

Role of stem cell-derived exosomes in cancer (Review)

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Abstract. Exosomes are small, extracellular membrane-enclosed vesicles that contain a variety of molecules, including proteins, DNA, mRNA and non-coding RNA; these vesicles have been defined as new tools for intercellular communication between cells. Numerous types of cells, including stem cells, secrete exosomes into the extracellular environment, and are significant communicators in the tumor microenvironment. Stem cells are a unique cell population defined by their ability to indefinitely self-renew, differentiate into a variety of cell lines, and form clonal cell populations. Stem cells also secrete large amounts of exosomes, which have demonstrated great potential in a variety of diseases. Increasing evidence has revealed that the mechanism of interaction between stem cells and human tumor cells involves the exchange of biological material through exosomes. In this review, the latest developments in the role of stem cell-derived exosomes in cancer are highlighted.

Contents

1. Introduction
2. Biogenesis, contents and secretion of exosomes
3. Uptake and functions of exosomes
4. Stem cells
5. Role of stem cell-derived exosomes in cancer
6. Conclusion

1. Introduction

Exosomes are small, lipid bilayer membrane vesicles of endocytic origin (30-100 nm in diameter). In recent years, there has been increasing interest in the relevance between biological and pathophysiological processes and these extracellular vesicles (EVs). Increasing evidence suggests that interactions between stem cells and human tumor cells involve the exchange of biological information via EVs, including exosomes (1). Stem cells secrete a large number of exosomes, which act as communicators in the tumor microenvironment and which play diverse roles in tumorigenesis, tumor angiogenesis and tumor metastases. However, the role of stem cell-derived exosomes in the pathophysiological processes of tumors has not been clarified until now. In this review, the recent findings with regard to the role of stem cell derived-exosomes in cancer are briefly summarized.

2. Biogenesis, contents and secretion of exosomes

Exosomes, which were first described in 1981, are derived from the internal vesicles of multivesicular bodies (MVBs) and consist of a lipid bilayer membrane surrounding a small amount of cytosol (2). Exosomes are secreted by all types of cells in culture and are observed in abundance in body fluids, including saliva, urine, blood and breast milk (3). In the present study, the current knowledge with regard to the biogenesis, contents and secretion of exosomes is summarized.

The formation of MVBs during exosome biogenesis is similar to the formation of MVBs during lysosome formation. First, the cell membrane is internalized to produce an endosome. Subsequently, the endosome forms inside a large number of small vesicles via invagination of portions of the endosome membrane. Such endosomes are called MVBs. Finally, exosomes are produced and packed with cytoplasmic contents, and the membrane of MVBs bulges inward and

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Abbreviations: MVBs, multivesicular bodies; MSCs, mesenchymal stroma/stem cells; CSCs, cancer stem cells; ESCRT, endosomal sorting complex required for transport; GTPase, guanosine triphosphatase; EVs, extracellular vesicles; VEGF, vascular endothelial growth factor; CLIC1, chloride intracellular channel-1; ERK, extracellular signal-regulated kinase

Key words: stem cell, exosomes, mesenchymal stem cell, cancer stem cell, multivesicular bodies, extracellular vesicles, tumor growth, angiogenesis, metastasis, therapy, biomarker

pinches off to create small membranous vesicles within the MVBs. The molecular mechanisms of exosome formation have been studied extensively; however, the exact mechanism of exosome packaging has not been fully clarified. Endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent signals have been suggested to be associated with the sorting of exosomes (4). ESCRT consists of four complexes and their associated proteins: ESCRT-0 identifies ubiquitinated proteins in the endosomal membrane, ESCRT-I and ESCRT-II direct endosomal membrane budding, and ESCRT-III facilitates separation from the endosomal membrane (5,6). Numerous studies have revealed that the ESCRT-0 protein hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) is necessary for exosome formation (7). ESCRT-I members, including HeLa-CIITA, MCF-7 and tumor susceptibility gene 101 (TSG101), are also associated with another ESCRT-independent mechanism of exosome biogenesis. Research has demonstrated that ALG-2-interacting protein X (Alix), an ESCRT-III-associated protein, promotes exosome biogenesis and, thus, intraluminal budding of vesicles in endosomes. Two proteins that are degradation products of the ESCRT-III complex, namely vacuolar protein sorting-associated protein 4 (VPS4) and charged multivesicular body protein 4 (CHMP4), are also involved in exosome biogenesis (6). However, certain studies have demonstrated that ESCRT-independent signals were also involved in exosome biogenesis. These pathways may involve lipids including sphingosine-1-phosphate and four spin-enriched microdomains or heat shock proteins (6).

After exosomes are formed, they contain >14,000 biomolecules, including proteins, RNAs and DNA (8). In the late 1990s, the first 'proteomic' analyses of the protein composition of dendritic cell-derived exosomes were performed (9). Biochemically, exosomes contain common marker proteins [e.g., tetraspanins, including cluster of differentiation (CD) 9, CD10, CD26, CD53, CD63, CD81 and CD82], which are present in the exosomal membrane. Exosomes also contain Alix and TSG101, which are involved in the formation of MVBs. Two cytoplasmic heat shock proteins, Hsc70 and Hsp90, have also been observed in exosomes (3,10). mRNAs and microRNAs (miRNAs/miRs) were definitively identified in exosomes for the first time by Valadi *et al* (11). Thakur *et al* observed that exosomes from cancer cells contained double-stranded DNA that could reflect the mutational status of the originated cells (12). It has also been demonstrated that RNA carried in exosomes may be delivered to target cells and that the expression of genes in target cells is influenced by miRNAs contained in exosomes (13). In addition, mRNAs and miRNAs from different cells may be cell-type specific.

Exosomes may be secreted via the fusion of MVBs and the cell membrane, followed by the release of the contents of the MVBs (exosomes) into the extracellular environment. Alternatively, the contents of MVBs are degraded through lysosomes. There are numerous studies on the secretion of exosomes, and various proteins associated with this process. Rab2b, Rab5a, Rab7, Rab9a, Rab11, Rab27a, Rab27b and Rab35, members of the Rab family of small guanosine triphosphatase (GTPase) proteins, have been demonstrated to accurately regulate the secretion of exosomes (14). Soluble NSF-attachment protein

receptor complexes are associated with the fusion of exosomes and the lipid bilayers (15). The accumulation of intracellular Ca^{2+} and intercellular pH has been observed to regulate the secretion of exosomes (16). In addition, heparanase over-expression promotes the secretion of exosomes (17). When exosomes are secreted, some of them are taken up by target cells localized near the cell of origin, while other exosomes are delivered to more distant sites through the blood or other biological fluids.

3. Uptake and functions of exosomes

In recent years, there has been increasing interest in intercellular communication via exosomes. A number of studies have attempted to determine the mechanism by which the cargo in exosomes is exchanged between exosomes and target cells. After exosomes are secreted, they may be taken up by the target cell via direct fusion with the plasma membrane, a receptor-ligand interaction, or endocytosis by phagocytosis (Fig. 1) (18,19). A number of biological molecules have significant roles in this process. Heat shock protein (HSP) 70, which is contained in exosomes, mediates the communication of cardioprotective signals to the heart and then activates a pathway downstream of toll-like receptor 4 (20). T-cell immunoglobulin- and mucin-domain-containing molecule, intercellular adhesion molecule 1 and heparan sulfate proteoglycans also influence the uptake of exosomes (16).

After exosomes are taken up by target cells, they play a vital role in cells. The primary function of exosomes in intercellular communication is the transfer of biologically active proteins, lipids and RNAs (21). Several studies have demonstrated that exosomes play crucial roles under normal and pathophysiological conditions, including lactation, the immune response, neuronal function and infectious diseases, as well as in the development and progression of liver disease, neurodegenerative diseases and cancer (22). Immune stimulation and tolerization are noted to be associated with exosomes; a previous study has suggested the potential use of exosomes in immunotherapy (23). Placental exosomes are involved in suppressive immunity during normal pregnancy (24). In addition, exosomes from human breast milk may contribute to the development of the infant immune system (23). The generation and progression of neurodegenerative diseases are also associated with exosomes. Exosomes transport proteins; thus, they may serve as a novel treatment approach or as new biomarkers in neurodegenerative diseases (24). In addition, exosomes have been promoted as specific therapeutic transporters for cardiovascular diseases (24), and are involved in the processes of infection biology, modulating the immune response and functioning as new acellular vaccines or infection biomarkers for infectious diseases (24). Additionally, exosomes are essential in the pathogenesis, diagnostics and therapeutics of liver diseases (24,25).

Increasing evidence has suggested that exosomes have significant roles in tumor growth, progression, metastasis and drug resistance (16). Tumor-derived exosomes regulate the formation of new blood vessels, which support tumor angiogenesis, and exosomes have crucial roles in tumor cell proliferation (16). In addition, exosomes induce the formation of the pre-metastatic niche, which regulates tumor

Table I. Functions of exosomes from different sources.

Conditions	Outcome	Reference
Normal conditions	Cell-cell communication	21
	Removal of unnecessary protein from cell	22
	Immune function	23,24,26
Liver diseases	Pathogenesis, diagnostics and therapeutics	23,25
Neurodegenerative diseases	Transporting proteins, a novel treatment approach or new biomarkers	24
Neurodegenerative diseases	New acellular vaccines or infection biomarkers for infectious diseases	24
Infectious diseases	New acellular vaccines or infection biomarkers for infectious diseases	24
Cancer	Growth	16
	Angiogenesis	16
	Progression	26
	Metastasis	26
	Drug resistance	26
	Manipulation of their microenvironment	26
	Immune response	26
	Diagnosis and therapeutics biomarker	16

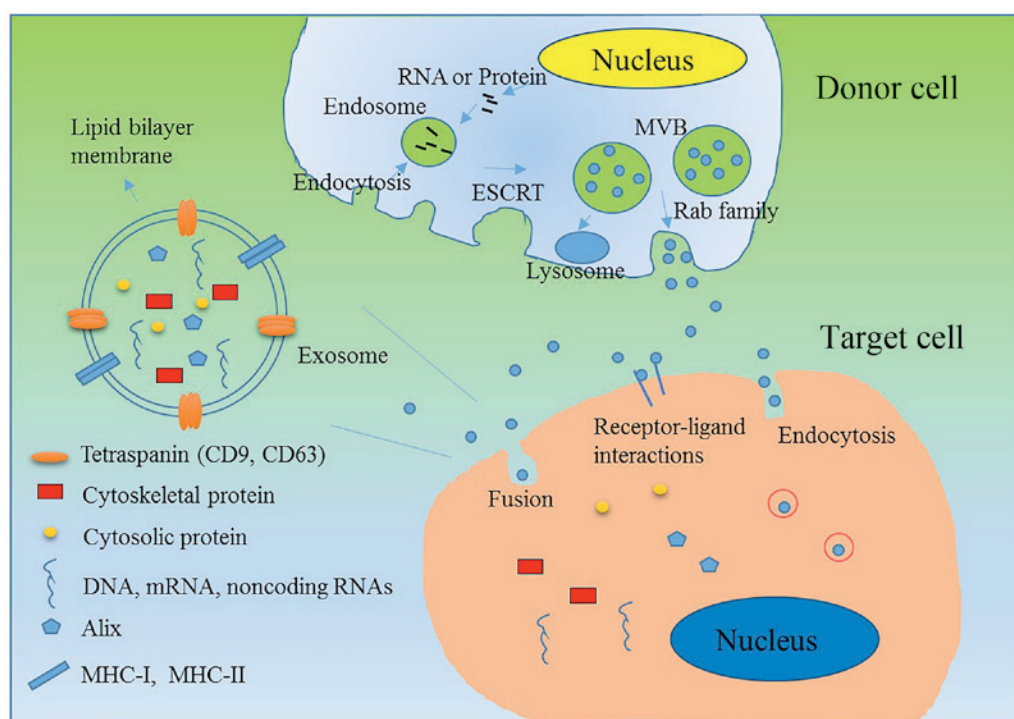


Figure 1. Biogenesis, contents, secretion and uptake of exosomes. Exosomes are small extracellular membrane-enclosed vesicles that contain a variety of molecules, including proteins, DNA, mRNA and non-coding RNA. Exosomes are initially formed by endocytosis to produce MVBs, and small membranous vesicles within the MVBs are created to produce exosomes. Exosomes contain RNAs/proteins of interest, including tetraspanins CD9 and CD63, cytosolic protein Rab family protein transmembrane molecules MHC I and MHC II, RNA and microRNAs. ESCRT-dependent and ESCRT-independent signals have been demonstrated to regulate the sorting of exosomes. When MVBs are produced, some of them fuse with the cell membrane and release their vesicles into the extracellular space to produce exosomes. Rab family members and soluble NSF-attachment protein receptor complexes play a key role in the secretion of exosomes. After exosomes are secreted, they may be taken up by target cells via direct fusion with the plasma membrane, a receptor-ligand interaction, or endocytosis by phagocytosis. MVB, multivesicular bodies; CD, cluster of differentiation; MHC, major histocompatibility complex; ESCRT, endosomal sorting complex required for transport; Alix, ALG-2-interacting protein X.

metastasis (26). Exosome-mediated mechanisms also clearly contribute to tumor cell drug resistance (26). Certain exosomes have a significant influence on the ability of tumors to evade immune surveillance; however, exosomes from different sources may enhance the immune response (26). Except for

the roles of exosomes in the pathogenesis of cancer, there are numerous studies on the use of exosomes as a new tool for cancer diagnosis and therapeutics (Table I).

In addition to the abovementioned studies, a number studies have examined the functions of exosomes (16,23,24,26);

however, which specific class of molecules contained in exosomes influences the target cells remains unclear.

4. Stem cells

Stem cells are a class of pluripotent cells that self-renew and differentiate into a variety of cell types. In this review, we focus on mesenchymal stem cells (MSCs) and cancer stem cells (CSCs).

MSCs, which originate from almost all vascularized organs and tissues, exhibit migratory capabilities and regenerative potential (27). MSCs may be isolated from various sources, including umbilical cord (UC), bone marrow (BM), liver, adipose tissue, multiple dental tissues and induced pluripotent stem cells (28,29). MSCs express CD44, CD73, CD90 and CD105, but not CD45, CD34 and CD14; these cells are characterized by their ability to adhere to plastics under standard cell culture conditions (30). MSCs affect the surrounding micro-environment by secreting factors, including growth factors, in an autocrine and paracrine manner. These cells decrease inflammation, promote angiogenesis, support tissue repair and suppress immunity (31). Since MSCs have the unique ability to home to damaged and cancerous tissue, they are of great interest in regenerative medicine and cancer therapy (32). MSCs also have a significant role in tumors. Previous studies have demonstrated that human MSCs (hMSCs) promote tumor growth and angiogenesis, in addition to providing stromal support (33,34), through autocrine and paracrine signaling (35,36). Although there are numerous studies of MSCs, the underlying mechanism of the correlation between MSCs and tumors remains largely unexplored. In recent years, a number of studies have noted that the interaction between MSCs and human tumor cells is associated with the exchange of biological material via EVs, including exosomes from MSCs (37-39). In the present review, MSC-derived exosomes are highlighted, which have a significant role in tumors, in order to realize the mechanism of this interaction.

During the past decade, CSCs have been increasingly identified in a number of malignancies. CSCs have stem-like characteristics and exhibit numerous features of embryonic or tissue stem cells (40). CSCs are associated with tumor initiation, metastasis, progression, invasion, recurrence and resistance to therapies. It is becoming widely accepted that CSCs play a central role in cancer cell biology; CSCs are essential for cancer initiation, formation and relapse. Previous studies have suggested that CSC-derived exosomes act as a vehicle to deliver genetic information and produce a favorable microenvironment for cancer development (41-43). In the present review, the role of CSC-derived exosomes in cancer is summarized.

5. Roles of stem cell-derived exosomes in cancer

Previous studies have demonstrated that stem cells generate a number of exosomes that may act as paracrine mediators by exchanging genetic information (38,39). There are certain differences between stem cell (MSC and CSC)-derived exosomes and other sources of exosomes. CSC-derived exosomes contain several proteins, including TSG101, Rab GTPases, annexins and signal transduction molecules (e.g.,

14-3-3, a heterotrimeric G protein, and Alix), that are potentially associated with their biogenesis, targeting and putative immunological function (44). In addition, MSC-derived exosomes are amenable to immortalization without compromising exosome production, and are not immunogenic. MSC-derived exosomes have intrinsic therapeutic properties that reduce tissue injury.

Tumor growth. Stem cell-derived exosomes have been noted to deliver gene regulatory information to target cells; this information regulates cell growth and angiogenesis by modulating a variety of cellular pathways. There have been a number of studies into the use of stem cell-derived exosomes to promote tumor cell proliferation in order to analyze the effects of cellular interactions between stem cells and various cancer cells. In recent years, studies have demonstrated that MSCs have a significant role in regulating tumor growth and metastasis (40,45). EVs, including exosomes isolated from hMSCs, were first completely biochemically and molecularly analyzed by Vallabhaneni *et al* (46). These authors used co-injection xenograft assays to demonstrate that the exosomes secreted from hMSCs support breast cancer cell proliferation and metastasis. They also observed that hMSC-derived EVs contain a large amount of miR-21 and 34a, which are tumor-supportive miRNAs, and ~150 different proteins, most of which are known tumor supportive factors, including platelet-derived growth factor receptor- β , tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2. The presence of bioactive lipids, including sphingomyelin, was verified in EVs, including exosomes, through lipidomic assays. Furthermore, metabolite assays identified the presence of lactic acid and glutamic acid in the EVs. In addition, Zhu *et al* revealed that MSC-derived exosomes enhanced vascular endothelial growth factor (VEGF) expression in tumor cells by activating the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, which promotes tumor growth (47). These authors were the first to demonstrate that exosomes from MSCs have a role in promoting tumor growth that is similar to that of the MSCs themselves, thus providing new insight into the actions of MSCs in tumor development and progression *in vivo*. Hernanda *et al* observed that exosomes (or microvesicles) secreted by MSCs may also be associated with tumor promotion (48). Subsequently, Yang *et al* demonstrated that the internalization of MSC-derived exosomes was involved in the acquisition of new tumor cell properties by altering cellular functionalities and providing the capability to re-organize the tumor microenvironment, which improves tumor growth (49). However, the effects of exosomes from different types of stem cells on cell proliferation may be completely different. Del Fattore *et al* demonstrated that BM- and UC-MSC-EVs (including exosomes) suppressed cell proliferation, while the opposite effect was observed with adipose tissue MSC-EVs (including exosomes) (50). In addition, microvesicles from human BM-derived MSCs inhibited tumor growth (51). Another experiment indicated that intra-tumoral injection of exosomes derived from miR-146-expressing MSCs significantly reduced glioma xenograft growth in a rat primary brain tumor model (52). miR-146 suppressed epidermal growth factor receptor (EGFR) expression through binding-targeting EGFR

Table II. Overview of functions of stem cell-derived exosomes in cancer.

Cancer type	Donor cells	Exosomal cargo	Target cells	Pathway involved	Application or outcome	Reference
Breast cancer	hMSCs	miR-21, miR34a, ~150 different proteins, sphingomyelin	MCF-7 breast cancer cells	Tumors co-injected with EVs exhibited higher angiogenesis	Supported breast cancer cell proliferation	46
Human gastric carcinoma	MSC	Unclear	SGC-7901 cells	VEGF ERK 1/2 pathway	Promoted tumor growth	47
Breast cancer and carcinoma of the ovary	MSC	MMP-2 protein	SCCOHT-1 cells, MCF-7 breast cancer cells	Altered cellular functionalities	Improved tumor growth and provided capability to re-organize tumor microenvironment	49
Malignant glial tumors	Umbilical cord MSC	Unclear	U87MG glioblastoma cells	Cell-cell communication	Decreased cell proliferation	50
Malignant glial tumors	Adipose tissue MSC	Unclear	U87MG glioblastoma cells	Cell communication	Improved tumor growth	50
Glioma	miR-146-expressing MSCs	miR-146	Glioma cells	miR-146 bound EGFR mRNA and suppressed EGFR expression	Reduced glioma xenograft growth	52
Multiple myeloma	Multiple myeloma BM-MSC	Lower miR-15a levels and higher content levels of CCL2	Multiple myeloma cells	Lower miR-15a expression in MM vs. normal BM-MSC-derived exosomes	MM BM-MSC-derived exosomes promoted MM tumor growth	54
Multiple myeloma	Normal BM-MSC	Suppressor miRNAs and normal level of CCL2	Multiple myeloma cells	Transfer of tumor suppressor miRNAs (including miR-15a)	Normal BM-MSC-derived exosomes inhibited growth of MM cells	54
Bladder tumor	hWJMSCs	Unclear	T24 cells	Downregulated phosphorylation of Akt protein kinase and upregulated cleaved caspase 3	Anti-proliferation and pro-apoptosis	55
Glioma	GASC	Unclear	Glioblastoma cell lines	Unclear	Supported tumor growth	42
Glioblastoma	GBM-derived CSCs	CLIC1	GBM cell lines	Stimulated cell growth through CLIC1	Novel regulator of GBM growth	43
Renal cancer	CD105-positive cancer stem cells	Proangiogenic mRNAs and miRNAs	HUVECs	mRNAs, including VEGF, FGF, angiopoietin1, ephrin A3, MMP-2 and MMP-9, and functional miRNAs, including miR-200c, miR-92 and miR-141, contribute to tumor angiogenesis	Contributed to triggering angiogenic switch and coordinating metastatic diffusion	39
Breast cancer cells	MSCs	miR-16	4T1 cells	Downregulated VEGF expression	Suppressed angiogenesis	59
Gastric cancer	MSC	miR-221	HGC-27	Regulated miR-221 expression	Promoted proliferation and migration	65

Table II. Continued.

Cancer type	Donor cells	Exosomal cargo	Target cells	Pathway involved	Application or outcome	Reference
Breast cancer	Adipose MSC	Genes associated with cell migration upregulated	Breast cancer cell line MCF-7	Wnt signaling pathway	Promoted migration and proliferation of breast cancer cell line MCF-7	66
Breast cancer	DCIS stem-like cells	miR-140 is downregulated in exosomes	Breast cancer cells	Downregulation of miR-140 removed inhibition of Wnt, Sox2 and Sox9 stem cell regulatory pathways	Lead to higher cancer stem cell populations and breast cancer progression	67
Breast cancer	DCIS stem-like cells	miR-21, miR-140 and miR-29a	MDA-MB-231 cells	miRNAs signaled through nearby immune cells via interaction with toll-like receptors to upregulate secretion of TNF and IL-6 secretion	Enhanced migratory capacity	68
Glioma	MSCs	miR-124 and miR-145	Glioma cells U87 and A172	miR-124 and miR-145 mimics decreased luciferase activity of respected reporter target genes, SCP-1 and Sox2	Decreased migration of glioma cells and self-renewal of GSCs	69
Breast cancer	BM-MSCs	miR-23b	BM2 cells	Increased miR-23b and decreased MARCKS expression	Promoted breast cancer cell dormancy in a metastatic niche	70
Osteosarcoma	BM-MSCs	miR-143	Human osteosarcoma cell line 143B	Delivered miR-143 to target cells	Reduced migration of osteosarcoma cells	71

hMSCs, human mesenchymal stem cells; miR/miRNA, microRNA; EV, extracellular vesicles; MSCs, mesenchymal stem/stroma cells; VEGF, vascular endothelial growth factor; ERK, extracellular signal-regulated kinase; MMP, matrix metalloproteinase; EGFR, epidermal growth factor receptor; BM, bone marrow; CCL2, chemokine (C-C motif) ligand 2; MM, multiple myeloma; hWJMSCs, human Wharton's jelly mesenchymal stem cells; GASC, glioma-associated stem cells; GBM, glioblastoma; CSCs, cancer stem cells; CLIC1, chloride intracellular channel-1; CD, cluster of differentiation; HUVECs, human umbilical vein endothelial cells; FGF, fibroblast growth factor; DCIS, ductal carcinoma *in situ*; Sox, sex-determining region Y-box 2; TNF, tumor necrosis factor; IL-6, interleukin 6; SCP-1, small carboxy-terminal domain phosphatase 1; GSCs, glioma stem cells; MARCKS, myristoylated alanine-rich C-kinase substrate.

mRNA, and reduced *in vitro* growth, migration and invasion of cancer (53). These studies revealed that miRNAs may be packaged into MSC exosomes, delivered to target tumor cells in culture, and reduce glioma cells, which suggested that the export of specific therapeutic miRNAs into MSC exosomes represented a new treatment strategy for malignant glioma. Additionally, although exosomes derived from multiple myeloma BM-MSCs have been noted to improve multiple myeloma progression, normal BM MSC-derived exosomes reduce tumor promotion (54). Exosomes from human umbilical cord Wharton's jelly MSCs reduced bladder tumor cell growth *in vitro* and *in vivo* (55). Thus, the effects of different sources of MSC-derived exosomes are uncertain.

Based on the aforementioned studies, it is known that exosomes secreted from MSCs may result in cell-to-cell transfer of mRNA, miRNA and proteins (56). However, the exact roles and mechanisms of MSC-produced exosomes in tumor biology remain largely elusive.

Cancer stem cell-derived exosomes also have a notable influence on tumor growth. It is known that human gliomas have a population of stem cells with a tumor-supporting ability; these cells are called glioma-associated stem cells (GASCs). Bourkoulas *et al* demonstrated that exosomes derived from GASCs support tumor growth and have a tumor-supporting phenotype (42). In addition, EVs, including exosomes from glioblastoma-derived CSCs, regulate tumor growth through chloride intracellular channel-1 (CLIC1) (43).

Thus, exosomes released from stem cells affect tumor growth (Table II); however, further studies of stem cell-derived exosomes are required.

Tumor angiogenesis. The formation of new blood vessels is necessary for tumor growth and development, and plays a significant role in tumor progression and metastasis. The process of angiogenesis is extremely complex; it generally includes vascular endothelial substrate degradation, vascular endothelial cell migration and endothelial cell proliferation, as well as the formation of vascular pipeline branches and a new basement membrane. Exosomes contain abundant angiogenic factors that regulate tumor angiogenesis. For example, MSC-derived exosomes promote tumor angiogenesis by increasing VEGF expression in tumor cells and activating ERK1/2 and p38 mitogen-activated protein kinase pathways (47). Other studies have demonstrated that placental MSC exosomes promote vascular network formation and improve microvascular endothelial cell migration in a concentration- and oxygen-dependent manner (57). In addition, EVs released by adipose mesenchymal stem cells (ASCs) may contribute to ASC-induced angiogenesis (58). In cancer stem cells, exosomes derived only from CD105-positive cancer stem cells conferred an activated angiogenic phenotype to normal human endothelial cells, stimulating their growth and vessel formation (39). The results defined a specific source of cancer stem cell-derived EVs that contribute to triggering the angiogenic switch and coordinating metastatic diffusion during tumor progression (39). The effects of exosomes from different types of stem cells on tumor angiogenesis are similar to the effects of stem cell-derived exosomes on tumor growth. However, their effect may be completely different. Lee *et al* observed that MSC-derived exosomes suppressed

angiogenesis by transferring anti-angiogenic molecules and serving as a significant mediator of cell-to-cell communication within the tumor microenvironment (59). These authors noted that MSC-derived exosomes inhibited tumor growth and angiogenesis in breast cancer by downregulating the expression of VEGF, which is a pro-angiogenic factor that is frequently overexpressed in cancer (60). miR-16, which is contained in MSC-derived exosomes, reduces the VEGF expression level in 4T1 cells. It has been demonstrated that miR-16 controls VEGF expression (61-63). In summary, exosomes are significantly associated with tumor angiogenesis.

Tumor metastases. Exosomes released from stem cells also contribute to tumor metastasis. A number of the key steps in tumor invasion and metastasis are associated with MSCs, including facilitating epithelial-mesenchymal transition and the induction of stem-like properties that allow cancer stem cells to increase their survivability through the circulation (64). A number of studies have examined the role of CSC- and MSC-derived exosomes in metastasis, tumor reseeding (self-seeding) and the formation of a pre-metastatic niche. Gastric cancer (GC) MSC-derived exosomes were observed to deliver miR-221 to HGC-27 cells, which facilitated the proliferation and migration of these cells (65). In addition, MSC-derived exosomes promoted Wnt signaling pathway activation to facilitate the migration and proliferation of the breast cancer cell line MCF-7 (66). The Wnt signaling pathway is characterized by the nuclear accumulation of β -catenin, which is involved in not only embryonic development but also tumor development (66). As for CSCs, Wolfson *et al* noted that exosomes derived from ductal carcinoma *in situ* (DCIS) stem-like cells contained lower levels of miR-140 compared with exosomes derived from a DCIS whole cell population, which could improve tumor growth and metastases (67). Dysregulation of miR-140 has an important role in regulating the transition of DCIS to invasive ductal carcinoma (IDC) (67). As the tumor grade increases, miR-140 is progressively down-regulated and plays a significant role in the stem cell regulatory pathways. Downregulation of miR-140 leads to higher CSC populations and breast cancer progression by removing tumor suppressive pathways (67). Other studies have characterized the exosomal exchange of miRNAs between DCIS stem-like cells and target cells; exosomes from DCIS stem-like cells were observed to enhance the migratory capacity via several miRNAs, including miR-140, miR-29a and miR-21, which are differentially expressed in the exosomes (68). These findings suggest that CSC-derived exosomes may improve tumor metastases.

However, there are certain contrary findings. Lee *et al* demonstrated that MSC-derived exosomes transferred specific miRNA mimics in a gap junction-dependent and contact-independent manner. miR-124 and miR-145 mimics vitally decrease the luciferase activity of their respective reporter target genes, including small carboxy-terminal domain phosphatase 1 (SCP-1) and sex-determining region Y-box 2 (Sox2), and decrease the migration of glioma cells and the self-renewal of glioma stem cells (69). Additionally, Ono *et al* noted that breast cancer BM-MSC-derived exosomes have more varied miRNAs than adult fibroblast-derived exosomes. BM-MSC-derived exosomes overexpress miR-23b, which

induces a dormant phenotype through suppressing a target gene, myristoylated alanine-rich C-kinase substrate, which encodes a protein that promotes cell cycling and motility (70). These findings suggest that exosomal transfer of miRNAs from the BM may promote breast cancer cell dormancy in a metastatic niche. Another study has also presented the same result; these authors observed that the delivery of miR-143 via MSC-derived exosomes significantly reduced the migration of osteosarcoma cells (71).

Taken together, these findings reveal that stem cell-derived exosome-mediated intercellular communication may be an essential mechanism for tumor metastasis.

Tumor therapy. Personalized medicine is used to identify patient- and tumor-specific factors that are useful for the identification of therapeutic options and the prognostic stratification of patients in order to maximize effectiveness and minimize treatment-associated toxicity (72). To achieve this goal, exosomes from stem cells, possibly MSCs, may provide a new approach to personalized medicine. Over the past few years, the potential of using MSCs in regenerative medicine has received increasing attention, and the use of MSCs in anticancer therapy has been extensively studied. A previous study suggested that MSC-derived exosomes may have a key role in not only regenerative medicine to repair damaged tissue but also tumor therapy (73). MSCs may communicate with cancer cells via gap junctional intercellular communication and via the secretion of exosomes (74,75). Chen and Lim revealed that intercellular communication between MSCs and tumor cells could be promoted by secreting microparticles, including exosomes, through the exosomal transfer of miRNAs (76). Additionally, in recent years, miRNAs have emerged as potential anti-cancer agents. Although miRNAs are an effective therapeutic method for cancer, the effectiveness of this approach requires targeted delivery. Exosomes work as delivery vehicles for nucleic acids or drugs; this novel approach has gained increasing interest due to the effective biocompatibility and biodistribution of exosomes. In addition, stem cells have an infinite capacity for reproducible production of exosomes as drug delivery vehicles, which may be essential for tumor therapy. Lim *et al* observed that exosomes transmit miRNAs from BM stroma to breast cancer cells in tumor cell quiescence. This study revealed that the transfer of miRNAs from BM stroma to breast cancer cells through exosomes may have a significant role in the dormancy of BM metastases through exosomes (77). Munoz *et al* revealed that MSC-derived exosomes could deliver anti-miR-9, which blocks miR-9, to glioblastoma multiforme (GBM) cells. Anti-miR-9 was involved in the expression of the drug efflux transporter P-glycoprotein, reversed the expression of the multidrug transporter, and sensitized the GBM cells to temozolomide, which increases cell death and caspase activity (78). These data demonstrated a potential role for MSCs in the functional delivery of synthetic anti-miR-9 to reverse the chemoresistance of GBM cells. Boelens *et al* revealed that stromal cell-derived exosomes stimulated the pattern recognition receptor retinoic acid-inducible gene I to activate signal transducer and activator of transcription 1-dependent antiviral signaling and activate NOTCH3 in breast cancer cells to expand breast cancer cell subpopulations that are adept at resisting therapy and

reinitiating tumor growth. These findings suggest a possible novel use of MSCs in the development of new 'biotech drugs' with increased efficacy and homing capacity (79). In addition, miR-122-modified adipose tissue-derived MSCs secrete exosomes to increase chemosensitivity (80). MSC-derived exosomes effectively silence the polo-like kinase 1 (PLK-1) gene by transporting PLK-1 small interfering RNA to bladder cancer cells (81). Pascucci *et al* were the first to demonstrate that MSCs act as a factory to develop drugs with a higher cell-target specificity through packaging and delivering active drugs, and suggested the possibility of using MSCs (82). These authors noted that paclitaxel (PTX) is incorporated by MSCs and released in exosomes. PTX is delivered to target cells and inhibits tumor growth through a simple procedure of exposing the cells to an extremely high concentration of PTX. Fuhrmann *et al* demonstrated that EVs, including exosomes, loaded with hydrophilic porphyrins induced a stronger phototoxic effect than free drugs in a cancer cell model; this approach may significantly improve cellular uptake and the therapeutic effect of phototoxic porphyrins *in vitro* (83). Significantly, these methods are simple and directly applicable to other drugs and vesicles, thus providing a new approach to cancer therapy.

EVs from other stem cells also have a potential role in tumor therapy. Fonsato *et al* demonstrated that EVs derived from human adult liver stem cells may inhibit HepG2 hepatoma and primary hepatocellular carcinoma cell growth and survival (Table III) (84). In summary, stem cell-derived exosomes have a significant role in anti-cancer treatment (Fig. 2).

Tumor biomarkers. Stem cell-derived exosomes provide an enriched source of biomarkers as they contain bioactive molecules that reflect the pathological state of the origin cells. Recently, CSCs have been used in cancer diagnostics and treatment. Exosomes released from rotenone-treated prostate and breast CSCs have specific biomolecular characteristics, including the expression of several exosomal markers, including CD9, CD63, CD81, Alix and TSG101. Thus, the release of exosomal markers may be highly relevant for biological activity and may be used as potential targets (44). In addition, GC-MSC-derived exosomes contain miR-221, which is a potential new biomarker for tumor diagnosis (65).

Taken together, these findings suggest that the constituents of exosomes deliver information from cell to cell (delivering proteins and nucleic acids), and may be used as a biomarker for targeted cancer therapy. There are a number of studies on the use of MSCs in regenerative medicine and anti-cancer treatments (85-87). Over the past few years, these studies have raised high expectations. However, safety concerns and tight regulations hamper their practical use in clinical settings. The use of stem cell-derived exosomes may have numerous advantages compared with cell-based approaches, and may improve the safety of MSCs in tumor therapy. However, further experiments are required to demonstrate the safety and feasibility of stem cell-derived exosomes.

6. Conclusion

In conclusion, studies have demonstrated that exosomes derived from various cell types, including stem cells, may

Table III. Exosomes in tumor therapy.

Cancer type	Donor cells	Exosomal cargo	Target cells	Application or outcome	Reference
Glioblastoma	MSCs	Anti-miR-9	Resistant GBM cells	Increase tumor death	78
Breast cancer	Stromal cells	Non-coding RNA and proteins	Breast cancer cells (1833)	Biotech drugs regulate therapy resistance	79
Hepatocellular carcinoma	miR-122-modified adipose tissue-derived MSCs	miR-122	HepG2	Increase chemosensitivity	80
Bladder cancer	MSCs	PLK-1 siRNA	Bladder cancer cell lines Pancreatic cell	Selective gene silencing of PLK-1	81
Pancreatic cancer	MSC with PTX	PTX	line sr4987	Deliver drugs	82
Breast cancer	hMSCs	Hydrophilic porphyrins	MDA-MB231 breast cancer (MDA) cells	Deliver drugs	83
Hepatocellular carcinoma	Liver stem cells	Antitumor miRNAs, including miR-451, miR-223 and miR-31	HepG2	Inhibit growth and survival	84

MSCs, mesenchymal stem cells; miR/miRNA, microRNA; siRNA, small interfering RNA; GBM, glioblastoma; PLK-1, polo-like kinase 1; PTX, paclitaxel; hMSCs, human mesenchymal stem cells.

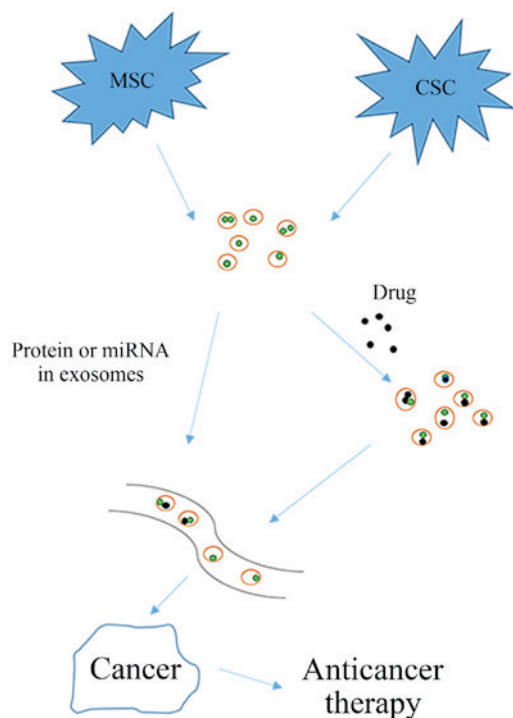


Figure 2. Proposed model for therapeutic application of exosomes. MSC- and CSC-derived exosomes may be used to mediate anti-proliferative or pro-apoptotic effects of anti-cancer therapy. These exosomes may also serve as effective drug delivery vehicles. MSCs, mesenchymal stroma/stem cells; CSCs, cancer stem cells; miRNA, microRNA.

act as mediators of cell-to-cell communication. The role of stem cell-derived exosomes in tumor development has been intensively studied due to the influence of exosomes on tumors and the significant therapeutic potential of stem cells.

MSC- and CSC-derived exosomes contain different protein and RNA profiles compared with their donor cells. Exosomes transfer their molecular contents, including numerous types of special proteins and RNA, between cells, thus influencing tumor behavior and proliferation. In addition, exosomes carry a number of the desirable attributes of a synthetic liposome vehicle, including the capacity to carry hydrophobic drugs, and are effective and safe drug delivery vehicles. In the present review, several aspects of stem cell-derived exosome biology in cancer, which has the ability to communicate with surrounding and distant cells, are discussed. MSC- and CSC-derived exosomes have a significant role in synergistically influencing cancer development, metastasis, progression and drug resistance. Although a number of studies have examined the role of stem cell-derived exosomes in cancer (46-49), the exact mechanisms of the effects of stem cell-derived exosomes on cancer have been largely unexplored and untested. The utility of exosomes as a delivery vehicle is also unclear. Additional studies of stem cell-derived exosomes are required to determine their roles in the pathogenesis of cancer and to provide a new tool for cancer diagnosis and therapeutics.

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