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# Extracellular vesicle-derived non-coding RNAs in osteosarcoma: regulatory mechanisms and clinical translation

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## Highlights:

- EV-derived miRNAs, lncRNAs, and circRNAs shape osteosarcoma progression.
- EV-ncRNAs are exploratory liquid-biopsy candidates for osteosarcoma.
- Standardized EV isolation and multicenter validation remain urgently needed.

**Abstract:** Osteosarcoma (OS) is the most common primary malignant bone tumor and remains clinically challenging because of early pulmonary metastasis, chemotherapy resistance, marked tumor heterogeneity, and the lack of reliable biomarkers for real-time disease monitoring. Extracellular vesicles (EVs) have emerged as key mediators of intercellular communication and promising, but still exploratory, liquid-biopsy platforms because of their stability in biofluids and information-rich molecular cargo. EV-encapsulated non-coding RNAs (ncRNAs), including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), participate in OS progression through source- and context-dependent regulatory networks. Representative oncogenic EV-miRNAs include OS cell-derived miR-675, which promotes migration and invasion by targeting calneuron 1 (CALN1), and BMSC-derived miR-21-5p, which enhances OS proliferation and invasion by suppressing phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) and activating phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) signaling. Conversely, tumor-suppressive miR-101 inhibits OS invasion and pulmonary metastasis through B-cell lymphoma 6 protein (BCL6)-associated PI3K/AKT and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling. EV-derived lncRNAs and circRNAs, including linc00852, LIFR antisense RNA 1 (LIFR-AS1), plasmacytoma variant translocation 1 (PVT1), metastasis associated lung adenocarcinoma transcript 1 (MALAT1), OIP5 antisense RNA 1 (OIP5-AS1), circ-0010220, and circular RNA derived from nuclear receptor interacting protein 1 (circNRIP1), regulate

malignant phenotypes through experimentally reported mechanisms such as the linc00852/AXL receptor tyrosine kinase (AXL) feedback loop, LIFR-AS1/miR-29a/nuclear factor I A (NFIA) signaling, OIP5-AS1/miR-153/ATG5 regulation, and circNRIP1/miR-532-3p/AKT3-mediated PI3K/AKT activation. Engineered EVs carrying therapeutic ncRNAs show strong clinical potential, but translation is limited by insufficient multicenter validation, inadequate disease controls, suboptimal delivery to pulmonary metastases, and non-standardized EV/ncRNA workflows. Overall, EV-derived ncRNAs remain promising but exploratory biomarkers and therapeutic candidates, requiring standardized methods and robust prospective multicenter validation.

**Keywords:** extracellular vesicles; non-coding RNAs; osteosarcoma; liquid biopsy; engineered EVs

## 1. Introduction

Extracellular vesicles (EVs) are membrane-enclosed nanostructures released by cells that serve as critical mediators of intercellular communication in both physiological and pathological processes [1]. Based on their size, origin, and biogenesis mechanisms, EVs are primarily classified into three categories: exosomes (40–150 nm in diameter), migrasomes (0.5–3 μm), and apoptotic bodies (1–5 μm) [2–4]. Exosomes, the most extensively studied EV subtype, are formed and secreted through the endosomal pathway. They carry diverse bioactive molecules, including proteins, lipids, and nucleic acids, and are implicated in tumor proliferation, metastasis, angiogenesis, and immune evasion [5,6]. Recently, migrasomes have emerged as a novel EV population generated by migrating cells, containing characteristic tubular reservoirs and small luminal vesicles (migrasome-associated nanoparticles, MANPs), and playing distinct roles in tumor microenvironment remodeling and immune modulation [7,8]. Apoptotic bodies, produced during programmed cell death, primarily mediate cell clearance and tissue homeostasis, though their specific contributions to cancer are now being recognized [9]. Crucially, EVs function not merely as passive carriers of molecular cargo but as active drivers of tumorigenesis that reprogram recipient cells and remodel the tumor microenvironment. Through targeted cargo delivery, they can prime pre-metastatic niches in distant organs, positioning them as valuable targets for cancer diagnosis and therapy [10,11]. It is important to note that EV nomenclature is still evolving; the operational classification based on size and biogenesis should be applied cautiously, as there is significant overlap in marker expression and isolation yields [2]. For studies where endosomal origin is not definitively proven, the generic term “EVs” is preferred over “exosomes”.

Osteosarcoma (OS) is the most common primary malignant bone tumor, exhibiting a bimodal age distribution (peaks at 18 and 60 years) and preferentially affecting the metaphysis of long bones—most notably the distal femur, proximal tibia, and proximal humerus [12,13]. The current standard treatment combines surgical resection with neoadjuvant and adjuvant chemotherapy, typically using cisplatin plus doxorubicin (AP) or high-dose methotrexate combined with cisplatin and doxorubicin (MAP) [14]. Although 5-year survival for primary OS has improved to 65%–70%, patients with metastatic or recurrent disease face grim prognoses of only 20%–30% [15]. Two core bottlenecks account for this poor outcome: chemotherapy resistance and a high propensity for hematogenous pulmonary metastasis, whose molecular underpinnings remain incompletely understood. Moreover, substantial tumor heterogeneity and the lack of reliable biomarkers for early diagnosis, treatment monitoring, and prognostic stratification compound these clinical challenges [16,17].

Liquid biopsy has recently emerged as a promising strategy for precision management of OS. Given the pressing clinical needs—absence of robust biomarkers and poorly understood mechanisms driving resistance/metastasis—liquid biopsy offers a non-invasive, real-time approach to molecular profiling. Extracellular vesicles, owing to their remarkable stability in biofluids and information-rich molecular cargo, represent particularly attractive liquid biopsy candidates [18,19]. Notably, EVs are enriched with diverse non-coding RNAs (ncRNAs)—including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs)—that play pivotal regulatory roles in tumorigenesis [20,21]. MiRNAs (~22 nucleotides) post-transcriptionally regulate gene expression by binding to target mRNA 3' untranslated regions and thereby modulate tumor cell proliferation, apoptosis, migration, and chemosensitivity [22,23]. For instance, EV-associated miR-675, miR-25-3p, and miR-101 have been implicated in OS metastasis and prognosis [24–26]. LncRNAs, transcripts exceeding 200 nucleotides, participate in tumor progression through chromatin remodeling, transcriptional regulation, post-transcriptional regulation, and, in some contexts, competing endogenous RNA (ceRNA)-related mechanisms [27]. EV-encapsulated lncRNAs may act as miRNA sponges and regulate downstream targets, thereby influencing malignant behavior in OS [28,29]. CircRNAs, characterized by covalently closed circular structures, possess exceptional stability and tissue-specific expression. Recently, EV-delivered circRNAs have attracted attention as potential regulators of OS progression, including through experimentally reported ceRNA-like mechanisms [30–32]. However, ceRNA activity should not be inferred solely from sequence complementarity or inverse expression correlations. Quantitative studies have shown that effective miRNA competition depends on miRNA abundance, competing target abundance, binding-site number, binding affinity, target-site spacing, and cellular context [33,34]. Therefore, the ceRNA axes discussed in this review should be interpreted as experimentally reported or proposed mechanisms in specific models, and stronger physiological support requires co-expression evidence, direct binding assays, loss- and gain-of-function experiments, rescue assays, and, where feasible, assessment of miRNA stoichiometry. Systematically summarizing the regulatory mechanisms and translational potential of EV-derived ncRNAs in OS is of critical importance. Accordingly, this review aims to comprehensively evaluate EV-derived miRNAs, lncRNAs, and circRNAs in osteosarcoma, with emphasis on their cellular sources, recipient-cell interactions, biological functions in tumor progression and microenvironmental remodeling, and translational potential as exploratory biomarkers and therapeutic targets, thereby providing theoretical insights and novel directions for precision management of osteosarcoma (Figure 1).

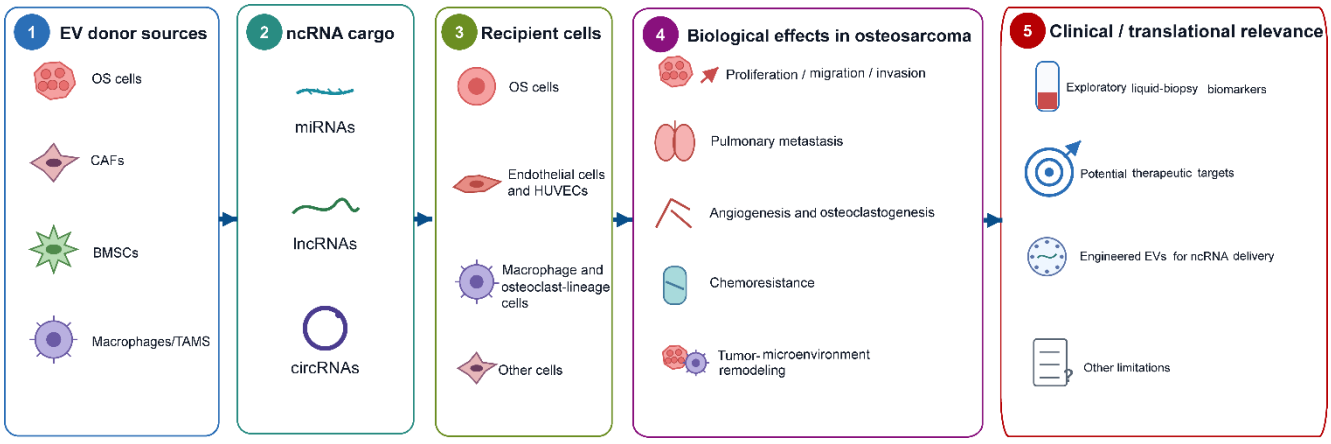

**Figure 1.** Schematic overview of EV-derived ncRNAs in osteosarcoma progression and clinical translation. OS cells and tumor microenvironment-associated cells, including CAFs, BMSCs, and macrophages or TAMs, release EVs containing ncRNA cargos, including miRNAs, lncRNAs, and circRNAs. These EV-associated ncRNAs can be delivered to OS cells, endothelial cells, macrophage/osteoclast-lineage cells, and other recipient cells, thereby contributing to OS proliferation, migration and invasion, pulmonary metastasis, angiogenesis and osteoclastogenesis, chemoresistance, and tumor-microenvironment remodeling. From a translational perspective, EV-associated ncRNAs are being investigated as exploratory liquid-biopsy biomarkers and potential therapeutic targets, while engineered EVs represent emerging platforms for ncRNA delivery. However, their clinical application remains limited and requires further validation. Abbreviations: CAFs, cancer-associated fibroblasts; BMSCs, bone marrow-derived mesenchymal stem cells; TAMs, tumor-associated macrophages; HUVECs, human umbilical vein endothelial cells.

## 2. EV miRNAs in osteosarcoma

Extracellular vesicle-derived miRNAs regulate the malignant biological behaviors of osteosarcoma through source- and context-dependent intercellular communication, affecting tumor cell proliferation, invasion, metastasis, chemoresistance, angiogenesis, and tumor-microenvironment remodeling (Table 1 and Figure 2). Owing to their stability in biofluids and non-invasive accessibility, EV-associated miRNAs are also being investigated as exploratory candidates for osteosarcoma diagnosis, prognostic assessment, and therapeutic monitoring.

### *2.1. miR-675*

miR-675 is a critical molecule promoting osteosarcoma metastasis. Studies have shown that EVs derived from metastatic osteosarcoma cells carry abundant miR-675, which directly targets and downregulates calneuron 1 (CALN1) expression, disrupts cellular calcium homeostasis and cytoskeletal regulation, thereby significantly enhancing the migration and invasion capabilities of osteosarcoma cells. Concurrently, elevated serum exosomal miR-675 levels are inversely correlated with reduced CALN1 expression in tumor tissues, collectively constituting the molecular signature of osteosarcoma metastasis. Clinical investigations have demonstrated that serum miR-675 levels are closely associated with pulmonary metastasis in osteosarcoma patients, with high-expression patients being more prone to distant metastasis. Therefore, combined detection of serum miR-675 and tumor tissue CALN1 expression can improve the accuracy of metastasis prediction, providing a basis for early identification of high-risk patients in clinical practice [24].

### *2.2. miR-331-3p*

miR-331-3p exhibits a dual role in osteosarcoma. On one hand, miR-331-3p present in EVs secreted by chemoresistant osteosarcoma cells can induce autophagy in recipient cells, thereby conferring drug resistance to non-resistant strains [35]. On the other hand, in osteosarcoma tissues and commercially available cell lines, miR-331-3p has been shown to suppress osteosarcoma progression through the BCL-2/Bax and Wnt/β-catenin signaling pathways [36]. This apparent discrepancy highlights that miR-331-3p is highly context-dependent: intracellular miR-331-3p may suppress OS progression in certain tissue or cell-line

models, whereas EV-encapsulated miR-331-3p derived from chemoresistant OS cells can transfer autophagy-mediated drug resistance to recipient cells. Thus, its biological interpretation should consider cellular localization, EV origin, recipient cell type, and treatment background.

### *2.3. miR-25-3p*

miR-25-3p exhibits both intracellular oncogenic and extracellular pro-tumorigenic functions, and is closely associated with the clinicopathological characteristics of osteosarcoma. On one hand, this molecule plays a pivotal role in the Hippo signaling pathway by regulating Merlin expression, thereby promoting osteosarcoma cell proliferation, migration, invasion, and cisplatin resistance [25]. On the other hand, it is highly enriched in tumor-derived EVs and effectively facilitates angiogenesis and endothelial cell invasion [37]. Serological analysis demonstrated that miR-25-3p could effectively distinguish osteosarcoma patients, suggesting its clinical value as an early diagnostic biomarker [38].

### *2.4. miR-21-5p*

miR-21-5p is detected in EVs derived from multiple cell types, but its biological interpretation in osteosarcoma should be stratified by EV donor source, recipient cell type, and experimental model. OS cell-derived EVs can be internalized by Raw264.7 macrophage-like cells and HUVECs, leading to increased miR-21-5p expression in recipient cells and promoting osteoclast differentiation and angiogenesis, respectively [39]. CAF-derived EV-miR-21-5p is clinically associated with advanced stage and poor prognosis and promotes OS cell proliferation and migration at least partly by suppressing ferroptosis [40]. BMSC-derived EV-miR-21-5p directly targets phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) and activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) pathway, thereby enhancing OS cell proliferation and invasion [41]. At present, direct evidence explaining why EV-miR-21-5p engages different downstream programs in different donor-recipient settings remains limited. This divergence may reflect donor-specific EV co-cargo, recipient-cell transcriptomic state, target-site abundance, RNA-binding proteins, RISC availability, and miRNA–target stoichiometry; however, these mechanisms have not been systematically tested in osteosarcoma EV models. Therefore, EV-miR-21-5p should be presented as a context-dependent EV-ncRNA rather than as a single linear pathway.

Collectively, EV-miR-21-5p exhibits marked donor-dependent functional divergence in osteosarcoma. OS cell-derived EV-miR-21-5p primarily remodels the tumor microenvironment by promoting osteoclast differentiation and angiogenesis, whereas CAF-derived EV-miR-21-5p promotes OS-cell proliferation and migration in association with ferroptosis suppression and adverse clinical features. In contrast, BMSC-derived EV-miR-21-5p directly engages the PIK3R1–PI3K/AKT/mTOR axis to enhance OS-cell proliferation and invasion. These source-specific effects should therefore not be interpreted as a single linear miR-21-5p pathway.

### *2.5. miR-101*

miR-101 exerts multifaceted effects in osteosarcoma. Its overexpression inactivates PI3K/AKT and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways through Rho-associated coiled-coil containing protein kinase 1 (ROCK1) downregulation, thereby suppressing

tumor growth and motility [42]. miR-101 expression is significantly lower in metastatic osteosarcoma specimens compared to non-metastatic counterparts, and osteosarcoma cells with high miR-101 expression form markedly fewer pulmonary metastatic nodules in nude mice than those with low expression. Mechanistic studies demonstrate that miR-101 directly targets B-cell lymphoma 6 protein (BCL6), reducing its mRNA and protein expression levels [26]. Additionally, clinical analyses reveal that serum exosomal miR-101 levels in osteosarcoma patients are lower than those in healthy controls, with even lower levels observed in metastatic patients. Downregulation of serum miR-101 is associated with shorter overall survival and relapse-free survival, suggesting its potential value as a circulating biomarker for osteosarcoma metastasis [43].

### *2.6. miR-1228*

miR-1228 is dramatically upregulated in EVs derived from cancer-associated fibroblasts in osteosarcoma. These miR-1228-enriched EVs are internalized by tumor cells and promote osteosarcoma migration and invasion. Mechanistic assays demonstrate that exosomal miR-1228 exerts pro-tumorigenic effects by directly targeting suppressor of cancer cell invasion (SCAI) [44].

### *2.7. miR-221-3p*

miR-221-3p is enriched in M2-TAM-derived EVs and promotes OS progression by targeting suppressor of cytokine signaling 3 (SOCS3) to activate the JAK2/STAT3 pathway, thereby enhancing proliferation, migration, and invasion while suppressing apoptosis. This effect can be reversed by JAK2/STAT3 inhibition, suggesting the miR-221-3p/SOCS3/JAK2/STAT3 axis as a potential therapeutic target for OS [45].

### *2.8. miR-1307*

miR-1307 is highly expressed in OS EVs, tissues, and cell lines, and directly targets the 3'-UTR of ADP-ribosylation factor GTPase-activating protein (ArfGAP) with GTPase domain, ankyrin repeat and PH domain 1 (AGAP1) to suppress its expression. Inhibition of exosomal miR-1307 attenuates its promoting effects on OS cell proliferation, migration, and invasion, suggesting that the miR-1307-AGAP1 axis may serve as a potential therapeutic target for OS [46].

### *2.9. miR-208a/208a-3p*

miR-208a-3p exerts oncogenic effects in osteosarcoma. This molecule is significantly upregulated in osteosarcoma tissues and cell lines; its overexpression promotes cell proliferation, migration, and invasion, whereas knockdown suppresses these malignant phenotypes. Mechanistic studies demonstrate that miR-208a-3p directly targets phosphatase and tensin homologue (PTEN) and programmed cell death 4 (PDCD4), activating PI3K/AKT and extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling pathways [47]. Furthermore, miR-208a carried by bone marrow mesenchymal stem cell-derived EVs can be internalized by osteosarcoma cells, promoting tumor progression through the same mechanisms [48]. Clinical analyses suggest that serum exosomal miR-208a-3p levels may serve as a potential biomarker for osteosarcoma diagnosis and prognostic evaluation [47].

**Table 1.** EV-derived miRNAs in osteosarcoma.

| ncRNA | EV source and experimental/clinical context | Functional category and biological effects | Clinical/translational relevance | Molecular mechanism | References |
|---|---|---|---|---|---|
| miR-675 | Metastatic OS cell-derived EVs; serum EV-associated miR-675 and tumor-tissue CALN1 examined in clinical samples | Oncogenic; promotes OS-cell migration, invasion, and metastatic potential | Exploratory circulating marker for pulmonary/metastatic risk prediction | Directly targets and downregulates CALN1, contributing to altered calcium homeostasis and cytoskeletal regulation | [24] |
| miR-331-3p† | Chemoresistant OS cell-derived EVs transferred to recipient OS cells; intracellular miR-331-3p also examined in OS tissues/cell-line models | Context-dependent/dual; EV-associated miR-331-3p promotes autophagy-mediated chemoresistance, whereas intracellular miR-331-3p has been reported to suppress OS progression | Potential indicator or therapeutic target for chemoresistance; interpretation depends on cellular localization and experimental context | EV context: induction of autophagy; intracellular context: regulation of the BCL-2/Bax and Wnt/β-catenin pathways | [35,36] |
| miR-25-3p | OS tissues and cell lines; tumor-derived EVs; serum/circulating miRNA analyses | Oncogenic/pro-tumorigenic; promotes proliferation, migration, invasion, cisplatin resistance, angiogenesis, and endothelial-cell invasion | Exploratory diagnostic and prognostic biomarker | Regulates Merlin/Hippo signaling; EV-associated miR-25-3p is additionally linked to angiogenic activity | [25,37,38] |
| miR-21-5p* | OS cell-derived EVs; recipient Raw264.7 macrophage-like cells and HUVECs | Pro-tumorigenic/TME-remodeling; promotes osteoclast differentiation and angiogenesis | Primarily mechanistic evidence for tumor-microenvironment remodeling; clinical biomarker value not established in this model | Increased recipient-cell miR-21-5p is associated with osteoclastogenic and angiogenic responses; a specific downstream target was not defined in the current manuscript | [39] |
| | CAF-derived EVs transferred to OS cells; clinical association with OS stage/outcome | Oncogenic; promotes OS-cell proliferation and migration and is associated with suppression of ferroptosis | Associated with advanced clinical stage and poor prognosis; potential stromal EV-associated therapeutic target | Promotes malignant behavior at least partly through ferroptosis suppression | [40] |
| | BMSC-derived EVs transferred to OS cells | Oncogenic; enhances proliferation and invasion | Potential therapeutic target for blocking BMSC–OS intercellular signaling | Directly targets PIK3R1 and activates the PI3K/AKT/mTOR pathway | [41] |

**Table 1.** *Cont.*

| ncRNA | EV source and experimental/clinical context | Functional category and biological effects | Clinical/translational relevance | Molecular mechanism | References |
|---|---|---|---|---|---|
| miR-101 | OS tissues and cell models; serum/plasma EV-associated miR-101; mouse pulmonary-metastasis models | Tumor-suppressive; inhibits OS growth, motility, invasion, and pulmonary metastasis | Exploratory circulating biomarker for metastasis and prognosis; also a therapeutic miRNA candidate | Targets ROCK1 and/or BCL6, with inhibition of PI3K/AKT and JAK/STAT signaling | [26,42,43] |
| miR-1228 | CAF-derived EVs transferred to OS cells | Oncogenic; promotes migration and invasion | Potential therapeutic target for blocking CAF-mediated OS progression | Directly targets SCAI | [44] |
| miR-221-3p | M2-TAM-derived EVs transferred to OS cells | Oncogenic/immunomicroenvironment-associated; enhances proliferation, colony formation, migration, and invasion while suppressing apoptosis | Potential therapeutic target for TAM-mediated OS progression | Targets SOCS3, resulting in activation of the JAK2/STAT3 pathway | [45] |
| miR-1307 | OS cell-derived EVs; OS tissues and cell lines | Oncogenic; promotes proliferation, migration, and invasion | Potential therapeutic target for suppressing OS progression | Directly targets the 3'-UTR of AGAP1 and suppresses AGAP1 expression | [46] |
| miR-208a/ miR-208a-3p | BMSC-derived EVs transferred to OS cells; serum EVs; OS tissues and cell lines | Oncogenic; promotes proliferation, migration, invasion, and tumor progression | Serum EV-associated miR-208a-3p is an exploratory diagnostic and prognostic biomarker candidate | Targets PTEN and PDCD4, with activation of PI3K/AKT and ERK1/2 signaling | [47,48] |

† Dual/context-dependent function: miR-331-3p shows opposite biological effects according to cellular localization and experimental context. EV-associated miR-331-3p derived from chemoresistant OS cells promotes autophagy-mediated chemoresistance, whereas intracellular miR-331-3p has been reported to exert tumor-suppressive activity.
* Donor-dependent functional heterogeneity: the biological effects of EV-miR-21-5p should be interpreted according to donor cell type, recipient cell type, and experimental context rather than as a single linear pathway.

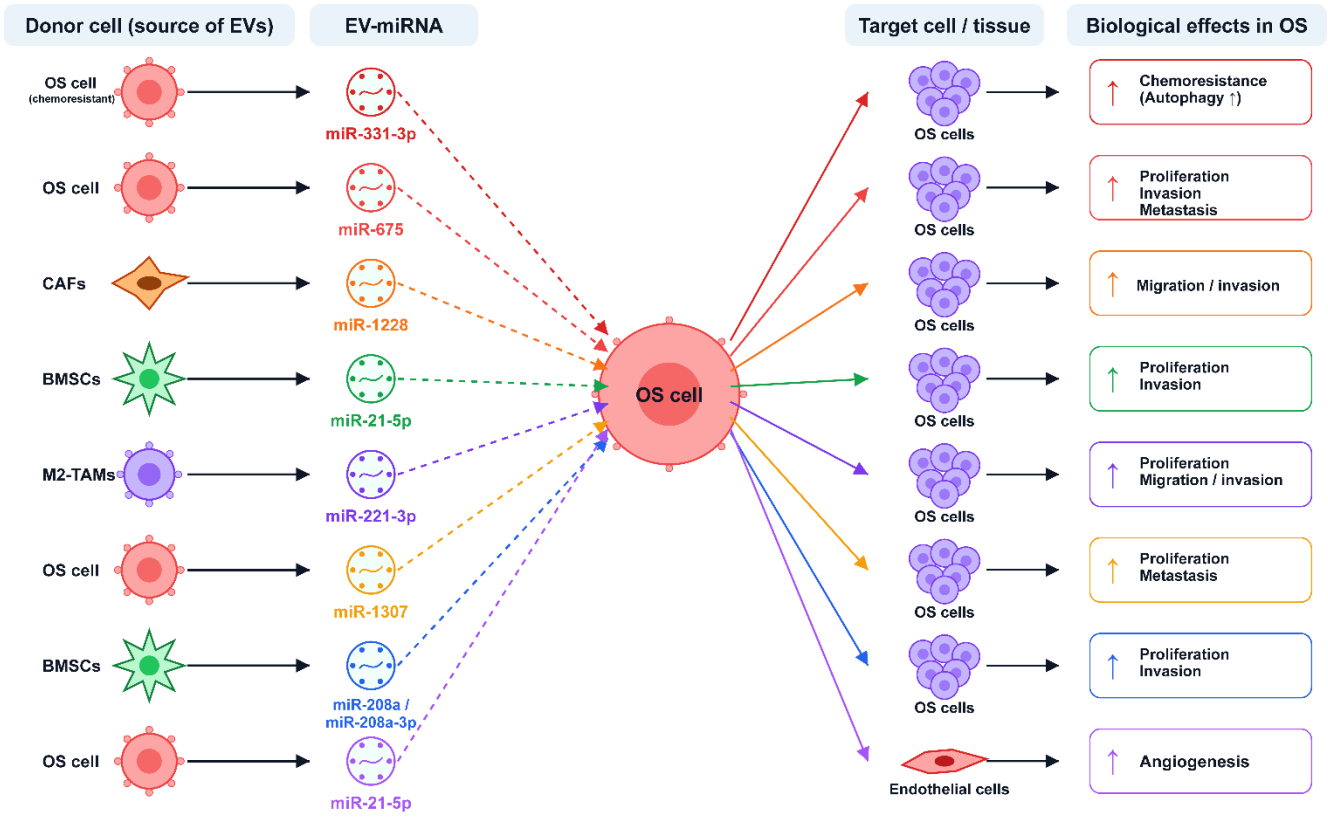


**Figure 2.** Source-dependent biological effects of EV-derived miRNAs in osteosarcoma. EV-associated miRNAs derived from OS cells and tumor-microenvironmental cells exert distinct biological effects according to their cellular origin and recipient cell type. Chemoresistant OS cell-derived EV-miR-331-3p promotes autophagy-associated chemoresistance; OS cell-derived EV-miR-675 and EV-miR-1307 promote malignant OS phenotypes; CAF-derived EV-miR-1228 enhances migration and invasion; BMSC-derived EV-miR-21-5p and EV-miR-208a/miR-208a-3p promote OS cell proliferation and invasion; and M2-TAM-derived EV-miR-221-3p facilitates OS progression. OS cell-derived EV-miR-21-5p also promotes angiogenesis through effects on endothelial cells. These source-dependent effects illustrate the functional heterogeneity of EV-miRNAs within the OS microenvironment. Abbreviations: M2-TAMs, M2-polarized tumor-associated macrophages.

## 3. EV lncRNAs in osteosarcoma

LncRNA is a molecule with a length of more than 200 nucleotides and is associated with numerous human malignant tumors. Its abnormal expression can give rise to diseases. Many of them have been proven to promote or inhibit tumor progression through complex pathways at the transcriptional and/or post-transcriptional levels, particularly playing a distinctive role in tumor proliferation, metastasis, invasion, apoptosis, and angiogenesis [27]. Compared with RNA that encodes proteins, lncRNA shows almost no evolutionary conservation among different species and exhibits specific expression patterns in specific tissues or cells [49]. LncRNAs are connected with every feature of cancer cell, encompassing cell proliferation, migration, invasion, apoptosis, sensitive of chemotherapy and metabolic regulation (Table 2). Researches in recent years have verified that these lncRNAs either promote or inhibit tumor progression through regulatory approaches such as direct engagement in transcription or involvement in post-transcriptional modifications. This might be associated with their capabilities of participating in

chromosome cycling, chromatin modification, DNA transcription, and the interactions between mRNA and proteins, *etc.* [50].

### *3.1. Linc00852*

Linc00852 in osteosarcoma-derived EVs enhances AXL receptor tyrosine kinase (AXL) expression/activation, and AXL signaling in turn upregulates linc00852 transcription, driving tumor proliferation, invasion, and metastasis. Hence, serum exosomal linc00852 is a novel biomarker for diagnosis and monitoring of osteosarcoma, and targeting this feedback loop represents a potential therapy [51].

### *3.2. LncRNA LIFR-AS1*

LIFR-AS1 in macrophage-derived EVs promotes osteosarcoma cell proliferation and invasion while inhibiting apoptosis by sponging miR-29a and upregulating the expression of its target gene nuclear factor I A (NFIA). This LIFR-AS1/miR-29a/NFIA axis reveals a novel mechanism underlying osteosarcoma progression and provides a potential therapeutic target for osteosarcoma treatment [52].

### *3.3. LncRNA PVT1 and MALAT1*

LncRNA plasmacytoma variant translocation 1 (PVT1) is widely expressed in EVs derived from BMSC. Studies have shown that lncRNA PVT1 promotes the proliferation and migration of osteosarcoma cells by directly binding to the oncogenic protein ETS-related gene (ERG) to inhibit its ubiquitination and degradation, as well as by sponging miR-183-5p to upregulate ERG expression [53]. Similarly, lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1), present in extracellular vesicles derived from BMSC-EVs, promotes the proliferation, invasion, and migration of osteosarcoma cells by regulating the MALAT1/miR-143/neurensin 2 (NRSN2) axis and activating the Wnt/β-catenin signaling pathway [54].

### *3.4. OIP5-AS1*

LncRNA OIP5 antisense RNA 1 (OIP5-AS1) is highly expressed in osteosarcoma cells and their secreted EVs. It upregulates autophagy related 5 (ATG5) expression by sponging miR-153, thereby promoting osteosarcoma cell proliferation, migration, invasion, and angiogenesis, while inhibiting autophagy [55]. Clinical sample analysis shows that the expression levels of lncRNA OIP5-AS1 are significantly elevated in tumor tissues and serum, and its high expression is closely associated with lung metastasis, chemoresistance (tumor necrosis rate < 90%), and poor patient survival. When combined with hsa_circ_0004674 detection, this lncRNA is superior to the traditional marker alkaline phosphatase (ALP) in predicting chemosensitivity and metastasis [56], suggesting that it may serve as a potential biomarker for the diagnosis, prognosis assessment, and therapeutic monitoring of osteosarcoma.

## 4. EV circRNAs in osteosarcoma

CircRNAs are a class of covalently closed circular non-coding RNA molecules generated through back-splicing, characterized by high stability, tissue-specific expression, and evolutionary conservation. CircRNAs primarily function as miRNA sponges to exert ceRNA activity, regulate parental gene

expression, and participate in protein translation regulation. In recent years, the discovery of circRNAs in extracellular vesicles and their role in osteosarcoma have attracted widespread attention (Table 2).

### *4.1. Circ-0010220*

Circ-0010220 is derived from BMSC-EVs. It epigenetically silences catenin beta interacting protein 1 (CTNNBIP1) by recruiting enhancer of zeste homolog 2 (EZH2) and activates the Wnt/β-catenin pathway, thereby enhancing tumor cell proliferation, migration, and invasion. Knockdown of this circRNA or inhibition of β-catenin reverses these effects. This circRNA constitutes the BMSC-EVs/Wnt/β-catenin signaling axis and serves as a key regulator of osteosarcoma progression as well as a potential therapeutic target [28].

### *4.2. CircNRIP1*

Circular RNA derived from nuclear receptor interacting protein 1 (circNRIP1) from BMSC-derived EVs is highly expressed in OS. It sponges miR-532-3p to activate AKT3 and the PI3K/AKT pathway, boosting OS cell proliferation, migration, and invasion. In mice, BMSC-EVs carrying circNRIP1 accelerate tumor growth. Thus, circNRIP1 is a key driver of OS progression and a promising diagnostic and therapeutic target [57].

**Table 2.** EV-derived lncRNAs and circRNAs in osteosarcoma.

| ncRNA | EV source and experimental/ clinical context | Functional category and biological effects | Clinical/translational relevance | Molecular mechanism | References |
|---|---|---|---|---|---|
| linc00852 | OS cell-derived EVs transferred to OS cells; serum EV-associated linc00852 evaluated clinically | Oncogenic; promotes proliferation, invasion, and metastasis | Exploratory diagnostic and monitoring biomarker; potential therapeutic target | Forms a positive-feedback loop with AXL: EV-delivered linc00852 enhances AXL expression/ activation, whereas AXL signaling promotes linc00852 transcription | [51] |
| LIFR-AS1* | Macrophage-derived EVs transferred to OS cells | Oncogenic; promotes proliferation and invasion and suppresses apoptosis | Potential therapeutic target for macrophage-mediated OS progression | Reported LIFR-AS1/miR-29a/NFIA ceRNA-like axis, resulting in increased NFIA expression | [52] |
| PVT1* | BMSC-derived EVs transferred to OS cells | Oncogenic; multimodal regulation; promotes OS-cell proliferation and migration | Potential stromal EV-associated therapeutic target | Stabilizes ERG by inhibiting its ubiquitination/ degradation; additionally regulates a reported PVT1/ miR-183-5p/ ERG ceRNA-like axis | [53] |

**Table 2.** *Cont.*

| ncRNA | EV source and experimental/ clinical context | Functional category and biological effects | Clinical/translational relevance | Molecular mechanism | References |
|---|---|---|---|---|---|
| MALAT1* | BMSC-derived EVs transferred to OS cells | Oncogenic; promotes proliferation, invasion, and migration | Potential target for suppressing BMSC-EV-mediated OS progression | Reported MALAT1/ miR-143/NRSN2 ceRNA-like axis, associated with activation of the Wnt/β-catenin pathway | [54] |
| OIP5-AS1* | OS cell-derived EVs; OS tissues, adjacent tissues, and patient serum | Oncogenic/multifunctional; promotes proliferation, migration, invasion, and angiogenesis while modulating autophagy | Associated with lung metastasis, chemoresistance, and poor survival; combined detection with hsa_circ_0004674 may improve prognostic/ chemosensitivity prediction | Reported OIP5-AS1/miR-153/ATG5 ceRNA-like axis; upregulates ATG5 and regulates angiogenesis and autophagy | [55,56] |
| circ-0010220 | BMSC-derived EVs transferred to OS cells | Oncogenic; enhances proliferation, migration, and invasion | Potential therapeutic target for blocking BMSC-EV-driven OS progression | Recruits EZH2 to epigenetically silence CTNNBIP1, thereby activating the Wnt/ β-catenin pathway | [28] |
| circNRIP1* | BMSC-derived EVs transferred to OS cells; mouse tumor models | Oncogenic; promotes proliferation, migration, invasion, and tumor growth | Potential diagnostic and therapeutic target | Reported circNRIP1/ miR-532-3p/AKT3 ceRNA-like axis, resulting in activation of the PI3K/AKT pathway | [57] |

*The indicated ceRNA-like relationships were demonstrated in the cited experimental models.

## 5. Therapeutic applications of engineered EV-encapsulated ncRNAs

Native extracellular vesicles face critical translational barriers, including rapid clearance, poor targeting specificity, and limited cargo capacity [58]. Engineered extracellular vesicles address these limitations through genetic, chemical, and physical strategies that confer precise targeting, enhanced drug loading, and controlled release [59,60]. These engineered systems have shown substantial clinical promise in oncology, neurodegeneration, and cardiovascular repair. Future advances in scalable purification, good manufacturing practice (GMP) manufacturing, and intelligent delivery platforms—such as nanomotor-driven systems—position engineered extracellular vesicles as a cornerstone modality in precision medicine for treating intractable diseases [61,62].

Engineered EVs serve as versatile nanocarriers for diverse non-coding RNAs, eliciting potent anti-osteosarcoma effects through multiple mechanistic pathways (Table 3). AD-MSC-derived engineered EVs enriched with miR-101 directly target and suppress BCL6 mRNA expression, thereby inactivating PI3K/AKT and JAK/STAT signaling cascades to inhibit osteosarcoma cell invasion, migration, and pulmonary metastasis [26]. Concurrently, cyclic Arg-Gly-Asp-D-Tyr-Lys peptide (c(RGDyK))-modified

engineered EVs enable targeted delivery of lncRNA MEG3, whose intrinsic tumor-suppressive properties substantially attenuate malignant progression [63]. Furthermore, Asn-Gly-Arg peptide (NGR)-modified cancer-associated fibroblast-derived EVs loaded with circ_0004872 encode a functional 109-amino acid peptide that promotes autophagy-dependent ferroptosis, enhances chemosensitivity, and effectively circumvents chemoresistance in osteosarcoma [64].

**Table 3.** Engineered EV-encapsulated ncRNAs.

| ncRNA | EV source and engineering strategy/ experimental context | Functional category and biological effects | Clinical/translational relevance | Molecular mechanism | References |
|---|---|---|---|---|---|
| miR-101 | Engineered AD-MSC-derived EVs enriched with miR-101; OS cell and pulmonary-metastasis models | Tumor-suppressive therapeutic cargo; inhibits OS-cell invasion and migration and reduces pulmonary metastasis | Preclinical therapeutic-delivery candidate for metastatic OS | Directly targets and suppresses BCL6, resulting in inhibition of PI3K/AKT and JAK/STAT signaling | [26] |
| lncRNA MEG3 | c(RGDyK)-modified engineered EVs carrying lncRNA MEG3; OS models | Tumor-suppressive therapeutic cargo; attenuates malignant OS progression | Tumor-targeted EV-based lncRNA delivery strategy; preclinical therapeutic candidate | Tumor targeting is enhanced by c(RGDyK) modification; the detailed downstream molecular mechanism of MEG3 is not specified in the current manuscript | [63] |
| circ_0004872* | NGR-modified CAF-derived EVs carrying circ_0004872; OS chemoresistance models | Therapeutic/peptide-encoding circRNA cargo; induces autophagy-dependent ferroptosis, enhances chemosensitivity, and counteracts chemoresistance | Preclinical strategy for overcoming OS chemoresistance | circ_0004872 encodes a functional 109-amino-acid peptide, which promotes autophagy-dependent ferroptosis | [64] |

Abbreviations: AD-MSCs, adipose-derived mesenchymal stem cells.

*circ_0004872 should be specifically annotated as a peptide-encoding circRNA, because its reported therapeutic activity involves production of a functional 109-amino-acid peptide rather than a conventional ceRNA mechanism.

Although engineered EVs represent a promising therapeutic platform, several OS-specific translational barriers remain unresolved. First, the source of therapeutic EVs requires careful consideration. Autologous EVs may reduce immunogenicity and immune rejection, but their individualized production is time-consuming and difficult to standardize, particularly for OS patients receiving intensive chemotherapy or with impaired immune status. In contrast, allogeneic EVs are more amenable to scalable manufacturing and off-the-shelf application, but require rigorous evaluation of donor variability, immune compatibility, residual cellular contaminants, and long-term safety. Second, cargo-loading strategies may

affect both EV integrity and ncRNA function. Physical loading approaches, including electroporation, sonication, and freeze–thaw cycles, can disrupt EV membranes and may induce RNA aggregation, degradation, or structural damage. This concern is particularly relevant to circRNA-based therapeutics, because disruption of circular topology or higher-order RNA structure may compromise circRNA stability, miRNA-sponging capacity, peptide-encoding potential, or other biological functions.

A particularly important unresolved challenge is whether systemically administered engineered EVs can achieve sufficient and durable accumulation within pulmonary metastatic lesions. Intravenously administered EVs are frequently sequestered by macrophage-rich organs such as the liver and spleen, whereas current OS studies provide limited quantitative evidence of lesion-level EV deposition in lung metastases. Importantly, the presence of a tumor-targeting ligand or an observed antimetastatic effect does not by itself demonstrate pharmacologically meaningful delivery to metastatic nodules, and whole-lung EV signals cannot distinguish true metastatic-lesion accumulation from nonspecific retention in normal pulmonary tissue. Future studies should therefore compare unmodified and ligand-engineered EVs in orthotopic and experimental lung-metastasis OS models using time-resolved biodistribution, lesion-level imaging, quantitative tumor-to-liver/spleen ratios, intralesional penetration, cargo-retention analyses, and pharmacodynamic target-engagement assays. These studies are needed to determine whether engineered EVs reach pulmonary metastases at therapeutically effective levels rather than merely exhibiting improved systemic targeting. In parallel, standardized GMP-compatible manufacturing, quality control, storage-stability testing, dosing optimization, and rigorous preclinical safety evaluation will be essential before engineered EV-encapsulated ncRNAs can be translated into routine clinical application for OS.

## 6. Conclusions

Extracellular vesicle-derived ncRNAs contribute to osteosarcoma progression and tumor-microenvironment remodeling through source- and context-dependent mechanisms. EV-associated miRNAs regulate proliferation, migration, invasion, pulmonary metastasis, angiogenesis, and chemoresistance, with miR-21-5p illustrating marked donor-dependent functional heterogeneity. EV-associated lncRNAs and circRNAs act through diverse mechanisms, including experimentally reported ceRNA-like interactions, feedback regulation, protein stabilization, epigenetic regulation, and peptide encoding. These findings establish EV-ncRNAs as mechanistically relevant but heterogeneous regulators of osteosarcoma biology.

From a clinical perspective, EV-associated ncRNAs remain exploratory rather than validated biomarkers. Existing studies are generally limited by small cohorts, heterogeneous disease controls, insufficient external validation, and variable EV isolation and ncRNA-detection workflows [65,66]. Large prospective multicenter studies using standardized sampling, MISEV-aligned EV characterization, MINSEQE/MIQE-aligned ncRNA analysis, predefined cutoffs, and independent validation will therefore be required before EV-ncRNA biomarkers can be incorporated into clinical practice.

Engineered EVs also provide a promising preclinical platform for ncRNA delivery, but translation will require scalable GMP-compatible manufacturing, reproducible cargo loading, rigorous safety assessment, and quantitative demonstration of therapeutically meaningful delivery to pulmonary metastatic lesions. Future studies should integrate multi-omics characterization with investigation of the tumor immune microenvironment while prioritizing robust biodistribution and clinical validation. Collectively,

EV-derived ncRNAs have substantial translational potential in osteosarcoma, but their clinical application will depend on methodological standardization and high-quality prospective evidence.

## Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI and AI-assisted tools only to improve language, readability, structural clarity, and figure preparation. Specifically, Claude and ChatGPT were used for language polishing and narrative refinement. The authors reviewed, edited, and verified all AI-assisted outputs and take full responsibility for the integrity, originality, accuracy, and scientific content of the manuscript.

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## Authors' contribution

Conceptualization, D.S. and G.Z.; methodology, D.S.; software, D.S. and Z.W.; validation, D.S. and D.S.; formal analysis, D.S.; investigation, G.F.; resources, G.Z.; data curation, T.B.; writing—original draft preparation, D.S.; writing—review and editing, D.S. and G.Z.; visualization, D.S.; supervision, G.Z. and G.F.; project administration, G.Z. and Y.W.; funding acquisition, G.Z. and Y.W. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors have no conflicts of interest to declare or other disclosures.

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