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Cellular crosstalk in the bone marrow niche

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Abstract

The bone marrow niche is a special microenvironment that comprises elements, including hematopoietic stem cells, osteoblasts, and endothelial cells, and helps maintain their characteristic functions. Here, we elaborate on the crosstalk between various cellular components, hematopoietic stem cells, and other cells in the bone marrow niche. We further explain the mechanism of preserving equilibrium in the bone marrow niche, which is crucial for the directional regulation of bone reconstruction and repair. Additionally, we elucidate the intercommunication among osteocytes, the regulation of osteoblast maturation and activation by lymphocytes, the deficiency of megakaryocytes that can markedly impair osteoblast formation, and the mechanism of interaction between macrophages and mesenchymal stem cells in the bone marrow niche. Finally, we discussed the new immunotherapies for bone tumors in the BM niche. In this review, we aimed to provide a candid overview of the crosstalk among bone marrow niche cells and to highlight new concepts underlying the unknown mechanisms of hematopoiesis and bone reconstruction. Thus, this review may provide a more comprehensive understanding of the role of these niche cells in improving hematopoietic function and help identify their therapeutic potential for different diseases in the future.

Keywords Niche, Haematopoietic stem cells, Bone marrow, Bone reconstruction and repair

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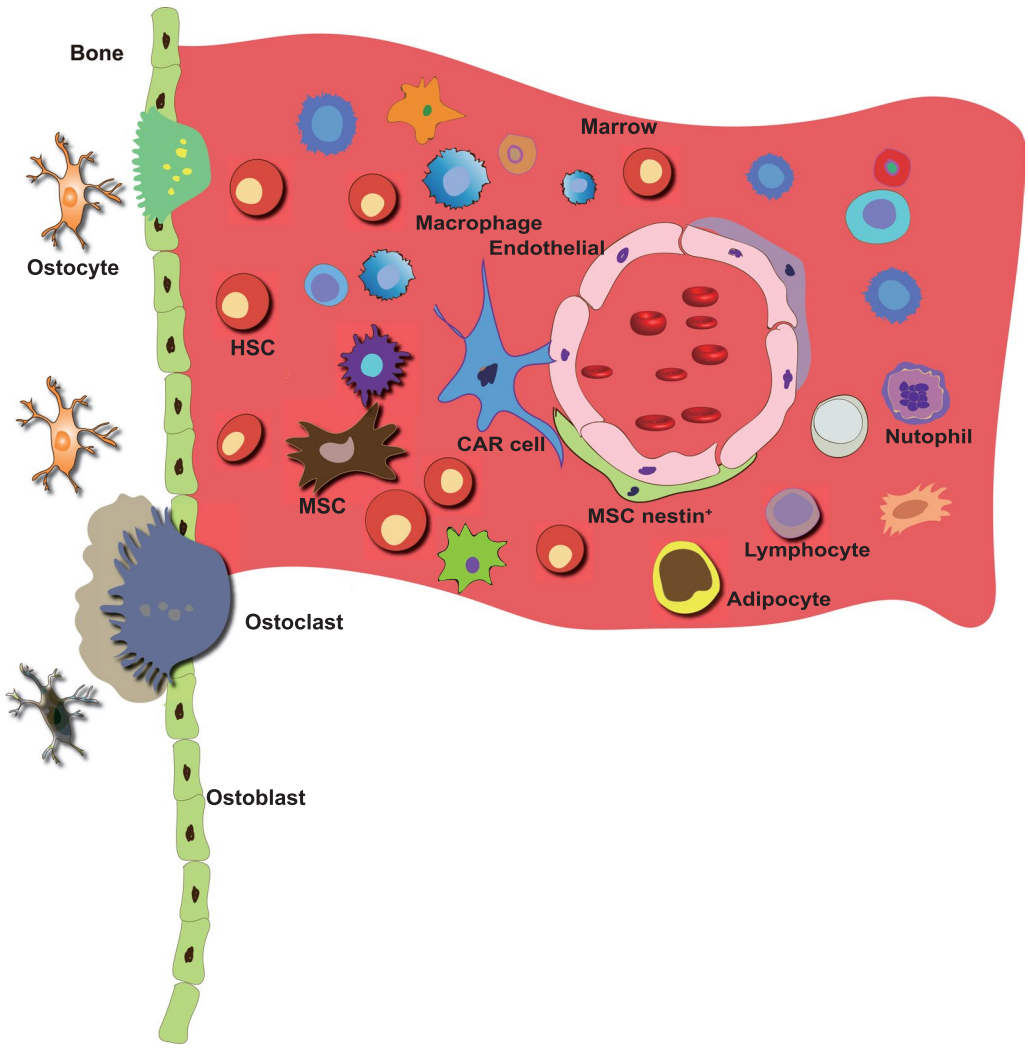
Graphical Abstract



Introduction

Osteoporosis is a representative disease of bone remodeling imbalance caused by aging or disease status [1]. Currently, no effective treatment exists to reverse bone metabolism imbalance and induce the directional reconstruction and repair of bone [2]. Nevertheless, the precise elucidation and analysis of cellular activities during bone remodeling and repair, improvement of hematopoietic function, and restoration of bone formation will greatly aid in the treatment of bone diseases and bone degradation in the rapidly increasing aging population in China and even in the global population.

The bone marrow (BM) niche is a special microenvironment that is composed of hematopoietic stem cells (HSCs), osteoblasts, adipocytes, and endothelial cells and helps maintain the unique functions of the BM [3]. The BM niche ensures hematopoietic homeostasis by controlling the proliferation, self-renewal, differentiation, and migration of HSCs and progenitor cells, as well as the responses to emergencies and injuries [4]. This niche comprises a unique matrix microenvironment required to support the hematopoietic function of HSCs [4]. The BM niche cells exert their effects on HSCs via three pathways: intercellular communication, cell adhesion, and extracellular matrix (ECM) remodeling [5]. Among the extrinsic factors released by these HSC-supporting cells, the chemokine stromal cell-derived factor 1 [SDF-1, also known as chemokine [C-X-C motif] ligand 12 [CXCL12]) and the cytokine stem cell factor (SCF) are essential regulators that manage HSCs in the BM [6]. Over the

last decade, remarkable progress has been achieved in deciphering the BM niche and the specific cellular and molecular elements that regulate HSC activity, identifying the major cellular components within the BM niche, and determining the mechanisms of cytokine-mediated cellular interaction. However, our understanding of the BM niche is still in its early stages. Therefore, in this review, we summarize the cellular components and intercellular crosstalk in the BM niche to provide a basis for the further investigation of the role of the BM niche in the maintenance of hematopoietic homeostasis and facilitate subsequent research on the targeted treatment of diseases.

Role of bone marrow niche in maintaining the balance of bone remodeling

In modern mammals, the BM consists of many different cell types, ECM, secreted factors (such as cytokines, chemokines, and growth factors), and metabolic signals [7] (Fig. 1). The HSCs in the BM promote bone remodeling/repair by dynamically interacting with a complex network of stromal cells, including by the reciprocal molecular crosstalk among inflammatory, endothelial, and niche cells, such as mesenchymal stem cells and schwann cells [8, 9]. Furthermore, osteoblast function during bone repair relies on signals from the surrounding niche cells, which provide a specific microenvironment and integrate signals to mediate appropriate stem cell responses, including osteogenic/chondrogenic differentiation, depending on the need of the organism [10]. Osteogenesis is related to the H-type endothelial cell subtype of capillary endothelial cells [10]. H-type cells express platelet and endothelial cell adhesion molecule 1 (PECAM1) and intrinsic mucin, targeting the molecular signals of bone progenitor cells through the Notch signaling pathway, reducing the expression of secreted phosphoprotein 1 (Spp1), RUNX family transcription factor 2 (Runx2), and Osterix (Osx), changing the BM niche of the injury site, and causing bone progenitor cells to differentiate into osteoblasts [11]. Inflammatory macrophages play an important role in endochondral ossification during fracture healing [10]. The influx of inflammatory cells leads to the secretion of bone morphogenetic protein 4 (BMP4), vascular endothelial growth factor (VEGF), interleukin 6 (IL6), and C–C motif chemokine ligand 2 (CCL2), which promote osteogenic differentiation and bone repair by interacting with newly formed bone progenitor cells [12]. In addition, sensory and sympathetic

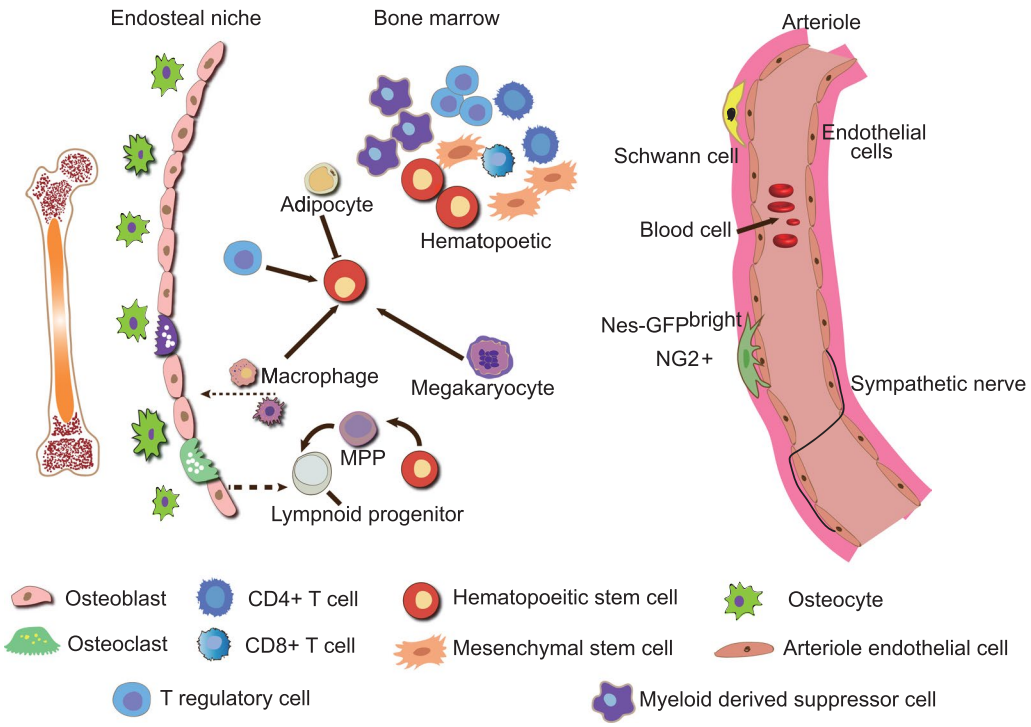


Fig. 1 Components of the HSPC bone marrow microenvironment. The bone marrow cavity contains multiple cell types from different lineages, and hematopoietic stem cells (HSCs) maintain hematopoiesis. Hematopoietic stem cells are predominantly found in perivascular niches (including periarterial, perisinusoidal, and perivascular in the transition zone). Osteoblastic niches are located near the periosteum, where they communicate locally through cell–cell interactions and remotely through cytokines or exosomes released into the bone marrow. HSC, hematopoietic stem cell; CAR, CXCL12-rich reticulocyte; ECM, extracellular matrix

nerves play a crucial role in bone homeostasis and bone repair [10]. Nerve growth factor (NGF) expressed by nerve cells and VEGF expressed by Schwann cells are upregulated during bone repair [13].

Overall, the dynamic equilibrium of the bone microenvironment regulates the regeneration and repair of bone, with the BM being the main site for the balance between bone regeneration and hematopoiesis. Thus, the bone microenvironment is important in the control and maintenance of the HSC niche. Table 1 summarizes the various cells of the bone microenvironment and their main markers in the HSC niche, as well as the main molecular crosstalk effects and cellular functions with HSCs (Table 1). Moreover, the interaction of endothelial cells and osteoblasts and the niche function are essential for maintaining bone health and coordinating bone repair and regeneration [14]. In addition, BM macrophages, megakaryocytes (MKs), CXCL12-abundant reticular (CAR) cells, and other cells that constitute the cellular components of the BM niche are vital in maintaining the functional balance of the niche [14, 15]. Aged skeletal stem cells can create an inflammatory degenerative niche [16]. In bone, cell-to-cell interactions maintain the dynamic microenvironment of the bone, wherein the crosstalk between osteoblast and vascular lineage cells and inflammatory cells is particularly essential for bone repair and remodeling [17]. Furthermore, the niche interacts with bone cells during homeostasis to preserve their undifferentiated properties. However, the changing niche environment after bone injury causes precursor cells to differentiate into osteoblasts and repair the damaged tissue [10].

Cellular crosstalk in the bone marrow niche

Interaction of hematopoietic stem cells with other cells in their niche

Mesenchymal stem cells and hematopoietic stem cells

BM mesenchymal stem cells (MSCs) are a group of cells that perform multiple functions and are located around blood vessels as well as directly on blood vessel surfaces along with sympathetic nerves [17]. The origins of MSC development are still being investigated, wherein MSCs have been initially thought to be derived from the mesoderm [18]. Furthermore, studies have shown that MSCs with positive (CD73, CD90, and CD105) and negative (CD14, CD19, CD34, CD45, and HLA-DR) markers can differentiate into other cell types, such as osteoblasts, chondrocytes, neural cells, muscle cells, and adipocytes,

Table 1 Different cell types, markers and functions within the niche

Cells	Markers	Main molecules	Function	Reference
Mesenchymal stem cells	CD73+, CD90+, CD105+, CD14–, CD19–, CD34–, CD45–, HLA-DR–, Terl119–, CD31–, CD51+, Sca1–, PDGFR+	CXCL12, SCF, Osteopontin, VCAM-1, Angiopoietin-1	Support and regulation of HSC quiescence, proliferation, differentiation, HSC mobilization	[19–22]
CAR cells	CD45–, CD31– ,Sca-1–, PDGFR+	CXCL12, SCF, Foxc1, Ebf1/3	Support and regulation of HSC quiescence, proliferation, differentiation, HSC mobilization	[6, 27–33, 40]
Osteoblasts	CD45–, Terl19–, CD31–, CD51+, PDGFR–, Sca1–	Osteopontin, N-cadherin, TPO/MPL, Angiopoietin 1, Wnt5a, 5-FU	Support of HSC quiescence	[28, 29, 42–49]
Endothelial cells	CD45–, CD31+, CD144+, Terl119–	CXCL12, SCF, Notch ligands, E-selectin, Del-1, Pleiotrophin	Support of HSC proliferation and expansion Hematopoietic regeneration after irradiation	[52–57]
Megakaryocytes	CD41+, CXCR4, Mpl	CXCL4, TGF-B, Thrombopoietin, FGF1, TPO	Support of HSC quiescence, HSC expansion after irradiation	[67–77]
Macrophages	CD68+, CD169+	VCAM-1, DARC, TGF-β	HSC retention in niche Support of HSC quiescence	[75, 78, 81, 82, 84–86]
Lymphocytes	Foxp3+	IL-10, CDC39, CD105, Adenosine	Protection of HSC from immune attack Support of HSC quiescence	[87–94]
Adipocytes	ADIPOQ, FABP4, Leptin	MCP-1, CXCL12, SCF, IL-8, LIF, CSF3, Adiponectin, Leptin	Support of HSC survival, proliferation and differentiation Hematopoietic regeneration after irradiation	[97–103]
Sympathetic nerve fibers	Tyrosine hydroxylase	Noradrenaline	HSCs mobilization	[113]
Parasympathetic nerve fibers	Choline acetyltransferase	Acetylcholine	HSC retention in niche, homing	[114]

as well as secrete various growth factors, cytokines, and chemokines [19, 20]. Commonly used MSCs include BM-derived MSCs, fat-derived MSCs, and umbilical cord blood-derived MSCs [21]. Moreover, the substances secreted by MSCs are important in immune regulation, cell migration, proliferation, differentiation, and tissue repair.

Several MSC populations, including Nes-GFP$^+$, NG2$^+$, LEPR$^+$ MSCs, or CAR cells, promote the maintenance of HSCs by releasing factors such as CXCL12 or SCF [19, 20]. MSCs are one of the main elements that promote the self-renewal niche of HSCs, where MSCs in the BM and cord blood express high levels of N-cadherin (NCAD), vascular cell adhesion molecule 1 (VCAM-1), neural cell adhesion molecule 1 (NCAM-1), and integrins [22]. Moreover, the interaction between MSCs and HSCs is multifaceted and plays a prominent role in supporting HSCs, including the transmission of the Wnt and Notch signaling pathways [23]. This balance between the HSCs and MSCs guarantees self-renewal and maintenance of the HSC niche. BMP4, a member of another signaling pathway secreted by MSC, influences HSCs during embryonic development, within which the conditional inactivation of the BMP4 receptor results in an increased HSC population in the BM [24]. Additionally, BMP4 can affect HSCs directly or through mediators such as Shh (Sonic hedgehog), in which Shh induces cytokine-dependent proliferation of HSCs, e.g., high concentrations of the Shh protein support the proliferation of cord blood HSCs [25]. The platelet derived growth factor receptor (PDGFR)-α$^+$ CD51$^+$ subpopulation of nestin (Nes)$^+$ cells have also been found to be rich in major HSC maintenance genes, supporting the notion of the co-separation of niche activity from MSC activity [26]. These findings suggest that cell-to-cell contact is one of the main mechanisms for maintaining HSC self-renewal by MSCs.

CAR cells and hematopoietic stem cells

CAR cells, also known as leptin receptor (LEPR)-expressing cells or CXCL12- and high SCF-expressing reticular cells, are a population of MSCs adjacent to blood sinuses [6]. The CAR cell subset is the main cellular component of the hematopoietic stem and progenitor cell (HSPC), creating a niche for HSCs capable of regulating HSC self-renewal, proliferation, and migration [27] (Fig. 2). CAR

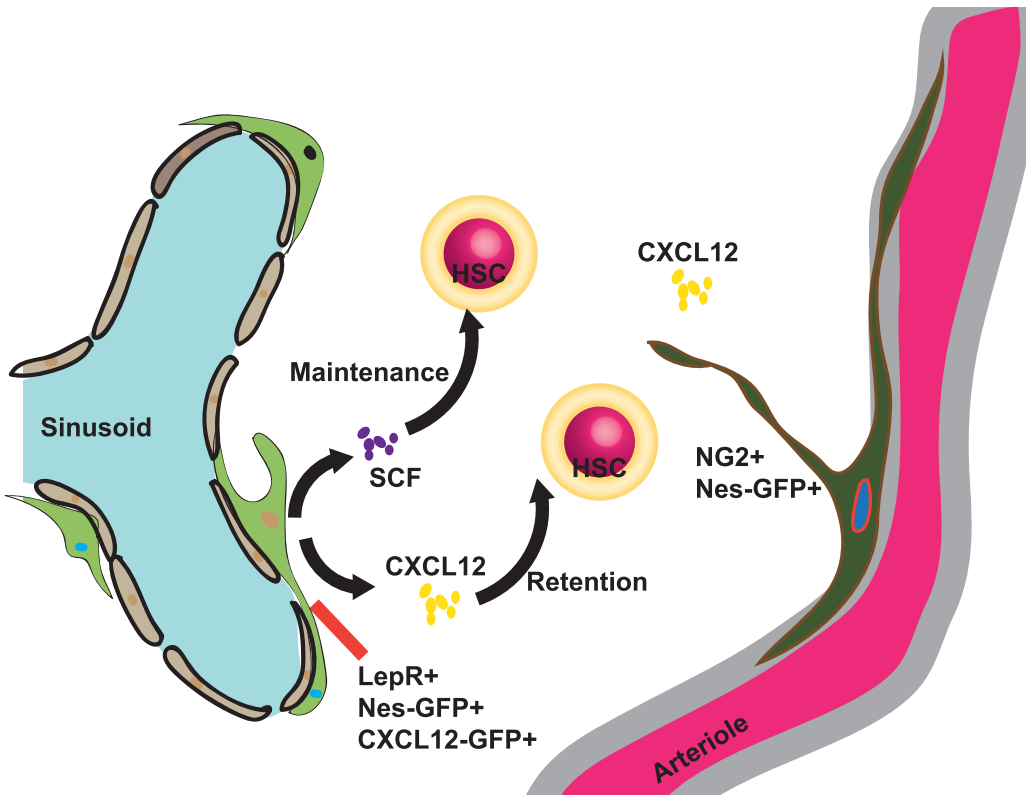


Fig. 2 Model showing the CAR cells, comprising the bone marrow hematopoietic stem cell (HSC) niche. CAR cells rich in LEPR$^+$, Nes-GFP$^+$, and CXCL12-GFP$^+$in the sinusoids secrete SCF to maintain HSCs in the niche and CXCL12 to achieve self-renewal of HSCs, while MSCs containing NG2$^+$and Nes-GFP$^+$ in arteriole vessels can only secrete CXCL12 to achieve self-renewal of HSCs

cells were the first cells found to colocalize with HSCs in the BM [28]. Although CAR/LEPR$^+$ MSCs constitute approximately 0.3% of the BM cells (including almost all colony-forming unit [CFU]-fibroblasts), they represent the major source of adipocytes and osteoblasts in the BM of adult mice and are required for HSC maintenance [28]. However, the CAR-expressing cell types are not well defined, with studies suggesting that these cells express genes including nestin, anti-myxovirus-1, LEPR, transcription factor damage-related homeobox (Prx-1), and osteogenesis-related transcription factor osterix (OSX) [29]. CAR cells, osteoblasts, and MSCs are niche components closely related to HSCs and perform important functions in immune cell function and hematopoiesis homeostasis [30]. Furthermore, CAR cells inversely regulate HSCs to create a niche and simultaneously generate osteoblasts to ensure sufficient physical space for HSCs [30].

Interestingly, CAR cells with high expression of CXCL12 and SCF make up the majority of the perisinusoidal niche, and deleting CXCL12 or SCF from all MSCs, in particular, leads to the depletion of HSC populations [6]. Moreover, CAR cells highly express lipid and osteogenic factors, such as peroxisome proliferator-activated receptor γ (PPAR-γ) and Runx, and can differentiate into osteoblasts and adipocytes [28, 31]. Studies have reported that mouse CAR cells undergo short-term ablation when CXCL12 and SCF production decreases, resulting in decreased HSCs in the niche [30]. MSC populations in the periarteriolar niche, including NG2$^+$ MSCs, have different effects on maintaining HSC function. However, Asada et al. demonstrated that the main source of SCF to maintain HSCs in the BM was sinus gland LEPR$^+$ CAR cells, not the NG2$^+$ cells in the periarteriolar niche [27]. Similarly, experiments in LEPR-Cre mice confirmed the importance of SCF in MSCs [32]. MSCs were found to secrete CXCL12 and many other factors (e.g., SCF, VCAM-1, angiopoietin-1, IL-6, IL-11, thrombopoietin (TPO), fms related receptor tyrosine kinase 3 (Flt-3) ligands, granulocyte colony stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and BMP4), which typically affect HSC populations and their hematopoietic function [32, 33]. In addition, deleting CXCL12 negatively affected the HSC population by redistributing the remaining HSCs population in the BM, whereas depleting CXCL12 in the LEPR$^+$ MSC of the sinus gland did not affect the HSC population. Nes$^+$ MSCs are located extremely close to Schwann cells and participate in the mobilization of HSCs via the sympathetic nervous system (SNS) [34]. In contrast, the knockout of nestin decreased the number of MSCs and increased HSC proportions within the spleen [35]. Furthermore, the co-cultivation of MSCs with HSCs increases the survival and expansion of HSCs within the spleen [35]. Additionally, the role of periarteriolar stromal cells in resting-state HSCs was confirmed by the recovery of mice with myelosuppression, polyinosinic-polycytidylic acid therapy, or Pml knockout, all of which significantly altered the distance between HSCs and arterioles, allowing HSC overproliferation, HSC population depletion, and migration from the arterioles [34, 36]. Many studies have demonstrated the role of osteoblast lineage cells in HSCs, indicating that hematopoiesis regulation by osteoblasts depends on their differentiation stage [37]. Self-renewing osteoprogenitors in the BM can also form a supportive HSC niche [38]. Nes$^+$ MSCs have been demonstrated to produce CXCL12 and SCF and colocalize with HSCs, and even lead to HSC mobilization when the numbers of Nes$^+$ MSCs are reduced, suggesting that osteogenesis and HSC maintenance in the BM are negatively associated [6].

Studies have also demonstrated the involvement of the early B cell factor (Ebf) in B-cell differentiation and bone development [39], as well as the preferential expression of Ebf3 (an Ebf family member) in CAR cells [40]. Using a conditional knockout mouse model, the specific knockout of Ebf3 expression in CAR/LEPR$^+$ cells was found to significantly reduce the number of long-term repopulating HSCs, pre-erythroid cells of erythroid progenitors (MEPs), common lymphoid progenitors, and granulocyte/monocyte progenitors, as well as reduce the number of functional HSCs and cause the increased differentiation of most Ebf3$^{-/-}$ CAR cells into the osteoblastic lineage [40]. A researcher has identified CAR cells subtypes (i.e., Adipo-CAR and Osteo-CAR cells) using single-cell sequencing and determined the localization of these subtypes and elucidated the source of pro-hematopoietic factors [29]. What's more, a study has revealed that the proportion of CAR cells in ClC-3$^{-/-}$ mice increased by 14.28%, whereas the number of HSCs decreased by 11.81% [41]. Additionally, the expression of the transcription factor Ebf3 was decreased, while the ECM receptor signaling pathway was enhanced, suggesting that ClC-3 chloride channels may regulate Ebf3 expression, thereby mediating the participation of the HSC niche in the bone remodeling process [40, 41]. Overall, the osteogenic and adipogenic differentiation potentials of CAR cells are inhibited by the expression of the transcription factors Ebf1/3, ensuring that these specialized MSCs remain undifferentiated and allowing for marrow cavity maintenance by controlling CXCL12 and SCF expression as well physically preserving the HSC niche [28].

Osteoblasts and hematopoietic stem cells

Osteoblasts are important components of the HSC niche [28]. These cells localize as anchored cell populations within the bone, producing large amounts of extracellular proteins, such as type I collagen, osteocalcin (OCN), and alkaline phosphatase [29]. A recent in vitro co-culture study showed that osteoblast adhesion favored HSC self-renewal [42]. Moreover, CFU assays and thymidine suicide techniques demonstrated the enrichment of HSPCs near the intraosseous niche [3]. Osteoblasts also synthesize and express many HSC-influencing factors, such as TPO, angiopoietin-1, and osteopontin, which prevent the increase in the HSC population [28, 43]. As mentioned earlier, SDF-1/CXCL12 and SCF, which are extrinsic factors released by HSC-supporting cells, are important regulators of HSC maintenance and retention in the BM [5, 9].

Additionally, $NCAD^+$ osteoblasts on the endosteal surface of the bone can interact with HSC precursors via a mechanism that may support HSCs through the BMP signaling pathway [24]. In contrast, the ablation of $NCAD^+$ osteoblasts has been shown to cause irreversible changes in HSC function during homeostasis and regeneration [22]. $NCAD^+$ osteoblasts can also control HSC status and function through noncanonical Wnt signaling [22, 44]. Furthermore, osteoblast ablation results in decreased HSC proportions in the bone with a concomitant increase in extramedullary hematopoiesis, whereas an increase in osteoblast numbers leads to increased HSCs [15, 28]. Interestingly, osteoblasts not only affect HSCs but also modify their well-differentiated progeny [28]. Osteoblasts influence the status of silenced HSCs through the $Tie2^+$/angiopoietin-1 and TPO/MPL signaling pathways, which facilitate the interaction between HSCs and niche components that promote quiescence and self-renewal of these cells [33, 45].

These results show that although the proportion of osteoblasts is important for maintaining HSC function, it is not the only determining factor [42]. Polycomb genes can regulate cell fate, including Bmi-1 and Mel-18 [42]. When HSCs divide, self-renewal cells attached to osteoblasts have high Bmi-1 expression and low Mel-18 expression and when stem cells leave the osteoblastic niche undergo differentiation, while the expression of the aforementioned proteins is opposite in differentiated cells [42]. Osteoblasts also play an important role in regulating HSC stress response. For example, injecting mature osteoblasts with 5-fluorouracil (5-FU) led to cell death, along with reduced osteoblast gene expression in the stromal cells and increased wnt family member 5a (Wnt5a) expression in some osteoblasts [46]. This increased expression of Wnt5a in these bone lineage cells may reduce the proliferation rate of HSC subsets, thereby protecting them from DNA damage and apoptosis after 5-FU chemotherapy [47]. The specific mechanism involves the interaction of Wnt5a with Ryk, a Wnt ligand receptor, to maintain HSC quiescence and protect HSCs after myeloablative stress [47]. However, 5-FU did not exert any influence in the intraosseous niche of aged mice [48], suggesting that stress response changes with age. Overall, the synthesis and expression of osteoblasts are influenced by HSCs, while osteoblasts mediate the self-renewal and differentiation direction of HSCs through extracellular proteins, cytokines, signaling pathways, stress responses, and proportions.

Endothelial cells and hematopoietic stem cells

Endothelial cells are a major type of BM non-hematopoietic cells that create an HSC niche primarily involved in controlling hematopoietic cell trafficking and homing [49]. Early study has found that human BM endothelial cells support the proliferation of hematopoietic cells in vitro cultures, indicating the role of endothelial cells in maintaining hematopoietic progenitor cells [50]. Furthermore, these cells are located in the niche and in direct contact with HSCs [11]. Moreover, the co-transplantation of endothelial progenitor cells with HSCs has been revealed to promote endothelial cell recovery as well as hematological and immunological reconstitution [11]. Studies investigating endothelial cells in the HSC niche have mainly focused on arteriolar endothelial cells (AECs) and sinusoidal endothelial cells (SECs) [51].

These two groups of cells function differently, but both cell types produce SCF required for hematopoietic stem cell survival [51]. AECs, along with CAR cells synthesize the ECM protein Del-1, block SEC regeneration by producing neutralizing antibodies against Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) or VE-cadherin and prevent hematopoietic cell reconstitution [52, 53]. Conversely, SECs synthesize only a small fraction of Del-1 [54]. Moreover, SECs are required for regenerative hematopoiesis, dependent on VEGFR2 for their regeneration, and produce high amounts of CXCL12 and E-selectin [54]. Additionally, the deletion of CXCL12 or SCF in $Tie2^+$ endothelial cells has been shown to reduce HSC numbers [11, 49, 55]. Early study has found that human BM endothelial cells support the proliferation of hematopoietic cells in vitro cultures, indicating the role of endothelial cells in maintaining hematopoietic progenitor cells [50]. Furthermore, Kiel et al. demonstrated an association between HSCs expressing CD150, CD48, and CD41 and SECs [56].

HSC proliferation is promoted via the expression of E-selectin (exclusively found only on endothelial cells), whereas E-selectin antagonists induce HSC dormancy and self-renewal [54, 57]. Studies have suggested that

the endosteal niche provides a hypoxic environment that maintains HSCs in a dormant state, whereas the vascular niche enables the proliferation and differentiation of HSCs in aits comparatively higher oxygen-containing environment [58, 59]. Lampreia et al. demonstrated that endothelial cells also stimulate HSC self-renewal by synthesizing Notch ligands [60]. In particular, the conditional deletion of Jagged-1, an endothelial cell-generated Notch ligand, in the endothelial cells causes depletion of the HSC pool and a severe decline in hematopoiesis [60, 61]. Jagged-1 is also involved in the regeneration of hematopoiesis after myeloablation [61, 62]. Lastly, erythroid precursors have been reported to differentiate into endothelial cells to restore the endothelial cells necessary for vascular network production [63]. Besides, endothelial cells and LEPR cells produce high levels of soluble SCF in the early postnatal bone marrow [55]. Membrane bound SCF from endothelial cells promotes the maintenance of HSCs in the early postnatal bone marrow, while SCF from LEPR cells is used to maintain HSCs and restricted hematopoietic progenitor cells in adult bone marrow [55]. Thus, co-culturing endothelial cells with hematopoietic progenitors may contribute to the expansion of the latter cells, helping to maintain HSCs in the niche.

Megakaryocytes and hematopoietic stem cells

MKs are platelet-producing cells mainly found in the BM [64]. These cells are important regulators of hemostasis and vascular integrity, as well as key components in thrombosis and inflammatory responses [65].However, MK function is not restricted to platelet production. Complex experimental studies in recent years have suggested that MKs are crucial regulators of BM HSCs. Bruns et al. demonstrated that MKs govern quiescence in the HSC niche [66]. Gene expression analysis studies have revealed that MKs in the BM are a source of chemokine motif ligand 4 (CXCL4, also known as platelet factor 4 [PF4]), which regulates HSC cell cycle activity [66]. In line with this finding, injecting mice with CXCL4 decreased HSC numbers due to their increased quiescence, whereas $CXCL4^{-/-}$ mice exhibited increased HSC numbers and proliferation [67].

Another study also demonstrated the association between HSCs and MKs, showing that MK ablation leads to HSC proliferation [68]. The ablation of MKs results in decreased levels of the biologically active transforming growth factor (TGF)-β1 protein in the BM and reduced levels of nuclear-localized phosphorylated SMAD2/3 (pSMAD2/3) in HSCs [69]. These findings suggest that MKs mediate HSC cell cycle activity via TGF-β1, and the absence of TGF-β1 conditions in MKs increases HSC activation and proliferation [68–70]. Thus, TGF-β1 as a major signal generated by MKs to maintain HSC quiescence. Consequently, considering that the MK ablation or loss of TGF-β1 conditions in MKs induce quiescence in HSCs, MKs involved in the maintenance of HSC quiescence. Similarly, further studies confirmed the loss of HSC quiescence after MK depletion and indicated that TPO secreted by MKs was involved in HSC maintenance [71]. In addition to the secretion of CXCL4 and TGF-β1, MKs are major producers of TPO [66, 70, 71]. Moreover, extensive studies have shown that TPO and its receptor c-MPL regulate hematopoietic activity [71, 72]. A study on MK-depleted mice found that reduced MK production led to a reduction in both the number and quiescent phase of HSCs [64]. The disturbance of HSC quiescence in the BM was achieved by ablating the MK populations in the mice [64]. Furthermore, destroying MKs may decrease TPO concentrations within the BM because of the production of TPO by MKs [72]. This concept was supported by the regaining of HSC function in TPO-injected MK-ablated mice.

However, considering MKs may control HSC proliferation through secreted proteins, the calcium ion-dependent C-type lectin-like receptor 2 (CLEC-2) may stimulate MKs to produce TPO [73]. Therefore, direct contact between MKs and HSCs may not be essential. Additionally, Ludin et al. have shown that a subset of α-smooth muscle actin $(SMA)^{+}$ monocytes and macrophages maintain HSPCs [74]. It is important to determine the interaction of HSCs with megakaryocytes or $\alpha\text{-}SMA^{+}$ monocytes and macrophages by deep confocal imaging of α-catulin-GFP^{+}-c-kit^{+} HSCs [74, 75]. So, MK cells can indirectly mediate HSC proliferation through the secretion of extracellular proteins CLEC-2 and SMA. Under stress conditions, fibroblast growth factor 1 (FGF1) signaling in MKs temporarily dominates TGF-β inhibitory signaling to stimulate HSC proliferation [76]. Furthermore, MKs close to HSCs are activated by CXCL4, whereas TGF-β1 secretion regulates their quiescence [67]. Thus, FGF1 produced by MKs also promotes HSC expansion in stress conditions. All these observational results indicate that MK is an ecological niche cell derived from HSC, which can directly or indirectly dynamically regulate the function of HSC.

Macrophages and hematopoietic stem cells

Similar to other niche components, macrophages directly influence HSCs in the BM niche, and macrophages in the splenic niche retain HSCs via VCAM-1 adhesion molecules [77]. Macrophages also have a unique and indispensable role in the organization of BM erythroid islands, a highly specific erythropoietic niche that contains a specialized central macrophage surrounded by erythrocytes [78]. In these structures, the central macrophage

promotes erythroblast maturation in several ways, including mitochondrial clearance by tunneling nanotubes [79]. Several studies have established that G-CSF treatment leads to HSC mobilization, production of granulocytes, inhibition of macrophages and osteoblasts [80, 81], and activation of norepinephrine secretion by sympathetic neurons of the BM microenvironment [82].

Certain macrophage populations in the BM have been found to exhibit α-SMA and increased expression of the cyclooxygenase (COX)−2 [83]. These macrophages can resist radiation-induced cell death and further upregulate COX-2 expression in response to stress [84]. In this mechanism, COX-2-derived prostaglandin E2 (PGE2) may limit reactive oxygen species production by inhibiting the Akt kinase and activating the increased expression of the stromal cell chemokine CXCL12, thereby preventing HSPC exhaustion required for HSC quiescence [74].

Another function of macrophages is to inhibit HSCs from entering the quiescence phase via the interaction of CD82 with HSCs through the Duffy antigen receptor for chemokines (DARC)/CD234 on the macrophages [85]. This interaction activates TGF-β1/SMAD signaling and inhibits the proliferation and differentiation of the HSCs [85]. Moreover, macrophages have been recognized as a pool of cells with regulatory properties because they exert humoral effects on Nes^+ cells via unidentified cytokines and induce these cells to secrete CXCL12, thereby maintaining HSCs in the niche [84]. Furthermore, macrophages have been suggested to promote HSC retention in their BM niches via Nes^+ stromal cells, as evidenced by the macrophage depletion in the BM causes HSC mobilization in the blood [80]. From this, it can be seen that macrophages directly affect the HSC niche, retaining HSCs through VCAM-1 adhesion molecules and maintaining their quiescence through stress response.

Lymphocytes and hematopoietic stem cells

A subset of early lymphoid progenitors has been reported to reside in the endosteal niche and is maintained by factors produced by osteoblasts [86]. Other lymphoid progenitors are found in the perivascular niches created partly by $LEPR^+$ cells [9]. Among the numerous lymphocytes colocalizing with HSCs, $Foxp3^+$ regulatory T (Treg) cells play a significant role in protecting HSCs from immune attack in the niche [9]. In particular, allogeneic HSCs (as well as syngeneic HSCs) survive in unirradiated mice for at least 1 month after transplantation, whereas the depletion of Treg cells results in the rapid loss of allogeneic HSCs [87]. Furthermore, Treg cells in the BM show elevated production of IL-10, which directly affects MSC function [88, 89]. Thus, IL-10 secreted by Treg cells regulates hematopoiesis via another mechanism, indicating that this cytokine is required for HSCs maintenance [89]. Moreover, BM Treg cells are migratory in nature and home in the BM [39]. After Treg cell depletion, the function and phenotype of MSCs and HSCs are impaired. This Treg depletion-associated impairment of MSC and HSC functions results in the reduced hematopoietic supportive capacity of the niche [89]. HSPCs are also lost after $Foxp3^+$ Treg cell depletion [87]. This loss of HSPCs may be linked with the clear colocalization of HSPCs with Treg cells and their accumulation at the endosteal surface of the calvaria and trabecular bone.

Moreover, the highly CD150-expressing subpopulation of Treg cells in the BM produced elevated levels of extracellular adenosine via the cell-surface ectoenzyme CD39 [90]. The generated adenosine then enhances Treg cells activity, protects HSCs from oxidative stress, and maintains HSC quiescence [39]. Furthermore, the co-transplantation of this Treg cell subset, instead of other Treg cell subsets, was found to promote better engraftment of HSCs in allogeneic hosts [90]. Studies have indicated that perisinusoidal $LEPR^+$ cells seem poised to undergo adipogenic differentiation, whereas periarteriolar $LEPR^+$ cells appear poised to undergo osteogenic differentiation [29, 46, 91]. The adenosine produced by these $LEPR^+$ cells upon differentiation may act in opposite directions by activating immunity and decreasing the immune privilege of the niche [92]. However, scarce evidence exists on the potential role of B lymphocytes in the BM niche. Nevertheless, recent studies have suggested that acetylcholine, a neurotransmitter abundantly produced by B cells, limits hematopoiesis in vivo [93].

Therefore, Treg cells and B lymphocytes in the BM niche can be considered as immune privileged sites and protective zones for HSCs. Furthermore, Treg cells can create a privileged zone in the bone marrow, protecting HSCs from oxidative stress and keeping them quiescent to protect HSCs in the niche after transplantation.

Adipocytes and hematopoietic stem cells

Adipocytes are the most abundant stromal components of the adult BM and are considered negative hematopoiesis regulators [94]. There is a well-established interrelationship between hematopoiesis and adipose tissue within the human BM [95]. This understanding is derived from the fact that the red BM at birth is largely devoid of adipocytes and exhibits highly active hematopoiesis [95]. In contrast, the red marrow, when replaced with yellow marrow in adults, is enriched in adipose tissue and characterized by reduced hematopoietic activity [96]. After bone marrow transplantation (BMT), the destruction of hematopoietic function in HSCs by mouse bone marrow adipocytes via affecting lipid raft/TGF-β signaling

pathway [95]. Furthermore, this view was supported by findings showing that hematopoietic recovery after irradiation is significantly accelerated in mice unable to produce adipocytes or after adipogenesis inhibition by PPAR-γ antagonists [97].Additionally, Wilson et al. have demonstrated that PPAR-γ knockout mice devoid of adipocytes display severe extramedullary hematopoiesis [98]. This finding may be related to the observed dysregulation of the CXCL12/CXCR4 axis, implying that adipocytes may be involved in HSC retention or mobilization [98]. One such related mechanism may be a positive feedback loop in which adipocytes, via the strong secretion of monocyte chemotactic protein-1 (MCP-1), stimulate MSC differentiation into new adipocytes and negatively affect HSCs [99].

Numerous studies have revealed that niche components produce adiponectin, while HSCs express adiponectin receptors [100]. Moreover, adiponectin increases HSC proliferation via a p38 MAPK-dependent pathway and maintains their undifferentiated state [101]. Consequently, adiponectin deficiency in mice impairs the ability to restore hematopoiesis after chemotherapy [101]. Moreover, after MSC exposure to radiotherapy or chemotherapy, the adipocyte numbers increase due to enhanced adipogenic differentiation of the MSCs [102]. This substantial increase in the number of adipocytes hinders hematopoietic regeneration, which can be used as diagnostic criteria for BM hypoplasia [102]. Although leptin, another adipokine secreted by adipocytes independently, exerts a negligible effect on the survival and proliferation of mouse and human HSCs in vitro [103]. Leptin exhibits its pleiotropic effects on MSC differentiation into adipocytes in vivo [103]. In addition, leptin demonstrates a synergistic effect with SCF by stimulating the proliferation of primitive hematopoietic progenitor cells in vitro during the formation of HSCs and progenitor cell colonies [55].

In the BM, adipocytes produce hematopoietic factors, including CXCL12, IL-8, colony-stimulating factor 3 (CSF3), and leukemia inhibitory factor (LIF), comparable to MSCs [104]. Furthermore, BM adipocytes have been shown to promote HSC regeneration and hematopoiesis after irradiation or 5-FU treatment [105]. Adipocytes and their precursors, which comprise a small $LEPR^+$ cell subpopulation, produce the required SCF for hematopoietic recovery [105]. SCF from these $LEPR^+$ cells (but not from endothelial cells or osteoblasts) also activate regeneration, whereas the conditional deletion of SCF in the adipocytes inhibits hematopoietic regeneration [105]. In contrast, in previous comparative studies, obese mice showed a significant increase in lymphocytes and adipocytes in the bone marrow, leading to an increase in the expression of leptin mRNA in bone tissue, indicating that bone marrow adipocytes enhance hematopoietic function by secreting leptin [98]. Similarly, high-fat diet induced obesity leads to an increase in the number of bone marrow precursor cells in HSCs [96]. Thus, adipocytes formed under hematopoietic stress create large amounts of leptin and SCF, representing an emergency response that provides the factors necessary for the survival and expansion of HSCs.

Neural and hematopoietic stem cells

Autonomic nerve fibres extensively innervate the bone and BM. The physiological role of the SNS involves regulating bone formation, hematopoiesis, and cancer progression [106, 107]. Furthermore, the BM is co-innervated by the SNS and sensory nerves, which envelop the arterioles and directly contact periarterial Nes^-GFP^+ stromal cells [108]. Méndez Ferrer et al. further showed that SNS neural connectivity plays a key role in the BM, including the mobilization of HSCs from the BM via circadian rhythms [109]. In this mechanism, sympathetic nerve fibres release the neurotransmitter norepinephrine, which exerts its influence via the β3 adrenergic receptors [110]. The activation of the SNS exerts control on HSCs from the BM and downregulates CXCL12 levels during the daytime, leading to activation of HSC activation and enhanced production of neutrophil and inflammatory monocytes [111]. The SNS may also play a multidimensional role in the homeostatic BM by targeting different cellular components [111]. The bone is innervated by fibres of the parasympathetic nervous system (PNS) and considering that the PNS and SNS are known to have opposing roles in most organ systems, the PNS may antagonize sympathetic regulation in the bone (and possibly the BM) [112]. The parasympathetic cells produce the neurotransmitter acetylcholine that transmits signals via nicotinic or muscarinic receptors [113]. Researchers have demonstrated that the parasympathetic signals promote bone mass accumulation by mediating nicotinic receptor activation, regulating osteoblast proliferation, and osteoclast apoptosis [114].

Moreover, the cholinergic nervous system counteracts the sympathetic noradrenergic system by inhibiting it at night, thereby reducing HSC outflow [115]. Additionally, the cholinergic system maintains HSC quiescence in the endosteal niche during proliferative stress via CXCL12 production [116]. However, the type 1 muscarinic receptor (CHRM1), a receptor for the parasympathetic neurotransmitter acetylcholine in the hypothalamus, has been found to regulate G-CSF-induced HSC mobilization from the BM through glucocorticoid release [117].

Glial cells, which include Schwann cells, in the PNS envelop the axons of the peripheral nerves [118]. Schwann cells consist of myelinating and non-myelinating

cells [119]. The myelinating Schwann cells that supply myelin to the nerves express myelin basic protein (MBP) but not glial fibrillary acidic protein (GFAP) [120]. In contrast, non-myelinating Schwann cells (which wrap around the sympathetic nerves in the BM) express GFAP but not MBP [120]. Schwann cells are part of the BM niche, producing functionally active TGF-β and leading to the phosphorylation of the SMAD2/3 signaling pathways that affect cell cycle progression [121]. During this process, regulating the activation of latent TGF-β signaling promotes HSC quiescence and maintenance directly in the BM [121].

Furthermore, Wallerian degeneration and the clearance of $GFAP^+$ Schwann cells in the BM were shown to be induced by the transection of postganglionic sympathetic nerves [122]. Additionally, decreased neuregulin-1 signaling due to nerve injury can trigger Schwann cell apoptosis [123]. Moreover, non-myelinating Schwann cells were demonstrated to have the most prominent role in the HSC quiescent phase, with the loss of the autonomic nerves of the BM reducing the number of these secreting Schwann cells and leading to the disappearance of HSCs [124]. Therefore, the excitation of the sympathetic nervous system controls the rhythm of HSC mobilization, while the cholinergic nervous system promotes the stillness and maintenance of HSCs in BM by regulating TGF-β signaling.

Crosstalk between osteoblasts and osteoclasts

Extracellular vesicles in osteoblasts (also known as small osteoblast vesicles [SOVs]) are secreted and trapped by mature osteoblasts (MOBs) [125]. Studies have indicated that a fraction of MOB-derived SOVs limits bone formation and stimulates osteoclast genesis [125]. Moreover, the intercellular communication between osteoblasts and osteoclasts is enabled by SOVs via the upregulation of RANKL in SOV-treated osteoblasts [126]. These results explain the negative regulation of bone formation by SOVs through MOBs and the positive regulation of bone resorption, thus facilitating the "reciprocal reversal phase" via microRNA-mediated mechanisms [126]. Furthermore, SOVs not only have a direct effect on osteoclasts but also exhibit a direct effect on the BM [127]. For example, the inhibition of osteoblast production and enhanced osteoclast genesis in the BM have been revealed in HSCs co-cultured with SOVs [127]. Moreover, osteoclasts were demonstrated not to affect HSC maintenance in osteoclast differentiation models with cytokine deficiency as well as in c-Fos-deficient and RANKL-deficient mice models [126, 128, 129]. However, osteoclasts were found to promote HSC migration from the BM into the blood circulation when the endosteum was disrupted [129].

Osteoclast–osteoblast communication occurs in basic multicellular units (BMUs) during the initiation, transition, and termination phases of bone remodeling. During the initiation phase, hematopoietic precursors are recruited to BMUs [130, 131]. These precursors then express cell surface receptors, including c-Fms, RANK, and osteoclast-associated receptors, followed by their differentiation into osteoclasts after contact with ligand-expressing osteoblasts [132, 133]. Osteoclasts and osteoblasts communicate using three pathways: cell–cell contact, diffusible paracrine factors, and the cell-bone matrix [134]. Furthermore, EphrinB2 expression on osteoclasts and EphB4-mediated bidirectional signaling in osteoblast precursors are important components of osteoclast–osteoblast communication [135]. Reverse signaling in osteoclasts through EphrinB2 decreases c-Fos and NFATc1 activity and inhibits osteoclast function, whereas positive signaling in osteoblast precursors via EphB4 reduces RhoA activity, thereby enhancing osteoblast differentiation [136]. These interactions most likely occur during the termination phase in the bone remodeling compartment, where bone remodeling is achieved by osteoblastic bone formation and bone matrix mineralization (Fig. 3).

Crosstalk between MKs and bone cells

The lack of MKs can substantially impair bone formation. Further research suggests that the increased secretion of TGF-β by MKs promotes the proliferation and differentiation of osteoblasts, as well as the formation of $CD31^+$ $Emcn(Endomucin)^+$ blood vessels [69]. Additionally, treatment with MKs or TPO significantly mitigates radioactive bone injury in mice via the direct or indirect increase in TGF-β1 levels in the BM [70]. MK-derived TGF-β1 is also involved in inhibiting apoptosis and promoting DNA damage repair in radiation-exposed osteoblasts [70].

However, the effect of MKs on osteoblast differentiation remains controversial because MKs have been shown to inhibit osteoblast differentiation. Recently, Lee et al. demonstrated that an MK-conditioned medium could promote osteoblast amplification while inhibiting its differentiation [137], partly by modulating Pyk2 phosphorylation, a negative regulatory process of osteoblast differentiation [138]. Furthermore, MKs migrate to their ecological niche in the bone through TPO signals and secrete platelet-derived growth factor receptor-β (PDGF-β) to promote osteoblast expansion and ecological niche remodeling [139]. In addition, MKs inhibit osteoblast differentiation to maintain its hematopoiesis-enhancing activity (HEA) during steady-state conditions [140].

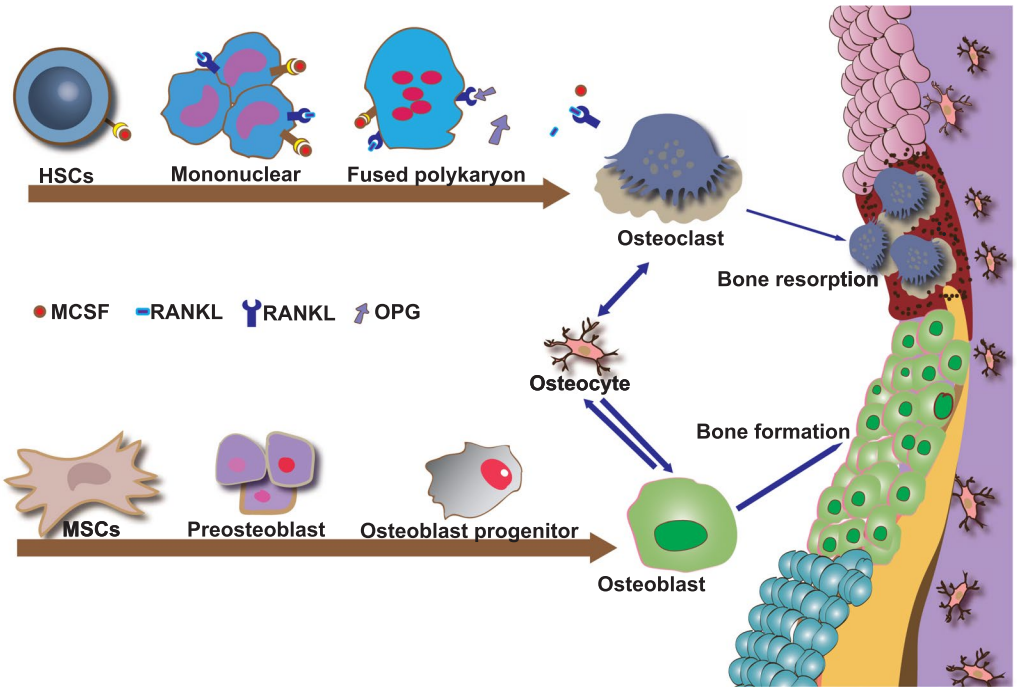


Fig. 3 Schematic illustration of the evolution of osteoblasts and osteoclasts during bone formation and bone remodeling. HSCs first evolve into mononuclear, then into fused polykaryon, and finally form osteoclasts through the RANKL pathway and OPG mediation, achieving bone resorption. MSCs first evolve into preosteoblasts, then osteoblast progenitor cells, and finally form osteoblasts, achieving bone formation

Studies have shown that MKs inhibit bone resorption and exert bone-protective effects by stimulating bone formation, making MKs an ideal therapeutic target for metabolic bone diseases. Moreover, immature osteoblasts have higher HEA than mature osteoblasts [37, 141]. In vitro studies in aged mice have demonstrated that MKs inhibit the differentiation of osteoclast precursors into osteoclasts, thereby curbing bone resorption [137, 142]. Furthermore, the mice exhibited a decrease in the MK potential to stimulate osteoblast expansion despite the increase in the number of MKs [143]. Facts prove that hematopoietic ecological niche remodeling was necessary for increased success in HSC transplantation after total-body irradiation in mice [144].

Osteomacs (OMs) have been reported to play a role in increased mouse survival after irradiation, with an interaction between OMs and MKs in the irradiated HSC transplantation [69]. Interestingly, MKs require physical contact with OMs rather than HSPC support to enhance their effectiveness [69]. In support of this finding, some studies have suggested that MKs regulate the hematopoietic niche through protein secretion and physical contact [145]. Furthermore, the number of OMs and osteoblasts in aged mice also decreased, along with a decrease in HSC transplantation and myeloid bias [146]. Finally, MKs will likely lose their HEA potential even though the number of MKs increases.

Crosstalk between lymphocytes and bone cells

Lymphocytes mediate the regulation of osteoblast maturation and activation. IL-17A and IL-17F secreted by Treg cells strongly promote osteoblast differentiation in MSCs after binding to bone morphogenetic protein-2 (BMP-2) [17, 147]. Researchers have revealed that some cytokines, including interferon-γ secreted by $CD4^+$ T lymphocytes, can promote the differentiation of MSCs into osteoblasts [148]. Furthermore, the activity of TGF-β, which is also produced by $CD4^+$ T lymphocytes, negatively correlates with osteoblast differentiation [149]. Both patients with osteoporosis and oophorectomy (OVX)-induced osteoporosis animal models demonstrate increased TNF-α expression in T cells and osteoblast apoptosis [150]. Additionally, osteoblasts may play a role in antigen presentation, activating T cells as helper cells and promoting T cell differentiation and maturation [150].

Similarly, B cells also exhibit a regulatory effect on osteoblast function. Studies on rheumatoid arthritis have found that B cells produce osteoblast-inhibiting factors, such as CCL3 and TNF, and activate the ERK and NF-κB signaling pathway that inhibits osteoblast differentiation, thereby suppressing bone formation in RA [151, 152]. In addition, RANKL produced by B cells promotes osteoclast production, directly disrupts bone metabolism balance, and causes postmenopausal osteoporosis [152, 153]. In an in vitro model of human osteoclastogenesis,

human B lymphocytes have been shown to stimulate osteoclastogenesis inhibitory factor (OPG) production by secreting TGF—β, thereby inhibiting osteoclast formation [152]. Compared with young mice, B lymphocytes isolated from bone marrow and spleen produce significantly higher levels of RANKL and OPG in elderly mice [152]. In addition, Notch signaling transduction is also considered a mediator of the impact of activated B lymphocytes on osteoblast generation [153]. Conversely, IL-7 and CXCL12 secretion by osteoblasts can induce B-cell differentiation in HSCs [154].

In addition, lymphocytes regulate the chondrocyte differentiation process by secreting certain cytokines. Chondrocytes mainly produce large amounts of ECM to form cartilage and preserve normal cartilage function [155]. Additionally, IL-6 secreted by activated T cells can induce the production of tissue inhibitors of metalloproteinases, thereby playing a role in cartilage protection [153]. Furthermore, Kuhl et al. demonstrated that IL-3 secreted by T cells could increase the expression of the chondrocyte-specific genes sex-determining region of Y chromosome (SRY)-box transcription factor 9 (SOX9) and type IIa collagen [155]. SOX9 is a key transcription factor in chondrocyte development and maturation, participating in the entire differentiation process of chondrocytes [156]. Researchers have also found that TGF-β positively influences cartilage remodeling [157]. Studies have shown that T cell-secreted TGF-β can increase the mRNA expression of chondrocyte differentiation-related genes (including SOX9) and facilitate chondrocyte differentiation [158]. In contrast, chondrocytes can directly inhibit T lymphocyte proliferation, which restricts the expression of the IL-2 receptor α-chain (CD25) in $CD4^+$ T cells [159].

Crosstalk between macrophages and mesenchymal stem cells

In the BM niche, macrophages are key regulators in inflammatory response initiation, propagation, and resolution phases during tissue regeneration [34]. Macrophages promote HSC retention by regulating MSCs and, along with MSCs, can co-regulate the physiological processes in the body [160]. Macrophages have been reported to have an extensive adaptive phenotypic and functional shift that may exacerbate and resolve inflammation during tissue repair [161, 162]. The mechanism of macrophages in bone healing has been gradually elucidated over recent years, with results indicating the presence of three main types of niche macrophages: M0, M1, and M2 type [163, 164]. M0 macrophages exhibit a strong ability to enhance the osteogenesis of MSCs [163]. M1 macrophages only participate in the early osteogenic phase, an inflammatory response regulated by MSCs in the early phase of bone injury, and are absent in the later phase of bone mineralization [163]. Compared with M1 macrophages, M2 macrophages release more BMP-2, stimulating the nuclear transfer of RUNX2 by activating the SMAD1 signaling pathway and upregulating ALP and OCN expression in MSCs, consequently inducing MSCs to form thick cell sheets [164]. Moreover, MSCs support osteogenesis promotion by M2 macrophages, which in turn is conducive to the osteogenic differentiation of MSCs [165]. Therefore, M2 macrophages have a relatively greater ability to promote tissue regeneration.

MSCs are the main functional cells of the bone healing process [166]. The upregulation of CD54 production in MSCs after contact with M1 macrophages increases the inhibitory effect on T-cell proliferation [167]. Moreover, MSCs can induce immune tolerance through TSG-6-dependent paracrine effects and the intercellular interactions between MSCs and proinflammatory macrophages [168]. Macrophage-derived oncostatin M has been reported to mediate crosstalk between $Nes^{-}GFP^{+}$ MSCs and macrophages [169]. In general, M1 macrophages trigger apoptosis of MSCs and inhibit MSC growth in vitro, whereas M2 macrophages support MSC growth, proliferation, and migration [170].

New immunotherapies for bone tumors in the BM niche

Osteosarcoma

Osteosarcoma (OS) is a common primary malignant bone tumor that usually occurs at the metaphyseal end of a long bone and is clinically characterized by localized pain and swelling [171]. The age of onset of osteosarcoma has a bimodal pattern, with incidence peaking in the second decade of life and late adulthood, with a 5-year survival rate of approximately 70% without metastases and 30% at the time of diagnosis [172]. The current standard of care for osteosarcoma includes chemotherapy, amputation, etc., and although chemotherapy has significantly advanced the treatment of osteosarcoma, the 5-year survival rate in drug-resistant forms of osteosarcoma is still less than 20% [173]. In recent years, the rise of immunotherapy has provided a new strategy for the treatment of osteosarcoma [173].

The tumor microenvironment (TME) of bone tumors interacts with the BM niche [171]. In OS, osteoclasts control the occurrence of osteoclasts and coordinate bone remodeling through the RANKL/RANK/OPG signaling pathway [173]. Recent studies have shown that cGAS/STING signaling can promote the production of type 1 interferon and enhance the antigen presentation of T cells, which can exert immunostimulatory effects [174]. Cytokines such as IL-2 activate natural killer cells (NK) and cytotoxic T lymphocytes (CTLs) to enhance

the immune system's attack on tumors and are used to treat osteosarcoma [175]. About cytotoxic T cell lymphocyte antigen 4 (CTLA-4) expressed on regulatory T cells (Tregs), is a co-inhibitory cytokine of the B7 ligand of the CD28 receptor during T cell activation [176]. In addition, the anti-PD-1 antibody durvalumab (ClinicalTrials.gov Identifiers: NCT02815995) and the anti-PD-L1 antibody nivolumab (ClinicalTrials.gov Identifiers: NCT02304458) are used to restore the anti-tumor activity of T cells by blocking the PD-1/PD-L1 pathway, thereby achieving the goal of treating osteosarcoma [177]. Tumor-associated macrophages (TAMs) are the main tumor-infiltrating immune cells of TME. Macrophages, which are part of BM niche, are associated with poor prognosis when their expression genes are elevated, resulting in increased infiltration of TAMs [178]. Current targeted therapies for TAM such as inhibition of signaling pathways such as CSF-1/CSF-1R, CCL2/CCR2, and CD47/SIRPα are still being evaluated clinically, and further studies are needed to determine their translational benefit in sarcoma patients [179].

In recent years, a research group has confirmed the tumorigenic effect of ephrin type-A receptor 2 (EphA2) in OS cells, with higher expression in tumors with higher Huevo's grade than lower ones, and higher expression in OS cells with worse prognosis and higher recurrence rate [180, 181]. The activity of EphA2 was inhibited by treatment with two receptor tyrosine kinase inhibitors, pazopanib and trametinib [180, 181]. This finding revealed that the downregulation of EphA2 expression had a significant inhibitory effect on cell proliferation and migration [181]. In addition, studies have shown that HER2 is a protein on the surface of OS cells, and that T cells have been genetically engineered to become CAR cells that express HER2 and that these CAR-T cells are able to specifically recognize and kill HER2-expressing OS cells [181, 182]. An antibody–drug conjugate consisting of trastuzumab, a monoclonal antibody against HER2, and deruxtecan, a topoisomerase I inhibitor, is being evaluated for the treatment of recurrent osteosarcoma [182]. These advances have shown the potential of immunotherapy in the treatment of osteosarcoma, and although the current efficacy of immunotherapy in osteosarcoma is still limited, these studies provide the basis for possible therapeutic breakthroughs in the future.

Chondrosarcoma

Chondrosarcoma (ChS) is a malignant tumour that originates in chondrocytes, and it is the second most common type of osteosarcoma[183]. Although surgical resection is the preferred treatment for ChS, conventional chemotherapy and radiation therapy often do not respond well to tumors that have a high metastatic potential or are difficult to remove by surgery, resulting in a generally poor prognosis [184]. As a result, the rise of immunotherapy has provided an emerging therapeutic target for ChS.

Recent studies have found that the expression level of PD-L1 in ChS may be closely related to the aggressiveness of tumors and the survival rate of patients [185]. This finding suggests that PD-1/PD-L1 inhibitors are promising as a new strategy for the treatment of ChS. ChS cells are able to use certain mechanisms to evade recognition and attack by the immune system, which may lead to alterations in the tumor microenvironment that would otherwise be immunogenic [177, 185]. TAMs in ChS cells induce immunosuppression by expressing the colony-stimulating factor 1 receptor (CSF1R), also known as CD115 [184]. Activation of CSF1R is mediated by two ligands secreted by ChS cells: CSF-1 and IL-34 [179]. The involvement of these ligands leads to the release by macrophages of tumor growth-promoting cytokines, such as TGF-β and IL-10, which further transform the immune microenvironment into a state conducive to tumor growth, making it more susceptible to progression and recurrence [179]. Similarly, the expression of EphA2 was significantly higher in the dedifferentiated ChS cell samples with a poor prognosis than in the samples with a better prognosis, and the specific inhibition of EphA2 affected the survival rate of ChS cells [186].

In addition, recent studies have shown that the expression level of FBXO22 in recurrent ChS is significantly increased, which may be related to the recurrence and progression of tumors [187]. Studies have shown that the inhibition of FBXO22 can not only reduce the proliferation and migration of ChS cells, but also promote apoptosis [187]. More interestingly, the inhibition of FBXO22 also increased the expression of PD-L1, which provides a new therapeutic strategy for immunotherapy [187]. However, not all patients with chondrosarcoma will respond to immunotherapy, so it is important to predict which patients are most likely to benefit from this treatment.

Ewing sarcoma

Ewing sarcoma (ES) is a rare, aggressive sarcoma that occurs more frequently in adolescents and young adults, ranking third among the most common primary bone tumors [188]. Current multi-agent chemotherapy and local therapies have a poor prognosis for patients with metastatic or recurrent disease, including current treatments for ES in addition to first-line chemotherapeutic agents (e.g., vincristine, doxorubicin, cyclophosphamide, etoposide, etc. [189]), gene therapy (e.g., EWSR1::FLI1 [190]), PARP inhibitors (e.g., irinotecan and temozolomide [191, 192]), and quadrivalent DR5 agonist antibodies (e.g., INBRX-109 [193]). In recent years, emerging

cellular immunotherapy methods, including immune checkpoint inhibitors and CAR-T cell therapy, have attracted much attention in ES [188].

For combined macrophage-induced innate immunotherapy for ES, the researchers found that ES cells evade macrophage phagocytosis by simultaneously overexpressing CD47 and downregulating cell surface calreticulin (csCRT) [194]. The study proposes the use of a combination of CD47 blockers (magrolimab, MAG) and chemotherapeutic agents (e.g., doxorubicin, DOX) to enhance macrophage phagocytosis of ES cells, thereby inhibiting tumor growth and metastasis [194].

As with OS and ChS, EphA2 expression was significantly increased in ES compared to normal tissues, suggesting that EphA2 may be closely associated with tumor aggressive behavior [186, 195]. CAV-1 plays an important role in the angiogenesis of a variety of tumors as a key membrane transport regulator in the process of tumor angiogenesis [196]. In addition, the complex formed by EphA2 and CAV-1 can enhance the expression and release of basic fibroblast growth factor (bFGF), thereby promoting endothelial cell migration [195, 197]. Notably, the phosphorylation of EphA2 at Ser897 is closely related to tumor aggressiveness [195].

A comprehensive treatment strategy targeting EphA2 overexpression may be an effective approach for the evaluation of patients with advanced ES. Future successful use of immunotherapies in Ewing's sarcoma may rely on a combination strategy to enhance anti-tumor immunity while restoring the immunosuppressive tumor microenvironment.

Giant cell tumor of bone

Giant cell tumor of bone (GCTB) is an aggressive tumor characterized by osteolytic destruction, which usually occurs in the long metaphysis and epiphysis and spine between the ages of 20 and 40 years, with a high recurrence rate after surgical treatment, frequent local recurrence, and rare metastasis [198]. In the course of immunotherapy for GCTB, denosumab inhibits the activity of tumor cells by targeting the nuclear factor kappa B receptor activating factor ligand (RANKL), which can inhibit the activity of osteoclasts, thereby reducing the destruction of bone by GCTB cells [199–201].

The long-term safety and recurrence of denosumab in the treatment of GCTB are also of concern to investigators. However, Denosumab's inhibition of osteoclast activity may lead to morphological changes in GCTB cells during treatment, which may be similar to those characteristic of malignant tumor cells [202]. Denosumab has been reported to cause serious complications such as mandibular osteonecrosis, and long-term use may affect the normal development and healing process of bones [198]. In vitro studies have shown that denosumab causes an inhibitory response to tumor stromal cells rather than a cytotoxic response, which means that tumor cells may remain alive after treatment, increasing the risk of tumor recurrence after discontinuation of the drug [203]. The use of denosumab prior to curettage of tumors may increase the risk of GCTB recurrence [204]. The frequency of PD-L1 hyperexpression and CD47-SIRPα$^+$ cell infiltration in relapsed lesions treated with denosumab was significantly higher than that in primary lesions or relapsed lesions not treated with denosumab [204]. PD-L1 and CD47-SIRPα immune checkpoint inhibitors may provide clinical benefit in patients with GCTB whose lesions have recurred after denosumab treatment [204].

In recent years, transcriptome analysis by RNA sequencing has found that the expression of Siglec-15 in GCTB tissues is high, which is significantly associated with Campanacci stage, tumor recurrence and poor prognosis [205]. Knockdown of Siglec-15 decreases the proliferation, migration, and invasion of GCTB stromal cells [205]. CXCL8 may be a gene downstream of Siglec-15 and may be associated with the CXCL2, CXCL3, CXCL5, CXCL10, CXCL11, ADORA1, BDKRB1, C5AR1, and NMU genes [205]. It is important to note that although immunotherapy methods have shown potential in laboratory studies and early clinical trials, most are still in the research and development stage, and more clinical trials are needed to verify the safety and efficacy of these treatments and determine the optimal treatment strategy.

Current methods for reshaping the BM niche of hematopoietic stem cells

The artificial niche based on biomaterials and the interactions of hematopoietic stem cells, bone marrow mesenchymal stem cells, endothelial cells, and other cells has potential clinical significance in promoting bone regeneration and treating blood diseases. The development of biomaterials has made it possible to achieve the expansion and/or differentiation of HSCs in vitro, mimicking the BM niche microenvironment by using two- or three-dimensional (3D) culture systems [206]. 2D in vitro culture revealed that the key ECM proteins and cellular ligands that promote HSC amplification are sensitive not only to biochemistry but also to the physical properties of their environment [207]. Recent studies have found that the use of poly-L-lysine (PLL)-coated biomaterials enhances the proliferation of CD34+HSCs and promotes the differentiation of HSCs into erythrocyte progenitor cells [208]. In 3D culture systems, hydrogels and inorganic bone-like mock scaffolds are currently widely used, and co-culture of CD34+HSCs and MSCs in BM has been shown to be effective in amplifying HSCs

and secreting ECM proteins [208, 209]. HSCs proliferate higher than 2D co-cultures of 3D-MSCs, 3D-osteoblasts, and other cell types using HSCs in 3D mixed co-cultures with BMMSCs and OBs in human-derived bone scaffolds [206]. In addition, in a study on HSC differentiation, three-dimensional encapsulation of cells in pullulan (polysaccharide-based) hydrogel delayed the differentiation of HSCs into megakaryocytes and prolonged the viability and stem cell properties of HSCs [210].

Co-culture of HSCs with MSCs has been shown to be similar to native BM niche [211]. Studies have shown that encapsulation in hydrogels with low diffusivity (high collagen concentration, low Lin + BM cells) increases the total number of raw HSCs [209, 211]. The use of bioengineering methods, such as static and dynamic culture methods and microfluidic technology, not only promotes the understanding of the complex biological characteristics of HSCs in physiological and pathological states, but also brings new insights into the proliferation and bone repair of HSCs in the future. In the future, the preparation of materials for HSC amplification in BM niche will require higher ECM concentrations, higher material stiffness, uniform physical properties and molecular composition, predictable and controllable mechanical properties, and reproducible and adjustable degradation rates. In addition, the use of bioengineering methods, such as static and dynamic culture methods and microfluidic technology, not only promotes the understanding of the complex biological characteristics of HSCs in physiological and pathological states, but also brings new understanding of the proliferation and bone repair of HSCs in the future.

Conclusions

The cellular components of the endosteal and vascular BM niches interact with HSCs, and the population-specific crosstalk in the niche is regulated by chemokines, such as CXCL12, SCF, and TGFβ, as well as by intracellular Notch, Wnt, and Shh signaling pathways along with certain other factors. In the BM niche, crosstalk occurs among cells, and the niche cells control the niche function and influence each other. However, no consensus exists on how HSC interaction with the BM microenvironment enables them to perform their vital functions. Although the interactions between the numerous elements that support HSC survival, division, and differentiation processes in the ecological niche have been understood through the production of some major HSC regulatory factors by BM niche cells, there is still a lack of experimental research progress on therapeutic drugs in clinical practice. Future studies should aim to understand the different types of BM niche cells completely, determine the interaction mechanism of the cellular components that constitute the BM niche, and reveal the mechanism that regulates BM hematopoietic homeostasis. Furthermore, the molecular mechanism of HSC regulation of hematopoiesis and bone dynamic conversion balance in the BM niche must be uncovered. In the future, researchers will benefit from the widespread application of HSC-specific humanized mice, single-cell RNA sequencing, mass cytometry, whole bone sections, and in vivo animal imaging. These developments will allow the investigation of the effect and involvement of these niche cells in the occurrence and progression of tumor diseases, the elucidation of the molecular mechanisms controlling these processes, and the examination of their potential as targets for therapeutics.

Abbreviations

BM	Bone marrow
HSCs	Hematopoietic stem cells
ECM	Extracellular matrix
CXCL12	Chemokine (C-X-C motif) ligand 12
SCF	Stem cell factor
RUNX	Family transcription factor
BMP4	Bone morphogenetic protein 4
VEGF	Vascular endothelial growth factor
NGF	Nerve growth factor
MKs	Megakaryocytes
LEPR	Leptin receptor
CAR cells	CXCL12-abundant reticular cells
PDGFR	Platelet derived growth factor receptor
PPAR-γ	Peroxisome proliferator-activated receptor γ
TPO	Thrombopoietin
SNS	Sympathetic nervous system
PNS	Parasympathetic nervous system
Ebf	Early B cell factor
NCAD	N-cadherin
AECs	Arteriolar endothelial cells
SECs	Sinusoidal endothelial cells
TGF	Transforming growth factor
Treg cells	$Foxp3^+$ regulatory T cells
GFAP	Glial fibrillary acidic protein
MBP	Myelin basic protein
SOVs	Small osteoblast vesicles
MOBs	Mature osteoblasts
BMUs	Basic multicellular units
OMs	Osteomacs
OPG	Osteoprotegerin
SOX9	Sex-determining region of Y chromosome (SRY)-box transcription factor 9
OS	Osteosarcoma
ChS	Osteochondroma
ES	Ewing sarcoma
GCTB	Giant cell tumor of bone
TME	Tumor microenvironment
TAMs	Tumor-associated macrophages

Author contributions

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