Exosomal non-coding RNAs have a significant effect on tumor metastasis

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Exosomes are produced by the majority of eukaryotic cells and are capable of transporting a variety of substances, including non-coding RNAs, between cells. Metastasis is a significant cause of death from cancer. Numerous studies have established an important role for exosomal non-coding RNAs in tumor metastasis. Exosomal non-coding RNAs from a variety of cells have been shown to affect tumor metastasis via several mechanisms. Exosomes transmit non-coding RNAs between tumor cells, fibroblasts, endothelial cells, and immune cells within the tumor microenvironment. Exosomal non-coding RNAs also have an effect on epithelial-mesenchymal transition, angiogenesis, and lymphangiogenesis. Exosomes derived from tumor cells have the ability to transport non-coding RNAs to distant organs, thereby facilitating the formation of the metastatic niche. Due to their role in tumor metastasis, exosomal non-coding RNAs have the potential to serve as diagnostic or prognostic markers as well as therapeutic targets for tumors. The purpose of this paper is to review and discuss the mechanisms of exosomal non-coding RNAs, their role in tumor metastasis, and their clinical utility, aiming to establish new directions for tumor metastasis, diagnosis, and treatment research.

INTRODUCTION

Vesicles are produced and released frequently in eukaryotic cells. Extracellular vesicles (EVs) are capable of transporting a variety of substances, including proteins, nucleic acids, and lipids, between adjacent or distant cells, serving as a vital mode of information and material exchange between cells.[1] EVs are mixtures of different subtypes of vesicles, such as ectosomes and exosomes. Ectosomes, which include microvesicles, microparticles, and large vesicles, are formed and released directly from the plasma membrane via outward budding. These EVs are formed from endosomes and range in diameter from 50 nm to 1 mm. Endosomes form early-sorting endosomes (ESEs), late-sorting endosomes (LSEs), and multivesicular bodies (MVBs) in the cytoplasm, and exosomes are formed when intraluminal vesicles (ILVs) in MVBs are released from the cell. Exosomes are about 40 nm–160 nm in diameter.[2] Certain proteins are present in varying concentrations in different subtypes of EVs. CD9, CD63, and CD81 are abundant in exosomes, but they are also present in other vesicles. Syntenin-1, TSG101, ADAM10, and EHD4 were all found to be highly expressed in small EVs. Syntenin-1 and TSG101 are specifically distributed in exosomes and thus serve as exosome markers. GP96, actinin-4, and mitofilin are abundant in large vesicles and thus can be used as markers for them.[3] At present, exosomes have been purified from nearly all mammalian cell types. Exosomes derived from antigen-presenting cells were discovered in 2002 to promote the activation of CD4$^+$ and CD8$^+$ T cells and the induction of a specific immune response.[4] Since then, an increasing number of studies have established that exosomes play a role in a variety of physiological processes. In addition, exosomes have also been implicated in the progression of diseases. Exosomes, as components of the tumor microenvironment, also play a critical role in tumors. Tumor cells can exchange bioactive substances with other cells via exosomes, thereby affecting tumor progression.[5] Exosomes, as intercellular messengers, play a critical role in tumor progression.

Tumor metastasis is a characteristic of cancer, and over 90% of cancer deaths are due to metastasis, not the primary site of the tumor.[6,7] It is a clinical challenge to treat patients with tumor metastasis and improve their survival time and quality of life. At the moment, the mechanisms underlying tumor metastasis are unclear. It is a very complex process that involves the tumor microenvironment, angiogenesis, immunity, and drug resistance, among other factors. Exosomes, as a ubiquitous mode of intercellular communication, play a significant role in the interactions of tumor cells with other cells and the stroma as well as in the process of tumor metastasis. Exosomes derived from tumor cells, for example, contain a number of immunosuppressive proteins that aid in tumor immune escape.[8] However, research on the role and mechanism of exosomes in tumor metastasis remains in its early stages.

Non-coding RNA is a type of nucleic acid that can be encased in exosomes and transported between tumor cells and other cells.

MicroRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) are the most common types of non-coding RNAs. They are involved in the regulation of various cellular activities, despite the fact that they do not encode proteins.[9] miR-27b can prevent tumor cells from metastasizing in gastric cancer.[10] lnc-PTAR regulates miR-101 and ZEB1 in ovarian cancer, promoting metastasis.[11] circ-0008305 has been shown to inhibit transforming growth factor β (TGF-β)-induced epithelial-mesenchymal transition (EMT) and metastasis in non-small cell lung cancer.[12] Exosomes can transport a variety of non-coding RNAs to recipient cells, affecting various aspects of tumor progression, including the microenvironment, immunity, angiogenesis, lymph angiogenesis, and the formation of a pre-metastatic niche. In this paper, we will discuss the role of exosomal non-coding RNAs in tumor metastasis.

NON-CODING RNAs ARE PACKAGED INTO EXOSOMES DURING DOUBLE INVAGINATION OF THE CELL MEMBRANE

Exosome production requires a double invagination of the cell membrane (Figure 1). For the first time, the membrane invaginates, forming a cup-shaped structure that eventually forms ESEs composed of cell membrane surface and extracellular components. The newly formed ESEs can fuse with the cell's already existing ESEs. ESEs mature and become LSEs. The second invagination occurs in LSEs, which results in the formation of MVBs that contain ILVs. Double invagination results in the entry of intracellular components into ILVs. MVBs can fuse with and be degraded by lysosomes. If MVBs are not degraded, they can be transported to and fuse with the cell membrane. Then, ILVs are released from the cell's surface in the form of exosomes.[2,13]

Both extracellular and intracellular components, including non-coding RNA in the cytoplasm, can enter exosomes via the double membrane invagination process associated with exosome formation. The exact mechanism by which non-coding RNA enters exosomes is unknown. Apart from passively wrapping themselves around other substances in the cell, some studies have suggested that RNA-binding proteins (RBPs) are involved in the process by which exosomes sort non-coding RNAs, such as HnRNPA2B1, selectively (Figure 1). RBPs can recognize specific sequences on target RNAs and aid in cytoplasmic RNA transport, and RNA can enter exosomes selectively when bound to RBPs. HnRNPA2B1 is capable of recognizing and binding to the specific miR-198 sequence and thereby mediating miR-198 exosome packaging.[14] Due to its specific binding to HnRNPA2B1, lnc-NMAT2 can be sorted into exosomes.[15] YBX1 is another type of RBP involved in the packaging of miRNAs into exosomes. Both YBX1 and miR-223 possess binding sites, and YBX1 is homologous to the exosome surface molecule CD63. With the aid of YBX1, miR-223 can be selectively sorted into exosomes.[16] However, the exact mechanism by which RBPs incorporate non-coding RNA into exosomes during exosome generation is unknown. In addition, miRNA was found to be packaged into exosomes via post-transcriptional uridylation of the 3′-terminal strand.[17] This suggests that post-transcriptional modification of miRNA may be involved in exosome

sorting. To summarize, the mechanism by which exosomes encapsulate RNA is unknown, and more research is required.

THE MECHANISM OF EXOSOMAL miRNAs IN TUMOR METASTASIS

miRNAs are a class of non-coding RNAs with an approximate length of 21 nt that are derived from introns of protein-coding genes or RNA-polymerase-II-specific independent gene transcripts.[18] The process of miRNA synthesis begins with the synthesis of the miRNA precursor with a hairpin structure, which is then exported to the cytoplasm for trimming into miRNA/miRNA double strands, one of which functions as a mature miRNA. Mature miRNAs and other proteins combine to form the miRNA-induced silencing complex (miRISC). In miRISC, miRNAs have a seed region containing nucleotides 2–8 at their 5′ ends that bind specifically to the target mRNA. The Watson-Crick base pairing between the miRNA seed region and the target mRNA's 3′ untranslated region (3′ UTR) is required for miRNA target recognition and driving function. The seed region directs mature miRNAs to specifically target mRNA for post-transcriptional repression, which includes mRNA translational repression and mRNA degradation. Complementarity between miRNA and mRNA is critical in determining the type of post-transcriptional inhibition. Complete pairing of miRNA and mRNA target sites facilitates mRNA degradation, whereas incomplete pairing promotes mRNA translation repression. Translational suppression is widely accepted as the default and most prevalent mechanism by which miRNA suppresses mRNA. Both mechanisms can exist when miRNA and mRNA are completely paired.[19] This is a relatively specific mechanism by which miRNAs regulate target gene expression.

Exosomes transport miRNA from the cytoplasm of tumor cells to other cells, where they regulate target genes via the mechanism described above. miR-105 can be transported to endothelial cells via exosomes derived from breast cancer cells. miR-105 contains a sequence that can bind to the 3′ UTR of the ZO-1 mRNA. After treatment with exosomal miR-105, the levels of ZO-1 in human microvascular endothelial cells (HMVECs) decreased, indicating that miR-105 can enter and reduce the level of ZO-1 in HMVECs. By inhibiting the expression of ZO-1 and destroying the adhesion between endothelial cells, exosomal miR-105 increases the permeability of the vascular wall.[20] Similarly, exosomes from breast cancer cells contain a high level of miR-122. miR-122 can bind to the 3′ UTR of pyruvate kinase mRNA, thereby reducing pyruvate kinase's mRNA and protein levels and impairing the glucose metabolism of receptor cells.[21] In addition, miR-122 was found to be highly expressed in the serum exosomes of hepatocellular carcinoma (HCC) patients, according to the sequencing data.[22] Numerous exosomal miRNAs have been implicated in the process of tumor metastasis (Table 1; Figure 2).

THE MECHANISM OF EXOSOMAL lncRNAs IN TUMOR METASTASIS

lncRNAs are a class of non-coding RNAs that are approximately 200 nt in length and lack an open reading frame.[42] While lncRNAs cannot be translated into proteins, they can be transmitted between cells via
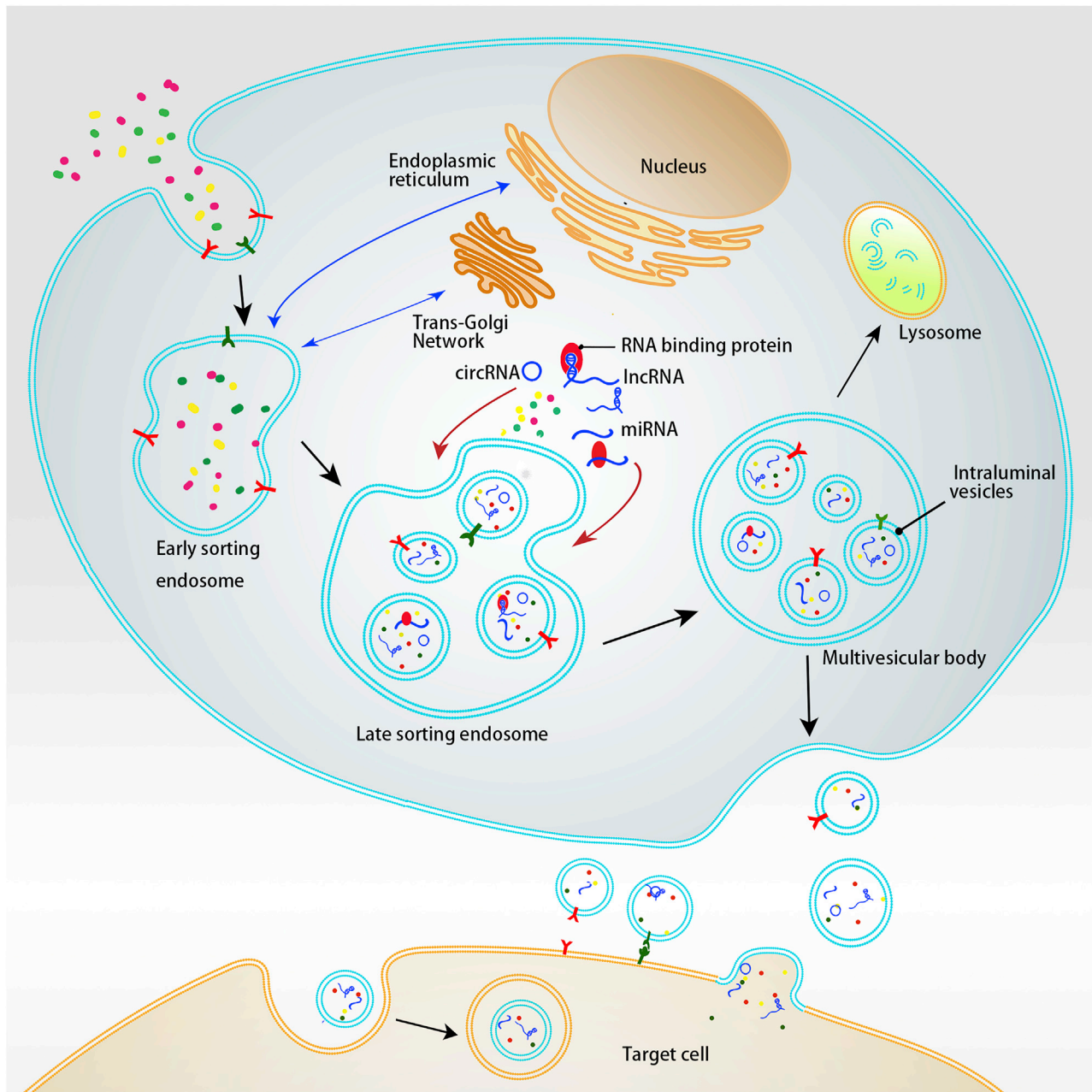
Figure 1. Biogenesis of exosomes

Early-sorting endosomes (ESEs) are formed when extracellular substances, such as lipids, proteins, ions, and cell-surface proteins, enter the cell via membrane invagination. The ESEs' inner surface is formed by the outer side of the cell membrane. ESEs can fuse with the endoplasmic reticulum, the trans-Golgi network, or their derived vesicles or other existing ESEs. The membrane of ESEs invaginates a second time to form LSEs, and during this second invagination, ILVs are formed, with the ILVs' outer side being formed by the outer side of the cell membrane. Non-coding RNAs are incorporated into ILVs along with other cytoplasmic constituents. In this process, RBP can bind miRNA or lncRNA and is associated with the selective sorting of non-coding RNAs. LSEs generate MVBs with a defined collection of ILVs. MVBs can fuse with lysosomes, which are eventually degraded. Undegraded MVBs migrate to the inner side of the cell membrane and fuse with it, while ILVs contained in MVBs are released as exosomes outside the cell. Exosomes eventually enter recipient cells via a variety of mechanisms, and exosomal components, such as non-coding RNAs, are also transported into the recipient cell.

exosomes and may affect tumor metastasis (Table 1; Figure 2). Exosomal lncRNAs have been shown to play a critical role in a variety of tumor types. Exosomal lncRNAs function in a manner distinct from miRNAs. Exosomal lncRNAs function in a variety of ways, including miRNA sponging, mRNA stability reduction, interaction with RBPs, promoter combing, and epigenetic regulation in the nucleus.[15,43,44]

Table 1. The role of exosomal non-coding RNA in tumor metastasis

Exosomal non-coding RNA	Donor cell	Recipient cell	Role in tumor metastasis
miR-21, miR-10b[23]	HCC cell	HCC cell	promoting HCC cells migration, invasion, and EMT
miR-155[24]	gastric cancer cell	human umbilical vein endothelial cell (HUVEC)	promoting angiogenesis of HUVECs
miR-196a-1[25]	gastric cancer cell	gastric cancer cell	enhancing invasion ability of gastric cancer cells
miR-499a-5p[26]	lung adenocarcinoma cell	lung adenocarcinoma cell	promoting cancer cell migration and epithelial-mesenchymal transition (EMT)
miR-1229[27]	colorectal cancer (CRC) cell	HUVEC	promoting tubulogenesis of HUVECs
miR-25-3p[28]	CRC cell	HUVEC	promoting vascular permeability and angiogenesis to induce vascular leakiness
miR-25-3p, miR-92a-3p[29]	liposarcoma cell	macrophage	inducing IL-6 release from macrophages
miR-106a[30]	gastric cancer cell	peritoneal mesothelial cell	inducing peritoneal mesothelial cell damage, apoptosis, migration, and mesothelial-to-mesenchymal transition
miR-125b-5p[31]	melanoma cell	macrophage	inducing the tumor-promoting phenotype of tumor-associated macrophages
miR-1910-3p[32]	breast cancer cell	breast cancer cell	promoting migration of breast cancer cells
miR-9[33]	nasopharyngeal carcinoma cell	HUVEC	inhibiting HUVECs tube formation and migration
lnc-FMR1-AS1[34]	esophageal CSC	non-CSC	promoting stemness phenotypes of non-CSCs
lnc-UCA1[35]	CRC cell	CRC cell	promoting proliferation and migration of CRC cells
lnc-MALAT1[36]	CRC cell	CRC cell	promoting invasion and migration
lnc-RUNX2-AS1[37]	myeloma cell	mesenchymal stem cell	inhibiting the osteogenesis of mesenchymal stem cells
lnc-PTENP1[38]	normal cell	bladder cancer cell	suppressing bladder cancer cell invasion and migration
circ-MMP2[39]	HCC cell	normal liver cell	promoting the malignant phenotype formation of normal liver cells
circ-0000284[40]	cholangiocarcinoma cell	surrounding normal cell	promoting the migration and proliferation of surrounding normal cells and suppressing their apoptosis
circ-SATB2[41]	non-small cell lung cancer cell	lung cancer cell and bronchial epithelial cell	promoting the migration and invasion of lung cancer cells and inducing abnormal proliferation of bronchial epithelial cell

lncRNAs serve as competitive endogenous RNAs (ceRNAs) and have an important regulatory function. ceRNAs can bind specifically to miRNAs, and the specific sites on miRNAs where ceRNAs bind are known as miRNA response elements (MREs),[45] which are also present in mRNAs. lncRNAs and mRNAs bind to miRNAs competitively, reducing miRNA-mediated inhibition of downstream mRNAs and forming an lncRNA-miRNA-mRNA regulatory network. In osteosarcoma, exosomes derived from bone marrow mesenchymal stem cells transported and increased the expression of lnc-PVT1 in osteosarcoma cells. lnc-PVT1 acts as a ceRNA, sponging miR-183-5p and reducing miR-183-5p's inhibitory effect on its target, ERG, thereby promoting ERG expression, which in turn promotes osteosarcoma cell proliferation and migration.[43] Certain lncRNAs can combine directly with mRNAs without involving miRNAs. lnc-APC1 can bind to specific sites on Rab5b mRNA in colorectal cancer (CRC). In this study, silencing lnc-APC1 prolonged the half-life of

Rab5b mRNA, indicating that lnc-APC1 can regulate the stability of Rab5b mRNA, thereby reducing exosome production from CRC cells and thereby inhibiting tumor metastasis and angiogenesis.[44]

Certain exosomal lncRNAs are internalized by target cells and translocated into the nucleus to perform their functions. Exosomal lncRNAs have the ability to interact directly with DNA and regulate transcription. In bladder cancer, tumor cells produce exosomal lnc-LNMAT2, which is internalized by human lymphatic endothelial cells (HLECs) and interacts physiologically with the PROX1 promoter's P3 region in the nucleus. The lnc-LNMAT2 and PROX1 promoters form a triple-stranded RNA-DNA structure and enhance PROX1 transcription.[15] PROX1 is a lymphatic vascular marker that is required for the differentiation of endothelial cells into HLECs. PROX1 inhibition results in the absence of lymphatic vessels and abnormal development or function of lymphatic vessels.[46] Exosomal lnc-LNMAT2

Figure 2. Illustration of important non-coding RNAs transported via exosomes between different cells in tumor metastasis

Exosomes transport non-coding RNAs between tumor cells and other types of cells within the tumor microenvironment, including immune and non-immune cells. CAF-derived exosomes transfer lnc-H19, miR-34a, miR-92, miR-148b, and miR-181d-5p to tumor cells, whereas tumor-cell-derived exosomes transfer miR-1247-3p, miR-375, and lnc-Gm26809 to fibroblasts to promote fibroblast activation. Tumor-cell-derived exosomes deliver lnc-H19, miR-103, circ-IARS, and circ-100338 to endothelial cells, where they regulate endothelial cell adhesion and vascular permeability. Tumor-cell-derived exosomes deliver non-coding RNAs to neutrophils, T cells, DC cells, NK cells, and TAMs. NK cells and macrophages can also deliver exosomal non-coding RNAs into tumor cells via exosomes. miRNAs, lncRNAs, and circRNAs can be transported to recipient tumor cells via tumor-cell-derived exosomes to promote EMT. Exosomal non-coding RNAs derived from tumors can be internalized by HUVECs and HLECs to promote angiogenesis and lymphangiogenesis. Tumor-cell-derived exosomes deliver non-coding RNAs to distant organs, such as the lung, bone, and brain, where they affect the cells and promote the formation of the pre-metastatic niche.

activates PROX1 in endothelial cells, promoting differentiation of endothelial cells into lymphatic endothelial cells, as well as lymphatic formation and extension. Increased lymphatic vessel formation in tumors promotes lymph node metastasis.[15] In the nucleus, lncRNAs can collaborate with other molecules to regulate histone modification, which is required for epigenetic transcriptional regulation to occur. HnRNPA2B1 has the ability to bind to target DNA and participate in epigenetic regulation of H3K4 trimethylation.[47] Exosomal lnc-LNMAT2 binds to the PROX1 promoter region in the nucleus and recruits hnRNPA2B1, which promotes H3K4 trimethylation on the PROX1 promoter and activates PROX1 expression.[15]

In addition, exosomal lncRNAs can interact directly with proteins. Exosomal lnc-91H may directly interact with HNRNPK in CRC, promoting tumor cell migration and invasion.[48] HNRNPK has been linked to tumor metastasis.[49] In CRC cells, the lnc-RPPH1 and TUBB3 proteins interact directly. The TUBB3 protein contains a unique domain that interacts with specific regions of lnc-RPPH1, promoting CRC cell migration and invasion. In addition, lnc-RPPH1 can inhibit the ubiquitination of TUBB3 and increase its stability.[50] Thus, while some exosomal lncRNAs may not affect protein expression at the transcription or translation levels, they can affect protein stability via regulating ubiquitination and degradation.

THE MECHANISM OF EXOSOMAL circRNAs IN TUMOR METASTASIS

circRNAs are non-coding circular RNAs that lack a PolyA tail and a 5′ end and are generated by backward splicing or exon skipping of the pre-mRNA.[51] Certain circRNAs have been identified as being transmitted and functioning between cells via exosomes during tumor metastasis (Table 1; Figure 2). circRNAs can act as sponges for miRNAs or RBPs, and some can be translated into peptides.[52]

circRNAs derived from exosomes act primarily as ceRNAs in tumors by sponging miRNAs. circRNAs contain miRNA-binding sites that regulate gene expression by interacting with and affecting miRNAs. circ-PDE8A in exosomes was found to be negatively correlated with the prognosis of patients with pancreatic tumors and to promote tumor cell proliferation, migration, and invasion. Due to the presence of miR-338-binding sites on circ-PDE8A, circ-PDE8A has been identified as a miR-338 sponge. MACC1 is one of miR-338's targets,[53] has been implicated in tumor metastasis, and is a key regulator of MET signaling. MET, a tyrosine kinase receptor, is regarded as an oncogene and is a transcriptional target of MACC1.[54] MET signaling is primarily mediated by the RAS-mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)-AKT pathways and has a direct effect on cell proliferation, migration, adhesion, and cytoskeleton organization.[55] MACC1 and MET levels are decreased when miR-338 levels are increased. circ-PDE8A overexpression increases MACC1 and MET levels, implying that circ-PDE8A regulates the miR-338/MACC1/MET axis.[53] Similarly, exosomal circ-RanGAP1 regulates miR-877-3p/VEGFA expression in gastric cancer by acting as a miRNA sponge.[56] circ-WHSC1 and circ-PUM1 can be transported via tumor-derived exosomes and act as miRNA sponges, promoting tumor progression in ovarian cancer.[57,58]

Several studies have reported that exosomal circRNAs can interact directly with proteins, promoting tumor metastasis. circ-100338 was highly expressed in tumor-derived exosomes and was found to promote angiogenesis and metastasis in HCC. circ-100338 has the ability to bind to a wide variety of proteins, including RBPs, transcription factors, and enzymes. NOVA2 is one of the specific RBPs and is linked to angiogenesis; its interaction with circ-100338 may account for its angiogenesis-promoting effect.[59] Exosomal circ-ABCC1 has been shown to interact directly with β-catenin, mediating β-catenin's entry into the nucleus and activation of the Wnt pathway, thereby promoting CRC metastasis.[60] Although some circRNAs can be translated into peptides, the mechanism by which exosomal circRNAs promote tumor metastasis via peptide translation remains unknown. In conclusion, exosomal circRNAs promote tumor metastasis mainly via interaction with miRNAs.
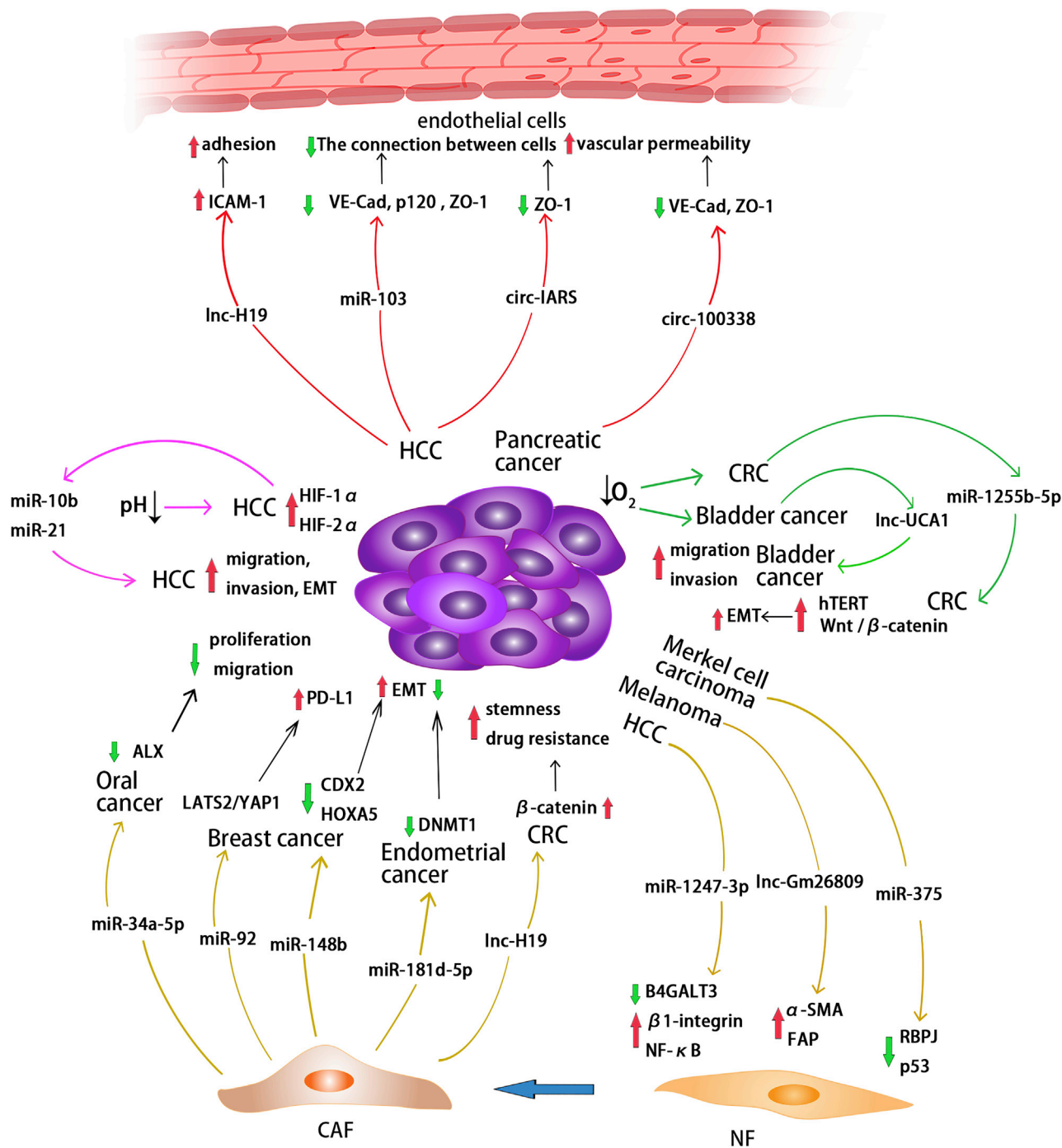
EXOSOMAL NON-CODING RNA AND THE NON-IMMUNE TUMOR MICROENVIRONMENT

Rather than existing independently, tumor cells interact with their microenvironment to form a whole, analogous to "seeds" and "soil." The tumor microenvironment contains a diverse array of cells and non-cellular elements. The tumor microenvironment can be classified as immune dominated by immune cells or non-immune dominated by fibroblasts and endothelial cells. Tumor cells and neighboring cells can exchange non-coding RNAs via exosomes within the tumor microenvironment. We begin this section by discussing the communication between tumor cells and the non-immune microenvironment via exosomes (Figure 3); the immune microenvironment will be discussed later in the section on exosomal non-coding RNAs and tumor immunity.

Endothelial cells mediate angiogenesis in the tumor microenvironment. Adhesion between tumor cells and endothelial cells is a critical step in the progression of tumor cells into blood vessels. Exosomes can transport lncRNAs between cells, enhancing endothelial cell monolayer adhesion. In HCC, exosomal lnc-H19 derived from CD90$^+$ cancer stem cells (CSCs) increases ICAM-1 expression in endothelial cells, thereby enhancing the adhesion ability of endothelial cells. This is critical for CSCs' entry into vessels.[61] After adhering to the endothelial cells, tumor cells must pass through the vessel wall to spread into the bloodstream and distant organs, as well as penetrate the vessel walls when implanted in distant organs. The connection between endothelial cells has a direct effect on vascular permeability. Exosomes secreted by HCC cells express a high level of miR-103, which downregulates VE-cadherin, p120, and ZO-1, all of which are important adhesion molecules within and between endothelial cells. As a result, exosomal miR-103 increases vascular permeability and promotes tumor metastasis.[62] Pancreatic-cancer-secreted exosomal circ-IARS can inhibit ZO-1 expression in endothelial cells, increasing vascular permeability and promoting tumor metastasis.[63] HCC exosomes carrying circ-100338 can be internalized by human umbilical vein endothelial cells (HUVECs) and downregulate VE-cadherin and ZO-1 expression in HUVECs, thereby enhancing vascular permeability.[59] These findings suggest that non-coding RNA contained in exosomes secreted by tumor cells can promote metastasis by regulating endothelial cell surface adhesion molecules, thereby destroying endothelial cell-to-cell connections and increasing vascular permeability.

Cancer-associated fibroblasts (CAFs) can secrete a variety of cell factors that affect tumor progression. When activated by tumors, normal fibroblasts differentiate into CAFs, which can alter the tumor microenvironment and interact with tumor cells. Exosomal non-coding RNAs secreted by tumor cells contribute to the transformation of fibroblasts into CAFs.[64,65] Exosomal miR-1247-3p derived from cancer cells specifically targets B4GALT3 in fibroblasts and regulates the β1-integrin/nuclear factor κB (NF-κB) axis, activating fibroblasts to transform into CAFs in HCC.[64] Similarly, exosomal miR-375 can downregulate its targets, RBPJ and p53, promoting fibroblast polarization to CAFs in Merkel cell carcinoma.[66] RBPJ, also known as CSL, is a Notch signaling molecule that inhibits CAF activation.[67,68] Exosomes secreted by melanoma cells and their contents of lnc-Gm26809 upregulate the specific CAF markers, α-SMA and FAP, in fibroblasts.[65] CAFs, on the other hand, can secrete exosomes and interact with tumor cells. Primary CAF and NF cells from CRC and

Figure 3. Exosomal non-coding RNAs and the non-immune tumor microenvironment

Exosomes derived from HCC, melanoma, and Merkel cell carcinoma deliver non-coding RNAs to regulatory targets and signaling pathways in fibroblasts, thereby promoting the transformation of NFs into CAFs. Exosomal non-coding RNAs derived from CAFs also regulate corresponding molecules, thereby affecting the progression of tumor cells in oral cancer, breast cancer, endometrial cancer, and CRC. Exosomes derived from HCC contain lnc-H19, which regulates ICAM-1 and promotes endothelial cell adhesion. Exosomes derived from HCC and pancreatic cancer contain non-coding RNAs that inhibit intercellular adhesion molecules and improve vascular permeability. In HCC, tumor cells in an acidic environment secrete exosomal miR-10b and miR-21 to promote tumor cell migration, invasion, and EMT. In addition, exosomal non-coding RNAs derived from CRC and bladder cancer cells in hypoxic environments promote tumor EMT, migration, and invasion.

normal colorectal tissues were cultured, and exosomes of CAFs and NFs were collected. RNA sequencing revealed significant differences in the expression profiles of lncRNA and short non-coding RNA (including miRNA) in CAF and NF exosomes. Furthermore, it is suggested that these non-coding RNAs play a role in the interaction of fibroblasts and cancer cells.[69] Through exosomes, CAFs deliver lnc-H19 to CRC cells, which sponges miR-141 and activates the β-catenin pathway, enhancing the stemness and resistance of CRC cells to oxaliplatin.[70] CAF-derived exosomal miR-181d-5p can regulate CDX2 and HOXA5 in breast cancer cells, thereby promoting EMT of cancer cells.[71] On the other hand, CAF-derived exosomal non-coding RNAs can act as tumor suppressors. CAFs transport miR-34a-5p to adjacent tumor cells via exosomes and regulate ALX expression in oral cancer. ALX deficiency can increase intercellular adhesion and inhibit tumor cell growth, metastasis, invasion, and EMT.[72,73] miR-34a-5p inhibits ALX expression and the proliferation, motility, and EMT of oral cancer cells. The miR-34a-5p/ALX axis has an effect on tumor cell EMT via the AKT/GSK-3β/β-catenin/Snail signaling pathway.[74] Similarly, exosomal miR-34 derived from CAFs has been reported to inhibit tumor invasion in gastric cancer.[75] In addition, RNA sequencing of exosomes extracted from the urine of prostate cancer patients and normal controls revealed a decreased level of miR-34a expression in patients' urine exosomes.[76] miR-148b in CAF-derived exosomes was significantly reduced in endometrial cancer and can be transferred to tumor cells via exosomes and inhibit DNMT1, which promotes EMT of endometrial cancer. As a result, CAF-derived exosomal miR-148b suppresses tumor cell invasion.[77] CAF-derived exosomal non-coding RNAs can promote tumor immune escape. In breast cancer cells treated with CAF-derived exosomes, miR-92 and PD-L1 expression levels have increased. miR-92 targets LATS2, which interacts with YAP1, and YAP1 can regulate the transcription of PD-L1, implying that CAF-derived exosomes inhibit T cell function by regulating PD-L1 levels in breast cancer cells via the miR-92/LATS2/YAP1 pathway.[78]

The tumor microenvironment, in addition to cells, contains a variety of non-cellular components that affect tumor progression, such as oxygen and pH. Under normal conditions, the aerobic respiration of cells provides the majority of the energy required for cell activity. In solid tumors, the absence of blood vessels results in a hypoxic environment, which is widely regarded as one of the most fundamental tumor microenvironmental pressures. It does not promote the tumor's rapid growth.[79] circ-133 is abundant in anoxic tumor cells in CRC. Exosomal circ-133 derived from hypoxic tumor cells has the ability to enter normoxic tumor cells and promote their migration via the miR-133a/GEF-H1/RhoA axis.[80] Exosomal lnc-UCA1 derived from hypoxic bladder cancer cells promotes bladder cancer cell migration and invasion.[81] Under hypoxia in CRC, the content of miR-1255b-5p in exosomes derived from cancer cells decreased, resulting in an increase in the expression of human telomerase reverse transcriptase, which activates Wnt/β-catenin and promotes EMT of tumor cells.[82]

The increased glycolysis in tumor cells, hypoxia within the solid tumor, and abnormal blood flow in the tumor microenvironment all contribute to an imbalance in the pH of the tumor microenvironment, making the extracellular environment acidic. It is advantageous for tumor metastasis. Acidic conditions have been shown to increase miR-21 and miR-10b levels in tumor-cell-derived exosomes in HCC by increasing HIF-1α and/or HIF-2α. Under acidic conditions, high miR-21 and miR-10b levels in exosomes promote the migration, invasion, and EMT of tumor cells.[23] miR-21 has been demonstrated to promote tumor metastasis in CRC, breast cancer, and lung cancer.[83–85] In gastric cancer, exosome miR-21 has been found to promote peritoneal metastasis via mesothelial-to-mesenchymal transition of gastric cancer cells.[86] Furthermore, miR-10b was found to be highly expressed in the plasma exosomes of patients with lung squamous cell carcinoma, pancreatic ductal carcinoma, and gastric cancer.[87–89] As a result, both hypoxic and acidic tumor microenvironments promote tumor metastasis.

EXOSOMAL NON-CODING RNA AND TUMOR IMMUNITY

Tumor cells can affect a variety of immune cells via exosomal non-coding RNAs (Figure 4).

Macrophages comprise the majority of immune cells in the tumor microenvironment.[90] Macrophages are classified into two subtypes: classically activated M1 and transformed M2. M1 macrophages are capable of secreting pro-inflammatory factors and exerting anti-tumor activity. M2 macrophages have the ability to promote tumor metastasis, angiogenesis, and growth. Exosomes secreted by tumor cells promote macrophage transformation from M1 to M2. After macrophages internalized exosomes derived from CRC cells, the levels of M2 macrophage markers increased significantly without any significant changes in the levels of M1 macrophage markers, indicating that exosomes secreted by CRC cells promote macrophage M2 polarization. The level of lnc-RPPH1 in the exosomes of CRC cells is responsible for this effect. lnc-RPPH1 was found to be overexpressed in CRC tissues in seven CRC patients with liver metastasis via next-generation sequencing, and lnc-RPPH1 was found to be abundant in serum exosomes in these patients. High lnc-RPPH1 levels in exosomes can upregulate the expression of M2 markers in macrophages.[50] Breast-cancer-cell-derived exosomal lnc-BCRT1 has the ability to promote macrophage M2 polarization, enhancing macrophage migration and chemotaxis and promoting TGF-β secretion.[91] Cancer cells with activated CXCL12/CXCR4 can deliver upregulated miRNAs to macrophages via exosomes, activating the PI3K/AKT signaling pathway and regulating PTEN expression to promote M2 polarization in CRC.[92] CRC-derived exosomal miR-934 induces M2 macrophage polarization by downregulating PTEN and activating the PI3K/AKT signaling pathway. In addition, M2 macrophages can increase miR-934 expression in CRC cells by activating the CXCL13/CXCR5/NF-κB/p65/miR-934 feedback loop, which promotes the formation of CRC liver metastases.[93] Hypoxia can enhance exosome secretion and miR-301a expression in pancreatic cancer. Exosomes containing a high level of miR-301a induced M2 macrophage polarization by downregulating PTEN and activating PI3Kg.[94] Tumor-associated macrophages (TAMs) can also affect
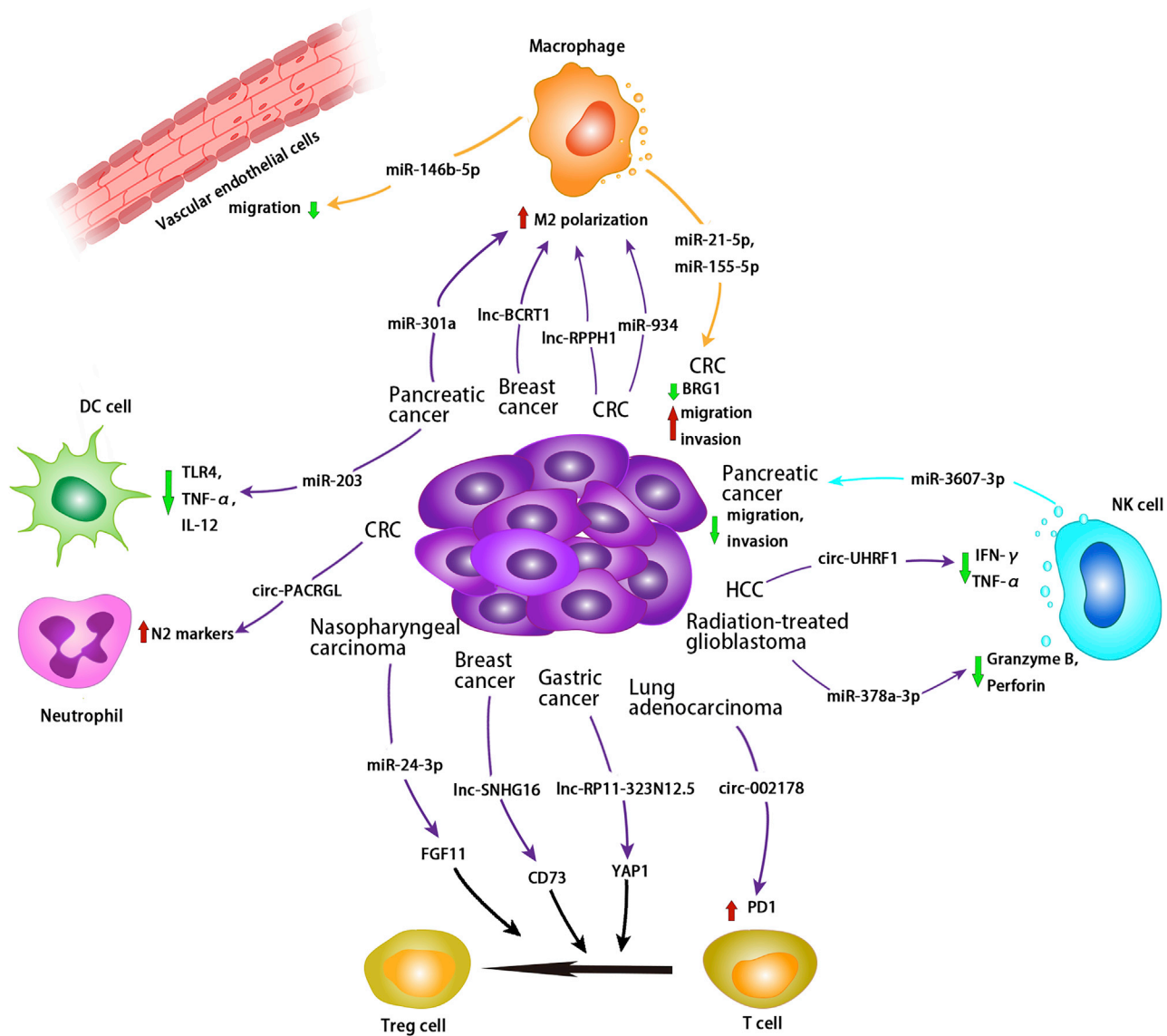
Figure 4. Exosomal non-coding RNAs and the immune tumor microenvironment

Exosomal non-coding RNAs from tumor cells can be internalized by macrophages and regulate their targets in macrophages, promoting M2 polarization in CRC, breast cancer, and pancreatic cancer cells. TAM-derived exosomal miR-21-5p and miR-155-5p downregulate BRG1, thereby promoting tumor migration and invasion in CRC. Macrophage-derived exosomal miR-146b-5p can inhibit vascular endothelial cell migration. Tumor-cell-derived exosomal non-coding RNAs regulate T cell differentiation into Treg cells in nasopharyngeal carcinoma, breast cancer, and gastric cancer. In addition, exosomal circ-002178 derived from lung adenocarcinoma has been shown to increase PD1 expression on T cells. Radiation-treated-glioblastoma-derived exosomal miR-378a-3p suppresses granzyme B and perforin expression in NK cells, whereas HCC-derived exosomal circ-UHRF1 suppresses IFN-γ and TNF-α expression in NK cells. However, exosomal miR-3607-3p derived from NK cells inhibits pancreatic tumor growth. Exosomal miR-203 derived from pancreatic cancer cells inhibits DC cell function by suppressing TLR4, TNF-α, and IL-12 expression. Exosomal circ-PACRGL derived from CRC cells increases N$_2$ markers in neutrophils.

tumor cells by secreting exosomes. Exosomes derived from M2 macrophages contain significant levels of miR-21-5p and miR-155-5p. These two miRNAs are delivered to colon cancer cells via exosomes and inhibit BRG1 expression, hence promoting tumor cell migration and invasion.[95] TAMs can deliver miR-95 to prostate cancer cells via exosomes. The complementary pairing of mir-95 and the 3′ UTR of JunB mRNA inhibits JunB mRNA translation, and miR-95 promotes

EMT in prostate cancer by inhibiting JunB expression.[96] In addition, TAMs can interact with other cells within the tumor microenvironment. In ovarian cancer, exosomal miR-146b-5p produced by TAMs has been shown to inhibit endothelial cell migration by modulating TRAF6/NF-κB/MMP2 expression in endothelial cells. The inhibitory effect of TAM-derived exosomes on endothelial cell migration was reversed when exosomes from ovarian epithelial cancer cells
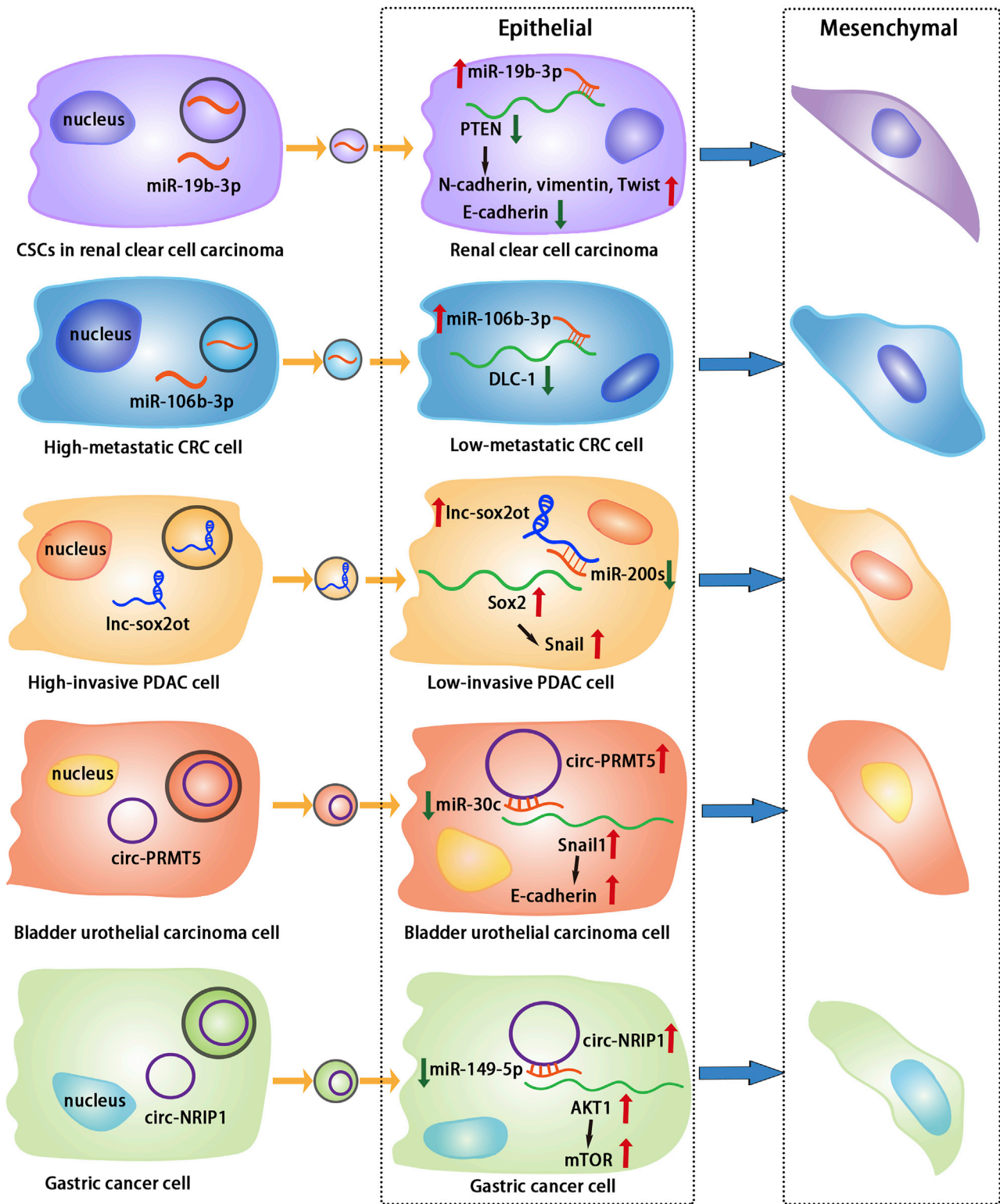
were added. The authors speculated that lncRNA contained in tumor exosomes restored endothelial cells' migration ability.[97] This study indicates that cell communication in the tumor microenvironment is a complex network, with various cells exerting inconsistent effects via exosome communication.

Natural killer (NK) cells are essential components of the innate immune system because they are capable of secreting cytokines. NK cells act as immunomodulators and directly kill cells via cytotoxicity. They are important immune cells that act as inhibitors of tumor growth in the tumor microenvironment. Exosomes derived from NK cells are rich in miR-3607-3p in pancreatic cancer, and these exosomes can inhibit pancreatic cancer cell migration and invasion, as miR-3607-3p in exosomes can directly regulate interleukin-26 (IL-26) in cancer cells.[98] On the other hand, exosomal miR-378a-3p from radiation-treated glioblastoma cells can enter NK cells, resulting in a decrease in the expression of granzyme B/perforin, the primary cytotoxic effector molecule of NK cells. This indicates that tumor-derived exosomes inhibit the immune killing ability of NK cells.[99] Cancer-derived exosomal circ-UHRF1 inhibited interferon (IFN)-γ and tumor necrosis factor alpha (TNF-α) secretion by NK cells via miR-449c/TIM-3 in HCC. circ-UHRF1/miR-449c/TIM-3 is specific to NK cells and has no effect on CD8$^+$ T cells. On NK cells, inhibiting tumor exosomal circ-UHRF1 enhanced resistance to anti-PD1 therapy.[100] These findings suggest tumor cells and NK cells can fight each other via exosomes during tumor progression, potentially affecting tumor development.

Exosomal non-coding RNA can also affect T cells. Exosomes derived from tumors not only inhibit proliferation but also affect T cell subtype differentiation. miR-24-3p is abundant in exosomes derived from nasopharyngeal carcinoma. miR-24-3p inhibited ERK and STAT phosphorylation by inhibiting FGF11 in T cells following exosome treatment, resulting in decreased T cell proliferation, inhibition of Th1 and Th17 differentiation, and induction of regulatory T (Treg) cell differentiation.[101] Th1 and Th17 cells stimulate the immune response, whereas Treg cells suppress the immune response. In breast cancer, V δ 1T cells are the predominant Treg cell type, and exosomes from breast cancer cells transfer lnc-SNHG16 to V δ 1T cells. lnc-SNHG16 can upregulate CD73 expression via the miR-16-5p/Smad5 axis, and CD73$^+$V δ 1T cells inhibit the immune response of breast cancer.[102] In gastric cancer, lnc-RP11-323N12.5 can be transferred to T cells via exosomes and combined with c-myc to increase YAP1 levels in T cells, promoting Treg cell differentiation and immunosuppression.[103] Exosomal non-coding RNAs can also impair T cell function by upregulating the expression of immunosuppressive molecules on their surface. Lung adenocarcinoma-derived

exosomal circ-002178 sponged miR-28-5p and increased PD-1 on the surface of T cells. On the other hand, circ-002178 expression was significantly higher in lung adenocarcinoma cells than in normal tissues, promoting PD-L1 expression in tumor cells.[104] The high level of PD-1/PD-L1 inhibited the immune killing effect of CD8$^+$ T cells.

In addition, exosomal miR-203 derived from pancreatic cancer can inhibit dendritic cell function by downregulating TLR4.[105] In CRC, tumor-derived exosomal circ-PACRGL can promote tumor cell migration and invasion. After neutrophils were treated with CRC-derived exosomes, circ-PACRGL increased the expression of N$_2$ markers via the miR-1423p/miR-506-3p-TGF-β1 axis and regulated N1-N$_2$ neutrophil differentiation.[106] N$_2$ neutrophils have been shown to promote tumor metastasis. Taken together, tumor-derived exosomes have the potential to inhibit the immune response to tumor cells by transporting non-coding RNA to various immune cells, which may be one of the mechanisms by which tumors evade the immune system.
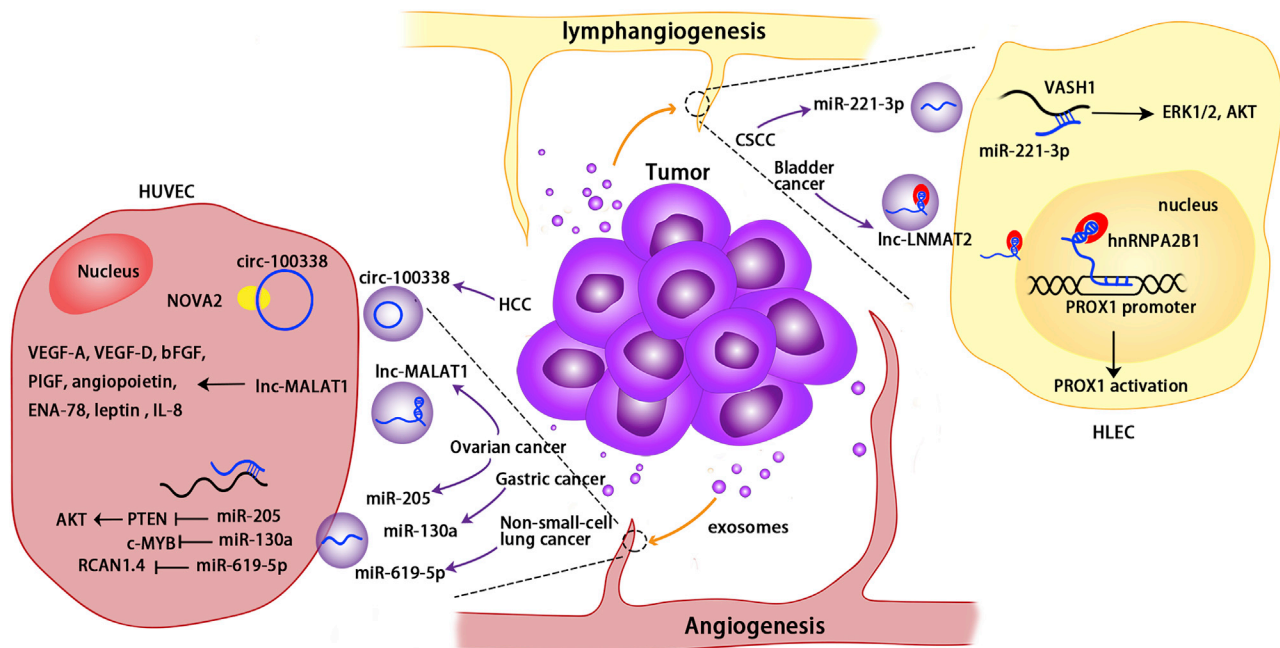
EXOSOMAL NON-CODING RNA AND EMT

EMT is critical for tumor invasion. During the initial stages of tumor metastasis, epithelial tumor cells undergo transformation into cells with interstitial characteristics. Tumor cells lose their polarity and intercellular connections, which facilitate migration and invasion. Numerous EMT drivers, including SNAIL1 and SNAIL2, have been shown to be significantly associated with disease recurrence and survival in many tumor types, indicating that EMT is associated with the prognosis of patients with malignancies.[107] EMT is characterized by a decrease in E-cadherin and an increase in vimentin and N-cadherin expression. Exosomal non-coding RNAs can affect tumor metastasis by regulating EMT-related pathways and transcription factors (Figure 5).

Exosomes derived from CSCs were found to transport miR-19b-3p into renal clear cell carcinoma. miR-19b-3p can regulate PTEN to significantly upregulate the expression of N-cadherin, vimentin, and Twist and downregulate the expression of E-cadherin in renal clear cell carcinoma cells, thereby promoting EMT.[108] Patients with CRC metastasis had significantly increased serum exosomal miR-106b-3p levels. Exosomal miR-106b-3p can promote EMT of CRC cells by suppressing DLC-1, a factor that promotes CRC cell invasion and lung metastasis in vivo.[109]

Not only exosomal miRNAs but also some exosomal lncRNAs can influence the EMT process in tumors. Tumor-derived exosomes were found to promote tumor invasion and stem cell characteristics in PDAC and showed a significant increase in lnc-Sox2ot. When the

Figure 5. Exosomal non-coding RNAs have an effect on EMT
Tumor-cell-derived exosomal non-coding RNAs regulate target molecules and signaling pathways to promote tumor cell EMT. In renal clear cell carcinoma, CSCs-derived exosomal miR-19b-3p suppresses PTEN expression in cancer cells, promoting EMT. Exosomes from highly metastatic CRC cells transfer miR-106b-3p to low-metastatic CRC cells and inhibit DLC-1, thereby promoting EMT. Exosomal lnc-sox2ot secreted by highly invasive PDAC cells promotes low-invasive PDAC cell EMT via miR-200s/Sox2/snail. In bladder urothelial carcinoma, cancer cells secrete exosomal circ-PRMT5 and promote EMT in recipient cancer cells via miR-30c/snail1/E-cadherin. Exosomal circ-NRIP1 from gastric cancer cells promotes EMT via miR-149-5p/AKT1/mTOR.

Figure 6. Exosomal non-coding RNAs have an effect on angiogenesis and lymphangiogenesis

Cancer-cell-derived exosomal non-coding RNAs regulate targets and pathways in vascular and lymphatic endothelial cells, thereby promoting angiogenesis and lymphangiogenesis. Exosomal circ-100338 from HCC cells can be internalized by HUVECs and promote angiogenesis via regulating NOVA2. Cancer-derived exosomal lnc-MALAT1 and miR-205 derived from ovarian cancer cells can enter HUVECs and regulate their targets, promoting angiogenesis. Exosomal miR-130a from gastric cancer cells promotes angiogenesis via c-MYB in HUVECs. Exosomal miR-619-5p from non-small cell lung cancer cells can enter HUVECs and regulate RCAN1.4 to promote angiogenesis. Exosomal lnc-LNMAT2 derived from bladder cancer cells can enter HLECs' nuclei and activate PROX1 via hnRNPA2B1, thereby promoting lymphangiogenesis. In CSCC, miR-221-3p from cancer cells can be internalized and inhibit VASH1, thereby promoting lymphangiogenesis via ERK1/2 and AKT.

tumor cells were treated with exosomes from the tumor, the lnc-Sox2ot expression increased in the receptor cells. lnc-Sox2ot regulated Sox2 expression in tumor cells by sponging miR-200 family members and altering the expression of E-cadherin, N-cadherin, and vimentin.[110] Similarly, lnc-ZFAS1 expression is high in exosomes derived from gastric cancer. Exosomes can be internalized into gastric cancer cells, and lnc-ZFAS1 in exosomes regulates Slug, Snail, Twist, and ZEB1 in tumor cells, thereby promoting tumor cell EMT and metastasis.[111]

The role of exosomal circRNA in EMT has also garnered attention. circ-PRMT5 has been shown to be highly expressed in bladder urothelial carcinoma and to promote tumor cell invasion and EMT. circ-PRMT5 was highly expressed in patient serum and urine exosomes, with a positive correlation between its level and lymph node metastasis.[112] RNA sequencing detected the high expression of 33 circRNAs in gastric tumorous tissues, with circ-NRIP1 being the most abundant. Serum exosomal circ-NRIP1 from patients was also highly expressed. Exosomes from gastric cancer cells expressing a high level of circ-NRIP1 can promote the expression of EMT markers in tumor cells.[113] These findings suggest that tumor cells may release exosomal circRNA to neighboring tumor cells, thereby promoting EMT and enhancing tumor invasiveness and metastasis.

EXOSOMAL NON-CODING RNAs AFFECT ANGIOGENESIS AND LYMPHANGIOGENESIS

Angiogenesis is an important characteristic of tumors.[6] Angiogenesis enables malignant tumor cells to migrate away from their primary sites to the circulatory system or distant organs. Exosomes play a role in tumor angiogenesis. Exosomes from lnc-APC1-silenced CRC cells, for example, promote angiogenesis in endothelial cells by activating the MAPK pathway.[44]

Exosomal non-coding RNA can stimulate endothelial cells to form blood vessels (Figure 6). In gastric cancer, miR-130a was found to be transported from cancer cells to HUVECs via exosomes. miR-130a directly binds and inhibits c-MYB in HUVECs, whereas c-MYB inhibits the proliferation, migration, and tube formation of HUVECs. As a result, gastric cancer cells have the ability to secrete exosomes in order to promote angiogenesis.[114] Exosomes from ovarian cancer cells contain miR-205 and can be internalized by HUVECs via a lipid-raft-related pathway. In endothelial cells, exosomal miR-205 regulated the PTEN-AKT pathway. Treatment with tumor-derived exosomal miR-205 significantly enhanced the proliferation, migration, and angiogenesis of HUVECs.[115] miR-619-5p expression was increased in exosomes derived from non-small cell lung cancer cells, and exosomal miR-619-5p was found to regulate the level of RCAN1.4 in HUVECs, thereby promoting

angiogenesis.[116] In addition, ovarian-cancer-derived exosomes can transport lnc-MALAT1 to HUVECs, resulting in a significant increase in lnc-MALAT1 expression in HUVECs. lnc-MALAT1 may contribute to angiogenesis in part by modulating the expression of VEGF-A, VEGF-D, bFGF, PlGF, angiopoietin, ENA-78, leptin, and IL-8. Certain circRNAs contained in exosomes have been shown to promote angiogenesis as well.[117] circRNA-100338 was found to be highly expressed in HCC-cell-derived exosomes. The treatment of HUVECs with tumor-cell-derived exosomes enhanced their proliferation and tubule formation, and the serum exosomal circ-100338 level in patients with HCC may be a predictor of lung metastasis.[59]

Along with vascular metastasis, lymphatic metastasis is a significant mode of tumor dissemination. The lymphatic vessels surrounding the tumor serve as conduits for tumor cells to spread from their primary sites to regional lymph nodes. Exosomal non-coding RNAs have been shown to affect lymphangiogenesis (Figure 6). lnc-LNMAT2 was found to be significantly overexpressed in muscle-invasive and lymph-node-positive bladder cancer, as determined by next-generation sequencing. Exosomal lnc-LNMAT2 derived from bladder cancer cells can directly interact with the PROX1 promoter sequence and increase PROX1 transcription in HLECs' nucleus. In addition, lnc-LNMAT2 and RBP can promote H3K4 trimethylation of the PROX1 promoter.[15] PROX1 is involved in the formation of lymphatic vessels, regulates endothelial cell differentiation, and induces lymphatic endothelial cell germination and extension.[118] Exosomal lnc-LNMAT2 promotes lymphangiogenesis by upregulating PROX1 in lymphatic endothelial cells.[15] Lymphatic metastasis is the main mechanism of cervical cancer metastasis. In cervical squamous cell carcinoma (CSCC), miR-221-3p was found to be transferred via exosomes from cancer cells to HLECs, promoting HLEC migration and tubule formation. Exosomal miR-221-3p inhibits vasohibin-1, a negative regulator of lymphangiogenesis, and increases ERK1/2 and AKT phosphorylation levels in CSCC cells, thereby promoting lymphangiogenesis and lymphatic metastasis.[119] In addition, cancer-derived exosomes can transport miR-221-3 from CSCC cells to vascular endothelial cells, promoting angiogenesis.[120] In summary, exosomal non-coding RNAs are involved in both vascular and lymphatic metastasis.
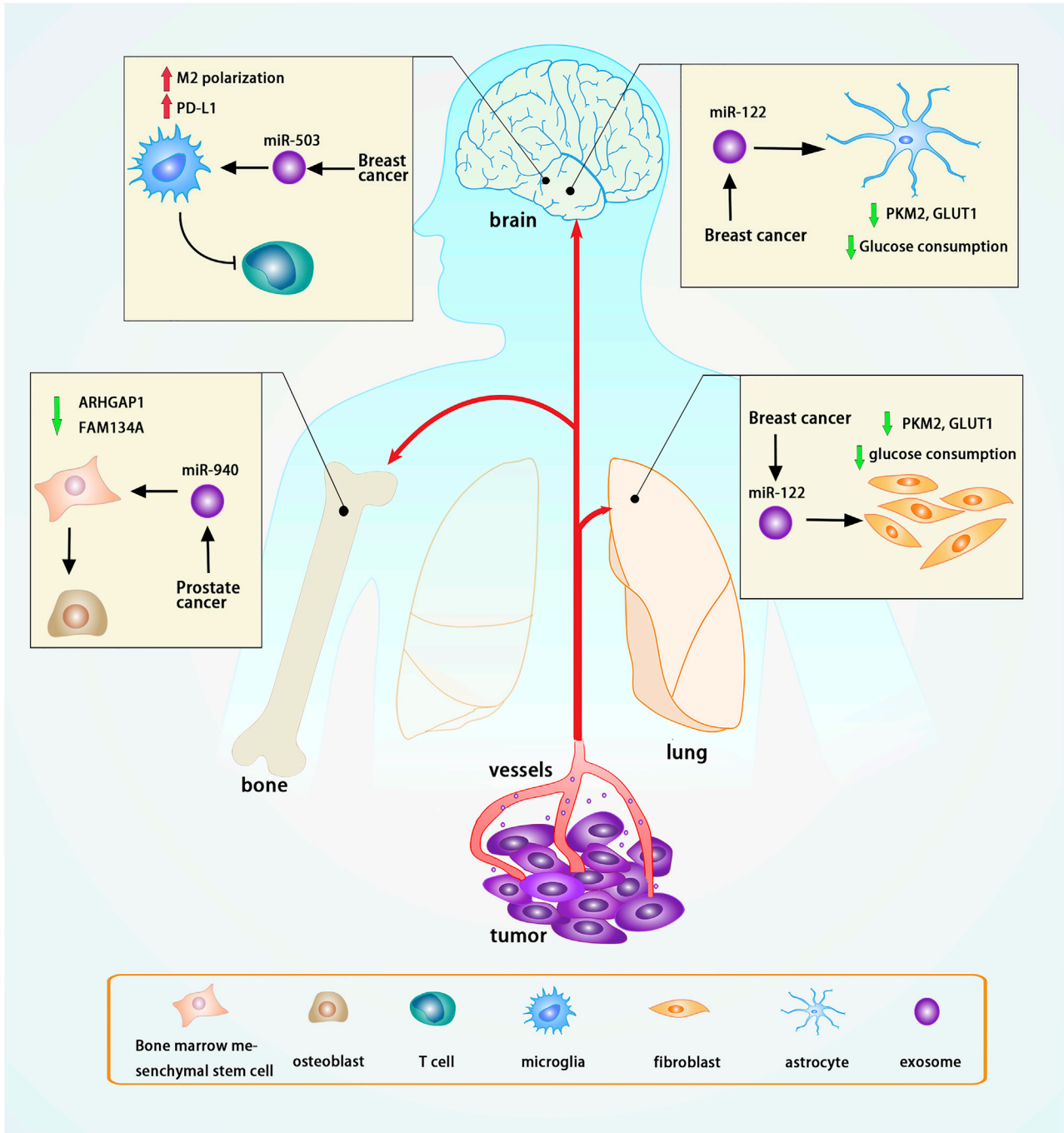
EXOSOMAL NON-CODING RNA AND DISTANT METASTASIS

Tumor cells can spread in different ways to distant organs. To colonize and grow in new organs, tumor cells need an optimal microenvironment. It has long been observed that tumors do not appear to randomly select organs to metastasize to but rather exhibit a tendency to travel to and grow in specific organs based on the original tumor type.[121] Tumor-derived exosomes play a role in organ-specific metastasis.[122] Tumor cells release exosomes during this process to prepare recipient organs and establish a microenvironment conducive to metastasis. Tumor-derived exosomes remained in organs where cancer cells metastasize, promoting the formation of a pre-metastatic niche.[122]

Exosomal non-coding RNA affects the formation of the metastatic niche in metastasis-prone organs (Figure 7). Exosomes derived from cancer cells promote the migration and proliferation of lung fibroblasts in breast cancer. Sequencing results revealed that treatment with cancer-derived exosomes increased the content of certain lncRNAs in lung fibroblasts, implying that cancer-secreted exosomes may deliver lncRNA to lung fibroblasts to participate in pre-metastatic niche formation.[123] Prostate cancer frequently metastasizes to the bone, and osteogenic lesions are common in prostatic bone metastases. miR-940 promotes the differentiation of human mesenchymal stem cells (HMCs) into osteoblasts. HMCs can internalize exosomal miR-940 derived from prostate cancer cells, promoting osteogenic differentiation via regulating ARHGAP1 and FAM134A. In addition, exosomes derived from cancer cells enhanced the osteogenic ability of host cells in xenograft models. miR-940 expression was higher in prostate cancer patients with bone metastasis than in normal prostate epithelial cells or bone tissues.[124] This study suggests that exosomal miR-940 derived from prostate cancer promotes bone metastasis. miR-122 was detected to be highly expressed in exosomes derived from breast cancer cells. Exosomal miR-122 inhibits glucose uptake in lung fibroblasts and brain astrocytes by downregulating PKM2 and GLUT1 while increasing the glucose supply to cancer cells. *In vivo*, tumor-derived exosomal miR-122 reduced glucose utilization in niche tissues and promoted tumor cell metastasis.[21] This indicated that exosomes from tumor cells reduced glucose uptake by niche cells, resulting in glucose redistribution in the pre-metastatic niche and promoting tumor cell growth in the metastatic organs. In breast cancer, the level of lnc-XIST was found to be negatively correlated with brain metastasis. lnc-XIST silencing increases exosomal miR-503 secretion, which induces M1-M2 polarization in microglia. This conversion upregulates PD-L1 expression in microglia, thereby inhibiting T cell proliferation. Microglia reprogramming by exosomal miR-503 promotes the brain metastasis of breast cancer.[125] These findings imply that exosomal non-coding RNA derived from tumor cells can alter the microenvironment in distant organs, thereby facilitating tumor metastasis.

CLINICAL APPLICATION OF EXOSOMAL NON-CODING RNA IN TUMOR

Interestingly, non-coding RNAs have the potential to serve as novel biomarkers for tumor diagnosis and prognosis.[126–128] However, isolating non-coding RNA from body fluids for diagnostic purposes is challenging because RNA degrades rapidly outside tissue and cells. Exosomes provide a similar intracellular environment for non-coding RNAs, protecting RNAs and maintaining their stability. Extracting exosomal RNA from body fluids for diagnostic purposes represents a novel strategy for clinical diagnosis. For instance, exosomal miR-30d-5p and let-7d-3p were found to be expressed at low levels in the serum of cervical cancer patients but were differentially expressed in cervical tumors and adjacent normal tissues. Therefore, serum exosomal miR-30d-5p and let-7d-3p may be used as non-invasive screening biomarkers for cervical cancer.[129] Exosomal lnc-HOTTIP is highly expressed in the plasma of patients with gastric cancer and is correlated with tumor node metastasis (TNM) stage, invasion

Figure 7. Exosomal non-coding RNAs and distant metastasis

Exosomal non-coding RNAs derived from tumor cells can be transported to distant organs and promote the formation of the pre-metastatic niche. In breast cancer, miR-122 can be transported to the lung and brain via cancer-cell-derived exosomes and inhibits PKM2 and GLUT1 expression, thereby decreasing glucose consumption in lung fibroblasts and brain astrocytes. Exosomal miR-503 derived from breast cancer promotes M2 polarization of microglia and increases PD-L1 expression on the microglial surface, inhibiting T cells. Exosomal miR-940 from prostate cancer regulates ARHGAP1 and FAM134A expression in bone marrow mesenchymal stem cells, promoting their differentiation into osteoblasts.

depth, and overall survival. Therefore, plasma exosomal lnc-HOTTIP is suggested as a potential diagnostic and prognostic marker for gastric cancer.[130] Furthermore, serum exosomal circRNAs have been shown to distinguish cancer patients from healthy individuals and can serve as biomarkers for tumor diagnosis.[131] The researchers analyzed circ-PNN expression in the serum of 50 patients with CRC and 50 healthy subjects and revealed that circ-PNN is useful in diagnosing CRC and may be used as a potential biomarker for CRC detection.[132]

On the other hand, exosomal non-coding RNAs have the potential to be used as a novel tumor-treatment strategy. Non-coding RNAs play a critical role in tumor progression, implying that they may be therapeutic targets. For instance, clinical trials have been conducted to determine the efficacy of miR-34 mimics in tumors.[133] Exosomes have a number of advantages as drug carriers and are currently being investigated for use in tumor therapy. Exosomes, when injected into the circulatory system of mice, are less clear and are more efficiently taken up by tumor cells than liposomes.[134] Due to the fact that exosomes are derived from tissues or cells, they are largely nontoxic. Although nucleic acid drugs targeting non-coding RNAs represent a novel strategy for tumor therapy, they need carriers to avoid degradation. Exosomes as carriers for nucleic acid drugs regulating noncoding RNAs have garnered considerable attention in recent years. Intravenous injection of miR-126-loaded exosomes into lung cancer cells in mice effectively internalized them, and miR-126 inhibited lung cancer progression by disrupting the PTEN/PI3K/AKT signaling pathway in cancer cells.[135] In gastric cancer, miR-214 is associated with chemoresistance to cisplatin, and intravenous injection of an antisense inhibitor of miR-214-loaded exosomes sensitizes the tumors' response to cis-diamminedichloroplatinum in mice.[136] Furthermore, exosomes can be modified artificially to enhance the targeting of nucleic acid drugs delivered via exosomes. In breast cancer cells, the transmembrane domain of the recombinant-tissue-derived growth factor receptor was fused to the GE11 peptide to enable exosomes to efficiently target EGFR[+] breast cancer cells and deliver let-7a to inhibit cancer cells.[137]

DISCUSSION

In this review, we explored the possible mechanisms by which exosomes package non-coding RNAs. In addition, we discussed the role of important exosomal non-coding RNAs discovered recently in the tumor microenvironment, tumor immunity, angiogenesis, lymphangiogenesis, and distant metastasis (Table 1; Figure 2).

Exosomes are small vesicles secreted by cells that contain a variety of substances and mediate intercellular communication.[2,5] Exosomes, which act as a "bridge" for cell-to-cell communication, transport non-coding RNAs and are critical in the progression of tumors. As demonstrated in our review, this view is shared by a large number of researchers. Although non-coding RNAs play an important role in tumorigenesis and have the potential to be used as diagnostic markers and therapeutic targets, overcoming RNA's extracellular instability is difficult in clinical application. Exosomes, which are vesicles with phospholipid bilayers secreted by cells, have been shown to protect non-coding RNA and enhance its extracellular stability. Exosomes can be extracted not only from blood but also from urine, saliva, and other bodily fluids.[138–140] Numerous studies have indicated that exosomal non-coding RNAs may be useful for non-invasive or minimally invasive tumor diagnosis.[129–131,141]

Exosomes can evade circulatory clearance, accumulate at the tumor site, and are essentially nontoxic.[134,142] Thus, exosomes as RNA vectors have a number of advantages in the treatment of cancer. Exosomes modified to contain a high level of tumor-suppressive or short hairpin RNAs (shRNAs) and small interfering RNAs (siRNAs) cancer-promoting non-coding RNAs can be used to inhibit tumor progression.[136,143,144] In this way, exosomes can be used to treat cancer. However, challenges remain in the clinical application of exosomal non-coding RNA. There are currently no convenient and rapid methods for extracting a large number of high-purity exosomes, limiting the use of exosomal non-coding RNAs in clinical diagnosis and treatment. Existing methods for exosome extraction do not completely separate exosomes from other types of vesicles, which casts doubt on the clinical utility of exosomes. Additional research on the production and regulation of exosomes may be needed to overcome these obstacles.

Exosomal non-coding RNA is involved in tumor metastasis, and because exosomes have many advantages as RNA carriers, exosomal non-coding RNA may be useful for tumor diagnosis and treatment. With the advancement of exosome and non-coding RNA research and the development of exosome extraction technology, we believe that exosomal non-coding RNA will have a broad range of applications in the near future.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.X. and H.C.; funding acquisition, J.X. and H.C.; writing – original draft preparation, D.W. and W.Z.; writing – reviewing and editing, D.W. and W.Z.; visualization, C.Z. and L.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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