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REVIEW

Osteoclasts in Osteosarcoma: Mechanisms, Interactions, and Therapeutic Prospects

Yi-Ming Gao[1], Yan Pei[1], Fei-Fei Zhao[2], Ling Wang[1]

[1]Department of Orthopedic Oncology, The Third Hospital of Hebei Medical University, Shijiazhuang, Hebei, People's Republic of China; [2]Department of Orthopedics, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei, People's Republic of China

Correspondence: Ling Wang, Tel +86031188603682, Email wangling2016uw@126.com

Abstract: Osteosarcoma is an extremely malignant tumor, and its pathogenesis is complex and remains incompletely understood. Most cases of osteosarcoma are accompanied by symptoms of bone loss or result in pathological fractures due to weakened bones. Enhancing the survival rate of osteosarcoma patients has proven to be a long-standing challenge. Numerous studies mentioned in this paper, including in-vitro, in-vivo, and in-situ studies have consistently indicated a close association between the symptoms of bone loss associated with osteosarcoma and the presence of osteoclasts. As the sole cells capable of bone resorption, osteoclasts participate in a malignant cycle within the osteosarcoma microenvironment. These cells interact with osteoblasts and osteosarcoma cells, secreting various factors that further influence these cells, disrupting bone homeostasis, and shifting the balance toward bone resorption, thereby promoting the onset and progression of osteosarcoma. Moreover, the interaction between osteoclasts and various other cells types, such as tumor-associated macrophages, myeloid-derived suppressor cells, DCs cells, T cells, and tumor-associated fibroblasts in the osteosarcoma microenvironment plays a crucial role in disease progression. Consequently, understanding the role of osteoclasts in osteosarcoma has sparked significant interest. This review primarily examines the physiological characteristics and functional mechanisms of osteoclasts in osteosarcoma, and briefly discusses potential therapies targeting osteoclasts for osteosarcoma treatment. These studies provide fresh ideas and directions for future research on the treatment of osteosarcoma.

Keywords: osteosarcoma, osteoclasts, bone remodeling, osteoimmunology, bone microenvironment

Introduction

Osteosarcoma is an extremely rare form of cancer that primarily affects children and adolescents.[1] It is characterized by the development of bone tumors, and there is a high incidence in teenagers and a second peak in adults older than 65, which is often associated with chronic bone diseases such as Paget's disease.[2,3] The global occurrence of osteosarcoma is approximately 4.8 cases per million individuals.[2,4] The tumor typically emerges in areas of the body where bones are undergoing rapid growth, such as the distal femur, proximal tibia, and proximal humerus.[5,6] The causes of osteosarcoma are multifaceted. In children, the development of osteosarcoma is often associated with the rapid bone growth and genetic alterations, specifically those involving genes such as RB1, TP53, and REQL4, among others. The abnormal alterations in these genes in young patients can contribute to an increased risk of developing osteosarcoma.[7] Furthermore, the risk of developing osteosarcoma is higher in males than in females.[8] Additionally, radiation exposure can contribute to the development of osteosarcoma, and there is an increased risk observed in individuals who have received radiation therapy for other types of cancer.[9] According to the American Cancer Society's Cancer Statistics Center, approximately one out of every ten cases of osteosarcoma occurs in individuals older than 65. The 5-year survival rate for osteosarcoma is 68%.[10] One of the major challenges in treating this cancer is its tendency to metastasize. Cancer metastasis refers to the spread of cancer cells from the primary tumor to other parts of the body through the bloodstream or lymphatic system. If cancer cells enter the bloodstream, they can potentially spread to other parts of the body.[11–13] The process of metastasis in osteosarcoma is intricate, and the lung is the most commonly affected organ in this phenomenon.[1,14]

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Osteoclasts are the only cells in the human body that are responsible for bone resorption, and they originate from the fusion of monocyte precursors. These cells are characterized by their large size and can contain anywhere from 2 to 50 nuclei.[15] Their primary function is to maintain bone homeostasis and participate in bone remodeling, often by interacting with osteoblasts.[16] When the balance between bone resorption by osteoclasts and bone formation by osteoblasts shifts toward excessive resorption, bones may experience significant loss, leading to an increased risk of fractures.[17,18] This imbalance can occur in various conditions, including osteosarcomas, and often result in varying degrees of bone destruction. In osteosarcoma, the activity of osteoclasts is enhanced, leading to the binding of receptor activator of nuclear factor kappa-B ligand (RANKL) on osteoblasts to the receptor activator of nuclear factor kappa-B (RANK) expressed by osteoclast precursor cells. This interaction activates the NF-κB pathway and promotes osteoclast differentiation. Ultimately, the increase in osteoclast activity in osteosarcomas plays a crucial role in the process of bone destruction observed in these tumors.[19,20]

In this review, osteoclasts and the characteristics and functions of osteoclasts in osteosarcoma are described in detail. In addition, we discuss the interactions between osteoclasts and cancer-associated macrophages, osteoclasts and cancer-associated fibroblasts, and osteoclasts and myeloid-derived suppressor cells in the osteosarcoma microenvironment.

Overview of Osteoclasts

Origin of Osteoclasts

Osteoclasts are derived from the myeloid system and are primarily responsible for bone matrix absorption. The existence of these multinucleated cells near bone structures was first observed in the nineteenth century, and they were initially referred to as Myeloplaques, although their function was unknown. In 1872, Kolliker named these cells "Ostoklasts" and suggested that they played a crucial role in bone resorption. In 1873, Rindfleisch discovered multinucleated cells in bone resorption pits, which are now known as "Howship's lacunae", although it was not initially believed that these giant cells caused absorption. It took researchers a significant amount of time to establish the importance of osteoclasts in bone resorption. The origin of osteoclasts has been a subject of debate for centuries.[21] In 1949, there were three main theories regarding the precursor cells involved in fusion. The first suggested that osteoclasts originated from the fusion of sessile connective tissue, the second proposed that osteoclasts were composed of osteoblasts, and the third suggested that osteoclasts originated from monocytes and macrophages in the hematopoietic system. The similarities between osteoclasts and macrophages in terms of motility, membrane characteristics, and staining patterns provided support for the last argument. This viewpoint has been repeatedly validated by researchers, confirming that osteoclast precursor cells are mononuclear macrophages originating from the hematopoietic system.[22,23] In 1975, Walker conducted an experiment demonstrating that splenic and myeloid cells from wild-type mice produced cells that were capable of bone resorption, further supporting the hematopoietic origin of osteoclasts.[24] Recent studies have also shown that osteoclasts can arise from the competitive differentiation of bone marrow progenitor cells and from tumor-associated macrophages.[25] Tumor-associated macrophages can be classified as M1 and M2 cells, and M1 macrophages exert proinflammatory effects, while M2 macrophages are anti-inflammatory cells. M1 macrophages can secrete TNF-α, IL-6, and IL-1β, which can promote osteoclast formation. Macrophages exhibit high plasticity and perform diverse functions depending on their specific tissue environments. The traditional M1/M2 macrophage classification may not fully describe their roles in bone tissue. In bone homeostasis, M1 macrophages are generally associated with the early inflammatory phase, while M2 macrophages become more prominent during later remodeling and anti-inflammatory stages. M1 and M2 macrophage polarization critically regulates bone resorption by osteoclasts throughout the bone homeostasis process. Osteoclasts are also recognized as a resident macrophage subset involved in bone tissue regulation.[26] Overall, osteoclasts play a crucial role in bone resorption, and they originate from mononuclear macrophages in the hematopoietic system, as confirmed by various studies.[22,27]

Factors Regulating Osteoclast Differentiation

Multiple cytokines and signaling pathways are involved in the regulation of osteoclast formation and differentiation. One of the major pathways is the RANKL/RANK/OPG signaling pathway.[28] Osteoblasts produce RANKL, which stimulates

osteoclast formation and enhances their bone resorption capacity. RANK is the receptor for RANKL, and its activation promotes osteoclast differentiation, maturation, and lifespan.[29] OPG is a soluble protein that competitively inhibits the binding of RANKL and RANK, thus preventing osteoclast formation and increasing bone resorption, which is crucial for bone remodeling.[29] Both M-CSF (macrophage colony-stimulating factor) and RANKL are essential for osteoclast formation, highlighting the importance of the M-CSF signaling pathway in osteoclast differentiation. Osteoblasts produce IL-34, which binds to the M-CSF receptor (C-FMS) and stimulates osteoclast differentiation.[30] Recent research has uncovered the significance of the KDM4B-CCAR1-MED1 axis in osteoclast differentiation and bone homeostasis. RANK-RANKL signaling increases KDM4B protein levels through transcriptional and posttranslational regulation. KDM4B serves as a key regulator of osteoclast formation and is necessary for RANKL-induced osteoclast formation.[31] In addition to these signaling pathways and cytokines, there are numerous other factors that play crucial roles in osteoclast generation and differentiation. The mechanisms underlying the regulation of these signaling pathways and cytokines in osteoclasts are still being investigated.[32–35] Osteoclasts play a crucial role in many bone-related diseases, including fractures, bone tumors, osteoporosis, and osteoarthritis. Determining the specific mechanisms by which osteoclasts affect the progression of these bone-related diseases and identify key therapeutic targets will have profound impacts on these conditions.[36]

Bone Remodeling

In healthy bones, bone homeostasis is maintained through a balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation. Osteoclasts play a key role in bone resorption, which involves the degradation of bone matrix that may be damaged during aging or due to diseases. This process is facilitated by the secretion of cytokines by osteoclasts.[35] Furthermore, osteoclasts can influence osteoblast differentiation through the secretion of soluble factors.[37] Recent research has demonstrated that osteoclasts play a role in promoting blood vessel formation, which further regulates bone formation.[38] The fusion of tartrate-resistant acid phosphatase (TRAP)+ monocytes results in the formation of multinucleated osteoclasts. These TRAP+ monocytes, which are also known as osteoclast precursor cells, not only promote the generation of H-type blood vessels but also secrete more platelet-derived growth factor-BB (PDGF-BB) to stimulate bone formation[39] The interaction between osteoclasts and osteoblasts is referred to as bone remodeling, and it is a continual regenerative process in bones. Disruption of this process can lead to an imbalance between osteoclasts and osteoblasts, which can contribute to various bone diseases, including osteoporosis, rheumatoid arthritis, bone tumors, and osteopetrosis, among others. Overall, osteoclasts play a crucial role in maintaining bone homeostasis by mediating bone resorption, influencing osteoblast differentiation, and participating in the regulation of blood vessel formation. Disruptions in this delicate balance can result in a variety of bone-related disorders.[40–44]

Osteoclasts in Osteosarcoma

In bone neoplasms, which often present as lytic lesions with bone destruction, there is a disruption in the balance between bone resorption and bone formation. Patients with osteosarcoma commonly experience bone loss, and an increase in osteoclast activity appears to be the primary cause of this bone loss, as evidenced by higher TRAP activity in osteosarcoma patients than in healthy individuals.[45] Osteoclasts are involved in multiple stages of bone resorption, including attachment to bone, cytoskeletal reorganization, and the formation of the sealing zone.[46] Osteoclasts typically reside in the resorption zone of the bone surface, which is known as the Howship cavity or lacuna. In this region, TRAP, cathepsin K (CTSK), and matrix metalloproteinase 9 (MMP-9) are transported from the ruffled border to the Howship lacuna, leading to bone degradation.[47]

The interaction between osteoclasts, osteosarcoma cells, and osteoblasts contributes to the development of osteosarcoma and promotes a vicious cycle.[48] Osteosarcoma cells can produce parathyroid hormone-related protein (PTHrP), which activates osteoblasts. These activated osteoblasts, in turn, indirectly activate osteoclasts by producing receptor activator of nuclear factor kappa-B ligand (RANKL). Osteoblasts and osteosarcoma cells can also directly generate RANKL, which activates osteoclasts. Additionally, osteosarcoma cells can activate osteoblasts. This malignant cycle involving osteoclasts, osteosarcoma cells, and osteoblasts contributes to the progression of osteosarcoma (Figure 1).[49] Recent studies have revealed novel mechanisms of osteoclast formation in osteosarcoma patients. Osteosarcoma cell-

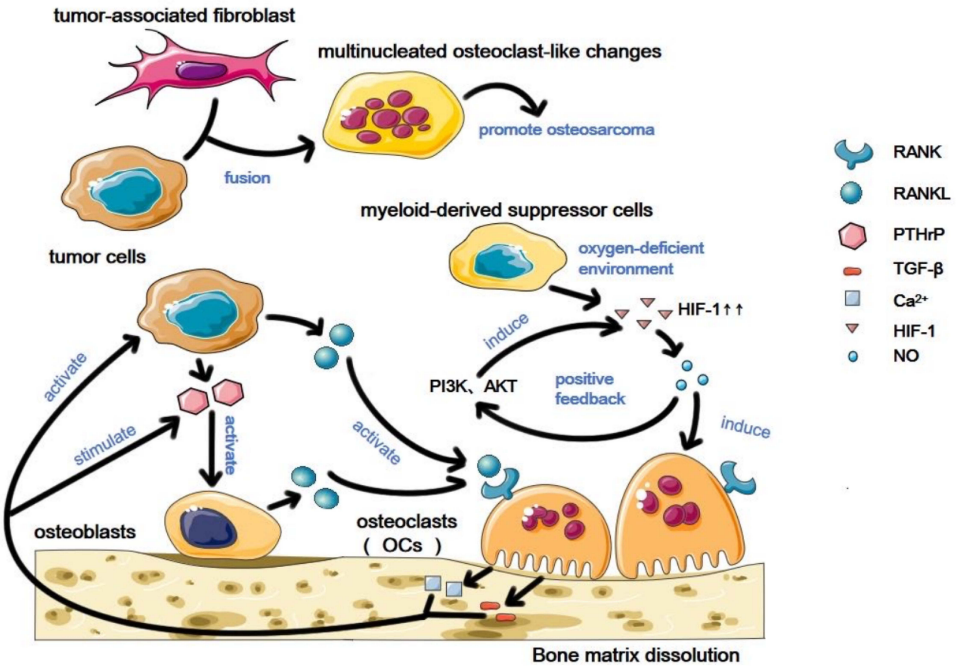

Figure 1 Osteoclasts in the osteosarcoma microenvironment. Osteosarcoma cells and osteoblasts in the tumor microenvironment affect osteoclasts through direct and indirect ways, leading to changes in osteoclasts. Osteoclasts produce related factors, which in turn regulate osteosarcoma cells and osteoblasts, and then activated or stimulated osteosarcoma cells and osteoblasts affect osteoclasts in a direct or indirect way, forming a vicious cycle. Osteosarcoma cells and tumor-associated fibroblasts melt in the osteosarcoma microenvironment and undergo multi-nucleated osteoclast-like changes. The myeloid-derived suppressor cells produce high levels of HIF-1 in hypoxia environment, thus producing NO-mediated osteoclast differentiation, and NO can also mediate HIF-1 increase through PI3K and AKT pathways, thus forming a positive feedback.

derived small extracellular vesicles (sEVs) transport exosomes to osteoclasts. These sEVs deliver miR-19a-3p, which promotes osteoclast differentiation and enhances osteoclast function through the PTEN/PI3K/Akt signaling pathway, resulting in bone destruction.[50] Another study demonstrated that exosomal miR-501-3p derived from osteosarcoma promoted osteoclast differentiation by modulating the PTEN/PI3K/Akt/NFATc1 signaling pathway. PTEN mediates the increase in the key transcription factor NFATc1 during osteoclast differentiation driven by exosomal miR-501-3p derived from osteosarcoma, exacerbating both intra- and extrabone loss. This research confirms the potential of osteosarcoma-derived exosomal miR-501-3p as a prognostic marker and therapeutic target that has the ability to predict the state of bone loss in patients.[51] Furthermore, osteoclasts not only contribute to bone loss in osteosarcoma but also influence tumor metastasis. Reduced osteoclast activity in osteosarcoma has been associated with increased lung metastasis and enhanced osteosarcoma cell migration. The absence of osteoclasts may cause osteosarcoma cells to migrate away from the bone, indicating that osteoclasts play a critical regulatory role in the progression and metastasis of osteosarcoma.[52] It is possible that the absence of osteoclasts can cause osteosarcoma cells to migrate away from the bone. This finding lends credence to the hypothesis that osteoclasts are a critical regulatory factors in the progression of osteosarcoma and play an important role in the metastasis of osteosarcoma.

Functional analysis of bone resorption and bone formation in osteosarcoma has provided insights into disease progression. Variation in osteoclast markers can partially reflect the advancement of osteosarcoma. TRAP is a specific marker of osteoclasts and serves as an excellent indicator of bone resorption and osteoclast activity.[53] Second, cancer metastasis has been linked to osteoclast-secreted cathepsin K (CTSK), which degrades collagen. Collagen is a major component of the nonmineralized bone protein matrix, and CTSK degradation has been linked to cancer metastasis.[54]

The factors regulating osteoclasts in osteosarcoma are complex and diverse. The MMP family is responsible for an important regulatory function in the osteosarcoma microenvironment.[55] MMP9 is expressed by osteoclasts and is an essential type of gelatinase associated with osteoclast migration. MMP9 deficiency causes defects in various skeletal functions, and multiple cytokines are good markers of osteoclasts. IL-6 and TNF-α are important regulators of osteoclasts

and play important roles in bone resorption. The secretion of TNF-α by osteosarcoma cells can regulate osteoclast differentiation and increase osteoclast activity. IL-6 also plays an important role in the differentiation and function of osteoclasts. IFN-γ is a cytokine that plays a significant role in bone metabolism.[56] IFN-γ affects osteoblasts and osteoclasts and is involved in osteosarcoma cells division and proliferation of osteosarcoma cells. In addition, there are certain microRNAs whose secretion affects osteoclasts and are involved in a variety of pathological processes, such as the formation of tumors, proliferation, invasion, and metastasis. In primary osteosarcoma, osteoclasts cause bone destruction and promote osteosarcoma development and metastasis.

Overall, osteoclasts play a significant role in osteosarcoma by contributing to bone loss, promoting tumor development, and influencing metastasis. Understanding the interaction between osteosarcoma cells and osteoclasts is crucial for developing more effective treatment approaches for osteosarcoma. Targeting osteoclasts holds promise as a therapeutic strategy, and further research in this area will provide valuable insights into osteosarcoma treatment.

Interactions Between Osteoclasts and Other Cells in the Osteosarcoma Microenvironment

Osteoclasts and Tumor-Associated Macrophages

Immune cells play a crucial role in the tumor microenvironment and exert immunosuppressive effects.[57] Macrophages are the predominant immune cells present in the osteosarcoma tumor microenvironment. Tumor-associated macrophages can be classified as M1 and M2 cells and exert opposing effects on inflammation and tumor development that are not yet fully understood. However, the activation of tumor-associated macrophages contributes to the progression of osteosarcoma, and M2 tumor-associated macrophages are continuously recruited during the development of human osteosarcoma.[58] The level of transforming growth factor in M2 macrophages has been shown to impact patient survival rates. Studies have shown that the Wnt signaling pathway influences macrophage activation in osteosarcoma.[59,60] Activation of the Wnt pathway reduces the concentration of RANKL, thereby inhibiting osteoclast formation. Understanding the polarization of macrophages and their role in osteosarcoma, as well as targeting tumor-associated macrophages, could have implications in osteosarcoma treatment. Other studies have also indicated that macrophages are involved in the fusion of osteoclasts. Siglec-15, a novel immunosuppressive molecule, inhibits the activity of T cells.[61] The tumor microenvironment contains macrophages associated with the tumor, and these tumor-associated macrophages, along with Siglec-15-expressing tumor cells, inhibit T-cell activation, thereby promoting tumor growth and metastasis. Knockdown of Siglec15 has been shown to inhibit osteoclast formation in macrophage lines.[62] Siglec15 can bind to secreted TLR2, which is a cell recognition signal, and initiate cell fusion prior to osteolysis, as discovered by Dou et al. This connection also influences tumor growth (Figure 2).[63]

Osteoclasts and Myeloid-Derived Suppressor Cells(MDSCs)

Myeloid-derived suppressor cells (MDSCs) are a diverse group of myeloid cells that are highly expressed in both human and mouse tumor models. These cells possess potent immunosuppressive functions and serve as crucial negative regulators of chronic inflammation and the tumor immune response.[64,65] Recent research has shown that elevated levels of MDSCs in the bone marrow are associated with pathological conditions such as inflammatory cancers. MDSCs can stimulate tumor development and metastasis.[66] Given their abundance and interactions with various cells in the bone microenvironment, these cells may also have an impact on bone-related diseases.[67] In 2013, Sawant et al discovered that MDSCs were progenitor cells of osteoclasts and that nitric oxide (NO) played a crucial role in the osteoclastic differentiation of MDSCs.[68] Therefore, MDSCs also play a significant role in regulating osteoclasts and promoting cancer-induced osteolysis. In the clinic, several drugs targeting MDSCs have been investigated for the treatment of osteosarcoma. For example, metformin has been shown to induce CD11b+ cell-mediated growth inhibition of osteosarcoma, which includes MDSCs (Figure 1).[69] Additionally, studies have shown that the majority of MDSCs infiltrating osteosarcoma tumors express CXCR4. In a mouse osteosarcoma model, the CXCR4 antagonist AMD3100 exerted a synergistic effect with an anti-PD-1 antibody, suggesting a potential novel treatment strategy for osteosarcoma patients.[70] Considering the intricate association between MDSCs and osteoclasts, it is plausible to hypothesize that

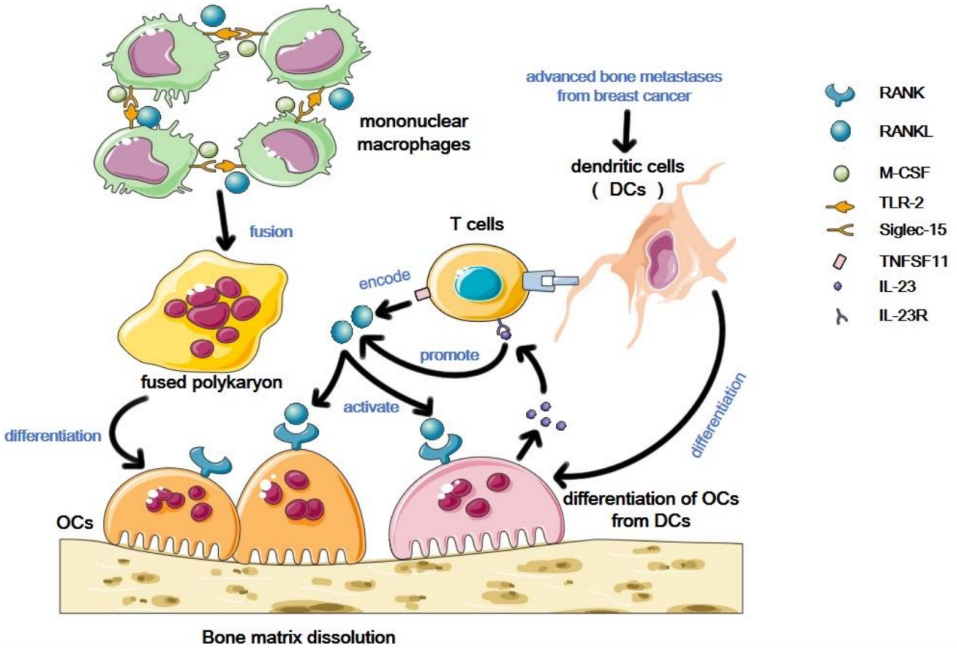

Figure 2 Osteoclasts in the bone marrow microenvironment. In the bone marrow microenvironment, stimulated by RANKL and M-CSF, TLR2 binds to SIGLEC-15, resulting in the fusion of mononuclear macrophages into polykaryotes and the final differentiation into osteoclasts. T cell expression of TNFSF11 encoding RANKL can also activate osteoclasts. In addition, DCs has been found in advanced bone metastases from breast cancer to differentiate into DC-OCs upon contact with T cells. Such osteoclasts can secrete IL-23, and binding to IL-23R on T cells also promotes the production of activated osteoclasts in RANKL.

MDSCs may play a role in controlling osteoclasts in the osteosarcoma microenvironment. Therefore, targeting the development of osteosarcoma could also serve as a strategy for modulating osteoclasts and treating this disease.

The microenvironment of osteosarcoma is complex and diverse, and the interaction between osteosarcoma cells and other cells in the tumor microenvironment may become a breakthrough in the development of new therapies for osteosarcoma (Table 1).

Table 1 Interactions Between Osteoclasts and Other Cells in the Osteosarcoma Microenvironment

Type of Study	Type of Immune Cells	Type of Ligand/Receptor	Chemical Available Agonists/Antagonists	Reference
In vitro	Mononuclear	RANKL/TNFSF11	LiCl	[58]
In-vitro, in-vivo	Macrophages	Sialyl-Tn carbohydrate ligand	IFN-γ	[59]
In-vitro, in-vivo	Macrophages	Siglec-15	NP-159 (Siglec-15 neutralizing antibody)	[60]
In-vitro, in-vivo	Macrophages	Sialylated TLR2	SialEXO 23 α2,3 specific sialidase	[61]
In-vitro, in-vivo	Myeloid-derived suppressor cell, tumor-associated macrophages	CD11b cells	Metformin (Met)	[67]
In-vivo, in-situ	Myeloid-derived suppressor cell	CXCL12	AMD3100(a CXCR4 antagonist)	[68]
In vitro, in-situ	Tumor-associated fibroblasts	α-Smooth muscle actin-positive fibroblasts	Still unclear	[69]
In vitro	T cells	RANKL/TNFSF11	Anti-CD3/CD28, PMA/Ionomycin	[70]
In-vitro, in-vivo	Dendritic cells, T cells	IL-23, IL-23R, RANKL	Anti-IL23 monoclonal antibody	[71]

Osteoclasts and Other Cells

During the pathogenesis of osteosarcoma, there are additional cell types that can interact with osteoclasts. The osteosarcoma stroma, which plays a supportive role in cancer development, includes fibroblasts, which are an important component. In osteosarcoma, fibroblasts differentiate into tumor-associated fibroblasts (CAFs) or "myofibroblasts." The exact role of these cells in relation to multinucleated osteoclasts is still unclear, but it has been observed that the fusion of CAFs with osteosarcoma cells is closely associated with the formation of multinucleated giant cells observed in osteosarcoma (Figure 1).[71] RANKL, which is essential for osteoclast differentiation, is encoded by the tumor necrosis factor (TNF) superfamily member 11 (TNFSF11). Therefore, studying the expression of TNFSF11 and the production of RANKL by relevant cells is important to support osteoclast differentiation and promote bone resorption in osteosarcoma. T-cell activation induces TNFSF11 expression and regulates osteoclastogenesis by affecting RANKL production (Figure 2).[72] Furthermore, Monteiro et al discovered that dendritic cells (DCs) could differentiate into osteoclasts during the development of osteolytic lesions. This pathway generates DC-OCs, which maintain a pro-osteoclastogenic antitumor T-cell phenotype, contributing to osteolysis. Understanding the interactions between fibroblasts, CAFs, T cells, DCs, and osteoclasts in osteosarcoma is important for unraveling the complex mechanisms of bone resorption and tumor progression. Further research in this area can provide valuable insights for developing targeted therapeutic strategies for osteosarcoma (Figure 2).[73]

Osteoclasts in the Treatment of Osteosarcoma

Surgery, radiotherapy, chemotherapy, and immunotherapy are commonly used treatment options for osteosarcoma. Immunotherapy involves using the patient's immune system to recognize and eliminate osteosarcoma cells, using medications to target immune checkpoints, monoclonal antibodies, CAR T-cell therapy, and similar treatments. Targeted drugs can also be beneficial in the treatment of osteosarcoma. These drugs inhibit the growth of new blood vessels within the tumor, thereby aiding in the fight against osteosarcoma. Examples of these drugs include Nexavar and Votrient. When considering the role of osteoclasts in the clinical treatment of osteosarcoma, two main aspects are important: inhibiting osteoclast formation induced by the tumor and inducing osteoclast-specific apoptosis in response to bone resorption.[74–76] Siglec-15, which is predominantly expressed in osteoclasts, can modulate the effect of RANKL on osteoclast cell lines. Anti-Siglec-15 antibodies can stimulate PDGF-BB secretion through various mechanisms. Targeting Siglec-15 promotes intramembranous and endochondral osteogenesis, and anti-Siglec-15 antibodies have potential in the treatment of bone tumors.[62] Additionally, secreted TLR2 acts as a receptor for Siglec-15, initiating the fusion of osteoclastic precursor cells by binding to sialylated TLR2.[63] The expression of Siglec-15 is regulated by M-CSF, and changes in TLR2 are induced by RANKL, suggesting that the formation of osteoclast fusion recognition signals requires the recognition of osteoclast and fusion control signals by M-CSF and RANKL, thus offering potential therapeutic targets for bone diseases associated with abnormal osteoclast activity.[76] Patients with osteosarcoma often experience pathological fractures, and bisphosphonates can serve as an adjunctive therapy. Bisphosphonates inhibit osteoclast activity, preventing bone resorption. Furthermore, the monoclonal antibody denosumab targets the RANKL protein and promotes bone growth. This agent is being studied as a potential treatment for osteosarcoma.[77] In addition to the research and development of therapeutic drugs, recent studies have also used advanced techniques such as comprehensive single-cell sequencing, somatic cell sequencing, and gene expression data analysis to analyze osteoclasts in osteosarcoma. As a result, 11 significantly meaningful osteoclast differentiation-related genes (ODRGs) were identified. These associated genes hold crucial prognostic importance for osteosarcoma and have the potential to become therapeutic targets in the future.[78] Moreover, a plethora of clinical trials exploring osteosarcoma are underway, promising potential benefits for patients (Table 2).

Conclusions and Perspectives

Osteoclasts are vital bone-related cells that play a crucial role in normal bone physiology. These cells are responsible for bone remodeling, allowing bones to grow and regenerate, and removing aging or damaged bone tissue. However, the relationship between osteoclasts and osteosarcoma is complex. Within the tumor microenvironment of osteosarcoma,

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Table 2 Osteosarcoma Treatment Related Clinical Trials

Research ID	Research Name	Primary Research Objective	Interventions	Status	Trial Phase	Primary Completion	Enrollment
NCT00586846	Phase II Study of Chemotherapy and Pamidronate for the Treatment of Newly Diagnosed Osteosarcoma	This study is designed to test the safety and feasibility of the simultaneous administration of a biphosphonate with chemotherapy for the treatment of osteosarcoma in newly diagnosed patients.	Drug: Cisplatin Drug: Doxorubicin Drug: Methotrexate	Completed	Phase 2	2009–02	40
NCT02470091	Denosumab in Treating Patients With Recurrent or Refractory Osteosarcoma	This phase II trial studies how well denosumab works in treating patients with osteosarcoma that has come back (recurrent) or does not respond to treatment (refractory). Immunotherapy with monoclonal antibodies, such as denosumab, may help the body's immune system attack the cancer, and may interfere with the ability of tumor cells to grow and spread.	Biological: Denosumab Other: Laboratory Biomarker Analysis Other: Pharmacological Study	Active, not recruiting	Phase 2	2023-09-30	56
NCT00330421	Sorafenib in Treating Patients With Soft Tissue Sarcomas (Extremity Sarcoma Closed to Entry as of 5/30/07)	This phase II trial is studying how well sorafenib works in treating patients with soft tissue sarcoma. Sorafenib may stop the growth of soft tissue sarcoma by blocking blood flow to the tumor and blocking some of the enzymes needed for tumor cell growth.	Drug: sorafenib tosylate Procedure: therapeutic conventional surgery Other: laboratory biomarker analysis Other: pharmacological study Procedure: computed tomography Procedure: dynamic contrast-enhanced magnetic resonance imaging	Completed	Phase 2	2008–07	15
NCT00880542	Sorafenib and Ifosfamide in Treating Patients With High-Grade Soft Tissue Sarcoma or Bone Sarcoma That Can Be Removed by Surgery	This phase II trial is studying the side effects of giving sorafenib together with ifosfamide and to see how well it works in treating patients with high-grade soft tissue sarcoma or bone sarcoma that can be removed by surgery.	Drug: sorafenib Drug: Ifosfamide	Terminated	Phase 2	2010–11	7

NCT01946529	Therapeutic Trial for Patients With Ewing Sarcoma Family of Tumor and Desmoplastic Small Round Cell Tumors	This protocol will study treatment for Ewing sarcoma family of tumors (ESFT) and desmoplastic small round cell tumor (DSRCT). Participants with ESFT will be divided into two treatment groups, A or B, based on tumor characteristics. Group A (standard risk) participants have tumor that is not in the pelvis, has not spread to other parts of the body, and are less than 14 years of age. Because previous clinical trials have shown that standard treatment is very effective for children whose tumors have these characteristics, these participants will receive standard treatment. Group B (high risk) participants are 14 years of age or older or have tumor in the pelvis, or the tumor has spread to other parts of the body. Participants with DSRCT in the abdomen and/or pelvis or with tumor that cannot be removed by surgery alone or has spread to other parts of the body will be included in Group B. Participants in this group are considered high risk because there is a greater chance of tumor recurring following standard treatments currently in use. All participants will be followed and evaluated for 10 years following completion of therapy.	Drug: vincristine Drug: doxorubicin Drug: cyclophosphamide	Active, not recruiting	Phase 2	2026–07	24

(*Continued*)

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Table 2 (Continued).

Research ID	Research Name	Primary Research Objective	Interventions	Status	Trial Phase	Primary Completion	Enrollment
NCT02357810	Pazopanib Hydrochloride and Topotecan Hydrochloride in Treating Patients With Metastatic Soft Tissue and Bone Sarcomas	The purpose of this clinical research study is to learn if pazopanib when given in combination with topotecan can help to control sarcomas. The safety of this drug combination will also be studied. Pazopanib hydrochloride and topotecan hydrochloride may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth.	Drug: Pazopanib Hydrochloride Drug: Oral Topotecan Hydrochloride Other: Laboratory Biomarker Analysis	Completed	Phase 2	2021-10-12	178
NCT02180867	Radiation Therapy With or Without Combination Chemotherapy or Pazopanib Before Surgery in Treating Patients With Newly Diagnosed Non-rhabdomyosarcoma Soft Tissue Sarcomas That Can Be Removed by Surgery	This randomized phase II/III trial studies how well pazopanib, when combined with chemotherapy and radiation therapy or radiation therapy alone, work in the treatment of patients with newly diagnosed non-rhabdomyosarcoma soft tissue sarcomas that can eventually be removed by surgery. Radiation therapy uses high energy x-rays to kill tumor cells. Drugs used in chemotherapy, such as ifosfamide and doxorubicin, work in different ways to stop the growth of tumor cells, either by killing the cells, by stopping them from dividing, or by stopping them from spreading. Pazopanib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. It is not yet known whether these therapies can be safely combined and if they work better when given together in treating patients with non-rhabdomyosarcoma soft tissue sarcomas.	Drug: Doxorubicin Drug: Doxorubicin Hydrochloride Drug: Ifosfamide Drug: Pazopanib Drug: Pazopanib Hydrochloride Radiation: Radiation Therapy Procedure: Therapeutic Conventional Surgery	Active, not recruiting	Phase 2 Phase 3	2019-06-30	140

NCT01532687	Gemcitabine With or Without Pazopanib in Treating Patients With Refractory Soft Tissue Sarcoma	This randomized phase II trial studies how well gemcitabine hydrochloride works with or without pazopanib hydrochloride in treating patients with refractory soft tissue sarcoma. Drugs used in chemotherapy, such as gemcitabine hydrochloride, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. Pazopanib hydrochloride may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Pazopanib hydrochloride may also stop the growth of tumor cells by blocking blood flow to the tumor. It is not yet known whether gemcitabine hydrochloride is more effective with or without pazopanib hydrochloride in treating patients with soft tissue sarcoma.	Drug: Gemcitabine Drug: Gemcitabine Hydrochloride Other: Laboratory Biomarker Analysis Drug: Pazopanib Drug: Pazopanib Hydrochloride Other: Placebo Administration	Completed	Phase 2	2019-10-31	54

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various cytokines secreted by tumor cells stimulate osteoclast activity. This leads to a vicious cycle of osteolytic lesion formation, contributing to tumor progression. Advancements have been made in the treatment of osteosarcoma in recent decades, but there is still a need for more effective therapies, especially for refractory cases. Bone metastasis is a common occurrence in advanced cancer, causing symptoms such as bone pain, fractures, and tumors. Denosumab, an inhibitor of RANKL, a critical factor in osteoclast formation that interferes with the RANK/RANKL/OPG signaling pathway, which is necessary for osteoclast activation. By inhibiting bone destruction mediated by osteoclasts, denosumab can inhibit tumor development.[79] The impact of osteoclasts on bone-related diseases is well-established. Various complications associated with osteosarcoma afflict patients, significantly inconveniencing their lives and even posing health threats. Over the next five years, building upon the current research foundation, proactive exploration of the mechanisms by which osteoclasts function in osteosarcoma, as well as their relationship with various osteosarcoma-related complications, could effectively prevent and treat osteosarcoma. Early detection and treatment stages of osteosarcoma can aid in preventing pathological bone destruction resulting from increased osteoclast activity. Therefore, understanding the functions and mechanisms of osteoclasts in osteosarcoma is of paramount importance for advancing the clinical treatment of this disease. Further research efforts should be focused on investigating the specific roles of osteoclasts in osteosarcoma development and developing more targeted therapeutic approaches.[80] Exploring more precise personalized therapeutic strategies in the field of osteosarcoma to target the specific mechanisms underlying the role of osteoclasts in disease progression and develop precision-targeted treatments for pathological osteoclasts within osteosarcoma is of paramount importance. For instance, the development of novel drugs to intervene in osteoclast activity can reduce the risk of bone pain and fractures. Additionally, the analysis of patients' genetic characteristics and tumor markers can help identify specific osteoclast-targeted treatment methods for each patient. Through personalized treatment, determining the optimal treatment regimen can maximize treatment efficacy.

Furthermore, investigating the relationship between tumor-associated immune cells and osteoclasts within osteosarcoma, and leveraging the interplay between tumor-associated immune cells and osteoclasts indirectly regulates osteosarcoma, mitigates disease progression, and enhances patient survival. Current research efforts aim to target osteoclasts to influence bone development to treat osteosarcoma. Continued research into the roles and mechanisms of osteoclasts in osteosarcoma is crucial for advancing treatment options for this disease. These precision-targeted treatments targeting osteoclasts may be a milestone breakthrough in the field of osteosarcoma treatment over the next 5 years, providing patients with more effective treatment options, alleviating symptoms and improving their quality of life. This presents an exciting and promising outlook for the future.

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Disclosure

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