Advancements in research regarding the influence of the tumor microenvironment on the proliferation and metastasis of osteosarcoma (Review)

TAO SHI[1*], JIANPING KANG[2*], QIYANG WANG[3*], JIAMEI SONG[4] and XIAOFENG HE[1]

[1]Department of Pain, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, P.R. China; [2]Department of Orthopedic Surgery, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, P.R. China; [3]Department of Orthopedic Surgery, Yunnan Province Spinal Cord Disease Clinical Medical Center, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan 650000, P.R. China; [4]Department of Reproductive Medicine, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, P.R. China

Received June 3, 2025; Accepted October 6, 2025

DOI: 10.3892/ol.2025.15373

Abstract. Osteosarcoma (OS), a common primary malignant bone tumor in children and young adults, is significantly influenced by a complex tumor microenvironment (TME) that includes bone cells, stromal cells, vascular cells, immune cells and a mineralized extracellular matrix (ECM). In recent years, the role of the TME in OS progression has garnered increasing attention. The TME not only provides the physical and biochemical support necessary for tumor cell growth, invasion and metastasis, but also promotes the maintenance of the malignant tumor phenotype through mechanisms such as immune suppression, angiogenesis and metabolic reprogramming. The present review discusses how various cellular and non-cellular components within the OS TME interact to drive tumor progression. Consequently, targeting the TME has emerged as a promising therapeutic strategy to overcome the limitations of conventional treatments, particularly for metastatic or recurrent OS. The present review underscores the potential of TME-targeted therapies and highlights the need for further research into the heterogeneity of the TME to improve clinical outcomes.

Correspondence to: Dr Xiaofeng He, Department of Pain, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Wuhua, Kunming, Yunnan 650000, P.R. China
E-mail: kmhxf912060@126.com

Dr Jiamei Song, Department of Reproductive Medicine, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Wuhua, Kunming, Yunnan 650000, P.R. China
E-mail: songjiamei@kmmu.edu.cn

*Contributed equally

Key words: osteosarcoma, tumor microenvironment, tumor-associated immune cells, bone marrow mesenchymal stem cells, extracellular matrix, hypoxia

Contents

1. Introduction
2. TME of OS
3. Vascular microenvironment
4. Hypoxia and the TME of OS
5. Translational challenges and future directions in OS TME research

1. Introduction

Osteosarcoma (OS) is one of the most common primary malignant bone tumors in children and young adults, and its occurrence and development are influenced by various factors. In recent years, advancements in the understanding of OS and the refinement of therapeutic approaches have led to an increase in the 5-year survival rate for patients with tumors, now ranging from 60 to 80% (1). Nevertheless, the mortality rate associated with OS remains elevated compared with other malignant bone tumors, particularly among patients with metastatic or recurrent OS, where the long-term survival rate frequently falls below 30% (2).

OS tumors develop within a multifaceted and dynamically evolving tumor microenvironment (TME) that encompasses bone cells, stromal cells, vascular cells, immune cells and a mineralized extracellular matrix (ECM). The intricate interactions between OS cells and their surrounding microenvironment are critical in influencing various aspects of tumor biology, including progression, apoptosis, invasion, metastasis, angiogenesis and responses to therapeutic interventions (3). The TME is not a passive bystander but an active participant in oncogenesis; it provides critical support for tumor cell growth and dissemination and promotes the maintenance of

the malignant phenotype through key mechanisms such as immune evasion, induction of angiogenesis and metabolic reprogramming (4).

Specific components of the OS TME, including osteocytes, tumor-associated immune cells, bone marrow (BM) mesenchymal stem cells (MSCs) and the ECM, interact to create a hypoxic and vascularized niche conducive to proliferation and metastasis (5). Recognition of the pivotal role of the TME has driven the development of novel therapeutic strategies. For instance, inhibitors targeting immunosuppressive cells or immune checkpoints have achieved breakthroughs in certain solid tumors and are being explored in OS clinical trials (6). Additionally, approaches such as targeting cytokines secreted by cancer-associated fibroblasts (CAFs) or blocking abnormal angiogenesis are under investigation as potential combination therapies to enhance traditional treatment efficacy (7). Although still in the early stages, these TME-targeted therapies hold promise to overcome chemoresistance and improve survival.

The present review addresses the multiple roles of the TME in OS development, examining how its various components influence tumor proliferation, metastasis and treatment response; it also discusses the emerging paradigm of targeting the TME, highlighting the need for further research into its heterogeneity to disrupt the pro-tumorigenic cycle and offer new hope for patients with OS.

2. TME of OS

OS tumors arise within a complex and dynamic microenvironment that comprises both cellular and non-cellular components. This environment consists of a heterogeneous population of bone cells, which includes osteoblasts (OBs), osteoclasts and osteocytes, alongside stromal cells such as MSCs and fibroblasts. Furthermore, it encompasses vascular cells, including endothelial cells and pericytes, as well as immune cells, which comprise BM cells and lymphocytes, in addition to a mineralized ECM (8).

Under typical physiological conditions, the interactions among bone, vascular and stromal cells occur through paracrine signaling and cellular communication, which are essential for maintaining bone homeostasis (8). BM cells represent the primary cell type within TME of OS (8). Recent single-cell analyses have uncovered a multitude of ligand-receptor interactions among OS tumors, BM and OBs, leading to the identification of 21 ligand-receptor gene pairs that exhibit a significant correlation with survival outcomes (9). The TME not only fosters a conducive environment for the proliferation of tumor cells but also secretes various factors, including cytokines, chemokines and growth factors, which facilitate tumor cell metastasis (8). Fig. 1 illustrates the complex composition of the OS TME, encompassing major cellular components such as osteocytes, stromal cells and immune cells, alongside non-cellular elements including the ECM, vascular networks and hypoxic conditions; it also depicts interactions between tumor cells and select components within the OS TME.

Osteocytes. Osteocytes constitute an essential element of the bone microenvironment, which encompasses three principal categories of bone cells: OBs, osteoclasts and osteocytes. These cells interact with stromal and immune cells by secreting a variety of cytokines and chemokines that facilitate tumor growth, invasion and metastasis. OBs originate from multipotent MSCs, and their development can be augmented by particular cytokines, including interferon (IFN)-γ, interleukin (IL)-12 and IL-13 (8). Conversely, their function may be inhibited by factors including tumor necrosis factor (TNF)-α, TNF-β, IL-1, IFN-α, IL-4 and IL-7 (10). OBs also have a notable role in influencing the formation, differentiation or apoptosis of osteoclasts through various signaling pathways, including the osteoprotegerin/receptor activator of nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK), RANKL/leucine-rich repeats containing G protein-coupled receptor 4/RANK, Ephrin 2/ephB4 and Fas/FasL pathways (11). Osteocytes can secrete a variety of soluble factors, including growth differentiation factor 15, TGF-β, chemokines CXC-motif chemokine ligand (CXCL) 1 and CXCL2, as well as vascular endothelial growth factor (VEGF). They additionally synthesize RANKL, colony-stimulating factor 1 (CSF-1), high-mobility group box 1 (HMGB1) and IL-11, all of which promote osteoclastogenesis and the process of bone resorption (12). In OS, osteocytes activate the CXCL12/CXCR4 signaling axis by producing CXCL12, which is linked to tumor metastasis. Osteoclasts are integral to the initiation and metastasis of OS (13). Among the cytokines involved, CSF-1 and soluble RANKL are crucial for the differentiation and activation of OBs). CSF-1 is responsible for the regulation of both the proliferation and survival of pre-osteoclasts (14). By contrast, RANKL, which is synthesized by OBs and various other bone cells (15), interacts with its receptor RANK located on the surface of osteoclast precursors (16). This interaction regulates the differentiation and maturation of osteoclast precursors via paracrine signaling mechanisms. In the context of OS, heightened osteoclast activity plays a significant role in the proliferation of OS cells and the degradation of bone tissue. This mechanism leads to the liberation of pro-tumorigenic factors, such as insulin-like growth factor 1 (IGF-1) and from the bone matrix, thereby promoting tumor proliferation and metastasis (17).

Tumor-associated immune cells. Within the microenvironment of OS, monocytes and macrophages constitute 70-80% of the total BM cell population (18). Various cell types, including tumor-associated macrophages (TAMs), T cells, B cells, natural killer (NK) cells and BM MSCs, present in the tumor immune microenvironment significantly influence tumor development, invasion and metastasis. Recent investigations into TAMs have emerged as a focal point of research.

TAMs constitute the most prevalent immune cell population within the OS microenvironment, and account for >50% of all infiltrating immune cells, alongside other cell types including dendritic cells (DCs), lymphocytes and myeloid-derived cells. Collectively, these cells form the primary components of the OS immune microenvironment (19). Research indicates that TAMs are prevalent in most high-grade OS biopsy specimens, and an increase in TAMs is associated with reduced metastasis and extended survival (20); however, the underlying mechanisms remain unclear. Immune cells within the TME can either promote or inhibit OS, contingent upon the immune environment and the specific cell types involved (21). For

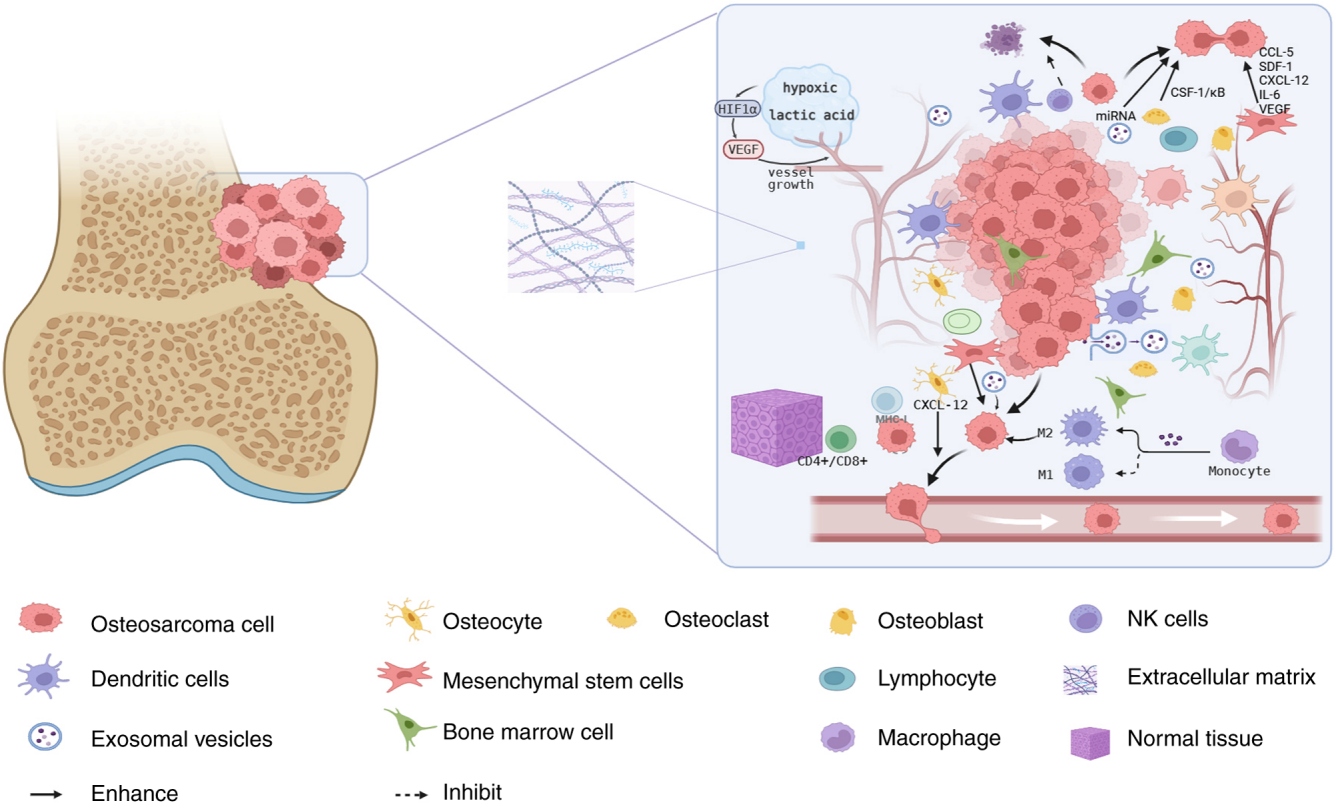

Figure 1. Microenvironment of osteosarcoma is composed of cellular and non-cellular components, including bone cells (osteoblasts, osteoclasts and osteocytes), stromal cells (mesenchymal stem cells and fibroblasts), immune cells (bone marrow and lymphocytes), mineralized ECM, rich microcirculation vessels and a hypoxic environment. Other components include CTCs, EVs and various signaling molecules. The tumor microenvironment not only provides a favorable growth environment for tumor cells, but also secretes various factors, including various cytokines, chemokines and growth factors, promoting tumor cell metastasis. The figure was created by BioRender. OSc, osteosarcoma cell; Ocytes, osteocyte; OCs, osteoclast; OBs, osteoblast; TAMs, tumor-associated macrophages; DCs, dendritic cells; MST, mesenchymal stem cell; Lymph, lymphocyte; ECM, extracellular matrix; EV, exosomal vesicles; BMC, bone marrow cell; NT, normal tissue; MSC, multipotent stem cell; CTCs, circulating tumor cells.

instance, certain immune cells, including T cells, B cells and NK cells, can exert antitumor effects, while others, such as myeloid-derived suppressor cells (MDSCs), M2 macrophages and regulatory T cells (Tregs), may facilitate tumor growth and metastasis. Consequently, elucidating the intricate mechanisms of interaction between OS and immune cells is essential for advancing research into novel immunotherapies (17).

TAMs. TAMs originate from peripheral monocytes and are recruited to the tumor site by various chemokines and cytokines, including C-C motif ligand 2, CSF-1 and VEGF. Upon entering the tumor, TAMs can exhibit two primary phenotypes: The pro-inflammatory M1 phenotype and the immunosuppressive M2 phenotype. The M1 phenotype is associated with protective responses and is typically linked to a favorable prognosis in patients with OS (22). Conversely, the M2 phenotype can be induced by various stimuli within the immune microenvironment, particularly through cytokines IL-4 and IL-13 that signal via the signal transducer and activator of the transcription 6 (STAT6) pathway, as well as IL-10 and glucocorticoids. M2-type TAMs secrete a range of cytokines that promote the migration and invasion of OS cells while inhibiting the immune function of T lymphocytes (23). M1 and M2 TAMs can undergo interconversion under the influence of key cytokines and signaling pathways, such as the Th2 cytokines (IL-4 and IL-13) via the STAT6 pathway, as well as IL-10 and TGF-β. This macrophage polarization is dynamic. Research has demonstrated that polarized M2-type TAMs play a significant role in the proliferation and metastasis of OS. For example, M2-type TAMs can facilitate the migration and invasion of OS cells by triggering an autocrine signaling loop that activates HMGB1 expression within the tumor cells. Furthermore, HMGB1 has been shown to encourage the polarization of M1-type TAMs into M2-type TAMs, thereby establishing a positive feedback mechanism that exacerbates the development and progression of OS (24).

T cells. T cells are integral to both cellular and humoral immunity and comprise a wide array of functionally specialized subsets, including T helper cells (Th1, Th2, Th9, Th17, Th22 and follicular helper T cells), cytotoxic T lymphocytes (CTLs) and Tregs. This diversity is crucial for orchestrating the antitumor immune response in OS, with the composition and balance of these infiltrating T cell subsets determining the immunological outcome. In OS, tumor-infiltrating lymphocytes are predominantly located in areas expressing human leukocyte antigen class I, while $CD4^+$ and $CD8^+$ T cells primarily aggregate at the interface between lung metastases and normal tissue (25). Studies have indicated that the density of T cells in metastatic lesions is significantly greater than that in primary and recurrent lesions, suggesting that T cells may also serve as potential prognostic indicators (26,27). Analysis of biopsy tissues and peripheral blood from patients with primary OS has revealed that T cell levels in biopsy

specimens exceed those in peripheral blood, suggesting that the immune microenvironment within tumor lesions is suppressive, potentially inhibiting T cell immune activity through TAMs, further suggesting that T cells may serve as an auxiliary biomarker for clinical diagnosis (28). Furthermore, the depletion of $CD163^+$ macrophages, a hallmark immunosuppressive subset in the OS TME, has been shown to enhance T cell growth and pro-inflammatory factor production *in vitro*. Therefore, targeting these cells represents a promising approach to reprogram the immunosuppressive OS TME and potentiate antitumor immunity (28). The prognostic value of T cells is well-established across various cancer types, with studies confirming that specific T-cell signatures, such as the CD8 T-cell signature, are robust predictors of patient survival (29,30). Furthermore, prognostic models based on T-cell-related genes have been successfully constructed for cancer types such as hepatocellular carcinoma (29,30).

B cells. B cells can be classified into three categories: Naive B cells, memory B cells and effector B cells/plasma cells, with the latter being the primary source of antibodies. Regulatory B cells, a subset of B cells, exert immunosuppressive effects by inhibiting $CD4^+$ T cells, CTLs, macrophages and DCs through the secretion of inhibitory cytokines such as IL-10, TGF-β and IL-35, as well as the expression of membrane surface regulatory molecules such as FasL and CD1d (31). Regulatory B cells also promote the conversion of T cells into T lymphocytes, thereby diminishing the antitumor immune response (32)]. Current research on B cells in OS remains limited; however, a recent pan-cancer immune-infiltration analysis showed that patients with high B-cell abundance exhibit a significantly improved overall survival, and the proportion of activated B cells ($CD19^+CD27^+$) correlates positively with metastasis-free survival in OS (33). Thus, the presence of effector B cells may serve as a favorable prognostic indicator. Antibodies play a crucial role in the regulation of tumor growth and metastasis through various mechanisms, including antibody-dependent cell-mediated cytotoxicity, regulatory effects, complement activation, tumor cell receptor blockade and alterations in tumor cell adhesion (34,35). Nonetheless, some studies have also indicated that certain antibodies may bind to antigens on the surface of tumor cells, thereby obstructing their cytotoxic effects (32,36).

NK cells. NK cells are a type of innate lymphocyte characterized by the expression of the intracellular transcription factor E4 BP 4+ and are identified as $CD3^-$ $CD19^-$ $CD56^+$ $CD16^+$. NK cells have been shown to not only directly eliminate tumor cells but also regulate tumor progression and metastasis (37,38). They can induce tumor cell death in the TME by releasing perforin, granzyme and TNF-α, as well as expressing FasL (39). The programmed cell death protein-1 (PD-1)/programmed cell death ligand 1 (PD-L1) axis plays a role in modulating the antitumor effects of NK cells. Research has demonstrated that blocking the PD-1/PD-L1 axis with PD-L1 antibodies enhances the cytotoxic activity of NK cells against human OS cells by inhibiting NK cell toxicity through the secretion of granzyme B (40). Comprehensive analyses of immune infiltration in the OS microenvironment have revealed that male patients exhibit a higher presence of NK cells compared with female patients (41). Additionally, it has been observed that NK cells are suppressed in the OS microenvironment, with increased expression of TGF-β (42). This suppression may involve the inhibition of the activating receptor natural killer group 2 member D and a reduction in perforin release by NK cells, thereby promoting angiogenesis, bone remodeling and cellular migration.

BM MSCs. BM MSCs are classified as multipotent stem cells that significantly contribute to the development of OS tumors through the modulation of immune responses and the facilitation of cell fusion and differentiation (43). MSCs and OBs are regarded as potential precursors to OS cells (33). Research suggests that MSCs play a crucial role in mediating the bidirectional crosstalk between OS tumor cells and the TME through the secretion of a variety of cytokines, chemokines, ILs and other signaling molecules (44). MSCs are actively involved in the paracrine signaling mechanisms of OS tumor cells, influencing multiple facets of tumor behavior, such as angiogenesis, proliferation, invasion, metastasis, immune modulation and resistance to chemotherapy (45). Furthermore, MSCs facilitate the growth, metastasis, and angiogenesis of OS by releasing an array of chemokines, including CCL5, stromal-derived factor 1, CXCL12, IL-6 and VEGF (45).

MSC-derived extracellular vesicles have been shown to enhance the proliferation, invasion and migration of OS cells via the metastasis-associated lung adenocarcinoma transcript 1/microRNA (miR)-143/Neurensin-2/Wnt/β-catenin signaling pathway, as well as through miR-655-mediated β-catenin signaling (46). Research indicates that TGF-β is significantly upregulated in patients with OS (47). Furthermore, OS cells enhance the secretion of extracellular vesicles that contain TGF-β. This process subsequently stimulates the release of IL-6, which is activated by signal transducer and activator of transcription 3 (STAT3) from MSCs, thereby facilitating lung metastasis (48). Additionally, single-cell RNA sequencing has uncovered a considerable heterogeneity among MSCs associated with OS (18).

MSCs facilitate the proliferation and metastasis of OS cells through two primary non-immune mechanisms. First, the interaction between OS cells and MSCs is mediated by IL-8 and aquaporin 1. Second, aberrant gene expression, including retinoblastoma, c-Myc, TP53, KRas and Indian Hedgehog, contributes to the transformation of MSCs into OS cells (49). Furthermore, studies have demonstrated that when MSCs are located within the microenvironment of OS cells, they have the capacity to differentiate into CAFs (50,51). This differentiation notably contributes to the increased proliferation, migration and invasion of OS cells. Under the influence of MSCs, OS cells are capable of inducing the migration and invasion of endothelial cells, which in turn promotes angiogenesis (52). From an immunological perspective, MSCs secrete anti-inflammatory factors and inhibit pro-inflammatory substances, thereby assisting OS cells in evading immune surveillance, particularly through autocrine or paracrine exosomal mechanisms. Lagerwei *et al* (53) demonstrated that MSCs suppress T cell proliferation and immune responses by releasing exosomal vesicles (EVs) containing miRNA/RNA and proteins. Additionally, Zhang *et al* (54) reported that MSC-derived EVs express TGF-β and TGF-β1. Moreover, MSC-derived exosomes can enhance OS tumorigenesis and metastasis through the induction of autophagy, as supported by evidence from other studies (55,56).

Circulating tumor cells (CTCs). OS cells are present not only within the tumor tissue but also in the bloodstream, where they are identified as CTCs (57). CTCs demonstrate the capacity to circumvent localized therapeutic approaches, including surgical resection and radiation therapy, and can remain present in small numbers during systemic treatments. This persistence ultimately facilitates the metastasis and recurrence of OS (57,58). Current research suggests that CTCs play a unique role within the immune microenvironment associated with OS. For instance, Zhang *et al* (59) demonstrated that the inhibition of IL-6 can suppress the proliferation of OS cells and decrease the presence of CTCs. *In vitro* studies revealed that IL-6 activates the Janus kinase (JAK)/STAT3 and mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathways. While both pathways facilitate the proliferation of OS cells, only the JAK/STAT3 pathway is implicated in promoting cell migration (59-61). A clinical study has shown that the level of IL-6 in OS samples is significantly upregulated compared with normal bone tissue, indicating that IL-6 plays an important role in the progression of OS (62). Furthermore, MSCs have been shown to enhance OS proliferation and metastasis through the secretion of IL-6 (63,64). Liu *et al* (65) further identified that IL-8 also contributes to the progression of OS. Their research involved isolating and culturing CTCs from patients, revealing that IL-8 promotes tumor growth and lung metastasis in both *in vitro* and *in vivo* models, indicating that targeting IL-8 may yield antitumor effects. Although the precise mechanisms governing the interaction between CTCs and tumor immunity remain unclear, CTCs may represent promising targets for therapeutic intervention and serve as biomarkers for predicting clinical outcomes.

Exosomes. Exosomes are a key subtype of EVs. EVs are membrane-bound structures that are released into the ECM following the fusion of intracellular multivesicular bodies with the cell membrane. These vesicles encapsulate a diverse array of biomolecules, including long non-coding RNAs, miRNAs, proteins, lipids and metabolites. Notably, cancer cells tend to produce a greater quantity of exosomes compared with normal cells, and these exosomes are implicated in various aspects of tumor biology, including tumor initiation, progression, immune evasion and drug resistance (66). Evidence suggests that EVs play a critical role in the onset, advancement and metastasis of OS (15). Specifically, exosomes can facilitate tumor growth by influencing endothelial cells to enhance angiogenesis, while also mediating intercellular interactions and participating in the regulation of cellular functions and the transmission of biological information, which in turn affects the expression and secretion of specific cytokines (67,68). Furthermore, exosomes can modify signaling pathways in recipient cells, thereby promoting the metastasis of cancer cells (69). Concurrently, exosomes can activate multiple signaling pathways that lead to the development of drug resistance in previously drug-sensitive cells or assist cancer cells in expelling cytotoxic agents (70). Research has also indicated that exosomes play a role in modulating the immune response, aiding tumor cells in evading immune surveillance and facilitating cancer metastasis. For instance, exosomes have been shown to promote lung metastasis in OS by releasing PD-L1 (9). Recent studies have advanced our understanding of the role of exosomal miRNAs in OS (71,72). For instance, in the context of OS, it has been demonstrated that exosomal miRNAs, such as miR-148a-3p, can stimulate the release of pro-angiogenic factors and induce angiogenesis by influencing the activity of osteoclasts and endothelial cells (73). Additionally, exosomes can enhance the invasiveness of OS through immune modulation; the surface of exosomes is adorned with tumor-associated antigens that interact with antigen-presenting cells, thereby inducing tumor-specific cytotoxic T cell immune responses (74). Moreover, it has been observed that exosomes secreted by metastatic OS cells can induce M2 polarization via TGF-β2, further promoting tumor invasion and metastasis (75). In a study, Shimbo *et al* (76) encapsulated synthetic therapeutic miRNA-143 within exosomes and administered it into the OS microenvironment, resulting in a significant reduction in OS cell migration. The investigation of exosomes has emerged as a prominent area of research and ongoing advancements in this field have the potential to yield novel breakthroughs in the clinical management of OS.

ECM. The ECM is a complex, mesh-like structure comprised of collagen, proteoglycans, glycoproteins and glycosaminoglycans, including hyaluronic acid. This matrix not only supplies essential nutrients and structural support to tumor cells but also plays a critical role in the construction and remodeling of the ECM, thereby influencing the physical and chemical characteristics of the TME (77). Cytokines and the ECM serve as pivotal mediators within this microenvironment, capable of modulating various biological behaviors of tumor cells, including proliferation, apoptosis, invasion and metastasis (78). OS is known to produce a large amount of ECM, which significantly impacts tumor invasiveness and the response to therapeutic interventions (66). Specific ECM components, such as collagen, fibronectin and laminin, are implicated in aberrant signaling pathways and structural irregularities that facilitate sarcoma growth and metastasis through diverse mechanisms, including integrin-mediated signaling activation, the promotion of EMT and the enhancement of cell migration and invasion (79). Furthermore, the ECM has the capacity to regulate the IGF axis, which is instrumental in modulating OS growth and conferring resistance to conventional chemotherapy agents (66). Targeting the ECM in the treatment of OS presents promising potential; for instance, ECM-like hydrogels can be utilized for the delivery of therapeutic agents, thereby offering a novel platform for OS treatment and bone regeneration (80). Additionally, ECM-associated factors, such as neurotrophic EGF-like molecule 1, have emerged as promising candidates for novel therapeutic strategies aimed at impeding OS progression (20). Evidence suggests that the invasiveness of OS can be mitigated by targeting the underlying mechanisms of ECM degradation and angiogenesis (14,81). Therapeutic strategies include suppressing key enzymes such as matrix metalloproteinases (MMPs; including MMP-2 and MMP-9) to prevent ECM breakdown and employing monoclonal antibodies or small-molecule inhibitors against pro-angiogenic factors such as VEGF to block new blood vessel formation (14,81). The interplay between the ECM and CAFs is also known to foster the development of OS (82). This cooperative relationship drives disease progression by creating

a stiffened, pro-fibrotic microenvironment that supports tumor cell survival, proliferation and invasion. The critical nature of this interplay is evidenced by studies showing that therapeutic strategies designed to destroy CAFs and disrupt the ECM can effectively suppress OS tumor growth (82). Moreover, ECM components, including EVs, hold potential as biomarkers for the diagnosis and prognosis of OS (83). Engineered AttIL 12-T cells, which are tumor-specific T cells with IL-12 anchored to their cell membrane, have demonstrated enhanced efficacy by targeting CAFs within the ECM, disrupting the tumor stroma and promoting T cell infiltration, indicating promising avenues for future research (82). This engineering approach involves uniformly tethering the IL-12 cytokine onto the surface of the adoptively transferred T cells, which allows for dose-controlled and localized delivery of IL-12 directly to the tumor site (82). In conclusion, the significant role of the ECM in OS is increasingly becoming a focal point for therapeutic strategies.

3. Vascular microenvironment

Tumor-associated blood vessels constitute a critical element of the TME. The establishment of a robust vascular network is critical for the growth and metastasis of tumors, as it provides essential oxygen and nutrients while also facilitating the spread of cancer cells. The presence of environmental stressors, such as hypoxia and acidosis, disrupts the balance between pro-angiogenic and anti-angiogenic factors, resulting in an increased expression of pro-angiogenic elements, including hypoxia-inducible factors (HIF) and VEGF, which together enhance tumor angiogenesis (84). Although the specific mechanisms governing neovascularization in OS are not yet fully understood, it is noteworthy that OS typically develops near the epiphyseal regions of long bones, where H-type endothelial cells known to promote angiogenesis are abundant (85). This suggests a potential involvement of these cells in the neovascularization process associated with OS. VEGF is crucial in modulating the growth, differentiation and development of new blood vessels within endothelial cells, which in turn has a notable impact on tumor proliferation and migration (86). By enhancing tumor angiogenesis, VEGF guarantees the supply of vital oxygen and nutrients to tumor cells, thereby supporting the processes of tumor initiation and progression (86,87). Notably, VEGF expression, particularly its isoform VEGF-A, is correlated with advanced tumor stages and metastatic potential (88). Compared with normal bone tissue, the expression of its receptor, VEGFR-2, is markedly elevated in OS, with high levels of VEGFR-2 expression associated with unfavorable prognostic outcomes (89). Gene amplification of VEGF, especially VEGF-A, has been documented in patients with OS and corroborated at the protein level. Empirical research has established a positive association between increased expression of VEGF and both tumor stage and the occurrence of metastasis (90). Consequently, a significant increase in vascular density may serve as a biomarker distinguishing primary OS tumors in patients with metastasis from those with non-metastatic disease. Furthermore, tumor-derived exosomes facilitate intercellular communication and angiogenesis by transporting pro-angiogenic factors and angiogenesis-related miRNAs (91). Tumor vascular endothelial cells have consistently been recognized as a vital target for anticancer therapies. Therefore, the application of anti-angiogenic agents, particularly those directed against VEGF, has the potential to selectively inhibit neovascularization and enhance progression-free survival in patients with cancer (92). Bevacizumab, an antibody targeting VEGF, has demonstrated notable efficacy in clinical settings when administered in conjunction with chemotherapy agents or immune checkpoint inhibitors (93,94). Research indicates that the VEGFR-2 tyrosine kinase inhibitor Apatinib can attenuate the activity of the Y chromosome sex-determining region box transcription factor-2 via the STAT3 signaling pathway, thereby mitigating doxorubicin-induced chemoresistance in OS (95). However, prolonged anti-angiogenic treatment may induce tumor hypoxia and promote invasive behavior, potentially leading to therapeutic resistance (96).

4. Hypoxia and the TME of OS

The rapid expansion of tumors beyond the oxygen supply capacity of surrounding blood vessels results in localized hypoxia, which is characterized by diminished oxygen levels and elevated lactate concentrations within the TME (97). This hypoxic and acidic milieu fosters the expression of angiogenic factors, enabling invasive OS cells to utilize vascular mimicry for the formation of angiogenic microchannels (98). Hypoxia triggers a range of cellular mechanisms, primarily governed by the transcription factor HIF-1α. Under normoxic conditions, HIF-1α undergoes rapid degradation; however, in hypoxic environments, it remains active, facilitating processes such as tumor growth, invasiveness, angiogenesis, metastasis and resistance to therapy (99,100). Empirical studies have demonstrated that in rapidly proliferating tumor tissues, HIF-1 facilitates a metabolic shift in hypoxic tumor cells from the more efficient oxidative phosphorylation to the less efficient glycolytic pathway, thereby sustaining energy production (101). This metabolic adaptation, referred to as the Warburg effect, leads to an increased production of lactate, which modifies the TME and promotes the proliferation, invasion and migration of tumor cells (102).

Research has indicated a direct correlation between lactate levels in tumors and the incidence of distant metastasis, suggesting that lactate accumulation may serve as a predictive biomarker for tumor metastasis and patient survival rates (103). Beyond being a mere metabolic byproduct, lactate functions as a signaling molecule. Zhang *et al* (104) identified lactylation as a significant epigenetic modification capable of regulating the transcription of numerous oncogenes and tumor suppressor genes, thereby revealing a universal mechanism of metabolic regulation that broadly influences tumor progression. Additionally, Lee *et al* (105) discovered a hypoxia-regulated protein, N-myc downstream regulated gene-3 (NDRG-3), which operates independently of HIF-1α. In the TME, reduced oxygen availability and hypoxia-induced glycolysis, which results in lactate production, can enhance the expression of NDRG-3 protein through the activation of the Raf/ERK signaling pathway. This process subsequently facilitates tumor angiogenesis and cellular proliferation.

Subsequent research has demonstrated that hypoxic conditions can expedite tumor progression by influencing the immune response, cytokine production, growth factors

and ILs, thereby promoting tumor immune evasion (106). Specifically, in hypoxic environments, immune responses are diminished due to a decrease in the infiltration and functionality of $CD8^+$ T cells, as well as the impaired maturation and activity of DCs and NK cells. Additionally, there is an induction of M2 polarization in TAM, along with an increase in the activity of Tregs and MDSCs (107). Furthermore, lactic acid plays a role in tumor immune resistance by facilitating M2 polarization, enhancing the activity of $CD8^+$ T cells and elevating PD-1 expression in Tregs (108,109).

He *et al* (110) demonstrated the inhibition of OS metastasis through the application of nanomaterials designed to deliver oxidants that degrade β-catenin within the HIF-1α/Bcl-2/adenovirus E1B 19-kDa interacting protein 3/LC3B-mediated mitochondrial autophagy pathway. Zheng *et al* (111) found that hypoxia significantly influences metabolic reprogramming; their research indicated that the inhibition of long non-coding RNA DLGAP 1-AS 2 can suppress aerobic glycolysis via the miR-451a/hexokinase 2 axis, thereby impeding OS progression. In conclusion, the hypoxic microenvironment in OS is garnering increasing attention for its pivotal role in orchestrating tumor progression, metastasis, immune regulation, metabolic alterations and therapeutic responses. Comprehensive research into its complexities presents promising opportunities for the development of innovative treatment strategies for OS.

5. Translational challenges and future directions in OS TME research

Despite extensive research regarding TME heterogeneity, including insights gained from animal models (112) and findings from *in vitro* experiments (113,114), effectively translating these findings into clinical practice remains challenging. First, studies have shown that the TME of OS exhibits high heterogeneity, with significant variations observed across patients, subtypes and even different regions within the same tumor, rendering a one-size-fits-all targeted strategy impractical (21,112). This heterogeneity extends to the dynamic nature of the TME, demanding therapeutic approaches capable of adapting to evolving tumor characteristics over time (21). Furthermore, there is a current lack of preclinical models that accurately mimic the human OS TME (112). Traditional animal xenograft models, along with cell line cultures, often fail to reproduce the full complexity of human-specific cell-cell interactions and the immune microenvironment, limiting the translational potential of laboratory discoveries (115,116).

Moreover, therapies targeting the TME may induce unintended systemic toxicity or immune-related adverse events. This necessitates particularly cautious evaluation in adolescent and pediatric patients, as this predominant OS patient population is especially vulnerable due to their ongoing physical development, which can affect organ function, drug metabolism and the long-term impact of treatments on growth and fertility (117). Existing clinical trials primarily focus on single-target therapies, overlooking the synergistic effects of multi-signaling networks within the TME, which may limit therapeutic efficacy. The potential for developing resistance to targeted therapies poses another significant challenge, as cancer cells can adapt and find alternative pathways for survival. Furthermore, the complexity of TME interactions means targeting one component may have unintended effects on others, potentially leading to treatment resistance or other adverse reactions (21). Developing reliable biomarkers to monitor the TME and predict treatment response remains an active research area. Identifying these biomarkers is crucial for achieving more personalized and effective treatment regimens.

In summary, while the TME presents a promising target for OS therapy, addressing its heterogeneity, developing accurate preclinical models, managing potential toxicity and overcoming resistance mechanisms are key challenges that must be overcome to successfully translate these insights into clinical practice. Future efforts must prioritize multi-omics integration analyses, develop humanized organoids or patient-derived xenograft models and advance personalized therapeutic strategies targeting both the TME and tumor cells simultaneously. Only through such a comprehensive approach can true lab-to-bedside translation be achieved.

In conclusion, the TME represents a highly intricate network system characterized by the coexistence and interaction of various cellular and non-cellular components, which are integral to the proliferation, invasion and metastasis processes in OS. Within the TME, diverse cell types, including TAMs, MSCs and immunosuppressive T cells, contribute to the invasive progression of OS through the secretion of numerous cytokines and the activation of associated signaling pathways. Concurrently, the high metabolic activity, extensive vascular network and hypoxic conditions of the tumor further intensify its invasiveness and metastatic capabilities. At present, the treatment of OS has encountered significant challenges, primarily the limited improvement in survival outcomes for patients with metastatic or recurrent disease, the high degree of inter- and intra-tumoral heterogeneity that complicates targeted therapy and the plateaued efficacy of conventional chemotherapy regimens (118). Future advancements are anticipated to involve the application of single-cell sequencing and spatial transcriptomics to conduct in-depth analyses of TME heterogeneity, thereby elucidating critical regulatory mechanisms (119,120). Moreover, the development of targeted therapies and immunotherapeutic strategies that specifically address the TME is a promising avenue for research (121,122). Additionally, the integration of artificial intelligence and multi-omics data analysis is expected to yield innovative approaches for the personalized treatment of OS (123). Through interdisciplinary collaboration, efforts to disrupt the malignant cycle perpetuated by the TME may offer new therapeutic prospects for patients with OS.

Acknowledgements

Not applicable.

Funding

This work was supported by a project of the Department of Education of Yunnan Province (grant no. 2025J0346) and an intramural project of the Second Affiliated Hospital of Kunming Medical University (grant no. 2022yk05).

Availability of data and materials

Not applicable.

Authors' contributions

TS, JK and QW designed the study. TS, JK and JS performed the literature search and analysis. TS and JK wrote the draft; TS and JS prepared the figure. QW, JS and XH critically revised the manuscript. XH conceived the intellectual framework. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhao X, Wu Q, Gong X, Liu J and Ma Y: Osteosarcoma: A review of current and future therapeutic approaches. Biomed Eng Online 20: 24, 2021.
2. Moukengue B, Lallier M, Marchandet L, Baud'huin M, Verrecchia F, Ory B and Lamoureux F: Origin and therapies of osteosarcoma. Cancers (Basel) 14: 3503, 2022.
3. Tsagozis P, Gonzalez-Molina J, Georgoudaki AM, Lehti K, Carlson J, Lundqvist A, Haglund F and Ehnman M: Sarcoma tumor microenvironment. Adv Exp Med Biol 1296: 319-348, 2020.
4. Wei L and Wang J: Tumor-associated macrophages promote pre-metastatic niche formation in ovarian cancer. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 40: 1138-1145, 2024 (In Chinese).
5. Fang J, Lu Y, Zheng J, Jiang X, Shen H, Shang X, Lu Y and Fu P: Exploring the crosstalk between endothelial cells, immune cells, and immune checkpoints in the tumor microenvironment: new insights and therapeutic implications. Cell Death Dis 14: 586, 2023.
6. Devaraji M and Varghese Cheriyan B: Immune-based cancer therapies: Mechanistic insights, clinical progress, and future directions. J Egypt Natl Canc Inst 37: 62, 2025.
7. Malone MK, Smrekar K, Park S, Blakely B, Walter A, Nasta N, Park J, Considine M, Danilova LV, Pandey NB, *et al*: Cytokines secreted by stromal cells in TNBC microenvironment as potential targets for cancer therapy. Cancer Biol Ther 21: 560-569, 2020.
8. Zeng J, Peng Y, Wang D, Ayesha K and Chen S: The interaction between osteosarcoma and other cells in the bone microenvironment: From mechanism to clinical applications. Front Cell Dev Biol 11: 1123065, 2023.
9. Chen F, Liu J, Yang T, Sun J, He X, Fu X, Qiao S, An J and Yang J: Analysis of intercellular communication in the osteosarcoma microenvironment based on single cell sequencing data. J Bone Oncol 41: 100493, 2023.
10. Amarasekara DS, Kim S and Rho J: Regulation of osteoblast differentiation by cytokine networks. Int J Mol Sci 22: 2851, 2021.
11. Chen X, Wang Z, Duan N, Zhu G, Schwarz EM and Xie C: Osteoblast-osteoclast interactions. Connect Tissue Res 59: 99-107, 2018.
12. Anloague A and Delgado-Calle J: Osteocytes: New kids on the block for cancer in bone therapy. Cancers(Basel) 15: 2645, 2023.
13. Behzatoglu K: Osteoclasts in tumor biology: Metastasis and epithelial-mesenchymal-myeloid transition. Pathol Oncol Res 27: 609472, 2021.
14. Yang Q, Liu J, Wu B, Wang X, Jiang Y and Zhu D: Role of extracellular vesicles in osteosarcoma. Int J Med Sci 19: 1216-1226, 2022.
15. Jerez S, Araya H, Hevia D, Irarrázaval CE, Thaler R, van Wijnen AJ and Galindo M: Extracellular vesicles from osteosarcoma cell lines contain miRNAs associated with cell adhesion and apoptosis. Gene 710: 246-257, 2019.
16. Feng X and Teitelbaum SL: Osteoclasts: New insights. Bone Res 1: 11-26, 2013.
17. Zhang G, Jian A, Zhang Y and Zhang X: A New signature of sarcoma based on the tumor microenvironment benefits prognostic prediction. Int J Mol Sci 24: 2961, 2023.
18. Zhou Y, Yang D, Yang Q, Lv X, Huang W, Zhou Z, Wang Y, Zhang Z, Yuan T, Ding X, *et al*: Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma. Nat Commun 11: 6322, 2020.
19. Cersosimo F, Lonardi S, Bernardini G, Telfer B, Mandelli GE, Santucci A, Vermi W and Giurisato E: Tumor-associated macrophages in osteosarcoma: From mechanisms to therapy. Int J Mol Sci 21: 5207, 2020.
20. Qin Q, Gomez-Salazar M, Tower RJ, Chang L, Morris CD, McCarthy EF, Ting K, Zhang X and James AW: NELL1 regulates the matrisome to promote osteosarcoma progression. Cancer Res 82: 2734-2747, 2022.
21. Zhu T, Han J, Yang L, Cai Z, Sun W, Hua Y and Xu J: Immune microenvironment in osteosarcoma: Components, therapeutic strategies and clinical applications. Front Immunol 13: 907550, 2022.
22. Xu J, Ding L, Mei J, Hu Y, Kong X, Dai S, Bu T, Xiao Q and Ding K: Dual roles and therapeutic targeting of tumor-associated macrophages in tumor microenvironments. Signal Transduct Target Ther 10: 268, 2025.
23. Huang Q, Liang X, Ren T, Huang Y, Zhang H, Yu Y, Chen C, Wang W, Niu J, Lou J and Guo W: The role of tumor-associated macrophages in osteosarcoma progression-therapeutic implications. Cell Oncol (Dordr) 44: 525-539, 2021.
24. Hou C, Lu M, Lei Z, Dai S, Chen W, Du S, Jin Q, Zhou Z and Li H: HMGB1 positive feedback loop between cancer cells and tumor-associated macrophages promotes osteosarcoma migration and invasion. Lab Invest 103: 100054, 2023.
25. Ligon JA, Choi W, Cojocaru G, Fu W, Hsiue EH, Oke TF, Siegel N, Fong MH, Ladle B, Pratilas CA, *et al*: Pathways of immune exclusion in metastatic osteosarcoma are associated with inferior patient outcomes. J Immunother Cancer 9: e001772, 2021.
26. Judge SJ, Darrow MA, Thorpe SW, Gingrich AA, O'Donnell EF, Bellini AR, Sturgill IR, Vick LV, Dunai C, Stoffel KM, *et al*: Analysis of tumor-infiltrating NK and T cells highlights IL-15 stimulation and TIGIT blockade as a combination immunotherapy strategy for soft tissue sarcomas. J Immunother Cancer 8: e001355, 2020.
27. Kleinberger M, Çifçi D, Paiato C, Tomasich E, Mair MJ, Steindl A, Spiró Z, Carrero ZI, Berchtold L, Hainfellner J, *et al*: Density and entropy of immune cells within the tumor microenvironment of primary tumors and matched brain metastases. Acta Neuropathol Commun 13: 34, 2025.
28. Lu X, Liu M, Yang J, Que Y and Zhang X: Panobinostat enhances NK cell cytotoxicity in soft tissue sarcoma. Clin Exp Immunol 209: 127-139, 2022.
29. Shigehara K, Kawai N, Shirosaki T, Ebihara Y, Murai A, Takaya A, Tokita S, Sasaki K, Shijubou N, Kubo T, *et al*: The size of CD8+ infiltrating T cells is a prognostic marker for esophageal squamous cell carcinoma. Sci Rep 15: 26638, 2025.
30. Chen L, Huang H, Huang Z, Chen J, Liu Y, Wu Y, Li A, Ge J, Fang Z, Xu B, *et al*: Prognostic values of tissue-resident $CD8^+$T cells in human hepatocellular carcinoma and intrahepatic cholangiocarcinoma. World J Surg Oncol 21: 124, 2023.
31. Noy R and Pollard JW: Tumor-associated macrophages: From mechanisms to therapy. Immunity 41: 49-61, 2014.
32. Kinker GS, Vitiello GAF, Ferreira WAS, Chaves AS, Cordeiro de Lima VC and Medina TDS: B cell orchestration of anti-tumor immune responses: A matter of cell localization and communication. Front Cell Dev Biol 9: 678127, 2021.
33. Cao Y, Kong L, Zhai Y, Hou W, Wang J, Liu Y, Wang C, Zhao W, Ji H and He P: Comprehensive analysis of TRIM56's prognostic value and immune infiltration in pan-cancer. Sci Rep 15: 13673, 2025.
34. Lo Nigro C, Macagno M, Sangiolo D, Bertolaccini L, Aglietta M and Merlano MC: NK-mediated antibody-dependent cell-mediated cytotoxicity in solid tumors: Biological evidence and clinical perspectives. Ann Transl Med 7: 105, 2019.

35. Capuano C, Pighi C, Battella S, De Federicis D, Galandrini R and Palmieri G: Harnessing CD16-mediated NK cell functions to enhance therapeutic efficacy of tumor-targeting mAbs. Cancers (Basel) 13: 2500, 2021.
36. Rodríguez-Nava C, Ortuño-Pineda C, Illades-Aguiar B, Flores-Alfaro E, Leyva-Vázquez MA, Parra-Rojas I, Del Moral-Hernández O, Vences-Velázquez A, Cortés-Sarabia K and Alarcón-Romero LDC: Mechanisms of action and limitations of monoclonal antibodies and single chain fragment variable (scFv) in the treatment of cancer. Biomedicines 11: 1610, 2023.
37. Khodayari H, Khodayari S, Ebrahimi E, Hadjilooei F, Vesovic M, Mahmoodzadeh H, Saric T, Stücker W, Van Gool S, Hescheler J and Nayernia K: Stem cells-derived natural killer cells for cancer immunotherapy: Current protocols, feasibility, and benefits of ex vivo generated natural killer cells in treatment of advanced solid tumors. Cancer Immunol Immunother 70: 3369-3395, 2021.
38. Shin E, Bak SH, Park T, Kim JW, Yoon SR, Jung H and Noh JY: Understanding NK cell biology for harnessing NK cell therapies: Targeting cancer and beyond. Front Immunol 14: 1192907, 2023.
39. Prager I and Watzl C: Mechanisms of natural killer cell-mediated cellular cytotoxicity. J Leukoc Biol 105: 1319-29, 2019.
40. Zhang ML, Chen L, Li YJ and Kong DL: PD-L1/PD-1 axis serves an important role in natural killer cell-induced cytotoxicity in osteosarcoma. Oncol Rep 42: 2049-2056, 2019.
41. Yang H, Zhao L, Zhang Y and Li FF: A comprehensive analysis of immune infiltration in the tumor microenvironment of osteosarcoma. Cancer Med 10: 5696-5711, 2021.
42. Lazarova M and Steinle A: Impairment of NKG2D-mediated tumor immunity by TGF-β. Front Immunol 10: 2689, 2019.
43. Chang X, Ma Z, Zhu G, Lu Y and Yang J: New perspective into mesenchymal stem cells: Molecular mechanisms regulating osteosarcoma. J Bone Oncol 29: 100372, 2021.
44. Antoon R, Overdevest N, Saleh AH and Keating A: Mesenchymal stromal cells as cancer promoters. Oncogene 43: 3545-3555, 2024.
45. Zheng Y, Wang G, Chen R, Hua Y and Cai Z: Mesenchymal stem cells in the osteosarcoma microenvironment: Their biological properties, influence on tumor growth, and therapeutic implications. Stem Cell Res Ther 9: 22, 2018.
46. Zhu G, Xia Y, Zhao Z, Li A, Li H and Xiao T: LncRNA XIST from the bone marrow mesenchymal stem cell derived exosome promotes osteosarcoma growth and metastasis through miR-655/ACLY signal. Cancer Cell Int 22: 330, 2022.
47. Chen C, Xie L, Ren T, Huang Y, Xu J and Guo W: Immunotherapy for osteosarcoma: Fundamental mechanism, rationale, and recent breakthroughs. Cancer Lett 500: 1-10, 2021.
48. Tu B, Du L, Fan QM, Tang Z and Tang TT: STAT3 activation by IL-6 from mesenchymal stem cells promotes the proliferation and metastasis of osteosarcoma. Cancer Lett 325: 80-88, 2012.
49. Deng Q, Li P, Che M, Liu J, Biswas S, Ma G, He L, Wei Z, Zhang Z, Yang Y, *et al*: Activation of hedgehog signaling in mesenchymal stem cells induces cartilage and bone tumor formation via Wnt/β-catenin. Elife 8: e50208, 2019.
50. Feng L, Chen Y and Jin W: Research progress on cancer-associated fibroblasts in osteosarcoma. Oncol Res 33: 1091-1103, 2025.
51. Duan W, Niu X, Liu Y and Tian W: Rab27b-mediated CAFs derived exosomal miR-22-3p suppresses ferroptosis and promotes cisplatin resistance in osteosarcoma. Cell Signal: Sep 17, 2025 (Epub ahead of print).
52. Lin L, Huang K, Guo W, Zhou C, Wang G and Zhao Q: Conditioned medium of the osteosarcoma cell line U2OS induces hBMSCs to exhibit characteristics of carcinoma-associated fibroblasts via activation of IL-6/STAT3 signalling. J Biochem 168: 265-271, 2020.
53. Lagerweij T, Pérez-Lanzón M and Baglio SR: A preclinical mouse model of osteosarcoma to define the extracellular vesicle-mediated communication between tumor and mesenchymal stem cells. J Vis Exp: 56932, 2018.
54. Zhang Q, Fu L, Liang Y, Guo Z, Wang L, Ma C and Wang H: Exosomes originating from MSCs stimulated with TGF-β and IFN-γ promote Treg differentiation. J Cell Physiol 233: 6832-6840, 2018.
55. Ruksha T and Palkina N: Role of exosomes in transforming growth factor-β-mediated cancer cell plasticity and drug resistance. Explor Target Antitumor Thery 6: 1002322, 2025.
56. Su Z, Fang X and Duan H: The paradoxical role of stem cells in osteosarcoma: From pathogenesis to therapeutic breakthroughs. Front Oncol 15: 1643491, 2025.
57. Yang C, Liu C, Xia C and Fu L: Clinical applications of circulating tumor cells in metastasis and therapy. J Hematol Oncol 18: 80, 2025.
58. Fu Y, Xu Y, Liu W, Zhang J, Wang F, Jian Q, Huang G, Zou C, Xie X, Kim AH, *et al*: Tumor-informed deep sequencing of ctDNA detects minimal residual disease and predicts relapse in osteosarcoma. EClinicalMedicine 73: 102697, 2024.
59. Zhang Y, Ma Q, Liu T, Guan G, Zhang K, Chen J, Jia N, Yan S, Chen G, Liu S, *et al*: Interleukin-6 suppression reduces tumour self-seeding by circulating tumour cells in a human osteosarcoma nude mouse model. Oncotarget 7: 446-458, 2016.
60. Tian H, Cao J, Li B, Nice EC, Mao H, Zhang Y and Huang C: Managing the immune microenvironment of osteosarcoma: The outlook for osteosarcoma treatment. Bone Res 11: 11, 2023.
61. Thuya WL, Cao Y, Ho PC, Wong AL, Wang L, Zhou J, Nicot C and Goh BC: Insights into IL-6/JAK/STAT3 signaling in the tumor microenvironment: Implications for cancer therapy. Cytokine Growth Factor Rev 85: 26-42, 2025.
62. Dou B, Chen T, Chu Q, Zhang G and Meng Z: The roles of metastasis-related proteins in the development of giant cell tumor of bone, osteosarcoma and Ewing's sarcoma. Technol Health Care 29: 91-101, 2021.
63. Paino F, La Noce M, Di Nucci D, Nicoletti GF, Salzillo R, De Rosa A, Ferraro GA, Papaccio G, Desiderio V and Tirino V: Human adipose stem cell differentiation is highly affected by cancer cells both in vitro and in vivo: Implication for autologous fat grafting. Cell Death Dis 8: e2568, 2017.
64. Perrot P, Rousseau J, Bouffaut AL, Rédini F, Cassagnau E, Deschaseaux F, Heymann MF, Heymann D, Duteille F, Trichet V and Gouin F: Safety concern between autologous fat graft, mesenchymal stem cell and osteosarcoma recurrence. PLoS One 5: e10999, 2010.
65. Liu T, Ma Q, Zhang Y, Wang X, Xu K, Yan K, Dong W, Fan Q, Zhang Y and Qiu X: Self-seeding circulating tumor cells promote the proliferation and metastasis of human osteosarcoma by upregulating interleukin-8. Cell Death Dis 10: 575, 2019.
66. Tzanakakis GN, Giatagana EM, Berdiaki A, Spyridaki I, Hida K, Neagu M, Tsatsakis AM and Nikitovic D: The role of IGF/IGF-IR-signaling and extracellular matrix effectors in bone sarcoma pathogenesis. Cancers (Basel) 13: 2478, 2021.
67. Duan SL, Fu WJ, Jiang YK, Peng LS, Ousmane D, Zhang ZJ and Wang JP: Emerging role of exosome-derived non-coding RNAs in tumor-associated angiogenesis of tumor microenvironment. Front Mol Biosci 10: 1220193, 2023.
68. Li Y, Jiang D, Zhang ZX, Zhang JJ, He HY, Liu JL, Wang T, Yang XX, Liu BD, Yang LL, *et al*: Colorectal cancer cell-secreted exosomal miRNA N-72 promotes tumor angiogenesis by targeting CLDN18. Am J Cancer Res 13: 3482-3499, 2023.
69. Ning XY, Ma JH, He W and Ma JT: Role of exosomes in metastasis and therapeutic resistance in esophageal cancer. World J Gastroenterol 29: 5699-5715, 2023.
70. Pan W, Miao Q, Yin W, Li X, Ye W, Zhang D, Deng L, Zhang J and Chen M: The role and clinical applications of exosomes in cancer drug resistance. Cancer Drug Resist 7: 43, 2024.
71. Tian W, Niu X, Feng F, Wang X, Wang J, Yao W and Zhang P: The promising roles of exosomal microRNAs in osteosarcoma: A new insight into the clinical therapy. Biomed Pharmacother 163: 114771, 2023.
72. Chen CC and Benavente CA: Exploring the impact of exosomal cargos on osteosarcoma progression: Insights into therapeutic potential. Int J Mol Sci 25: 568, 2024.
73. Li X, Rong K, Huang Y, Zhao Z, Xu C, Lin L, Zhang Y, Yan Y, Huang W, Zhang Y, *et al*: Suppression of miR-148a-3p can promote bone healing by enhancing angiogenesis-osteogenesis coupling through the PI3K/Akt pathway. FASEB J 39: e70930, 2025.
74. Pu F, Chen F, Zhang Z, Liu J and Shao Z: Information transfer and biological significance of neoplastic exosomes in the tumor microenvironment of osteosarcoma. Onco Targets Ther 13: 8931-8940, 2020.
75. Wolf-Dennen K, Gordon N and Kleinerman ES: Exosomal communication by metastatic osteosarcoma cells modulates alveolar macrophages to an M2 tumor-promoting phenotype and inhibits tumoricidal functions. Oncoimmunology 9: 1747677, 2020.
76. Shimbo K, Miyaki S, Ishitobi H, Kato Y, Kubo T, Shimose S and Ochi M: Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. Biochem Biophys Res Commun 445: 381-387, 2014.
77. Hu Q, Zhu Y, Mei J, Liu Y and Zhou G: Extracellular matrix dynamics in tumor immunoregulation: From tumor microenvironment to immunotherapy. J Hematol Oncol 18: 65, 2025.

78. Pattabiram S, Gangadaran P, Dhayalan S, Chatterjee G, Reyaz D, Prakash K, Arun R, Rajendran RL, Ahn BC and Aruljothi KN: Decoding the tumor microenvironment: Insights and new targets from single-cell sequencing and spatial transcriptomics. Curr Issues Mol Biol 47: 730, 2025.
79. Cui J, Dean D, Hornicek FJ, Chen Z and Duan Z: The role of extracelluar matrix in osteosarcoma progression and metastasis. J Exp Clin Cancer Res 39: 178, 2020.
80. Tian H, Wu R, Feng N, Zhang J and Zuo J: Recent advances in hydrogels-based osteosarcoma therapy. Front Bioeng Biotechnol 10: 1042625, 2022.
81. Yu Y, Li K, Peng Y, Zhang Z, Pu F, Shao Z and Wu W: Tumor microenvironment in osteosarcoma: From cellular mechanism to clinical therapy. Genes Dis 12: 101569, 2025.
82. Hu J, Lazar AJ, Ingram D, Wang WL, Zhang W, Jia Z, Ragoonanan D, Wang J, Xia X, Mahadeo K, *et al*: Cell membrane-anchored and tumor-targeted IL-12 T-cell therapy destroys cancer-associated fibroblasts and disrupts extracellular matrix in heterogenous osteosarcoma xenograft models. J Immunother Cancer 12: e006991, 2024.
83. Loi G, Stucchi G, Scocozza F, Cansolino L, Cadamuro F, Delgrosso E, Riva F, Ferrari C, Russo L and Conti M: Characterization of a bioink combining extracellular matrix-like hydrogel with osteosarcoma cells: Preliminary results. Gels 9: 129, 2023.
84. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
85. Fan J, Xie Y, Liu D, Cui R, Zhang W, Shen M and Cao L: Crosstalk between H-type vascular endothelial cells and macrophages: A potential regulator of bone homeostasis. J Inflamm Res 18: 2743-2765, 2025.
86. Lee C, Kim MJ, Kumar A, Lee HW, Yang Y and Kim Y: Vascular endothelial growth factor signaling in health and disease: From molecular mechanisms to therapeutic perspectives. Signal Transduct Target Ther 10: 170, 2025.
87. Shah FH, Nam YS, Bang JY, Hwang IS, Kim DH, Ki M and Lee HW: Targeting vascular endothelial growth receptor-2 (VEGFR-2): Structural biology, functional insights, and therapeutic resistance. Arch Pharm Res 48: 404-425, 2025.
88. Corre I, Verrecchia F, Crenn V, Redini F and Trichet V: The osteosarcoma microenvironment: A complex but targetable ecosystem. Cells 9: 976, 2020.
89. Liu K, Ren T, Huang Y, Sun K, Bao X, Wang S, Zheng B and Guo W: Apatinib promotes autophagy and apoptosis through VEGFR2/STAT3/BCL-2 signaling in osteosarcoma. Cell Death Dis 8: e3015, 2017.
90. Lammli J, Fan M, Rosenthal HG, Patni M, Rinehart E, Vergara G, Ablah E, Wooley PH, Lucas G and Yang SY: Expression of vascular endothelial growth factor correlates with the advance of clinical osteosarcoma. Int Orthop 36: 2307-2313, 2012.
91. Li Y, Lin S, Xie X, Zhu H, Fan T and Wang S: Highly enriched exosomal lncRNA OIP5-AS1 regulates osteosarcoma tumor angiogenesis and autophagy through miR-153 and ATG5. Am J Transl Res 13: 4211-4223, 2021.
92. Assi T, Watson S, Samra B, Rassy E, Le Cesne A, Italiano A and Mir O: Targeting the VEGF pathway in osteosarcoma. Cells 10: 1240, 2021.
93. Wang C, Duan L, Zhao Y, Wang Y and Li Y: Efficacy and safety of bevacizumab combined with temozolomide in the treatment of glioma: A systematic review and meta-analysis of clinical trials. World Neurosurg 193: 447-460, 2025.
94. Han X, Qin S, Liu S and Li Z: Intracavitary perfusion with bevacizumab plus cisplatin versus cisplatin alone for malignant pleural effusion in lung cancer patients: A meta-analysis of randomized controlled trials. World J Surg Oncol 23: 278, 2025.
95. Lowery CD, Blosser W, Dowless M, Renschler M, Perez LV, Stephens J, Pytowski B, Wasserstrom H, Stancato LF and Falcon B: Anti-VEGFR2 therapy delays growth of preclinical pediatric tumor models and enhances anti-tumor activity of chemotherapy. Oncotarget 10: 5523-5533, 2019.
96. Haibe Y, Kreidieh M, El Hajj H, Khalifeh I, Mukherji D, Temraz S and Shamseddine A: Resistance mechanisms to anti-angiogenic therapies in cancer. Front Oncol 10: 221, 2020.
97. Zhou J, Lan F, Liu M, Wang F, Ning X, Yang H and Sun H: Hypoxia inducible factor-1α as a potential therapeutic target for osteosarcoma metastasis. Front Pharmacol 15: 1350187, 2024.
98. Ren K, Zhang J, Gu X, Wu S, Shi X, Ni Y, Chen Y, Lu J, Gao Z, Wang C and Yao N: Migration-inducing gene-7 independently predicts poor prognosis of human osteosarcoma and is associated with vasculogenic mimicry. Exp Cell Res 369: 80-89, 2018.
99. Zhang QJ, Liu CH, Wang K, Mao XY, Dong Y, Zang MY, Zhang W, Yu QS and Hao L: Hypoxia-induced HIF-1α/VASN promotes bladder cancer progression. Sci Rep 15: 21635, 2025.
100. Zeng X, Liu S, Yang H, Jia M, Liu W and Zhu W: Synergistic anti-tumour activity of ginsenoside Rg3 and doxorubicin on proliferation, metastasis and angiogenesis in osteosarcoma by modulating mTOR/HIF-1α/VEGF and EMT signalling pathways. J Pharm Pharmacol 75: 1405-1417, 2023.
101. Vander Heiden MG and DeBerardinis RJ: Understanding the intersections between metabolism and cancer biology. Cell 168: 657-669, 2017.
102. Huang Y, Fan H and Ti H: Tumor microenvironment reprogramming by nanomedicine to enhance the effect of tumor immunotherapy. Asian J Pharm Sci 19: 100902, 2024.
103. Zhu L, Lin Z, Wang K, Gu J, Chen X, Chen R, Wang L and Cheng X: A lactate metabolism-related signature predicting patient prognosis and immune microenvironment in ovarian cancer. Front Endocrinol (Lausanne) 15: 1372413, 2024.
104. Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M, *et al*: Metabolic regulation of gene expression by histone lactylation. Nature 574: 575-580, 2019.
105. Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, Lee KM, Kang M, Jang YJ, Yang SJ, Hong YK, *et al*: A lactate-induced response to hypoxia. Cell 161: 595-609, 2015.
106. Wu Q, You L, Nepovimova E, Heger Z, Wu W, Kuca K and Adam V: Hypoxia-inducible factors: Master regulators of hypoxic tumor immune escape. J Hematol Oncol 15: 77, 2022.
107. Mortezaee K and Majidpoor J: The impact of hypoxia on immune state in cancer. Life Sci 286: 120057, 2021.
108. Feng Q, Liu Z, Yu X, Huang T, Chen J, Wang J, Wilhelm J, Li S, Song J, Li W, *et al*: Lactate increases stemness of CD8 + T cells to augment anti-tumor immunity. Nat Commun 13: 4981, 2022.
109. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin YT, Togashi Y, Kamada T, Irie T, Okumura G, Kono H, *et al*: Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. Cancer Cell 40: 201-218. e9, 2022.
110. He G, Nie JJ, Liu X, Ding Z, Luo P, Liu Y, Zhang BW, Wang R, Liu X, Hai Y and Chen DF: Zinc oxide nanoparticles inhibit osteosarcoma metastasis by downregulating β-catenin via HIF-1α/BNIP3/LC3B-mediated mitophagy pathway. Bioact Mater 19: 690-702, 2023.
111. Zheng C, Li R, Zheng S, Fang H, Xu M and Zhong L: The knockdown of lncRNA DLGAP1-AS2 suppresses osteosarcoma progression by inhibiting aerobic glycolysis via the miR-451a/HK2 axis. Cancer Sci 114: 4747-4762, 2023.
112. Wang Z, Ma L, Xu J and Jiang C: Editorial: Genetic and cellular heterogeneity in tumors. Front Cell Dev Biol 12: 1519539, 2024.
113. Oh JM, Park Y, Lee J and Shen K: Microfabricated organ-specific models of tumor microenvironments. Annu Rev Biomed Eng 27: 307-333, 2025.
114. Yu Z, Gan Z, Wu W, Sun X, Cheng X, Chen C, Cao B, Sun Z and Tian J: Photothermal-triggered extracellular matrix clearance and dendritic cell maturation for enhanced osteosarcoma immunotherapy. ACS Appl Mater Interfaces 16: 67225-67234, 2024.
115. Rodrigues J, Sarmento B and Pereira CL: Osteosarcoma tumor microenvironment: The key for the successful development of biologically relevant 3D in vitro models. In Vitro Model 1: 5-27, 2022.
116. Liu X, Fang J, Huang S, Wu X, Xie X, Wang J, Liu F, Zhang M, Peng Z and Hu N: Tumor-on-a-chip: From bioinspired design to biomedical application. Microsyst Nanoeng 7: 50, 2021.
117. Spalato M and Italiano A: The safety of current pharmacotherapeutic strategies for osteosarcoma. Expert Opin Drug Saf 20: 427-438, 2021.
118. Lu J, Tang H, Chen L, Huang N, Hu G, Li C, Luo K, Li F, Liu S, Liao S, *et al*: Association of survivin positive circulating tumor cell levels with immune escape and prognosis of osteosarcoma. J Cancer Res Clin Oncol 149: 13741-13751, 2023.
119. Nasir I, McGuinness C, Poh AR, Ernst M, Darcy PK and Britt KL: Tumor macrophage functional heterogeneity can inform the development of novel cancer therapies. Trends Immunol 44: 971-985, 2023.
120. Sharma A, Raut SS, Shukla A, Gupta S, Mishra A and Singh A: From tissue architecture to clinical insights: Spatial transcriptomics in solid tumor studies. Semin Oncol 52: 152389, 2025.
121. Alnaqbi H, Becker LM, Mousa M, Alshamsi F, Azzam SK, Emini Veseli B, Hymel LA, Alhosani K, Alhusain M, Mazzone M, *et al*: Immunomodulation by endothelial cells: Prospects for cancer therapy. Trends Cancer 10: 1072-1091, 2024.

122. Li Y, Huang Z and Yin Y: Heterogeneity of tumor-associated macrophages in colorectal cancer: Origins, classification, and immunotherapeutic implications. Pathol Res Pract 272: 156082, 2025.
123. Xu C, Yang J, Xiong H, Cui X, Zhang Y, Gao M, He L, Fang Q, Han C, Liu W, *et al*: Machine learning and multi-omics analysis reveal key regulators of proneural-mesenchymal transition in glioblastoma. Sci Rep 15: 19731, 2025.