a panel of miRNAs, including miR-1246, miR-4644, miR-3976, and miR-4306, were found to be markedly increased [42]. Comparison of the exosomal miRNA profiles revealed a strong similarity among the patients with metastatic uveal melanoma and three clusters of miRNAs were expressed in the exosomes from liver perfusates of the patients with liver metastasis [20]. Microarray analysis showed that the serum levels of seven exosomal miRNAs (let-7a, miR-1229, miR-1246, miR-150, miR-21,

miR-223, and miR-23a) were significantly elevated in primary colorectal cancer patients, even at an early stage, but reduced after surgical resection of tumors, implicating that the exosomal miRNA signatures may be promising biomarkers for colorectal cancer [43].

It was reported that the levels of exosomal miRNAs in tumors exhibited a strong correlation with those in peripheral blood of human ovarian cancer, implicating that the signatures of circulating exosomal miRNAs may reflect those of the originating tumor cells and could potentially be used as surrogate diagnostic markers for biopsy profiling [40]. However, there are contradictory reports regarding whether the miRNA profiles in peripheral blood are parallel with the profiles in tumors. A microarray analysis of mRNA populations revealed a poor overall correlation between the mRNAs in glioblastoma cells and the mRNAs in the microvesicles isolated from the cells [44]. Another study demonstrated that mesenchymal stem cell (MSC)-derived exosomes exhibited different protein and RNA profiles compared to their donor cells [45], suggesting that the exosomal RNAs are not simply copies of cellular RNAs and also supporting that a selective enrichment mechanism of RNAs exists in exosomes. Several recent studies indicated that tumor cells may selectively discard miRNAs via exosomes, such as tumor-suppressor miRNAs that restrict metastatic progression. Characterization of miRNA profiles showed that the tumor suppressor miRNA let-7 family was abundant in the intracellular and extracellular fractions in a metastatic gastric cancer cell line. Selective enrichment and secretion of let-7 miRNA family via exosomal transport may reflect an oncogenic mechanism by which aggressive cancer cells maintain tumorigenic and metastatic properties [46]. In metastatic bladder cancer cells, the release of tumor-suppressor miRNAs (miR-23b, miR-224, and miR-921) was increased and silencing of Rab27a or Rab27b halted the secretion of miR23b and

miR-921. The exosome-mediated secretion of tumor-suppressor miRNAs may facilitate activation of a metastasis cascade [47]. Nevertheless, the mRNA and miRNA profiles of exosomes provide valuable information of the donor cells and exosomal cargos have been suggested as potential diagnostic biomarkers and prognostic indicators for cancers [48, 49].

In the future, prospective cohort studies on the molecular signatures of the exosomes derived from the samples of cancer patients are needed to identify and validate specific exosomal molecules that associated with metastasis, progression, and drug resistance (Table. 1).

3. Microvesicles with macro-roles in tumor malignancy

Tumors are composed of multiple cell types and extracellular matrix (ECM). Tumor cell proliferation, survival, migration, invasion, and metastasis are maintained through intercellular communication. Exosomes, as effective information deliverers, regulate intercellular communication, which triggers intracellular signal-transducing mechanisms, and affect tumor development and progression (Fig. 1).

3.1. Tumor-derived exosomes promote angiogenesis

Tumors require a blood supply for their massive expansion and neoangiogenesis is a crucial step for cancer growth and dissemination. Tumor-associated fibroblasts and myofibroblasts are considered as rate-limiting determinants for tumor angiogenesis and progression. Tumor-derived exosomes could induce fibroblasts and adipose tissue-derived MSCs to differentiate into myofibroblast cells, which are transiently present in normal wound healing process but remain persistently in the stromal

compartment of tumors, influencing tumor angiogenesis [50-53]. In addition, tumor-derived exosomes expressed a high level of TGF- β , which is capable of driving the differentiation of fibroblasts into myofibroblasts. TGF- β -containing exosomes also induced fibroblasts to produce fibroblast growth factor 2 (FGF2) that mediates tumor vascularization [50].

Hypoxia, which has profound consequences for malignant progression, is a specific feature of malignant tumor microenvironment. Reduction in tissue oxygen tension often leads to neovascularization. Hypoxia could induce the secretion of angiogenic factors by the exosomes in tumor cells, promoting tumor angiogenesis and metastasis. The exosomes secreted by human leukemia cell line K562 exposed to hypoxia promoted tube formation by human umbilical vein endothelial cells (HUVECs) [54]. Under hypoxic conditions, miR-210 was highly expressed in the exosomes from metastatic cancer cells and the exosomal miR-210 enhanced angiogenesis of endothelial cells [54, 55]. Studies by using the materials of patients with glioblastoma multiforme (GBM) revealed an enrichment of hypoxia-regulated mRNAs and proteins in the exosomes. GBM cell-derived exosomes were able to activate VEGF receptor 2, which elicits an angiogenic response, stimulate endothelial cell proliferation and survival, and induce microvascular sprouting [27]. The functional roles of the exosomes in hypoxia-dependent cross-talk between malignant and stromal cells were also demonstrated by experimental studies [27].

Tumor exosomes may modulate the process of neovascularization by directly affecting proliferation of endothelial cells and stimulating angiogenic activities of endothelial cells. It was reported that the exosomal Tspan8-CD49d complex could be internalized into endothelial cells. The uptake of exosomes by endothelial cells induced VEGF-independent regulation of a series of angiogenesis-related genes,

accompanied by enhanced endothelial cell proliferation, migration, sprouting, and endothelial progenitor cell maturation [14]. The experimental study demonstrated that the exosomes from K562 cells were internalized by HUVECs during tubular differentiation on Matrigel and angiogenesis were induced by K562 cell-derived exosomes. Addition of the exosomes generated from LAMA84 chronic myelogenous leukemia (CML) lines to HUVECs induced the expression of intercellular cell adhesion molecule-1, vascular cell adhesion molecule 1, and interleukin-8. The treatment with the exosomes from CML cells enhanced endothelial cell motility and stimulated reorganization into tubes of HUVECs cultured on Matrigel [56, 57].

The recent study showed that the exosomes from human CD34-positive stem cells mediated proangiogenic paracrine effects. The CD34-positive exosomes, which contained high levels of pro-angiogenic miRNAs (miR-126 and miR-130a), mediated the formation of vessel-like endothelial structures of HUVECs *in vitro* by enhancing HUVEC viability and proliferation and stimulated angiogenesis *in vivo* [58]. In another study, the tumor-initiating cells expressing MSC marker CD105 in human renal cell carcinoma produced the microvesicles expressing a set of pro-angiogenic mRNAs, such as VEGF, FGF2, MMP-2, and MMP-9. The CD105 positive exosomes conferred an activated angiogenic phenotype to normal human endothelial cells and stimulated vessel formation in SCID mice [59].

3.2. Exosomes *regulate pro-metastasis niche formation and ECM degradation*

It has been demonstrated that tumors actively shed exosomes into extracellular milieu and these exosomes travel to surrounding cells and distant sites, including bone marrow, lungs, lymph nodes, and other organs, where they participate in the establishment of pro-metastasis niche and degradation of ECM [26, 60-62].

Primary acute myelogenous leukemia (AML) and AML cell lines released the exosomes, in which diverse RNA species including mRNAs and miRNAs relevant to AML pathogenesis were enriched. The AML exosomes transferred leukemia-associated mRNAs and miRNAs to bone marrow stromal cells and altered proliferative, angiogenic, and migratory responses of stromal and hematopoietic progenitor cells. It was shown that insulin-like growth factor-I receptor carried by the exosomes was transferred into bone marrow stromal cells upon internalization and induced insulin-like growth factor-I receptor signaling in bone marrow stromal cells [63]. Another study demonstrated that the exosomes released from CML cells stimulated bone marrow stromal cells to produce proinflammatory chemokine IL-8, which, in turn, promoted CML cell survival, adhesion to stroma, and migration [64]. In orthotopic nude mouse models, it was demonstrated that the exosomes from breast cancer cells were released into circulating blood and lung and internalized by pulmonary stromal cells. Trafficking of proteins, mRNAs, and miRNAs from tumor cells to stromal cells may contribute to the establishment pre-metastatic niche [62]. In addition, the exosomes mediated remodeling of lymph nodes, thereby facilitating lymphatic metastasis. Homing of melanoma exosomes to sentinel lymph nodes may impose molecular signals for cancer cell recruitment, ECM deposition, and vascularization [61]. The exosomes derived from highly metastatic melanomas were able to induce vascular leakiness at pre-metastatic sites, reprogram bone marrow progenitors, and promote the metastatic behavior of tumor cells by educating bone marrow progenitors through transferring oncoprotein receptor tyrosine kinase MET [26].

ECM remodeling is essential for tumor cell invasion and metastasis. Focal degradation of ECM is the first step in the invasion of cancer cells. Tumor exosomes

play an active role in remodeling of ECM by degrading matrix protein and influencing intercellular cross-talk between tumor and stromal cells. The exosomes derived from a variety of tumors, including breast cancer, renal cancer, fibrosarcoma, and melanoma, contained high level of MMPs or MMP activators. The amount of MMPs in the exosomes from highly metastatic tumor cells were higher than non-metastatic tumor cells [65]. Experimental studies demonstrated that the exosomes derived from rat highly metastatic pancreatic carcinoma were rich in proteases uPAR, MMPs, ADAMs, and HAdase. The exosomal MMPs were functionally active in degrading the components of ECM and the matrix protein type I and IV collagens, laminins, and fibronectin could be efficiently degraded by the exosomes. Tumor-derived exosomes also expressed high levels of adhesion molecules and selectively bound to the matrix proteins [66]. Exosomal CD44 and $\alpha 6\beta 4$ directed selective targeting their ligands hyaluronic acid and laminin, inducing degradation of ECM components by functional proteases enriched in exosomes and promoting proliferation of stromal cells [66]. Besides directly inducing ECM remodeling through their cargoes, exosomes modulate the activities of recipient cells, indirectly promoting ECM degradation. The exosomes from gastrointestinal stromal tumors contained active oncogenic protein tyrosine kinase KIT. The gastrointestinal stromal tumors-derived exosomal KIT functionally activated its downstream mediators ERK1/2 and AKT in myometrial smooth muscle cells and triggered the phenotypic conversion of progenitor smooth muscle cells to tumor-promoting cells, resulting in enhanced adhesion of the cells to ECM proteins, release of MMPs, and degradation of type 1 collagen in matrix [67].

Metastatic tumor cells can form protruding adhesive structures with ECM proteolytic activity called invadopodia, which adhere to ECM and dissolve collagens,

laminins, and fibronectin. Invadopodia were key docking sites for exosome-containing multivesicular endosomes [68]. Rab27a has been identified as a critical docking factor for multivesicular endosomes [5]. It was shown that exosomes dynamically localized to invadopodia in head and neck squamous cell carcinoma cells and controlled invadopodia biogenesis and proteolytic invasion via delivery of membrane-type matrix metalloproteinase-1 and other proteinases into invadopodia. Reduction of exosome secretion by specific shRNAs targeting Rab27a in tumor cells impaired the formation and activities of invadopodia in ECM degradation. On the other hand, exosome secretion was enhanced by invadopodia via providing exosome-targeting sites, suggesting a positive feedback loop between exosome secretion and invadopodia formation [68].

Both pre-metastatic niche formation and ECM degradation facilitate tumor cell migration and metastasis. More recently, an interesting discovery of the vesicular structure called “migrasome”, observed by using transmission electron microscopy and confocal microscopy, may shed a new light on the exosome-associated mechanisms for tumor metastasis. Migrasomes were released during cellular migration by breaking the retraction fibers from cells and the contents in the migrasomes could be taken up by surrounding cells. It was suggested that some of the exosomes may actually be released by migrasomes [69]. It is imaginable that the migrasome-mediated intercellular communication may be an important mechanism for tumor cell invasion, migration, and metastasis.

3.3. Stroma cell-derived exosomes promote tumor metastasis and progression

Tumor-associated stroma cells (such as fibroblasts and inflammatory cells) are able to produce exosomes, which shuttle proteins or miRNAs into adjacent tumor cells. It

was shown that the exosomes produced by tumor-associated fibroblasts were taken up by breast cancer cells and stimulated the invasiveness and metastasis of breast cancer cells. Co-injection of breast cancer cells with fibroblasts into mice enhanced metastasis, during which Wnt-planar cell polarity signaling in breast cancer cells and the exosomal CD81 in fibroblasts were considered to be critical, implicating an intercellular communication mediated by the fibroblast-derived exosomes stimulate breast cancer invasion and metastasis [18] It was also reported that the exosomes from macrophages may induce invasive behavior of tumor cells by shuttling functional miRNAs to tumor cells. MiR-223-containing exosomes released by IL-4-activated macrophages could be internalized by co-cultivated SKBR3 and MDA-MB-231 breast cancer cells and the exosome-shuttled miR-223 promoted the invasiveness of breast cancer cells *in vitro* [70].

MSCs are pluripotent progenitor cells and function as stromal cells in tumor tissues. It was found that the exosomes isolated from primary bone marrow MSC of patients with multiple myeloma (MM) contained lower content of the tumor suppressor miR-15a and higher levels of oncogenic proteins, cytokines, and protein kinases compared with the exosomes from normal bone marrow MSCs. These exosomes could be transferred to MM cells, induce tumor growth, and promote dissemination of tumor cells to the bone marrow in an animal model of MM. In contrast, the exosomes derived from normal bone marrow MSCs inhibited the growth of tumor cells [71]. Another study showed that MSC-derived exosomes enhanced the VEGF expression in tumor cells through activating ERK1/2 pathway. Co-injection of MSC-derived exosomes with gastric cancer cells into mice subcutaneously promoted tumor growth and increased tumor blood supply as well [72]. However, there are controversial reports concerning the roles of MSCs in tumor progression. Lee et al.

reported that the exosomes released by MSCs significantly downregulated the expression of VEGF by shuttling exosomal miR-16 into breast cancer cells, leading to suppression of angiogenesis both *in vitro* and *in vivo* [45]. The intercellular interactions mediated by MSC-derived exosomes and consequent impact on tumor metastasis and progression remain to be further explored.

3.4 Exosomes are involved in inducing drug resistance of tumor cells

Recent studies indicate that exosomes impair the sensitivity of tumor cells to chemotherapeutics by exchanging drug-resistant information from resistant cells to sensitive cells. Experimental studies demonstrated that treatment of A549 lung cancer cells with cisplatin induced the secretion of exosomes [73] and that platinum resistant ovarian cancer cells were found to release more exosomes than drug-sensitive cells [74], implicating that release of exosomes may be a mechanism of drug-resistance.

It was observed that a series of cellular miRNAs (miR-196a, miR-21, miR-222, miR-29a, miR-100, and miR-30a) implicated in therapy resistance pathways were abundant in the exosomes from docetaxel-resistant MCF-7 cells. Addition of the exosomes into drug-sensitive tumor cells significantly increased resistance of the recipient cells to docetaxel [75, 76]. In another study, it was found that the exosomes from drug resistant MCF-7 cells carried high level of P-glycoprotein (P-gP), which mediates drug-resistance in a wide variety of tumors, and induced drug resistance in drug-sensitive MCF-7 cells [77]. In prostate cancer cells, increased resistance was attributed to exosomal transfer of MDR-1/P-glycoprotein [78].

Stromal cells drove therapy resistance pathways by orchestrating the crosstalk between stromal and cancer cells via exosomes. Bone marrow stromal cell-derived exosomes could reduce the sensitivity of MM cells to Btz, a proteasome inhibitor, by

increasing MM viability [79]. Another study showed that the exosomes released from stromal cells instigated the pattern recognition receptor RIG-I that activated Stat1-dependent antiviral signaling and induced Notch3 activation in breast cancer cells. The paracrine antiviral and juxtacrine Notch3 pathways contributed to the expansion of therapy-resistant tumor-initiating cells [80].

Dormancy is a mechanism for tumor cell escaping from drug attack and dormant tumor cells are often unresponsive to traditional chemotherapies. Bone marrow MSC-derived exosomes induced dormancy by delivering miR-23b into breast cancer cells, resulting in downregulation of the expression of MARCKS that promotes cell cycling and motility [81].

Exosomes may influence the efficacy of chemotherapeutics either by directly binding to drugs or by exporting drugs from tumor cells, protecting cancer cells from being attacked. It was found that the exosomes purified from the supernatants of melanoma cells contained cisplatin in its native form. The experimental study using human tumor xenografts demonstrated that tumor cells actively eliminated cisplatin via exosomes in a pH-dependent manner. Treatment with anti-acidic drug reduced the plasmatic levels of tumor-derived exosomes and increased cellular uptake of cisplatin, suggesting a mechanism by which human melanoma eliminates anti-cancer drugs via exosomes [82].

Rituximab, a chimeric monoclonal antibody against CD20, is used to treat hematological cancers. By its Fc portion rituximab mediates antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity after ligation to CD20 on the surface of malignant cells. Lymphoma cell-derived exosomes contained abundant CD20, which may competitively bind to rituximab, consume complements, and thus protect tumor cells from rituximab-induced complement-dependent

cytotoxicity [83]. ATP-binding cassette (ABC) transporter A3 (ABCA3), an intracellular ABC transport protein with multidrug resistance, is localized within the limiting membranes of lysosomes and multivesicular bodies. The intracellular ABCA3 was found to mediate subcellular drug sequestration and the level of ABCA3 was associated with unfavorable treatment outcome in AML [84]. Pharmacological blockade and silencing of ABCA3 enhanced the sensitivity of lymphoma cells to rituximab, indicating that the ABCA3-dependent pathway of exosome secretion regulates resistance of cancer cells to either chemotherapeutics or therapeutic antibody rituximab [83]. A similar mechanism was observed in breast cancer cells overexpressing HER2. The exosomes released by the HER2-overexpressing SKBR3 and BT474 cells contained an activated full-length HER2 molecule. The HER2-positive exosomes inhibited the antiproliferation activity of therapeutic antibody trastuzumab targeting HER2, implicating a potential role of exosomes in modulating sensitivity of breast cancer cells to trastuzumab [85].

4. Perspectives of exosomes in clinical application

Tumor exosomal protein and miRNA expression profiles and their changes have been considered as indicative of tumor progression and metastasis. Human body fluids, in which exosomes are abundant, have been conventionally used as the main source of biomarkers for cancer diagnosis and prognosis. The isolation of exosomes from patient samples is relatively easy and minimally non-invasive, indicating the feasibility to use exosomes as biomarkers for disease diagnostic purposes [40, 44, 86]. However, how to screen out real tumor biomarkers in exosomes remains an important task. Many exosomal proteins or miRNAs proposed as biomarkers in cancer cell lines should be validated in the materials from cancer patients. More studies are needed to

further characterize exosomal proteins and miRNAs and to assess their value in cancer diagnosis and treatment. Further unraveling the role of exosomes under physiological and pathological conditions will assist in biomarker discovery and exploit the application of scientific approaches in clinical diagnosis. In addition, the currently used protocols for exosome isolation are extensive and time-consuming. Faster and more convenient methods need to be established.

MiRNAs and siRNAs have been proposed to be potent candidates in cancer therapy, but their clinical application is hindered by the lack of ideal delivery systems. The evidence that miRNAs can be functionally delivered to target cells via exosomes is shedding new light on targeted gene silencing in cancer [87]. Exosomes are not only bioavailable but also resistant to metabolic processes. Exosomes carrying let-7a significantly suppressed tumor growth in mice [88]. Curcumin incorporated into exosomes were more stable and more concentrated in blood [89]. Intravenously injection of engineered exosomes loaded with doxorubicin, a common chemotherapy drug in cancer treatment, resulted in inhibition of tumor growth *in vivo* [90]. The membrane-permeability of exosomes makes them possible to deliver small molecules. SiRNAs or drugs delivered by exosomes could be successfully transported across blood-brain barrier in mice [91-93]. The utilization of exosomes as a therapeutic delivery vehicle in antitumor therapy has attracted considerable attention.

However, there are still several questions to answer. First, appropriate donor cells, which are capable of producing exosomes on a large scale, should be determined. Second, the strategies to obtain a large amount of donor cells and exosomes should be established. Third, potential immune responses induced by MHC in allogeneic engineered cells or by the proteins fused to exosomes should be avoided. Fourth, the therapeutic agents should be efficiently introduced into exosomes. Fifth,

pharmacokinetics of exosomes should be optimized to prolong their retention time at particular tissues and avoid normal clearance mechanisms. Intensive studies and technologic improvements in enhancing exosome production and reducing immunogenicity will facilitate the use of this “natural” delivery vehicle in clinical therapy.

5. Conclusions

Exosomes have a wide range of biological functions. As cell-to-cell communicators, they deliver various effectors or signaling micro-molecules and play intriguing roles in regulating diverse biological processes. They are also contributors to cancer metastasis and progression. Detection of exosomes may represent a very promising strategy to gain pathogenic information and select specific biomarkers for the diagnosis and prognosis of cancer. Modified exosomes targeting to specific organs may allow systemic administration of miRNAs, siRNAs, and chemotherapeutics in cancer or other diseases. Currently, only a small part of knowledge related to exosomes is available. Extensive studies will definitely improve the understanding of exosome functions in tumor metastasis, progression, and treatment.

Conflict of interest

We declare that there is no conflict of interest.

Acknowledgements

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Table 1. Potential biomarkers in the exosomes from the biofluids of cancer patients

Tumor	Patient material	Exosomal proteins	Exosomal miRNAs	Ref.
Ovarian cancer	plasma/serum	claudin-4, TGF- β 1, MAGE3/6, EpCAM, CD24, ADAM10, CD9, EMMPRIN	miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205 and miR-214	[22, 24, 40, 94],
	ascites	CD24, EpCAM, L1CAM, ADAM10, EMMPRIN, TCR, CD20, HLA-DR, B7-2, HER2/neu, CA125, Histone H2A, FasL, TRAIL	ND	[22, 23, 95]
Prostate cancer	urine	PCA-3, TMPRSS2:ERG, PSA, PSMA,	miR-107, miR-574-3p,	[28, 29, 96]
	plasma/serum	survivin	miR-145, miR-141, miR-375	[96, 97]
Colorectal cancer	plasma/serum	ND	let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a	[43]
	ascites	CEACAM5, CD97, CD9, Tspan8, plexin, EpCAM, trophoblast glycoprotein	ND	[98]
Esophagus adenocarcinoma	plasma/serum	ND	miR-21,miR-223-5p, miR-223-3p, miR-483-5p, miR-409-3p, miR-196b-5p, miR-192-5p, miR-146a-5p, miR-126-5p	[38, 39, 99]
Uveal melanoma	liver perfusates	ND	miR-216a, miR217, miR129-5p, miR-203, miR-9*, miR125a-5p, miR-25, miR-125b, miR-335, miR-19a, miR-9, miR-370, miR-210, miR-320a, miR-124, miR-107, miR-486-5p	[20]
Non-small cell lung cancer	pleural effusion	Abi-1, basigin, caveolin-1, EGFR, Grb2, ras family proteins, Src, claudin 1, annexin IV, aquaporin 1, Ppase, CEACAM5, CEACAM6	ND	[100]
Breast cancer	plasma/serum	CD95L, survivin, survivin-AEx3,survivin-2B	miR-195, let-7, miR-101, miR-372, miR-373 (receptor negative breast cancer)	[37, 101-103]
Bladder cancer	urine	Nras, EPS8L1, EPS8L2, Mucin 4, EHD4, GsGTP, resistin,RAI1, EDIL-3, TACSTD2	ND	[30, 104, 105], ,
Glioblastoma multiform	plasma	MMP-9, Caveolin-1, IL-8, PDGF AA/AB, PTX-3, CD26, PAI-1, TGF- β , EGFRvIII	miR-21	[25, 27, 44]
Melanoma	plasma/serum	CD63, Caveolin-1,TYRP2, MET, MDA-9, GRP78	miR-125b	[16, 26, 106, 107]
Pancreatic cancer	serum	CD44v6, MET, CD104, EpCAM, Tspan8	miR-1246, miR-4644, miR-3976 and miR-4306	[42]
Gastric cancer	serum	GRN	ND	[108]
Hepatocellular carcinoma	serum	ND	miR-21, miR-718	[41, 109]