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REVIEW ARTICLE

Effects of extracellular vesicle-derived noncoding RNAs on pre-metastatic niche and tumor progression

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Abstract A pre-metastatic niche (PMN) is a protective microenvironment that facilitates the colonization of disseminating tumor cells in future metastatic organs. Extracellular vesicles (EVs) play a role in intercellular communication by delivering cargoes, such as noncoding RNAs (ncRNAs). The pivotal role of extracellular vesicle-derived noncoding RNAs (EV-ncRNAs) in the PMN has attracted increasing attention. In this review, we summarized the effects of EV-

Abbreviations: BMDCs, bone marrow-derived cells; BMMs, bone marrow macrophages; CCL2, C–C motif chemokine ligand 2; circRNAs, circular RNAs; CRC, colorectal cancer; CSCs, cancer stem cells; EV-ncRNAs, extracellular vesicle-derived noncoding RNAs; EVs, extracellular vesicles; GLUT1, glucose transporters; HCC, hepatocellular carcinoma; HDAF, human adult dermal fibroblasts; HMEX, human melanoma-derived exosomes; HSCs, hepatic stellate cells; lncRNAs, long ncRNAs; MDSCs, myeloid-derived suppressor cells; miRNAs, micro RNAs; ncRNAs, noncoding RNAs; NK, natural killer; OXPHOS, oxidative phosphorylation; PCa, prostate cancer; PMN, pre-metastatic niche; snRNA, small nuclear RNA; TAF, tumor-associated fibroblasts; TME, tumor microenvironment; α-SMA, alpha-smooth muscle actin; 3′-UTR, the 3′ untranslated region.

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Pre-metastatic niche;
Tumor progression

ncRNAs on the PMN in terms of immunosuppression, vascular permeability and angiogenesis, inflammation, metabolic reprogramming, and fibroblast alterations. In particular, we provided a comprehensive overview of the effects of EV-ncRNAs on the PMN in different cancers. Finally, we discussed the promising clinical applications of EV-ncRNAs, including their potential as diagnostic and prognostic markers and therapeutic targets.

Background

Currently, metastasis is the dominant factor in the poor prognosis and high mortality of patients with malignant tumors.[1,2] In the early stages of the majority of cancers, there are no specific clinical signs or symptoms, and tumors metastasize before the patient is diagnosed.[3] The molecular mechanisms underlying the development of tumor metastasis need further studies, such as alterations in the tumor microenvironment, entry of tumor cells into the circulation, and colonization of metastatic sites. The term "pre-metastatic niche (PMN)" was first proposed in 2005,[4] which opened up new horizons for better interpretation of tumor metastasis mechanisms. In the future, PMN-targeted therapeutic strategies might become an emerging cancer management modality and greatly enhance the survival rate of cancer patients.

Extracellular vesicles (EVs) are secreted by almost all kinds of cells and are divided into three categories according to their diameter, apoptosomes (500—2000 nm), microvesicles (20—1000 nm), and exosomes (20—100 nm).[5—7] More EVs are released from tumor cells than normal cells.[6] EVs not only interact with other components of the tumor microenvironment to promote tumor progression[8] but are also transported to distant organs to promote tumor metastasis through the following mechanisms: regulating cancer cell stemness,[8] enhancing tumor cell migration and invasion,[9] stimulating angiogenesis,[10] and inducing immunosuppression and chemoresistance.[11] In recent years, the effect of EV-ncRNAs on tumor progression has received growing attention. However, their effects and mechanisms in PMN formation have not been elucidated. In this review, we summarize the roles of EV-ncRNAs in different tumor PMNs and evaluate their clinical applications.

Pre-metastatic niche in tumors

The PMN is a microenvironment created by the primary tumor at specific metastatic sites that is conducive to tumor cell colonization and survival,[12] and it is essential for the formation of metastatic lesions. The concept of PMNs was first put forward in 2005, and subsequently, many basic studies on PMNs were performed. The main components of the PMN include primary tumor-derived cancer cells, EVs, bone marrow-derived cells (BMDCs), and host stromal cells and the factors they secrete. The main steps of PMN formation are priming, licensing, initiation, and progression. In the different phases of the PMN, various components interact with each other to determine its characteristics of immunosuppression, inflammation, angiogenesis, vascular permeability, lymphangiogenesis, organotropism, and reprogramming.[4,13,14] Dynamic changes in PMN composition bring tumor cells into a quiescent state and reactivate them at the appropriate time. In other words, they regulate the dormancy of cancer cells.[15,16]

Although EVs have been confirmed to be responsible for the survival of cancer cells migrating to the PMN,[17—19] the molecular mechanisms underlying the regulation of PMN establishment by EV cargoes remain largely unknown. Thoroughly investigating PMN drivers will provide numerous potential intervention targets for advanced cancer patients.

EV-ncRNAs and tumor progression

EVs include exosomes, microvesicles, and apoptotic bodies.[20] Microvesicles and apoptotic bodies are directly derived from the cell membrane.[21] The formation of exosomes involves several steps: early endosomal formation, formation of multiple intraluminal vesicles, formation of multivesicular endosomes, lysosome degradation, the fusion of multivesicular endosomes with the cell membrane, and eventual release of multivesicular endosomes to the outside of cells to form exosomes.[22] Although EVs were initially regarded as cellular metabolic garbage, they act as key carriers of intercellular communication.[23] EVs contain an abundance of proteins, lipids, DNAs, mRNAs, and ncRNAs.[24] EVs affect the receptor cell phenotype either by fusing with the membrane to induce receptor—ligand interactions or by being endocytosed.[25,26]

Increasing evidence suggests that tumor cells manipulate other cells in the tumor microenvironment (TME) by secreting EVs, which in turn facilitate cancer progression.[27] For example, EVs derived from hepatoblastoma cells promote the differentiation of bone marrow mesenchymal stem cells into cancer stem cells (CSCs) and result in cancer progression.[28] Due to their isotypic affinity for parental cells, exosomes are used as anti-cancer drug carriers for cancer treatment. In a mouse model of triple-negative breast cancer, transporting therapeutic miRNAs into tumors by modified exosomes led to significantly improved survival.[29]

Studies have shown that ncRNAs, including long ncRNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs), impact cancer progression. miRNAs exert post-transcriptional regulation of specific genes by targeting the 3′ untranslated region (3′- UTR) of mRNA.[30] LncRNAs regulate

chromatin dynamics, gene expression, growth, differentiation, and tumor development by interacting with RNAs, proteins, and DNAs.[31,32] CircRNAs have been demonstrated to function as miRNA sponges and to combine with proteins and be translated into proteins.[33] ncRNAs are enriched in tumor cell-derived EVs and are involved in malignant tumorigenesis, metastasis, drug resistance, immunosuppression, and angiogenesis. For example, EV-miR-150-5p favors lung cancer progression in hypoxic microenvironments by modifying the phenotype of natural killer (NK) cells,[34] EV-lncRNA-AGAP2-AS1 promotes cervical cancer cell proliferation by increasing sirtuin1 expression through competitive binding of miR-3064-5p,[35] and EV-circ_SLC19A1 activates the extracellular signal-regulated kinase 1/2 pathway to regulate prostate cancer (PCa) growth and invasion.[36] In addition, mesenchymal cell-derived EV-ncRNAs similarly exhibit tumor-regulating effects. For instance, NK-derived exosomal miRNA-186 directly inhibits the expression of MYCN, AURKA, TGFBR1, and TGFBR2 in neuroblastoma, providing a new potential target for immunotherapy.[37]

EV-ncRNAs are more abundant than protein-coding genes and are detectable in the plasma, urine, semen, and amniotic fluids of cancer patients, and these characteristics make them potentially reliable biomarkers for cancer diagnosis, metastasis, and prognosis.[38]

EV-ncRNAs cause immune suppression in the PMN

The immune system is the main killer of tumors, and immune deregulation in the PMN aids in the survival of tumor cells. EVs are basic forms of communication between the primary tumor and distant secondary sites. Tumor EVs exert an influence on T cells, macrophages, NK cells, and neutrophils, and EV-ncRNAs regulate the proliferation, differentiation, apoptosis, recruitment, and cytotoxicity of these immune cells. They even affect the interactions between immune cells.[39–43] Ultimately, this leads to the immune escape of cancer cells.

To investigate the effect of EV-ncRNAs on the immunosuppressive microenvironment of the PMN, mice were injected intravenously with tumor-derived EVs. One study reported that exosomal miRNA200b-3p directly inhibits phosphatase and tensin homolog, raising the expression of C–C motif chemokine ligand 2 (CCL2) in lung tissue through the AKT/NF-κB axis.[44] CCL2 contributes to the PMN throughmediating overexpression of endogenous Toll-like receptor 4 (TLR4) ligands, such as S100A8,[45] and recruiting myeloid-derived suppressor cells (MDSCs)[46,47] (Fig. 1B). In another study, BMDC EV-miR-92a activated hepatic stellate cells (HSCs) by enhancing the TGF-β pathway by targeting SMAD7. Activated HSCs promote the transendothelial migration of MDSCs to create an immunosuppressive microenvironment in the hepatic PMN of lung cancer[12] (Fig. 1A). In addition, HSCs convert mature peripheral blood mononuclear cells into MDSCs in a CD44-dependent manner.[48] These findings support the pivotal actions of MDSCs in PMN formation.[49] In breast cancer-bearing mice, MDSCs inhibit $CD8^+$ T-cell and NK cell function by producing reactive oxygen species and arginase 1.[50] MDCSs secrete exosomes, cytokines, chemokines, and growth factors that induce vascular leakage, extracellular matrix (ECM) remodeling, and immune disruption to promote the establishment of the PMN.[13,51,52]

In addition, the macrophage-regulated immune response is another important factor in tumorigenesis and metastasis.[53] Colorectal cancer (CRC)-derived exosomal miR-934 promoted liver metastasis by inducing macrophage M2 polarization.[54] EV-miR-21 binds to toll-like receptor 7 (TLR7) on macrophages, inducing polarization of macrophages toward a proinflammatory phenotype and the secretion of interleukin-6 (IL-6). The EV-miR-21-TLR7-IL6 axis promotes the pre-hepatic metastatic niche in CRC[55] (Fig. 1D). In addition, miR-378a-3p, which is enriched in EVs by hnRNPA2B1, is transported into bone marrow macrophages (BMMs), subsequently accelerating bone PMN shaping in PCa. Mechanistically, EV-miR-378a-3p increased Angptl2 expression by facilitating nuclear translocation of NFATC1 through inhibition of Dyrk1a[56] (Fig. 1E).

Furthermore, neutrophils trigger a pro-metastatic inflammatory microenvironment by suppressing innate and adaptive immunity.[57,58] In addition, the immune function and phagocytosis of lung epithelial cells contribute to the recruitment of neutrophils to the PMN.[59] At the molecular level, EV-snRNAs up-regulate TLR3 expression in lung epithelial cells through the NF-κB and MAPK pathways, thereby promoting chemokine production, neutrophil recruitment, and PMN formation[18] (Fig. 1C).

The "abscopal effect" of radiation therapy on tumors is partly mediated by the activation of the immune system, which lays the theoretical foundation for combining radiation therapy with immunotherapy.[60–62] There is evidence of significant changes in the phenotype of EVs and the miRNAs and proteins they contain after radiation treatment.[63–66] Whether the "abscopal effect" is due to changes in EVs and whether radiation inhibits the PMN by altering EV cargoes are valuable topics for future research. In addition, drugs cause dysregulation of EV cargoes. Long-term use of immunosuppressants increases tumor susceptibility in American kidney transplant recipients.[67] However, rapamycin has anti-tumor effects by inhibiting proliferation and anti-angiogenic activity. The expression of miR-6127, miR-6746-5p, and miR-6787-5p is significantly elevated in rapamycin-treated CRC cell EVs compared to untreated CRC cell EVs. Rapamycin down-regulated miR-6127 and miR-6746-5p and up-regulated miR-6787-5p in cyclosporine A-treated CRC cells. The above miRNAs inhibit the activation of lung fibroblasts in the PMN. The results of the bioinformatic analysis suggest that miR-6127, miR-6746-5p, and miR-6787-5p down-regulate histone genes that regulate the PMN.[68] Thus, whether an immunosuppressant inhibits or promotes cancer may depend on the particular conditions.

In summary, BMDCs and immune cells are continuously recruited to the secondary site, where they interact with cells to collectively transform the secondary site into a PMN that supports the ensuing metastasis. Reversing the immunosuppressive microenvironment of the PMN with miRNA inhibitors offers new prospects for the currently limited immunotherapeutic measures. Prior to clinical application, a tremendous amount of basic and clinical research is necessary.

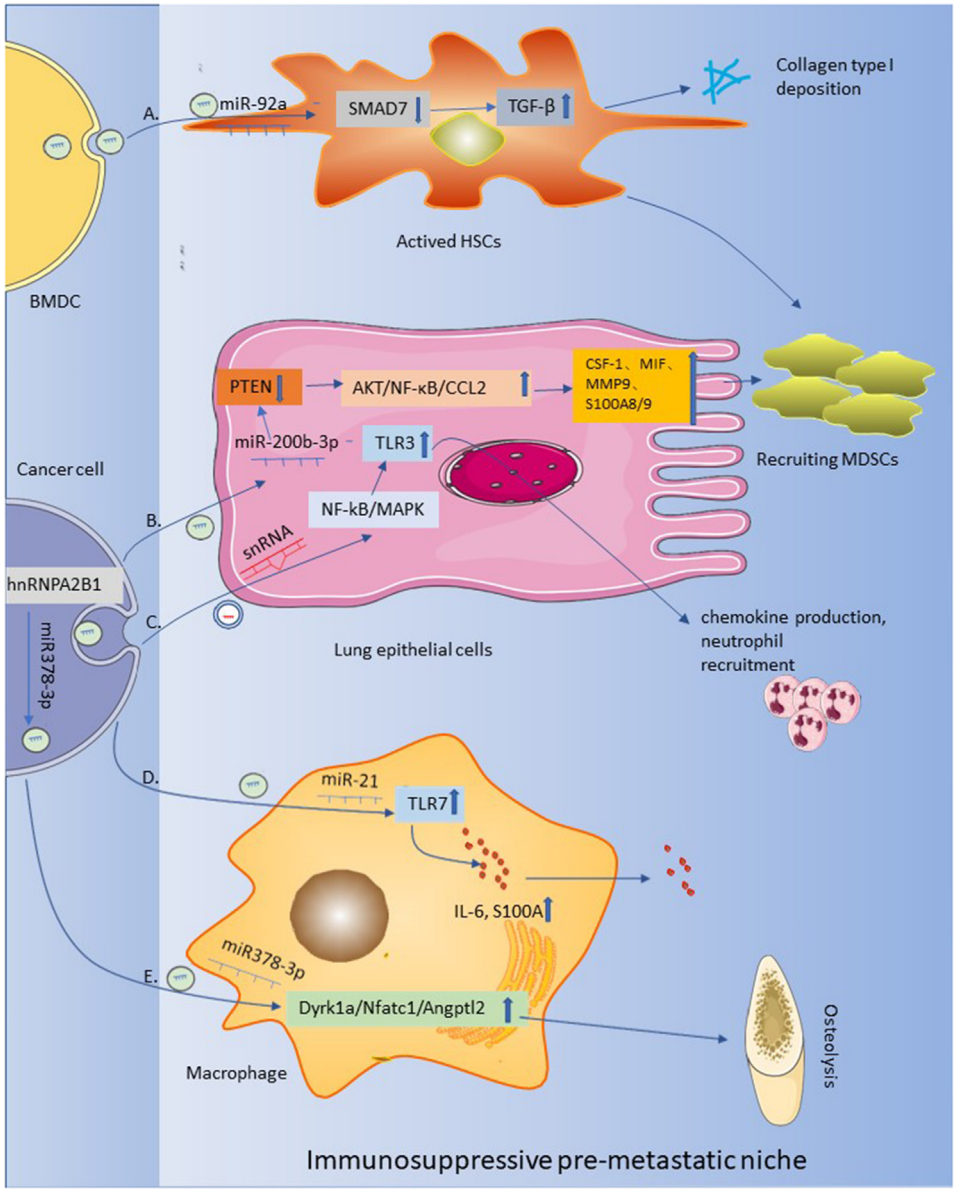

Figure 1 Effects of EV-ncRNAs on immune suppression in the PMN. **(A)** EV-miR-02a derived from BMDCs leads to the activation of HSCs and recruitment of MDSCs in the liver PMN. **(B)** Cancer cell exosomal miRNA200b-3p recruits MDSCs by the PTEN/AKT/NF-κB axis in the lungs. **(C)** EV-snRNAs result in chemokine production, neutrophil recruitment, and PMN formation in the lungs. **(D)** The EV-miR-21-TLR7-IL6 axis causes a pre-hepatic metastatic niche in CRC. **(E)** EV-miR-378a-3p is transported into BMMs to shape PMNs through the Dyrk1a/NFATC1/Angptl2 axis.

EV-ncRNAs regulate vascular permeability and angiogenesis in the PMN

To ensure the supply of nutrients for rapid tumor growth, the PMN initiates angiogenesis and increases vascular permeability. In this way, $VEGFR1^{+}$ hematopoietic progenitor cells, immune cells, stromal cells, and tumor cells in circulation easily extravasate from the blood vessels into the PMN.[4,14] EV-ncRNAs have earned notoriety for promoting angiogenesis and vascular permeability (Fig. 2).

ZO-1 is one of the most prominent elements in the tight junctions between endothelial cells. miR-105 is transferred to endothelial cells by exosomes secreted by breast cancer cells, thereby targeting ZO-1 to strengthen vascular permeability in distant organs.[69] Exosomal miRNAs (miR-638, miR-663a, miR-3648, and miR-4258) secreted by HuH-7M cells stimulate vascular permeability and trigger the intrahepatic PMN by suppressing the expression of VE-cadherin and ZO-1 in endothelial cells.[38] In addition, miR-25-3p was shown to be enriched in exosomes secreted by CRC. miR-25-3p not only enhances VEGFR2 promoter activity by targeting KLF2 to promote angiogenesis but also weakens the expression of tight junction molecules (ZO-1, occludin and claudin5) by targeting KLF4 to promote vascular permeability.[70] In a mouse model of CRC, exosomal miR-25-3p significantly enhanced vascular leakage and metastasis

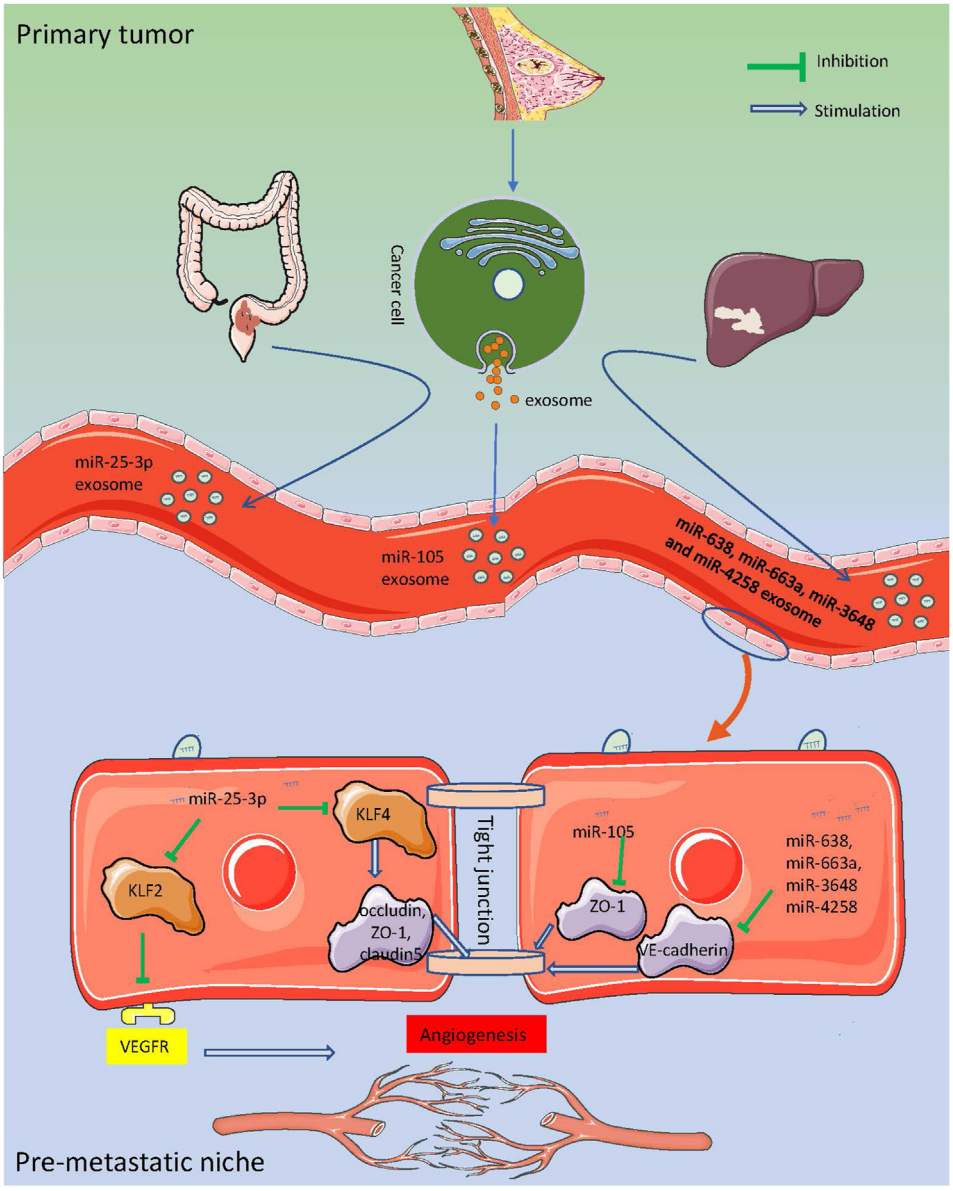

Figure 2 Effects of EV-ncRNAs on vascular permeability and angiogenesis in the PMN. EV-ncRNAs target vascular endothelial cell tight junction proteins, leading to increased vascular permeability and stimulating vascular neovascularization by stimulating VEGFR in the PMN.

in the liver and lung. In addition, in the serum of patients with metastatic CRC, exosomal miR-25-3p levels were significantly elevated compared to those in nonmetastatic patients.[70] Therefore, exosomal miR-25—3p participates in PMN formation and serves as a potential blood biomarker for CRC metastasis.

In summary, these studies indicate that the EV-mediated transfer of miRNA between cancer cells and vascular endothelial cells is essential for the PMN.

EV-ncRNAs modulate inflammatory factors in the PMN

Inflammation encourages cancer cells to grow and metastasize.[71] In the priming phase of the PMN, primary tumors become inflammatory. Chronic inflammation leads to the recruitment of immune cells to future metastatic organs to establish a favorable microenvironment.[14]

S100 protein is a common feature of the inflammatory PMN.[49] Exosomal S100A8/A9 not only promotes the polarization of M2 macrophages in mice with breast cancer[72] but also regulates serum amyloid A expression in pre-metastatic endothelial cells and alveolar macrophages, thereby attracting $CD11b^{+}$ bone marrow cells to pre-metastatic lungs.[73] In addition, tumor cell EV-ncRNAs boost the accumulation of S100 in the PMN. *In vivo*, CRC-EVs miR-21 elevates the production and secretion of IL-6 and S100A family members in mouse liver to provide a favorable environment for CRC liver metastasis.[55] *In vitro*, breast cancer-derived exosomal microRNA-200b-3p forces alveolar epithelial type II cells to up-regulate cytokines, such as cluster-stimulating

factor 1 (CSF-1), macrophage migration inhibitory factor (MIF), matrix metallopeptidase 9 (MMP9), and S100A8/9, to promote inflammatory PMN formation.[44]

In addition, IL-6 is one of the most essential proinflammatory cytokines in the TME and is secreted by macrophages, fibroblasts, and other cells. CRC-derived EV-miR-21 increases IL-6 synthesis and release by targeting the TLR7 pathway, polarizing macrophages, and thereby preparing an inflammatory positive feedback pathway in the liver PMN that allows CRC cell colonization.[55] It has been proven that the signal transducer and activator of transcription 3 (Stat3) is directly associated with miR-21,[74] and Stat3 activates macrophages to augment IL-6 secretion. The increased IL-6 induces miR-21 to activate Stat3.[55] Overexpression of Snail promotes metastasis by accelerating the epithelial—mesenchymal transition. In a study where the EV-miRNA profile between HT29-Snail (overexpressing Snail) cells and epithelial-like HT29 cells was compared, HT29-Snail EVs showed the up-regulation of let-7i, miR-205, and miR-130b, and the down-regulation of miR-1246, miR-3131, miR-375, miR-552- 3p, and miR-552—5p. HT29-Snail EVs lead to IL-8 secretion in lung macrophages and foster the inflammatory PMN probably through alterations in EV-miRNAs.[75] Similar work supported the role of inflammation regulated by EV-ncRNAs in the PMN. For example, immunofluorescence confirmed that Lewis lung carcinoma (LLC)-derived exosomes are predominantly phagocytosed by lung fibroblasts, leading to the expression of the inflammatory factors IL6, CCL1, CCL2, CCL5, and CXCL2. A miR-3473b inhibitor significantly reduced the expression of exosome-mediated inflammatory factors in fibroblasts.[76] CRC exosomal miR-10a regulates the expression levels of IL-6, IL-8, and IL-1β in primary normal human lung fibroblasts.[77] Although the mechanism is not well defined, these inflammatory factors accelerate the colonization of tumor cells. Blocking the inflammatory TME can delay PMN formation.

EV-ncRNAs induce PMN formation by regulating cellular metabolic reprogramming

The TME undergoes a series of metabolic reprogramming steps to meet the metabolic needs of tumor progression.[78,79] Tumors show significantly increased consumption of glucose through the glycolytic pathway.[80] miRNAs can directly regulate energy metabolism in tumor cells. For example, miR-155 up-regulates glycolysis,[81] while miR-210 down-regulates oxidative phosphorylation (OXPHOS).[82] Incremental extracellular acidification of the TME due to glycolysis imposes a huge burden on the immune response and leads to T lymphocyte incapacitation.[83] Initial acidification of the local environment is considered a marker of PMN formation.

The energy metabolism of normal stromal cells in distant organs is reprogrammed by tumor exosomes. Reprogrammed fibroblasts produce high-energy fuels, such as lactic acid, via aerobic glycolysis for utilization by cancer cells.[84,85] Coincubation of human adult dermal fibroblasts (HDAFs) with human melanoma-derived exosomes (HMEXs) increases aerobic glycolysis of HDAFs and decreases OXPHOS, resulting in increased extracellular acidification. This makes the PMN a sanctuary for metastatic tumor cells.[86] Transfecting inhibitors of miR-155 and miR-210 significantly reversed the metabolic reprogramming of HADFs, which confirmed that this effect was a consequence of HMEX miR-155 and miR-210[86] (Fig. 3A). In addition, glucose redistribution enhances the availability of nutrients to the PMN. CAFs supply energy metabolites to cancer cells via monocarboxylate transporter proteins, which are dependent on glycolysis.[87] Glucose transporters (GLUT1) are regulated by the PI3K/AKT/mTOR pathway and are responsible for fundamental levels of glucose uptake in all cells.[88] Breast cancer EV-miR-122 inhibits the glucose uptake of normal breast epithelial cells, lung fibroblasts, and brain astrocytes. Mechanistically, miR-122 binds to the 3′-UTR of pyruvate kinase (PKM) and inhibits GLUT1 transcription by reducing the localization of p-PKM2 in the nucleus. Interestingly, miR-122 inhibits the proliferation of primary breast cancer cells by down-regulating GLUT1, while it promotes metastasis in the brain and lung. Moreover, inhibition of miR-122 restored glucose uptake in the brain and lung and reduced PMN formation *in vivo*[89] (Fig. 3C).

Two other studies have reported that CSC EV-miRNAs target energy metabolism in the microenvironment to influence PMN formation. A total of 1839 miRNAs were identified in PCa bulk cells and CSC exosomes, of which 19 miRNAs were significantly differentially expressed. Their target genes regulate glucose metabolism, protein synthesis, and degradation.[90] According to bioinformatic analysis, the biological processes regulated by miRNAs in EVs of renal CSCs include metabolic processes, transcription, nucleic acid binding, and cell adhesion.[91] Although these are only bioinformatic predictions, they provide new clues for subsequent research.

Cellular metabolic reprogramming has fascinated oncologists, and stopping or reversing energy metabolic reprogramming in the TME is of great benefit to cancer patients.

EV-ncRNAs induce PMN formation by moderating fibroblasts

Fibroblasts are one of the most plentiful stromal cells in the TME. Normal fibroblasts are deemed to possess tumor progression inhibitory effects in the early stages. As time progresses, the tumor suppressive activity of fibroblasts is lost, and the fibroblasts induce more tumorigenesis and metastasis through the following mechanisms[92—94]: (i) promoting tumor cell proliferation and increasing CSC properties; (ii) driving angiogenesis by recruiting endothelial cells and increasing tumor vascularization; (iii) increasing cancer aggressiveness by disrupting the basement membrane; (iv) increasing suppressive immune cell infiltration; (v) inducing the Warburg effect; and (vi) inducing tumor drug resistance.[95] Fibroblasts are transformed into tumor-associated fibroblasts (TAFs) by EV-ncRNAs.[96,97] Reliable markers for the identification of TAFs include alpha-smooth muscle actin (α-SMA), vimentin, fibroblast activation protein, fibroblast-specific protein 1, and platelet-derived growth factor receptor (alpha and beta).[2,98—100]

Fibroblasts are routinely utilized as a tool to model PMNs *in vitro*. Normal fibroblasts transfected with miR-100-5p and miR-21-5p showed increased expression of MMP-2, -9,

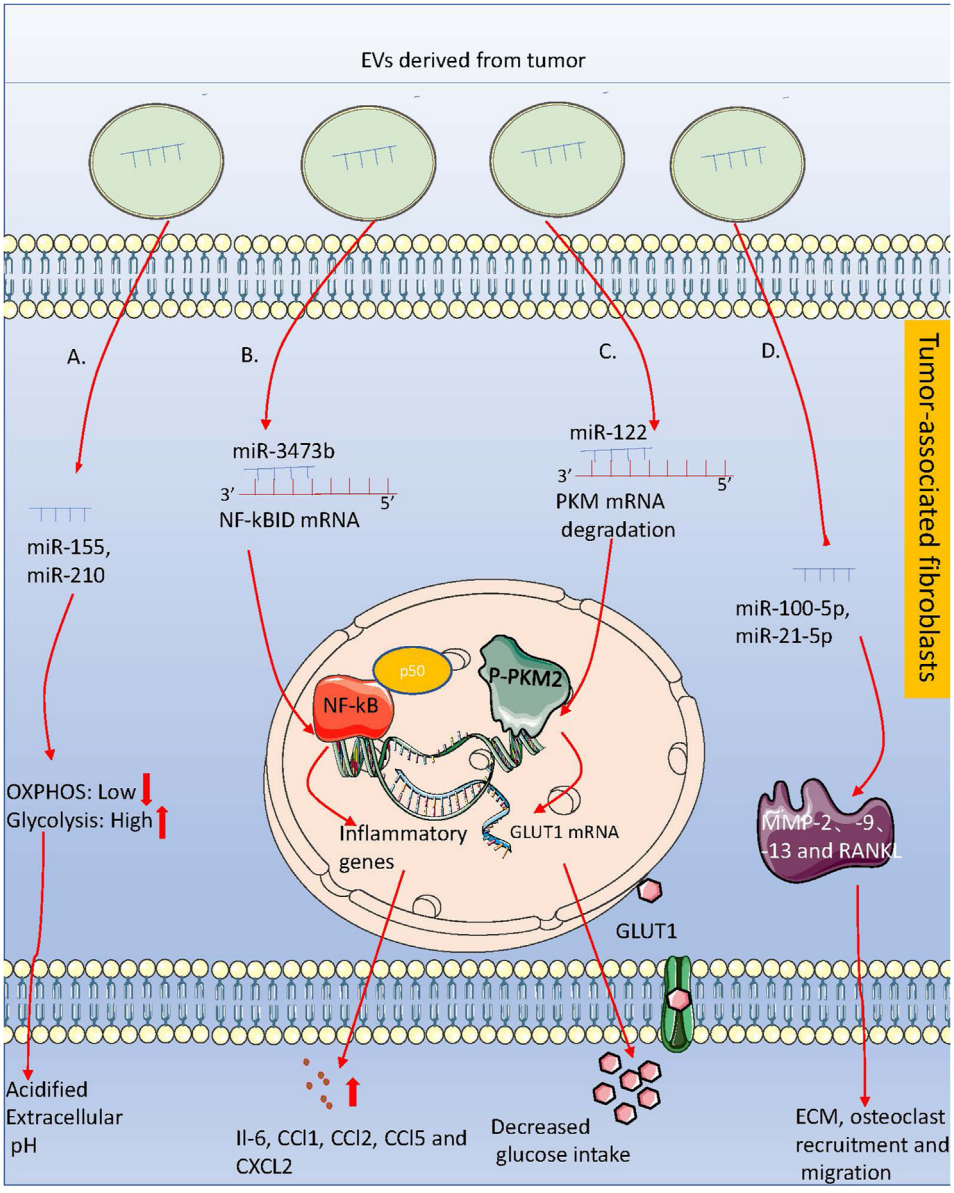

Figure 3 Effects of EV-ncRNAs on cellular metabolic reprogramming and fibroblast moderation. **(A)** HMEX miR-155 and miR-210 increase aerobic glycolysis of HDAFs and decrease OXPHOS. **(B)** Exosomal miR-3473b activates the NF-κB pathway in TAFs resulting in the secretion of inflammatory molecules. **(C)** EV-miR-122 inhibits glucose uptake by TAFs. **(D)** miR-100-5p and miR-21-5p lead to ECM remodeling and osteoclast recruitment and migration.

and -13 and osteoclast recruitment and migration-related protein (RANKL), which support PMN formation[90] (Fig. 3D). The proliferation and migration abilities of fibroblasts treated with exosomes have been found to be augmented.[77,101] Tumor-derived exosomal miR-1247-3p has been shown to activate CAFs, thereby promoting lung metastasis in hepatocellular carcinoma (HCC).[102] NF-κB is critical for the inflammatory microenvironment regulated by TAFs.[103] miR-3473b contained in LLC-derived exosomes, not the miR-3473b mimic, activated the NF-κB signaling pathway in α-SMA^{+} fibroblasts to induce tumor cell colonization in the lung, which suggests that exosomal miR-3473b activation of the NF-κB pathway is exosome-dependent[76] (Fig. 3B). In addition, the HADF metabolic status is altered by the HMEX miR-155 and miR-210, which increase extracellular matrix acidification to facilitate PMN formation.[86] Fibroblasts incubated with breast cancer cell exosomes are characterized by aberrant lncRNA expression. Differentially expressed lncRNAs regulate mRNAs that are involved in the TGF-beta signaling pathway, pentose phosphate pathway, Hedgehog signaling pathway, metabolic pathway, and complement and coagulation cascades.[101]

Unexpectedly, EV-miR-6127, -miR-6746-5p, and -miR-6787-5p inhibit fibroblast activation. They are predicted to down-regulate histones involved in chromatin organization, DNA packaging, and the cell cycle. They are hypothesized to reduce pulmonary PMN formation under rapamycin treatment in patients with posttransplant CRC.[68]

The above research findings provide unique insight into the prevention of metastasis, from the perspective of TAF suppression.

The function of EV-ncRNAs in inducing PMN formation in different tumors

CRC

CRC is the third most commonly diagnosed cancer in males and the second most commonly diagnosed cancer in females worldwide.[104] The metastatic organs for CRC are primarily the liver and lungs, which account for the majority of CRC-related deaths.[105] Recently, several CRC EV-ncRNAs have been described to affect PMN formation at CRC metastasis sites through multiple mechanisms. For example, exosomal miR-25-3p significantly entices hepatic and pulmonary vascular leakage in mice.[70] Lung fibroblasts are a functional target of CRC EV-ncRNAs. Exosomal miR-10a reprograms the activity of NHLFs and modifies the levels of inflammatory factors.[77] Obvious up-regulation of EV-miR-6127, -miR-6746-5p, and -miR-6787-5p under rapamycin treatment restrained lung fibroblasts to suppress PMN formation in posttransplant CRC.[68] In addition, CRC EV-miRNAs evoke PMN formation in the liver and lung by inducing macrophages to polarize toward the M2 phenotype.[55,75] These studies provide potential targets for intervention to prevent early CRC metastasis.

HCC

HCC is the most frequent primary liver cancer.[106] After surgical resection, chemotherapy, and radiofrequency ablation treatment, intrahepatic metastasis and recurrence are among the leading causes of death from HCC.[107] This is mainly due to the specific hemodynamics of the liver. HuH-7M exosomal miRNAs play a role in initiating PMN formation in the HCC liver by disrupting endothelial cell tight junctions and increasing the permeability of vascular endothelial cells.[38] To discover HCC markers superior to AFP, more EV-ncRNAs in the liver cancer PMN need to be identified.

Pancreatic cancer

Pancreatic cancer is the worst cancer in terms of prognosis; it is typically detected with extensive lymphatic metastases, its morbidity rate is approximately equal to its mortality rate, and it has a 5-year survival rate of less than 5%.[108,109] The role of EV-miRNAs in pancreatic cancer metastasis is gradually being emphasized.[110] Exosomes obtained from metastatic rat pancreatic cancer BSp73ASML cells are taken up by lymph node stromal cells (LnStr) in a CD44v-dependent manner. miR-494 and miR-542-3p up-regulate the transcription of MMP2, MMP3, and MMP14 by targeting cadherin-17, contributing to the establishment of PMNs.[111] Investigating how CD44v regulates exosomal miRNA profiles may be a promising therapeutic strategy to stop PMN establishment.

Lung cancer

Lung cancer is the malignancy with the highest morbidity and mortality worldwide, and more than 70% of lung cancer patient deaths result from metastasis.[106,112] The adrenal glands, bones, brain, and liver are all organs for lung cancer metastasis, each of which requires a supportive microenvironment at its destination to allow the arriving cancer cells to survive.[18] ECM remodeling, collagen type I overexpression, and g-MDSC accumulation in the liver have been proven to be generated from EV-miR-92a secreted by BMDCs, increasing the adhesion of LLC cells in mouse livers.[12] In addition, metastasis is strongly influenced by CAFs (102). LLC-derived exosomal miR-3473b is transported into lung fibroblasts, inducing intrapulmonary colonization of lung tumor cells by blocking the NF-κB inhibitor delta's capability to interact with p50.[76,113] Furthermore, LLC-derived exosomal snRNA initiates the pulmonary inflammatory cascade response to establish a PMN.[18]

Breast cancer

Breast cancer is the second most common cause of cancer-related death in females worldwide.[114] Bone metastases occur most frequently in breast cancer, and osteolytic metastases and related complications occur in 80% of patients with advanced breast cancer.[115] Cancer cell—osteoclast interactions regulate breast cancer bone metastasis.[116] miR-21 has been reported to be associated with breast cancer progression[117] and osteoclast development.[118] Mechanistically, miR-21 targets the programmed cell death 4 (PDCD4) mRNA 3′-UTR to prompt osteoclastogenesis.[119] Similarly, an animal study showed that exosomal miR-21 from SCP28 cells promoted osteoclastogenesis but not osteoblastogenesis while forming PMNs in bone.[120]

In addition, the lung is another common metastatic organ of breast cancer. EV-ncRNAs manipulate lung PMN formation through multiple mechanisms. For example, breast cancer secretes microRNA-200b-3p to promote the immunosuppressive microenvironment in the lung[44]; miR-105 induces vascular permeability by up-regulating ZO-1; miR-122 impairs the uptake of glucose by stromal cells in the PMN[89]; and lncRNAs are involved in PMN formation by activating lung fibroblasts.[101]

PCa

Similarly, the bulk of deaths in PCa patients is ascribed to bone metastases. Primary tumor cells must undergo invasion of surrounding tissues, intravasation, migration, survival in the circulation, extravasation, angiogenesis, and formation of a premetastatic microenvironment before bone metastases can form.[121] BMMs have been characterized as contributors to bone metastasis.[122] The BMM Dyrk1a/Nfatc1/Angptl2 axis is activated by miR-378a-3p-containing EVs to boost osteolysis and promote PMN formation in bone in PCa.[56] In addition, miR-100-5p and miR-21-5p are overexpressed in PCa exosomes and are involved in osteoblast differentiation in preparation for PMN formation.[90] Underlying molecular regulatory mechanisms may provide ideas for intervention in the PMN in PCa.

Melanoma

Melanoma is a highly metastatic skin cancer, and once metastasis occurs, the 5-year survival rate is less than 15%.[123] It is now better recognized that EVs have a complex association with metastasis and that melanoma exosomes access distal organs to recruit BMDCs to promote PMN formation and make the site vulnerable to metastasis.[124] Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) in melanoma EVs modulates the expression of mRNAs, proteins, and miRNAs to affect the PMN. Mice treated with IGF2BP1-down-regulated cell-derived EVs showed far less intrapulmonary fibronectin deposition and deposition of $CD45^+$ cells.[125] In addition, suppressing exosomal miR-155 and miR-210 inhibits PMN formation by reversing HMEX-induced extracellular acidification of HADFs.[86]

The above findings confirm the role of EV-ncRNAs in the PMN and may provide novel channels for the development of metastasis inhibitors.

Mechanisms by which distinct ncRNAs in EVs facilitate PMN formation

Tumor-derived EVs have been reported to contain ncRNAs involved in the metastatic process. miRNAs are considered to exert crucial roles in angiogenesis regulation,[126] migration invasion,[127] and the immune response.[128] In recent years, the role of EV-miRNA in guiding the PMN has gained great attention. The lung, liver, and bone are usually target organs for a large number of carcinoma metastases. The establishment of the PMN is accomplished in part by the following mechanisms mediated by EV-miRNAs: angiogenesis and vascular permeability, immunosuppression, energy metabolism reprogramming, up-regulation of inflammatory molecules, matrix remodeling, macrophage differentiation, osteoclast activation, and conversion of fibroblasts to CAFs.

In addition, lncRNAs in EVs have been reported to be associated with metastasis.[129] lncRNAs are another subset of ncRNAs that are more than 200 nucleotides in length. A significant number of lncRNAs are expressed abnormally and are engaged in breast cancer lung PMN. High-throughput sequencing results indicate that there are 64 lncRNAs with increased expression and 8 lncRNAs with decreased expression in WI-38 cells and HFL1 cells treated with MDA-MB-231-secreted exosomes compared to MCF-10A-serected exosomes.[101] However, how these aberrant lncRNAs function in PMN formation needs to be further investigated.

Furthermore, snRNA is a ligand for TLR3 in lung epithelial cells and is enriched in exosomes. Activated TLR3 initiates a prometastatic inflammatory response in the lung.[18]

Finally, circRNA is a special closed-loop structure RNA with high stability that is conserved between species. Although not in an EV-dependent manner, circIKBKB significantly enhances the ability of BC cells to induce the formation of bone PMNs by promoting osteoclastogenesis through activation of the NF-κB pathway. CircRNAs enriched in EVs play a unique role in tumors. In the future, the role of EV-circRNAs in the PMN will be gradually revealed.

In conclusion, different kinds of ncRNAs derived from EVs have been identified to promote PMN formation, and the role of circRNAs in PMN formation will be investigated prominently in the future.

EV-ncRNAs as tumor biomarkers and therapeutic targets

Although improvements in cancer screening and management protocols have largely improved the prognosis of cancer, patients with cancer experience a dramatic reduction in survival rate once metastasis occurs. Therefore, the development of new predictive and diagnostic tools for metastasis is a challenge.

Recently, circulating EVs were described as a diagnostic and prognostic tool for patients with various cancers.[130,131] miRNAs are protected from nuclease degradation by EVs and are therefore stable in the circulation.[132] miR-21,[120] miR-122,[89] and miR-200b-3p[44] secreted by tumors are up-regulated in the serum of patients with metastasis and can be used as biomarkers to predict the metastases of breast cancer. In addition, the exosomal miR-21 level may be an independent prognostic factor for overall survival and disease-free survival in patients with advanced CRC.[133] EV-miR-21 and miR-25-3p expression levels are higher in the circulation of patients with liver metastasis than in patients without metastasis and are positively correlated with CRC liver metastasis.[55,70] Furthermore, EV-ncRNAs released from CRC cells can be used as biomarkers for CRC staging.[75] The expression level of miR-10a was down-regulated in the serum and cancer tissues of CRC patients and was negatively correlated with the depth of infiltration of CRC.[77]

Key molecules in the PMN are not only used as metastatic and prognostic biomarkers but also as potential therapeutic targets for cancer patients. For example, scientists have developed a nanoparticle carrying siS100A4, named "CBSA/siS100A4@Exosome", that efficiently targets the lung PMN due to the affinity of the exosomes. It exhibits outstanding inhibition of lung metastasis in triple-negative breast cancer mice.[134]

In short, as more in-depth knowledge of the PMN is obtained, more reliable liquid biopsy biomarkers and targeted drugs to combat metastasis will be developed.

Conclusions

EV-ncRNAs exert various functions in the formation of the PMN. EV-ncRNAs regulate not only angiogenesis and vascular permeability, immunosuppression, energy metabolism reprogramming, the expression level of inflammatory molecules, and matrix remodeling, but also the conversion of fibroblasts to CAFs. However, there are still multiple topics that need to be studied: (i) the mechanism of specific enrichment of ncRNAs in EVs; (ii) the mechanism of the particular dissemination of EV-ncRNAs to the PMN; (iii) whether circRNAs have roles similar to those of miRNAs in the PMN; (iv) whether EV-ncRNAs undergo dynamic changes in different stages of the PMN; and (v) how the theoretical basis of the roles of EV-ncRNAs in the PMN can be translated into clinical diagnosis and treatment.

To conclude, despite much progress in terms of understanding the cellular and molecular interactions within the PMN, numerous underlying mechanisms remain elusive. Targeting PMN-promoting molecules and cells to inhibit PMN formation and prevent metastasis may be a promising strategy for tumor therapy. More efforts need to be made to identify early diagnostic biomarkers and develop better interventions. Cancer patients will certainly benefit from research in this area, with improved survival rates and better quality of life.

Author contributions

YL, ZS, and CW provided direction and guidance throughout the preparation of this manuscript. ZC and QW wrote and edited the manuscript. JL, WW, and WY reviewed and made significant revisions to the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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