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Review Article

Functions and therapeutic potentials of exosomes in osteosarcoma

Jiaji Yue[a], Zhe-Sheng Chen[b], Xiang-Xi Xu[c], Shenglong Li[d,*]

[a]Department of Bone and Joint Surgery, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong, 518000, PR China
[b]College of Pharmacy and Health Sciences, St. John's University, Queens, NY, USA
[c]Department of Radiation Oncology, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA
[d]Department of Bone and Soft Tissue Tumor Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang, Liaoning, 110042, PR China

***Correspondence:** lishenglong@cancerhosp-ln-cmu.com (S. Li)
Tel.: +86-24-3191-6813

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ABSTRACT

Osteosarcoma, a primary malignant tumor of the skeleton, has a morbidity of 2.5 per 1 million people. The epiphysis of extremities is typically affected. Osteosarcoma has a high likelihood of early metastasis, rapid progression, and poor prognosis. The survival rate of patients with metastatic or recurrent osteosarcoma remains low; therefore, novel diagnostic and therapeutic methods are urgently needed. Exosomes, extracellular vesicles 30–150 nm in diameter, are secreted by various cells and are widely present in various body fluids. Exosomes are abundant in biologically active components, such as proteins, nucleic acids, and lipids. Exosomes participate in numerous physiological and pathological processes via intercellular substance exchange and signaling. This review presents the novel findings regarding exosomes in osteosarcoma diagnosis, prognosis, and therapeutics.

Keywords: Exosomes, Osteosarcoma, Biomarkers, Functions, Therapeutic potential

1. INTRODUCTION

Osteosarcoma (OS) is a primary malignant bone tumor originating from primitive osteogenic mesenchymal cells in adolescents and young adults under the age of 20 years [1]. Although the quality of life of patients with OS has significantly improved over the past few decades, its etiology remains unclear. Studies aimed at determining causes of OS have typically focused on multiple factors, including genetics, epidemiology, and the environment [2]. Research has identified associations with secondary OS in patients with Paget disease, electrical burns, trauma, exposure to beryllium, exposure to alkylating agents, FBJ virus, osteochondromatosis, enchondromatosis, fibrous dysplasia, orthopedic prosthetics, or bone infarction and infection. Additionally, OS has been reported to correlate with exposure to ionizing radiation, radium, and archaic contrast agents, such as thorotrast [3]. Furthermore, several genetic aberrations have been identified in cases of primary OS, including hereditary retinoblastoma, Li-Fraumeni syndrome, Rothmund-Thompson syndrome, Bloom syndrome, and Werner syndrome [4]. Radiographs of OS present osteogenic, osteolytic, or mixed bone destruction at the lesion. "Codman's triangle" and sun-exposed periosteal reaction [5] are typical radiographic features. MRI accurately depicts OS on the basis of tumor cell differentiation and proliferation [6]. Radionuclide scans can determine whether bone metastases occur in OS [7]. Frozen biopsies are used for rapid intraoperative diagnosis, and paraffin sections are used for obtaining accurate histological findings postoperatively [8]. High levels of serum alkaline phosphatase and lactate dehydrogenase are predictive of poor prognosis [9]. Treatments for OS include neoadjuvant chemotherapy, surgical resection, chemotherapy, and interventional therapy [10]. In addition, cellular immunotherapy, gene therapy, and stem cell therapy have made some progress in recent years [11]. However, these methods remain in experimental stages. Approximately 18% of patients present micrometastases at the time of diagnosis, and the 5-year survival rate remains poor for patients with metastasis and recurrence [12]. Treatment outcomes remain suboptimal, owing to the tendency of OS to

remain asymptomatic, and to show early onset metastasis, and high malignancy. The 5-year survival rate of patients with OS without chemotherapy is below 30%. The leading cause of death is lung metastasis [13]. The 2-year survival rate of patients with OS with pulmonary metastasis is less than 25%, and the survival period after treatment shows a plateau, thus making breakthrough efficacy with traditional treatment regimens challenging [14]. Therefore, the underlying mechanisms of OS development and metastasis must be determined to enable the discovery of novel markers for clinical detection and effective therapeutic targets.

Exosomes have been reported to be involved in regulating cellular behavior by transferring cargoes (proteins, DNA, RNA, and lipids) intercellularly. Increasing evidence indicates that exosomes have high potential to promote OS progression and development; moreover, the therapeutic potential of exosomes in OS is receiving increasing attention. Exosomes are membranous vesicles 30–100 nm in diameter originating from late endosomes, which are formed by inward budding of the limited multivesicular body (MVB) membrane [15]. Exosomes were first identified as double-layered lipid structures containing no organelles in blood erythrocytes [16]. Exosomes contain various nucleic acids and evolutionarily conserved proteins [17], which transmit biological information through cellular communication for biological processes and disease progression [18]. In lung adenocarcinoma (LUAD), LINC00273 is induced by M2 macrophages and exosomal LINC00273 was transferred into LUAD cells to recruit NEDD4, thereby promoting LATS2 ubiquitination, inhibiting the Hippo pathway and YAP-induced RBMX transcription, and ultimately resulting in LUAD malignancy [19]. Anlotinib-resistant non-small-cell lung cancer (NSCLC) cells promote the proliferation of parental NSCLC cells by transferring functional miR-136-5p from anlotinib-resistant NSCLC cells to parental NSCLC cells via exosomes. Exosomal miR-136-5p can lead to anlotinib resistance in NSCLC cells by targeting PPP2R2A and promoting activation of the AKT pathway [20]. Exosomes secreted by various cells in OS enable intercellular communication of ncRNAs and protein components, thus effectively regulating the tumor microenvironment, and promoting proliferation and metastasis. In addition, exosomes' stability in the circulatory system supports their diagnostic and therapeutic potential. This article reviews the biological properties of exosomes and their roles in the diagnosis and treatment of OS.

2. EXOSOME FORMATION AND BIOLOGICAL CHARACTERISTICS

Extracellular vesicles (EVs) are universally found in cells, and carry proteins, genetic material, and metabolites [21]. On the basis of their size and release mechanism, EVs are classified into exosomes (30–150 nm in diameter), microvesicles/extranuclear granulosomes (100–1000 nm in diameter), and apoptotic vesicles (50–1500 nm in diameter) [22]. Exosome formation involves dual invagination of the protoplasmic membrane and the formation of intracellular multivesicular bodies (MVBs), which contain intraluminal vesicles (ILVs) [23]. The endoplasmic reticulum also contributes to early endonucleosome formation [24]. Invagination of late endosomal membranes results in the formation of ILVs within large MVBs, which fuse with lysosomes or autophagosomes, thereby leading to cargo degradation, or fuse with the plasma membrane and release the contained ILVs as exosomes [25]. Exosomes are present in almost all body fluids, including plasma, urine, ascites, and breast milk [26].

2.1 Exosome formation

Exosome formation is activated by endosomal endocytosis, wherein the endosomal limiting membrane undergoes multiple deformations and invaginates, thus generating ILVs. The ILVs transform into MVBs with dynamic subcellular structures. MVBs are generated at the endosomal limiting membrane through either an endosomal sorting complex required for transport (ESCRT) mechanism or a non-ESCRT mechanism [27]. The ESCRT mechanism involves recognition of cytoplasmic protein complexes with ubiquitinated modified membrane proteins. The ESCRT-0 complex plays a vital role in the generation of multivesicular bodies by binding and clustering ubiquitinated proteins. The ESCRT-I complex recognizes and passes ESCRT-0 to ESCRT II. TSG101 in ESCRT I identifies disulfide bonds and consequently induces endosomal membrane invagination; shearing of the bud neck via ESCRT III then leads to the formation of MVBs [28]. In the absence of ESCRT, MVB formation is initiated by the accessory protein ALG-2 interacting protein X (Alix), which directly binds the intracellular bridging protein syntenin and participates in exosome formation [29]. The abundant tetratransmembrane protein can facilitate the production of these ESCRT-nondependent MVBs [30]. MVB fusion with lysosomes induces the degradation and recirculation of their cargo. Cholesterol abundance in MVBs plays an essential role in regulating their sorting: cholesterol-rich MVBs are targeted to the cell membrane for release as exosomes, whereas low-cholesterol MVBs are targeted for transport to lysosomes [31].

2.2 Exosome mechanisms in biological function

Exosome-mediated intercellular transmission relies on membrane receptors. Exosomes activate receptors on recipient cells, thereby activating the uptake of exosomes through cytokinesis [32]. Studies have focused on exploring the functions of cell-derived exosomes and the use of exosomes for disease treatment [33]. Target cell specificity may be determined by specific interactions between proteins enriched on the surfaces of exosomes and receptors on the membranes of recipient cells [34]. Known mediators include transmembrane tetraspanins, integrins, and extracellular matrix components [35].

2.3 Exosomes' potential in tumor diagnosis and treatment

Exosomes primarily regulate the exclusion of redundant and nonfunctional cellular components [36]. Exosomes are intercellular linkers that transport proteins, lipids, and nucleic acids to target cells in various biological processes, such as angiogenesis, antigen presentation, apoptosis, and inflammation [37]. The specific cell components in the exosomes reflect cellular origin and physiological state, and show significant disease specificity, thus making them ideal biomarkers. Exosomes are involved in various cancer-associated processes, including proliferation, apoptosis, angiogenesis, and metastasis; consequently, they may serve as noninvasive biomarkers for cancer diagnosis [38, 39]. For example, miR-21, miR-222, and miR-124-3p in serum exosomes are detectable in early tumor progression after surgical treatment of patients with high-grade glioma [40]. Moreover, miR-21, miR-451, and miR-636 in urinary exosomes of patients with prostate cancer closely correlate with preoperative prostate-specific antigen levels; thus, urinary exosomal microRNAs (miRNAs) may potentially serve as noninvasive markers to predict prostate cancer metastasis and prognosis [41]. Plasma exosomal miR-363-5p has shown high diagnostic performance in discriminating patients with LN (+) versus LN (–) breast cancer. Elevated miR-363-5p expression levels have been found to indicate lower overall survival [42]. The therapeutic potential of exosomes is associated primarily with targeted drug delivery and biomedical regeneration. Exosomes have great potential in treating diseases, owing to their nontumorigenic, bactericidal, and low immunogenicity characteristics [43]. Ligand enrichment on engineered exosomes can induce or inhibit signaling in receptor cells, or can target exosomes to specific cells [44]. Exosomes loaded with chemotherapeutic agents have shown promise for antineoplastic drugs delivery with low toxicity and high tolerance [45].

3. EXOSOMES IN OS PROGRESSION

Exosomes can transmit intercellular signals that regulate proliferation and metastasis. Exosomes promote tumor proliferation and metastasis by inducing epithelial-mesenchymal transition, and accelerating tumor neovascularization and immunosuppression through regulating the microenvironment and transformation of cancer-associated fibroblasts [46, 47]. Exosomes have major roles in regulating proliferation, invasion metastasis, and OS angiogenesis, by participating in intercellular contacts and modulating cellular signaling.

3.1 Exosomes in OS proliferation

The potential to proliferate indefinitely is the fundamental feature of cancer cells [48]. OS cells express growth factor receptors and rarely show negative feedback regulation, thus resulting in continuous activation of signal stimulation, and unlimited cell division and proliferation [49]. Exosomes participate in various processes in the proliferation of OS (**Table 1**). For example, miR-208a from bone marrow-derived mesenchymal stem cell (BMSC)-derived exosomes has been found to promote OS cell proliferation and inhibit apoptosis by suppressing PDCD4 expression, and activating the ERK1/2 and Hippo pathways. BMSC-derived exosomal miR-206 inhibits cell proliferation by targeting TRA2B [50]. In addition, BMSC-derived exosomes encapsulate PVT1 and translocate it to OS cells. PVT1 promotes tumor growth and metastasis by binding miR-183-5p, thus increasing ERG expression [51]. The MALAT1/miR-143/NRSN2/Wnt/β-catenin axis is another crucial target through which BMSE-EVs promote proliferation [52]. ADSC exosomes deliver COLGALT2 to OS cells, thus leading to OS malignancy [53]. BMSC-derived exosomes promote OS proliferation and metastasis via the LCP1/JAK2/STAT3 pathway. Moreover, targeting the miR-135a-5p/LCP1 axis inhibits OS progression [54]. MG-63-cell-derived exosomes promote the proliferation of OS and inhibit apoptosis. Hic-5 from MG-63-cell-derived exosomes interacts with Smad4 and regulates Wnt/β-catenin signaling by decreasing TCF/LEF activity [55]. OS-cell-derived exosomal miR-1307 promotes OS cell proliferation by inhibiting AGAP1 expression; consequently, the miR-1307-AGAP1 axis may serve as a potential therapeutic target for OS [56]. In patients with OS, exosomal miR-15a expression is diminished in plasma exosomes. Moreover, exosomal miR-15a has been found to inhibit the GATA2/MDM2 axis via the p53 signaling pathway, thereby inhibiting the proliferation and invasion of OS cells in vitro [57].

3.2 Exosomes in OS metastasis

Epithelial-mesenchymal transition is a biological phenomenon in which epithelial cells lose their epithelial properties and acquire a mesenchymal phenotype. In this process, epithelial features are diminished. Cells change from polygonal to spindle-shaped fibroblast-like morphology; show loss of cell polarity and decreased adhesion; and acquire invasion and metastasis ability [58]. Exosomes are essential in the invasive metastasis of OS (**Table 1**). For example, miR-143 is transferred to OS cells via exosomes and significantly inhibits tumor invasiveness [59]. Highly invasive OS cells secrete exosomal miR-675, which suppresses CALN1 expression in recipient cells. The expression of exosomal miR-675 in the serum in patients with OS is strongly correlated with prognosis [60]. Mazumdar et al. have found that both highly metastatic 143-B cells and weakly metastatic SAOS-2-cell-derived EVs induce the recruitment of bone marrow cells to the lungs, and that components in exosomes may inhibit remote metastasis of OS [61]. In OS, the Rab22a-NeoF1 fusion protein is assimilated into exosomes. The exosomal Rab22a-NeoF1 fusion protein promotes formation of the premetastatic lung niche by recruiting

Table 1 | Biological functions of exosomes in the proliferation and metastasis of osteosarcoma.

Exosomal target	Parent cell	Target cell	Mechanism	Biological function	Ref.
Proliferation and metastasis					
miR-208	BMSCs	Osteosarcoma cells	PDCD4/ERK1/2	Increase the viability, migration, and clonogenicity of OS	[95]
miR-206	BMSCs	Osteosarcoma cells	TRA2B	Promote OS cell proliferation, migration, and invasion, and induce apoptosis	[50]
MALAT1	BMSCs	Osteosarcoma cells	MALAT1/miR-143/NRSN2/Wnt/β-catenin	Promote OS cell proliferation, migration, and invasion	[52]
PVT1	BMSCs	Osteosarcoma cells	PVT1/miR-183-5p/ERG	Promote OS growth and metastasis	[51]
ATG5	BMSCs	Osteosarcoma cells	/	Promote OS cell proliferation, migration, and invasion	[96]
COLGALT2	ADSCs	Osteosarcoma cells	/	Promote OS cell proliferation, migration, and invasion	[97]
Linc00852	Osteosarcoma cells with high AXL expression	Osteosarcoma cells with low AXL expression	Linc00852/miR-7-5p/AXL	Promote cell proliferation, migration, and invasion	[98]
LCP1	BMSCs	Osteosarcoma cells	miR-135a-5p/LCP1/JAK2/STAT3	Induce the proliferation and metastasis of OS cells	[54]
Hic-5	MG-63	MG-63 and HOS cells	Hic-5/smad4-TCF/LEF-Wnt/β-catenin	Promote cell proliferation and inhibit cell apoptosis	[55]
miR-1307	Osteosarcoma cells	Osteosarcoma cells	AGAP1	Promote OS cell proliferation, migration, and invasion	[56]
miR-15a	Serum-derived exosomes	Osteosarcoma cells	miR-15a/p5/GATA2/MDM2	Promote OS cell proliferation and invasion	[99]
miR-769-5p	BMSCs	Clinical specimens	DUSP16/JNK/p38 MAPK	Promote OS proliferation and metastasis	[100]
SHNG17	CAFs\NFs	HOS cells	miR-2861	Promote OS proliferation and metastasis	[101]
miR-143	/	Osteosarcoma cells	/	Inhibit cell invasion	[59]
miR-675	Osteosarcoma cells	hFOB1.19	CALN1	Promote cell migration and invasion	[25]
Rab22a-NeoF1/PYK2	PYK2-positive osteosarcoma cells	Macrophages	RhoA	Facilitate pre-metastatic niche formation	[62]
miR-1307	Osteosarcoma cells	Osteosarcoma cells	AGAP1	Promote cell proliferation, migration, and invasion	[62]
LCP1	BMSCs	Osteosarcoma cells	miR-135a-5p/Nrdp1/JAK2/STAT3	Promote OS proliferation and metastasis	[54]
LIFR-AS1	Macrophages	Osteosarcoma cells	miR-29a/NFIA	Promote cell proliferation and invasion, and restrain cell apoptosis	[102]
Angiogenesis					
miR-25-3p	/	Osteosarcoma cells	DKK3	Promote capillary formation and the invasion of vascular endothelial cells	[42]
EWSAT1	/	Osteosarcoma cells	/	Increase sensitivity/reactivity of vascular endothelial cells	[103]
OIP5-AS1	Osteosarcoma cells	Osteosarcoma cells	miR-153/ATG5	Increase angiogenesis	[66]

Table 1 | Continued

Exosomal target	Parent cell	Target cell	Mechanism	Biological function	Ref.
miR-199a-5p	Osteosarcoma cells	HUVECs	VEGFA	Inhibit the growth and angiogenesis of osteosarcoma	[104]
miR-148a-3p and miR-21-5p	Osteosarcoma cells	Raw264.7 and HUVECs	/	Influence osteoclastogenesis, bone resorption, and tumor angiogenesis	[69]
Immunosuppressive					
miR-148a-3p and miR-21-5p	Osteosarcoma cells	Raw264.7 and HUVECs	/	Influence osteoclastogenesis, bone resorption, and tumor angiogenesis	[69]
Tim-3	MG63	Macrophages	/	Induce M2 type differentiation of macrophages	[72]

bone-marrow-derived macrophages [62]. OS-cell-derived exosomal miR-1307 promotes proliferation, migration, and invasion by regulating AGAP1 expression, thus indicating the inhibitory roles of miR-1307 in the malignant progression of OS [56].

3.3 Exosomes in OS angiogenesis

Proangiogenic and antiangiogenic factors have major roles in the formation of blood vessels [63]. Tumor cells require nutrient supply and metabolite excretion for survival and development [64]. Tumor-derived exosomes are critical mechanisms that promote angiogenesis (**Table 1**). In OS tissues, an increase in miR-25-3p promotes tumor proliferation, metastasis, and drug resistance by inhibiting DKK3. EWSAT1 promotes OS angiogenesis by transfering it into the exosome-driven vascular endothelial cell, thus increasing the secretion and the sensitivity/responsiveness to angiogenic factors [65]. OS cells with high exosome abundance regulate OS tumor angiogenesis and autophagy through miR-153 and ATG5, by secreting exosomal lnc-OIP5-AS1, which is taken up by adjacent OS cells [66].

3.4 Exosomes in OS the immune response

Exosomes participate in the immune response and regulate immunocompetence [67]. Tumor-cell-derived exosomes carry tumor-associated antigens and stimulate immune cells to generate antitumor immune responses. However, they can interfere with immune recognition, and inhibit T cells and immune-associated cells, thereby accelerating tumor cell immune escape and metastasis [17]. Immune cells derived from the tumor microenvironment regulate proliferation and metastasis through exosomes [68]. Exosomes also have critical roles in the tumor immune microenvironment of OS (**Table 1**). Exosomal miR-1228 secreted by cancer-associated fibroblasts (CAFs) promotes OS invasion and migration by targeting SCAI. This miRNA may serve as a potential therapeutic target for OS [42]. Exosomes enhance tube formation in endothelial cells and increase the expression of angiogenic markers. Next-generation sequencing has revealed that specific miRNAs, such as miR-148a and miR-21-5p, have essential roles in the tumor microenvironment [69]. The exosomes of metastatic OS cells secrete TGFβ2, which is taken up by tumor-associated macrophages, thus promoting the M2 phenotype and contributing to immunosuppression and tumorigenesis [70]. OS-cell-derived EVs promote myofibroblast/cancer-associated fibroblast differentiation, smooth muscle actin expression, and fibronectin production. In addition, they significantly promote the invasiveness of human lung fibroblasts [71]. OS-derived exosomes induce M2 polarization of macrophages via Tim-3, thereby promoting OS invasion and metastasis [72]. Exosomal Col6a1 converts normal fibroblasts into CAFs through the secretion of proinflammatory cytokines. Activated CAFs promote OS cell invasion and migration by mediating the TGF-β/COL6A1 signaling pathway [73]. The macrophage-derived exosomal long noncoding RNA LIFR-AS1 promotes the malignant progression of OS by binding miR-29a and consequently increasing NFIA expression [74].

4. APPLICATION POTENTIAL OF EXOSOMES IN OS

Exosomes contain various biologically active molecules in circulation and mediate remote intercellular interaction [75]. Tumor-derived exosomes contain multiple proteins, genetic material, lipids, and other molecules that reflect tumor physiological and pathological status [76]. The specific lipid bilayer structure of exosomes protects RNA molecules from degradation [77]. Therefore, detecting tumor exosomes provides major advantages in liquid biopsy. Exosomes have good application potential for early diagnosis, assessment of treatment efficacy, and monitoring of prognosis in various diseases. They have become new and ideal biomarkers, and may possibly serve as targeted drug carriers in clinical diagnosis and treatment.

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4.1 Potential of exosomes in OS diagnosis

Exosomes are essential in the early diagnosis and prognostic assessment of OS. Eight novel miRNAs have been identified by next-generation sequencing in three distinct OS cell lines, five of which are present in the circulating exosomes of patients with OS [57]. EV-miR-101 expression levels are significantly lower in OS patients. In plasma from patients with OS metastasis, EV-miR-101 is lower than that in patients without metastasis and thus may serve as a potential diagnostic marker for OS [78]. Ye et al. have revealed that the expression levels of miR-92a-3p, miR-130a-3p, miR-195-3p, miR-335-5p, and let-7i-3p are significantly upregulated in the exosomes of patients with OS and therefore may serve as potential diagnostic markers [79]. HSATI, HSATII, LINE1-P1, and Charlie 3 are overexpressed at the RNA level in serum exosomes from patients with OS and thus may be potential OS biomarkers [80]. Exosome-derived SENP1 in patients with OS is closely correlated with tumor size, location, necrosis rate, lung metastasis, and surgical staging. Higher plasma exosome-derived SENP1 levels indicate poorer disease-free survival and overall survival [81]. Seven exosomal proteins have been identified as potential biomarkers of OS lung metastasis [82]. In addition, SERS and MALDI-TOF MS exosomes have shown great potential in the rapid diagnosis of OS [83].

4.2 Potential of exosomes in OS treatment

Exosomes have great potential in the treatment of OS. Multidrug-resistant OS cells secrete exosomes containing MDR-1 messenger RNA and P-glycoprotein, thus promoting doxorubicin resistance in sensitive cells. Exosomes targeting drug-resistant OS cells may inhibit the malignant progression of OS [84]. Compared with exosomes from normal osteoblasts, OS-derived exosomes contain immunomodulatory substances that significantly decrease T cell proliferation rates and promote T regulatory phenotypes, thereby facilitating OS progression [10, 85]. The miRNAs miR-135b, miR-148a, miR-27a, and miR-9 are highly expressed in the serum exosomes in patients with OS and may potentially be reliable biomarkers of chemotherapy sensitivity [16, 86]. Exosome-loaded doxorubicin has been found to enhance cellular uptake efficiency and antitumor effects in the OS MG63 cell line, while showing low cytotoxicity, thus potentially providing a good targeting regimen for OS [87]. OS cells promote OS lung metastasis by releasing exosomes containing PD-L1 and N-calcineurin. In addition, the expression levels of exosomal PD-L1 and N-calcineurin in the serum in patients

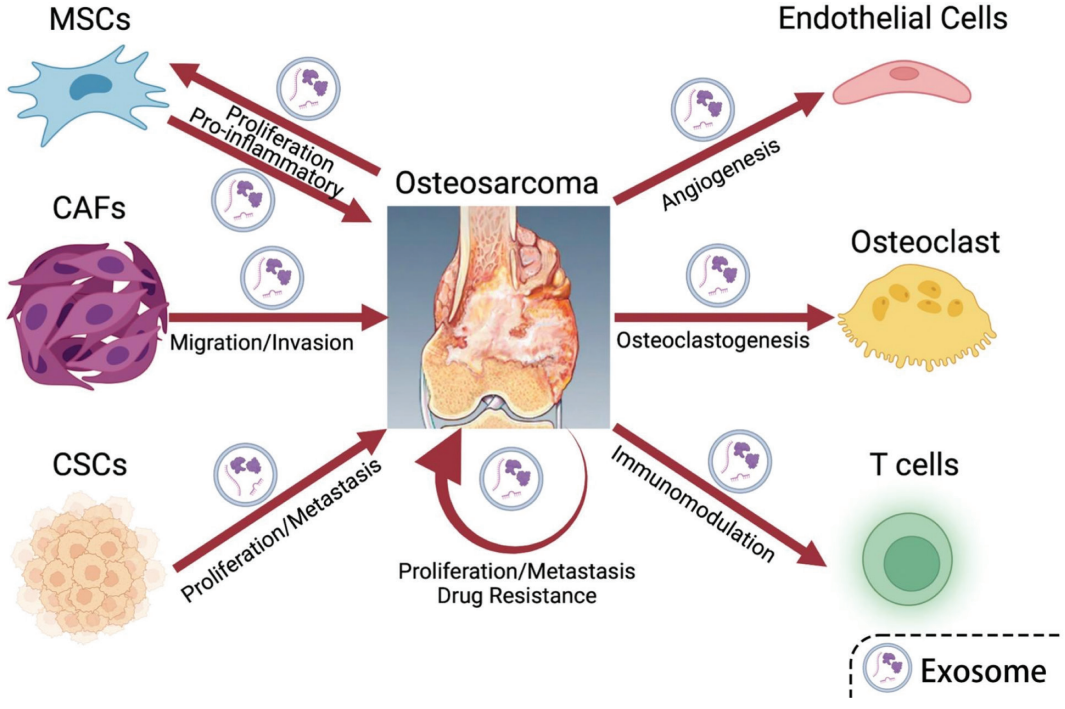

Figure 1 | The interaction of OS and related cells through exosomes.
Mesenchymal stem cells, CAFs, and CSCs secrete exosomes containing specific proteins and genetic material, which promote the proliferation, metastasis, and invasion of OS. OS cells generate exosomes targeting specific cells that promote angiogenesis, osteoclastogenesis, and immunomodulation of the target cells. OS promotes drug resistance, proliferation, and metastasis through exosome secretion. (Created in Biorender.com.)

with OS have been found to predict pulmonary metastasis progression [88]. Exosomes from cisplatin-resistant OS cells decrease the expression of multidrug resistance-associated protein 1 and P-glycoprotein in MG63 and U2OS cells; increase chemosensitivity to cisplatin; and inhibit apoptosis through exosomal-hsa_circ_103801 [89]. Moreover, exosomes from drug-resistant HMPOS-2.5R cell lines have been found to transfer drug resistance to drug-sensitive HMPOS cells, thereby decreasing the therapeutic sensitivity of OS [90].

5. CONCLUSIONS

Early diagnosis is critical for promoting good prognosis and survival in tumor patients [91]. Exosomes are stable and widespread in all tissues, organs, and body fluids, and are released by all types of cells (**Figure 1**) [92]. Tumor exosomes regulate tumor progression, angiogenesis, metastasis, and immune escape by interacting with other cells in the tumor microenvironment [93]. Standard methods for liquid biopsy are needed to isolate exosomes quickly, easily, and specifically. Exosomes are a promising biomarker for the diagnosis of OS, predicting prognosis, and monitoring treatment response in real time. Therefore, large multicenter studies are needed to develop the validity of liquid biopsies. For study of biological functions, whether exosomes have similar regulatory functions in vivo and in vitro is impossible to determine. For therapeutic purposes, exosome-derived cells should be carefully selected to ensure the safety of the treatment. Erythrocytes are the most promising exosome-producing cells, because they are readily available in blood banks, do not contain nuclei, and lack genetic material. Beyond their potential as biomarkers, exosomes may support new research directions in the precision treatment of tumors [87]. To improve the effectiveness of antitumor drug therapy, development of drug-loading systems remains a key challenges. As natural therapeutic carriers, exosomes contain bioactive molecules and can avoid immune rejection [94]; in addition they enable exogenous drugs to maintain stability in vivo. These advantages make exosomes an ideal loading system providing a new paradigm for drug delivery, and are expected to be an important tool for the development of precision medicine for tumors. Han et al. have constructed fusion gene iRGD-Lamp2b-modified mesenchymal stem cells to isolate and purify exosomes and loaded anti-miRNA-221 oligonucleotides into exosomes. AMO-loaded exosomes have been found to effectively inhibit the proliferation and clonal formation of colon cancer cells in vitro [51].

This review discussed the biological functions of exosomes in the progression of OS and clinical applications. Exosomes from OS promote malignant progression by regulating tumor metastasis, angiogenesis, tumor immunity, and drug resistance. Exosomes therefore provide new potential therapeutic targets.

ABBREVIATIONS

Osteosarcoma, OS; microRNAs, miRNAs; non-small-cell lung cancer, NSCLC; extracellular vesicles, EVs; multivesicular bodies, MVBs; luminal vesicles, ILVs; endosomal sorting complex required for transport, ESCRT; bone marrow-derived mesenchymal stem cells, BMSCs; cancer-associated fibroblasts, CAFs.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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