Journal of Inflammation Research

Dovepress
Taylor & Francis Group

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REVIEW

Pathophysiological Insights Into the Role of Osteoclasts in Osteoarthritis: Mechanisms, Therapeutic Targets, and Future Directions

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Abstract: Osteoarthritis (OA) is the most prevalent musculoskeletal issue. In the absence of effective pharmacological interventions, advanced stages of this disease frequently necessitate joint replacement surgery, thereby imposing a substantial socioeconomic burden. An increasing number of studies suggest that subchondral bone osteoclasts are crucial for the onset of arthritis, even before the formation of cartilage lesions. Osteoclasts are the only type of cells responsible for bone resorption and are integral to the etiology of OA. Subchondral osteoclasts accelerate OA progression by mediating cartilage damage, promoting angiogenesis, and mediating neuropathic pain. With advancements in knowledge of bone biology and focused medicines, OA therapeutics for osteoclasts are gradually being revealed. This article presents an examination of the function and processes that regulate subchondral osteoclasts in OA, detailing recent breakthroughs in targeted therapy for osteoarthritis involving subchondral osteoclasts. The aim of this study is to address the current knowledge gap in OA treatment and promote the advancement of innovative therapeutic approaches. Notably, combining single-cell RNA sequencing (scRNA-seq) with traditional therapeutic approaches to investigate the gene expression patterns of osteoclasts in OA from both temporal and spatial dimensions may lead to the discovery of novel OA treatment targets.

Keywords: osteoarthritis, osteoclast, pain, angiogenesis, single-cell sequencing

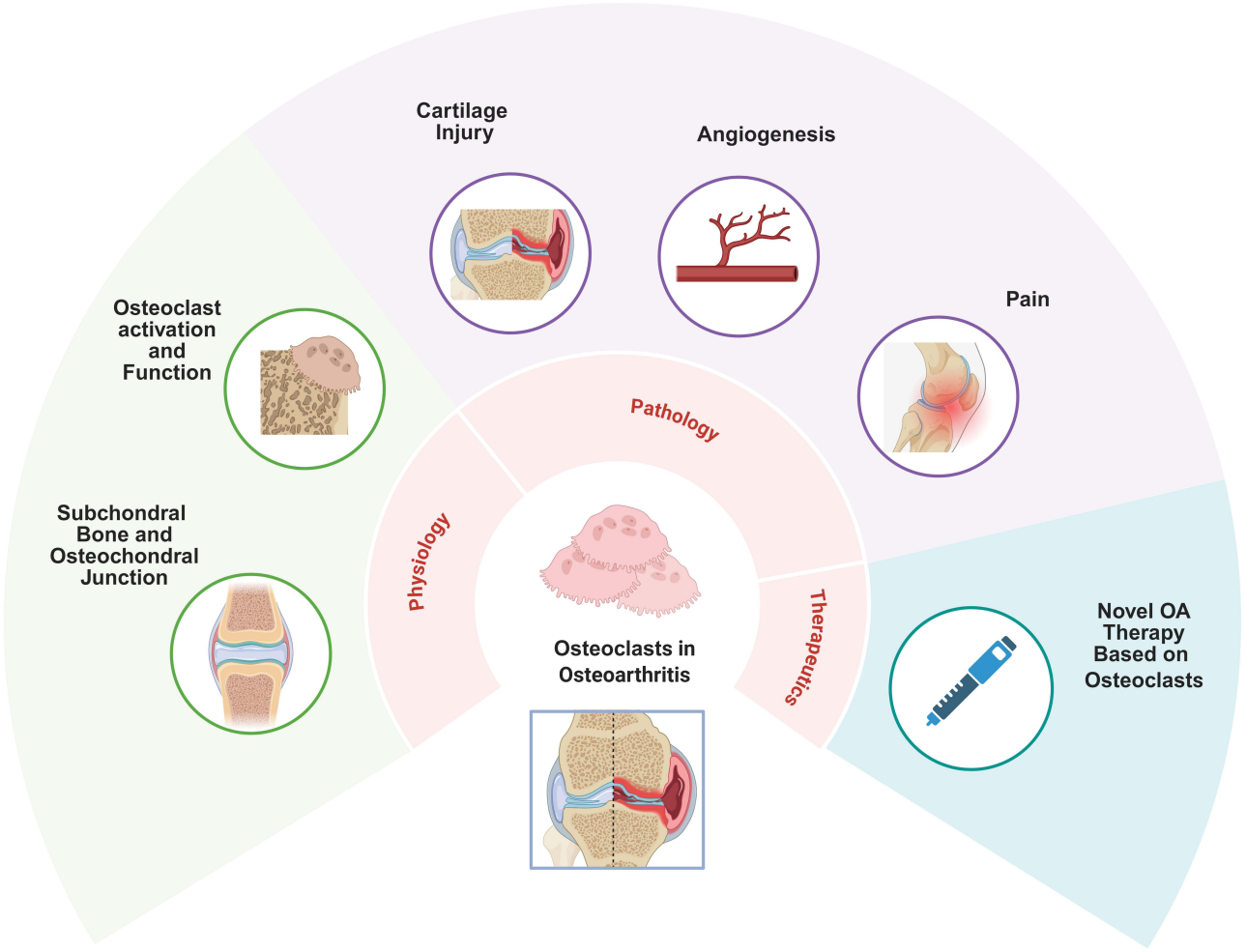
Introduction

Osteoarthritis (OA) has become a common degenerative joint disease characterized by deterioration of the articular cartilage, inflammatory reactions, and metabolic abnormalities in bone cells.[1,2] Subchondral bone is usually regarded as a crucial factor in the progression of OA during the development of arthritis lesions, as it is integral to the overall joint structure.[3] Increasing amounts of data indicate that irregular remodeling of subchondral bone transpires prior to the deterioration of articular cartilage and contributes to the acceleration of cartilage degeneration. Additionally, subchondral bone may act as the primary cause of pain for sufferers, making it crucial in the pathophysiology of this condition.[4,5] Among them, osteoclasts (OCs), a type of cell that is vital to the resorption and remodeling of tissue from the bone, are closely correlated with metabolic disorders of subchondral bone.[6,7]

Research has shown that the activity of OCs in subchondral bone increases substantially, leading to OCs surpassing the activity of osteoblasts (OBs), resulting in an imbalance between OCs and OBs in bone coupling, thereby causing bone destruction and joint degeneration.[8,9] In addition, OC-specific eQTL (expression quantitative trait locus) dataset analyses have been used to identify alleles that may be associated with the development of OA.[10] The above evidence suggests that aberrant activation of OCs is a key component in the pathogenesis of OA. In recent years, increasing evidence has shown that OCs also have complex effects on angiogenesis and neural invasion in the pathogenesis of OA.[11,12] However, the precise regulatory

Received: 3 March 2025
Accepted: 10 August 2025
Published: 17 August 2025

Graphical Abstract



mechanisms and specific functions of OCs in subchondral bone in OA remain inadequately understood. Consequently, it is of paramount importance to research the mechanisms involving OCs that regulate the pathological processes of OA. In addition, on the therapeutic side, intervention strategies for OCs offer new hope for the treatment of OA.[13,14]

This review offers a thorough examination and discussion of the significant recent advances in the study of subchondral bone OCs in OA research. The focus of this review is on the pathophysiology of OCs, such as their involvement in cartilage damage, angiogenesis, and nerve invasion. We aim to summarize the existing research findings, explore their potential applications in the treatment and prevention of OA and reveal the potential of single-cell RNA sequencing (scRNA-seq) technology in this field. By providing a comprehensive understanding of the mechanics of subchondral bone OCs, we aim to offer novel directions and strategies for the treatment of osteoarthritis.

Subchondral Bone Structure and Osteoclast Physiology

Subchondral Bone and Osteochondral Junction

The distal bone component of calcified cartilage is generally referred to as subchondral bone, which can be further defined as the subchondral bone plate and subchondral bone trabeculae. It is located beneath the articular cartilage, is connected to the cartilage, and is crucial for the mechanical function and stress distribution of the joint. The articular

cartilage and subchondral bone are mechanical and functional units, with the latter serving as a supportive foundation for the former and absorbing the substantial burdens that are applied to weight-bearing joints.[15] The structure of subchondral bone is unique and contains abundant blood vessels, nerves, and other cells. It has good metabolic activity and repair capabilities and can regulate the differentiation and growth of chondrocytes.[16,17]

The subchondral bone is intimately linked to the underlying cartilage, constituting the osteochondral junction, or cartilage–bone junction. The osteochondral junction is a crucial structure that links cartilage and bone, serves to protect joints and sustain the body through robust adhesion and optimal stress distribution.[18] In addition, the osteochondral connection also promotes the transport of nutrients and oxygen, maintaining the metabolic function of the cartilage. In certain diseases and injuries, the osteochondral junction may be damaged or destroyed, leading to impaired function of cartilage and bone as well as biomechanical changes.[17,19] Research indicates that OCs can degrade cartilage–bone connections through matrix metalloproteinases (MMPs) and cysteine protease-dependent pathways, resulting in diminished bone density.[20]

Biological Characteristics of Osteoclasts

OCs originate from pluripotent hematopoietic stem cells, which further differentiate into myeloid progenitor cells. These progenitor cells can then differentiate into megakaryocytes, granulocytes, monocytes/macrophages, or OCs.[21,22] The number of nuclei in each osteoclast ranges from 10 to 100, and the quantity of nuclei affects the rate of bone resorption by the osteoclast for a given volume of bone.[23] Mature, multinucleated OCs are present only at sites of bone resorption. The cytoskeleton of mature OCs can form podosome-like structures to facilitate their movement toward resorption sites. This actin-rich, podosome-like cytoskeleton can be observed in developing OCs, and its structure can be independently analyzed using fluorescence recovery after photobleaching (FRAP) techniques and immunofluorescence microscopy.[24]

The fully formed F-actin ring surrounding OCs is an important marker for demonstrating their functional activity and bone resorption potential.[22] Mature OCs present elevated levels of tartrate-resistant acid phosphatase (TRAP). TRAP is regarded as a definitive histological marker of adult OCs. Mature OCs also express significant indicators, such as matrix metalloproteinase 9 (MMP9) and the integrin αvβ3. Nonetheless, multinucleated cells and the TRAP enzyme are extensively utilized to detect and monitor the development of OCs.[25]

The procedures associated with OC fusion are thought to transpire as outlined below: (a) migration, (b) recognition, (c) cell–cell adhesion, and (d) membrane fusion.[23] In the presence of macrophage colony-stimulating factor (M-CSF), mononuclear pre-OCs differentiate into multinucleated OCs. M-CSF and RANKL, both of which are expressed by OBs, are involved in the differentiation of OCs.[26] By binding to the RANK receptor on the surface of OCs, it directly stimulates osteoclast activation. This interaction between RANKL and the RANK receptor induces a signaling cascade, thereby promoting the expression of genes associated with osteoclastogenesis,[27] such as dendritic cell-specific transmembrane protein (DC-STAMP), nuclear factor of activated T cells 1 (NFATc1),[28] TRAP, and CTSK, which can resorb and degrade bone tissue[29] (Figure 1).

Overview of Osteoclasts as Therapeutic Targets for OA

Recent studies have demonstrated the critical importance of subchondral bone in the development of OA.[30] Studies have shown that pathological changes in subchondral bone can be detected even before the appearance of cartilage lesions.[7,31] The initial observable changes associated with OA typically involve osteoclast activation and alterations in the turnover rate of subchondral bone under the influence of abnormal mechanical stress.[32,33] Numerous studies have suggested that the early stages of OA can be defined by transient activation of OCs, a change that precedes phenotypic alterations in cartilage degradation.[34] There is increasing recognition that therapeutic strategies targeting OCs may represent a significant pathway for treating OA.[35] As the mechanisms underlying the role of OCs in OA are gradually untangled, osteoclast-based treatments for OA are increasingly being proposed.[6,7,36–38] OCs play multifaceted roles in the pathogenesis of OA, including mediating cartilage damage, participating in joint fibrocartilaginous changes, promoting angiogenesis, and inducing pain. The interplay of these effects collectively contributes to the progression of OA (Figure 2 and Table 1).

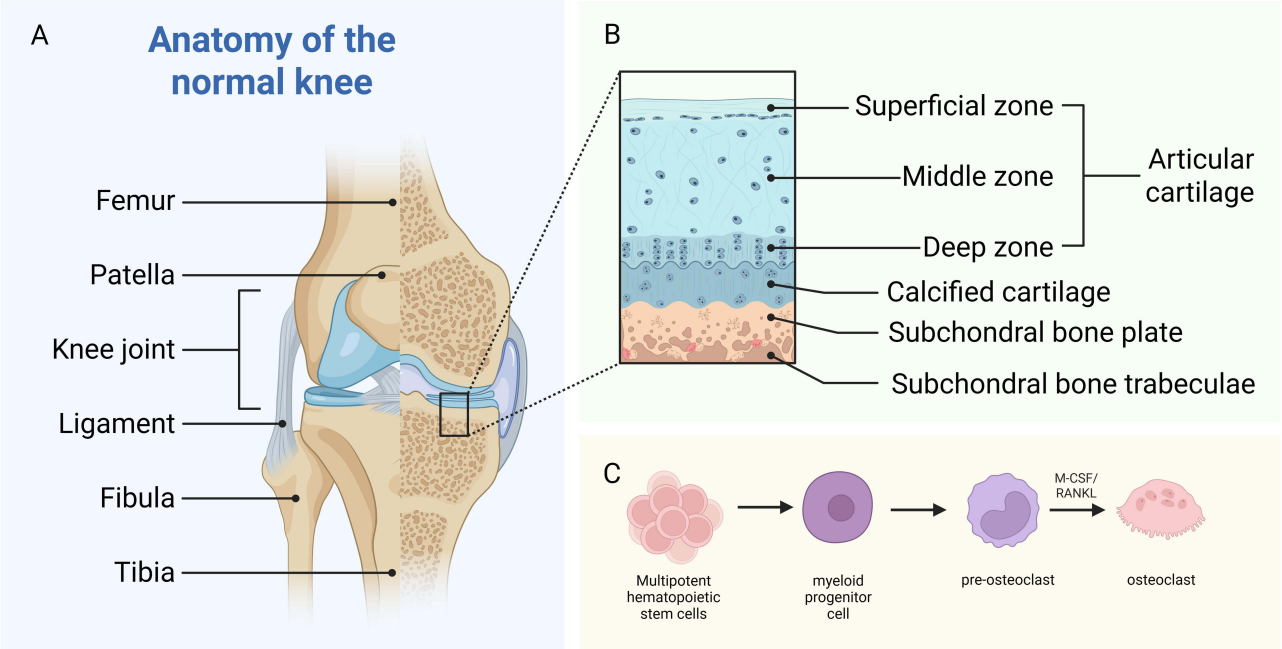


Figure 1 Schematic representation of subchondral bone structure and osteoclast formation (**A**) The anatomical structure of a healthy knee; (**B**) The inset shows a magnified view of the microstructure of subchondral bone; (**C**) The process of OC formation. Created in BioRender. Chen, S. (2025) https://BioRender.com/a42j210.
Abbreviations: M-CSF: Macrophage colony-stimulating factor; RANKL: Receptor Activator of Nuclear Factor-κB Ligand.

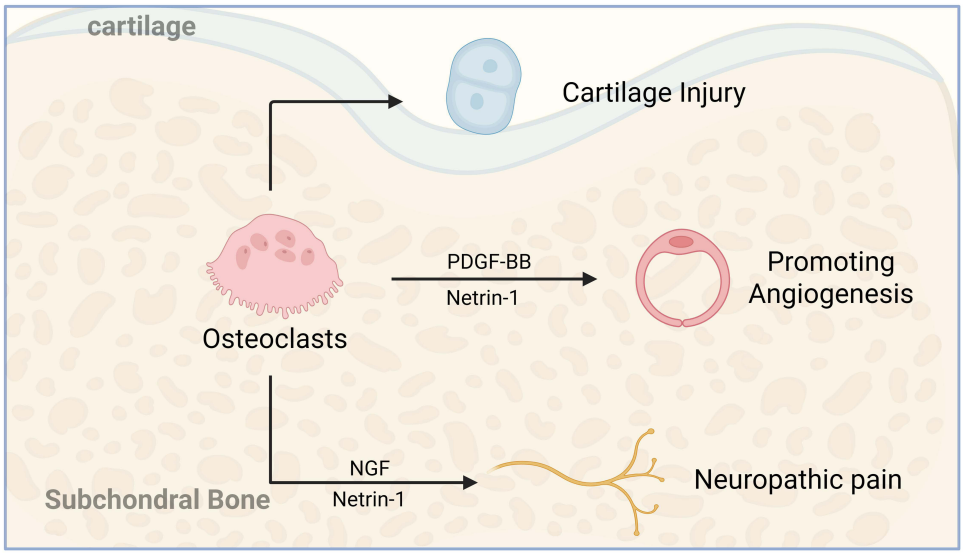


Figure 2 Diagram of the role of OCs in regulating cartilage damage, angiogenesis, and pain in the OA microenvironment. Created in BioRender. Chen, S. (2025) https://BioRender.com/fb5aame.
Abbreviations: PDGF-BB, Platelet-Derived Growth Factor-BB; NGF, Nerve Growth Factor.

Mediating Cartilage Injury

OA is a degenerative joint condition characterized by the deterioration of articular cartilage and subchondral bone, with cartilage degradation being crucial to the course of the disease. Recent studies have shown that OCs contribute to articular cartilage degradation through direct secretion of catabolic factors and indirect modulation of biomechanical stress[41] (Figure 3).

Emerging research indicates that exosome-mediated communication between OCs and chondrocytes plays a significant role in OA pathogenesis. OC-derived exosomes transport microRNAs (miRNAs) to chondrocytes,

Table 1 Overview of the Various Mechanisms of Action of OCs

Potential Therapeutic Approach and Target	Type and Outcome	Effect	Reference
Direct contact	Cartilage damage	OC precursors have the ability to interact with chondrocytes of normal phenotype, directly contact hypertrophic chondrocytes, and migrate into cartilage through invasive vascularization.	[39]
Degradation of osteochondral junction and articular cartilage	Cartilage damage	The capability of OCs to degrade articular cartilage and osteochondral junctions in an MMP-dependent as well as caspase-dependent ways	[20]
Exosomes	Cartilage damage	OCs deliver miRNA to chondrocytes via exosomes, targeting Smad2 to promote cartilage matrix degradation	[40]
Exosomes	Cartilage damage	Delivery of miRNA targeting TIMP-2/3 resistance molecules in chondrocytes reduces cartilage matrix degradation in resistance to OA	[34]
Disruption of mechanical homeostasis indirectly leads to articular cartilage degradation	Cartilage damage	OC-mediated bone resorption induces a substantial increase in TGF-β1 levels, which in turn drives the acquisition of osteoprogenitor cells to subchondral bone, thereby causing aberrant bone remodelling and osteosclerosis. Ongoing cartilage injury remains due to of subchondral bone alterations how modify its biomechanical properties, conveying shear forces to the cartilage layer.	[41]
Disruption of mechanical homeostasis indirectly leads to articular cartilage degradation	Cartilage damage	The medial and lateral compartments descend asymmetrically due to the absence of a bone barrier similar to the fibula on the lateral side and the absence of robust soft tissue support around the medial tibial plateau. This exacerbates the imbalance of joint stress.	[42,43]
PDGF-BB	Angiogenesis	PDGF-BB can be secreted by OCs, which binds to PDGFR-β and activates the PDGF-BB/PDGFR-β signalling pathway, which is essential for angiogenesis.	[44]
Netrin-1	Angiogenesis	Netrin-1 is considered to be a potent vascular mitogen that can activate CD146 receptors on endothelial cell membranes and promote angiogenesis	[45]
NGF	Causes pain	OCs mediate OA pain by secreting nerve growth factor (NGF) to recruit sensory nerves.	[46]
Netrin-1	Causes pain	Netrin-1 stimulates the growth of axons in DRG neurones and the sensory innervation of subchondral bone through its receptor, DCC.	[47]
Increased Sensitivity	Causes pain	OCs secrete protons from the bone resorption site by acidifying the extracellular bone microenvironment via the α3 vacuolar proton ATPase. The acidic environment activates and upregulates pH-sensitive acid-sensitive nociceptors, transient receptor potential vanilloid subfamily member 1 (TRPV1), and acid-sensing ion channel 3 (ASIC3), thereby instructing pain the neurones in the peripheral part.	[48,49]
Inflammatory environment	Causes pain	When pathological inflammation occurs, OC precursor cells directly secrete or indirectly activate proinflammatory T cells to produce proinflammatory cytokines (such as IL-6, IL-1β, and TNF-α), thereby increasing OC bone resorption and mediating inflammatory pain.	[50]

specifically targeting Smad2, thereby facilitating cartilage matrix degradation.[40] Furthermore, these exosomes may diminish cartilage matrix integrity by delivering miRNAs that suppress tissue inhibitors of metalloproteinases (TIMP-2/3) in chondrocytes.[34] Targeted silencing of genes associated with OC activation has been shown to mitigate cartilage

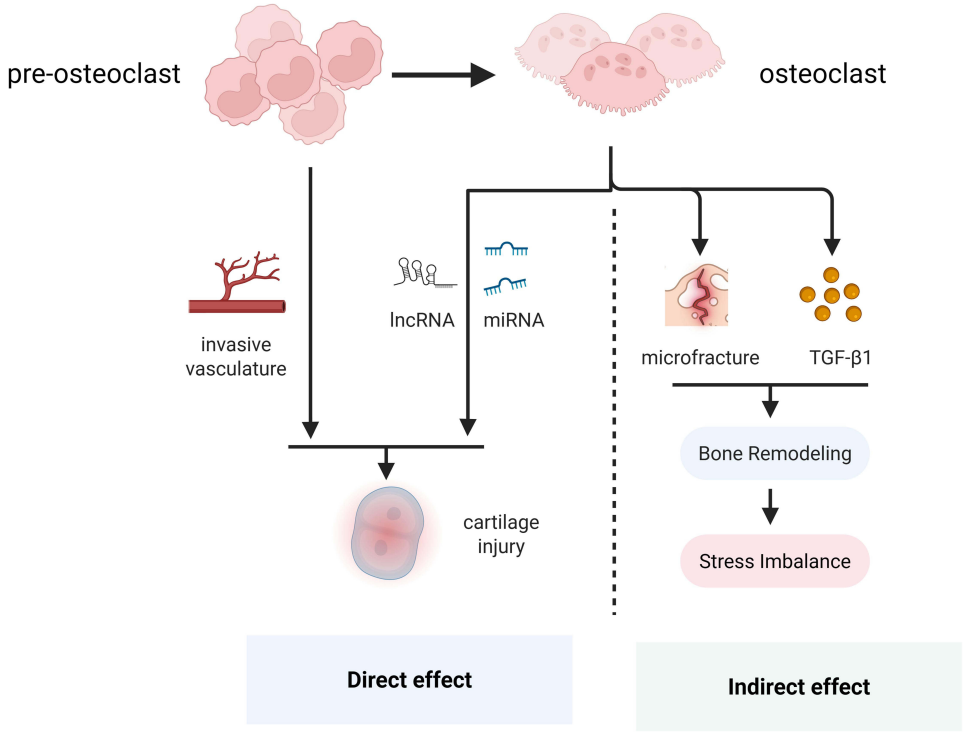


Figure 3 Direct and indirect factors of cartilage damage mediated by OCs. Pre-osteoclasts can directly mediate cartilage injury through vascular invasion. Alternatively, mature osteoclasts may directly mediate cartilage damage by secreting lncRNA or miRNA. In addition, mature osteoclasts can mediate subchondral bone microfracture by affecting the mechanical stress of subchondral bone and secrete TGF-β1 to mediate bone remodeling, leading to stress imbalance in cartilage and indirectly affecting cartilage homeostasis. Created in BioRender. Chen, S. (2025) https://BioRender.com/s82g938.
Abbreviations: LncRNA, Long non-coding RNA; miRNA, microRNA; TGF-β1, Transforming Growth Factor-β1.

degradation in OA by preemptively inhibiting pathological changes in subchondral bone.[12] Dai et al discovered that the exosomal let-7a-5p, which is derived from pre-OCSs and adult OCs, regulates Smad2, thereby promoting the hypertrophic differentiation of chondrocytes.[51] Additionally, Hu et al proposed that OC precursors can migrate to cartilage via invasive vasculature, directly contacting hypertrophic chondrocytes and interacting with chondrocytes of a normal phenotype.[41]

Moreover, OCs indirectly contribute to articular cartilage degradation by disrupting mechanical homeostasis. Under typical physiological conditions, subchondral bone dynamically modulates mechanical stresses applied to the joint through the coordinated functions of OCs and OBs.[52] As OA progresses, OC-mediated microfractures in subchondral bone alter its biomechanical properties, shear stresses applied to the cartilage layer result in persistent cartilage damage. Moreover, OC-mediated bone resorption leads to a substantial increase in TGF-β1 levels, resulting in aberrant bone remodeling and osteosclerosis.[53] In addition, in the early stages of OA, TGF-β1 can promote subchondral bone angiogenesis, such as the establishment of H-type tubes ($CD31^{hi}EMCN^{hi}$), which are closely related to osteoprogenitor cells.[54] These H-type channels also impede subchondral bone transformation and impact bone microarchitecture, bone cysts and bone spurs.[55] The expression of TGF-β1 in osteoclasts is markedly elevated in a time-dependent and dose-dependent manner in response to mechanical stimulation.[56] Moreover, the apoptosis of chondrocytes increases when these cells are cultured with OCs.[56] In addition, intraperitoneal injection of TGF-β1R inhibitors can reverse chondrocyte apoptosis and reduce cartilage degradation in OA model rats.[56] The lack of robust soft tissue support around the medial tibial plateau, coupled with the absence of a bony barrier akin to the fibula on the lateral side, results in asymmetric subsidence between the medial and lateral compartments, exacerbating joint stress imbalance.[42,43,57] Mechanical stress-induced upregulation of TGF-β1 in OCs is implicated in cartilage degradation and chondrocyte death in OA.[56,58]

Promoting Angiogenesis

Under normal physiological conditions in bone joints, both HIF-1α and the DLL4/Notch signaling pathway play critical roles in positively regulating type H vasculogenesis, which subsequently promotes subchondral bone formation. This process is indispensable for maintaining the normal structure and function of bone joints, ensuring joint stability, and supporting load-bearing capacity.[59–61] However, in the complex pathological context of OA, the osteogenic process mediated by type H vasculogenesis tends to differ from that in the normal state, contributing to the detrimental progression of OA.

In a healthy bone environment, type H vasculogenesis serves as a precisely regulated physiological process that accurately matches the growth, repair, and metabolic demands of bone tissue. HIF-1α stabilizes and activates a series of target genes in a hypoxic environment, coordinating with the DLL4/Notch signaling pathway to finely control vasculogenesis. This guidance ensures the orderly extension of new blood vessels into bone-forming regions, providing adequate nutritional and oxygen support for osteoblast recruitment, differentiation, and bone matrix synthesis. Simultaneously, it facilitates the timely removal of metabolic waste, thereby maintaining a normal and robust balance between bone formation and remodeling in the subchondral bone.[59]

In OA, there are microfractures at the osteochondral junction, creating a conduit from the subchondral bone plate to the noncalcified cartilage. The fluid, cells, and cytokines beneath the articular cartilage subsequently continue to increase, leading to abnormal angiogenesis in the subchondral bone.[62] In the past few years, the rich blood supply of subchondral bone has been considered a marker and drug target for OA.[63] OA is characterized by inflammation and cartilage injury, and angiogenesis may contribute to the destruction of joints.[64,65] An earlier diagnostic characteristic of human OA is increased angiogenesis in subchondral bone, which is accompanied by vascular invasion into ischemic cartilage.[65–69] Animal studies have consistently shown that the increased thickness of the subchondral bone plate ultimately injures the articular cartilage, possibly resulting from aberrant subchondral bone angiogenesis and osteogenesis.[70–72]

In the early stage of OA, the overactivation of OCs in the subchondral bone promotes type H ($CD31^{hi}EMCN^{hi}$) angiogenesis, leading to changes in the oxygen microenvironment of the joint, which is a critical factor in the progression of OA.[73] Maintaining a hypoxic microenvironment in the subchondral bone significantly impedes the progression of OA.[11] The interaction between H-type vascular activation-mediated chondrocytes and subchondral bone is crucial for the progression of OA. H-type blood vessels mediate cartilage matrix homeostasis by secreting MMP-9 and MMP-2, and excessive angiogenesis can lead to osteogenesis.[74]

Furthermore, significant changes, such as elevated levels of inflammatory factors and abnormal mechanical stress, occur in the microenvironment within bone joints. The combined effects of these factors may interfere with the normal function of HIF-1α and the DLL4/Notch signaling pathway, resulting in uncontrolled type H angiogenesis.[75,76] Although bone mass increases, bone strength does not improve owing to insufficient bone mineralization, leading to a lack of mechanical properties of subchondral bone, thereby causing damage to articular cartilage and exacerbating the vicious cycle of OA.[77] Moreover, the abnormal expansion of neovascularization may also carry inflammatory factors deep into the bone tissue, further aggravating the inflammatory response, forming a vicious cycle and promoting the progression of OA. Interestingly, bevacizumab (an anti-VEGF antibody) can reduce the formation of subchondral H-type blood vessels in an OA model and delay the progression of OA.[78] Notably, OCs appear to produce various angiogenic factors, such as platelet-derived growth factor-BB (PDGF-BB)[79,80] and netrin-1,[81,82] which can directly stimulate the formation of new blood vessels.

PDGF-BB

Preosteoclasts, as progenitors of osteoclasts, exhibit restricted bone resorption activity. Bone marrow TRAP+ mononuclear cells and osteoclasts can release PDGF-BB, which is essential for angiogenesis and is linked to osteogenesis to sustain bone homeostasis in healthy mice.[80] PDGF-BB serves as the ligand for platelet-derived growth factor receptor β. The interaction of PDGF-B with PDGFR-β initiates PDGF-BB/PDGFR-β signaling.[44] PDGF-BB/PDGFR-β signaling is crucial for angiogenesis and is associated with the stability of newly created vessels, which regulate the cellular mechanisms involved in osteogenesis. Similarly, PDGF-BB secreted by monocytic preosteoclasts promotes type

H angiogenesis to maintain normal bone formation and bone remodeling.[80,83,84] Knockdown of PDGF-BB in TRAP+ mononuclear cells impairs angiogenic osteogenesis junctions,[80] whereas if PDGF-BB expression is increased in TRAP+ mononuclear cells, bone formation and even fracture healing are enhanced.[85] In addition, abnormal overexpression of PDGF-BB in pre-OCs leads to an imbalance in vascular and skeletal homeostasis.[44,86] Su et al recently reported that increased secretion of PDGF-BB by preosteoclasts fosters aberrant angiogenesis-dependent bone formation in subchondral bone, hence contributing to the pathophysiology of OA pathogenesis.[44,87]

Netrin-1

In addition to common angiogenic factors, OCs are able to secrete netrin-1, which is considered a potent vascular mitogen.[81,82] In addition to its role in axon guidance, netrin-1, a neuronal guidance molecule, can activate the CD146 receptor on the endothelial cell membrane to promote angiogenesis.[45] Netrin-1 particularly promotes the proliferation, migration, and adhesion of vascular cells with efficacies comparable to those of VEGF, PDGF, or fibronectin.[81]

OA Pain

Joint pain is a cardinal symptom of OA. Abnormal subchondral bone remodeling has been identified as a major factor in OA pain.[88] Research has indicated that magnetic resonance imaging (MRI) of subchondral bone marrow edema-like lesions is strongly correlated with OA pain.[5] Elevated OC activity facilitates the recruitment of mesenchymal stem cells within the bone marrow, which subsequently undergo aberrant subchondral bone formation. A close interconnection between the nervous system and the skeletal system has been established.[4] The sensory nerve supply of subchondral bone during OA progression is contingent upon OC activity.[47] Subchondral bone OCs engage in the recruitment of sensory nerves through the secretion of nerve growth factor (NGF)[46] and Netrin-1,[47] thereby facilitating the mediation of OA pain.

NGF

NGF is instrumental in both acute and chronic pain conditions, particularly those linked to inflammation.[89,90] Previous studies have confirmed that anti-NGF therapy has potential in treating OA-related pain.[91,92] CRISPR-induced deletion of NGF mitigated osteoarthritis pain in a murine model of medically produced osteoarthritis.[93]

Netrin-1

OCs can also induce subchondral sensory nerve axon growth by secreting Netrin-1[83]. Previous studies have shown that Netrin-1 is important for subchondral bone remodeling, the induction of calcitonin gene-related peptide (CGRP)-positive sensory innervation, and pain in OA.[47] Mechanistically, Netrin-1 facilitates axon development in DRG neurons and sensory innervation of subchondral bone via its receptor DCC.[47] Knockdown of Netrin-1 in OCs reduces OA pain behavior.[47,94]

Increased Sensitivity

Furthermore, a substantial body of clinical evidence has demonstrated that persistent continuous nociceptive input from OA joints induces sensitization of both the central and peripheral nervous systems.[95] Central sensitization of the spinal cord, coupled with the dysregulation of ascending and descending pathways between the brain and spinal cord, as well as local cytokines, chemokines, and inflammatory mediators in OA joints, contributes to heightened pain sensitivity (hyperalgesia) and the perception of pain from nonpainful stimuli (allodynia).[48,96–98] OC resorption can generate an altered milieu in the subchondral bone, thereby peripherally sensitizing pain neurons. OCs acidify the extracellular bone microenvironment through the vacuolar proton ATPase, facilitating the secretion of protons from the site of bone resorption.[99,100] The acidic environment enhances and activates pH-sensitive nociceptors, specifically transient receptor potential vanilloid subfamily member 1 (TRPV1) and acid-sensing ion channel 3 (ASIC3),[49] which in turn triggers the release of neuropeptides and excitatory amino acids from nerve endings, ultimately causing pain in the cerebral cortex. In this process, the local ATP level and H+ concentration increase are coupled, and ATP binding to adenine receptors in the bones can also induce pain signals.

Inflammatory Environment Induces Pain

When pathological inflammation occurs, OC precursor cells directly secrete or indirectly activate proinflammatory T cells to produce proinflammatory cytokines (such as IL6, IL1β and TNF-α), thereby increasing OC bone resorption and mediating skeletal inflammatory pain.[50] Among them, IL-1β can directly act on nociceptors by interacting with TRPV1 and IL-1β receptors, to lower the pain threshold, or by increasing prostaglandin synthesis, to indirectly sensitize nociceptors and induce pain. Moreover, TNF-α and IL6 can promote Na+ influx in spinal dorsal horn neurons by activating the p38-MAPK pathway and lowering the excitability threshold.

Intercommunication between the nervous system and the skeletal system has become increasingly documented, underscoring their mutual interdependence.[4] The sensory nerve supply of subchondral bone during the progression of OA is closely associated with OC activity.[47] There is increasing recognition that targeting the subchondral bone and peripheral nerves may represent an alternative strategy for curtailing the progression of OA.[101]

Novel OA Therapy Based on Osteoclasts

A more comprehensive understanding of ovarian cancer biology and genetics is believed to facilitate the development of more targeted therapeutic strategies.[10,36] In recent years, biotherapy has received considerable attention, with stem cell-based therapies being among the most researched strategies. Mesenchymal stem cells (MSCs) and their secreted extracellular vesicles (EVs) have garnered widespread attention for the treatment of OA.[102–105] Exosomes facilitate intercellular communication via endocytosis, ligand–receptor interactions, direct membrane fusion, or signaling pathways.[102]

As research continues to expand, EVs have become key for mediating OC activity and treating OA. Research has shown that exosome-derived miR-212-3p from OCs targets the TGF-β1/Smad2 signaling pathway, inhibiting the anabolic metabolism of chondrocytes in OA and accelerating catabolic metabolism.[40] Exosomes derived from dental pulp stem cells alleviate knee OA by inhibiting TRPV4-mediated OC activation.[106] Bone marrow mesenchymal stem cells and their derived exosomes alleviate cartilage damage and pain in sodium iodoacetate-induced experimental OA in rats.[107] Li et al reported that human articular cartilage stem cells derived from TNFAIP3 could partially inhibit the formation and activation of subchondral bone OCs, indicating a beneficial effect in OA.[108]

In addition, some emerging therapeutic approaches are gradually enriching the osteoclastology of OA: a new antioxidant, PDA-PEG nanoparticles prepared by Wu et al, could alleviate early OA by inhibiting the generation of OCs and angiogenesis in the subchondral bone.[37] The traditional Chinese medicine Sanqi can inhibit OC fusion by suppressing the ERK/c-fos/NFATc1 pathway, thereby delaying osteoclastogenesis and subsequently inhibiting abnormal angiogenesis and neuropathic pain.[38] The EP4 receptor antagonist HL-43 can inhibit the secretion of Netrin-1, recruit CGRP-positive sensory neurons to the subchondral bone to alleviate OA pain, and reduce the H-type vasculature in the subchondral bone by inhibiting the secretion of PDGF-BB by OCs to treat OA.[109] The STING inhibitor C-176 can reduce articular cartilage and subchondral bone damage in a DMM mouse OA model by inhibiting OC differentiation.[14] Diosgenin inhibits the differentiation and bone resorption function of OCs, thereby suppressing subchondral bone loss in OA mice and indirectly protecting the articular cartilage.[13] Dihydroartemisinin reduces osteoclast development and bone resorption by obstructing the NF-κB, MAPK, and NFATc1 signaling pathways, hence mitigating OA.[110,111]

Single-Cell Sequencing

Single-cell sequencing (scRNA-seq) technology has rapidly developed in recent years, providing a powerful tool for comprehensively dissecting cellular heterogeneity, and has been considered a breakthrough tool for advancing the science of entire organisms.[112] In the context of bone biology, scRNA-seq can be used to elucidate the cellular basis of the occurrence and development of OA,[113,114] elucidate intricate and infrequent cell populations, reveal regulatory interactions among genes, and monitor various cell lineages throughout OA development.[115]

Wang et al revealed the trajectory of OC formation and intercellular communication with immune cells in the context of osteoporosis, suggesting that the bone immune microenvironment plays an important role in the formation and activation of OCs.[116] Hu et al identified the cell subtypes of the OA tibial plateau and described two unique endothelial

cell populations distinguished by exosome synthesis and the inflammatory response or vascular function and angiogenesis via single-cell sequencing. They also described three OC subtypes associated with vascularization, matrix production, and matrix mineralization, providing important insights into the pathophysiology of OA.[114] Tsukasaki et al identified the importance of CD11c and Cited2 in the in vitro activation of OCs, refining the molecular mechanisms of OC activation through the use of scRNA-seq.[117] However, possibly due to the lower number of OCs than other cells, there is still a lack of scRNA-seq analysis of OCs in the subchondral bone of OA patients. As methods for the specific isolation of OCs, such as magnetic bead sorting,[34] continue to advance, scRNA-seq of OCs in OA subchondral bone will likely emerge in the future, contributing to the diagnosis and treatment of OA.

Summary and Outlook

In summary, the role of subchondral bone OCs in OA has emerged as a crucial area of research, shedding light on the complex interplay among bone remodeling, cartilage degeneration, and pain mechanisms. These findings highlight that OCs not only are integral to the pathophysiology of OA through mediating cartilage injury and promoting angiogenesis but also serve as potential therapeutic targets for innovative treatments. Current therapeutic strategies, including biotherapies involving mesenchymal stem cells and their exosomes, show promise in mitigating OC-related pathological changes. Future research should focus on leveraging advanced techniques such as scRNA-seq to deepen our understanding of OC heterogeneity and its interactions within the joint microenvironment. This could lead to targeted interventions that specifically address the multifaceted nature of OA, ultimately advancing the field of OA treatment.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by a grant from the central government guides local funds for scientific and technological development (No. YDZJSX20231A062) and Shanxi Province scientific and technological achievements transformation guidance project(No. 202204021301067), as well as National Natural Science Foundation of China (Grant No. 82350710800, 82374470), and Shenzhen Medical Research Fund B2302005, and NHMRC, APP1163933.

Disclosure

The authors declare that they have no competing interests. Graphical abstract Created in BioRender. Chen, S. (2025) https://BioRender.com/b69db0n.

References

1. De Roover A, Escribano-Núñez A, Monteagudo S, Lories R. Fundamentals of osteoarthritis: inflammatory mediators in osteoarthritis. *Osteoarthritis Cartilage*. 2023;31(10):1303–1311. doi:10.1016/j.joca.2023.06.005
2. Zhang W, Ouyang H, Dass CR, Xu J. Current research on pharmacologic and regenerative therapies for osteoarthritis. *Bone Res*. 2016;4:15040. doi:10.1038/boneres.2015.40
3. Hu Y, Chen X, Wang S, Jing Y, Su J. Subchondral bone microenvironment in osteoarthritis and pain. *Bone Res*. 2021;9(1):20. doi:10.1038/s41413-021-00147-z
4. Xiao Y, Han C, Wang Y, et al. Interoceptive regulation of skeletal tissue homeostasis and repair. *Bone Res*. 2023;11(1):48. doi:10.1038/s41413-023-00285-6
5. Kwoh CK. Clinical relevance of bone marrow lesions in OA. *Nat Rev Rheumatol*. 2013;9(1):7–8. doi:10.1038/nrrheum.2012.217
6. Wang H, Yuan T, Wang Y, et al. Osteoclasts and osteoarthritis: novel intervention targets and therapeutic potentials during aging. *Aging Cell*. 2024;23(4):e14092. doi:10.1111/acel.14092
7. Chen W, Wang Q, Tao H, et al. Subchondral osteoclasts and osteoarthritis: new insights and potential therapeutic avenues. *Acta Biochim Biophys Sin*. 2024;56(4):499–512. doi:10.3724/abbs.2024017
8. Faqeer A, Wang M, Alam G, et al. Cleaved SPP1-rich extracellular vesicles from osteoclasts promote bone regeneration via TGFβ1/SMAD3 signaling. *Biomaterials*. 2023;303:122367. doi:10.1016/j.biomaterials.2023.122367

9. Ota K, Quint P, Weivoda MM, et al. Transforming growth factor beta 1 induces CXCL16 and leukemia inhibitory factor expression in osteoclasts to modulate migration of osteoblast progenitors. *Bone*. 2013;57(1):68–75. doi:10.1016/j.bone.2013.07.023
10. Mullin BH, Zhu K, Brown SJ, et al. Leveraging osteoclast genetic regulatory data to identify genes with a role in osteoarthritis. *Genetics*. 2023;225(2):iyad150. doi:10.1093/genetics/iyad150
11. Zhang H, Wang L, Cui J, et al. Maintaining hypoxia environment of subchondral bone alleviates osteoarthritis progression. *Sci Adv*. 2023;9(14): eabo7868. doi:10.1126/sciadv.abo7868
12. Kim GM, Kim J, Lee JY, Park MC, Lee SY. IgSF11 deficiency alleviates osteoarthritis in mice by suppressing early subchondral bone changes. *Exp Mol Med*. 2023;55(12):2576–2585. doi:10.1038/s12276-023-01126-6
13. Ding H, Ding H, Mu P, Lu X, Xu Z. Diosmetin inhibits subchondral bone loss and indirectly protects cartilage in a surgically-induced osteoarthritis mouse model. *Chem. Biol. Interact*. 2023;370:110311. doi:10.1016/j.cbi.2022.110311
14. Yu ZC, Fu R, Li Y, Zhao DY, Jiang H, Han D. The STING inhibitor C-176 attenuates osteoclast-related osteolytic diseases by inhibiting osteoclast differentiation. *FASEB J*. 2023;37(4):e22867. doi:10.1096/fj.202201600R
15. Sharma AR, Jagga S, Lee SS, Nam JS. Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. *Int J Mol Sci*. 2013;14(10):19805–19830. doi:10.3390/ijms141019805
16. Kon E, Boffa A, Andriolo L, et al. Subchondral and intra-articular injections of bone marrow concentrate are a safe and effective treatment for knee osteoarthritis: a prospective, multi-center pilot study. *Knee Surg Sports Traumatol Arthrosc*. 2021;29(12):4232–4240. doi:10.1007/s00167-021-06530-x
17. Aj B, Tm C, D h K Sr, Ma B, Pg C. A systematic review of the relationship between subchondral bone features, pain and structural pathology in peripheral joint osteoarthritis. *Arthritis Res Therapy*. 2015;17(1). doi:10.1186/s13075-015-0735-x
18. L Z, X G, W C, et al. Variation characteristics of stress distribution in the subchondral bone of the knee joint of judo athletes with long-term stress changes. *Front Endocrinol*. 2023:13. doi:10.3389/fendo.2022.1082799
19. Roemer FW, Neogi T, Nevitt MC, et al. Subchondral bone marrow lesions are highly associated with, and predict subchondral bone attrition longitudinally: the MOST study. *Osteoarthritis Cartilage*. 2010;18(1):47–53. doi:10.1016/j.joca.2009.08.018
20. Löfvall H, Newbould H, Karsdal MA, et al. Osteoclasts degrade bone and cartilage knee joint compartments through different resorption processes. *Arthritis Res Ther*. 2018;20(1):67. doi:10.1186/s13075-018-1564-5
21. Kondo M, Wagers AJ, Manz MG, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu Rev Immunol*. 2003;21:759–806. doi:10.1146/annurev.immunol.21.120601.141007
22. Søe K, Delaisse JM, Borggaard XG. Osteoclast formation at the bone marrow/bone surface interface: importance of structural elements, matrix, and intercellular communication. *Semin Cell Dev Biol*. 2021;112:8–15. doi:10.1016/j.semcdb.2020.05.016
23. Søe K. Osteoclast fusion: physiological regulation of multinucleation through heterogeneity-potential implications for drug sensitivity. *Int J Mol Sci*. 2020;21(20):7717. doi:10.3390/ijms21207717
24. Pal K, Tu Y, Wang X. Single-molecule force imaging reveals that podosome formation requires no extracellular integrin-ligand tensions or interactions. *ACS Nano*. 2022;16(2):2481–2493. doi:10.1021/acsnano.1c09105
25. Anwar A, Sapra L, Gupta N, Ojha RP, Verma B, Srivastava RK. Fine-tuning osteoclastogenesis: an insight into the cellular and molecular regulation of osteoclastogenesis. *J Cell Physiol*. 2023;238(7):1431–1464. doi:10.1002/jcp.31036
26. Kitaura H, Marahleh A, Ohori F, et al. Osteocyte-related cytokines regulate osteoclast formation and bone resorption. *Int J Mol Sci*. 2020;21 (14):5169. doi:10.3390/ijms21145169
27. Liang J, Liu L, Feng H, et al. Therapeutics of osteoarthritis and pharmacological mechanisms: a focus on RANK/RANKL signaling. *Biomed Pharmacother*. 2023;167:115646. doi:10.1016/j.biopha.2023.115646
28. Yang JG, Sun B, Wang Z, et al. Exosome-targeted delivery of METTL14 regulates NFATc1 m6A methylation levels to correct osteoclast-induced bone resorption. *Cell Death Dis*. 2023;14(11):738. doi:10.1038/s41419-023-06263-4
29. Lu J, Wang M, Wang Z, Fu Z, Lu A, Zhang G. Advances in the discovery of cathepsin K inhibitors on bone resorption. *J Enzyme Inhib Med Chem*. 2018;33(1):890–904. doi:10.1080/14756366.2018.1465417
30. Z X, M L, G H, et al. Osteoclasts secrete leukemia inhibitory factor to promote abnormal bone remodeling of subchondral bone in osteoarthritis. *BMC Musculoskeletal Disorders*. 2022;23(1). doi:10.1186/s12891-021-04886-2
31. Barton KI, Shekarforoush M, Heard BJ, et al. Use of pre-clinical surgically induced models to understand biomechanical and biological consequences of PTOA development. *J Orthop Res*. 2017;35(3):454–465. doi:10.1002/jor.23322
32. Bertuglia A, Lacourt M, Girard C, Beauchamp G, Richard H, Laverty S. Osteoclasts are recruited to the subchondral bone in naturally occurring post-traumatic equine carpal osteoarthritis and may contribute to cartilage degradation. *Osteoarthritis Cartilage*. 2016;24(3):555–566. doi:10.1016/j.joca.2015.10.008
33. Karsdal MA, Leeming DJ, Dam EB, et al. Should subchondral bone turnover be targeted when treating osteoarthritis? *Osteoarthritis Cartilage*. 2008;16(6):638–646. doi:10.1016/j.joca.2008.01.014
34. Liu J, Wu X, Lu J, et al. Exosomal transfer of osteoclast-derived miRNAs to chondrocytes contributes to osteoarthritis progression. *Nat Aging*. 2021;1(4):368–384. doi:10.1038/s43587-021-00050-6
35. Zhou F, Han X, Wang L, et al. Associations of osteoclastogenesis and nerve growth in subchondral bone marrow lesions with clinical symptoms in knee osteoarthritis. *J Orthop Translat*. 2022;32:69–76. doi:10.1016/j.jot.2021.11.002
36. Veis DJ, O'Brien CA. Osteoclasts, Master Sculptors of Bone. *Annu Rev Pathol*. 2023;18:257–281. doi:10.1146/annurev-pathmechdis-031521-040919
37. Wu Z, Yuan K, Zhang Q, Guo JJ, Yang H, Zhou F. Antioxidant PDA-PEG nanoparticles alleviate early osteoarthritis by inhibiting osteoclastogenesis and angiogenesis in subchondral bone. *J Nanobiotechnology*. 2022;20(1):479. doi:10.1186/s12951-022-01697-y
38. Fang C, Guo JW, Wang YJ, et al. Diterbutyl phthalate attenuates osteoarthritis in ACLT mice via suppressing ERK/c-fos/NFATc1 pathway, and subsequently inhibiting subchondral osteoclast fusion. *Acta Pharmacol Sin*. 2022;43(5):1299–1310. doi:10.1038/s41401-021-00747-9
39. Tonna S, Poulton IJ, Taykar F, et al. Chondrocytic ephrin B2 promotes cartilage destruction by osteoclasts in endochondral ossification. *Development*. 2016;143(4):648–657. doi:10.1242/dev.125625
40. Dai J, Hu Z, Zeng F, et al. Osteoclast-derived exosomal miR-212-3p suppressed the anabolism and accelerated the catabolism of chondrocytes in osteoarthritis by targeting TGF-β1/Smad2 signaling. *Arch Biochem Biophys*. 2024;751:109827. doi:10.1016/j.abb.2023.109827

41. Hu W, Chen Y, Dou C, Dong S. Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis. *Ann Rheum Dis*. 2021;80(4):413–422. doi:10.1136/annrheumdis-2020-218089
42. Wang J, Lv HZ, Chen W, Fan MK, Li M, Zhang YZ. Anatomical adaptation of fibula and its mechanism of proximal partial fibulectomy associated with medial compartment knee osteoarthritis. *Orthop Surg*. 2019;11(2):204–211. doi:10.1111/os.12437
43. Dong T, Chen W, Zhang F, Yin B, Tian Y, Zhang Y. Radiographic measures of settlement phenomenon in patients with medial compartment knee osteoarthritis. *Clin Rheumatol*. 2016;35(6):1573–1578. doi:10.1007/s10067-015-3146-0
44. Su W, Liu G, Liu X, et al. Angiogenesis stimulated by elevated PDGF-BB in subchondral bone contributes to osteoarthritis development. *JCI Insight*. 2020;5(8). doi:10.1172/jci.insight.135446
45. Tu T, Zhang C, Yan H, et al. CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. *Cell Res*. 2015;25(3):275–287. doi:10.1038/cr.2015.15
46. Nwosu LN, Mapp PI, Chapman V, Walsh DA. Blocking the tropomyosin receptor kinase A (TrkA) receptor inhibits pain behaviour in two rat models of osteoarthritis. *Ann Rheum Dis*. 2016;75(6):1246–1254. doi:10.1136/annrheumdis-2014-207203
47. Zhu S, Zhu J, Zhen G, et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. *J Clin Invest*. 2019;129 (3):1076–1093. doi:10.1172/JCI121561
48. Segond von Banchet G, Boettger MK, König C, Iwakura Y, Bräuer R, Schaible HG. Neuronal IL-17 receptor upregulates TRPV4 but not TRPV1 receptors in DRG neurons and mediates mechanical but not thermal hyperalgesia. *Mol Cell Neurosci*. 2013;52:152–160. doi:10.1016/j.mcn.2012.11.006
49. Yoneda T, Hiasa M, Nagata Y, Okui T, White FA. Acidic microenvironment and bone pain in cancer-colonized bone. *Bonekey Rep*. 2015;4:690. doi:10.1038/bonekey.2015.58
50. Nagae M, Hiraga T, Wakabayashi H, Wang L, Iwata K, Yoneda T. Osteoclasts play a part in pain due to the inflammation adjacent to bone. *Bone*. 2006;39(5):1107–1115. doi:10.1016/j.bone.2006.04.033
51. Dai J, Dong R, Han X, et al. Osteoclast-derived exosomal let-7a-5p targets Smad2 to promote the hypertrophic differentiation of chondrocytes. *Am J Physiol Cell Physiol*. 2020;319(1):C21–C33. doi:10.1152/ajpcell.00039.2020
52. Klose-Jensen R, Hartlev LB, Boel LWT, et al. Subchondral bone turnover, but not bone volume, is increased in early stage osteoarthritic lesions in the human hip joint. *Osteoarthritis Cartilage*. 2015;23(12):2167–2173. doi:10.1016/j.joca.2015.06.001
53. Luo P, Yuan QL, Yang M, Wan X, Xu P. The role of cells and signal pathways in subchondral bone in osteoarthritis. *Bone Joint Res*. 2023;12 (9):536–545. doi:10.1302/2046-3758.129.BJR-2023-0081.R1
54. Zhen G, Wen C, Jia X, et al. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. *Nat Med*. 2013;19(6):704–712. doi:10.1038/nm.3143
55. Scharstuhl A, Glansbeek HL, van Beuningen HM, Vitters EL, van der Kraan PM, van den Berg WB. Inhibition of endogenous TGF-beta during experimental osteoarthritis prevents osteophyte formation and impairs cartilage repair. *J Immunol*. 2002;169(1):507–514. doi:10.4049/jimmunol.169.1.507
56. Zhang RK, Li GW, Zeng C, et al. Mechanical stress contributes to osteoarthritis development through the activation of transforming growth factor beta 1 (TGF-β1). *Bone Joint Res*. 2018;7(11):587–594. doi:10.1302/2046-3758.711.BJR-2018-0057.R1
57. Wang J, Chen W, Hou ZY, Lyu HZ, Zhu YB, Zhang YZ. Law of dynamic deformation of bone. *Chin Med J*. 2019;132(21):2636–2637. doi:10.1097/CM9.0000000000000483
58. Bailey KN, Nguyen J, Yee CS, Dole NS, Dang A, Alliston T. Mechanosensitive control of articular cartilage and subchondral bone homeostasis in mice requires osteocytic transforming growth factor β signaling. *Arthritis Rheumatol*. 2021;73(3):414–425. doi:10.1002/art.41548
59. Li S, Cai X, Guo J, et al. Cell communication and relevant signaling pathways in osteogenesis-angiogenesis coupling. *Bone Res*. 2025;13(1):45. doi:10.1038/s41413-025-00417-0
60. Shi S, Ou X, Liu C, Wen H, Ke J. Research progress of HIF-1a on immunotherapy outcomes in immune vascular microenvironment. *Front Immunol*. 2025;16:1549276. doi:10.3389/fimmu.2025.1549276
61. Nakano T, Fukuda D, Koga JI, Aikawa M. Delta-like ligand 4-notch signaling in macrophage activation. *Arterioscler Thromb Vasc Biol*. 2016;36(10):2038–2047. doi:10.1161/ATVBAHA.116.306926
62. Pesesse L, Sanchez C, Henrotin Y. Osteochondral plate angiogenesis: a new treatment target in osteoarthritis. *Joint Bone Spine*. 2011;78 (2):144–149. doi:10.1016/j.jbspin.2010.07.001
63. Fernández-Torres J, Martínez-Nava GA, Gutiérrez-Ruíz MC, Gómez-Quiroz LE, Gutiérrez M. Role of HIF-1α signaling pathway in osteoarthritis: a systematic review. *Rev Bras Reumatol Engl Ed*. 2017;57(2):162–173. doi:10.1016/j.rbre.2016.07.008
64. M K, P T, W J, et al. Nanoparticle-based inhibition of vascular endothelial growth factor receptors alleviates osteoarthritis pain and cartilage damage. *Sci Adv*. 2024;10(7). doi:10.1126/sciadv.adi5501
65. Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. *Rheumatology*. 2005;44(1):7–16. doi:10.1093/rheumatology/keh344
66. Suri S, Gill SE, Massena de Camin S, Wilson D, McWilliams DF, Walsh DA. Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis. *Ann Rheum Dis*. 2007;66(11):1423–1428. doi:10.1136/ard.2006.063354
67. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet*. 2019;393(10182):1745–1759. doi:10.1016/S0140-6736(19)30417-9
68. Walsh DA, McWilliams DF, Turley MJ, et al. Angiogenesis and nerve growth factor at the osteochondral junction in rheumatoid arthritis and osteoarthritis. *Rheumatology*. 2010;49(10):1852–1861. doi:10.1093/rheumatology/keq188
69. Fransès RE, McWilliams DF, Mapp PI, Walsh DA. Osteochondral angiogenesis and increased protease inhibitor expression in OA. *Osteoarthritis Cartilage*. 2010;18(4):563–571. doi:10.1016/j.joca.2009.11.015
70. Lu J, Zhang H, Cai D, et al. Positive-feedback regulation of subchondral H-type vessel formation by chondrocyte promotes osteoarthritis development in mice. *J Bone Miner Res*. 2018;33(5):909–920. doi:10.1002/jbmr.3388
71. Saito M, Sasho T, Yamaguchi S, et al. Angiogenic activity of subchondral bone during the progression of osteoarthritis in a rabbit anterior cruciate ligament transection model. *Osteoarthritis Cartilage*. 2012;20(12):1574–1582. doi:10.1016/j.joca.2012.08.023
72. Cui Z, Crane J, Xie H, et al. Halofuginone attenuates osteoarthritis by inhibition of TGF-β activity and H-type vessel formation in subchondral bone. *Ann Rheum Dis*. 2016;75(9):1714–1721. doi:10.1136/annrheumdis-2015-207923
73. Qin X, Xi Y, Jiang Q, Chen C, Yang G. Type H vessels in osteogenesis, homeostasis, and related disorders. *Differentiation*. 2023;134:20–30. doi:10.1016/j.diff.2023.09.005

74. Romeo SG, Alawi KM, Rodrigues J, Singh A, Kusumbe AP, Ramasamy SK. Endothelial proteolytic activity and interaction with non-resorbing osteoclasts mediate bone elongation. *Nat Cell Biol*. 2019;21(4):430–441. doi:10.1038/s41556-019-0304-7
75. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. *J Neuroimmune Pharmacol*. 2014;9(2):142–160. doi:10.1007/s11481-014-9531-7
76. Min JH, Lee CH, Ji YW, et al. Activation of Dll4/notch signaling and hypoxia-inducible factor-1 alpha facilitates lymphangiogenesis in lacrimal glands in dry eye. *PLoS One*. 2016;11(2):e0147846. doi:10.1371/journal.pone.0147846
77. Wang Y, Xu J, Zhang X, et al. TNF-α-induced LRG1 promotes angiogenesis and mesenchymal stem cell migration in the subchondral bone during osteoarthritis. *Cell Death Dis*. 2017;8(3):e2715. doi:10.1038/cddis.2017.129
78. Barranco C. Osteoarthritis: animal data show VEGF blocker inhibits post-traumatic OA. *Nat Rev Rheumatol*. 2014;10(11):638. doi:10.1038/nrrheum.2014.173
79. Cui Z, Wu H, Xiao Y, et al. Endothelial PDGF-BB/PDGFR-β signaling promotes osteoarthritis by enhancing angiogenesis-dependent abnormal subchondral bone formation. *Bone Res*. 2022;10(1):58. doi:10.1038/s41413-022-00229-6
80. Xie H, Cui Z, Wang L, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat Med*. 2014;20(11):1270–1278. doi:10.1038/nm.3668
81. Park KW, Crouse D, Lee M, et al. The axonal attractant netrin-1 is an angiogenic factor. *Proc Natl Acad Sci U S A*. 2004;101(46):16210–16215. doi:10.1073/pnas.0405984101
82. Layne K, Ferro A, Passacquale G. Netrin-1 as a novel therapeutic target in cardiovascular disease: to activate or inhibit? *Cardiovasc Res*. 2015;107(4):410–419. doi:10.1093/cvr/cvv201
83. Gao B, Deng R, Chai Y, et al. Macrophage-lineage TRAP+ cells recruit periosteum-derived cells for periosteal osteogenesis and regeneration. *J Clin Invest*. 2019;129(6):2578–2594. doi:10.1172/JCI98857
84. Rindone AN, Liu X, Farhat S, et al. Quantitative 3D imaging of the cranial microvascular environment at single-cell resolution. *Nat Commun*. 2021;12(1):6219. doi:10.1038/s41467-021-26455-w
85. Zhen G, Dan Y, Wang R, et al. An antibody against Siglec-15 promotes bone formation and fracture healing by increasing TRAP+ mononuclear cells and PDGF-BB secretion. *Bone Res*. 2021;9(1):47. doi:10.1038/s41413-021-00161-1
86. Santhanam L, Liu G, Jandu S, et al. Skeleton-secreted PDGF-BB mediates arterial stiffening. *J Clin Invest*. 2021;131(20):e147116. doi:10.1172/JCI147116
87. Clarke J. PDGF-BB is the key to unlocking pathological angiogenesis in OA. *Nat Rev Rheumatol*. 2020;16(6):298. doi:10.1038/s41584-020-0423-3
88. Zhang W, Robertson WB, Zhao J, Chen W, Xu J. Emerging trend in the pharmacotherapy of osteoarthritis. *Front Endocrinol*. 2019;10:431. doi:10.3389/fendo.2019.00431
89. McMahon SB. NGF as a mediator of inflammatory pain. *Philos Trans R Soc Lond B Biol Sci*. 1996;351(1338):431–440. doi:10.1098/rstb.1996.0039
90. Barthel C, Yeremenko N, Jacobs R, et al. Nerve growth factor and receptor expression in rheumatoid arthritis and spondyloarthritis. *Arthritis Res Ther*. 2009;11(3):R82. doi:10.1186/ar2716
91. Sanga P, Katz N, Polverejan E, et al. Efficacy, safety, and tolerability of fulranumab, an anti-nerve growth factor antibody, in the treatment of patients with moderate to severe osteoarthritis pain. *Pain*. 2013;154(10):1910–1919. doi:10.1016/j.pain.2013.05.051
92. Tiseo PJ, Kivitz AJ, Ervin JE, Ren H, Mellis SJ. Fasinumab (REGN475), an antibody against nerve growth factor for the treatment of pain: results from a double-blind, placebo-controlled exploratory study in osteoarthritis of the knee. *Pain*. 2014;155(7):1245–1252. doi:10.1016/j.pain.2014.03.018
93. Zhao L, Huang J, Fan Y, et al. Exploration of CRISPR/Cas9-based gene editing as therapy for osteoarthritis. *Ann Rheum Dis*. 2019;78(5):676–682. doi:10.1136/annrheumdis-2018-214724
94. Ashraf S, Mapp PI, Burston J, Bennett AJ, Chapman V, Walsh DA. Augmented pain behavioural responses to intra-articular injection of nerve growth factor in two animal models of osteoarthritis. *Ann Rheum Dis*. 2014;73(9):1710–1718. doi:10.1136/annrheumdis-2013-203416
95. Suokas AK, Walsh DA, McWilliams DF, et al. Quantitative sensory testing in painful osteoarthritis: a systematic review and meta-analysis. *Osteoarthritis Cartilage*. 2012;20(10):1075–1085. doi:10.1016/j.joca.2012.06.009
96. Jin X, Gereau RW. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. *J Neurosci*. 2006;26(1):246–255. doi:10.1523/JNEUROSCI.3858-05.2006
97. von Banchet GS, Fischer N, Uhlig B, Hensellek S, Eitner A, Schaible HG. Molecular effects of interleukin-1β on dorsal root ganglion neurons: prevention of ligand-induced internalization of the bradykinin 2 receptor and downregulation of G protein-coupled receptor kinase 2. *Mol Cell Neurosci*. 2011;46(1):262–271. doi:10.1016/j.mcn.2010.09.009
98. Woolf CJ, Safieh-Garabedian B, Ma QP, Crilly P, Winter J. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. *Neuroscience*. 1994;62(2):327–331. doi:10.1016/0306-4522(94)90366-2
99. Xu J, Cheng T, Feng HT, Pavlos NJ, Zheng MH. Structure and function of V-ATPases in osteoclasts: potential therapeutic targets for the treatment of osteolysis. *Histol Histopathol*. 2007;22(4):443–454. doi:10.14670/HH-22.443
100. Qin A, Cheng TS, Lin Z, et al. Prevention of wear particle-induced osteolysis by a novel V-ATPase inhibitor saliphenylhalamide through inhibition of osteoclast bone resorption. *PLoS One*. 2012;7(4):e34132. doi:10.1371/journal.pone.0034132
101. Sun Q, Li G, Liu D, et al. Peripheral nerves in the tibial subchondral bone: the role of pain and homeostasis in osteoarthritis. *Bone Joint Res*. 2022;11(7):439–452. doi:10.1302/2046-3758.117.BJR-2021-0355.R1
102. You B, Zhou C, Yang Y. MSC-EVs alleviate osteoarthritis by regulating microenvironmental cells in the articular cavity and maintaining cartilage matrix homeostasis. *Ageing Res Rev*. 2023;85:101864. doi:10.1016/j.arr.2023.101864
103. J Z, Z S, L W. Harnessing stem cell-derived extracellular vesicles for the regeneration of degenerative bone conditions. *Int j Nanomed*. 2023;18. doi:10.2147/IJN.S424731
104. José alcaraz M. Control of articular degeneration by extracellular vesicles from stem/stromal cells as a potential strategy for the treatment of osteoarthritis. *Biochem Pharmacol*. 2024;228:116226. doi:10.1016/j.bcp.2024.116226
105. N Z, Z S, L S, et al. Exosomes: roles and therapeutic potential in osteoarthritis. *Bone Res*. 2020;8:8. doi:10.1038/s41413-020-0100-9

106. Fu Y, Cui S, Zhou Y, Qiu L. Dental pulp stem cell-derived exosomes alleviate mice knee osteoarthritis by inhibiting TRPV4-mediated osteoclast activation. *Int J Mol Sci*. 2023;24(5):4926. doi:10.3390/ijms24054926
107. He L, He T, Xing J, et al. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. *Stem Cell Res Ther*. 2020;11(1):276. doi:10.1186/s13287-020-01781-w
108. Li ZL, Li XT, Hao RC, et al. Human osteoarthritic articular cartilage stem cells suppress osteoclasts and improve subchondral bone remodeling in experimental knee osteoarthritis partially by releasing TNFAIP3. *Stem Cell Res Ther*. 2023;14(1):253. doi:10.1186/s13287-023-03411-7
109. Jiang W, Jin Y, Zhang S, et al. PGE2 activates EP4 in subchondral bone osteoclasts to regulate osteoarthritis. *Bone Res*. 2022;10(1):27. doi:10.1038/s41413-022-00201-4
110. Ding D, Yan J, Feng G, Zhou Y, Ma L, Jin Q. Dihydroartemisinin attenuates osteoclast formation and bone resorption via inhibiting the NF-κB, MAPK and NFATc1 signaling pathways and alleviates osteoarthritis. *Int J Mol Med*. 2022;49(1):4. doi:10.3892/ijmm.2021.5059
111. Zhou L, Liu Q, Yang M, et al. Dihydroartemisinin, an anti-malaria drug, suppresses estrogen deficiency-induced osteoporosis, osteoclast formation, and RANKL-induced signaling pathways. *J Bone Miner Res*. 2016;31(5):964–974. doi:10.1002/jbmr.2771
112. Shapiro E, Biezuner T, Linnarsson S. Single-cell sequencing-based technologies will revolutionize whole-organism science. *Nat Rev Genet*. 2013;14(9):618–630. doi:10.1038/nrg3542
113. Gu Y, Hu Y, Zhang H, Wang S, Xu K, Su J. Single-cell RNA sequencing in osteoarthritis. *Cell Prolif*. 2023;56(12):e13517. doi:10.1111/cpr.13517
114. Hu Y, Cui J, Liu H, et al. Single-cell RNA-sequencing analysis reveals the molecular mechanism of subchondral bone cell heterogeneity in the development of osteoarthritis. *RMD Open*. 2022;8(2):e002314. doi:10.1136/rmdopen-2022-002314
115. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med*. 2018;50(8):1–14. doi:10.1038/s12276-018-0071-8
116. Wang Y, Wang Q, Xu Q, Li J, Zhao F. Single-cell RNA sequencing analysis dissected the osteo-immunology microenvironment and revealed key regulators in osteoporosis. *Int Immunopharmacol*. 2022;113(Pt A):109302. doi:10.1016/j.intimp.2022.109302
117. Tsukasaki M, Huynh NCN, Okamoto K, et al. Stepwise cell fate decision pathways during osteoclastogenesis at single-cell resolution. *Nat Metab*. 2020;2(12):1382–1390. doi:10.1038/s42255-020-00318-y