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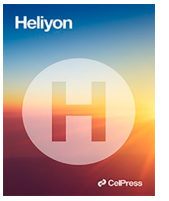

Review article

The bone nonunion microenvironment: A place where osteogenesis struggles with osteoclastic capacity

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ABSTRACT

Bone nonunion is a common and serious orthopedic disorder, the occurrence of which is associated with a disruption of the dynamic balance between osteoblasts and osteoclasts during bone repair. However, the critical molecular mechanisms affecting this homeostasis are not well understood, and it is essential to investigate the specific components of this mechanism and to restore the balance between osteoblasts and osteoclasts to promote bone repair. First, we defined this complex local environmental factor as the "bone nonunion microenvironment" and identified the importance of the "struggle" between osteoblasts and osteoclasts, which is the most essential element in determining the process of *repair*. On this basis, we also explored the cellular factors that influence osteogenesis and the molecular signals that influence the balance between *osteoclast* and osteoblasts, which are important for restoring homeostasis. Further, we explored other factors involved in osteogenesis, such as the biomechanical environment, the nutritional environment, the acid-base environment, and the temperature environment, which are important players in osteogenesis. In conclusion, we found that the balance between osteoblasts and osteoclasts is the essence of bone healing, which is based on the "bone nonunion microenvironment". Therefore, investigating the role of the bone nonunion microenvironment in the system of osteoblast-osteoclast "struggle" provides an important basis for further understanding of the mechanism of nonunion and the development of new therapeutic approaches.

1. Introduction

Fracture is the most common trauma in orthopedics, and surveys have shown that new fracture cases have risen by more than 30 % globally in the last two decades, and the incidence is still increasing year by year [1]; the probability of postoperative nonunion or delayed healing of fracture (collectively referred to as bone nonunion) is also 5–10 % of all fractures, and can be as high as 30 % [2], which is a healthcare issue concerning the quality of life of patients suffering from fracture around the globe. The diagnostic criteria used *widely* in academia *is* still from the US Food and Drug Administration (FDA): the fracture has not healed at 9 months after the

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fracture and the imaging *results* of the last 3 months do not show signs of healing [3]. *The data suggest that bone nonunion are more common in older individuals, which may be related to age-related declines in blood supply* [4]. *Similarly, research also indicates a high correlation between osteoporosis, a condition that causes weak bone tissue and may make fracture healing more challenging, and bone nonunion* [5]. Fracture healing is a complex process that requires the synergy of multiple factors, such as age, health status and choice of treatment [6]. They are generally categorized into systemic, local, and medical factors, among which local factors may play a major role [7]. Local conditions such as changes in blood flow, growth factor levels, mechanical stability, and infections can all be involved in the healing process [8]. We *describe* this complex of factors that *facilitate the emergence* of nonunion as the "bone nonunion microenvironment"*(BNM)*.

The BNM is a complex environment composed of cells, tissues, blood, and various factors around the fracture, which is an important reason for interfering with the balance of osteogenesis and *osteoclastic* capacity, and has the following characteristics: excessive release of inflammatory factors and prolonged inflammatory response to inhibit osteoblasts differentiation [9]; imbalance in the metabolism of extracellular matrix (including collagen (*COL*), fibronectin) leading to the loss of bone matrix [10]; the ability of bone marrow mesenchymal stem cells (BMSCs) differentiate into osteoblasts and chondrocytes is *diminished* [11]; the nutrition (especially minerals and proteins) and oxygen provided by the blood for bone cells proliferation are greatly *decreased* [12]; the massive increase of immune cells (mainly referring to macrophages and neutrophils) and immune factors causes excessive bone resorption [13]; and other physicochemical factors, such as temperature and pH, interfere with bone cells proliferation, differentiation, and migration [14]. In conclusion, *the* BNM is a complex environmental system that contains important mechanisms for the occurrence of bone nonunion.

Although there has been some research on the pathogenic mechanisms of nonunion, our detailed understanding is still limited [15]. *There are still many unanswered questions, such as the molecular and cellular mechanisms of nonunion occurrence and the specific causes of abnormal blood supply*. The balance between osteogenesis and osteoclastic capacity is critical in the bone healing. It mainly consists of the formation of new bone and the breakdown and resorption of old bone. These two processes are mutually restrained and together maintain the dynamic balance of bone tissue. *This* balance of osteogenesis and osteoclast capacity *was named* "struggle".

Specifically, the activity and number of osteoblasts increase significantly after bone repair is initiated to promote bone formation and these osteoblasts are mainly derived from BMSCs and periosteal tissue. At the same time, osteoblasts stimulate the metabolism of the fracture end, cell proliferation and differentiation, and the formation of new bone through the secretion of cytokines and growth factors (e.g. transforming growth factor-β, bone morphogenetic protein). On the other hand, osteoclasts *(OC)* break down and resorb old bone to remove *necrotic* tissue from the fracture and creating space for new bone. However, when the cells are in an abnormal environment, this balance is disturbed, and a "struggle" develops. For example, if osteogenesis is insufficient, new bone formation is slowed down or stopped, *and overpowered OC can lead to* the resorption of old bone exceeds the capacity of osteogenesis, which ultimately leads to slow healing or nonunion (Fig. 1).

At present, the specific mechanism by which this local complex factor affects the "struggle" between osteoblasts and osteoclasts is not clear, so further investigate the mechanism by which the BNM leads to the imbalance between osteogenesis and osteoclasts *is necessary*. *This* is important for *preventing and curing* nonunion by regulating the BNM to restore the balance between osteogenesis and

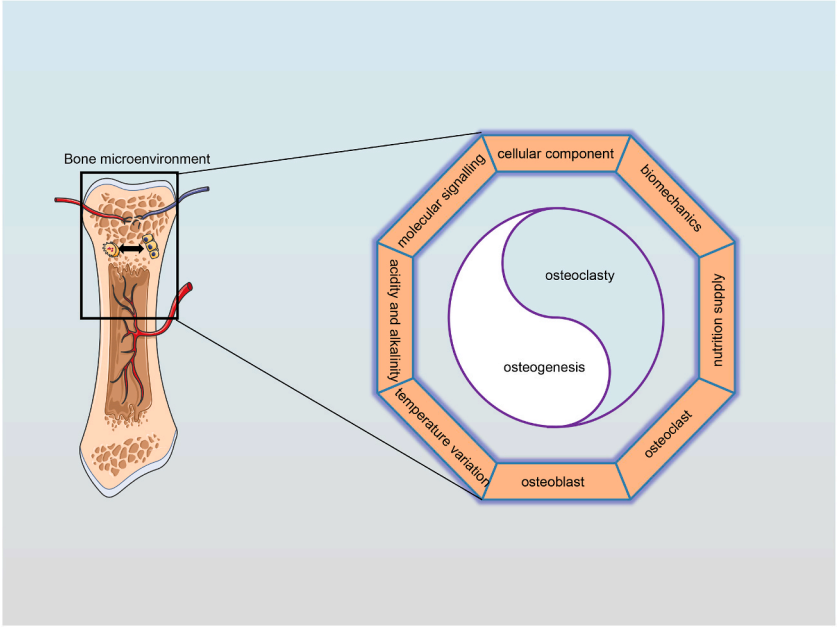

Fig. 1. A necessary prerequisite for bone repair when osteoblasts and osteoclasts are in balance. The balance between the two is regulated by the local microenvironment, such as other cells in the microenvironment, molecular signals, nutritional factors, biomechanics, and acid-base conditions. In addition, there are other factors (e.g., temperature) that can be categorized as "bone nonunion microenvironment."

osteoclasts.

2. Cellular components of the BNM

2.1. Characterization of osteoblasts and osteoclasts

Osteoblasts are mainly derived from *pre-osteoblasts* formed by the differentiation of progenitor cells in the bone marrow, which are the main functional cells for bone formation and are responsible for the synthesis and mineralization of the bone matrix, thus forming the basic skeletal structure. As an initiator of the differentiation of progenitor cells into osteoblasts, the expression of Runt-associated transcription factor 2 (RUNX2) implies the beginning of osteoblast formation [16]. It promotes osteoblast formation by encoding a series of factors such as osteocalcin, sclerostin and bone morphogenetic protein (BMP) [17]. Co-expression of alkaline phosphatase (ALP) and type I collagen then indicates the complete formation of osteoblasts, which are then transformed to the bone matrix with the involvement of numerous factors [18]. In addition, osteoblasts themselves have an active *secretory* function, and the autocrine factors it synthesizes, including type I collagen, osteocalcin, osteonectin, and osteopontin can promote the formation and mineralization of the bone matrix [10]; there are also some paracrine factors, such as insulin-like growth factor 1/2(IGF 1/2) [19], fibroblast growth factor, and interleukin-1 [20], and they have a positive osteogenesis differentiation.

Osteoclasts are giant multinucleated cells originating from hematopoietic stem cells in the bone marrow. Upon initiation of bone repair, osteoclasts begin to activate upon contact with the bone matrix, with reorganization of the cytoskeleton, formation of cell polarity, and the emergence of membrane regions specific for bone resorption [21]. The RANKL/RANK/OPG pathway is recognized as a major pathway regulating osteoclast formation, interestingly, this receptor for nuclear factor κ-B ligand (RANKL) can also be activated at the membrane of osteoblasts, thus promoting osteoclast formation [22]. During bone repair, osteoblasts degrade and remove necrotic bone tissue by secreting cathepsin K (CTSK) [23], and they can also regulate the activity of osteoblasts [10]. Besides, mediating the migration of stem cells from bone marrow to blood circulation is also an important physiological function of osteoclasts [24].

2.2. Osteoblasts and osteoclasts interact in the BNM

There are multiple modes of coupling between osteoblasts and osteoclasts. On molecular biological level, it is the mutual contact of transmembrane proteins of the two cells. Initially, researchers found that the two are balanced by the release of growth factors such as IGF 1/2 and CTSK embedded in the bone matrix to promote bone formation [25], but later *research has* demonstrated that this is not the only connection. Existing research has *shown* that osteoblasts and osteoclasts also directly regulate bone formation through multiple pairs of transmembrane proteins, such as EFNB2-EPHB4, FAS-FASL and NRP1-SEMA3A [26]. It has been found that at the *onset of repair*, osteogenesis signals are transmitted from tyrosine kinase receptor B4 (EPHB4) on the surface of osteoblasts to hepcidin B2 (EFNB2) on the surface of osteoclasts, which inhibits C-FOS/NFATC1 production by osteoclasts, thereby shifting the balance toward bone formation [27]. *c-Fos and NFATc1 are key factors in osteoclasts differentiation, and their synergistic action promotes the translocation of NFATc1 from the cytoplasm into the nucleus and the transcription of osteoclast-specific genes such as CTSK* [28]. *The Fas/FasL system provides an important apoptotic mechanism for a variety of cells, including immune cells and OC, and research has shown that the Fas/FasL pathway can be induced to participate in apoptotic homeostasis of osteogenesis and osteoclasts by caspases, as well as by calmodulin* [29]. Osteoblasts and osteoclasts are also *connected* through the tumor necrosis factor receptor family member FAS and its receptor FASL, which is based on the principle that the FAS-FASL signaling cascade reaction mediates apoptosis of osteoclasts, so that osteoclast activity and bone resorption are increased when FASL of the osteoblasts is inhibited by the abnormal environment [30]. Additionally, Sema3A is a power secretory osteoprotective factor. Research have shown that semaphorin 3A (SEMA3A), which is generated by osteoblast spectrum cells, can stimulates bone production via the wnt/β-catenin signaling pathway and inhibits *osteoclast* differentiation by binding to neurociliin-1 (NRP1) [31]. In addition, several indirect factors, including complement component 3a (C3a), osteoprotegerin (OPG), and macrophage colony-stimulating factor (M-CSF), convey the communication between the two (described in more detail in section 3). Because of the intricacy of the BNM, there appear to be more possible targets among the elements impacting the balance between *osteoblasts* and osteoclasts that are worth investigating as essential conditions for deciding whether new bone develop.

Since inflammation and *immune* response happen first, dysregulation of these processes is the main microenvironmental factor impeding osteogenesis following nonunion. *Research has indicated that while pro-inflammatory factors (*e.g.*, TNF-α and IL-1β) persist and stimulate RANKL, which leads to excessive osteoclast activation, reduced or unaffected osteoblast differentiation, and inhibition of bone formation, appropriate inflammatory signals promote osteoblast proliferation and maintain bone homeostasis* [32]. *Similarly*, macrophages, neutrophils, and T cells in the immune system play a major role in maintaining the equilibrium between *osteoclasts* and osteoblasts. Remarkably, bone homeostasis is influenced by macrophage polarization states in two separate ways. Pro-inflammatory macrophages, *also known as* M1-type macrophages, induce increased osteoclast activity and local inflammation by secreting significant levels of pro-inflammatory cytokines, such as TNF-α and IL-6 [13]. A local ischemic and hypoxic environment of nonunion appears to inhibit the differentiation of M2-type macrophages, even though these macrophages, also called anti-inflammatory macrophages, can promote inflammation to subside [33]. This results in the failure to promote osteoblast activity. A balance between osteogenesis and osteoclasts toward bone resorption can also be achieved by immune-derived pro-inflammatory substances (e.g., IL-1) in the local microenvironment by stimulating osteoclast activity through the RANKL [34]. These demonstrate in detail how the immunological and inflammatory milieu fundamentally upsets the equilibrium between osteogenesis and osteoclastogenic potential, eventually resulting in the formation of nonunion.

Since oxidative stress levels, pH, and temperature fluctuate after the initiation of nonunion, these variables may also be part of the BNM that influences the balance of osteogenesis and osteoclastic capability. The degree of vascular disruption following a fracture determines how much oxygen and nutrients are directly blocked from the area, which causes localized oxidative stress and metabolic disruptions. Studies have indicated that hypoxia stimulates the synthesis of osteoclast cytokines, including growth differentiation factor-15 (GDF-15) and RANKL, which in turn increases osteoclast development [35]. *Hypoxia-inducible factor-1α (HIF-1α) is the oxygen-regulated isoform of the HIF-1 transcription factor. In healthy settings, HIF-1α encourages osteoblast differentiation and proliferation by controlling the expression of genes that are important for these processes, such as alkaline phosphatase and BMP* [36]. *HIF-1α expression is downregulated in cells due to metabolic reprogramming of osteoblasts under hypoxic circumstances, which prevents osteoblast development into bone* [37]. *HIF-1α could stimulate osteoclast activation and creation by controlling the expression of genes linked to osteoclast differentiation, such as osteoclast-stimulating factor (OSF) and RANKL* [38]. *This process accelerates the breakdown of the bone matrix.* Simultaneously, a hypoxic environment might cause dysregulation of lactate metabolism, which raises local acidity. Ultimately, body acidosis stimulates the development of osteoclasts by means of RANKL *pathway* [39]. *And interestingly, there is also related research showing that excessive hypoxia produces severely limiting effects on osteoclast growth unrelated to hypoxia-inducible factors* [40]. *This study identifies the range of physiological oxygen tension in osteoclasts at the cellular level, allowing for a clearer line of research on osteoblast and osteoclast interactions in hypoxic environments* [41]. As *individuals* age, *it is widely recognized* that *there is a natural decline in core body temperature*. What is less well understood is how these temperature fluctuations affect osteogenesis. A possible explanation for the imbalance between *osteoblasts* and osteoclasts is that a local temperature drop brought on by altered blood supply and metabolism strongly suppresses osteoblast differentiation, whereas osteoclasts are largely unaffected [42].

In conclusion, it is *clear* that the various complex factors that comprise the microenvironment surrounding *bone nonunion can* disturb the equilibrium between osteogenesis and osteoclastic capability via distinct mechanisms. Whether *these factors promote* osteoclastic capability or *hinder* osteogenesis, *their ultimate consequence is the impediment of new bone formation*. Studying the specific processes by which the BNM influences the equilibrium between osteogenesis and osteoclastic capability is essential and crucial.

2.3. The role of other cells in the BNM

As an important source of many kinds of bone cells, the differentiation of BMSCs determines the formation of various kinds of bone cells (including chondrocytes and adipocytes), but it is also deeply regulated by the BNM. Research has found that macrophages in the early stage of fracture are induced to polarize into M1 by inflammatory factors, which further increases the secretion of pro-

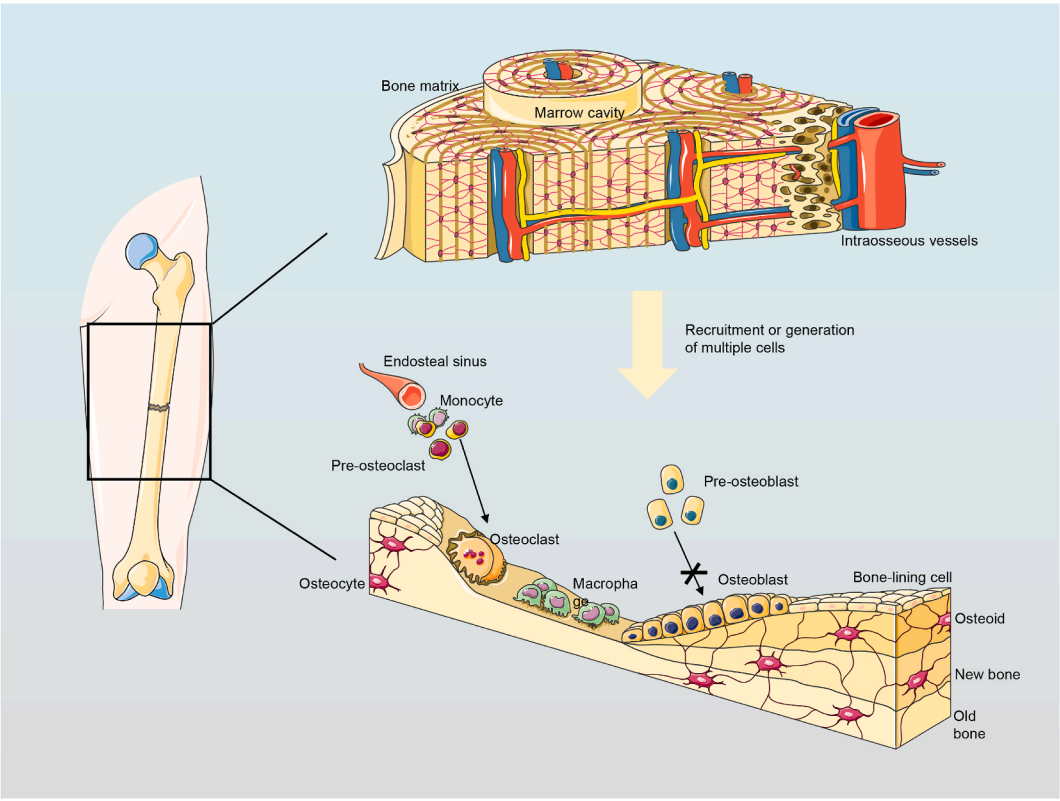

Fig. 2. In addition to osteoblasts and osteoclasts, other cellular components of the bone nonunion microenvironment affect bone repair, such as ***pre-osteoblasts***, ***pre-osteoclasts***, and immune cells.

inflammatory factors and hinders the differentiation of BMSCs into osteogenesis [43], which is essentially similar to the fact that persistent inflammation hinders the differentiation of BMSCs [44]. In the hypoxic environment, chondrocytes can undergo apoptosis ***through the action of*** HIF-1α and HIF-2α, ***which enhance glycolysis and chondrocyte maturation*** [45]. ***Moreover***, matrix metalloproteinase (MMPS) activated by various inflammatory factors can also degrade chondrocytes outside their matrix. Furthermore, chondrocytes undergo apoptosis in an acidic environment because to the action of the main CTSK [46], but chondrocytes also degrade bone matrix due to the highly active acid-sensitive ion channels (ASICs) [47]. As opposed to the complex BNM, the same environment can also mediate the death of bone cells and block the osteogenesis process through different pathways and modalities. There are some other cells that can affect the osteogenesis through direct or indirect ways, such as adipocytes can secrete many cytokines including leptin, resistin and lipocalin, among which leptin can directly block apoptosis of osteoblasts and promote their proliferation [48]; peroxidase in the microenvironment can activate resistin receptor γ and thus inhibit the differentiation of BMSCs into osteoblasts [11]; lipocalin can inhibit the role of osteoblasts through the OPG/RANK/RANKL pathway, but promote osteogenesis [49].

Parallel to this, it is necessary to consider how other cells, including smooth muscle, vascular endothelium, and neural cells, affect osteogenesis (Fig. 2). It is ***clear*** that cellular elements play a multifaceted function in the BNM. Numerous cells can affect the balance between osteogenesis and osteoclast competence to affect the process of bone formation locally following a fracture; however, the mechanisms by which these cells act vary depending on the microenvironment. A thorough grasp of the function and regulatory processes of these cells can serve as a crucial theoretical foundation for the creation of more potent therapies for nonunion.

3. Molecular signaling in the BNM

In addition to the previously discussed molecular interactions between osteoblasts and osteoclasts, various growth factors, cytokines, and hormonal interactions within the microenvironment play a crucial role in disturbing the balance between osteogenesis and osteoclastic activity. These factors exert their effects through diverse signaling pathways, directly or indirectly affecting the equilibrium.

3.1. Osteoblast-associated molecules and signaling

The first thing is the function of osteoblast-associated cytokines, which are essential to the process of bone production. As the most well-known members of the transforming growth factor-β family, BMPs can directly stimulate the production of osteoblasts. ***Research has found that BMP, as an autologous bone graft substitute or biomaterial companion, has a favorable healing-promoting effect on nonunion*** [50]. As one of the most traditional members, BMP-2 can further stimulate osteogenesis by boosting the production of the downstream osteogenesis-related protein ALP via the BMP-2/Smad/RUNX2 signaling pathway [51]. Interestingly, BMP-7 activates the PI3K/Akt pathway to inhibit osteoblast death in addition to inducing the synthesis of ALP, a hallmark of osteoblast development [52]. But the inflammatory and acidic milieu of bone nonunion greatly reduces these inducing capacities. Furthermore, BMP-4, 5, and 6 as well as additional members of the BMP family have been demonstrated to be osteogenesis regulators.

It is believed that osteoblasts can release the glycoprotein known as OPG. ***Notably, abnormal expression of OPG has been shown to be associated with dysregulated probiotic flora and sex hormones, capable of leading to impaired bone remodeling*** [53]. OPG overexpression primarily suppresses osteoclast development and differentiation via the RANK/RANKL pathway, which subsequently and indirectly encourages BMSC differentiation toward osteogenesis [54]. It has been discovered that reduced OPG expression raises RANKL expression and osteoclast numbers, indicating that OPG functions as a kind of inhibitor on osteoclast differentiation [55]. The predominant manifestations include a notable decrease in bone density and a decrease in OPG expression resulting from variations in postmenopausal hormone levels [56]. This implies that metabolic hormone dysregulation in the BNM is one of the key elements contributing to problems with osteogenesis. Conversely, we may observe that it would be advantageous to further encourage bone growth by controlling hormone levels [56].

Macrophage colony-stimulating factor (M-CSF) is a cytokine with multiple biological functions, which play a role in osteoblast and osteoclast differentiation, respectively, and has been widely used clinically to intervene in microbial infections, inflammation, and immune response processes. By interacting to tyrosine kinases in the osteoblast precursor's membrane, M-CSF activates and phosphorylates osteoblasts and creates a binding site for phosphatidylinositol 3-kinase (PI3K) [57]. The RANK in bone marrow precursors is upregulated by M-CSF because of the immune cells' massive aggregation in the BNM. This promotes the differentiation of bone marrow precursor cells and drum-breaking precursor cells and speeds up bone resorption [22]. Furthermore, M-CSF can contribute to the last phases of osteoclast development by directly binding to the highly active RANK/RANKL pathway in the BNM or indirectly activating the Akt/ERK pathway in tandem with RANKL [58]. It follows that a key element disrupting the equilibrium of bone healing is the quantity of macrophage colony components in the microenvironment, which causes distinct osteoclast differentiation capabilities.

The Wnt pathway can control several cellular processes in disorders linked to the heart and bones, as well as cell behavior and destiny. This has been more thoroughly studied in relation to these disorders. Among them, the Wnt/β-catenin pathway is one of the most classical pathways regulating osteoblast differentiation and bone formation. The release of β-catenin from cells in a complex with phosphorylated glycogen synthase kinase-30 (GSK-30), axonin, etc., and the initiation of downstream target genes, such as RUNX-2, which mediates processes like apoptosis, are the outcomes of Wnt signaling, which is triggered by the interaction of secreted Wnt ligand with Fzd and Lrp surface receptors [59]. Through various bone formation-associated factors like RNUX2, osteogenesis-associated transcription factors osteixl (OSX), and OPG, the Wnt/β-catenin signaling pathway promotes the differentiation of BMSCs into ***pre-osteoblasts*** and osteoblast [60]. The ability of Wnt to be activated by Fzd and Lrp is significantly reduced if the

local metabolic environment [61], including glucose metabolism, amino acid metabolism, and fatty acid metabolism, is dysregulated. Additionally, the activation of rapamycin complex 1 (mTORC1) by multiple Wnt proteins (including Wnt1, 7, and 16) to promote bone formation, as well as the activation of mTORC2 in response to mechanical loading, are inhibited [62].

Other osteogenesis chemical signals include LGR4/RANKL/RANK, SEMA3A/Nrp, and Lysophosphatidic acid (LPA), which influence the production of bone by controlling the activity of osteoblasts and/or osteoclasts. Leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4), for instance, is one of the RANKL receptors whose expression reduces osteoclast activity [63]. Blocking osteoclast differentiation through the SEMA3A/Nrp1/DAP12 axis is one way that semaphorins (particularly SEMA3A), might do this [31]. It has been shown that the bioactive phospholipid LPA stimulates osteogenesis through RhoA/ROCK1/β-catenin [64], however it has also been demonstrated that LPA causes morphologic alterations in mature osteoclasts and prevents osteoclast apoptosis [65]. Further investigation is required to elucidate the precise mechanism.

In summary, osteoblast molecules are the signals most directly involved in bone formation, and although they are not the only determinants of osteogenesis, and some can even influence osteoclast function, their variable diversity in the BNM makes them a more important uncertainty.

3.2. Osteoclast-associated molecules and signaling

As is previously known, the primary route for osteoclast differentiation is the RANKL/RANK pathway. The inducible factor stimulates osteoclast differentiation by binding to the membrane receptor RANK on osteoclast precursors and presenting RANKL in the membrane of osteoblasts [22]. ***M-CSF is the*** most closely linked to the RANKL/RANK pathway, it is mainly secreted by monocyte macrophages to promote osteoclast differentiation, and so is the most common member of osteoclast-associated molecular signaling [57]. Sphingosine 1 phosphate (S1P) is a key molecule secreted by osteoclast precursors, which on the one hand promotes osteoblast activation and RANKL activation, and further promotes ***osteoclast genesis*** through the RANKL/RANK pathway [66]. ***Furthermore***, S1P can also resist the deficiency of osteoclast CTSK action, enabling osteoclasts to maintain bone resorption activity under conditions of CTSK inhibition [67]. ***These findings strongly indicate that factors influencing the RANKL/RANK pathway can serve as critical*** molecular signals ***in the*** regulation of osteoclasts.

Semaphorin (SEMA) is a key axon guidance protein during the development of the nervous system, however, some members of the SEMA family have been found to play important regulatory roles in osteoclast differentiation and bone resorption. Research has found

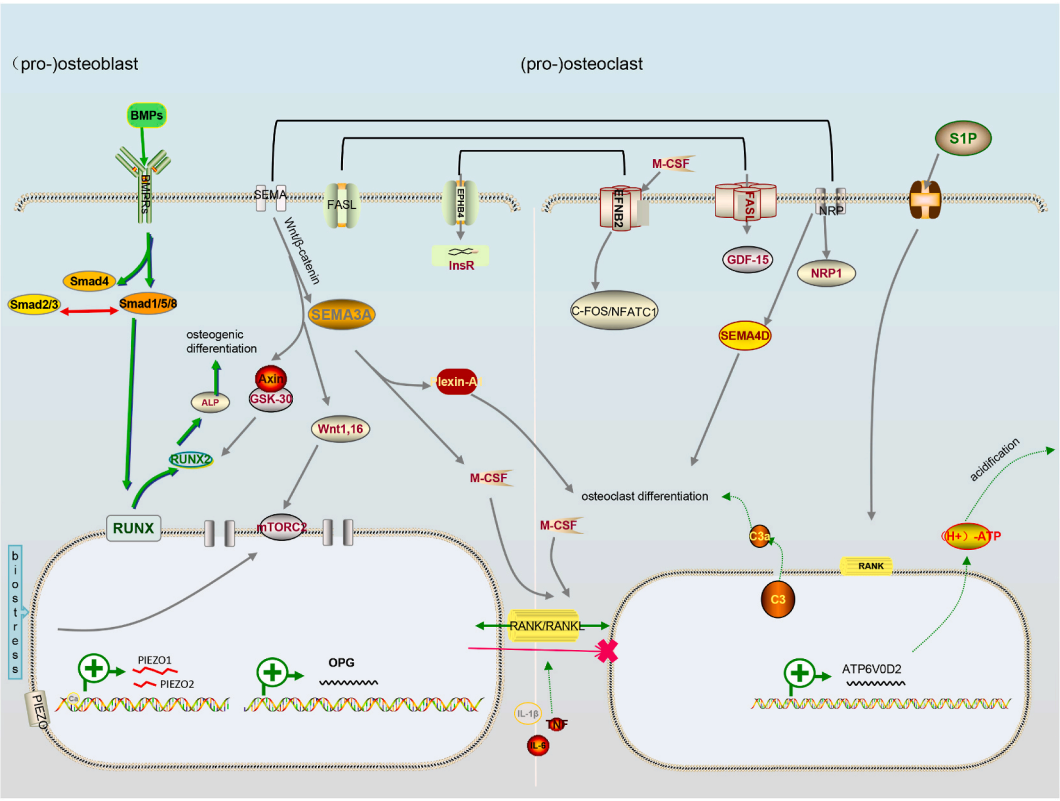

Fig. 3. Molecular signaling in the bone nonunion microenvironment is a direct pathway to the bone repair process, with osteoblast-associated signaling promoting bone formation and osteoclast signaling promoting bone resorption. In addition, there are complex pathway associations between the two (e.g., RANKL/RANK).

that SEMA4D is highly expressed in osteoblasts, which on the one hand directly enhances osteoclast activity and thus inhibits bone nodulation [68], and on the other hand inhibits the expression of osteoblast-related proteins (e.g., ALP) by binding to Plexin-B1 (PLXNB1) on the surface of the osteoblasts and activating the small GTPase RHOA [69]. Interestingly, another member of the SEMA family, SEMA3A has been shown to play the opposite role, with SEMA3A inhibiting M-CSF induced differentiation of osteoclast precursors into osteoclasts via the RhoA pathway, but it also competes via the SEMA3A/Nrp1/Plexin-A1 axis to inhibit the PlexinA1-TREM1-DAP1 complex formation, thereby preventing osteoclast differentiation [70]. Thus, the direction of these regulatory mechanisms in the dysregulated BNM such as inflammation and immunity is an important research hotspot.

Complement Component C (C3) is considered a key peptide structure for osteoclast maturation, and its expression is significantly upregulated during ***osteoclast genesis***. Research has found that osteoclast derived C3 can also be cleaved into the active fragment C3a to promote ALP expression in osteoblasts [71]. However, the expression of C3 is markedly upregulated when the BNM is dysregulated locally by hormone levels (particularly sex hormones), which accelerates bone loss [26]. In this context, the addition of C3aR agonists reverses the situation by converting C3 into more C3a, which further increases ALP and osteogenesis [71]. Consequently, we can draw inspiration from the regulation of the equilibrium between osteogenesis and osteoblasts through the modulation of hormone levels in the BNM.

In addition, ATP6V0D2 (ATPase H+ transporter V0 subunit D2) is a gene encoding a vesicular (H+)-ATPase transporter protein found mainly in endosomal organelles such as vacuoles, lysosomes, and endosomes. It has been found that ATP6V0D2 may be associated with osteoclast maturation, because in ATP6V0D2-deficient mice, there is a reduction in mature giant osteoclasts without altered osteoblast differentiation [72]. More importantly, the V-ATPase also targets the extracellular environment responsible for acidifying parts of the cell, which may be hugely linked to the low pH of the BNM [73]. Collagen Triple Helix Repeat Containing 1 (CTHRC1) is a soluble protein released by mature osteoclasts that targets and induces osteoblast differentiation. Normally, CTHRC1 expression is upregulated when mature osteoclasts are exposed to hydroxyapatite and calcium, whereas its deficiency leads to a reduction in bone mass, which is important for maintaining bone mass under physiological conditions [26]. Therefore, their role in the dysregulated microenvironment also deserves our attention.

Apart from the osteoclast signaling molecules, other inflammatory and immune-related variables play crucial regulatory functions for osteoclasts, as monocyte macrophages are the primary source of osteoclasts. Some researchers have shown that osteoclasts, like a variety of immune cells (e.g., natural killer cells, T lymphocytes), are co-regulated by several pro- and anti-inflammatory factors [74]. For example, research has found that interleukin (e.g., IL-1β, IL-6) and tumor necrosis factors (TNF-α) pro-inflammatory factors regulate osteoclast resorption through the RANKL/OPG pathway, whereas anti-inflammatory factors (e.g., IL-4, IL-10) have the opposite effect [75].

In summary, many molecular cues govern osteoclast activity and eventually impact osteoclast development, maturation, and bone resorption by either identical or distinct routes (Fig. 3). The objectives we need to concentrate on, which dictate the course of osteogenesis and osteoclast homeostasis development, are the intricate BNM and alterations in the interaction of various molecular signals. Additionally, these chemical cues play a significant role in the BNM.

4. Biomechanical factors in the BNM

Biomechanics plays a paramount role in the functioning of bones within the human body. It encompasses various forms of mechanical forces, including tension, torsion, shear, and leverage, among others. The mechanical properties of different parts of the bone and muscle determine the movement, load, direction, and force of the organism. Bone tissue is very sensitive to mechanical stress stimuli, and the differentiation of osteoblasts and osteoclasts, as well as their bone resorption or formation functions are closely related to mechanical stress [76]. Wolfe's law also states that lack of mechanical stress leads to bone microstructural degradation, mass loss and metabolic disorders. Therefore, the role between mechanical stress and the bone microenvironment is reciprocal [77]. As early as 2010, Ardem Patapoutian discovered and named the mechanical stress-sensitive proteins PIZEO (PIEZO1 and PIEZO2), of which PIEZO1 is expressed by non-sensory tissues and can sense a variety of mechanical stresses including hydrostatic pressure, shear stress, and membrane stretch. In 2021, David Julius and Ardem Patapoutian revealed another innovative mechanism for the role of PIEZO receptors in biological stress changes [78], and PIEZO proteins are becoming an important mediator in research on ***mechanic transduction*** regulation of bone repair.

Research has found that PIEZO1 deficiency in the osteoblast lineage (including BMSCs, osteoblasts, and ***pre-osteoblasts***) prevents COL2 and COL9 production by decreasing the nuclear localization of the sensor YAP, leading to an increase in osteoclast activity without substantial effects on osteoclast bone resorption function [76]. However, knockdown of Piezo1 from osteoblasts and osteocytes did not eliminate the skeletal response to mechanical stimuli, suggesting that PIEZO1 is not the only ***transducers*** [79]. Although PIEZO1 and PIEZO2 gene expression was detected in bone tissue, PIEZO1 expression was significantly higher than PIEZO2 in osteoblasts and osteocytes [80]. In addition, research has found that appropriate local tension-compression stress stimulation given to fracture patients can promote fracture healing, as evidenced by a significant increase in the volume and density of bone scabs and promote fibrous scab osteogenesis [81]. Similarly, research has also found that post-fracture stress stimulation can promote the expression of Cbf-α1, osteocalcin, OPG, etc. in osteoblasts and inhibit the expression of RANKL in osteoclasts [82]. These clearly show that the local biostability of the fracture is lost when nonunion occurs, and that aberrant protein production associated with mechanical stress also plays a significant role in the milieu around nonunion.

In conclusion, biomechanical stability is crucial for bone healing. Mechanistically, disturbances in mechanosensitive pathways and their associated proteins are important contributors to the imbalance between osteogenesis and osteoclastic ability, and they are also important to assess the BNM. ***Thus, investigating the role of mechanosensitive pathways is critical for elucidating the underlying***

mechanisms of bone nonunion.

5. Other regulators of the BNM

Inadequate nutrition supply is a major contributor to the imbalance between osteoblasts and osteoclasts, which is one of the negative effects immediately coming from significant vascularization shortage following fracture. For instance, a major reduction in the blood's calcium supply during nonunion causes an imbalance in the metabolism of calcium and phosphorus, an overexpression of RANKL on the surface of osteoblasts, and a decrease in vitamin D secretion, all of which contribute to an increase in resorption of bone [12]. According to research, both iron excess and deficiency can directly or indirectly impair iron metabolism, which can result in reduced bone mineralization and osteogenesis. However, bringing iron levels back within the normal range can encourage the production of bone matrix and bone mineralization [83].

The local environment quickly becomes acidic due to the anaerobic fermentation of sugar, which produces lactic acid generation and buildup. Fractures are localized due to vascular damage, generating an ischemia and hypoxic hematoma. Simply said, an imbalance in the acid-base environment may either improve or worsen many different types of cell activity. Studies have indicated that when the pH of the surrounding environment falls below 6.9, the osteoblast functions are impacted, which includes reduced collagen production, decreased ALP activity, and inhibited mineralization [14]. However, osteoclast absorption is increased. Studies have revealed that osteoblasts secrete more interleukin-6 (IL-6) and cathepsin B in acidic environments, which suggests that acidic environments may stimulate localized inflammatory responses, and influence bone metabolism [78].

Changes in the temperature microenvironment are an accurate indicator of the type or location of a lesion because they represent the body's metabolic release of energy. The factors that typically influence the temperature microenvironment are immune transporters, cellular metabolism, blood perfusion, and inflammatory response [15]. According to certain studies, there is a kinetic link between local temperature and thermal changes during the bone regeneration; that is, distinct healing phases exhibit distinct temperature variations [84]. For example, bone nonunion occurs with a general increase in local temperature, which affects cell activity and migration during healing by influencing the activity and migration of cells such as osteoblast and BMSC, as well as local oxygen saturation, and ultimately proliferation in the osteoblast spectrum [85].

In conclusion, unfavorable changes in other environmental elements may also contribute to the formation of bone nonunion. Nutritional circumstances, acidic and alkaline environments, and temperature factors can all have a substantial influence on bone healing. Furthermore, because the local environment is a complex system, there are a multitude of possible elements that might impact

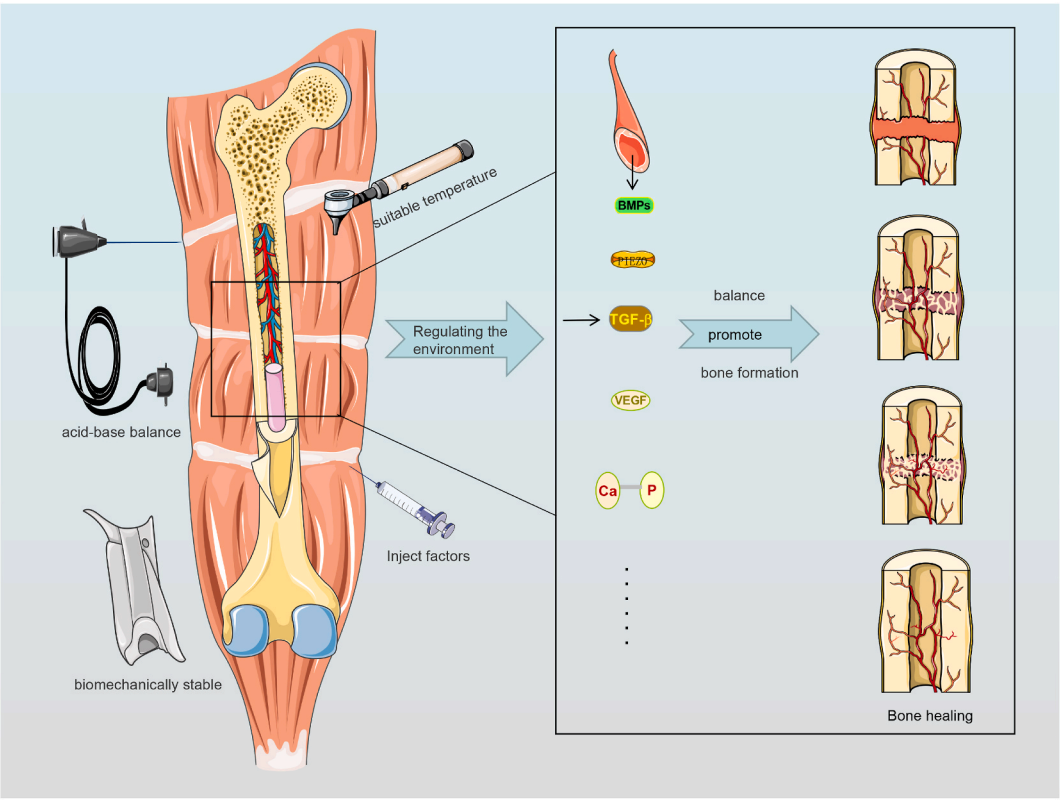

Fig. 4. Repair of bone ***nonunion*** can be facilitated by modulating a range of microenvironments such as neutralizing acidity, stabilizing biomechanics, and appropriate anti-inflammatory therapy.

the bone healing process. All the elements that make up the microenvironment around bone nonunion must be taken into consideration as they have the potential to impact the crucial balance between osteogenesis and osteoblasts (Fig. 4).

6. Summary and conclusion

Bone nonunion is a significant post-fracture complication characterized by a challenging healing process and a high morbidity rate. The physical, chemical, and biological responses at the fracture site are exceedingly unstable and unpredictable due to the incredibly complex processes that influence bone healing and cause nonunion. ***Consequently,*** we refer to the numerous elements influencing fracture healing as the "bone nonunion microenvironment" and consider the summary of this microenvironment to be a crucial resource for future elaboration on the mechanism behind the occurrence of nonunion. The balance between osteoblasts and osteoclasts, which is the most crucial requirement for fracture healing—that is, osteogenesis by osteoblasts must exceed bone resorption by osteoclasts for the bone repair process to move toward positive osteogenesis—is, first and foremost, the most significant component of the BNM. The primary signals that are particularly engaged are the ways in which osteoblasts and osteoclasts couple, such as EFNB2-EPHB4, FAS-FASL, and NRP1-SEMA3A [26]. Furthermore, as an important source of osteoblasts that can indirectly gauge the status of bone repair, it is imperative to investigate the growth and differentiation of osteoblast lineage cells in the BNM, specifically BMSCs [43], chondrocytes [47], and adipocytes [48]. Second, further research is required to determine the variables influencing the "struggle" between osteoblasts and osteoclasts.

In essence, the balance between osteogenesis and osteoclasts is determined by the composition of the surrounding factors, such as the most basic osteogenesis (including but not limited to BMPs [51], OPG [54], and M-CSF [57]) and the molecular signals associated with osteoclasts (including but not limited to RANKL/RANK [22], SEMA [68], and C3 [71]). Furthermore, it is impossible to ignore the subject of biomechanical variables in bone formation, and we also need to pay close attention to the biomechanical environment in the BNM. Changes to the mechanical milieu affect the development conditions of cells within the osteoblast spectrum, and the identification of PIZEO receptors and biomechanical sensors has prompted further investigation into bone disorders [78]. Next, we should concentrate on the disruption of osteogenesis and osteoblast homeostasis caused by external influences, including temperature, acid-base, and nutritional variables, all of which are crucial for the local metabolism of bone damage. More research is required to determine whether there are any more unidentified variables interfering with the process of bone regeneration in BNM.

In briefly, we refer to this crucial element influencing the equilibrium between osteogenesis and osteoblasts as the "bone nonunion microenvironment" as it contains a variety of elements, signals, and cell development and activity. Secondly, we discovered that the characteristics of the BNM consist of a methodical arrangement of factors such as temperature, nutrient environments, acid-base environments, osteoblast and osteoclast homeostasis, and molecular signals related to osteogenesis and osteoclasts. Ultimately, we must investigate the modifications in the "struggle" between osteoblasts and osteoclasts inside the intricate milieu of bone nonunion. These are the theoretical resources that will help us understand the pathophysiology of bone nonunion and investigate more potent treatment options.

Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Kang Cheng: Writing – review & editing, Writing – original draft, Visualization, Software. **Silong Gao:** Visualization, Software. **Yongliang Mei:** Resources, Investigation. **Daqian Zhou:** Resources, Formal analysis. **Chao Song:** Software, Methodology. **Daru Guo:** Writing – review & editing, Investigation. **Yunqing Hou:** Methodology, Conceptualization. **Zongchao Liu:** Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] G.B.D.F. Collaborators, Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019, Lancet Healthy Longev 2 (9) (2021) e580–e592.
[2] B. Wildemann, et al., Non-union bone fractures, Nat. Rev. Dis. Prim. 7 (1) (2021) 57.
[3] J.D. Thomas, J.L. Kehoe, *Bone nonunion*, in *StatPearls*, Treasure Island (FL) ineligible companies. Disclosure: James Kehoe declares no relevant financial relationships with ineligible companies (2024).
[4] R. Tian, et al., Prevalence and influencing factors of nonunion in patients with tibial fracture: systematic review and meta-analysis, J. Orthop. Surg. Res. 15 (1) (2020) 377.
[5] B. Dozza, et al., Nonunion fracture healing: evaluation of effectiveness of demineralized bone matrix and mesenchymal stem cells in a novel sheep bone nonunion model, J Tissue Eng Regen Med 12 (9) (2018) 1972–1985.
[6] J.A. Nicholson, et al., Fracture nonunion in long bones: a literature review of risk factors and surgical management, Injury 52 (Suppl 2) (2021) S3–S11.
[7] M. Rupp, et al., Diaphyseal long bone nonunions - types, aetiology, economics, and treatment recommendations, Int. Orthop. 42 (2) (2018) 247–258.

[8] R. Zura, et al., Epidemiology of fracture nonunion in 18 human bones, JAMA Surg 151 (11) (2016) e162775.
[9] K. Yokota, et al., Characterization and function of tumor necrosis factor and interleukin-6-induced osteoclasts in rheumatoid arthritis, Arthritis Rheumatol. 73 (7) (2021) 1145–1154.
[10] E.F. Eriksen, Cellular mechanisms of bone remodeling, Rev. Endocr. Metab. Disord. 11 (4) (2010) 219–227.
[11] C. Xie, Q. Chen, Adipokines: new therapeutic target for osteoarthritis? Curr. Rheumatol. Rep. 21 (12) (2019) 71.
[12] J. Delgado-Calle, T. Bellido, The osteocyte as a signaling cell, Physiol. Rev. 102 (1) (2022) 379–410.
[13] M. Locati, G. Curtale, A. Mantovani, Diversity, mechanisms, and significance of macrophage plasticity, Annu. Rev. Pathol. 15 (2020) 123–147.
[14] F. Perut, et al., Citrate supplementation restores the impaired mineralisation resulting from the acidic microenvironment: an in vitro study, Nutrients 12 (12) (2020).
[15] E.A. Repasky, S.S. Evans, M.W. Dewhirst, Temperature matters! And why it should matter to tumor immunologists, Cancer Immunol. Res. 1 (4) (2013) 210–216.
[16] T. Komori, Runx2, an inducer of osteoblast and chondrocyte differentiation, Histochem. Cell Biol. 149 (4) (2018) 313–323.
[17] G. Chen, C. Deng, Y.P. Li, TGF-beta and BMP signaling in osteoblast differentiation and bone formation, Int. J. Biol. Sci. 8 (2) (2012) 272–288.
[18] C. Halling Linder, K. Enander, P. Magnusson, Glycation contributes to interaction between human bone alkaline phosphatase and collagen type I, Calcif. Tissue Int. 98 (3) (2016) 284–293.
[19] G. Xi, C.J. Rosen, D.R. Clemmons, IGF-I and IGFBP-2 stimulate AMPK activation and autophagy, which are required for osteoblast differentiation, Endocrinology 157 (1) (2016) 268–281.
[20] Y.H. Cheon, et al., WHI-131 promotes osteoblast differentiation and prevents osteoclast formation and resorption in mice, J. Bone Miner. Res. 31 (2) (2016) 403–415.
[21] D.J. Hadjidakis, I.I. Androulakis, Bone remodeling, Ann. N. Y. Acad. Sci. 1092 (2006) 385–396.
[22] B.F. Boyce, Advances in the regulation of osteoclasts and osteoclast functions, J. Dent. Res. 92 (10) (2013) 860–867.
[23] J.F. Charles, A.O. Aliprantis, Osteoclasts: more than 'bone eaters', Trends Mol. Med. 20 (8) (2014) 449–459.
[24] C.S. Bahney, et al., Cellular biology of fracture healing, J. Orthop. Res. 37 (1) (2019) 35–50.
[25] M. Gomarasca, G. Banfi, G. Lombardi, Myokines: the endocrine coupling of skeletal muscle and bone, Adv. Clin. Chem. 94 (2020) 155–218.
[26] J.M. Kim, et al., Osteoblast-osteoclast communication and bone homeostasis, Cells 9 (9) (2020).
[27] M. Blank, N.A. Sims, Cellular processes by which osteoblasts and osteocytes control bone mineral deposition and maturation revealed by stage-specific EphrinB2 knockdown, Curr. Osteoporos. Rep. 17 (5) (2019) 270–280.
[28] Y. Zhang, et al., BHLHE40 promotes osteoclastogenesis and abnormal bone resorption via c-Fos/NFATc1, Cell Biosci. 12 (1) (2022) 70.
[29] N.S. Soysa, N. Alles, Positive and negative regulators of osteoclast apoptosis, BoneKEy Rep. 11 (2019) 100225.
[30] D. Zhao, et al., A novel lncRNA GM15416 regulates osteoblast apoptosis and differentiation through the c-Fos/Fas axis and mitigates osteoporosis, Int. J. Biol. Macromol. 254 (Pt 2) (2023) 127824.
[31] M. Hayashi, et al., Osteoprotection by semaphorin 3A, Nature 485 (7396) (2012) 69–74.
[32] Y. Xia, et al., TGFbeta reprograms TNF stimulation of macrophages towards a non-canonical pathway driving inflammatory osteoclastogenesis, Nat. Commun. 13 (1) (2022) 3920.
[33] O.R. Colegio, et al., Functional polarization of tumour-associated macrophages by tumour-derived lactic acid, Nature 513 (7519) (2014) 559–563.
[34] L. Gong, et al., The macrophage polarization regulates MSC osteoblast differentiation in vitro, Ann. Clin. Lab. Sci. 46 (1) (2016) 65–71.
[35] J.H. Park, N.K. Lee, S.Y. Lee, Current understanding of RANK signaling in osteoclast differentiation and maturation, Mol. Cell 40 (10) (2017) 706–713.
[36] Y. Miyauchi, et al., HIF1alpha is required for osteoclast activation by estrogen deficiency in postmenopausal osteoporosis, Proc. Natl. Acad. Sci. U. S. A. 110 (41) (2013) 16568–16573.
[37] J. Tao, et al., Spatiotemporal correlation between HIF-1alpha and bone regeneration, Faseb. J. 36 (10) (2022) e22520.
[38] K. Doi, et al., Role of lysine-specific demethylase 1 in metabolically integrating osteoclast differentiation and inflammatory bone resorption through hypoxia-inducible factor 1alpha and E2F1, Arthritis Rheumatol. 74 (6) (2022) 948–960.
[39] S. Stegen, G. Carmeliet, Hypoxia, hypoxia-inducible transcription factors and oxygen-sensing prolyl hydroxylases in bone development and homeostasis, Curr. Opin. Nephrol. Hypertens. 28 (4) (2019) 328–335.
[40] W. Da, L. Tao, Y. Zhu, The role of osteoclast energy metabolism in the occurrence and development of osteoporosis, Front. Endocrinol. 12 (2021) 675385.
[41] J.J. Patel, et al., Hypothermia inhibits osteoblast differentiation and bone formation but stimulates osteoclastogenesis, Exp. Cell Res. 318 (17) (2012) 2237–2244.
[42] L. Chen, et al., Amorphous calcium phosphate NPs mediate the macrophage response and modulate BMSC osteogenesis, Inflammation 44 (1) (2021) 278–296.
[43] X. Liu, et al., BMSC transplantation aggravates inflammation, oxidative stress, and fibrosis and impairs skeletal muscle regeneration, Front. Physiol. 10 (2019) 87.
[44] X. Zhou, et al., D-mannose alleviates osteoarthritis progression by inhibiting chondrocyte ferroptosis in a HIF-2alpha-dependent manner, Cell Prolif. 54 (11) (2021) e13134.
[45] X.Y. Yuan, et al., Design, synthesis and biological evaluation of inhibitors of cathepsin K on dedifferentiated chondrocytes, Bioorg. Med. Chem. 27 (6) (2019) 1034–1042.
[46] X. Wu, et al., The role of Ca(2+) in acid-sensing ion channel 1a-mediated chondrocyte pyroptosis in rat adjuvant arthritis, Lab. Invest. 99 (4) (2019) 499–513.
[47] I.R. Reid, P.A. Baldock, J. Cornish, Effects of leptin on the skeleton, Endocr. Rev. 39 (6) (2018) 938–959.
[48] H. Mukohira, et al., Mesenchymal stromal cells in bone marrow express adiponectin and are efficiently targeted by an adiponectin promoter-driven Cre transgene, Int. Immunol. 31 (11) (2019) 729–742.
[49] S. Chai, et al., Gushukang inhibits osteocyte apoptosis and enhances BMP-2/Smads signaling pathway in ovariectomized rats, Phytomedicine 64 (2019) 153063.
[50] C.E. Gillman, A.C. Jayasuriya, FDA-approved bone grafts and bone graft substitute devices in bone regeneration, Mater. Sci. Eng., C 130 (2021) 112466.
[51] J. Kureel, et al., miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation, Cell Death Dis. 5 (2) (2014) e1050.
[52] T. Ono, et al., RANKL biology: bone metabolism, the immune system, and beyond, Inflamm. Regen. 40 (2020) 2.
[53] N. Amin, et al., Probiotics and bone disorders: the role of RANKL/RANK/OPG pathway, Aging Clin. Exp. Res. 32 (3) (2020) 363–371.
[54] N. Udagawa, et al., Osteoclast differentiation by RANKL and OPG signaling pathways, J. Bone Miner. Metabol. 39 (1) (2021) 19–26.
[55] C.H. Cheng, L.R. Chen, K.H. Chen, Osteoporosis due to hormone imbalance: an overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover, Int. J. Mol. Sci. 23 (3) (2022).
[56] S.H. Mun, P.S.U. Park, K.H. Park-Min, The M-CSF receptor in osteoclasts and beyond, Exp. Mol. Med. 52 (8) (2020) 1239–1254.
[57] L. Chai, et al., Psoralen and bakuchiol ameliorate M-CSF plus RANKL-induced osteoclast differentiation and bone resorption via inhibition of AKT and AP-1 pathways in vitro, Cell. Physiol. Biochem. 48 (5) (2018) 2123–2133.
[58] L.Y. Lawson, et al., Osteoblast-specific wnt secretion is required for skeletal homeostasis and loading-induced bone formation in adult mice, J. Bone Miner. Res. 37 (1) (2022) 108–120.
[59] I.M.N. Molagoda, et al., Fisetin promotes osteoblast differentiation and osteogenesis through GSK-3beta phosphorylation at Ser9 and consequent beta-catenin activation, inhibiting osteoporosis, Biochem. Pharmacol. 192 (2021) 114676.
[60] R.C. Riddle, et al., Tsc2 is a molecular checkpoint controlling osteoblast development and glucose homeostasis, Mol. Cell Biol. 34 (10) (2014) 1850–1862.
[61] C.M. Karner, F. Long, Wnt signaling and cellular metabolism in osteoblasts, Cell. Mol. Life Sci. 74 (9) (2017) 1649–1657.
[62] J. Luo, et al., LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption, Nat. Med. 22 (5) (2016) 539–546.
[63] Y. Xie, et al., Lysophosphatidic acid receptor 4 regulates osteogenic and adipogenic differentiation of progenitor cells via inactivation of RhoA/ROCK1/beta-catenin signaling, Stem Cell. 38 (3) (2020) 451–463.

[64] D.M. Lapierre, et al., Lysophosphatidic acid signals through multiple receptors in osteoclasts to elevate cytosolic calcium concentration, evoke retraction, and promote cell survival, J. Biol. Chem. 285 (33) (2010) 25792–25801.
[65] J.M. Grewe, et al., The role of sphingosine-1-phosphate in bone remodeling and osteoporosis, Bone Res 10 (1) (2022) 34.
[66] S. Lotinun, et al., Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation, J. Clin. Invest. 123 (2) (2013) 666–681.
[67] P. Llamas-Granda, et al., Tenofovir modulates semaphorin 4D signaling and regulates bone homeostasis, which can Be counteracted by dipyridamole and adenosine A2A receptor, Int. J. Mol. Sci. 22 (21) (2021).
[68] E. Terpos, et al., Semaphorin 4D correlates with increased bone resorption, hypercalcemia, and disease stage in newly diagnosed patients with multiple myeloma, Blood Cancer J. 8 (5) (2018) 42.
[69] Z. Li, et al., The role of semaphorin 3A in bone remodeling, Front. Cell. Neurosci. 11 (2017) 40.
[70] K. Matsuoka, et al., Osteoclast-derived complement component 3a stimulates osteoblast differentiation, J. Bone Miner. Res. 29 (7) (2014) 1522–1530.
[71] X. Sun, et al., Pregnenolone inhibits osteoclast differentiation and protects against lipopolysaccharide-induced inflammatory bone destruction and ovariectomy-induced bone loss, Front. Pharmacol. 11 (2020) 360.
[72] K. Jahn, et al., Osteocytes acidify their microenvironment in response to PTHrP in vitro and in lactating mice in vivo, J. Bone Miner. Res. 32 (8) (2017) 1761–1772.
[73] M.B. Madel, et al., Immune function and diversity of osteoclasts in normal and pathological conditions, Front. Immunol. 10 (2019) 1408.
[74] Y. Yao, et al., The macrophage-osteoclast Axis in osteoimmunity and osteo-related diseases, Front. Immunol. 12 (2021) 664871.
[75] W. Li, et al., Osteocytes promote osteoclastogenesis via autophagy-mediated RANKL secretion under mechanical compressive force, Arch. Biochem. Biophys. 694 (2020) 108594.
[76] X. Li, et al., Stimulation of Piezo1 by mechanical signals promotes bone anabolism, Elife 8 (2019).
[77] J.M. Kefauver, A.B. Ward, A. Patapoutian, Discoveries in structure and physiology of mechanically activated ion channels, Nature 587 (7835) (2020) 567–576.
[78] L. Wang, et al., Mechanical sensing protein PIEZO1 regulates bone homeostasis via osteoblast-osteoclast crosstalk, Nat. Commun. 11 (1) (2020) 282.
[79] E. Sugisawa, et al., RNA sensing by gut Piezo1 is essential for systemic serotonin synthesis, Cell 182 (3) (2020) 609–624 e21.
[80] E.Y. Chao, N. Inoue, Biophysical stimulation of bone fracture repair, regeneration and remodelling, Eur. Cell. Mater. 6 (2003) 72–84. ; discussion 84-5.
[81] J. Jin, et al., Shear stress modulates osteoblast cell and nucleus morphology and volume, Int. J. Mol. Sci. 21 (21) (2020).
[82] Z. Chen, et al., Effects of zinc, magnesium, and iron ions on bone tissue engineering, ACS Biomater. Sci. Eng. 8 (6) (2022) 2321–2335.
[83] M.G. Ledesma-Colunga, et al., Shaping the bone through iron and iron-related proteins, Semin. Hematol. 58 (3) (2021) 188–200.
[84] D. Haluzan, et al., Thermal changes during healing of distal radius fractures-Preliminary findings, Injury 46 (Suppl 6) (2015) S103–S106.
[85] D.R. Epari, et al., Pressure, oxygen tension and temperature in the periosteal callus during bone healing–an in vivo study in sheep, Bone 43 (4) (2008) 734–739.