Extracellular vesicle long non–coding RNA-mediated crosstalk in the tumor microenvironment: Tiny molecules, huge roles

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Abstract
Emerging evidence has shown that dynamic crosstalk among cells in the tumor microenvironment modulates the progression and chemotherapeutic responses of cancer. Extracellular vesicles comprise a crucial form of intracellular communication through horizontal transfer of bioactive molecules, including long non–coding RNA (lncRNA), to neighboring cells. Three main types of extracellular vesicles are exosomes, microvesicles and apoptotic bodies, exhibiting a wide range of sizes and different bio genesis. Over the last decade, dysregulation of extracellular vesicle lncRNA has been revealed to remodel the tumor microenvironment and induce aggressive phenotypes of tumor cells, thereby facilitating tumor growth and development. This review will focus on extracellular vesicle lncRNA-mediated crosstalk between tumor cells and recipient cells, including tumor cells as well as stromal cells in the tumor microenvironment, and overview the mechanisms by which lncRNA are selectively sorted into extracellular vesicles, which may pave the way for their clinical application in cancer diagnosis and treatment.

KEYWORDS
biomarker, chemoresistance, extracellular vesicle, long non–coding RNA, therapeutics, tumor microenvironment, tumor progression
1 | INTRODUCTION

The tumor microenvironment (TME), composed of cancer cells, stromal cells and the extracellular matrix (ECM), creates a niche for their residence and interactions. The representative stromal cells include endothelial cells, mesenchymal stem cells, cancer-associated fibroblasts (CAF), adipocytes and infiltrating immune cells. It is now accepted that the reciprocal communication among cells in the TME plays a significant role in the ECM remodeling, angiogenesis, drug resistance, energy metabolism reprogramming and anti-tumor immune responses.

Tumor cells can exchange information with recipient cells through cell-to-cell contact, secretion of soluble factors, as well as release of extracellular vesicles (EV). EV, heterogeneous membrane-enclosed phospholipid vesicles, are implicated in cancer initiation, angiogenesis, tumor immunity and drug resistance. They are usually subdivided into three main types based on their size and biogenesis: exosomes (40-100 nm), microvesicles (50-1000 nm) and apoptotic bodies (800-5000 nm). Among these, exosomes, particles that are derived from endosomal origin, have drawn increasing attention in the field of cancer research. According to their endosomal origin, knockdown or overexpression experiments of ESCRT-pathway molecules like Rab27a, TSG101 and Hrs are necessary for determining exosomes. Particles only detecting surface markers or particle size are not defined as exosomes. Hence, we use the term small EV (sEV) instead of exosomes in the references which do not perform studies for determining EV as of endosomal origin.

Extracellular vesicles have emerged as extracellular messengers to regulate signaling pathways and gene expression by transferring diverse cargoes, including long non-coding RNA (lncRNA). LncRNA are defined as RNA transcripts longer than 200 nucleotides with a lack of protein-coding capacity, which modulate the occurrence and development of cancer. Recently, EV-enriched lncRNA have been shown to shape the local cellular microenvironment and mediate phenotypic alterations of cancer cells. In this review, we aim to summarize the EV lncRNA-mediated crosstalk between tumor cells and the recipient cells in the TME. The article further discusses the underlying mechanisms of cancer cells selectively sorting lncRNA into EV, and highlights the promising clinical applications of EV lncRNA in cancer diagnosis and treatment.

2 | EXTRACELLULAR VESICLE LONG NON-CODING RNA MEDIATE CROSSTALK BETWEEN TUMOR CELLS

As a key mediator of cell-to-cell communication, tumor-derived EV could package and transfer lncRNA to target cells, including neighboring tumor cells and stromal cells, thereby modulating their phenotypes and remodeling the TME. EV lncRNA mediating the progression and chemoresistance of tumor cells in the microenvironment are included in Table 1.

2.1 | Digestive system

2.1.1 | Small extracellular vesicle long non-coding RNA

Several lines of evidence support the notion that EV-mediated delivery of lncRNA is associated with the progression of gastrointestinal cancer. sEV-mediated overexpression of lncRNA ZFAS1 enhances the proliferation and migration of gastric cancer cells. According to Li et al, highly invasive pancreatic ductal adenocarcinoma (PDAC) cells package and transfer IncRNA-Sox2ot to recipient PDAC cells via sEV. Upon competitively binding to the miR-200 family, IncRNA-Sox2ot upregulates Sox2 expression and promotes the epithelial–mesenchymal transition (EMT) and stem cell properties, aiding in the progression and metastasis of PDAC. Moreover, circulating IncRNA-Sox2ot encapsulated in sEV is correlated with the TNM stage, lymphatic or vascular invasion, and the overall survival of PDAC patients, rendering it useful marker for pancreatic cancer prognosis. In contrast, EV lncRNA exert a strong influence on the chemotherapeutic responses of gastrointestinal cancer through diverse mechanisms. Transmitted from cisplatin-resistant gastric cancer cells to sensitive cancer cells, sEV lncRNA HOTTIP sponges miR-218 to activate HMGA1, and confers cisplatin resistance to sensitive cancer cells. Furthermore, expression of circulating lncRNA HOTTIP in sEV is significantly upregulated in cisplatin-resistant gastric cancer patients in contrast to the cisplatin-sensitive gastric cancer patients, indicating its potential use for the early diagnosis and treatment of gastric cancer. Like lncRNA HOTTIP, lncRNA PART1 is enriched in the gefitinib-resistant cells and correlated with poor response to gefitinib in esophageal squamous cell carcinoma. Kang et al discovered that gefitinib-resistant cells could deliver lncRNA PART1 to sensitive cells via sEV, which increases gefitinib-resistant potency by regulating miR-129/Bcl-2 pathway. In colorectal cancer (CRC), UCA1-containing sEV derived from cetuximab-resistant cells could be transferred to recipient cancer cells, enhancing UCA1 expression and cetuximab resistance in vitro.

2.1.2 | Large extracellular vesicles

Likewise, the malignant progression and chemoresistance of gastrointestinal cancers could be modulated by large EV lncRNA. IncRNA TUC339 could be exported by hepatocellular cancer (HCC) cells through large EV and taken up by adjacent counterparts, thus promoting HCC cell growth and suppressing cell adhesion to ECM. In addition to this, Takahashi et al observes that large EV-mediated transport of lincRNA-ROR plays a functional role in TGFβ-dependent chemoresistance in HCC. Knockdown of lincRNA-ROR promotes cell apoptosis in response to sorafenib, camptothecin or doxorubicin through p53 signaling, and simultaneously reduces expression of CD133 + tumor-initiating cells, further supporting targeting lincRNA-ROR to potentiate chemosensitivity in HCC.
### TABLE 1  EV IncRNA mediate the progression and chemoresistance of tumor cells in the TME

| System          | Tumor type  | EV IncRNA     | EV type | EV identification | Target | Function                                                                 | Reference |
|-----------------|-------------|---------------|---------|------------------|--------|--------------------------------------------------------------------------|----------|
| Digestive System| PDAC        | IncRNA-Sox2ot | sEV     | TEM, WB          | miR-200| Promote progression and metastasis                                       | 12       |
| Gastric cancer  |             | ZFAS1         | sEV     | TEM, NTA, WB     | /      | Promote cell proliferation and migration                                  | 10       |
| ESCC            | PART1       | HOTTIP        | sEV     | TEM, NTA, WB     | miR-218| Confer cisplatin resistance                                               | 13       |
| CRC             | UCA1        | TUC339        | Large EV| TEM, DGC         | /      | Confer gefitinib resistance                                               | 14       |
| HCC             |             | lincRNA-ROR   | Large EV| TEM, NTA         | p53 signaling | Confer sorafenib, camptothecin, or doxorubicin sensitivity                | 17       |
| Gynecological system | Breast cancer | AFAP1-AS1     | sEV     | TEM, NTA, WB     | ERBB2  | Confer trastuzumab resistance                                             | 18       |
|                 | AGAP2-AS1   | SNHG14        | sEV     | TEM, WB          | /      | Confer trastuzumab resistance                                             | 19       |
|                 | UCA1        | H19           | sEV     | TEM, NTA, WB     | /      | Confer gefitinib resistance                                               | 20       |
|                 | H19         | RP11-838N2.4  | sEV     | TEM, WB          | FOXO1  | Confer doxorubicin resistance                                             | 21       |
| Respiratory system | NSCLC      | H19           | sEV     | TEM, NTA, WB     | /      | Confer gefitinib resistance                                               | 22       |
|                 |             | Renal cancer  | IncARSR | TEM, NTA, WB     | miR-34/miR-449 | Confer soratinib resistance                                               | 23       |
|                 | Prostate cancer | PCSEAT   | sEV     | TEM, NTA, WB     | miR-143-3p/miR-24-2-5p | Confer gefitinib resistance                                               | 24       |
| Urogenital system | Bladder cancer | UCA1       | sEV     | TEM, NTA, WB     | /      | Confer gefitinib resistance                                               | 25       |
|                 | Renal cancer | IncARSR      | sEV     | TEM, NTA, WB     | miR-34/miR-449 | Confer soratinib resistance                                               | 26       |
| Neural system   | Pituitary adenoma | H19       | sEV     | TEM, NTA, WB     | 4E-BP1| Inhibit cell growth                                                      | 27       |
|                 | Glioblastoma | SBF2-AS1     | sEV     | TEM, NTA, WB     | miR-151a-3p/XRCC4 | Confer temozolomide resistance                                             | 28       |

*Note*: /, not disclosed; CRC, colorectal cancer; DGC, density gradient centrifugation; ERBB2, Erb-B2 receptor tyrosine kinase 2; ESCC, esophageal squamous cell carcinoma; EV, extracellular vesicles; FOXO1, forkhead box protein O1; HCC, hepatocellular cancer; IncRNAs, long non-coding RNA; NSCLC, non–small cell lung cancer; NTA, nanoparticle tracking analysis; PDAC, pancreatic ductal adenocarcinoma; sEV, small extracellular vesicles; TEM, transmission electron microscopy; TME, tumor microenvironment; WB, western blot; XRCC4, X-ray repair cross complementing 4.
Taken together, intercellular trafficking of IncRNA via EV modulates the development and chemoresistance of gastrointestinal cancer. EV IncRNA could function as not only appealing biomarkers for cancer diagnosis and prognosis but also attractive therapeutic targets to reverse chemoresistance. Clinical and preclinical trials regarding the utilization of EV IncRNA in cancer treatment have not yet been reported. It remains to be seen whether EV IncRNA-targeted therapy is a panacea for cancer, or a poison pill with serious side effects.

2.2 | Gynecological system

Dysregulation of EV IncRNA could regulate the resistance to chemotherapy in breast cancer through multiple mechanisms. In HER-2-positive breast cancer, sEV-containing IncRNA AFAP1-AS1 interacts with AU-binding factor 1 to upregulate the translation of Erb-B2 receptor tyrosine kinase 2 (ERBB2), resulting in dissemination of trastuzumab resistance.18 Recent studies have also proved that IncRNA AGAP2-AS1 and IncRNA-SNHG14 in sEV disseminate resistance to trastuzumab from trastuzumab-resistant cells to sensitive cancer cells.19,20 Dong et al performed a signal transduction reporter array to suggest that IncRNA-SNHG14 contained in sEV mediates trastuzumab resistance by inducing Bcl-2 expression and inhibiting Bax expression.20

Apart from trastuzumab, tamoxifen and doxorubicin are widely applied as first-line drugs for breast cancer.21,22 Xu et al found that sEV-mediated transfer of UCA1 from tamoxifen-resistant LCC2 cells could significantly induce the tamoxifen resistance in tamoxifen-sensitive MCF-7 cells.23 In addition, IncRNA H19 in sEV could be delivered from doxorubicin-resistant cells to sensitive cells, resulting in enhanced chemoresistance of doxorubicin.24 The involvement of EV IncRNA in chemoresistance has attracted huge attention among researchers, but their functional roles in cancer development and underlying molecular mechanisms remain to be elucidated in breast cancer.

2.3 | Respiratory system

Currently, resistance to tyrosine kinase inhibitors seriously compromises the effect of chemotherapy and results in cancer-related death in non–small cell lung cancer (NSCLC).25 However, the contribution of EV IncRNA to drug resistance in recipient cells is still poorly understood. Lei et al demonstrated that IncRNA H19 is responsible for the gefitinib resistance in NSCLC. In addition, sEV-mediated transport of IncRNA H19 confers gefitinib resistance to sensitive NSCLC cells.26 Treatment-sensitive NSCLC cells are endowed with resistance to erlotinib by internalizing sEV IncRNA RP11-838N2.4 from erlotinib-resistant cells.27 Acting as a transcription inhibitor, forkhead box protein O1 (FOXO1) could inactive IncRNA RP11-838N2.4 by binding to its promoter region in erlotinib-resistant cell lines.27

2.4 | Urogenital system

Intratumoral hypoxia is one of the most important features of the solid tumor microenvironment, posing an obstacle to rapid tumor growth.28 Hypoxia increases the transfer of sEV IncRNA-UCA1, promoting cell proliferation, invasion and migration in the recipient bladder cancer cells. The level of circulating IncRNA-UCA1 packaged in sEV is remarkably higher in bladder cancer patients than normal controls, and predicts bladder cancer with a sensitivity and specificity of 80% and 83.33%, respectively.29 However, large-scale clinical studies are still needed to validate its diagnostic or prognostic values. In renal cancer, Qu et al identified that sEV IncARSR facilitated AXL and c-MET expression through sponging miR-34/miR-449, disseminating sunitinib resistance to the sunitinib-sensitive cells.30 Locked nucleic acid-based therapy targeting IncARSR could partially restore the sunitinib response of renal cancer. Yang et al confirmed that sEV IncRNA PCSEAT positively regulates EZH2 expression to mediate the cellular proliferation and motility by sponging miR-143-3p and miR-24-2-5p in prostate cancer.31

2.5 | Neural system

Pituitary adenoma accounts for over 25% of all primary intracranial tumors.32 sEV-mediated delivery of IncRNA H19 inhibits the growth of distal pituitary adenoma cells by suppressing 4E-BP1 phosphorylation. Meanwhile, cabergoline could induce H19 expression and exert a synergic effect with sEV H19 in inhibiting pituitary tumor growth.32 In contrast to benign pituitary adenoma, glioblastoma in the most common primary malignant tumor in the brain.33 Zhang et al proposed that IncRNA SBF2-AS1 in temozolomide-resistant glioblastoma cells could be shuttled to peripheral sensitive cells via sEV and suppresses their temozolomide sensitivity by accelerating the DNA damage repair through the miR-151a-3p/X-ray repair cross complementing 4 (XRCC4) axis. Collectively, EV IncRNA play a pivotal role in chemoresistance of neural tumors; however, their biological effects in neural tumors remain a mystery.

3 | EXTRACELLULAR VESICLE LONG NON–CODING RNA MEDIATE CROSSTALK BETWEEN TUMOR CELLS AND STROMAL CELLS

3.1 | Mesenchymal stem cells

Mesenchymal stem cells (MSC) are frequent components in the TME, contributing greatly to the growth and development of cancer.34 Recently, it has been reported that EV IncRNA orchestrate intercellular communication between tumor cells and MSC (Figure 1).35 Wang et al demonstrated that a set of IncRNA are differentially expressed in adipose-derived mesenchymal stem cells (AD-MSC) stimulated with lung cancer-derived sEV, shedding
new light on the involvement of sEV lncRNA in the regulation of AD-MSC. ZHANG et al. could be transmitted from multiple myeloma (MM) cells to MSC, thereby suppressing the osteogenic property of MSC. EV lncRNA secreted from cancer cells also induce the normal fibroblast (NF)/cancer-associated fibroblasts (CAF) transformation. Moreover, cancer cell-derived lncRNA are shuttled from cancer cells to endothelial cells, thereby modulating angiogenesis and inducing lymphangiogenesis. In addition, cancer cells may transport lncRNA to natural killer cells as well as tumor-associated macrophages (TAM) via EV, resulting in enhanced cytotoxicity and immunosuppression, respectively. Reciprocally, stromal cell-derived lncRNA are also delivered to cancer cells by EV. For example, MSC-derived and CAF-derived lncRNA are transferred to cancer cells by EV, leading to cancer progression and chemoresistance. Moreover, TAM transmit lncRNA to cancer cells through EV, which induces aerobic glycolysis and apoptosis resistance

Reciprocally, EV lncRNA derived from MSC could also be transferred to tumor cells to regulate tumor progression. sEV LINC00461 positively modulates BCL-2 expression by competitively binding to miR-15a/miR-16, leading to enhanced cellular proliferation and reduced apoptosis of MM cells. ZHANG et al. indicated that lncRNA PVT1 could be encapsulated and transferred to osteosarcoma cells in bone marrow MSC-derived sEV, which promotes tumor growth and metastasis by inhibiting the ubiquitination of ERG protein and sponging miR-183-5p.

Proteasome inhibitors have been regarded as a first-line therapeutic strategy for MM patients over the past decade.39 However, proteasome inhibitors resistance severely impedes its therapeutic effect, and the underlying mechanism has yet to be explored. MSC could package and shuttle IncPSMA3 and IncPSMA3-AS1 to MM cells and confer proteasome inhibitor resistance to them.40 Mechanistically, IncPSMA3-AS1 and pre–PSMA3 form RNA–RNA duplex, thus promoting IncPSMA3 expression by increasing its stability.40 The authors also indicate that targeting IncPSMA3-AS1 could improve proteasome inhibitor resistance and the overall survival of MM in vivo.

3.2 | Cancer-associated fibroblasts

Cancer-associated fibroblasts, one of the most prominent stromal cells in the microenvironment, are associated with tumor growth, metastasis, angiogenesis, chemoresistance, ECM remodeling, metabolic reprogramming and immune evasion.41 CAF generally exert inherent support to tumor cells by cell-to-cell contact and release of soluble factors. However, recent studies have suggested that EV lncRNA are involved in crosstalk between tumor cells and CAF (Figure 1).42,43 Feng et al.44 revealed that sEV lncRNA, secreted by breast cancer cells, modulated the malignant transformation of lung fibroblasts, thus constructing a pre–metastatic niche and facilitating the tumor pulmonary metastasis. Ding et al.45 identified the participation of Inc-CAF in the normal fibroblast (NF)/CAF
transformation in oral squamous cell carcinoma (OSCC). OSCC-derived sEV deliver Inc-CAF to stromal fibroblasts and induce the CAF phenotype via IL-33, thereby facilitating the growth of OSCC. Similarly, Hu et al stated that sEV-mediated shuttling of IncRNA Gm26809 from melanoma cells transferred NF to CAF, which fuels the proliferation and migration of melanoma cells. In return, sEV IncRNA CCAL could be transferred from CAF to CRC cells and activate the Wnt/β-catenin signaling pathway through HUVR, resulting in suppression of CRC cell apoptosis and chemoresistance. sEV IncRNA H19 also stimulates the β-catenin signaling pathway by sponging miR-141, which induces the stemness and chemoresistance of CRC cells. In conclusion, these studies highlight the potential for preventing NF/CAF transformation and overcoming drug resistance by targeting EV IncRNA in the TME, although further studies are yet to be undertaken.

3.3 | Endothelial cells

Angiogenesis is an indispensable event for the growth and metastasis of solid tumors as well as an attractive target for cancer treatment. Recent evidence has revealed that cancer cell-secreted EV IncRNA could be shuttled to endothelial cells to regulate angiogenesis (Figure 1). LncRNA H19, shuttled from CD90+ liver cancer cells to endothelial cells, promotes its tube formation and cell-cell adhesive properties by enhancing the expression of VEGF and ICAM-1, respectively. In lung cancer, sEV IncRNA CCAT2 and IncRNA POU3F3 derived from glioma cells enhance endothelial cell migration, proliferation, tube formation in vitro and arteriogenesis in vivo. Furthermore, sEV IncRNA CCAT2 up-regulates vascular endothelial cell permeability upon TGF-β stimulation. It has also been reported that sEV IncRNA CCAT2 and IncRNA POU3F3 derived from glioma cells enhance endothelial cell migration, proliferation, tube formation in vitro and arteriogenesis in vivo.50,51

In epithelial ovarian cancer (EOC), Qiu et al suggested that transfer of Inc-MALAT1 from EOC cells to human umbilical vein endothelial cells (HUVEC) induces angiogenesis by upregulating the expression of angiogenesis-related genes. Wu et al found that sEV isolated from tumor-associated macrophages (TAM) in the ascites of EOC inhibited the migration of endothelial cells through the miR-146b-5p/TRAF6/NF-kB/MMP2 pathway. However, EOC-derived sEV IncRNA, ENST00000444164 and ENST00000437683, may reverse this effect of TAM on endothelial cells through activating the NF-κB pathway.53

In contrast, EV IncRNA derived from cancer cells could also exhibit anti-angiogenic properties. sEV IncRNA GASS suppresses HUVEC proliferation and tube formation as well as promotes their apoptosis by increasing PTEN expression and inhibiting PI3K/AKT activation, further supporting its role as a novel therapeutic target in lung cancer treatment. In addition, sEV IncRNA LNMAT2 could be transmitted from bladder cancer cells to human lymphatic endothelial cells (HLEC), and leads to lymphangiogenesis and lymphatic metastasis in bladder cancer. Based on the above evidence, EV IncRNA participate in the angiogenesis and lymphangiogenesis of endothelial cells, which may facilitate cancer metastasis and act as a potential therapeutic target.

3.4 | Natural killer cells

Natural killer (NK) cells are a subset of the type I innate lymphoid cells which play a critical role in host defense against viral infection and malignant transformation. It is well known that cell-to-cell contact and release of soluble factors mediate the interactions between NK and tumor cells. In the recent decade, increasing evidence has shown that EV could also mediate this bidirectional communications by transferring proteins and microRNA (miRNA). Nonetheless, there is a lack of scientific evidence supporting the involvement of EV IncRNA in this process. Fang et al found that upregulation of IncRNA GAS5 in activated NK cells suppressed the immune escape of liver cancer. Mechanistically, IncRNA GAS5 over-expression enhanced IFN-γ secretion, NK cell cytotoxicity and the percentage of CD107a+ NK cells through the miR-544/RUNX3 axis (Figure 1). It has also been reported that IncRNA GAS5-containing sEV are produced by NSCLC cells. Therefore, it may be possible that sEV-mediated transfer of IncRNA GAS5 is involved in the regulation of NK cell cytotoxicity, although further studies are required to validate this hypothesis.

3.5 | Tumor-associated macrophages

Macrophages that are recruited to the TME are termed TAM. TAM can promote cancer cell proliferation, induce immune evasion, enhance tumor invasion and tumor metastasis, and stimulate tumor angiogenesis. Functionally, TAM are categorized into M1 subtype with an anti-tumor role and M2 subtype with a tumor-supportive role. TAM generally exhibit an M2-like phenotype, which is associated with high tumor grades and poor prognosis in a variety of cancers. It has been reported that EV IncRNA from tumor cells can regulate the activation, polarization and function of TAM (Figure 1). In hepatocarcinoma cells, sEV LINC ROR regulates the inflammation of macrophages upon LPS stimulation by inhibiting the secretion of IL-10. Li et al demonstrated a novel role for HCC-derived sEV IncRNA TUC339 in macrophage activation and M1/M2 polarization. Overexpression of TUC339 reduces pro-inflammatory cytokine production, decreases co-stimulatory molecule expression, impairs phagocytosis and drives M2 polarization in the macrophage. In CRC, Liang et al demonstrated that sEV IncRNA RPHPH1 could be transferred to macrophages and mediated macrophage M2 polarization, thus promoting proliferation and metastasis of CRC cells. Compared to traditional tumor marker CEA and CA199, sEV IncRNA RPHPH1 displays greater specificity and sensitivity, which may support its role as a promising diagnostic marker of CRC.
In contrast, IncRNA could act as signal transducer from TAM to tumor cells via EV to reprogram tumor metabolism. Chen et al found that TAM-derived sEV IncRNA H19 interfered with the interaction of PHD2 and HIF-1α to stabilize HIF-1α, and promoted cancer aerobic glycolysis and apoptosis resistance. Reciprocally, glycolytic tumor cells released lactate to further induce HIF1α upregulation in TAM through the ERK-ELK1 signaling pathway. Moreover, HIF1α knockdown via short hairpin RNA (shRNA) inhibited glycolysis and chemoresistance of breast cancer cells in vivo, highlighting the prospect of EV IncRNA-based targeting therapy.

4 | ASSORTMENT AND UPTAKE OF EXTRACELLULAR VESICLE LONG NON-CODING RNA

Previous studies have suggested that noncoding RNA, mainly miRNA and IncRNA, are selectively sorted into EV; however, the underlying mechanisms remain largely unexplored. Based on current research, three possible mechanisms of miRNA sorting into EV have been proposed. First, RNA-binding proteins (RBP), especially heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), could mediate the selective loading of miRNA into sEV through the recognition of specific “EXOmotifs” on miRNA. Second, the levels of endogenous miRNA targets in response to cell activation may be involved in the miRNA sorting to sEV. Third, the 3′ end posttranscriptional modifications of miRNA are key factors for miRNA sorting into sEV. Koppers-Lalic et al confirmed that miRNA with 3′ end adenylation were relatively enriched in B cells, whereas those with 3′ end uridylation were overexpressed in sEV.

With regards to IncRNA, Qu et al used RNA pull-down and RNA immunoprecipitation assays to reveal that hnRNPA2B1 directs the specific loading of IncARSR into sEV in renal cancer. Subsequent studies verified that hnRNPA2B1 is responsible for the selective packaging of IncRNA AGAP2-AS1 into sEV in breast cancer as well as that of IncRNA H19 in NSCLC. Similarly, IncRNA LNMAT2 is exported into sEV by direct binding with hnRNPA2B1 through its specific sequence on 1930-1960 nt in bladder cancer. This indicates that hnRNPA2B1 plays a critical role in targeting selected IncRNA into EV. Does any other RBP contribute to this process? Are there other modulators involved in the process, such as endogenous RNA or posttranscriptional modifications of IncRNA? Because there are no relevant studies regarding the selective loading of IncRNA into microvesicles and apoptotic bodies, what are the differences in loading mechanisms among exosomes, microvesicles and apoptotic bodies? In vivo experiments and powerful imaging methods to track EV are urgently needed to elucidate the mechanisms of how cancer cells manipulate IncRNA into EV, which may offer new insights into EV-based RNA therapeutics.

After release into the TME, EV appear to be taken up by recipient cells through two different endocytic pathways; namely clathrin-dependent and clathrin-independent pathways. The latter category includes caveolin-dependent endocytosis, macropinocytosis, phagocytosis and lipid raft-mediated endocytosis. Ageta et al indicated that recipient cells could internalize EV through various mechanisms in a cell-context dependent manner. However, fewer studies have been conducted to elaborate the recipient cells’ mechanisms to take up EV, which merits further exploration in the future.

5 | CONCLUSION

Extracellular vesicles mediate cell-to-cell communication by transferring their molecular cargoes to recipient cells. Growing evidence has revealed that EV-mediated delivery of IncRNA modulates a variety of processes, such as immune response, chemosensitivity, tumor growth and development. As described above, tumor-derived EV IncRNA confer aggressive and chemoresistant phenotypes to neighboring counterparts in the TME. Meanwhile, EV IncRNA mediate the interaction between tumor and stromal cells, thereby remodeling the local environment to facilitate tumor growth and progression.

EV-containing IncRNA can reflect the biological and pathological state of nonparental cells, and the existence of EV keeps them stable and resistant to endogenous RNase. These traits make EV IncRNA valuable diagnostic and prognostic biomarkers in a variety of cancers. Further bioinformatics analyses could determine a panel of EV IncRNA, which more precisely predict cancer diagnosis and prognosis than any single EV IncRNA.

EV-containing IncRNA could also serve as therapeutic targets for cancer treatment. It is appealing to locally inhibit EV IncRNA release or uptake to modulate the drug resistance and eventually improve the prognosis of cancer. Manipulation of EV carrying small interfering RNA (siRNA) or shRNA to target EV IncRNA may open a new avenue for cancer treatment. Kamerkar et al demonstrated that bioengineered sEV derived from normal fibroblast-like mesenchymal cells deliver siRNA or shRNA to oncogenic KRASG12D, leading to cancer suppression and increased overall survival in PDAC. However, there are still several problems to be solved before its clinical application in cancer. For example, what are the standard procedures for EV isolation, purification, identification and IncRNA extraction? What are the exact mechanisms for cancer cells to selectively sort IncRNA into EV and how do the recipient cells take them up? What is the precise role of EV IncRNA in cancer progression and chemotherapeutic resistance? Since current studies mainly concentrate on sEV IncRNA in the TME, what are the functional differences between sEV and large EV IncRNA? Are EV IncRNA-based therapies able to treat cancer or do they cause severe side-effects? Therefore, applying EV IncRNA from bench to bedside is a challenging but intriguing endeavor that requires further exploration in the future.

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CONFLICT OF INTEREST
The authors report no conflict of interest.

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REFERENCES
1. Park SA, Surh YJ. Modulation of tumor microenvironment by chemopreventive natural products. Ann N Y Acad Sci. 2017;1401:65-74.
2. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21:309-322.
3. Shi Y, Du L, Lin L, Wang Y. Tumour-associated mesenchymal stem/stromal cells: emerging therapeutic targets. Nat Rev Drug Discovery. 2017;16:35-52.
4. Kozlova N, Grossman JE, Iwanicki MP, Muranen T. The interplay of the extracellular matrix and stromal cells as a drug target in stroma-rich cancers. Trends Pharmacol Sci. 2020;41:183-198.
5. Ma P, Pan Y, Li W, et al. Extracellular vesicles-mediated noncoding RNAs transfer in cancer. J Hematol Oncol. 2017;10:57.
6. Kagiya T. MicroRNAs and osteolytic bone metastasis: The roles of microRNAs in Tumor-induced osteoclast differentiation. J Clin Med. 2015;4:1741-1752.
7. Mohankumar S, Patel T. Extracellular vesicle long noncoding RNA as potential biomarkers of liver cancer. Brief Funct Genomics. 2016;15:249-256.
8. Kolat D, Hammad R, Bednarek AK, Pluciennik E. Exosomes as carriers transporting long noncoding RNAs: molecular characteristics and their function in cancer (Review). Mol Med Report. 2019;20:851-862.
9. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011;43:904-914.
10. Pan L, Liang W, Fu M, et al. Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression. J Cancer Res Clin Oncol. 2017;143:991-1004.
11. Cheng Y, Dai X, Yang T, Zhang N, Liu Z, Jiang Y. Low long noncoding RNA growth arrest-specific transcript 5 expression in the esoxomes of lung cancer cells promotes tumor angiogenesis. J Oncol. 2019;2019:2476175.
12. Li Z, Jiang P, Li J, et al. Tumor-derived exosomal Inc-Sox2ot promotes EMT and stemness by acting as a ceRNA in pancreatic ductal adenocarcinoma. Oncogene. 2018;37:3822-3838.
13. Wang J, Lu B, Su Y, Wang X, Bu J, Yao L. Exosome-mediated transfer of IncRNA HOTIP promotes cisplatin resistance in gastric cancer cells by regulating HMGA1/miR-218 axis. OncoTargets Ther. 2019;12:11325-11338.
14. Kang M, Ren M, Li Y, Fu Y, Deng M, Li C. Exosome-mediated transfer of IncRNA PART1 induces gefitinib resistance in esophageal squamous cell carcinoma via functioning as a competing endogenous RNA. J Exp Clin Cancer Res. 2018;37:171.
15. Yang YN, Zhang R, Du JW, et al. Predictive role of UCA1-containing exosomes in cetuximab-resistant colorectal cancer. Cancer Cell Int. 2018;18:164.
16. Kogure T, Yan IK, Lin WL, Patel T. Extracellular vesicle-mediated transfer of a novel long noncoding RNA TUC339: a mechanism of intercellular signaling in human hepatocellular cancer. Genes Cancer. 2013;4:261-272.
17. Takahashi K, Yan IK, Kogure T, Haga H, Patel T. Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer. FEBs Open Bio. 2014;4:458-467.
18. Han M, Gu Y, Lu P, et al. Exosome-mediated lncRNA AFAP1-AS1 promotes trastuzumab resistance through binding with AUF1 and activating ERBB2 translation. Mol Oncol. 2020;19:26.
19. Zheng Z, Chen M, Xing P, Yan X, Xie B. Increased expression of exosomal AGAP2-AS1 (AGAP2 Antisense RNA 1) in breast cancer cells inhibits trastuzumab-induced cell cytotoxicity. Med Sci Monit. 2019;25:2211-2220.
20. Dong H, Wang W, Chen R, et al. Exosome-mediated transfer of IncRNA SHHG14 promotes trastuzumab chemoresistance in breast cancer. Int J Oncol. 2018;53:1013-1026.
21. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365:1687-1717.
22. Sharifi S, Barar J, Hejazi MS, Samadi N. Doxorubicin changes Bax /Bcl-xL ratio, caspase-8 and 9 in breast cancer cells. Adv Pharm Bull. 2015;5:351-359.
23. Xu CG, Yang MF, Ren YQ, Wu CH, Wang LQ. Exosomes-mediated transfer of IncRNA UCA1 results in increased tamoxifen resistance in breast cancer cells. Eur Rev Med Pharmacol Sci. 2016;20:4362-4368.
24. Wang X, Pei X, Guo G, et al. Exosome-mediated transfer of long noncoding RNA H19 induces doxorubicin resistance in breast cancer. J Cell Physiol. 2020.
25. Westover D, Dugazagotia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol. 2018;29:i10-i19.
26. Lei Y, Guo W, Chen B, Chen L, Gong J, Li W. Tumorreleased IncRNA H19 promotes gefitinib resistance via packaging into exosomes in nonsmall cell lung cancer. Oncol Rep. 2018;40:3438-3446.
27. Zhang W, Cai X, Yu J, Lu X, Qian Q, Qian W. Exosome-mediated transfer of IncRNA RP11838N2.4 promotes erlotinib resistance in non-small cell lung cancer. Int J Oncol. 2018;53:527-538.
28. Gillies RJ, Verduzco D, Gatenby RA. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. Nat Rev Cancer. 2012;12:487-493.
29. Xue M, Chen W, Xiang A, et al. Hypoxic exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCA1. Mol Cancer. 2017;16:143.
30. Qu L, Ding J, Chen C, et al. Exosome-transmitted IncARSPr promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. Cancer Cell. 2016;29:653-668.
31. Yang X, Wang L, Li R, et al. The long non-coding RNA PCSEAT exosomal transfer in cancer. J Exp Clin Cancer Res. 2016;35:32.
32. Zhang Y, Liu YT, Tang H, et al. Exosome-transmitted lncRNA H19 promotes gefitinib resistance via packaging into exosomes in nonsmall cell lung cancer. Oncof Rep. 2018;40:3438-3446.
33. Zheng Y, Wang G, Chen R, Hua Y, Cai Z. Mesenchymal stem cells in the osteosarcoma microenvironment: their biological properties, influence on tumor growth, and therapeutic implications. Stem Cell Res Ther. 2018;9:22.
35. Wang S, Li X, Zha R, Han Q, Zhao RC. Lung cancer exosomes initiate global long non-coding RNA changes in mesenchymal stem cells. *Int J Oncol*. 2016;48:681-689.

36. Li B, Xu H, Han H, et al. Exosome-mediated transfer of Incrunk2-AS1 from multiple myeloma cells to MSCs contributes to osteogenesis. *Oncogene*. 2018;37:5508-5519.

37. Deng M, Yuan H, Liu S, Hu Z, Xiao H. Exosome-transmitted LINC00461 promotes multiple myeloma cell proliferation and suppresses apoptosis by modulating microRNA/BCL2 expression. *Cytottherapy*. 2019;21:96-106.

38. Zhao W, Qin P, Zhang D, et al. Long non-coding RNA PVT1 encapsulated in bone marrow mesenchymal stem cell-derived exosomes promotes osteosarcoma growth and metastasis by stabilizing ERG and sponging miR-183-5p. *Aging*. 2019;11:9581-9596.

39. Ito S. Proteasome inhibitors for the treatment of multiple myeloma. *Cancers*. 2020;12:265.

40. Xu H, Han H, Song S, et al. Exosome-transmitted PSMA3 and PSMA3-AS1 promote proteasome inhibitor resistance in multiple myeloma. *Clin Cancer Res*. 2019;25:1923-1935.

41. Chen X, Song E. Turning foes to friends: targeting cancer-associated fibroblasts. *Nat Rev Drug Discovery*. 2019;18:99-115.

42. Deng X, Ruan H, Zhang X, et al. Long noncoding RNA CCAL transferred from fibroblasts by exosomes promotes chemoresistance of colorectal cancer cells. *Int J Cancer*. 2020;14:1700-1716.

43. Ren J, Ding L, Zhang D, et al. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal LncRNA H19. *Theranostics*. 2018;8:3932-3948.

44. Feng T, Zhang P, Sun Y, et al. High throughput sequencing identifies breast cancer-secreted exosomal LncRNAs initiating pulmonary pre-metastatic niche formation. *Gene*. 2019;710:258-264.

45. Ding L, Ren J, Zhang D, et al. A novel stromal IncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. *Carcinogenesis*. 2018;39:397-406.

46. Hu T, Hu J. Melanoma-derived exosomes induce reprogramming of fibroblasts into cancer-associated fibroblasts via Gm26809 delivery. *Cell Cycle*. 2019;18:3085-3094.

47. Cook KM, Figg WD. Angiogenesis inhibitors: current strategies and future prospects. *CA Cancer J Clin*. 2010;60:222-243.

48. Coniglio A, Costa V, Lo Dico A, et al. CD90+ liver cancer cells modulate endothelial cell phenotype through the release of exosomes containing H19 IncRNA. *Mol Cancer*. 2014;15:1455.

49. Wu DM, Deng SH, Liu T, Han R, Zhang T, Xu Y. TGF-beta-mediated exosomal Inc-MMP2-2 regulates migration and invasion of lung cancer cells to the vasculature by promoting MMP2 expression. *Cancer Med*. 2018;7:5118-5129.

50. Lang HL, Hu GW, Zhang B, et al. Glioma cells enhance angiogenesis and inhibit endothelial cell apoptosis through the release of exosomes that contain long non-coding RNA CCAT2. *Oncol Rep*. 2017;38:785-798.

51. Lang HL, Hu GW, Chen Y, et al. Glioma cells promote angiogenesis by releasing exosomes containing long non-coding RNA POU5F3. *Eur Rev Med Pharmacol Sci*. 2017;21:959-972.

52. Qiu JJ, Lin XJ, Tang XY, Zheng TT, Lin YY, Hua KQ. Exosomal metastasis-associated lung adenocarcinoma transcript 1 promotes angiogenesis and predicts poor prognosis in epithelial ovarian cancer. *Int J Biol Sci*. 2018;14:1960-1973.

53. Wu Q, Wu X, Ying X, et al. Suppression of endothelial cell migration by tumor associated macrophase-derived exosomes is reversed by epithelial ovarian cancer exosomal IncRNA. *Cancer Cell Int*. 2017;17:62.

54. Chen C, Luo Y, He W, et al. Exosomal long noncoding RNA LNMAT2 promotes lymphatic metastasis in bladder cancer. *J Clin Investig*. 2020;130:404-421.

55. Eckert C, Klein N, Kornek M, Lukacs-Kornek V. The complex myeloid network of the liver with diverse functional capacity at steady state and in inflammation. *Front Immunol*. 2015;6:179.

56. Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol*. 2013;14:996-1006.

57. Soriani A, Vulpis E, Cuoio L, Santoni A, Zingoni A. Cancer extracellular vesicles as novel regulators of NK cell response. *Cytokine Growth Factor Rev*. 2020;51:19-26.

58. Fabbri M. Natural killer cell-derived vesicular miRNAs: a new anti-cancer approach? *Can Res*. 2020;80:17-22.

59. Fang P, Xiang L, Chen W, et al. LncRNA GAS5 enhanced the killing effect of NK cell on liver cancer through regulating miR-544/RUNX3. *Innate Immunology*. 2019;25:99-109.

60. Li C, Lv Y, Shao C, et al. Tumor-derived exosomal IncRNA GAS5 as a biomarker for early-stage non-small-cell lung cancer diagnosis. *J Cell Physiol*. 2019;234:20721-20727.

61. Zhu J, Zhi Q, Zhou BP, Tao M, Liu J, Li W. The role of tumor-associated macrophages in the tumor microenvironment: mechanism and functions. *Anticancer Agents Med Chem*. 2016;16:1133-1141.

62. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. *J Hematol Oncol*. 2017;10:58.

63. Galdiero MR, Garlanda C, Jaillon S, Marone G, Mantovani A. Tumor-associated macrophages and neutrophils in tumor progression. *J Cell Physiol*. 2013;228:1404-1412.

64. Xue Y, Tong L, Liu Anwei Liu F, et al. Tumor infiltrating M2 macrophages driven by specific genomic alterations are associated with prognosis in bladder cancer. *Oncopel*. 2019;42:581-594.

65. Lu Y, Guo L, Ding G. PD1(+) tumor associated macrophages predict poor prognosis of locally advanced esophageal squamous cell carcinoma. *Future Oncol*. 2019:15:4019-4030.

66. Li X, Li N. [LINC ROR from hepatocarcinoma cell-derived exosomes modulates inflammation in human macrophages]. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2019; 50: 177-181.

67. Li X, Lei Y, Wu M, Li N. Regulation of macrophage activation and polarization by HCC-derived exosomal IncRNA TUC339. *Int J Mol Sci*. 2018;19:2958.

68. Liang ZX, Liu HS, Wang FW, et al. LncRNA RPPH1 promotes colorectal cancer metastasis by interacting with TUBB3 and by promoting exosomes-mediated macrophage M2 polarization. *Cell Death Dis*. 2019:10:829.

69. Chen F, Chen J, Yang L, et al. Extracellular vesicle-packaged HIF-1alpha-stabilizing IncRNA from tumour-associated macrophages regulates aerobic glycolysis of breast cancer cells. *Nat Cell Biol*. 2019;21:498-510.

70. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun*. 2013;4:2980.

71. Shurtleff MJ, Temoce-Diaz MM, Karhilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. *eLife*. 2016;5:e19276.

72. Cheng J, Meng J, Zhu L, Peng Y. Exosomal noncoding RNAs in glioma: biological functions and potential clinical applications. *Mol Cancer*. 2020;19:66.

73. Squadrito ML, Baer C, Burdet F, et al. Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep*. 2018;8:1432-1446.

74. Koppers-Lalic D, Hackenberg M, Bijnsdorp IV, et al. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep*. 2014;8:1649-1658.

75. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. *J Extrcell Vesicles*. 2014;3:24641.

76. Ageta H, Tschida K. Post-translational modification and protein sorting to small extracellular vesicles including exosomes by ubiquitin and UBLs. *Cell Mol Life Sci*. 2019;76:4829-4848.
77. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. *Biochem Biophys Acta*. 2014;1841:108-120.

78. Shenoda BB, Ajit SK. Modulation of immune responses by exosomes derived from antigen-presenting cells. *Clin Med Insights Pathol*. 2016;9:1-8.

79. Zhao R, Zhang Y, Zhang X, et al. Exosomal long noncoding RNA HOTTIP as potential novel diagnostic and prognostic biomarker test for gastric cancer. *Mol Cancer*. 2018;17:68.

80. Kamerkar S, LeBleu VS, Sugimoto H, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546:498-503.

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