Molecular Therapy
Nucleic Acids
Review

Emerging Function and Clinical Values of Exosomal MicroRNAs in Cancer

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Exosomes are a subset of membrane-bound extracellular vesicles with diameters ranging from 30 to 100 nm. Exosomes enclose a variety of molecules, such as lipids, proteins, and non-coding RNAs. In the past decades, microRNAs (miRNAs) have attracted great attention in cancer research, as they play an important role in the occurrence and development of cancer. Increasing evidence indicates that tumor cells communicate with not only other tumor cells but also cells present in the tumor microenvironment via secretion and transfer of exosomal miRNAs. More importantly, exosomal miRNAs are found to serve as signaling molecules to regulate tumor growth, angiogenesis, metastasis, sensitivity to chemotherapy, and immune evasion. Deregulated expression of exosomal miRNAs is an early event in carcinogenesis and may reflect the malignant characteristics of cancer. Owing to the wide existence and high stability of exosomal miRNAs in body fluids, they may represent a novel class of non-invasive biomarkers for cancer. In this review, we highlight the recent advances on the functional role of exosomal miRNAs in cancer pathogenesis. We also discuss the potential clinical utility of exosome-shuttled miRNAs as biomarkers for the diagnosis and treatment of cancer.

Exosomes are 30- to 100-nm extracellular vesicles of endosomal origin, comprising a lipid bilayer and a great variety of bioactive molecules, such as nucleic acids, lipids, and proteins.[1] Exosomes are released from all cell types, including epithelial cells, immune cells, and tumor cells.[2–4] Notably, tumor cells secrete excessive amounts of exosomes compared with normal proliferating cells.[5,6] Exosomes form within intracellular multivesicular bodies (MVBs), and they are secreted upon fusion of MVBs with the plasma membrane.[7] Exosomes can be detected in almost all body fluids, including blood, urine, and saliva.[8–10]

Exosomes are critical vehicles for intercellular communication. Thus, they have multiple physiological roles, such as maintenance of cellular homeostasis through the release of intracellular harmful components and activation of immune responses through the delivery of antigens or activating ligands.[11,12] Emerging evidence demonstrates the crucial role of exosomes in tumor development. Exosomes can be trafficked between tumor cells or between tumor cells and the surrounding microenvironment.[13] Cancer-associated exosomes can promote tumor survival and growth.[14] They contribute to the establishment of a tumor-promoting niche by inducing angiogenesis, remodeling the extracellular matrix, and impairing the function of immune cells.[15–17] Additionally, exosomes can transmit drug-resistance characteristics among tumor cells.[18]

MicroRNAs (miRNAs) are the most extensively studied class of short non-coding RNAs (ncRNAs).[19] miRNAs regulate the expression of target genes at the post-transcriptional level, primarily via binding to fully or partially complementary sites within the 3′ UTR of target mRNAs.[20] miRNAs are involved in a variety of cellular processes, including cell proliferation, differentiation, and death.[21,22] Notably, miRNAs have been found to play an important role in the onset and progression of cancer, such as tumor growth, invasion, and metastasis.[23–26] Therefore, miRNAs may be promising biomarkers for cancer diagnosis and prognosis.[27] miRNAs are critical exosomal constituents, and exosomal miRNAs are confirmed to participate in the occurrence and development of cancer.[28] miRNA-containing exosomes can be shed from parental cells into the circulation,[29,30] demonstrating that exosomal miRNAs may serve as ideal non-invasive biomarkers for cancer. In this review, we summarize the current knowledge on the role of exosomal miRNAs in carcinogenesis and cancer progression. We also discuss the potential applications of exosomal miRNAs as cancer biomarkers in clinical practice.

Exosome Biogenesis

Exosomes are generated by both normal and pathological cells and are found in all body fluids.[31] Exosomes originate from the endosomal compartment.[32] The biogenesis of exosomes involves two steps.[33] First, the internalization of the cell membrane leads to the formation of early endosomes (Figure 1). Multiple intraluminal vesicles (ILVs) are then formed by the inward invagination of endosomal membranes, resulting in the formation of MVBs. During this process, cytosolic constituents, including nucleic acids, proteins, and lipids, can be sorted into ILVs. Upon fusion of MVBs with the plasma membrane, ILVs are released as exosomes into the extracellular milieu.

Some factors have been found to be involved in exosome biogenesis. The endosomal sorting complexes required for transport (ESCRTs)

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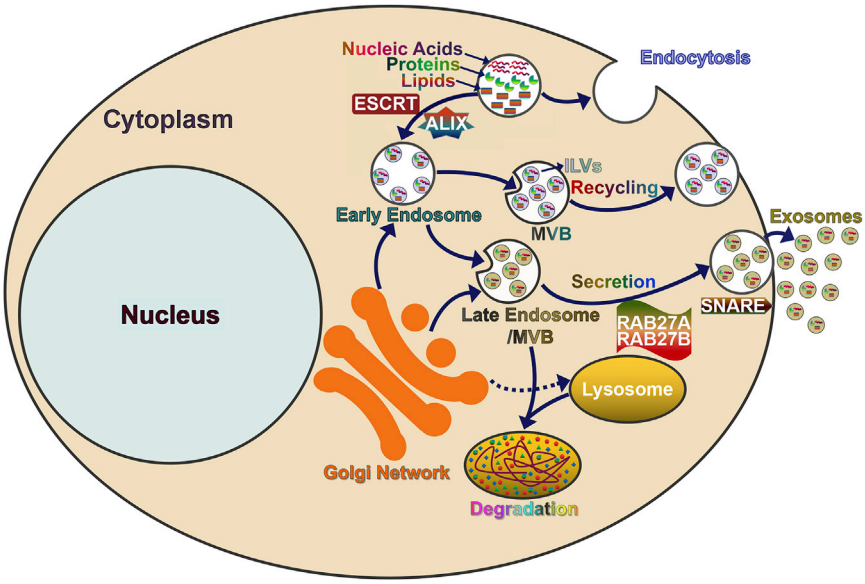

Figure 1. Schematic Representation of the Biogenesis and Secretion of Exosomes

Exosomes are formed as intraluminal vesicles (ILVs). The cargoes (nucleic acids, proteins, and lipids) are ingested by the cells through the endocytotic pathway and are then transported to early endosomes. The maturation of early endosomes gives rise to multivesicular bodies (MVBs), late endosomes containing numerous ILVs. During the process of ILV generation, nucleic acids (miRNAs, DNAs, and RNAs), proteins (cytoplasmic proteins, tetraspanins, and membrane receptors), and lipids (ceramides and cholesterol) are incorporated into exosomes. Several molecules are involved in the biogenesis of ILVs, such as ESCRT and ALIX. MVBs can fuse with the cellular membranes to release exosomes into the extracellular space. Alternatively, MVBs can fuse with lysosomes, which results in the degradation of engulfed contents. MVB fusion with the cellular membrane is a fine-tuned multistep process involving MVB trafficking along microtubules, docking at the cellular membrane, and fusion to the cellular membrane. Several Rab GTPases (Rab27a and Rab27b) are implicated in the transport of MVBs to the cellular membrane and in the release of exosomes. Additionally, SNARE complexes may facilitate the fusion of MVBs with the cellular membranes. Exosomal cargoes can be delivered to the recipient cells via endocytosis, fusion with the cellular membrane, or ligand-receptor interaction.

are essential for MVB biogenesis, in which cargo sorting is coupled to the invagination and detachment of ILVs.[34] ESCRT-0 is responsible for the assembly and gathering of cargoes. ESCRT-I and -II harbor various membrane-binding sites, and they can promote the internalization of an endosomal membrane. Thus, ESCRT-I and -II are capable of inducing the formation of a membrane bud, in which cargoes are confined. ESCRT-II then recruits ESCRT-III subunits, the vacuolar protein sorting-associated protein 20 (Vps20) and the eukaryotic sucrose non-fermenting protein 7 (Snf7), to the neck of the membrane bud. The ESCRT-III complex directs membrane scission from the cytoplasmic side of the bud. Following scission, cargoes are entrapped in ILVs while ESCR-III persists on the outside of the remaining membrane until it is recycled.

Moreover, accessory proteins, such as ALG-2-interacting protein X (ALIX) and tumor susceptibility gene 101 (TSG101), play a crucial role in cargo packaging and exosome biogenesis. ALIX not only encloses cargoes to enter internalized vesicles but also induces vesicle formation.[35] ALIX recruits the ESCRT-III complex, resulting in the formation of ILVs.[36] TSG101, a component of the ESCRT machinery, is required for epidermal growth factor (EGF)-stimulated MVB formation.[37] Accordingly, depletion of TSG101 may suppress MVB formation. Members of the Rab GTPase family (e.g., Rab27a and Rab27b) mediate the trafficking of MVBs to the site of the cellular membrane.[38,39] The formation and secretion of exosomes requires contractile machinery that is able to draw opposing membranes together prior to shearing the membrane connection and releasing ILVs into the extracellular milieu.[40] Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes may facilitate the fusion of MVBs with the cellular membrane.[41]

Potential Mechanisms for miRNA Sorting to Exosomes

Exosomal cargo represents a novel focus of investigation that would enrich our knowledge of the molecular mechanisms underlying the role of exosomes in cancer progression. A great variety of molecules can be incorporated into exosomes, including miRNAs, DNAs, RNAs, and proteins. Among these bioactive compositions, miRNAs have attracted substantial attention owing to their regulatory function in gene expression. miRNAs are short ncRNAs of approximately 19–24 nt in length.[42] miRNAs function to suppress the expression of protein-coding genes at the post-transcriptional level.[43–45] Moreover, miRNAs and their target genes constitute complicated regulatory networks that contribute to the fine-tuning of various biological processes, such as cell proliferation, differentiation, and death.[46,47] More importantly, miRNA dysregulation is found to be associated with cancer progression.[48–50]

miR-142-3p, miR-150, and miR-451 were found to be enriched in exosomes as compared to parent cells, demonstrating that these miRNAs were preferentially packaged into exosomes.[51] Likewise, members of the let-7 miRNA family were more abundant in exosomes derived from gastric cancer (GC) cells than those derived from other cancer cells.[52] Remarkably, some exosomal miRNAs (e.g., miR-21, let-7f, miR-20b, and miR-30e-3p) exhibited different expression levels in cancer patients compared with healthy controls.[53,54] Thus, cells adopt specific sorting mechanisms that direct miRNA loading into exosomes, leading to the selective encapsulation of miRNAs into cell-secreted exosomes.

Four potential pathways for sorting miRNAs into exosomes are proposed (Figure 2). The neural sphingomyelinase 2 (nSMase2)-dependent pathway was found to guide miRNAs sorting into exosomes.

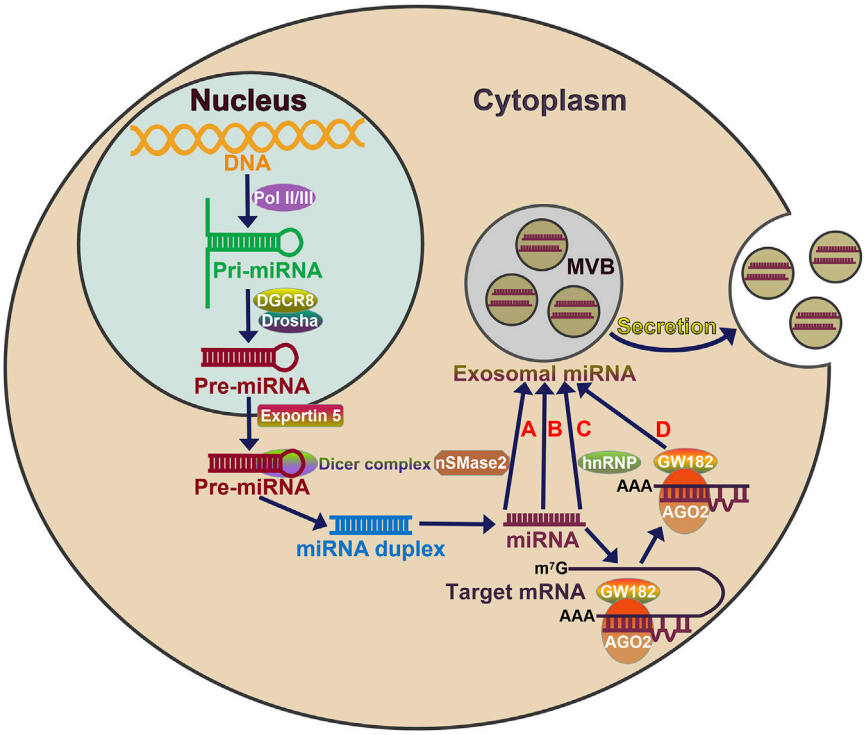

Figure 2. Biogenesis, Packaging, and Secretion of Exosomal miRNAs

miRNA genes are initially transcribed into primary miRNAs (pri-miRNAs) in the nucleus. A nuclear complex consisting of Drosha and DGCR8 cleaves the pri-miRNAs, resulting in the generation of precursor miRNAs (pre-miRNAs). Exportin 5 is responsible for nuclear export of the pre-miRNAs. In the cytoplasm, the pre-miRNAs are further processed by the Dicer complex into double-strand miRNAs (~22 nt). One strand of the miRNA duplex (the mature miRNA) is selected to be incorporated into the RNA-induced silencing complex (RISC). The main components of the miRISC include miRNA, miRNA-targeted mRNA, GW182, and Argonaute 2 (Ago2). The miRNA in the miRISC can bind to the complementary sequence in the 3′ UTR of its target mRNA, which causes mRNA destabilization and translation suppression. Mature miRNAs are generally sorted into exosomes via four potential pathways: A, nSMase2-dependent pathway; B, the 3′ end of the miRNA sequence-dependent pathway (the 3′ end of the miRNA sequence contains an important sorting signal and thus guides specific miRNAs to be packed into exosomes); C, the miRNA motif and sumoylated hnRNP-dependent pathway (three hnRNP family proteins, hnRNPA2B1, hnRNPA1, and hnRNPC, guide miRNA loading into exosomes, and, among them, sumoylated hnRNPA2B1 specifically recognizes the GGAG motif in the 3′ portion of miRNA sequences and governs the sorting of miRNAs into exosomes); and D, the miRISC-related pathway. The RISC can co-localize with MVBs. Ago2 and miRNA-targeted mRNA are also associated with the exosomal assortment of miRNAs. Finally, MVBs fuse with the cellular membrane and release exosomes containing miRNAs into the extracellular space.

nSMase2 was the first molecule found to be associated with miRNA packaging into exosomes.[55] Overexpression of nSMase2 caused an increase in the abundance of exosomal miRNAs.[56] In contrast, inhibition of nSMase2 expression decreased the levels of exosomal miRNAs. The second sorting mechanism involves the sumoylated heterogeneous nuclear ribonucleoprotein (hnRNP)-dependent pathway. Three hnRNP family proteins (hnRNPA2B1, hnRNPA1, and hnRNPC) bound to exosomal miRNAs and induced the loading of miRNAs into exosomes.[57] Specially, the sumoylated hnRNPA2B1 controlled the exosomal assortment of miRNAs by recognizing the GGAG motif in the 3′ part of miRNA sequences.

The third pathway depends on the 3′ end of the miRNA. The 3′ end of the miRNA sequence might contain an important sorting signal that contributed to guiding its incorporation into exosomes.[58] The last sorting pathway is mediated by the miRNA-induced silencing complex (miRISC). The primary components of miRISC were found to co-localize with MVBs.[59] Moreover, blockade of the turnover of MVBs into lysosomes resulted in excessive accumulation of miRISCs, while suppression of MVB formation caused the loss of miRISC and relieved miRNA-mediated gene silencing.[60] It was reported that exosomal miRNA sorting was controlled by cell activation-dependent changes of miRNA target abundance.[61] Artificially elevating the cellular levels of miRNAs or their target mRNAs benefited miRNA enrichment in MVBs. Additionally, Argonaute 2 (Ago2), a component of the RISC, was also implicated in the exosomal assortment of miRNAs. Knockout of Ago2 could reduce the abundance of some exosomal miRNAs, including miR-142-3p, miR-150, and miR-451.[51] Collectively, certain sequences present in miRNAs may favor their uptake into exosomes, and specific protein complexes may be involved in the loading of miRNAs into exosomes. Nevertheless, the sorting mechanisms of exosomal miRNAs remain unclear and deserve further investigation.

Roles of Exosomal miRNAs in Cancer

Exosomal miRNAs have been shown to exert multifaceted effects on cancer progression, including the regulation of cancer growth, angiogenesis and metastasis, domination of host immune responses, manipulation of cancer chemoresistance, and remodeling of the tumor microenvironment (Figure 3).

Exosomal miRNAs Affect Cancer Cell Growth

Proliferation is a crucial aspect of cancer progression that is commonly manifested by the deregulation of cell cycle-related proteins. Tumor growth is a contributing factor to the development of tumor angiogenesis and metastasis. Emerging evidence demonstrates that cancer-secreted exosomal miRNAs regulate cancer cell proliferation by targeting cell cycle-associated proteins or signaling pathways.

Exosomal miR-200b could be transmitted between colorectal cancer (CRC) cells, and it promoted the proliferation of recipient cells by

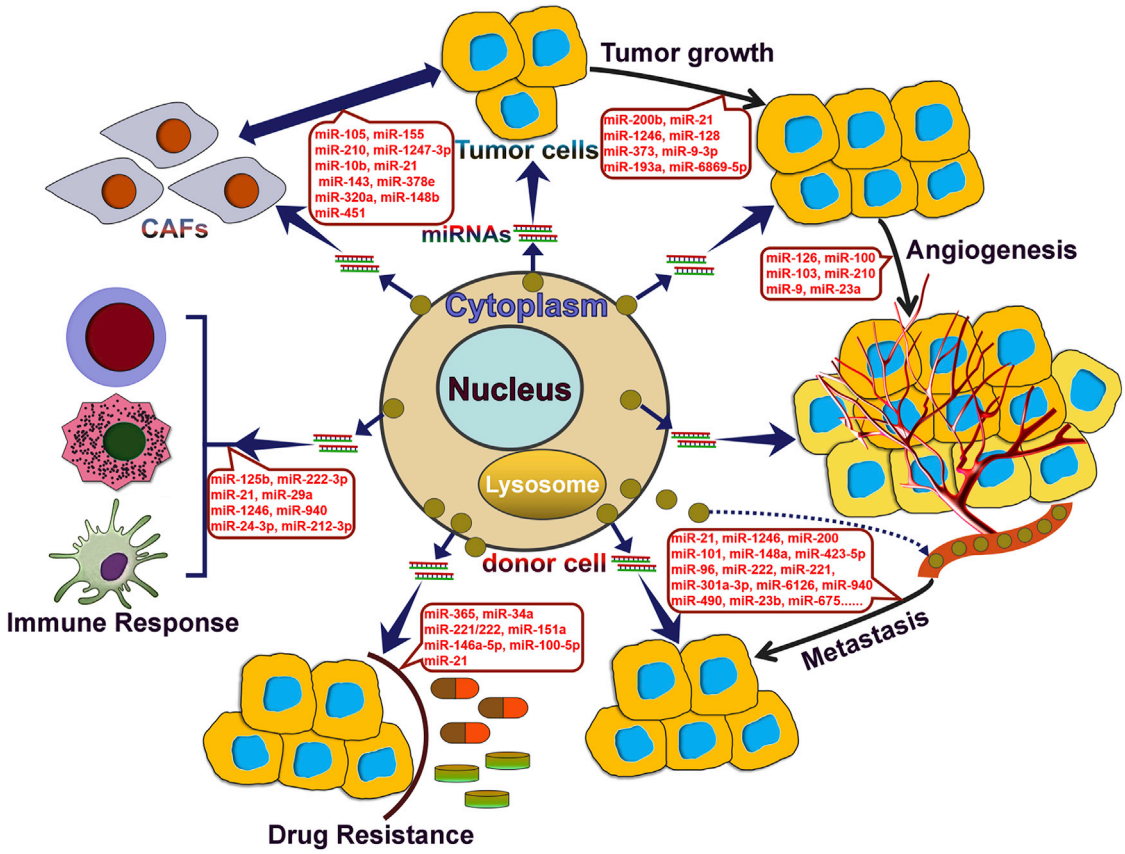

Figure 3. Summary of the Function of Exosomal miRNAs in Cancer

Exosomal miRNAs play multifaceted roles in tumor initiation and development. Tumor cells could transfer exosomal miRNAs to surrounding tumor cells. In recipient tumor cells, exosomal miRNAs can control cell proliferation, invasion, and metastasis by orchestrating their downstream targets. Exosomal miRNAs mediate a special communication between tumor cells and endothelial cells, thus playing an important role in tumor angiogenesis. Drug-resistant tumor cells can transmit the resistant phenotype to drug-sensitive tumor cells through the horizontal transfer of exosomal miRNAs. In terms of mechanism, exosomal miRNAs mainly control the apoptotic or autophagic pathways, hence conferring chemoresistance to recipient tumor cells. Exosomal miRNAs serve as key messengers for exchanging information between tumor cells and immune cells (macrophages, T cells, and dendritic cells), contributing to the formation of a tumor-promoting, immunosuppressive microenvironment. In addition, exosomal miRNAs are instrumental for the cross-talk between tumor cells and CAFs within the tumor microenvironment. Tumor-derived exosomal miRNAs are capable of reprogramming and activating CAFs, thus facilitating tumorigenesis and tumor development. Exosomal miRNAs derived from adjacent CAFs in turn modulate the malignant phenotype of tumor cells.

lowering p27 expression.[62] Exosomal miR-6869-5p could depress CRC cell proliferation and inhibit the production of inflammatory cytokines (interleukin-6 [IL-6] and tumor necrosis factor alpha [TNF-α]) in CRC cells by blocking the Toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB)-signaling pathway.[63] Inhibition of oncogenic miR-21 sorting into exosomes suppressed the growth and migration of hepatocellular carcinoma (HCC) cells.[64] Exosomal miR-9-3p restrained HCC cell proliferation by directly targeting fibroblast growth factor 5 (HBGF-5).[65] Exosomal miR-1246, enriched in metastatic breast cancer cells, could be transferred into non-malignant breast cells.[66] miR-1246 promoted the proliferation, migration, and drug resistance of breast cancer cells by downregulating cyclin-G2 (CCNG2). Exosomal miR-193a impeded the cell cycle progression and exerted an inhibitory effect on colon cancer cell proliferation by directly targeting cell cycle-associated protein 1 (Caprin1).[67]

Moreover, exosomal miRNAs can orchestrate the apoptotic signaling pathway in cancer cells. For instance, exosomal miR-128 reduced the expression of Bcl-2-associated X protein (Bax) in recipient breast cancer cells.[68] Exosomal miR-373 repressed the expression of estrogen receptor (ER) and inhibited the apoptosis of breast cancer cells.[69] Human adipose mesenchymal stem cell (hAMSC)-derived exosomal miRNAs could induce the apoptosis of human ovarian cancer (OC) cells by upregulating apoptotic proteins (Bax, caspase 3, and caspase 9) and downregulating anti-apoptotic Bcl-2.[70] Exosomal miR-101 promoted GC cell apoptosis by targeting anti-apoptotic myeloid cell leukemia-1 (Mcl-1).[71]

Exosomal miRNAs Modulate Cancer Cell Invasion and Metastasis

The epithelial-mesenchymal transition (EMT), a vital process in cancer cell invasion and metastasis, is characterized by the downregulation

of cell adhesion molecules (E-cadherin and β-catenin) with the concomitant acquisition of mesenchymal markers (N-cadherin and vimentin).[72] Exosomal miRNAs serve as significant regulators in the EMT process. Exosomal miR-21 significantly increased the expression of Snail and vimentin and decreased the expression of E-cadherin, contributing to the induction of EMT-related changes in oral squamous cell carcinoma (OSCC) cells.[73] Exosomal miR-1246 could repress the activities of N-cadherin and vimentin, hence restraining the EMT process in prostate cancer cells.[74] The miR-200 family derived from malignant colon cancer negatively modulated EMT-inducing transcription factors, zinc finger E-box-binding homeobox 1 (ZEB1) and Snail2 (Slug).[75] Exosomal miR-101 repressed GC cell invasion and migration by inhibiting ZEB1.[71]

Exosomal miRNAs serve a crucial role in tumor-endothelial crosstalk, thereby regulating angiogenesis and cancer progression. HCC cell-derived exosomal miR-103 enhanced vascular permeability and promoted tumor metastasis by targeting various endothelial junction proteins (VE-cadherin, p120-catenin, and zonula occludens 1) in endothelial cells.[76] Hypoxic leukemia cell-secreted exosomal miR-210 enhanced the angiogenic activity in endothelial cells by targeting the receptor tyrosine kinase ligand Ephrin-A3.[77] Oppositely, exosomal miR-126 served a negative role in leukemia cell motility and adhesion by lowering the expression of C-X-C motif chemokine ligand 12 (CXCL12) and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells.[78] Exosomal miR-210, released by metastatic breast cancer cells, could be transported to endothelial cells and enhanced the angiogenesis in recipient cells.[56] Exosomal miR-23a secreted by nasopharyngeal carcinoma (NPC) cells promoted the growth, migration, and tube formation of endothelial cells, and it also enhanced the outgrowth of blood vessels by inhibiting testis-specific gene antigen 10 (TSGA10).[79] In contrast, exosomal miR-9 suppressed NPC cell migration and angiogenesis by directly targeting *midkine* (*MDK*) and modulating the phosphoinositide-dependent protein kinase (PDK)/protein kinase B (Akt)-signaling pathway.[80] Exosomal transfer of miR-23a from hypoxic hepatocarcinoma cells induced the angiogenesis in recipient endothelial cells by targeting sirtuin 1 (SIRT1).[81] Similarly, exosomal miR-23a derived from hypoxic lung cancer cells suppressed the expression of prolyl hydroxylase (PHD) and tight junction protein zonula occludens-1 (ZO-1), thus enhancing vascular permeability and cancer trans-endothelial migration.[82]

Exosomal miRNAs are able to affect the invasive and metastatic behaviors of cancer cells. miR-148a delivered by exosomes promoted glioblastoma (GBM) cell proliferation and metastasis via activating the signal transducer and activator of transcription 3 (STAT3) pathway by targeting cell adhesion molecule 1 (CADM1).[83] Exosomal miR-423-5p could be internalized into GC cells and promoted cancer cell proliferation and migration by targeting suppressor of fused protein (SUFU).[84] Exosomal miR-96 directly targeted LIM domain-only protein 7 (LMO7), and it enhanced the proliferation and migration of lung cancer cells.[85] Liver kinase B1 (LKB1) was found to enhance lung cancer cell motility by reducing exosomal secretion of migration-suppressing miRNAs (miR-125a, miR-126, and let-7b).[86] miR-222 could be transferred between tumor cells, and it enhanced tumor malignancy in melanoma through activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway.[87] Exosomal miR-6126 limited the invasion and migration of OC cells by inhibiting integrin-β1, a vital modulator of cancer cell metastasis.[88] Exosomal miR-940 suppressed the proliferation, invasion, and migration of OC cells.[89] Mechanistically, miR-940 directly targeted the proto-oncogene tyrosine-protein kinase (SRC). The transfer of tumor-suppressive miR-940 by cancer-derived exosomes might enable OC cells to maintain their invasiveness and tumorigenic phenotype. Exosomal miR-490 derived from mast cell (MC) blocked the epidermal growth factor receptor (EGFR)/Akt/extracellular signal-regulated kinase (ERK) 1/2 pathway in recipient HCC cells, which led to the attenuation of HCC cell migration.[90] Exosomal delivery of miR-23b from the bone marrow inhibited the invasion and migration of breast cancer cells by inhibiting its target gene, *myristoylated alanine-rich C kinase substrate* (*MARCKS*).[91]

Exosomal miRNAs derived from metastatic cancer cells may affect the behavior of less aggressive cancer cells or even normal cells. The let-7 family of miRNAs was more abundant in exosomes from highly invasive OC cells than lowly invasive OC cells.[92] Exosome-mediated transfer of these miRNAs might be correlated with the invasive activity of OC cells. Exosomes derived from brain metastatic (BM) cancer cells could be taken up by non-BM cancer cells.[93] Deregulated exosomal miRNAs (miR-210, miR-19a, and miR-29c) might contribute to the increased adhesive and invasive abilities of non-BM cells. Exosomal miR-342-3p and miR-1246 derived from highly metastatic OSCC cells could be transferred to poorly metastatic OSCC cells and promote the growth, migration, and invasion of recipient cancer cells.[94] Remarkably, exosomal miR-1246 induced a highly metastatic phenotype in recipient OSCC cells by downregulating DENN/MADD domain-containing protein 2D (DENND2D). Exosomal miR-675 enriched in metastatic osteosarcoma (OS) cells could target calneuron1 (CALN1), and, thus, it modulated the invasion and migration of non-malignant fibroblast cells.[95] miR-10b derived from metastatic breast cancer cells could be delivered into normal mammary epithelial cells through exosomes.[96] The transferred miR-10b enhanced the invasive capability in mammary epithelial cells by reducing the expression of homeobox protein hox-D10 (HOXD10) and kruppel-like factor 4 (KLF4). miR-21 could be transferred from cigarette smoke extract (CSE)-transformed human bronchial epithelial (HBE) cells to normal HBE cells via exosomes.[97] Moreover, exosomal miR-21 activated STAT3 and increased the expression of vascular endothelial growth factor (VEGF), contributing to the induction of angiogenesis and malignant transformation in HBE cells. Taken together, these studies demonstrate that exosomes from aggressive cancer cells spread malignant properties by a horizontal transfer of miRNAs.

Cancer cells commonly initiate metastasis by breaking away from their adjacent cells and intruding into neighboring tissues after having undergone an EMT process that changes their characteristics to acquire motility and invasiveness.[98] The formation of blood vessels is essential for cancer growth and progression. Accumulating evidence reveals

that exosomal miRNAs play a regulatory role in tumor metastasis by controlling the EMT process, angiogenesis, and invasion. It can be concluded that the intercellular communication mediated by exosomal miRNAs may represent a vital mechanism for cancer progression.

Cancer-Derived Exosomal miRNAs Mediate Immunomodulation

Exosomal miRNAs function as critical mediators in the cross-talk between cancer cells and macrophages. Tumor-associated macrophages (TAMs) are important components of the tumor microenvironment and play a vital role in cancer pathogenesis. Macrophages can be categorized into two classes (M1 and M2) according to their function.[99] M1-polarized macrophages inhibit tumor growth whereas M2-polarized macrophages provide an immunosuppressive niche for tumor development and metastasis.[100]

Exosomal miR-let-7a-5p, miR-10a-5p, miR-1246, and miR-125b-5p derived from lung adenocarcinoma cells promoted macrophage repolarization toward a more pro-inflammatory, anti-tumor M1 phenotype.[101] Oppositely, exosomal miR-222-3p from epithelial ovarian cancer (EOC) induced macrophage polarization and differentiation to the M2 phenotype by fine-tuning the suppressor of cytokine signaling 3 (SOCS3)/STAT3 pathway, which further promoted the growth and metastasis of EOC.[102] Likewise, exosomal miR-940 enriched in hypoxic EOC cells performed a tumor-promoting function in EOC by driving macrophage activation toward the M2 phenotype.[103] Exosomal miR-301a-3p derived from hypoxic pancreatic cancer (PC) cells triggered the M2 polarization of macrophages via activation of the phosphatase and tensin homolog (PTEN)/PI3Kγ signaling pathway.[104] Consequently, exosomal miR-301a-3p increased the metastatic activity of PC cells. Exosomal miR-21 and miR-29a derived from non-small-cell lung cancer (NSCLC) could be taken up by TAMs.[105] In recipient cells, miR-21 and miR-29a could initiate a TLR-mediated pro-metastatic inflammatory response that supported tumor development. Exosomal miR-1246 enriched in colon cancer cells induced the reprogramming of macrophages into a tumor-supporting state.[106]

Tumor-derived exosomal miRNAs facilitate tumor immunosuppression by driving the dysfunction of immune cells. NPC-derived exosomal miRNAs (hsa-miR-24-3p, hsa-miR-891a, hsa-miR-106a-5p, hsa-miR-20a-5p, and hsa-miR-1908) impaired T cell proliferation and differentiation by targeting the mitogen-activated protein kinase 1 (MAPK1) and Janus kinase (JAK)/STAT pathways.[107] In another study, exosomal miR-24-3p was confirmed to block T cell proliferation and differentiation via regulating the ERK- and STAT-signaling pathways by directly targeting fibroblast growth factor 11 (FGF11).[108] PC-derived exosomal miR-212-3p could be delivered to dendritic cells.[109] Exosomal miR-212-3p suppressed the expression of regulatory factor X-associated protein (RFXAP) and major histocompatibility complex class II (MHC class II), which contributed to the immune tolerance of dendritic cells.

The immune cells, including macrophages, T cells, and dendritic cells, constitute a host defense system against cancer progression. Nevertheless, cancer cells have evolved to transfer their miRNAs to these immune cells via exosomes. Exosome-shuttled miRNAs play a critical role in reprogramming host immune cells, thereby facilitating immune dysfunction and cancer progression. The functional role of exosomal miRNAs in the reciprocal interplay between cancer cells and host immune system merits further investigation.

Exosomal miRNAs Modulate Cancer Cell Resistance to Chemotherapy

Exosomal miRNAs can alter the chemosensitivity of cancer cells by manipulating cellular signaling pathways. Exosomal miR-34a increased docetaxel sensitivity of prostate cancer cells partly by regulating Bcl-2.[110] Exosomal transfer of miR-151a sensitized temozolomide (TMZ)-resistant GBM cells to TMZ by targeting X-ray repair cross-complementing 4 (XRCC4).[111] The macrophage-derived exosomes (MDEs) markedly reduced the sensitivity of pancreatic ductal adenocarcinoma (PDAC) cells to gemcitabine.[112] Further study indicated that exosomal miR-365 repressed the activation of gemcitabine in cancer cells by upregulation of the triphospho-nucleotide pool and activation of the enzyme cytidine deaminase. Exosomal miR-221/222 released by tamoxifen-resistant breast cancer cells could effectively inhibit the expression of its target genes *p27* and *ERα*, thus conferring tamoxifen resistance to recipient cancer cells.[113]

The expression of miR-146a-5p was reduced in both NSCLC cells and exosomes during the process of cisplatin (DDP)-mediated drug resistance.[114] Overexpression of miR-146a-5p could attenuate the resistant property of DDP-resistant NSCLC cells (A549/DDP) via blocking cellular autophagy by directly targeting autophagy-related gene 12 (Atg12). On the contrary, exosomal miR-100-5p derived from A549/DDP enhanced DDP resistance in recipient NSCLC cells by targeting the mammalian target of rapamycin (mTOR).[115] Exosomal miR-21 could be transferred from M2 macrophages to GC cells, and it decreased the sensitivity of recipient cancer cells to DDP.[116] Mechanistically, exosomal miR-21 repressed cell apoptosis and activated the PI3K/Akt signaling pathway by targeting PTEN.

Cancer chemoresistance is still an unsolved problem in cancer treatment. A small quantity of drug-resistant cancer cells will persist and survive following chemotherapy, leading to tumor recurrence in patients. Drug-resistant cancer cells can disseminate the resistant phenotype to drug-sensitive cells via exosomes. This leads to the formation of a large population of cancer cells that are unaffected by drug therapy. Remarkably, exosomal miRNAs function to modulate drug susceptibility of cancer cells mainly by regulating cell cycle-associated proteins or modifying cellular apoptosis and autophagy pathways. The contribution of exosomal miRNAs to the development of cancer chemoresistance is an emerging research area that requires systematic exploration.

Exosomal miRNAs Are Involved in Shaping the Tumor Microenvironment

Cancer-derived exosomal miRNAs are important participants in the cross-talk between tumor cells and cancer-associated fibroblasts

(CAFs) within the tumor microenvironment. Breast cancer-secreted exosomal miR-105 could induce a metabolic program in CAFs by activating the MYC signaling.[117] Exosomal miR-105 favored persistent tumor growth by adapting CAFs to a different metabolic environment. Human melanoma-derived exosomes (HMEXs) could be taken up by human adult dermal fibroblasts (HADFs).[118] HMEX-derived miR-155 and miR-210 were essential for the promotion of glycolysis and suppression of oxidative phosphorylation (OXPHOS) in HADF cells. This promoted extracellular acidification and thus facilitated the formation of a pre-metastatic niche that supported tumor metastasis. Highly metastatic HCC cells could deliver miR-1247-3p to CAFs via exosomes.[119] Exosomal miR-1247-3p activated the β1-integrin/NF-κB signaling in fibroblasts by targeting β-1,4-galactosyltransferase III (B4GALT3). Activated CAFs promoted cancer progression through the secretion of pro-inflammatory cytokines (IL-6 and IL-8). CRC-derived exosomal miR-10b directly targeted the p110α catalytic subunit of PI3K (PIK3CA) and repressed the PI3K/Akt/mTOR signaling pathway in fibroblasts.[120] Consequently, exosomal miR-10b was capable of promoting CRC growth by activating the surrounding stromal cells to become CAFs.

On the other hand, exosomal miRNAs derived from adjacent stromal cells in the tumor microenvironment could modulate the malignant phenotype of cancer cells. For example, CAF-derived exosomes and their miRNA cargoes (miR-21, miR-143, and miR-378e) could induce the stemness and EMT phenotype in breast cancer cells.[121] Exosomal miR-21 secreted by fibroblasts promoted CRC metastasis.[122] Likewise, CAF- and cancer-associated adipocyte (CAA)-derived exosomal miR-21 caused the inhibition of cell apoptosis and induction of chemoresistance in OC by directly targeting apoptotic protease activating factor 1 (APAF1).[123] CAF-released exosomal miR-451 promoted the migration of adjacent esophageal cancer cells.[124] miR-320a could be transferred from CAFs to HCC cells via exosomes.[125] miR-320a inhibited HCC cell proliferation, migration and metastasis by inhibiting its downstream target Pre-B cell leukemia homeobox 3 (PBX3). Similarly, CAF-derived exosomal miR-148b could be transferred to endometrial cancer cells, and it inhibited cancer metastasis by targeting DNA methyltransferase1 (DNMT1).[126]

Cancer-derived exosomal miRNAs serve as signaling molecules to provide a suitable niche for cancer progression. Exosomal miR-503 promoted polarization of the microglia from a tumor-suppressive M1 to a tumor-promoting M2 phenotype.[127] This contributed to brain metastasis in breast cancer. Tumor cells could interact with multiple different cells, including osteoclasts, osteoblasts, and MSCs, leading to an osteolytic or osteoblastic phenotype.[128] Prostate cancer-derived exosomal hsa-miR-940 induced the osteogenic differentiation of MSCs by controlling the expression of *ARHGAP1* and *FAM134A*. hsa-miR-940 also promoted extensive osteoblastic lesions in the bone metastatic microenvironment. Exosomal let-7 miRNAs derived from metastatic GC cells exerted a tumor-suppressive function by targeting oncogenes, including *rat sarcoma* (*RAS*) and *high mobility group A2* (*HMGA2*).[52] Metastatic GC cells released exosomal let-7 miRNAs into the extracellular environment to maintain their oncogenic characteristics.

Exosomal miRNAs derived from the microenvironment in turn affect tumor development. Exosomes derived from TNF-like weak inducer of apoptosis (TWEAK)-stimulated macrophages (TMs) could be transferred to EOC cells.[129] TMs suppressed the metastasis of EOC cells via exosomal transfer of miR-7 to EOC cells. MSC-derived exosomal miR-100 possessed anti-angiogenic activity during tumorigenesis by inhibiting VEGF expression in recipient breast cancer cells.[130] Exosomal miR-140 released by preadipocytes inhibited the tumorigenesis and migration of breast cancer cells by targeting sex determining region Y-box 2 (SOX2)/SOX9.[131] It was reported that the oncogenic miR-221 derived from GC-derived MSCs (GC-MSCs) promoted the proliferation and migration of recipient GC cells.[132] Astrocyte-derived exosomal miR-19a could be transferred to brain metastatic tumor cells, and it promoted tumor growth through the downregulation of PTEN.[133] Therefore, targeting the tumor microenvironment might represent a promising therapeutic approach to prevent tumor progression.

The intercommunication between tumor cells and the surrounding microenvironment is significant for tumor occurrence and development. The tumor microenvironment is composed of multiple types of non-tumor cells, including fibroblasts, MSCs, and infiltrating immune cells, all of which can communicate with tumor cells. Exosomal miRNAs play a regulatory role in the establishment of a tumor-supportive or tumor-suppressive milieu by reprogramming the cells within the tumor microenvironment. However, the molecular mechanisms by which exosomal miRNAs remodel the tumor microenvironment are not fully elucidated and await deep exploration in the future.

Exosomal miRNAs Serve as Potential Biomarkers for Cancer Diagnosis and Prognosis

Exosomal miRNAs display different expression patterns between cancer patients and healthy individuals. Moreover, exosomal miRNAs can indicate tumor progression, severity, and aggressiveness. Exosomal miRNAs have potential as promising cancer biomarkers for clinical use. Exosomal miR-181b-5p, miR-361b-5p, miR-10b-5p, and miR-320b were able to efficiently distinguish NSCLC patients from non-NSCLC individuals.[134] These miRNAs might function as highly sensitive, non-invasive biomarkers for early NSCLC diagnosis. Exosomal miR-451a, miR-21, and miR-4257 were highly expressed in NSCLC patients compared with healthy individuals.[135,136] These miRNAs were strongly associated with tumor progression, recurrence, and poor prognosis in NSCLC patients. Summarizing, they served as potential predictive biomarkers for NSCLC.

Exosomal miR-21 and miR-1246 were upregulated in patients with esophageal squamous cell carcinoma (ESCC) compared with healthy controls.[137,138] They were positively associated with tumor aggressiveness and progression. Moreover, serum miR-1246 could distinguish ESCC patients from healthy controls with relatively high

sensitivity and specificity.[138] Thus, exosomal miR-21 and miR-1246 might serve as clinical biomarkers for the prediction of ESCC progression. Exosomal miR-21 level in the cerebrospinal fluid (CSF) of glioma patients was strikingly higher than that in healthy controls.[139] The expression of exosomal miR-21 was correlated with tumor metastasis, recurrence, and poor prognosis in glioma patients. It is likely that exosomal miR-21 may be a promising biomarker for multiple types of cancer. Further study is warranted to validate the clinical significance of exosomal miR-21 in cancer.

Exosomal miR-6803-5p, miR-17-5p, and miR-92a-3p were significantly elevated in the sera from CRC patients when compared to healthy controls.[140,141] In contrast, exosomal miR-548c-5p was obviously downregulated in CRC patients.[142] High levels of exosomal miR-17-5p and miR-92a-3p were remarkably correlated with the pathologic grade and stage of CRC patients.[140] Upregulated miR-6803-5p and downregulated miR-548c-5p were associated with tumor progression and poor prognosis in CRC patients. Therefore, these exosomal miRNAs might be promising non-invasive biomarkers for CRC diagnosis and prognosis.

Serum exosomal miR-301a level was positively associated with disease progression and overall survival in GBM patients.[143] This miRNA might function as an effective biomarker for glioma. Exosomal miR-223-3p level in the sera of patients with breast cancer was significantly higher than that of healthy controls.[144] Its expression was tightly associated with the malignancy of breast cancer, suggesting that exosomal miR-223-3p might be a useful biomarker for the early detection of invasive breast cancer. Exosomal miR-210 and miR-1233 were significantly upregulated in clear-cell renal cell carcinoma (ccRCC) patients compared with healthy individuals.[145] These miRNAs could distinguish ccRCC patients from healthy individuals with high sensitivity and specificity. Exosomal miR-210 and miR-1233 might be utilized as diagnostic biomarkers for renal cancer.

Additionally, the prognostic values of exosomal miRNAs have been investigated in several types of cancer. The serum levels of exosomal miR-200b and miR-200c could differentiate between benign and malignant EOC.[146] Upregulation of exosomal miR-200b and miR-200c correlated with tumor progression and poor overall survival in EOC patients. Exosomal miR-638 level was decreased in the sera of HCC patients.[147] Serum exosomal miR-638 was negatively correlated with HCC progression. Its downregulation predicted poor prognosis and low overall survival in HCC patients. Serum exosomal miR-638 might be a prospective circulating biomarker for HCC. Exosomal miR-4772-3p could discriminate colon cancer patients with recurrence from non-recurrent patients.[148] Exosomal miR-4772-3p might serve as a prognostic biomarker for colon cancer patients with tumor recurrence. Exosomal miR-let-7b and miR-18a were significantly correlated with poor outcomes in patients with multiple myeloma (MM).[149] miR-let-7i-5p, miR-26a-1-3p, and miR-615-3p were correlated with the overall survival of patients with metastatic RCC.[150] Therefore, exosomal miRNAs may represent non-invasive biomarkers for cancer prognosis.

Early diagnosis of cancer is critical to improve the survival rate of cancer patients. Therefore, discovering effective biomarkers is of great importance. At present, blood-based markers for cancer diagnosis, such as carcinoembryonic antigen (CEA) and α-fetoprotein (AFP), lack sufficient sensitivity, specificity, and accuracy.[151,152] Exosomal miRNAs display great promise as useful cancer biomarkers owing to their high stability, easy accessibility, and wide presence in body fluids. Large-scale studies are required to better define the potential clinical utility of exosomal miRNAs as diagnostic and prognostic biomarkers in cancer. The expression level of exosomal miRNAs is linked to tumor type, stage, and other clinical variables. Accordingly, it is critical to confirm the utilized range of exosomal miRNAs and figure out their associations with conventional cancer biomarkers. Moreover, standardized techniques for exosome separation and detection are not available. A set of stable inner reference genes for accurate quantification of exosomal miRNAs remains to be developed. These problems must be solved before the clinical utilization of exosomal miRNAs.

Exosome-Mediated miRNA Delivery for Cancer Treatment: Challenges and Future Prospects

Increasing evidence reveals that exosome-mediated delivery of miRNAs is tightly associated with carcinogenesis and cancer progression. Targeting the biogenesis of exosome may be a potential approach to treat cancer. Amiloride inhibited exosome production and blunted the T cell-suppressive function of myeloid-derived suppressor cells (MDSCs) *in vivo*, thereby enhancing the anti-tumor efficacy of the chemotherapeutic drug.[153] Prohibiting the uptake of exosomes by recipient cells may represent another strategy to impair exosome function. Tumor-derived exosomes containing oncogenic EGFR could be ingested by endothelial cells, and, thus, they supported tumor growth and angiogenesis.[154] Diannexin abrogated these effects via inhibiting the uptake of tumor-derived exosomes by endothelial cells. Nevertheless, exosome-based therapeutics may also interfere with the physiological function of non-tumor cell-derived exosomes. This results in severe and even unpredictable side effects. The use of exosomes as carriers to deliver tumor-suppressive miRNAs represents an alternative therapeutic strategy for the treatment of cancer.

A variety of miRNAs have been confirmed to play an inhibitory role in cancer progression.[155–157] Therefore, miRNAs hold promise as therapeutic targets for cancer. The major challenge of applying miRNAs for therapeutic purpose is that exogenous miRNAs are quickly degraded by ribonucleases that are highly active in the blood.[158] Exosome-shuttled miRNAs can be protected from enzymatic degradation and stably exist in the circulation.[159] Exosomes can deliver miRNAs to local or remote recipient cells. Notably, exosome-mediated delivery of miRNAs might provide a distant and more bioactive pool of circulating miRNAs compared to those transported by ribonucleoprotein complexes.[160] Due to their stable and non-toxic characteristics, exosomes would be ideal candidate carriers for the delivery of anti-tumor miRNAs.[161]

Nevertheless, several issues remain to be addressed before the clinical application of exosomes as anti-carcinogenic therapeutic tools.

It is necessary to identify the most robust cellular source of exosomes appropriate for clinical use. Exosomes can be obtained from multiple types of human cells. There is a lack of comparative studies on how the cellular origin of exosomes influences their therapeutic efficacy. Thus, it is uncertain which source is the most optimal in terms of exosome isolation. At present, the isolation methods of exosomes are expensive, time consuming, and labor intensive. Reproducible and low-cost techniques for mass production of exosomes are urgently required. The amount of nucleic acids in exosomes varies greatly from patient to patient. Moreover, distinct isolation approaches or different sources may give quite different results. It is critical to verify the physiologically relevant dosage of exosomal miRNAs required for intercellular communication. Further research is warranted to determine whether the compositional differences between exosomes derived from distinct origins reflect their functions.

A major hurdle in the clinical application of exosomes has been the lack of feasible approaches to achieve pure exosomes without contamination by similar-sized particles. Therefore, improved purification and identification protocols for exosomes need to be developed. The requirements for the characterization of pure exosomes should be determined. Given that exosomes contain a variety of proteins and carry MHC molecules, exogenous exosomes may elicit an immune reaction, leading to their rapid elimination in the host. Exosomes with low immunogenicity are more suitable for therapeutic applications. Future efforts should be directed to identify and remove pro-tumorigenic components from exosomes. It will be difficult to control the exact component of cell-derived natural exosomes. Alternatively, determination of the identity of exosomal composition is critical for large-scale manufacture of synthetic exosome mimetics that may have a promising applicability in gene therapy. Different recipient cells might display distinct responses to exosomes, suggesting that the receptor specificity is an important factor for the uptake of exosomes.[162] Identification of tumor cell-specific receptor repertoires for targeted delivery of miRNAs by exosomes is an essential step toward clinical cancer therapy. Furthermore, a better understanding of exosome biology and its role in cancer progression will be helpful to predict the safety and therapeutic efficacy of exosomes *in vivo*.

The impact of exosome-shuttled miRNAs on tumor progression was previously disclosed. Exosome-formed miR-143 could be transferred from MSCs to OS cells.[163] Exosomal miR-143 dramatically inhibited the migration of recipient OS cells. However, the delivery efficiency of exosome-formed miR-143 was low. Moreover, researchers failed to load miRNAs into exosomes *in vitro*.[164] Efficient encapsulation of candidate miRNAs in exosomes is a predominant challenge that remains to be overcome. The factors (e.g., solubility and charge distribution) that affect miRNA entrapment efficiency must be carefully delineated. Specifically, the methods for accurately defining the amount of loaded miRNA need to be ascertained. Moreover, the pathways by which anti-tumor miRNAs exert their function must be well determined to prevent the risk of off-target effects.

Exosomes loaded with miRNAs can be delivered either systemically or through local inoculation into the tumor tissue. For the treatment of easily accessible tumors (e.g., breast cancer and melanoma), exosomes can be delivered by local injection, attenuating the risk of off-target effects and dissemination. In contrast, for the therapeutic intervention on other types of tumors, exosomes should be delivered by systemic administration. An in-depth investigation into the pharmacokinetic profile and biodistribution of exosomes delivered by distinct routes and modalities is warranted before their clinical translation. Since the clearance of exosomes predominantly occurs in the liver and kidney,[165] the impacts of administrated exosomes on the physiological function of these organs deserve deep investigation. In addition, further studies are required to explore whether different administration routes could affect the efficacy of exosomal miRNA-mediated therapeutics.

Regular injections of miRNA-loaded exosomes may be essential for maintaining the miRNA effect in cancer patients. The verification of optimal administration times is worthwhile. To favor the tissue-specific delivery of miRNA cargoes, the surface of exosomes could be engineered to improve their tumor-targeting capability. Targeting ligands, peptides, and antibody fragments attached to the surface proteins of exosomes should be designed and produced. As the procedures for cloning and recombinant expression are time consuming, highly efficient targeting approaches should be developed. For instance, targeting peptides or ligands could be directly linked to the exosomal surface through chemical conjugation. Furthermore, iterative clinical trials must be conducted before the clinical application of miRNA-loaded exosomes. Collectively, applying exosome-delivered miRNAs to clinical cancer therapy is a challenging but intriguing effort that needs further investigation.

Conclusions

Exosomes are a novel class of intercellular signal mediators that are engaged in the occurrence and progression of cancer. Exosomes contain a wide range of biological molecules, including lipids, proteins, and nucleic acids. miRNAs play an important role in cancer progression and hold promise as diagnostic and prognostic biomarkers for cancer. The function of exosomal miRNAs in cancer pathogenesis has been a research hotspot over the past few years. Increasing evidence indicates that exosomal miRNAs serve a regulatory role in cancer growth, angiogenesis, invasion, and metastasis. Moreover, exosomal miRNAs can modulate the chemoresistance of cancer cells. For the purpose of retaining cancer cells' sensitivity to anti-cancer drugs, the mechanisms behind exosomal miRNA-regulated cancer chemoresistance need to be further delineated.

Exosomal miRNAs serve as signaling molecules in the communication between tumor cells and the tumor microenvironment. The detailed mechanisms underlying the biogenesis, secretion, and uptake of exosomes still await adequate elucidation. The regulatory role of exosomal miRNAs in the shaping of the tumor microenvironment is worthy of deeper exploration. Exosomal miRNAs can affect the expression of immune-related genes and reprogram the immune

cells. Nevertheless, the study on the involvements of exosomal miRNAs in modulating host immune responses is only in its infancy. Further studies are needed to gain a better understanding of the mechanisms underlying exosomal miRNA-mediated immunomodulation. Therefore, a great research effort is required to completely unveil the role of exosomal miRNAs in carcinogenesis and cancer progression. An in-depth investigation into the contributions of exosomal miRNAs to cancer pathogenesis will provide tremendous opportunities for the clinical translation of exosome-mediated miRNA delivery in targeted cancer therapy.

AUTHOR CONTRIBUTIONS

M.W. and K.W. conceived and designed the study. M.W. wrote the manuscript. F.Y. and H.D. prepared the figures. Y.W., P.L., and K.W. edited the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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