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Exosomes in cancer development and clinical applications

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Exosomes participate in cancer progression and metastasis by transferring bioactive molecules between cancer and various cells in the local and distant microenvironments. Such intercellular cross-talk results in changes in multiple cellular and biological functions in recipient cells. Several hallmarks of cancer have reportedly been impacted by this exosome-mediated cell-to-cell communication, including modulating immune responses, reprogramming stromal cells, remodeling the architecture of the extracellular matrix, or even endowing cancer cells with characteristics of drug resistance. Selectively, loading specific oncogenic molecules into exosomes highlights exosomes as potential diagnostic biomarkers as well as therapeutic targets. In addition, exosome-based drug delivery strategies in preclinical and clinical trials have been shown to dramatically decrease cancer development. In the present review, we summarize the significant aspects of exosomes in cancer development that can provide novel strategies for potential clinical applications.

KEYWORDS
biomarker, cancer malignancy, cancer therapy, drug resistance, exosome

1 | INTRODUCTION

Exosomes are nanosized vesicles that are actively secreted by almost all types of cells, including fibroblasts, endothelial cells, epithelial cells, neuronal cells, immune cells, as well as cancer cells.[1] Enriched with many bioactive molecules, such as nucleic acids, proteins, lipids, and metabolites, exosomes are endowed with the ability to relay signals between cells.[2] Indeed, exosomes have been investigated in many types of body fluid, such as bile, blood, breast milk, urine, cerebrospinal fluid, and saliva, suggesting that exosomes play multiple roles in regulating physiological responses.[3] Recently, the pathophysiological effects of exosomes on diseases, especially cancers, have emerged. Tumor-derived exosomes have reportedly been involved in the development of cancer malignancy by promoting cancer proliferation, establishing a premetastatic niche, and regulating drug resistance.[2] Clinically, exosomes functioning as diagnostic biomarkers, therapeutic targets, or even as anticancer drug-delivery vehicles have all been emphasized as a result of their unique biological and pathophysiological characteristics.[4] Here, we provide a comprehensive overview of exosomes in cell biology of cancer and discuss how exosome-based intercellular communications regulate cancer progression and metastasis. Additionally, we summarize the role of exosomes in clinical applications in relation to their molecular and biological characteristics.

2 | EXOSOMES

Since platelet-derived vesicles with coagulant properties were first investigated by Peter Wolf in 1967,[5] additional roles of extracellular vesicles (EV) were gradually discovered in the following years. In 1981, exosomes were first referred to as vesicles bearing enzymatic activity that are released by cells.[6] Later, Johnstone et al[7] indicated that exosomes are a consequence of the fusion between

multivesicular bodies (MVB) and the plasma membrane that directs the recycling of transferrin receptors during reticulocyte maturation. In 1996, exosomes derived from B lymphocytes were found to show antigen-presenting properties, enabling the induction of T-cell responses.[8] Similarly, antigen-presenting exosomes from dendritic cells were also found to suppress cancer progression.[9] Aside from participating in the immune system, comprehensive functions in various pathophysiological processes highlight the importance of exosomes in regulating cancer development and neurodegenerative diseases.[2]

2.1 | Characteristics of exosomes

Exosomes are nanoscaled extracellular vesicles (in general, their sizes range from 30 to 150 nm) released by almost all cell types.[2,4] Within the endosomal network, the biogenesis of exosomes is generated as intraluminal vesicles (ILV, also called pre-exosomes) by inward budding of the multivesicular body membrane.[2] Mechanisms of exosome biogenesis are highly regulated through several distinct pathways,[1] including ESCRT (endosomal sorting complexes required for transport)-dependent and ESCRT-independent pathways (Figure 1). In the

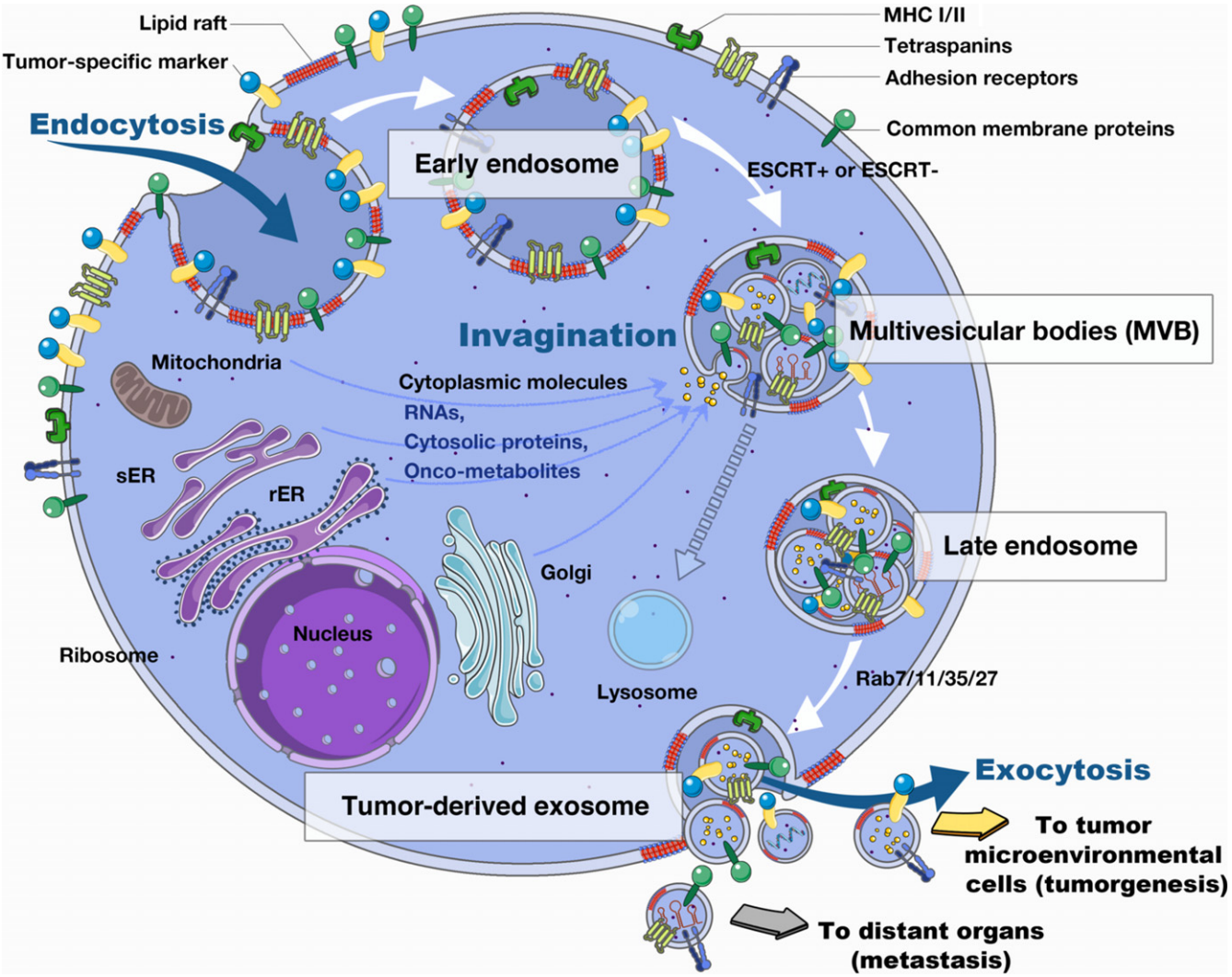

FIGURE 1 Biogenesis of exosomes. First, endocytosis could be mediated by either a clathrin-dependent pathway or a clathrin-independent pathway, which often actively occurs at the lipid raft containing a variety of tumor-specific receptors and signaling proteins (eg, growth factor receptors, oncoproteins) in addition to common membrane proteins, such as tetraspanins (eg, CD9, CD63, CD81), MHC I and II, and adhesion molecules (eg, integrins, cadherins). Using the endosomal network, the biogenesis of exosomes is achieved in an endosomal sorting complexes required for transport (ESCRT)-dependent or ESCRT-independent method. Accordingly, intraluminal vesicles (exosomes) show inward budding of the multivesicular bodies (MVB). Indeed, numerous cytoplasmic (eg, ubiquitin-related proteins, heat shock proteins, microRNAs [miRNAs], mRNAs, cytoskeleton proteins etc.) and nuclear molecules (eg, transcriptional factors, longnoncoding RNAs [lncRNAs], DNAs etc.) can be selectively loaded into MVB in a cancer type-specific and/or stage-specific way. Furthermore, multivesicular bodies are fused with the plasma membrane, leading to the release of exosomes toward the extracellular space in an exocytic way. Several Rab GTPases, including Rab11/35, Rab7, and Rab27, have been reported to be involved in exosome secretion. Finally, tumor-derived exosomes are transferred to the local tumor microenvironment and distinct organs to regulate tumorigenesis or metastasis, respectively. rER, rough endoplasmic reticulum; sER, smooth endoplasmic reticulum

ESCRT membrane-scission machinery, 4 multiprotein subcomplexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) are required for exosome biogenesis. Early ESCRT complexes (ESCRT-0, ESCRT-I, and ESCRT-III) recognize ubiquitinated cargo by their ubiquitin-binding subunits, leading to the formation of stable protein complexes within the cytoplasm. The ESCRT-III complex then transiently assembles on endosomes and conducts the vesicle scission.[10] Recently, some auxiliary components, including ATPase, vacuolar protein sorting-associated protein (VPS4), or ALG-2-interacting protein X (ALIX), have been indicated to participate in the regulation of ESCRT membrane-scission machinery.[10] In contrast, the budding or release of exosomes is regulated by lipids, such as sphingolipid ceramide[11] or sphingosine 1-phosphate within the ESCRT-independent pathway.[12]

Molecular constituents, such as nucleic acids, proteins, lipids, and metabolites, of exosomes vary depending upon their cells of origin, environmental conditions, developmental stages, epigenetic changes, and mechanisms of biogenesis. Various RNA species, including mRNAs, microRNAs (miRNAs), rRNAs, transfer RNAs (tRNAs), or long noncoding RNAs (lncRNAs), were also identified in exosomes,[13] leading to knowledge of epigenetic modification between cells and changes in biological activities and functions. Recently, dsDNAs appearing in tumor-derived exosomes were shown to reflect the mutational status of parental cancers.[14] Consistently, >10 kb fragments of cellular genomic DNA were found to bear mutations on oncogenes and tumor suppressor genes in tumor-derived exosomes.[15] Moreover, exosomes contain a variety of bioactive proteins originating from the plasma membrane, cytoplasm and, in most, but not all, parental cells. Specific proteins, such as ALIX, ESCRT complexes, or Rab GTPases, involved in the biogenesis of exosomes are conserved and enriched within these nanosized vesicles.[10] Other proteins, such as heat shock proteins (eg, HSP70 or HSP 90), tetraspanins (eg, CD9, CD63, and CD81), or integrins, are selectively packaged into exosomes to take part in intracellular assembly or exosome trafficking.[2] Exosomal protein profiles can reflect the enriched protein expression patterns as well as pathophysiological activities of parental cells. Since actively emanating from lipid rafts, the membrane composition of exosomes is relatively abundant in specific lipid species, including cholesterol, sphingomyelin, phosphatidylcholine, diacylglycerol, and ceramide, compared to their parent cells.[16] Some lipids and lipid-metabolizing enzymes, such as neutral sphingomyelinase (nSMase) or phospholipase D2 (PLD2), regulate the formation and release of exosomes.[11]

The heterogeneity of exosomes in association with exosome-mediated cell functions, distinct molecular cargo profiles, and the resultant biophysical characteristics have been investigated according to their distinct morphology and/or sizes. The latter has been used to discriminate large exosome vesicles (90-120 nm), small exosome vesicles (60-80 nm), or nonmembranous nanoparticles (also called exomeres, ~35 nm).[17] Intrinsically, distinct molecular signatures in exomeres and exosomes were further seen in proteomic, lipidomic, and glycomic analyses. Based on proteomic profiling, enzymes related to metabolism and hypoxia as well as microtubule and coagulation proteins are more abundantly expressed in exomeres compared to large or small membranous exosomal vesicles. In contrast, large and small exosome vesicles show enrichment in mitotic spindle and interleukin (IL)-2/STAT5 signaling, and proteins related to endosomal secretion, respectively.[17] Moreover, the difference in sialylated glycoproteins (ie, galectin-3-binding protein) between exosomes and exomeres is organ distribution.[17] Consistent with the variations in lipid compositions between exomeres and exosomes,[17] density gradient centrifugation showed that isolated exosomes are separated into 2 distinct populations: lower density exosomes (LD-Exo) and higher density exosomes (HD-Exo).[18] Intriguingly, LD-Exo-treated endothelial cells showed an upregulation of solute carrier family 38 member 1 (SLC38A1) compared to HD-Exo-treated endothelial cells.[18] Together, these studies suggested that distinct exosome subpopulations with unique compositions trigger diverse biological effects on recipient cells.

2.2 | Exosomes in cell-to-cell communication

Cell-to-cell communication is critical in maintaining physiological homeostasis and directs pathological manifestations. Rather than direct cell-cell contact or the release and uptake of extracellular signaling molecules, such as cytokines, growth factors, hormones, and extracellular matrix, exosomes are emerging as critical mediators in inter- and intracellular communications both locally and distantly.[4]

Enclosed by the lipid bilayer-membrane, exosomes provide a protective shield for vulnerable biological molecules. Indeed, the exosomal membrane structure encapsulates and protects miRNAs or proteins from degradation by RNases[19] or by proteinase,[20] respectively. Several studies have indicated that biological activity of exosomal molecules authentically modulates cell signaling events and biological processes of the recipient cells.[21] During cancer development, tumor-derived microvesicles (also called oncosomes) are capable of transmitting the oncogenic receptor EGFR vIII from aggressive brain cancer cells to another cancer cell lacking this oncogenic receptor activity.[22] In addition, lncRNAs are exchanged between gastric cancer cells through exosomes, triggering cancer progression.[23] Exosome-mediated cell-cell communication is not limited to cancer cells, rather it has also been shown within the tumor microenvironment locally and distantly.[24] Tumor-derived exosomes can transport transforming growth factor beta (TGF-β) from cancers to normal fibroblasts, subsequently driving fibroblast into myofibroblast differentiation.[25] In contrast, cancer-associated fibroblast (CAF)-derived exosomes modulate the metabolism of cancer cells by inhibiting the mitochondrial oxidative phosphorylation process in cancer cells.[26] Regarding the role of exosomes on long distance transfer of biological molecules between cells, malignant cancer cells, such as breast or pancreatic cancers, secrete exosomes containing bioactive molecules, such as telomerase activity[27] or macrophage migration inhibitory factor,[28] to the distant tumor-associated microenvironment and contribute to the formation of premetastatic niches.

3 | EXOSOMES IN CANCER MALIGNANCY

Exosome-mediated cell-cell communication is required in remodeling tumor microenvironments and forming premetastatic niches during cancer development (Figure 2). Bioactive molecules of exosomes derived from cancer cells or stromal cells provide the essential signals for reprogramming of various cells and architectures in tumor microenvironments or premetastatic niches (Table 1).

3.1 | Tumor-derived exosomes in cancer progression

Cancer progression is a dynamic and multistep process in which several well-studied signaling events aid in orchestrating the development of cancer malignancy. Tumor-derived exosomes have been indicated to actively regulate cancer progression by inducing autocrine/paracrine oncogenesis, reprogramming stromal cells, modulating the immune system, while also promoting angiogenesis.[24]

Transfer of oncogenic molecules within oncosomes between primary tumor results in morphological transformation and an increase in anchorage-independent growth in recipient cancer cells.[22] Likewise, tumor-derived exosomes exert antiapoptotic effects of TGF-β1 signaling in an autocrine way, which subsequently renders the promotion of cancer proliferation.[33] ZFAS1 lncRNA enclosed within exosomes is transferred from malignant cancers to ZFAS1-negative cancer subpopulations to enhance the proliferation of ZFAS1-negative cancer subpopulations.[23] Through this advantageous paracrine route, some metabolites, such as intermediates of the tricarboxylic acid (TCA) cycle, are often packaged into CAF-derived exosomes and are transferred from CAF to cancer cells.[26] Subsequently, metabolic cargoes carried within exosomes enable glycolysis modulation and glutamine-dependent reductive carboxylation in cancers, contributing to cancer growth.[26] Reciprocally, tumor-derived exosomes regulate

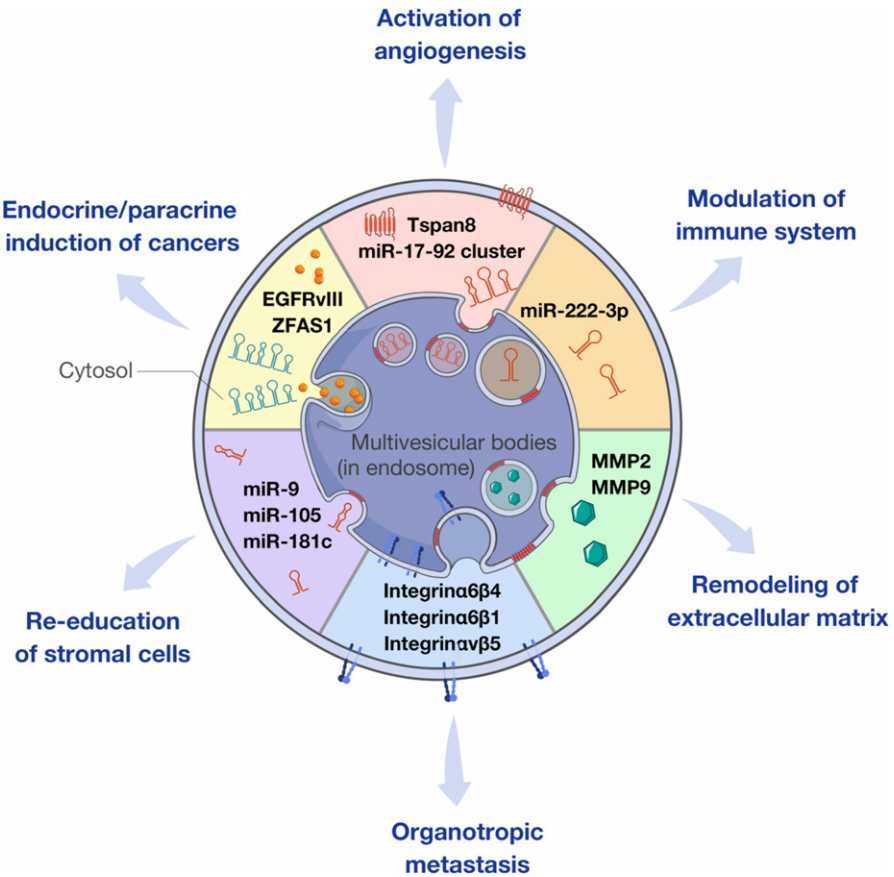

FIGURE 2 Summary of tumor-derived exosome-mediated functions. Tumor-derived exosomes regulate the autocrine/paracrine induction of cancers, activation of angiogenesis, modulation of the immune system, re-education of stromal cells, organotropic metastasis, and remodeling the extracellular matrix, contributing to cancer progression and metastasis. For example, tumor-derived exosomes transfer epidermal growth factor receptor (eEGFR) vIII oncogenic receptor or ZFAS1 lncRNA from aggressive cancers to nonaggressive cancers, inducing cancer progression. Also, tumor-derived exosomes that show tetraspanins or microRNA (miRNA) clusters induce endothelial migration and tube formation. Furthermore, tumor-derived exosomes containing miRNAs, such as miR-222-3p, induce polarization of M2 macrophages. Additionally, tumor-derived exosomes deliver miRNA, such as miR-9, miR-105, and miR-181c, from cancers to normal fibroblasts or vascular endothelial barriers, subsequently enhancing cancer malignancy. Moreover, integrins direct tumor-derived exosomes to specific distinct target organs, leading to metastatic organotropism. By delivery of extracellular matrix remodeling enzymes, tumor-derived exosomes contribute to cancer metastasis

TABLE 1 Functional effects of exosomal bioactive molecules in cancer development

Exosomal bioactive molecules	Type of bioactive molecule	Mechanism	Functional effect	Process	Cancer type	Reference
Delta-like 4	Protein	Inhibit Notch signal	Increase vessel branching and length	Modification of cancers and tumor microenvironment		29
EGFR vIII	Protein	Activate AKT and MAPK signal	Increase anchorage-independent growth		Glioma	22
Integrins	Protein	Activate Src and upregulate proinflammatory S100 genes	Direct exosomes to specific tissues	Metastatic organotropism	Breast cancer	30
MET	Protein	Activate MET signal	Increase prometastatic activity of bone marrow cells	Priming premetastatic niches	Melanoma	31
MIF	Protein	Activate TGF-β signal-induced fibronectin production	Increase liver premetastatic niche formation	Increase liver metastatic burden	Pancreatic cancer	28
TGF-β	Protein	Activate SMAD-related signal	Increase fibroblast FGF2 production	Trigger fibroblast to myofibroblast differentiation		25
TGF-β	Protein		Increase mesenchymal stem cell differentiation into myofibroblasts	Increase cancer proliferation and invasiveness	Prostate cancer	32
TGF-β1	Protein	Activate antiapoptotic and pro-survival signals	Increase proliferation and survival	Increase cancer growth	Chronic myeloid leukemia	33
Tspan8	Protein		Increase endothelial cell proliferation, migration, and sprouting	Increase angiogenesis	Adenocarcinoma	34
Snail and miR-146a	Protein and miRNA		Increase proliferation and drug resistance	Increase cancer proliferation and survival	Pancreatic cancer	35
miR-9	miRNA		Increase CAF-like property	Increase cancer growth	Breast cancer	36
miR-17-92 cluster	miRNA		Increase endothelial cell migration and tube formation	Increase angiogenesis	Leukemia	37
miR-21	miRNA	Regulate PTEN/PI3K/AKT signal	Inhibit apoptosis	Increase drug resistance	Gastric cancer	38
miR-105	miRNA	Downregulate tight junctions (ZO-1)	Destroy vascular endothelial barrier	Increase metastasis	Breast cancer	39
miR-181c	miRNA	Downregulate PDPK1/cofilin signal	Destroy blood-brain barrier	Increase brain metastasis	Breast cancer	40
miR-200	miRNA	Regulate gene expression and EMT	Increase cancer colonization in the lung	Increase metastasis	Breast cancer	41
miR-222-3p	miRNA	Regulate SOCS3/STAT3 pathway	Increase TAM polarization	Increase cancer progression	Epithelial ovarian cancer	42
ZFAS1	lncRNA	Regulate MAPK signal and EMT transcription factors	Increase cell cycle progression and EMT	Increase cancer growth and metastasis	Gastric cancer	23
hTERT mRNA	mRNA		Transform nonmalignant fibroblasts into telomerase-positive cells	Modification of cancer microenvironment		27

(Continues)

TABLE 1 (Continued)

Exosomal bioactive molecules	Type of bioactive molecule	Mechanism	Functional effect	Process	Cancer type	Reference
Amino acids, lipids, and TCA-cycle intermediates	Metabolites	Regulate mitochondrial oxidative phosphorylation, glycolysis, and glutamine-dependent reductive carboxylation	Downregulate mitochondrial function and upregulate glucose metabolism in cancers	Increase cancer growth	Prostate cancer	26

CAF, cancer-associated fibroblast; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FGF, fibroblast growth factor; lncRNA, long noncoding RNA; miRNA, micro RNA; TAM, tumor-associated macrophage; TCA, tricarboxylic acid; TGF, transforming growth factor.

endothelial angiogenic responses by promoting the formation of endothelial tubule networks, which could lead to cancer malignancy.[43]

Tumor exosomes direct stromal cell reprogramming and can also serve as a critical event driving cancer progression. In this regard, tumor-derived exosomes deliver miRNA, such as miR-9, that gives rise to the differentiation of fibroblasts into CAF with higher cell motility.[36] Moreover, miR-9 is also released from the CAF to enhance cancer progression.[36] Tumor-derived exosomes also trigger differentiation of mesenchymal stem cells (MSC) into myofibroblasts that show pro-angiogenic and pro-invasive characteristics.[32] The differentiated MSC then further promote cancer proliferation and invasion by secreting growth factors and matrix-regulating factors.[32]

Cancer cells require adequate oxygen and nutrients for growth and development. Tumor-induced angiogenesis supplies necessary oxygen and nutrients, while also serving to remove waste materials. Surface tetraspanins on tumor-released exosomes, such as Tspan8, can remotely activate resting endothelial cells, sprouting of endothelial cells and maturation of endothelial cell progenitors by upregulation of angiogenesis-related genes.[34] In addition, tumor-derived exosomes bearing miRNA clusters, such as miR-17-92 cluster, induce endothelial migration and tube formation.[37] Furthermore, neovascularization is observed as a result of increases in the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) that were prominently found in endothelial cells treated with tumor-derived exosomes.[44] Endothelial cells re-educated by tumor-derived exosomes show enhanced cell motility and tube formation ability.[44]

Accumulating evidence supports the involvement of exosomes in mediating communication between cancer cells and immune cells, such as macrophages, neutrophils, natural killer (NK) cells, dendritic cells, and T cells. Indeed, tumor-derived exosomes also regulate the polarization of macrophages. For example, macrophages that have acquired miR-222-3p within exosomes are capable of inducing polarization toward tumor-promoting M2 macrophages in a SOCS3/STAT3 signal-dependent way.[42] Additionally, tumor-derived exosomes facilitate tumorigenesis and cancer progression by enhancing differentiation of bone marrow-derived neutrophils and recruitment of neutrophils to cancer cells.[45] Clinically, exosomes collected from liquid biopsies of patients with acute myelogenous leukemia decreased the cytotoxicity of natural killer cells attributed to an increase in Smad phosphorylation and a decrease in NKG2D receptor expression.[46] This study suggests that tumor-derived exosomes facilitate cancer progression by attenuating immune responses.[46] Furthermore, tumor-derived microvesicles affect the functions of myeloid cells by ablating monocyte differentiation into dendritic cells, which subsequently suppresses the activity of T-cell proliferation and anticancer cytolytic functions.[47]

3.2 | Tumor-derived exosomes in cancer metastasis

Metastasis is the most common cause of cancer-related death. Multistep processes are required for cancer metastasis. In 1889, Stephen Paget postulated the "seed and soil" hypothesis, in which metastasis depends on the interaction between cancer cells (designated as the seed) and specific organ microenvironments (designated as the soil).[48] Metastatic cancer cells secrete soluble or vesicle-enclosed bioactive molecules that enable remodeling of extracellular matrix architecture and reprogramming of contributing cells in distant organ sites, such as bone marrow progenitor cells, CAF, tumor-associated macrophages (TAM), and tumor-associated neutrophils, toward establishing suitable premetastatic niches in advance of cancer metastasis.

According to Paget's "seed and soil" hypothesis, the nonrandom pattern of cancer metastasis was further elaborated by Hart and Fidler in 1980[49] observing that metastatic colonization was profoundly affected by interaction with the tumor microenvironment at specific target organs. Nevertheless, the regulatory mechanisms of organ-specific metastasis are poorly established. Until recently, a series of reports from Lyden's group substantiated that tumor-derived exosomes assist in priming premetastatic niches by activating bone marrow-derived vascular endothelial growth factor receptor 1 (VEGFR1)+ hematopoietic progenitor cells through the exchange of exosome-mediated MET oncoprotein.[31] Recently, the same group further indicated that integrin expression profiles of tumor-derived exosomes function as "ZIP codes" to direct exosomes to specific tissues/organs, leading to metastatic organotropism.[30] In light of proteomics and clinical relevance analyses, exosomal integrins α6β4 and α6β1 were positively correlated with lung metastasis, whereas exosome integrin αvβ5 was highly associated with liver metastasis.[30] Furthermore, tumor-derived exosomes induced the activation of Src and caused an upregulation of pro-inflammatory S100 genes in the recipient cells of target organs, leading to the establishment of premetastatic niches.[30]

In support of the above studies, several studies have also shown that tumor-derived exosomes deliver extracellular matrix remodeling enzymes such as MMP2 or MMP9, conferring degradation of extracellular matrixes and contributing to cancer invasion and metastasis.[50] Similarly, increase in TGF expression of Kupffer cells in the liver is achieved by MIF-1 contained within pancreatic cancer-derived exosomes, which subsequently leads to an increase in fibronectin production by hepatic stellate cells.[28] This remodeled microenvironment then further enhances the recruitment of bone marrow-derived macrophages, which contributes to the formation of premetastatic niche in the liver, providing suitable conditions for metastasis.[28]

The pathological relevance of tumor-derived exosomes on cancer metastasis is emphasized by their functional effect on the invasive capability of cancers.[51] Undoubtedly, invadopodia biogenesis and exosome secretion are concomitantly required for exosome-mediated cancer invasion.[52] Inhibition of exosome biogenesis or secretion by Rab27a knockdown significantly reduced the formation of mature invadopodia, as well as extracellular matrix digestion.[52] Interestingly, invadopodia are critical sites for exosome secretion, suggesting that exosomes regulate invasive activity in a synergistic way.[52] Furthermore, tumor-derived exosomes may transfer miRNAs from metastatic cancer cells to less metastatic cells, by which alterations in gene expression could facilitate metastasis within less metastatic cells.[41]

Brain metastasis is a leading cause of morbidity and mortality in cancer patients. Despite the highly selective permeability of the blood-brain barrier (BBB), metastatic cancer cells are still able to invade the central nervous system (CNS). Recent studies have shown that exosomes derived from metastatic cancer cells can disrupt the structure and function of the BBB.[39,40] miRNAs, such as miR-105 and miR-181c, within cancer exosomes are taken up by vascular endothelial cells and prompt the destruction of vascular endothelial barriers through targeting tight junction proteins or inducing abnormal localization of the cytoskeleton.[39,40] As a result, the destroyed vascular endothelial barriers permit cancer metastasis to the brain.

3.3 | Stroma-derived exosomes in cancer development

Active communications between stromal cells and cancer cells in facilitating cancer progression can also be achieved by exosome-mediated signaling activities. Regardless of how exosomes are secreted, either constitutively or induced upon signal activation, exosomes are released from stromal cells continuously to influence the pathogenicity of cancer cells.[50] Bioactive molecules, such as Notch ligands, can be transferred between endothelial cells and cancer cells in the tumor microenvironment.[29] Notch ligand Delta-like 4 is found within exosomes derived from endothelial cells. They inhibit Notch signaling in cancer cells or other endothelial cells, leading to the modification of cancers and the tumor microenvironment.[29] In addition, enormous numbers of exosomes were found to be released from CAF during chemotherapy.[35] Presumably, it is believed that such change is to promote survival, proliferation, and drug resistance of cancer cells, in part as a result of increased expression of chemoresistance-inducing factor, Snail.[35] Moreover, exosomes from TAM diminish chemotherapy sensitivity of cancers as a result of miR-21 activity.[38] These studies suggested that exosomes derived from tumor microenvironments play an active and essential role in the regulation of cancer chemo- and/or radiation resistance.

4 | EXOSOMES IN CLINICAL APPLICATIONS

Exosomes contribute to the pathophysiological development of cancers by delivering specific bioactive molecules crucial at various stages of cancer development, suggesting exosomes have the potential to serve as diagnostic biomarkers and therapeutic targets. The phenomenon of drug resistance associated with exosomes is emerging in various types of cancers. Therefore, understanding the mechanisms of exosome-mediated cancer therapeutic resistance should provide valuable information for precision cancer therapies. Moreover, exosomes are nonimmunogenic in nature and have been used as an anticancer drug delivery system, given that their membrane composition is similar to most of the cells in the body.

4.1 | Tumor exosomes as potential biomarkers

Cancer is actively evolving over time through each mutation and selection process that further promotes its malignancy. Being once removed from its parental cells, exosomal cargoes bare strong resemblance to the intracellular status of the original secreted cell. Thus, real-time detection of the changes within exosomal cargoes could provide insightful information for the fundamental prerequisite of precision medicine in terms of diagnosis, prognosis, and disease monitoring (Table 2). For example, early detection of cancer substantially improves the odds for successful therapeutic outcome and enhances survival rate; therefore, the unmet need for sensitive and precise biomarkers is vital. Advantages of identifying tumor markers within liquid biopsies are that it is minimally invasive, easily obtained, and rapid and economical relative to tissue biopsies. Moreover, the vast amount of dynamic information readily extracted from a patient is valuable in aiding identification of early detection biomarkers in cancer patients. In particular, lipid-based exosomes provide a more robust and enriched vehicle for vulnerable biological molecules in circulating fluids, such as serum, plasma, urine, and saliva. The stability of biological molecules in exosomes derived from blood plasma is high (over 90 days) under general storage conditions.[56] Moreover, the number of exosomes in body fluids is often found to be significantly higher in patients with disease.[56] To be noted, despite rapid and easy evaluation of cell-free DNAs (cfDNAs) and circulating tumor cells (CTC) within liquid biopsy, their characteristics in association with cancer development and progression are questionable and are limited in comparison with tumor exosomes. cfDNAs have been reported to carry characteristic mutations of the corresponding

TABLE 2 Exosomal bioactive molecules used as diagnostic and prognostic biomarkers in cancer

Biomarker	Type of biomarker	Type of body fluid	Analytical approach	Expression level	Cancer type	Reference
AR-V7 RNA	RNA	Plasma	PCR	Upregulated	Prostate cancer	53
EGFR vIII mRNA	mRNA	Serum	PCR	Upregulated	Glioblastoma	54
Gene	Gene	Urine	PCR	Gene expression	Prostate cancer	55
Integrins	Protein	Plasma	ELISA	Upregulated	Breast cancer	30
MIF	Protein	Plasma	ELISA	Upregulated	Pancreatic cancer	28
ZFAS1	Long noncoding RNA	Serum	PCR	Upregulated	Gastric cancer	23

EGFR, epidermal growth factor receptor.

primary cancers, whereas higher circulating tumor DNAs (ctDNAs) clearance processes are commonly observed in liver or kidney, implying a poor understanding of the stability and pathogenicity of cfDNAs.[57] Circulating tumor cells are extremely rarely observed in peripheral blood. Thus, to isolate and identify these rare circulating tumor cells among all other hematopoietic and immune cells is rather difficult and an effective isolating and detection approach is urgently needed.[58]

Recently, we found that specific integrin expression profiles of tumor-derived exosomes could function as "ZIP codes" that direct organotropic metastasis.[30] This is the first bioactive molecule that predicts organ-specific metastases of cancers, attributing to exosomal integrins as biomarkers of organotropic metastatic potential.[30] Similarly, a specific exosome signature, including tyrosinase-related protein-2 (TYRP2), very late antigen 4 (VLA-4), heat shock protein 70 (HSP70), HSP90 isoform, and MET, was identified to be abundantly expressed during the late stage of melanoma, serving as an alternative diagnosis of aggressive melanoma using liquid biopsies.[31] Clinically, exosomes derived from serum samples of patients with glioblastoma bear specific EGFR VIII, reinforcing tumor-derived exosomes as sources of biomarkers reflecting the status of parental cancer cells.[54] In addition, specific gene expressions of exosomes in urine are correlated with patients having high-grade prostate cancer, which sheds light on the advantages of exosomes in the diagnosis and prognosis of cancer development.[55] In contrast, androgen receptor splice variant 7 RNA expression in exosomes derived from patients with metastatic prostate cancer highly predicts the development of endocrine therapy resistance.[53] Together, these basic and clinical studies imply a potential role of exosomes in disease prognostication.

4.2 | Tumor exosomes as therapeutic targets

Cargoes of tumor-derived exosomes attribute to cancer development. Thus, alternative therapeutic strategies, such as blockage of exosome production, secretion, and exosome-mediated cell-cell communication, as well as ablation of specific active exosomal cargos, have been proposed as novel cancer interventions (Table 3). Indeed, inhibition of the ESCRT-dependent or the ESCRT-independent mechanism-mediated exosome biogenesis, such as syndecan/syntenin/ALIX signaling[60] or sphingomyelinases,[11] respectively, has shown detrimental effects in exosome production and on cancer progression. Another critical protein significant in exosome secretion, Rab27 small GTPase, is involved in regulating the docking of multivesicular endosomes onto the plasma membrane and the size of multivesicular endosomes.[61] Cancer proliferation and metastasis were hindered upon inhibiting Rab27a.[45]

An inhibitor of clathrin-mediated endocytosis, chlorpromazine, was shown to impede cancer malignancy in vitro by targeting the mechanism of exosome uptake by endocytosis or macropinocytosis.[62] Furthermore, the surface proteins of tumor-derived exosomes display specific glycosylation patterns that are involved in the regulation of exosome uptake by recipient cells.[59] Such a finding suggests that alteration in the glycosylation of exosomal proteins can be potent in cancer progression. Recently, a cancer treatment strategy for extracorporeal hemofiltration of exosomes from the circulation by an affinity plasmapheresis platform has been proposed,[63] suggesting that removal of exosome from the circulatory system provides an additional strategy for therapeutic reagents to block the oncogenic signal on cancers. Together, these studies suggest that various potential therapeutic strategies by intercepting biogenesis, secretion, or uptake of tumor-derived exosomes are promising means for the development of anticancer therapies.

4.3 | Roles of tumor exosomes in drug resistance

Drug resistance in cancer occurs from tumor exosomes through a drug efflux-dependent mechanism by pumping chemotherapeutic agents out of cancer cells. Intercellular transfer of exosomal miRNAs/proteins between drug-resistant cells and drug-sensitive cells leads to modified gene expression in the drug-sensitive cell population. Such change in gene expression endows the sensitive cells with an antiapoptotic ability when met with the drug, an ability that is also observed in drug-resistant cells.[69] Such phenomenon was observed in docetaxel-resistant tumors that secrete exosomes containing P-glycoprotein, a type of drug efflux pump protein. These exosomes were taken up by drug-sensitive cancer cells, and drug resistance within these sensitive cells was observed.[70]

Furthermore, exosomes derived from HER2-overexpressing breast cancers display activated HER2 protein that regulates the degree of sensitivity to trastuzumab, an anticancer drug. Moreover, activated HER2 protein from exosomes contributes to oncogenic signal-mediated cancer malignancy.[71] Other reports have shown that tumor-derived exosomes can also protect target cells by transporting

TABLE 3 Candidate mechanisms of cancer therapy by targeting/using exosome

Candidate mechanisms	Treatment approach	Status	Reference
Ablate exosomal cargo	Alternate exosomal glycosylation	In vitro	59
Block exosome production	Inhibit syndecan/syntenin/ALIX signal	In vitro	60
Block exosome production	Inhibit sphingomyelinase	In vitro	11
Block exosome secretion	Inhibit Rab27 small GTPase	In vitro	61
Block exosome-mediated cell-cell communication	Inhibit endocytosis and macropinocytosis	In vitro	62
Block oncogenic signal	Extracorporeal hemofiltration of exosomes		63
Cytotoxic effects in brain cancer	Exosome delivery of anticancer drugs across BBB	In vivo	64
Cytotoxic effects in cancer	Exosome delivery of anticancer drug	In vitro	65
Increase the targeting specificity of exosome	Specific RGD peptide-fused exosomes	In vivo	66
Immunotherapy	Dendritic cell-derived exosomes loaded with MAGE cancer antigens	Phase I clinical trial	67
Immunotherapy	IFN-γ-dendritic cell-derived exosomes loaded with MHC class I- and class II-restricted cancer antigens	Phase II clinical trial	68

ALIX, ALG-2-interacting protein X; BBB, blood-brain barrier; IFN, interferon; MAGE, melanoma antigen gene; RGD, Arg-Gly-Asp.

an abundance of proteins targeted by drugs and neutralizing the effects of the drug on target cells.[72] Similarly, cells of the tumor microenvironment also release exosomes that placate drug resistance in cancer cells. Stroma-derived exosomes have been shown to modulate the sensitivity of chemotherapy and radiation in cancer cells by regulating STAT1-dependent antiviral and NOTCH3 signaling.[73] Together, these studies highlight varied mechanisms of exosome-mediated drug resistance either through pumping anticancer drugs out of cells or transferring bioactive molecules between cells.

4.4 | Roles of exosomes in drug delivery

Resembling liposomes, naturally secreted exosome vesicles have garnered much attention as drug-delivery vehicles. First of all, the nanometric-sized exosomes can be easily transferred between cells. Second, the lipid bilayer-membrane structure of exosomes confers a protected environment for bioactive molecules from degradation in the extracellular milieu.[3] Third, exosomes show lower immunogenicity and toxicity than other drug-delivery strategies.[74] Last, exosomes bearing specific surface proteins, such as integrins, can direct themselves to specific organs.[30] These features of exosomes implicate that exosomes can be efficient drug-delivery vesicles for the delivery of anticancer agents, siRNAs, or proteins (Table 3). Interestingly, exosomes transfer anticancer drugs through the BBB, leading to cytotoxic effects in *Danio rerio* brain cancers.[64] Prevalently, exosomes loaded with anticancer drug derived from autologous cancers can be taken up by parental cancer cells through endocytosis, leading to increased cytotoxicity in parental cancer cells.[65] In terms of targeting specificity, αv integrin-specific RGD (Arg-Gly-Asp) peptide was fused on exosomes loaded with anticancer drug (ie, doxorubicin) to significantly improve exosome uptake by αv integrin-positive cancer cells, leading to inhibition of cancer growth.[66]

Intrinsically, exosomes have been recognized as novel cell-free vaccines in immunotherapy.[9] Cancer antigens loaded into exosomes derived from autologous dendritic cells facilitate anticancer immune responses (ie, induced natural killer, NK, cell effector functions) in patients with advanced non-small-cell lung cancer.[67] Further study used exosomes from interferon-γ-mature dendritic cells to accelerate anticancer immune responses in both NK and T cells.[68] Increase in NK cell activity and longer progression-free survival rate were observed in patients with advanced non-small-cell lung cancer.[68] Together, these studies suggested that exosomes function as potential drug-delivery vehicles or cell-free vaccines in anticancer therapies.

5 | CONCLUSION

Normal cell homeostasis relies upon the exchange of biological materials across the membranes and such transport is facilitated through vesicles that compartmentalize cargo to its appropriate destination. Exosomes are these vesicles that function as mediators of intercellular communication. They are ubiquitously discharged into the extracellular milieu and have unique features depending upon the secreted cell of origin. Past studies have suggested that, in an aberrant state, like cancer, exosomal protein withholds cargoes that disclose information regarding the state of the secreting cell, while also providing insights to the progression of the recipient cell. Exosome-mediated cell-to-cell communication has emerged as an indispensible regulatory process in cancer tumorigenesis and metastasis, as well as in chemotherapeutic resistance. Exosomes assist in the process of organotropic metastasis, and additional critical oncogenic signals also take part in reconciling the selectivity and functionality of exosome cargoes involved in organotropic metastasis. As a result of the complex regulatory mechanisms and cross-talk mediated by exosomes between cancer cells and stromal cells, details of these processes require further investigation. In addition, the origin and biological significance of heterogeneity in exosomes remain largely unknown because of a lack of analytical platforms and available technologies. At last, we can appreciate the reciprocal interaction of exosomes between cancer cells and stromal

cells. Exosomes are emerging as promising biomarkers and valuable therapeutic targets closely aligned with the development of precision medicine. Moreover, they can function as potential drug-delivery vehicles or cell-free vaccines, providing alternative strategies for exosome-based anticancer therapies. Together, comprehensive studies clarifying the roles of exosomes in various cancers and health states can revolutionize current diagnostic and therapeutic tools in medicine.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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