New perspective of skeletal stem cells

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ABSTRACT

Tissue-resident stem cells are a group of stem cells distinguished by their capacity for self-renewal and multilineage differentiation capability with tissue specificity. Among these tissue-resident stem cells, skeletal stem cells (SSCs) were discovered in the growth plate region through a combination of cell surface markers and lineage tracing series. With the process of unravelling the anatomical variation of SSCs, researchers were also keen to investigate the developmental diversity outside the long bones, including in the sutures, craniofacial sites, and spinal regions. Recently, fluorescence-activated cell sorting, lineage tracing, and single-cell sequencing have been used to map lineage trajectories by studying SSCs with different spatiotemporal distributions. The SSC niche also plays a pivotal role in regulating SSC fate, such as cell–cell interactions mediated by multiple signalling pathways. This review focuses on discussing the spatial and temporal distribution of SSCs, and broadening our understanding of the diversity and plasticity of SSCs by summarizing the progress of research into SSCs in recent years.

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Introduction

Bones are structurally sophisticated and functionally active organs with tremendous regenerative potential.[1, 2] Unfortunately, bone cell dysfunction is commonly associated with many diseases, such as age-related osteoporotic diseases, osteoarthritic diseases, fractures, blood diseases, and tumours.[3, 4] The original generation and functional maintenance of each tissue type in bone depend on the precise regulation of the corresponding stem cells.[5, 6] Limited anabolic pharmaceutical options require an advanced understanding of the stem cell regulatory mechanism in the skeletal system. In addition to haematopoietic stem cells that can produce various blood cells with long-term haematopoietic function *in vivo*, other stem cells in bone tissue may serve as the source cells of non-haematopoietic cell lines (such as bone, cartilage, vascular endothelium, and matrix).[6-8] The Longaker group[9, 10] first identified skeletal stem cells (SSCs) in mouse and human through a series of proof-of-principle studies and defined self-renewing cell populations in different species (human SSCs as $PDPN^+CD146^-CD73^+CD164^+$ and mouse SSCs as $CD45^-TER119^-TIE2^-ITGAV^+CD200^+$), which give rise to osteochondroprogenitors and support marrow development (**Table 1**). Notably, research on SSCs can provide a theoretical basis for multiple therapeutic techniques used to treat various bone and joint diseases, such as stem cell therapy, tissue replacement therapy, and organ transplantation. However, current research on SSCs still confronts significant challenges, both conceptually and technically, requiring collaboration and contribution by skeletal scientists and clinical workers. Therefore, this review will introduce the current research progress concerning SSCs from various perspectives.

Table 1. Differences in surface markers between hSSCs and mSSCs

	PDPN	CD146	CD73	CD164	THY1		AlphaV	Thy	6C3	CD105	CD200
hSSC	+	–	+	+		mSSCs	+	–	–	–	
hBCSP	+	+				Pre-BCSP	+	–	–	–	–
hOP-1	–	+			hi	BCSP	+	–	–	+	
hOP-2	–	+	–	–	lo	PCP	+	–	–	+	+
hCP-1	+	–	–	–		Thy	+	+	–	+	
hCP-2	+	–	–	+		BLSP	+	+	–	–	
hCP-3	+	–	+	+		6C3	+	–	+	+	
						HEC	+	–	–	–	

Note: Data are from the studies of Chan et al.[9, 10] 6C3: stromal cells; BCSP: bone, cartilage and stromal progenitor; BLSP: B-cell lymphocyte stromal progenitor; hBCSP: human bone, cartilage, and stromal progenitor cell; hCP: human chondrogenic progenitor; HEC: hepatic leukaemia factor expressing cell; hOP: human osteogenic progenitor; hSSC: human skeletal stem cell; mSSC: mouse skeletal stem cell; PCP: pro-chondrogenic progenitor; PDPN: podoplanin; pre-BCSP: pre-bone, cartilage and stromal progenitor; Thy: thymus cell antigen.

Search Strategy

Articles about SSCs, osteoprogenitors or bone marrow stromal cells (BMSCs) in bone development and bone repair were retrieved using the search terms: (skeletal stem cells) OR (osteoprogenitors) OR (bone marrow stromal cells) OR (periosteal stem cells) OR (circulating osteogenic precursor/circulating osteogenic cells) AND (growth plate) OR (periosteum/perichondrium) OR (craniofacial bone) OR (periodontal tissue). All these searches were performed on PubMed and Web of Science prior to November 2022. The results were further screened by title and abstract. Irrelevant articles were excluded. Finally, 133 articles were included in this review.

Current Approaches for Identifying Skeletal Stem Cells

The surface markers of SSCs constitute one of the most crucial identifiers in defining a subset of SSCs from multiple developmental stages to different anatomic regions. Various techniques are used to identify SSCs, including fluorescence-activated cell sorting, lineage tracing, and renal capsule transplantation.[11, 12] More recent studies have elucidated the hierarchy of SSC subset developmental trajectories, meanwhile taking advantage of single-cell sequencing transcriptomics and lineage tracing.[13, 14] Notably, lineage tracing in transgenic mice is the gold standard for tracking the differentiation and maintenance of specific cell populations *in vivo*.[11] Lineage tracing, as the name suggests, involves monitoring the spatiotemporal distribution of SSCs, and uses different site-specific recombinases.[12, 15, 16] The advantage of this method is that developmental processes can be assessed without any related damage.[17, 18] Furthermore, using *Cre-LoxP* technology, cells of interest can be permanently labelled and followed throughout the investigation.[19]

At present, the application of genetics is more focused on placing the reporter gene and the Cre-ERT transgene in the same mouse so that exogenous drugs can be used to induce the reporter gene at a specific time of expression.[17, 20] Marker molecules include modified *Rosa26* reporter gene alleles, such as *R26R*-lacZ (encoding β-galactosidase), *R26R*-YFP (encoding yellow fluorescent protein, YFP), *R26R*-tdTomato (encoding red fluorescent protein, tdTomato), and a tandem dimer of red fluorescent protein (DsRed and *R26R*-Confetti).[21-23] SSCs are currently identified using lineage tracing combined with stem cell surface marker molecules.[24, 25] However, identification and isolation of populations of SSCs by their expression of a specific gene promoter results in extensive heterogeneity,[26] and researchers are gradually pursuing more accurate experimental methods to identify and isolate SSCs.[12]

Discovery of Mouse Skeletal Stem Cells

The term "skeletal stem cells" has emerged to describe a group of stem cells contributing to bone growth and development.[27] Bianco et al.[27, 28] defined this population from anatomical and functional aspects as those cells residing in the postnatal bone marrow and producing cartilage, bone, marrow adipocytes, and stroma that supports haematopoiesis. However, this only described the cell capacity without clearly identifying how these cells could be profiled at the cellular level. Hence, how to specifically isolate these cells and how to perform detailed verification on which subset of bone cells constitutes true SSCs is generating attention from the research field.[27]

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The isolation of SSCs is based on similar approaches which emerged from the haematopoietic field based on fluorescence-activated cell sorting-related cell isolation combining colony-forming unit-fibroblast assay and renal capsule transplantation.[27, 29, 30] In 2015, Chan et al.[9] pioneered the use of these techniques to isolate and confirm SSCs in the long bones of postnatal mice, which were sorted and defined using a series of cell surface markers (CD45$^-$TER119$^-$TIE2$^-$ITGAV$^+$CD105$^-$CD200$^+$). Mouse SSCs (ITGAV$^+$CD200$^+$) are located at the top of the differentiation hierarchy and can give rise to bone, cartilage and stromal progenitors (ITGAV$^+$CD105$^+$). These stem cells, as crucial progenitor cells that regulate bone accrual, also possess the capacity for multi-directional differentiation and generate osteoprogenitor cells (ITGAV$^+$CD105$^+$Thy$^+$), chondrocyte progenitor cells (ITGAV$^+$CD200$^+$CD105$^+$Thy$^+$), stromal cells (6C3$^+$), and B lymphocytes (ITGAV$^+$Thy$^+$).[9]

Discovery of Human Skeletal Stem Cells

Chan et al.[10] identified hSSCs and mapped their entire differentiation based on their findings in rodent models. hSSCs were identified in the foetal long bones at 17 weeks and defined as a population expressing CD146$^-$PDPN$^+$CD73$^+$CD164$^+$ surface markers that had the ability to self-renew and were capable of continuous cell colony-forming unit formation.[10] Furthermore, they could differentiate into multilineage ossicles containing bone and cartilage matrix after being implanted into the murine renal capsule.[10] Human bone, cartilage, and stromal progenitor cells are initial descendants of hSSCs and consequently engender osteoprogenitor cells and chondroprogenitor cells which eventually turn into bone, cartilage, and stromal cells.[10] Analysis of limb buds and long bone samples from human embryos at 5- and 8-week gestation revealed a population of embryonic skeletal stem and progenitor cells (eSSPCs, defined as cells expressing PDGFRA$^{low/-}$PDPN$^+$CADM1$^+$) with the potential to differentiate into osteoblasts and chondrocytes by single-cell transcriptome sequencing.[31] Besides long bones, eSSPC were also found in calvarial samples, the development of which was primarily derived from intramembranous ossification, suggesting that it may play a key role in calvarial development.[31] These findings were confirmed by evidence suggesting that eSSPCs localised at the perichondrium possess self-renewal capacity and differentiation capability that maintain calvarial homeostasis.[10] Additionally, transcriptional regulatory network analysis revealed that the transcription factors forkhead box P1/2 (*FOXP1/2*) were enriched in eSSPCs.[31] Immunofluorescence staining showed that FOXP1/2$^+$ cells were mainly located at the perichondrium and inside the primary ossification centre, which can drastically impair intramembranous ossification as shown by research identifying Foxp1/2/4-regulated precursor cells as playing a crucial role in regulating murine periosteal bone formation.[31, 32]

Apart from developmental stage, it is plausible to believe that aging is also tightly correlated with SSC function.[33] Activated young hSSCs are clonally diverse and produce abundant skeletal lineage cells and bone marrow stroma, whereas lineage differentiation is skewed in aged hSSCs.[33] Aging triggers significant changes in the microenvironment that extrinsically shape the functions of SSCs, as does cellular senescence.[33] Current research on SSCs still has noted limitations, and the spatiotemporal characteristics and microenvironment of SSCs at multiple differentiation stages still need to be elucidated.

Skeletal Stem Cells In Long Bones

Skeletal stem cells in the growth plate

Growth plates are responsible for the longitudinal growth of long bones in children. These include slowly circulating cells (resting zone) that generate columns of proliferative chondrocytes (proliferative zone), which then give rise to hypertrophic chondrocytes (hypertrophic zone).[34] At the end of the hypertrophic zone, the growth plate cartilage is replaced by bone and marrow tissues through endochondral ossification.[35] Many studies have confirmed the existence of a subset of SSCs located in the growth plate and its surrounding structures, including the perichondrium (**Table 2**, and **Figure 1**).[34, 36-40] Additionally, other studies identified type 2 collagen alpha 1 chain (*Col2a1*), *Sox9*, and *Aggrecan* as growth plate SSC markers.[36, 37, 41] Li et al.[36, 41] found that chondrogenic progenitors from Col2a1$^+$ cells perform self-renewal in foetal and neonatal confetti rodents. Following the formation of secondary ossification centres, Col2a1$^+$ chondrocytes develop into stable unidirectionally-differentiated chondrocytes residing in the proliferative zone.[41] Col2a1$^+$ progenitors are also present in the growth plates and articular cartilage with a small distribution within the marrow cavity. These progenitors contribute to osteoblasts and chondrocytes and are partially responsible for forming CD31$^+$ blood vessels.[36] Consequently, the number and differentiation potential of Col2a1$^+$ progenitors in long bones and joints decreases during aging.[36] Although Chan et al.[9] defined SSCs in mouse and human long bone growth plates by cell surface markers, the specific spatial location of this group of stem cells in the growth plate is unclear. Thus, whether ITGAV$^+$ CD200$^+$ SSCs associate with Col2a1$^+$ progenitors that anatomically contribute to bone accrual remains unclarified.[9, 10] SSCs in the long bones of postnatal mice can be divided into early osteochondral stem cells and perivascular SSCs, both of which contain stem cell properties.[42] Furthermore, osteochondral stem cells (labeled by CD45$^-$TER119$^-$TIE2$^-$Thy1$^-$6C3$^-$CD105$^-$CD51$^+$) are involved in developing and regenerating long bones and can undergo multilineage differentiation *in vivo*.[42] In contrast, perivascular SSCs (distinguished by CD45$^-$CD31$^-$PDGFα^+Sca1$^+$CD24$^+$) in the bone marrow are functionally different in shaping the haematopoietic stem cell microenvironment and are a crucial source of bone marrow adipocytes.[43]

GLI-Kruppel family member GLI1 (Gli1) is a marker of osteogenic mesenchymal progenitors.[38] Gli1-positive cells are found below the growth plate and are responsible for the formation of cancellous bone; they are called "metaphyseal mesenchymal progenitors".[38] This population of cells is affected by hedgehog signalling and contributes to bone formation following fracture.[38] Parathyroid hormone-related protein (PTHrP) is specifically expressed in the quiescent zone of the long bone growth plate in postnatal mice as demonstrated by Mizuhashi et al.[37] The PTHrP$^+$ chondrocyte population

Table 2. Markers of skeletal stem cells in long bone growth plates

Gene	Transgenes	Source	Function	References
Col2a1	*Col2a1a-CreER*	Bone/cartilage	Contribute to osteoblasts and chondrocytes and the formation of CD31$^+$ blood vessels	36
Gli1	*Gli1-CreERT*	Bone/cartilage	Expressed in the quiescent zone of the long bone growth plate in postnatal mice	38
PTHrP	*PTHrP-CreER*	Bone/cartilage	Generate chondrocytes, osteoblasts, and mature cortical osteocytes during the repair of femoral fracture	37, 41
Sox9	*Sox9-CreERT*	cartilage	Differentiate into chondrocytes	35, 42
FoxA2	*FoxA2-CreER*	Bone/cartilage	Promote growth plate tissue regeneration, exhibit higher clonogenicity, and longevity	40
mTert	*mTert-rtTA*	Cartilage	Contribute to endochondral osteogenesis as chondrogenic osteoprogenitor cells	39

Note: CreER: Cre-estrogen receptor; Col2a1: type 2 collagen alpha 1 chain; FoxA2: forkhead box A2; Gli1: GLI-Kruppel family member GLI1; mTert: mouse Telomerase; PTHrP: parathyroid-associated protein; rtTA: reverse tetracycline transcriptional activator; Sox9: SRY (sex determining region Y)-box 9.

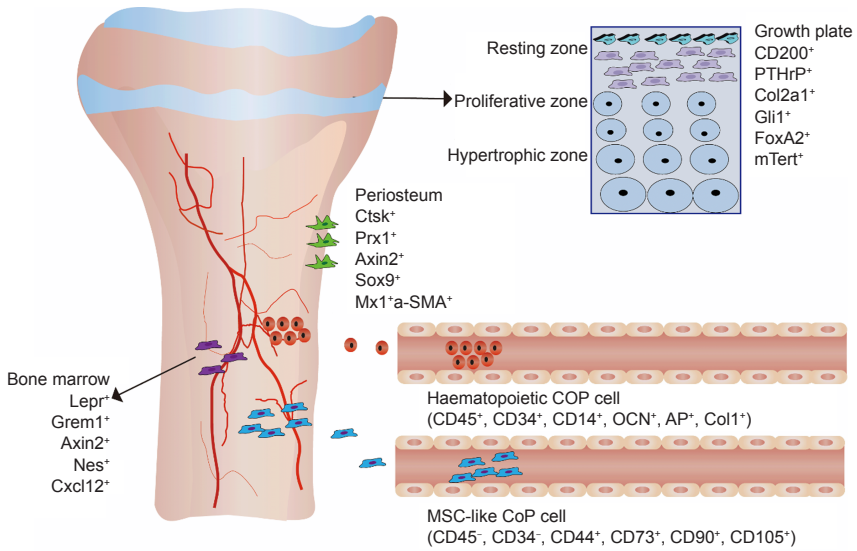

Figure 1. The distribution of SSCs in the growth plate, periosteum, bone marrow, and peripheral circulation shows different cell surface markers. The cell surface markers of SSCs on the growth plate are CD200$^+$, PTHrP$^+$ Col2a1$^+$, Gli1$^+$, FoxA2$^+$, and mTert$^+$. The cell surface markers of PSCs are Ctsk$^+$, Prx1$^+$ Axin2$^+$, Sox9$^+$, Mx1$^+$, and α-SMA$^+$. The cell surface markers of SSCs in the bone marrow cavity are Lepr$^+$, Nestin$^+$, Nes$^+$, Cxcl12$^+$, and Grem1$^+$. The cell surface markers of circulating osteogenic cells in the peripheral circulation are haematopoietic COP cells (CD45$^+$, CD34$^+$, CD14$^+$, OCN$^+$, AP$^+$, and Col1$^+$) and MSC-like COP cells (CD45$^-$, CD34$^-$, CD44$^+$, CD73$^+$, CD90$^+$, and CD105$^+$). Axin2: axis inhibition protein 2; AP: alkaline phosphatase; OCN: osteocalcin; Col1: type 1 collagen; Col2a1: type 2 collagen alpha 1 chain; COP: circulating osteogenic cell; Ctsk: cathepsin K; Cxcl12: chemokine (C-X-C motif) ligand 12; FoxA2: forkhead box A2; Gli1: GLI-Kruppel family member GLI1; Grem1: gremlin 1, DAN family BMP antagonist; Lepr: leptin receptor; MSC: mesenchymal stem cell; mTert: mouse Telomerase; Mx1: MX dynamin like GTPase 1; Nes: nestin; Prx1: paired related homeobox 1; PSC: periosteal stem cell; PTHrP: parathyroid-associated protein; Sox9: SRY (sex determining region Y)-box 9; SSCs: skeletal stem cells; α-SMA: α-smooth muscle actin.

expresses a panel of SSC markers and uniquely possesses stem cell properties under *in vitro* culture conditions. Furthermore, PTHrP$^+$ chondrocytes continue to form columnar chondrocytes in the proliferating zone over the long term.[37] As the hypertrophy process of these chondrocytes progresses, osteoblasts and BMSCs are simultaneously formed beneath the growth plate.[37] Importantly, these PTHrP$^+$ cells and their progeny are still observable in areas of cartilage growth at 3 and 6 months, and even 1 year of age in murine models.[37, 41] Additionally, PTHrP$^+$ SSCs in the resting zone are maintained in a Wnt-inhibitory environment. PTHrP$^+$ cell populations give rise to a specific type of chondrocytes by interacting with Indian hedgehog proteins released from the hypertrophic zone.[34] Compared to PTHrP$^+$ stem cells, the forkhead box A2-positive (FoxA2$^+$) SSC population persists in the quiescent zone of the growth plate for a longer time, and these cells do not mimic characteristics of PTHrP$^+$ stem cells that display higher clonogenicity and prolonged longevity. Notably, FoxA2$^+$ cells show improved chondrogenic ability and promote growth plate tissue regeneration following trauma.[40] Telomerase activity prevents cell senescence and maintains the cycling capacity of stem cells.[41] mTert$^+$ cells have been confirmed in the metaphysis, growth plate, and bone marrow. The mTert$^+$ cell population contributes to endochondral ossification as chondrogenic osteoprogenitors or even stem cells that play a temporal role in developmental stages.[41]

Skeletal stem cells in the periosteal region of long bones

The dimensional expansion of long bones requires a joint effort by different subsets of SSCs responsible for axial and transversal enlargement.[44, 45] Importantly, growth plates are responsible for the longitudinal extension of bones.[45] Cells in the periosteum contribute to bone thickening and cortical maintenance during bone development.[46] The periosteum also contains SSCs with bone regeneration ability controlled by periostin.[44] The discovery of these SSC populations relies on validating cell surface marker identification and stemness confirmation (**Table 3**, and **Figure 1**).[9, 14, 47-51] Another significant subset of periosteal stem cells (PSCs) is labeled by the cysteine protease cathepsin K (*Ctsk*), which is traditionally considered a critical marker of osteoclasts.[52] A distribution of Ctsk-positive mesenchymal stem cells (MSCs) was previously reported in the perichondral groove of Ranvier, suggesting that Ctsk is not just a marker for osteoclasts but also contributes to osteoblastic lineage cells.[53] Additionally, recent research by Debnath et al.[14] confirmed the existence of a group of Ctsk-positive PSCs in the periosteum of long bones and calvaria that holds self-renewal capacity and sits at the apex of the differentiation hierarchy through lineage tracing and serial transplantation studies. Ctsk-positive periosteal cells include three classes of SSCs ($CD200^+CD105^-$); bone, cartilage, stromal progenitor cells ($CD200^-CD105^-$), and bone, cartilage, stromal precursor cells ($CD200^-CD105^+$).[14] Furthermore, skull formation mainly depends on intramembranous ossification, which implies that $Ctsk^+$ cells are involved in maintenance of the calvarial osteogenic pattern. $Ctsk^+$ cells isolated from the periosteum can self-renew and differentiate into bone, cartilage, and adipocytes *in vitro*.[53] Pathologically, ablation of the tumour suppressor gene liver kinase b1 (*Lkb1*) in the $Ctsk^+$ cell population induces the spontaneous formation of neoplasm phenocopies of human osteosarcoma in mice, suggesting that $Ctsk^+$ cells may serve as stem cells and participate in the development of malignant tumours.[54] An identified transcriptome crucial to skeletal development, paired related homeobox 1 (*Prx1*), also labels mesenchymal cells with specific functions in limb bones.[55, 56] $Prx1^+$ cells isolated from the periosteum expressed multiple characteristic transcriptomes of BMSCs, such as *Pdgfra*, gremlin 1, DAN family BMP antagonist (*Grem1*), chemokine (C-X-C motif) ligand 12 (*Cxcl12*), and nestin (*Nes*).[55] Prx1-expressing cells extracted from the periosteum of postnatal long bones can differentiate into chondrocytes and osteoblasts.[55] Therefore, by transplanting $Prx1^+$ cells from the periosteum to the fracture site, $Prx1^+$ cells can differentiate into osteogenic progenitor cells with self-renewal ability.[56, 57] Furthermore, the number of $Prx1^+$ cells in the cranial suture niche also decreases during aging, and $Prx1^+$ cells are also proven to be majorly involved in restoring cranial defects.[57]

Sox9 is also understood to label groups of PSCs and has previously been studied during the process of endochondral ossification.[47,58] Intriguingly, recent studies revealed that extracellular nutrients determine endochondral ossification through the transcription factor forkhead box O (*FOXO*).[59] In the absence of fat tissue, *FOXO* determines the fate of skeletal progenitors by regulating *Sox9* expression.[59] $Sox9^+$ periosteal progenitors generate chondrocytes, osteoblasts, and mature cortical osteocytes during the repair of femoral fractures.[47] Sox9-expressing progenitors in the periosteum supply hybrid skeletal cells to form osteoarthritis osteophytes.[13] This indicates that $Sox9^+$ cells derived from the periosteum have stem cell properties and broadly participate in bone regeneration. Similarly, Ortinau et al.[48] reported the presence of $Mx1^+\alpha SMA^+$PSCs in postnatal mice, which also rapidly differentiate into chondrocytes and osteoblasts in the injured area with the periosteal amendment. Chemokine (C-C motif) ligand 5 receptors, C-C motif chemokine receptor (CCR)3 and CCR5, have also been demonstrated to label PSCs. CCR5 induces PSCs to migrate to the injured area in response to repair.[48, 60] Additionally, Gli1, apart from the growth plate labelling, also represents a population of PSCs that persist in the periosteal tissue generating chondrocytes and osteoblasts during fracture healing through transforming growth factor β/Smad2 signalling.[49]

The close relationship between PSCs and stem cells of the growth plate resting zone dynamically maintains skeletal homeostasis during both regenerative and developmental stages. Loss of *Hox11* function leads to reduced cartilage formation and delayed fracture healing.[61] With the loss of *Hox11* function, osteoblast maturation is defective, and osteocytes are abnormal.[50] Hox11-expressing cells are mainly found in the periosteum, the earliest stages of skeletal

Table 3. Markers of skeletal stem cells in the periosteum of long bones

Gene	Transgenes	Source	Function	References
Ctsk	*Ctsk-Cre*	Bone/cartilage/adipose	A marker of osteoclasts; distinguishing periosteal osteoprogenitor cell types	14, 52, 54
Prx1	*Prrx1-Cre*	Bone/cartilage	In the repair of cranial defects	55, 56
Sox9	*Sox9-CreERT*	Bone/cartilage	Generate chondrocytes, osteoblasts, and mature cortical osteocytes during the repair of femoral fracture	47
Mx1 *αSMA*	*Mx1-Cre* *αSMA-CreERT* *αSMA-GFP*	Bone/cartilage	Repairing new periosteum	48
Gli1	*Gli1-CreERT*	Bone/cartilage	Labels skeletal stem cells of the growth plate	49
Hoxa11	*Hoxa11-CreERT2* *Hoxa11-EGFP*	Bone/cartilage	Regulating differentiation of Hox-expressing skeletal stem cells into the osteolineage	50, 51

Note: Ctsk: cathepsin K; αSMA: α-smooth muscle actin.

development, and are capable of self-renewal.[51] Nes$^+$ cells are capable of tri-lineage differentiation, but they are not true SSCs as they lack the ability to self-renew.[62] Furthermore, Tsukasaki et al.[63] reported that PSCs are responsible for growth plate maintenance, long bone growth, and intramembranous osteogenesis. Notably, mice deficient in PSCs show defects in intramembranous and endochondral ossification, which reflects the importance of crosstalk between SSCs in the periosteum and growth plate.[63]

Skeletal Stem Cells in the Marrow Cavity

MSCs and BMSCs are a mixture of different subpopulations with significant heterogeneity in the bone marrow cavity.[64, 65] The earliest research on the existence of stem cells in bones was carried out in the 1960s when Friedenstein et al.[66] found that reticular tissue develops after transplantation of bone marrow fragments and bone marrow cell suspensions into diffusion chambers, and sometimes bone formation was also observed. Subsequently, Caplan[67] proposed the name "mesenchymal stem cells" to define the group of stem cells involved in forming bone and cartilage tissue in embryos and in repairing bone in adulthood. In 2005, The International Society for Cellular Therapy[68] proposed minimum criteria for defining human MSCs: 1) cells which are plastic-adherent under standard culture conditions; 2) cells which express the surface immune markers CD105, CD73 and CD90, but not CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and human leukocyte antigen-DR; and 3) cells with tri-lineage differentiation ability *in vitro*. The types of MSCs have been extended to bone marrow, adipose tissue, periosteum, synovial lining, muscle tissue, dental pulp, and umbilical cord.[69-72]

Since then, continuous progress has been made over the decades. Although MSCs can be extracted from many different tissues, they remain difficult to define due to their severe ambiguity regarding nature, identity, and function.[30] Notably, MSCs are highly heterogeneous cells with stem cell properties.[73] SSCs have significant biological and functional overlaps with MSCs, the main difference between them being that MSCs can differentiate into bone, cartilage and fat, but SSCs can only differentiate into bone and cartilage. Studies have proved that various markers in the bone marrow cavity can be used to label MSCs, including, but not limited to, *Grem1*, *Gli1*, leptin receptor (*Lepr*), *Nes*, early B cell factor 3 (*Ebf3*), and *Cxc112*. Nestin$^+$ MSCs are capable of expanding after being implanted into animal models (**Table 4**, and **Figure 1**).[74-79] Additionally, parathyroid hormone treatment promotes the differentiation of Nestin$^+$ MSCs into osteoblasts. 80 Mx1$^+$ stromal cells also rapidly respond to fractures by differentiating into osteoblasts at the injury site to accelerate bone healing.[81] However, neither Nestin$^+$ MSCs nor Mx1$^+$ stromal cells can differentiate into cartilage or adipose tissue.[80, 81] Grem1 expression identifies distinct connective tissue stem cells in bone and intestine. Grem1 can mark osteochondroreticular stem cells in the bone marrow.[82] Grem1-expressing cells have the ability to self-renew and to differentiate into osteogenic, cartilage, and reticular marrow stromal cells but not adipocytes.[82] Grem1$^+$ cells do not express *Nes* and *Cxcl12*, unlike SSCs around blood sinusoid vessels. Furthermore, Grem1$^+$ cells are essential for bone development and bone repair.[82, 83]

BMSCs can be divided into metaphyseal MSCs (mpMSCs) and diaphyseal MSCs. Among them, mpMSCs have a stronger ability to differentiate into osteoblasts and are an important source of diaphyseal MSCs. PDGFRβ$^+$ mpMSCs have multi-directional differentiation potential. Adult mouse mpMSCs can differentiate into adipocytes after injury.[84] Of note, diaphyseal MSCs include Lepr$^+$ mesenchymal cells and other stromal cells in the bone marrow cavity. The leptin receptor (LepR) is an MSC marker highly enriched in the bone marrow.[78, 79] Almost all LepR$^+$ cells are colony-forming unit fibroblasts in the bone marrow. Fate-mapping showed that LepR-Cre marked the vast majority of osteoblasts and adipocytes in the bone marrow.[79, 85] Indeed, LepR$^+$ cells, which are the source of most bone and fat cells in the adult bone marrow cavity, only gradually increase after birth.[79] LepR$^+$ cells are normally quiescent, but they enter a proliferative state after injury, and are involved in bone regeneration after irradiation and fracture healing.[79] Importantly, the Leptin–LepR signalling pathway is key in regulating adipogenesis and osteogenesis. The elevated expression of Leptin enhances adipogenesis and reduces the osteogenesis of bone marrow MSCs.[78] Shu et al.[86] also found that bone formation before and after adolescence is dominated by Aggrecan$^+$ growth plate chondrocytes and Lepr$^+$ bone marrow MSCs, which regulate bone growth and thickening, respectively. Furthermore, most of the Lepr$^+$

Table 4. Markers of skeletal stem cells in the bone marrow

Gene	Transgenes	Source	Function	References
Axin2	*Axin2-CreER*	Bone/cartilage/adipose	Osteoblasts, mesenchymal cells	74, 75
Cxcl12	*Cxcl12-CreER*	Bone/cartilage	Reactivate to form osteoblasts after injury	76
Mx1	*Mx1-CreER*	Bone/cartilage	Differentiate into cartilage and adipose tissue, respond to bone fractures	81
Nestin	*Nestin-CreER*	Bone/cartilage	Nestin$^+$ mesenchymal stem cells can self-renew and expand in transplantation experiments	77, 80
Lepr	*Lepr-Cre*	Bone/cartilage/adipose	Regulation of adipogenesis and osteogenesis	78, 79
Grem1	*Grem1-CreER*	Bone/cartilage	Mark osteochondroreticular stem cells, bone repair	82, 83

Note: Axin2: axis inhibition protein 2; CreER: Cre-estrogen receptor; Cxcl12: chemokine (C-X-C motif) ligand 12; Grem1: Gremlin 1, DAN family BMP antagonist; Lepr: leptin receptor; Mx1: MX dynamin like GTPase 1; Nes: nestin.

BMSCs originated from growth plate cartilage. This transition explains, from a stem cell perspective, how mammalian limb skeletons transition from rapid longitudinal growth to slow thickening of bone after adolescence.[86]

Skeletal Stem Cells In Craniofacial Bone

Skeletal stem cells in the periosteum of the cranium

Many studies have observed that many osteogenic precursor cells contribute to bone formation in the periosteum of the skull (**Table 5**, and **Figure 2**).[14, 48, 87-91] However, they have not been confirmed due to the lack of specific cell surface markers. Ctsk$^+$ PSCs are present not only in the periosteum of long bones but also in the periosteum of the calvaria.[14] Ctsk$^+$ PSCs are primarily involved in intramembranous osteogenesis but can also contribute to endochondral osteogenesis after injury.[14] Ortinau et al.[48] traced a group of long-term PSCs expressing Mx1 and α-smooth muscle actin (αSMA), which is responsible for periosteal osteoblastogenesis throughout life and can respond to bone defect injury in a mouse calvarial defect model. Notably, Mx1$^+$αSMA$^+$ PSCs specifically express the chemokine (C-C motif) ligand 5 receptors, CCR3 and CCR5, and chemokine (C-C motif) ligand 5 induces PSC migration *in vivo* to participate in bone healing.[48]

Table 5. Markers of skeletal stem cells in craniofacial bone

Craniofacial bone	Gene	Transgenes	Source	Function	References
Periosteum of the cranium	*Ctsk*	*Ctsk-Cre*	Bone/cartilage	Intramembranous osteogenesis	9
	Mx1	*Mx1-Cre*	Bone/cartilage	Participate in bone healing	48
	SMA	*αSMA-CreERT*	Bone/cartilage		
Calvarial sutures	*Gli1*	*Gli1-CreER*	Bone/cartilage	Cause premature craniosynostosis	81, 82
	Axin2	*Axin2-CreERT*	Bone/cartilage	Respond to orthodontic tension force	87
	Prx1	*Prx1-Cre*	Bone/cartilage	Scattered distribution in calvaria sutures	57
Teeth and periodontal tissue	*Gli1*	*Gli1-CreER*	Bone/cartilage	Regulated by Wnt pathway	88
	Axin2	*Axin2-CreERT*	Bone/cartilage	Primary progenitor cells of cementoblasts	89
	Prx1	*Prx1-Cre*	Bone/cartilage	Participate in angiogenesis.	90
Jaw bone	*Ctsk*	*Ctsk-Cre*	Bone/cartilage	Present on the periosteum of the jaw	91
	Ly6a		Bone/cartilage		

Note: Axin2: axis inhibition protein 2; Cre: Cre recombinase; CreER: Cre recombinase estrogen receptor; CreERT: Cre recombinase receptor tamoxifen; Ctsk: cathepsin K; Gli1: GLI-Kruppel family member GLI1; Ly6a: lymphocyte antigen 6 complex, locus A; Mx1: MX dynamin like GTPase 1; Prx1: paired related homeobox 1; SMA: smooth muscle actin; αSMA: α-smooth muscle actin.

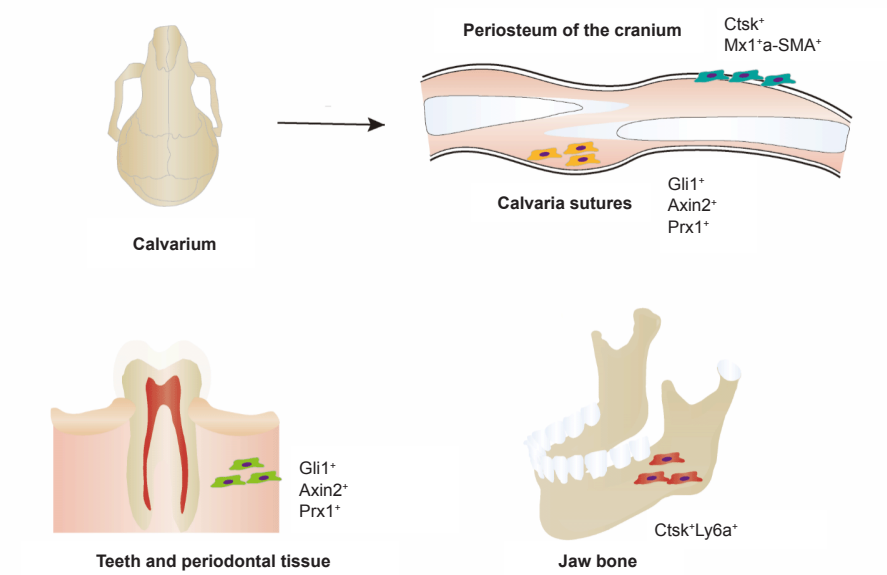

Figure 2. Distribution of SSCs in craniofacial bone, with different cell surfaces shown. The cell surface markers of SSCs in the cranial periosteum are Ctsk$^+$ and Mx1$^+$αSMA$^+$. The cell surface markers of SSCs in the calvarial sutures are Gli1$^+$, Prx1$^+$, and Axin2$^+$. The cell surface markers of SSCs in the teeth and periodontal tissue are Gli1$^+$, Prx1$^+$, and Axin2$^+$. The cell surface markers of SSCs in the jaw bone are Ctsk$^+$ and Ly6a$^+$. Axin2: axis inhibition protein 2; Ctsk: cathepsin K; Gli1: GLI-Kruppel family member GLI1; Ly6a: lymphocyte antigen 6 complex, locus A; Mx1: MX dynamin like GTPase 1; Prx1: paired related homeobox 1; SSC: skeletal stem cell; αSMA: α-smooth muscle actin.

Skeletal stem cells in calvarial sutures

The cranial sutures refer to the connection gaps between the craniofacial bones, which contain active osteogenic activity.[92] Ablation of the interstitial Gli1$^+$ cells causes premature craniosynostosis and cranial growth impairment in adult mice.[93] The number of Gli1$^+$ suture stem cells (SuSCs) is significantly reduced in Twist1$^{+/-}$ mice with craniosynostosis.[93] The Gli1$^+$ SuSC lineage is rapidly activated in the standard suture expansion model to contribute to bone formation regulated by the Wnt signalling pathway, and β-catenin conditional knockout restricts the activation of Gli1$^+$ SuSCs.[87] Takamitsu et al. confirmed that the Axin2$^+$ stem cell population in the cranial suture retained self-renewal and differentiation capabilities for a long time.[76] The bone morphogenetic protein (BMP) signalling pathway regulates the Axin2$^+$ stem cell population. From investigations, it was noted that Bmpr1a knockout mice with Axin2-expressing SuSCs lose osteogenic differentiation ability, resulting in craniosynostosis.[94] In contrast, the disruption of Rap1b in mice enhances osteogenic differentiation and impairs chondrogenic differentiation, resulting in abnormal skeletal development.[95] Importantly, *Rap1b* is downstream of *Axin2* in response to fibroblast growth factor and BMP signalling and is critical for balancing fibroblast growth factor and BMP signalling.[95] Furthermore, postnatal Prx1$^+$ SSCs are present in calvarial sutures and decrease with age. Unlike the ablation of Gli1$^+$ cells, the ablation of Prx1$^+$ cells does not lead to craniosynostosis and cranial growth disorder, which may be due to the scattered distribution of Prx1$^+$ cells and the widespread distribution of Gli1$^+$ cells.[57]

Skeletal stem cells in teeth and periodontal tissue

The teeth are attached to alveolar bone by periodontal ligaments. Gli1$^+$ cells are present in the molar periodontal ligament in mice. Gli1$^+$ periodontal ligament SSCs are distributed around nerve bundles and are regulated by the Wnt pathway.[88] Axin2$^+$ periodontal ligament cells also contribute to the formation of alveolar bone in response to orthodontic tension force. Ablation of Axin2$^+$ periodontal ligament cells predominantly blocks osteogenic patterns in tooth development.[89] Notably, Axin2$^+$ cells are the primary cells that form cementoblasts in the adult physiological state, while CD90$^+$ cells become the primary progenitor cells of cementoblasts in the pathological state of periodontal disease.[96] Prx1-expressing cells also exist in the periodontal ligament, contributing to postnatal periodontal development and regeneration. In the process of periodontal ligament reconstruction after tooth transplantation, Prx1$^+$ cells participate in angiogenesis.[90]

Skeletal stem cells in the jaw

The jaw SSC population possesses activity within multiple signalling pathways to achieve regeneration in a mouse model of mandibular distraction, such as focal adhesion kinase.[97] In the mouse models of mandibular distraction osteogenesis, this work also reflects the ability of SSCs to achieve regeneration in response to mechanotransduction.[97] Ctsk$^+$ stromal cells have also been confirmed to exist within the jaw bone marrow and periosteum.[91] Ding et al.[91] also found that the Ctsk$^+$Ly6a$^+$ subset of cells with Ly6a surface markers only exists on the periosteum of the jaw. Therefore, the migration of the Ctsk$^+$Ly6a$^+$ cell population can be activated when required for osteogenic differentiation of jaw defects, suggesting that the Ctsk$^+$Ly6a$^+$ population may be a critical progenitor subset in the process of jaw formation.[91]

Circulating Osteogenic Cells

The earliest reports regarding circulating SSCs suggested the presence of some circulating stem cells with osteogenic characteristics in the blood of breast cancer patients.[98] The term circulating SSCs was first coined by Kuznetsov et al.[99] who obtained osteogenic and adipogenic cell populations from four mammals, transplanted them subcutaneously into immunodeficient individuals, and found that they generated bone organoids. Osteoblast-lineage cells found in human circulation further convinced the research field of the existence of circulating SSCs.[100] Notably, osteoblastic cells undergo massive expansion during adolescence, which is closely related to bone formation.[100] Circulating SSCs are a group of blood-derived cells with osteogenic ability which can differentiate into MSCs. There are also many other terms, including circulating osteogenic precursor (COP) cells, circulating osteoprogenitors, circulating MSCs (cMSCs), and monocyte-derived mesenchymal precursors.[101] Among these, COP cells are most frequently reported.[101] This cell population expresses surface markers related to bone formation, such as osteocalcin (OCN), alkaline phosphatase (AP), and type 1 collagen. However, they do not express CD45, CD14, and the endogenous stem cell marker CD34.[101, 102] More evidence is still needed to determine the source of COP cells, of which bone marrow is the main source.[102] COP cells also have the characteristics of MSCs, including plasticity and cell surface expression of CD105, CD73, and CD90 (**Table 6**, and **Figure 3**).[103] They also lack expression of CD45, CD34, and CD14 and differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*.[104] It is also reported that COP cells consist of two subsets, one of which expresses haematopoietic cell line antigen markers, and the other possesses bone marrow mesenchymal stem and progenitor cell properties.[99, 105] The proportion of COP cells in healthy people is relatively low and stable and only increases during fracture healing or adolescence.[30, 100, 106, 107] COP cells are mobilised to participate in tissue repair under the influence of fractures, thyroid hormones, hypoxia, and other factors.[108-110] They are attracted by osteogenic expressed stromal cell-derived factor-1 and migrate to the fracture site to participate in the repair of bone tissue.[111] Other chemokines, including platelet-derived growth factor AB, insulin-like growth factor 1, and macrophage-derived chemokine, mobilise peripheral COP cells to migrate to the corresponding tissues to play their role, but how these chemokines are recognised and responded to is still unclear.[112]

Circulating progenitors are also called circulating healing cells and are characterised by Lin-cell surface markers.[113] These cells dramatically increase in the femoral fracture callus to contribute to fracture repair.[114] Bone marrow stromal cell antigen 2 (BST2)-expressing circulating healing cells derived

Table 6. Markers of circulating osteogenic cells in the peripheral circulation

Cell type	Markers	Function	References
Haematopoietic COP cells	$CD45^+CD34^+CD14^+OCN^+AP^+Col1^+$	Maintain a stable level in the peripheral circulation	69, 103, 107
MSC-like COP cells	$CD45^-CD34^-$ $CD44^+CD73^+CD90^+CD105^+$	Possess characteristics of mesenchymal stem cells	71, 99, 102, 106

Note: AP: alkaline phosphatase; Col1: type 1 collagen; COP: circulating osteogenic cell; MSC: mesenchymal stem cell; OCN: osteocalcin.

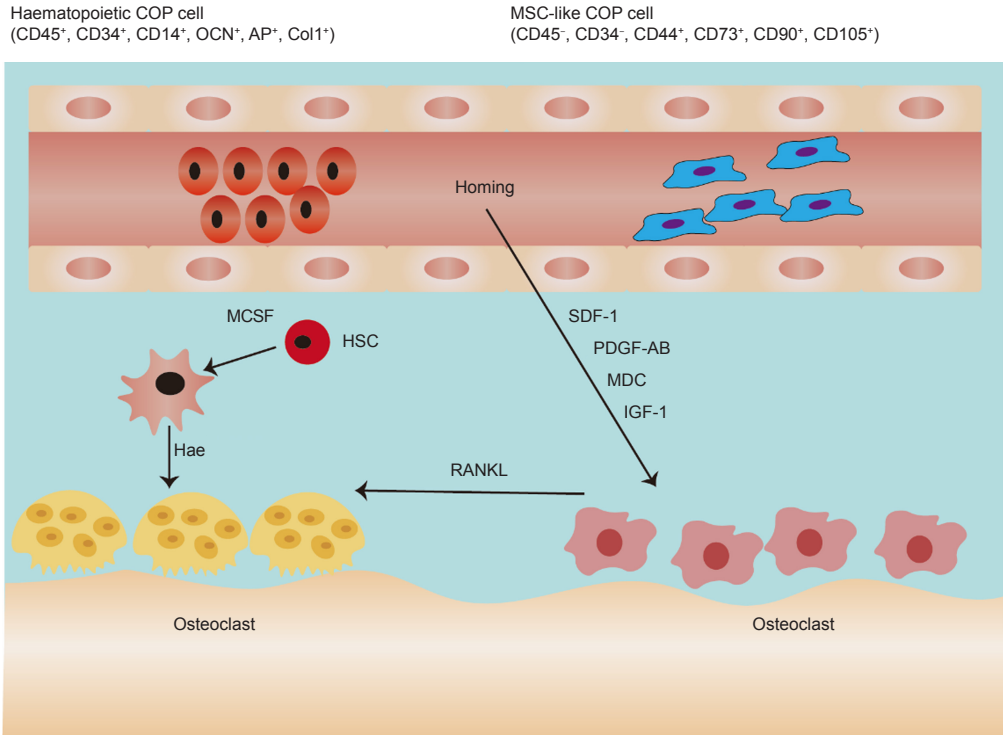

Figure 3. Circulating osteogenic cells contribute to bone formation through stem cell homing to receive IGF-1, PDGF-AB, SDF-1, and MDC. M-CSF stimulates HSCs to differentiate into monocytes and macrophages and then differentiate into osteoclasts to receive RANKL. COP: circulating osteogenic precursor cell; HSC: haematopoietic stem cell; IGF-1: insulin-like growth factor 1; M-CSF: macrophage colony-stimulating factor; MDC: macrophage-derived chemokine; MSC: mesenchymal stem cell; PDGF-AB: platelet derived growth factor AB; RANKL: receptor activator of nuclear factor kappa-B ligand; SDF-1: stromal cell-derived factor-1.

from bone marrow are circulating promoters that quickly accelerate fracture injury and repair damaged tissues.[115] BST2-expressing circulating healing cells also induce the activation of quiescent stem cells.[115] Additionally, $CD34^+$ cells derived from endothelial progenitor cells are induced under trauma to provide a suitable microenvironment for fracture healing through angiogenesis and osteogenesis to accelerate fracture healing.[116-118] Furthermore, $CD34^+OCN^+$ cells originating from the haematopoietic circulating population also contribute to the process of fracture repair.[103] Notably, CD31 is a marker of circulating endothelial precursor cells, and $CD31^+$ cells extracted from human peripheral blood and transplanted into a rat model of a bone defect promote bone tissue repair.[22, 23] Although circulating osteogenic cells appear to home upon injury and contribute to fracture healing, what has been discovered so far is not enough to comprehensively profile circulating SSCs. Furthermore, the origin and niche of the osteogenic cells in the blood remain unstudied.

Regulation of the Skeletal Stem Cell Niche

The functions of self-renewal and pluripotent differentiation of SSCs need to be carried out within a unique but supportive niche. The stem cell niche affects stem cell state (quiescent/proliferative states) as well as their lineage directions. A balanced niche is primarily required for the proper function of SSCs which then have the capacity to differentiate into skeletal tissues during bone accrual and regeneration.[119, 120] The availability of a sufficient number and appropriate function of SSCs are prerequisites for healthy tissue regeneration. Failing to maintain such a niche can drastically dampen the functioning

of SSCs. For instance, increased cellular senescence caused by systemic and local pro-inflammatory environments is the main reason for the decline in the number and function of SSCs.[121-123]

Chronic inflammation mediated by nuclear factor κB activation leads to dysfunctional stem cell pool regeneration because it induces cellular senescence.[122] Several signalling pathways have also been shown to regulate the SSC niche. Col2a1$^+$ growth plate chondrocytes are regulated by the hedgehog and mammalian target of rapamycin complex 1 signalling pathways.[41] CTSK$^+$ PSCs are regulated by the Wnt signalling pathway, and Wnt-responding cells have been traced to utilize the *Axin2* gene in mice (**Figure 4B**).[7, 124] The bone healing of diabetic patients is usually worse than that of healthy people, and alteration of the SSC niche may be a cause. Expression of Indian hedgehog controls SSC expansion in diabetic mice, and exogenous Indian hedgehog rescues defective bone healing caused by the altered SSC niche in diabetic mice.[125] A large number of studies have confirmed that BMP2 plays a vital role in maintaining bone development and growth.[126, 127] The BMP antagonist *Grem1* is a marker of bone marrow SSCs.[82] Although BMP2 is abundantly expressed in bone tissue, only Prx1$^+$ periosteal progenitors contribute to bone repair.[128] Exogenous recombinant BMP2 rapidly induces expansion of isolated SSCs, whereas the addition of exogenous recombinant transforming growth factor β or tumour necrosis factor α does not.[9]

Ambrosi et al.[121] further found that aging of SSCs alters the signalling of the bone marrow niche to distort the differentiation of bone and haematopoietic lineages, resulting in reduced bone regeneration capacity. The combination of BMP2 and macrophage-colony stimulating factor antagonists reverses this change and converts aging SSCs back to a younger state in mice (**Figure 4C**).[121]

Piezo1 is highly expressed in PSCs and their derived osteoblast lines and chondrocytes. Activation of piezo1 promotes the expression and nuclear localisation of yes-related protein in PSCs, thereby increasing β-catenin expression and nuclear localisation. Yes-related protein directly interacts with β-catenin to form a transcriptional yes-related protein/β-catenin complex, up-regulating osteogenic, chondrogenic and angiogenic factors (**Figure 4A**).[129] Platelet-derived growth factor-BB has also been shown to recruit PSCs for osteogenic differentiation.[130] In conclusion, the regulation of SSCs requires the regulation of many factors, but many still need to be explored.

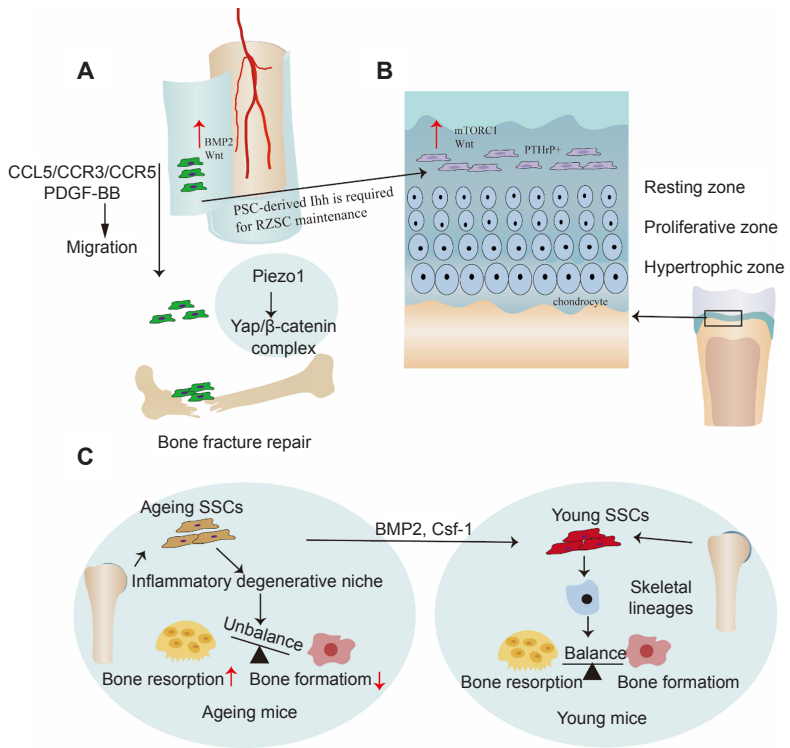

Figure 4. (A) Wnt and BMP2 are enriched in PSCs. PDGF-BB and CCL5/CCR3/CCR5 promote the migration of SSCs, while activation of Piezo1 promotes the expression and nuclear localisation of Yap in PSCs, and forms a transcriptional Yap/β-catenin complex which promotes fracture healing. (B) The Wnt and mTORC1 signalling pathways regulate SSCs of the growth plate. PSCs control growth plate SSCs through the Ihh signalling pathway. (C) Aged mice develop a pro-inflammatory microenvironment that disrupts the osteoclast–osteoblast balance. A combination of BMP2 and CSF1 antagonists reverses this change and activates aging SSCs in mice, returning them to a younger state. BMP2: bone morphogenetic protein 2; CCL5: chemokine (C-C motif) ligand 5; CCR3: C-C motif chemokine receptor 3; CCR5: C-C motif chemokine receptor 5; CSF1: colony stimulating factor 1; mTORC1: mechanistic target of rapamycin complex; PDGF-BB: platelet derived growth factor BB; PSC: periosteal stem cell; PTHrP: parathyroid hormone-related protein; Wnt: wingless-related integration site; Yap: yes-related protein.

Conclusions and Future Insights

The discovery of SSCs in mice and humans began a new chapter in bone research and an era of tissue-residing stem cell research. Researchers have successively used different cell markers to confirm the existence of SSCs at different sites, but many problems still have not been clarified. Recently, single-cell transcriptome sequencing analysis of 108 intervertebral disc nucleus pulposus, annulus fibrosus and endplate tissues revealed a group of PDGFRA and protein C receptor, endothelial (PROCR) nucleus pulposus progenitor cells, which were confirmed to have clonogenic and trilineage differentiation abilities *in vitro*.[131] Whether there are SSC populations in the pedicle of the spine and the vertebral body and what biological characteristics they exhibit in the function of the spine still require additional research for clarification. Furthermore, breast and prostate cancers are prone to bone metastasis, and the role of SSCs in this situation is still unclear. This review summarizes the research progress into SSCs in recent years. SSCs are far more complex than previously thought, and their characteristics still baffle scientists. Future research directions should focus on mapping the differentiation hierarchy of all SSCs and their niches in space and time based on their surface markers. The bridge between SSC dysfunction and the pathogenesis of skeletal diseases has not been clearly defined, and more research on this aspect is needed. Finally, the main factors determining stem cell fate commitment are discussed. Various stem cells have been used for clinical research, such as BMSCs, adipose stem cells, and umbilical cord stem cells. The elucidation of the regulatory mechanisms of SSCs at the cellular, molecular, and tissue structure levels is expected to provide new strategies for stem cell therapy and bring new hope for human health and life.

This review summarizes recent research progress on SSCs from initial identification to potential utilization. As one of the major groups of representative tissue-specific resident stem cells, SSCs refreshed our knowledge of skeletal development, bone homeostasis, and ossification-based regeneration. These important findings on SSCs also define the responsible group of cells that continuously restore bone quality from upstream. This ends the argument of whether a whole group of "stem cells", namely MSCs, should be hierarchically considered as the source of bone-forming cells. Although conceptual conflicts on how these stem cells should be entirely defined still exist, it is not negligible that the studies on SSCs have great therapeutic potential which will help overcome the limitations of recent osteoporotic treatments. Future research directions should focus on making the link between the dysfunction of SSCs and the pathogenesis of skeletal diseases to clearly define their pharmaceutical value. At present, various sources of cells have been taken advantage of, such as BMSCs, adipose-derived stromal cells, and umbilical cord stromal cells. The elucidation of the regulatory mechanism of SSCs from cellular to organic levels is expected to provide new strategies for developing stem cell therapy which will bring hope for future human health.

We have systematically summarized the literature on SSCs in recent years, but due to the huge amount of literature on SSCs, there may be omissions. In addition, this review has not thoroughly discussed the spatiotemporal distribution of SSCs due to the current lack of sufficient research regarding this point.

Author contributions

RX and NL designed the review; GY performed literature research and wrote the manuscript; ZL and XL revised and edited the manuscript. All authors approved the final version of this manuscript.

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Conflicts of interest statement

The authors declare no conflict of interest.

1. Zheng, C.; Chen, J.; Liu, S.; Jin, Y. Stem cell-based bone and dental regeneration: a view of microenvironmental modulation. *Int J Oral Sci.* **2019**, *11*, 23.
2. Zhu, L.; Luo, D.; Liu, Y. Effect of the nano/microscale structure of biomaterial scaffolds on bone regeneration. *Int J Oral Sci.* **2020**, *12*, 6.
3. Su, N.; Yang, J.; Xie, Y.; Du, X.; Chen, H.; Zhou, H.; Chen, L. Bone function, dysfunction and its role in diseases including critical illness. *Int J Biol Sci.* **2019**, *15*, 776-787.
4. Khosla, S.; Farr, J. N.; Tchkonia, T.; Kirkland, J. L. The role of cellular senescence in ageing and endocrine disease. *Nat Rev Endocrinol.* **2020**, *16*, 263-275.
5. Henkel, J.; Woodruff, M. A.; Epari, D. R.; Steck, R.; Glatt, V.; Dickinson, I. C.; Choong, P. F.; Schuetz, M. A.; Hutmacher, D. W. Bone regeneration based on tissue engineering conceptions - a 21st century perspective. *Bone Res.* **2013**, *1*, 216-248.
6. Gao, C.; Peng, S.; Feng, P.; Shuai, C. Bone biomaterials and interactions with stem cells. *Bone Res.* **2017**, *5*, 17059.
7. Anthony, B. A.; Link, D. C. Regulation of hematopoietic stem cells by bone marrow stromal cells. *Trends Immunol.* **2014**, *35*, 32-37.
8. Ng, A. P.; Alexander, W. S. Haematopoietic stem cells: past, present and future. *Cell Death Discov.* **2017**, *3*, 17002.
9. Chan, C. K.; Seo, E. Y.; Chen, J. Y.; Lo, D.; McArdle, A.; Sinha, R.; Tevlin, R.; Seita, J.; Vincent-Tompkins, J.; Wearda, T.; Lu, W. J.; Senarath-Yapa, K.; Chung, M. T.; Marecic, O.; Tran, M.; Yan, K. S.; Upton, R.; Walmsley, G. G.; Lee, A. S.; Sahoo, D.; Kuo, C. J.; Weissman, I. L.; Longaker, M. T. Identification and specification of the mouse skeletal stem cell. *Cell.* **2015**, *160*, 285-298.
10. Chan, C. K. F.; Gulati, G. S.; Sinha, R.; Tompkins, J. V.; Lopez, M.; Carter, A. C.; Ransom, R. C.; Reinisch, A.; Wearda, T.; Murphy, M.; Brewer, R. E.; Koepke, L. S.; Marecic, O.; Manjunath, A.; Seo, E. Y.; Leavitt, T.; Lu, W. J.; Nguyen, A.; Conley, S. D.; Salhotra, A.; Ambrosi, T. H.; Borrelli, M. R.; Siebel, T.; Chan, K.; Schallmoser, K.; Seita, J.; Sahoo, D.; Goodnough, H.; Bishop, J.; Gardner, M.; Majeti, R.; Wan, D. C.; Goodman, S.; Weissman, I. L.; Chang, H. Y.; Longaker, M. T. Identification of the human skeletal stem cell. *Cell.* **2018**, *175*, 43-56.e21.
11. Kretzschmar, K.; Watt, F. M. Lineage tracing. *Cell.* **2012**, *148*, 33-45.
12. Gulati, G. S.; Murphy, M. P.; Marecic, O.; Lopez, M.; Brewer, R. E.;

Koepke, L. S.; Manjunath, A.; Ransom, R. C.; Salhotra, A.; Weissman, I. L.; Longaker, M. T.; Chan, C. K. F. Isolation and functional assessment of mouse skeletal stem cell lineage. *Nat Protoc.* **2018**, *13*, 1294-1309.

13. Roelofs, A. J.; Kania, K.; Rafipay, A. J.; Sambale, M.; Kuwahara, S. T.; Collins, F. L.; Smeeton, J.; Serowoky, M. A.; Rowley, L.; Wang, H.; Gronewold, R.; Kapeni, C.; Méndez-Ferrer, S.; Little, C. B.; Bateman, J. F.; Pap, T.; Mariani, F. V.; Sherwood, J.; Crump, J. G.; De Bari, C. Identification of the skeletal progenitor cells forming osteophytes in osteoarthritis. *Ann Rheum Dis.* **2020**, *79*, 1625-1634.
14. Debnath, S.; Yallowitz, A. R.; McCormick, J.; Lalani, S.; Zhang, T.; Xu, R.; Li, N.; Liu, Y.; Yang, Y. S.; Eiseman, M.; Shim, J. H.; Hameed, M.; Healey, J. H.; Bostrom, M. P.; Landau, D. A.; Greenblatt, M. B. Discovery of a periosteal stem cell mediating intramembranous bone formation. *Nature.* **2018**, *562*, 133-139.
15. Han, X.; Zhang, Z.; He, L.; Zhu, H.; Li, Y.; Pu, W.; Han, M.; Zhao, H.; Liu, K.; Li, Y.; Huang, X.; Zhang, M.; Jin, H.; Lv, Z.; Tang, J.; Wang, J.; Sun, R.; Fei, J.; Tian, X.; Duan, S.; Wang, Q. D.; Wang, L.; He, B.; Zhou, B. A suite of new Dre recombinase drivers markedly expands the ability to perform intersectional genetic targeting. *Cell Stem Cell.* **2021**, *28*, 1160-1176.e7.
16. He, L.; Li, Y.; Li, Y.; Pu, W.; Huang, X.; Tian, X.; Wang, Y.; Zhang, H.; Liu, Q.; Zhang, L.; Zhao, H.; Tang, J.; Ji, H.; Cai, D.; Han, Z.; Han, Z.; Nie, Y.; Hu, S.; Wang, Q. D.; Sun, R.; Fei, J.; Wang, F.; Chen, T.; Yan, Y.; Huang, H.; Pu, W. T.; Zhou, B. Enhancing the precision of genetic lineage tracing using dual recombinases. *Nat Med.* **2017**, *23*, 1488-1498.
17. Wagner, D. E.; Klein, A. M. Lineage tracing meets single-cell omics: opportunities and challenges. *Nat Rev Genet.* **2020**, *21*, 410-427.
18. Hsu, Y. C. Theory and practice of lineage tracing. *Stem Cells.* **2015**, *33*, 3197-3204.
19. Zomer, A.; Steenbeek, S. C.; Maynard, C.; van Rheenen, J. Studying extracellular vesicle transfer by a Cre-loxP method. *Nat Protoc.* **2016**, *11*, 87-101.
20. Kristianto, J.; Johnson, M. G.; Zastrow, R. K.; Radcliff, A. B.; Blank, R. D. Spontaneous recombinase activity of Cre-ERT2 in vivo. *Transgenic Res.* **2017**, *26*, 411-417.
21. Muzumdar, M. D.; Tasic, B.; Miyamichi, K.; Li, L.; Luo, L. A global double-fluorescent Cre reporter mouse. *Genesis.* **2007**, *45*, 593-605.
22. Madisen, L.; Zwingman, T. A.; Sunkin, S. M.; Oh, S. W.; Zariwala, H. A.; Gu, H.; Ng, L. L.; Palmiter, R. D.; Hawrylycz, M. J.; Jones, A. R.; Lein, E. S.; Zeng, H. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci.* **2010**, *13*, 133-140.
23. Soriano, P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet.* **1999**, *21*, 70-71.
24. Matsushita, Y.; Ono, W.; Ono, N. Bone regeneration via skeletal cell lineage plasticity: All hands mobilized for emergencies: quiescent mature skeletal cells can be activated in response to injury and robustly participate in bone regeneration through cellular plasticity. *Bioessays.* **2021**, *43*, e2000202.
25. Ono, N.; Balani, D. H.; Kronenberg, H. M. Stem and progenitor cells in skeletal development. *Curr Top Dev Biol.* **2019**, *133*, 1-24.
26. Jeffery, E. C.; Mann, T. L. A.; Pool, J. A.; Zhao, Z.; Morrison, S. J. Bone marrow and periosteal skeletal stem/progenitor cells make distinct contributions to bone maintenance and repair. *Cell Stem Cell.* **2022**, *29*, 1547-1561.e6.
27. Bianco, P.; Robey, P. G. Skeletal stem cells. *Development.* **2015**, *142*, 1023-1027.
28. Bianco, P.; Kuznetsov, S. A.; Riminucci, M.; Gehron Robey, P. Postnatal skeletal stem cells. *Methods Enzymol.* **2006**, *419*, 117-148.
29. Bianco, P. "Mesenchymal" stem cells. *Annu Rev Cell Dev Biol.* **2014**, *30*, 677-704.
30. Bianco, P.; Cao, X.; Frenette, P. S.; Mao, J. J.; Robey, P. G.; Simmons, P. J.; Wang, C. Y. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat Med.* **2013**, *19*, 35-42.
31. He, J.; Yan, J.; Wang, J.; Zhao, L.; Xin, Q.; Zeng, Y.; Sun, Y.; Zhang, H.; Bai, Z.; Li, Z.; Ni, Y.; Gong, Y.; Li, Y.; He, H.; Bian, Z.; Lan, Y.; Ma, C.; Bian, L.; Zhu, H.; Liu, B.; Yue, R. Dissecting human embryonic skeletal stem cell ontogeny by single-cell transcriptomic and functional analyses. *Cell Res.* **2021**, *31*, 742-757.
32. Zhao, H.; Zhou, W.; Yao, Z.; Wan, Y.; Cao, J.; Zhang, L.; Zhao, J.; Li, H.; Zhou, R.; Li, B.; Wei, G.; Zhang, Z.; French, C. A.; Dekker, J. D.; Yang, Y.; Fisher, S. E.; Tucker, H. O.; Guo, X. Foxp1/2/4 regulate endochondral ossification as a suppresser complex. *Dev Biol.* **2015**, *398*, 242-254.
33. Ambrosi, T. H.; Goodnough, L. H.; Chan, C. K. F. Human skeletal stem cell aging. *Aging (Albany NY).* **2020**, *12*, 16669-16671.
34. Hallett, S. A.; Matsushita, Y.; Ono, W.; Sakagami, N.; Mizuhashi, K.; Tokavanich, N.; Nagata, M.; Zhou, A.; Hirai, T.; Kronenberg, H. M.; Ono, N. Chondrocytes in the resting zone of the growth plate are maintained in a Wnt-inhibitory environment. *Elife.* **2021**, *10*, e64513.
35. Wang, Y.; Middleton, F.; Horton, J. A.; Reichel, L.; Farnum, C. E.; Damron, T. A. Microarray analysis of proliferative and hypertrophic growth plate zones identifies differentiation markers and signal pathways. *Bone.* **2004**, *35*, 1273-1293.
36. Li, X.; Yang, S.; Yuan, G.; Jing, D.; Qin, L.; Zhao, H.; Yang, S. Type II collagen-positive progenitors are important stem cells in controlling skeletal development and vascular formation. *Bone Res.* **2022**, *10*, 46.
37. Mizuhashi, K.; Ono, W.; Matsushita, Y.; Sakagami, N.; Takahashi, A.; Saunders, T. L.; Nagasawa, T.; Kronenberg, H. M.; Ono, N. Resting zone of the growth plate houses a unique class of skeletal stem cells. *Nature.* **2018**, *563*, 254-258.
38. Shi, Y.; He, G.; Lee, W. C.; McKenzie, J. A.; Silva, M. J.; Long, F. Gli1 identifies osteogenic progenitors for bone formation and fracture repair. *Nat Commun.* **2017**, *8*, 2043.
39. Carlone, D. L.; Riba-Wolman, R. D.; Deary, L. T.; Tovaglieri, A.; Jiang, L.; Ambruzs, D. M.; Mead, B. E.; Shah, M. S.; Lengner, C. J.; Jaenisch, R.; Breault, D. T. Telomerase expression marks transitional growth-associated skeletal progenitor/stem cells. *Stem Cells.* **2021**, *39*, 296-305.
40. Muruganandan, S.; Pierce, R.; Teguh, D. A.; Perez, R. F.; Bell, N.; Nguyen, B.; Hohl, K.; Snyder, B. D.; Grinstaff, M. W.; Alberico, H.; Woods, D.; Kong, Y.; Sima, C.; Bhagat, S.; Ho, K.; Rosen, V.; Gamer, L.; Ionescu, A. M. A FoxA2^{+} long-term stem cell population is necessary for growth plate cartilage regeneration after injury. *Nat Commun.* **2022**, *13*, 2515.
41. Newton, P. T.; Li, L.; Zhou, B.; Schweingruber, C.; Hovorakova, M.; Xie, M.; Sun, X.; Sandhow, L.; Artemov, A. V.; Ivashkin, E.; Suter, S.; Dyachuk, V.; El Shahawy, M.; Gritli-Linde, A.; Bouderlique, T.; Petersen, J.; Mollbrink, A.; Lundeberg, J.; Enikolopov, G.; Qian, H.; Fried, K.; Kasper, M.; Hedlund, E.; Adameyko, I.; Sävendahl, L.; Chagin, A. S. A radical switch in clonality reveals a stem cell niche in the epiphyseal growth plate. *Nature.* **2019**, *567*, 234-238.
42. Ambrosi, T. H.; Sinha, R.; Steininger, H. M.; Hoover, M. Y.; Murphy, M. P.; Koepke, L. S.; Wang, Y.; Lu, W. J.; Morri, M.; Neff, N. F.; Weissman, I. L.; Longaker, M. T.; Chan, C. K. Distinct skeletal stem cell types orchestrate long bone skeletogenesis. *Elife.* **2021**, *10*, e66063.

43. Matic, I.; Matthews, B. G.; Wang, X.; Dyment, N. A.; Worthley, D. L.; Rowe, D. W.; Grcevic, D.; Kalajzic, I. Quiescent bone lining cells are a major source of osteoblasts during adulthood. *Stem Cells.* **2016**, *34*, 2930-2942.
44. Duchamp de Lageneste, O.; Julien, A.; Abou-Khalil, R.; Frangi, G.; Carvalho, C.; Cagnard, N.; Cordier, C.; Conway, S. J.; Colnot, C. Periosteum contains skeletal stem cells with high bone regenerative potential controlled by Periostin. *Nat Commun.* **2018**, *9*, 773.
45. Berendsen, A. D.; Olsen, B. R. Bone development. *Bone.* **2015**, *80*, 14-18.
46. Allen, M. R.; Hock, J. M.; Burr, D. B. Periosteum: biology, regulation, and response to osteoporosis therapies. *Bone.* **2004**, *35*, 1003-1012.
47. He, X.; Bougioukli, S.; Ortega, B.; Arevalo, E.; Lieberman, J. R.; McMahon, A. P. Sox9 positive periosteal cells in fracture repair of the adult mammalian long bone. *Bone.* **2017**, *103*, 12-19.
48. Ortinau, L. C.; Wang, H.; Lei, K.; Deveza, L.; Jeong, Y.; Hara, Y.; Grafe, I.; Rosenfeld, S. B.; Lee, D.; Lee, B.; Scadden, D. T.; Park, D. Identification of functionally distinct Mx1^{+}αSMA^{+} periosteal skeletal stem cells. *Cell Stem Cell.* **2019**, *25*, 784-796.e5.
49. Xia, C.; Ge, Q.; Fang, L.; Yu, H.; Zou, Z.; Zhang, P.; Lv, S.; Tong, P.; Xiao, L.; Chen, D.; Wang, P. E.; Jin, H. TGF-β/Smad2 signalling regulates enchondral bone formation of Gli1(+) periosteal cells during fracture healing. *Cell Prolif.* **2020**, *53*, e12904.
50. Song, J. Y.; Pineault, K. M.; Dones, J. M.; Raines, R. T.; Wellik, D. M. Hox genes maintain critical roles in the adult skeleton. *Proc Natl Acad Sci U S A.* **2020**, *117*, 7296-7304.
51. Pineault, K. M.; Song, J. Y.; Kozloff, K. M.; Lucas, D.; Wellik, D. M. Hox11 expressing regional skeletal stem cells are progenitors for osteoblasts, chondrocytes and adipocytes throughout life. *Nat Commun.* **2019**, *10*, 3168.
52. Lotinun, S.; Ishihara, Y.; Nagano, K.; Kiviranta, R.; Carpentier, V. T.; Neff, L.; Parkman, V.; Ide, N.; Hu, D.; Dann, P.; Brooks, D.; Bouxsein, M. L.; Wysolmerski, J.; Gori, F.; Baron, R. Cathepsin K-deficient osteocytes prevent lactation-induced bone loss and parathyroid hormone suppression. *J Clin Invest.* **2019**, *129*, 3058-3071.
53. Yang, W.; Wang, J.; Moore, D. C.; Liang, H.; Dooner, M.; Wu, Q.; Terek, R.; Chen, Q.; Ehrlich, M. G.; Quesenberry, P. J.; Neel, B. G. Ptpn11 deletion in a novel progenitor causes metachondromatosis by inducing hedgehog signalling. *Nature.* **2013**, *499*, 491-495.
54. Han, Y.; Feng, H.; Sun, J.; Liang, X.; Wang, Z.; Xing, W.; Dai, Q.; Yang, Y.; Han, A.; Wei, Z.; Bi, Q.; Ji, H.; Kang, T.; Zou, W. Lkb1 deletion in periosteal mesenchymal progenitors induces osteogenic tumors through mTORC1 activation. *J Clin Invest.* **2019**, *129*, 1895-1909.
55. Ouyang, Z.; Chen, Z.; Ishikawa, M.; Yue, X.; Kawanami, A.; Leahy, P.; Greenfield, E. M.; Murakami, S. Prx1 and 3.2kb Col1a1 promoters target distinct bone cell populations in transgenic mice. *Bone.* **2014**, *58*, 136-145.
56. Esposito, A.; Wang, L.; Li, T.; Miranda, M.; Spagnoli, A. Role of Prx1-expressing skeletal cells and Prx1-expression in fracture repair. *Bone.* **2020**, *139*, 115521.
57. Wilk, K.; Yeh, S. A.; Mortensen, L. J.; Ghaffarigarakani, S.; Lombardo, C. M.; Bassir, S. H.; Aldawood, Z. A.; Lin, C. P.; Intini, G. Postnatal calvarial skeletal stem cells expressing PRX1 reside exclusively in the calvarial sutures and are required for bone regeneration. *Stem Cell Reports.* **2017**, *8*, 933-946.
58. Murao, H.; Yamamoto, K.; Matsuda, S.; Akiyama, H. Periosteal cells are a major source of soft callus in bone fracture. *J Bone Miner Metab.* **2013**, *31*, 390-398.
59. van Gastel, N.; Stegen, S.; Eelen, G.; Schoors, S.; Carlier, A.; Daniëls, V. W.; Baryawno, N.; Przybylski, D.; Depypere, M.; Stiers, P. J.; Lambrechts, D.; Van Looveren, R.; Torrekens, S.; Sharda, A.; Agostinis, P.; Lambrechts, D.; Maes, F.; Swinnen, J. V.; Geris, L.; Van Oosterwyck, H.; Thienpont, B.; Carmeliet, P.; Scadden, D. T.; Carmeliet, G. Lipid availability determines fate of skeletal progenitor cells via SOX9. *Nature.* **2020**, *579*, 111-117.
60. Ortinau, L.; Lei, K.; Jeong, Y.; Park, D. Real-time imaging of CCL5-induced migration of periosteal skeletal stem cells in mice. *J Vis Exp.* **2020**, 10.3791/61162.
61. Rux, D. R.; Song, J. Y.; Swinehart, I. T.; Pineault, K. M.; Schlientz, A. J.; Trulik, K. G.; Goldstein, S. A.; Kozloff, K. M.; Lucas, D.; Wellik, D. M. Regionally restricted hox function in adult bone marrow multipotent mesenchymal stem/stromal cells. *Dev Cell.* **2016**, *39*, 653-666.
62. Tournaire, G.; Stegen, S.; Giacomini, G.; Stockmans, I.; Moermans, K.; Carmeliet, G.; van Gastel, N. Nestin-GFP transgene labels skeletal progenitors in the periosteum. *Bone.* **2020**, *133*, 115259.
63. Tsukasaki, M.; Komatsu, N.; Negishi-Koga, T.; Huynh, N. C.; Muro, R.; Ando, Y.; Seki, Y.; Terashima, A.; Pluemsakunthai, W.; Nitta, T.; Nakamura, T.; Nakashima, T.; Ohba, S.; Akiyama, H.; Okamoto, K.; Baron, R.; Takayanagi, H. Periosteal stem cells control growth plate stem cells during postnatal skeletal growth. *Nat Commun.* **2022**, *13*, 4166.
64. Abbuehl, J. P.; Tatarova, Z.; Held, W.; Huelsken, J. Long-term engraftment of primary bone marrow stromal cells repairs niche damage and improves hematopoietic stem cell transplantation. *Cell Stem Cell.* **2017**, *21*, 241-255.e6.
65. Shi, Y.; Kang, X.; Wang, Y.; Bian, X.; He, G.; Zhou, M.; Tang, K. Exosomes derived from bone marrow stromal cells (BMSCs) enhance tendon-bone healing by regulating macrophage polarization. *Med Sci Monit.* **2020**, *26*, e923328.
66. Friedenstein, A. J.; Piatetzky, S., II; Petrakova, K. V. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol.* **1966**, *16*, 381-390.
67. Caplan, A. I. Mesenchymal stem cells. *J Orthop Res.* **1991**, *9*, 641-650.
68. Horwitz, E. M.; Le Blanc, K.; Dominici, M.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F. C.; Deans, R. J.; Krause, D. S.; Keating, A.; International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy.* **2005**, *7*, 393-395.
69. Prieto González, E. A. Heterogeneity in adipose stem cells. *Adv Exp Med Biol.* **2019**, *1123*, 119-150.
70. De Micheli, A. J.; Laurilliard, E. J.; Heinke, C. L.; Ravichandran, H.; Fraczek, P.; Soueid-Baumgarten, S.; De Vlaminck, I.; Elemento, O.; Cosgrove, B. D. Single-cell analysis of the muscle stem cell hierarchy identifies heterotypic communication signals involved in skeletal muscle regeneration. *Cell Rep.* **2020**, *30*, 3583-3595.e5.
71. De Bari, C.; Dell'Accio, F.; Vanlauwe, J.; Eyckmans, J.; Khan, I. M.; Archer, C. W.; Jones, E. A.; McGonagle, D.; Mitsiadis, T. A.; Pitzalis, C.; Luyten, F. P. Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum.* **2006**, *54*, 1209-1221.
72. Bartold, M.; Gronthos, S.; Haynes, D.; Ivanovski, S. Mesenchymal stem cells and biologic factors leading to bone formation. *J Clin Periodontol.* **2019**, *46 Suppl 21*, 12-32.
73. McLeod, C. M.; Mauck, R. L. On the origin and impact of mesenchymal stem cell heterogeneity: new insights and emerging tools for single cell analysis. *Eur Cell Mater.* **2017**, *34*, 217-231.
74. Usami, Y.; Gunawardena, A. T.; Francois, N. B.; Otsuru, S.; Takano, H.; Hirose, K.; Matsuoka, M.; Suzuki, A.; Huang, J.; Qin, L.; Iwamoto, M.;

Yang, W.; Toyosawa, S.; Enomoto-Iwamoto, M. Possible contribution of Wnt-responsive chondroprogenitors to the postnatal murine growth plate. *J Bone Miner Res.* **2019**, *34*, 964-974.
75. Maruyama, T.; Jeong, J.; Sheu, T. J.; Hsu, W. Stem cells of the suture mesenchyme in craniofacial bone development, repair and regeneration. *Nat Commun.* **2016**, *7*, 10526.
76. Matsushita, Y.; Nagata, M.; Kozloff, K. M.; Welch, J. D.; Mizuhashi, K.; Tokavanich, N.; Hallett, S. A.; Link, D. C.; Nagasawa, T.; Ono, W.; Ono, N. A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. *Nat Commun.* **2020**, *11*, 332.
77. Ono, N.; Ono, W.; Mizoguchi, T.; Nagasawa, T.; Frenette, P. S.; Kronenberg, H. M. Vasculature-associated cells expressing nestin in developing bones encompass early cells in the osteoblast and endothelial lineage. *Dev Cell.* **2014**, *29*, 330-339.
78. Yue, R.; Zhou, B. O.; Shimada, I. S.; Zhao, Z.; Morrison, S. J. Leptin receptor promotes adipogenesis and reduces osteogenesis by regulating mesenchymal stromal cells in adult bone marrow. *Cell Stem Cell.* **2016**, *18*, 782-796.
79. Zhou, B. O.; Yue, R.; Murphy, M. M.; Peyer, J. G.; Morrison, S. J. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell.* **2014**, *15*, 154-168.
80. Méndez-Ferrer, S.; Michurina, T. V.; Ferraro, F.; Mazloom, A. R.; Macarthur, B. D.; Lira, S. A.; Scadden, D. T.; Ma'ayan, A.; Enikolopov, G. N.; Frenette, P. S. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature.* **2010**, *466*, 829-834.
81. Park, D.; Spencer, J. A.; Koh, B. I.; Kobayashi, T.; Fujisaki, J.; Clemens, T. L.; Lin, C. P.; Kronenberg, H. M.; Scadden, D. T. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell.* **2012**, *10*, 259-272.
82. Worthley, D. L.; Churchill, M.; Compton, J. T.; Tailor, Y.; Rao, M.; Si, Y.; Levin, D.; Schwartz, M. G.; Uygur, A.; Hayakawa, Y.; Gross, S.; Renz, B. W.; Setlik, W.; Martinez, A. N.; Chen, X.; Nizami, S.; Lee, H. G.; Kang, H. P.; Caldwell, J. M.; Asfaha, S.; Westphalen, C. B.; Graham, T.; Jin, G.; Nagar, K.; Wang, H.; Kheirbek, M. A.; Kolhe, A.; Carpenter, J.; Glaire, M.; Nair, A.; Renders, S.; Manieri, N.; Muthupalani, S.; Fox, J. G.; Reichert, M.; Giraud, A. S.; Schwabe, R. F.; Pradere, J. P.; Walton, K.; Prakash, A.; Gumucio, D.; Rustgi, A. K.; Stappenbeck, T. S.; Friedman, R. A.; Gershon, M. D.; Sims, P.; Grikscheit, T.; Lee, F. Y.; Karsenty, G.; Mukherjee, S.; Wang, T. C. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. *Cell.* **2015**, *160*, 269-284.
83. Pérez-Lozano, M. L.; Sudre, L.; van Eegher, S.; Citadelle, D.; Pigenet, A.; Lafage-Proust, M. H.; Pastoureau, P.; De Ceuninck, F.; Berenbaum, F.; Houard, X. Gremlin-1 and BMP-4 overexpressed in osteoarthritis drive an osteochondral-remodeling program in osteoblasts and hypertrophic chondrocytes. *Int J Mol Sci.* **2022**, *23*, 2084.
84. Sivaraj, K. K.; Jeong, H. W.; Dharmalingam, B.; Zeuschner, D.; Adams, S.; Potente, M.; Adams, R. H. Regional specialization and fate specification of bone stromal cells in skeletal development. *Cell Rep.* **2021**, *36*, 109352.
85. Zhou, X.; von der Mark, K.; Henry, S.; Norton, W.; Adams, H.; de Crombrugghe, B. Chondrocytes transdifferentiate into osteoblasts in endochondral bone during development, postnatal growth and fracture healing in mice. *PLoS Genet.* **2014**, *10*, e1004820.
86. Shu, H. S.; Liu, Y. L.; Tang, X. T.; Zhang, X. S.; Zhou, B.; Zou, W.; Zhou, B. O. Tracing the skeletal progenitor transition during postnatal bone formation. *Cell Stem Cell.* **2021**, *28*, 2122-2136.e3.
87. Jing, D.; Chen, Z.; Men, Y.; Yi, Y.; Wang, Y.; Wang, J.; Yi, J.; Wan, L.; Shen, B.; Feng, J. Q.; Zhao, Z.; Zhao, H.; Li, C. Response of Gli1(+) suture stem cells to mechanical force upon suture expansion. *J Bone Miner Res.* **2022**, *37*, 1307-1320.
88. Men, Y.; Wang, Y.; Yi, Y.; Jing, D.; Luo, W.; Shen, B.; Stenberg, W.; Chai, Y.; Ge, W. P.; Feng, J. Q.; Zhao, H. Gli1+ periodontium stem cells are regulated by osteocytes and occlusal force. *Dev Cell.* **2020**, *54*, 639-654.e6.
89. Wang, K.; Xu, C.; Xie, X.; Jing, Y.; Chen, P. J.; Yadav, S.; Wang, Z.; Taylor, R. W.; Wang, J.; Feng, J. Q. Axin2^{+} PDL cells directly contribute to new alveolar bone formation in response to orthodontic tension force. *J Dent Res.* **2022**, *101*, 695-703.
90. Gong, X.; Zhang, H.; Xu, X.; Ding, Y.; Yang, X.; Cheng, Z.; Tao, D.; Hu, C.; Xiang, Y.; Sun, Y. Tracing PRX1(+) cells during molar formation and periodontal ligament reconstruction. *Int J Oral Sci.* **2022**, *14*, 5.
91. Ding, Y.; Mo, C.; Geng, J.; Li, J.; Sun, Y. Identification of periosteal osteogenic progenitors in jawbone. *J Dent Res.* **2022**, *101*, 1101-1109.
92. Lattanzi, W.; Barba, M.; Novegno, F.; Massimi, L.; Tesori, V.; Tamburrini, G.; Galgano, S.; Bernardini, C.; Caldarelli, M.; Michetti, F.; Di Rocco, C. Lim mineralization protein is involved in the premature calvarial ossification in sporadic craniosynostoses. *Bone.* **2013**, *52*, 474-484.
93. Zhao, H.; Feng, J.; Ho, T. V.; Grimes, W.; Urata, M.; Chai, Y. The suture provides a niche for mesenchymal stem cells of craniofacial bones. *Nat Cell Biol.* **2015**, *17*, 386-396.
94. Maruyama, T.; Stevens, R.; Boka, A.; DiRienzo, L.; Chang, C.; Yu, H. I.; Nishimori, K.; Morrison, C.; Hsu, W. BMPR1A maintains skeletal stem cell properties in craniofacial development and craniosynostosis. *Sci Transl Med.* **2021**, *13*, eabb4416.
95. Maruyama, T.; Jiang, M.; Abbott, A.; Yu, H. I.; Huang, Q.; Chrzanowska-Wodnicka, M.; Chen, E. I.; Hsu, W. Rap1b is an effector of Axin2 regulating crosstalk of signaling pathways during skeletal development. *J Bone Miner Res.* **2017**, *32*, 1816-1828.
96. Xie, X.; Xu, C.; Zhao, L.; Wu, Y.; Feng, J. Q.; Wang, J. Axin2-expressing cells in the periodontal ligament are regulated by bone morphogenetic protein signalling and play a pivotal role in periodontium development. *J Clin Periodontol.* **2022**, *49*, 945-956.
97. Ransom, R. C.; Carter, A. C.; Salhotra, A.; Leavitt, T.; Marecic, O.; Murphy, M. P.; Lopez, M. L.; Wei, Y.; Marshall, C. D.; Shen, E. Z.; Jones, R. E.; Sharir, A.; Klein, O. D.; Chan, C. K. F.; Wan, D. C.; Chang, H. Y.; Longaker, M. T. Mechanoresponsive stem cells acquire neural crest fate in jaw regeneration. *Nature.* **2018**, *563*, 514-521.
98. Fernández, M.; Simon, V.; Herrera, G.; Cao, C.; Del Favero, H.; Minguell, J. J. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. *Bone Marrow Transplant.* **1997**, *20*, 265-271.
99. Kuznetsov, S. A.; Mankani, M. H.; Gronthos, S.; Satomura, K.; Bianco, P.; Robey, P. G. Circulating skeletal stem cells. *J Cell Biol.* **2001**, *153*, 1133-1140.
100. Eghbali-Fatourechi, G. Z.; Lamsam, J.; Fraser, D.; Nagel, D.; Riggs, B. L.; Khosla, S. Circulating osteoblast-lineage cells in humans. *N Engl J Med.* **2005**, *352*, 1959-1966.
101. Feehan, J.; Nurgali, K.; Apostolopoulos, V.; Al Saedi, A.; Duque, G. Circulating osteogenic precursor cells: building bone from blood. *EBioMedicine.* **2019**, *39*, 603-611.
102. Kumagai, K.; Vasanji, A.; Drazba, J. A.; Butler, R. S.; Muschler, G. F. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. *J Orthop Res.* **2008**, *26*, 165-175.

103. Kelly, R. R.; McDonald, L. T.; Pellegrini, V. D.; Cray, J. J.; Larue, A. C. Identification of circulating murine CD34(+)OCN(+) cells. *Cytotherapy*. **2018**, *20*, 1371-1380.
104. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. **2006**, *8*, 315-317.
105. Alm, J. J.; Koivu, H. M.; Heino, T. J.; Hentunen, T. A.; Laitinen, S.; Aro, H. T. Circulating plastic adherent mesenchymal stem cells in aged hip fracture patients. *J Orthop Res.* **2010**, *28*, 1634-1642.
106. Zvaifler, N. J.; Marinova-Mutafchieva, L.; Adams, G.; Edwards, C. J.; Moss, J.; Burger, J. A.; Maini, R. N. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res.* **2000**, *2*, 477-488.
107. Suda, R. K.; Billings, P. C.; Egan, K. P.; Kim, J. H.; McCarrick-Walmsley, R.; Glaser, D. L.; Porter, D. L.; Shore, E. M.; Pignolo, R. J. Circulating osteogenic precursor cells in heterotopic bone formation. *Stem Cells*. **2009**, *27*, 2209-2219.
108. Hong, H. S.; Lee, J.; Lee, E.; Kwon, Y. S.; Lee, E.; Ahn, W.; Jiang, M. H.; Kim, J. C.; Son, Y. A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. *Nat Med*. **2009**, *15*, 425-435.
109. Rochefort, G. Y.; Delorme, B.; Lopez, A.; Hérault, O.; Bonnet, P.; Charbord, P.; Eder, V.; Domenech, J. Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. *Stem Cells*. **2006**, *24*, 2202-2208.
110. Rubin, M. R.; Manavalan, J. S.; Dempster, D. W.; Shah, J.; Cremers, S.; Kousteni, S.; Zhou, H.; McMahon, D. J.; Kode, A.; Sliney, J.; Shane, E.; Silverberg, S. J.; Bilezikian, J. P. Parathyroid hormone stimulates circulating osteogenic cells in hypoparathyroidism. *J Clin Endocrinol Metab*. **2011**, *96*, 176-186.
111. Pignolo, R. J.; Kassem, M. Circulating osteogenic cells: implications for injury, repair, and regeneration. *J Bone Miner Res*. **2011**, *26*, 1685-1693.
112. Eggenhofer, E.; Luk, F.; Dahlke, M. H.; Hoogduijn, M. J. The life and fate of mesenchymal stem cells. *Front Immunol*. **2014**, *5*, 148.
113. Kyoizumi, S.; Kubo, Y.; Misumi, M.; Kajimura, J.; Yoshida, K.; Hayashi, T.; Imai, K.; Ohishi, W.; Nakachi, K.; Young, L. F.; Shieh, J. H.; Moore, M. A.; van den Brink, M. R.; Kusunoki, Y. Circulating hematopoietic stem and progenitor cells in aging atomic bomb survivors. *Radiat Res*. **2016**, *185*, 69-76.
114. Lo Sicco, C.; Tasso, R.; Reverberi, D.; Cilli, M.; Pfeffer, U.; Cancedda, R. Identification of a new cell population constitutively circulating in healthy conditions and endowed with a homing ability toward injured sites. *Sci Rep*. **2015**, *5*, 16574.
115. Lo Sicco, C.; Reverberi, D.; Villa, F.; Pfeffer, U.; Quarto, R.; Cancedda, R.; Tasso, R. Circulating healing (CH) cells expressing BST2 are functionally activated by the injury-regulated systemic factor HGFA. *Stem Cell Res Ther*. **2018**, *9*, 300.
116. Kawakami, Y.; Matsumoto, T.; Mifune, Y.; Fukui, T.; Patel, K. G.; Walker, G. N.; Kurosaka, M.; Kuroda, R. Therapeutic potential of endothelial progenitor cells in the field of orthopaedics. *Curr Stem Cell Res Ther*. **2017**, *12*, 3-13.
117. Kuroda, R.; Matsumoto, T.; Kawakami, Y.; Fukui, T.; Mifune, Y.; Kurosaka, M. Clinical impact of circulating CD34-positive cells on bone regeneration and healing. *Tissue Eng Part B Rev*. **2014**, *20*, 190-199.
118. Ma, X. L.; Sun, X. L.; Wan, C. Y.; Ma, J. X.; Tian, P. Significance of circulating endothelial progenitor cells in patients with fracture healing process. *J Orthop Res*. **2012**, *30*, 1860-1866.
119. Uccelli, A.; Moretta, L.; Pistoia, V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol*. **2008**, *8*, 726-736.
120. Gattazzo, F.; Urciuolo, A.; Bonaldo, P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim Biophys Acta*. **2014**, *1840*, 2506-2519.
121. Ambrosi, T. H.; Marecic, O.; McArdle, A.; Sinha, R.; Gulati, G. S.; Tong, X.; Wang, Y.; Steininger, H. M.; Hoover, M. Y.; Koepke, L. S.; Murphy, M. P.; Sokol, J.; Seo, E. Y.; Tevlin, R.; Lopez, M.; Brewer, R. E.; Mascharak, S.; Lu, L.; Ajanaku, O.; Conley, S. D.; Seita, J.; Morri, M.; Neff, N. F.; Sahoo, D.; Yang, F.; Weissman, I. L.; Longaker, M. T.; Chan, C. K. F. Aged skeletal stem cells generate an inflammatory degenerative niche. *Nature*. **2021**, *597*, 256-262.
122. Josephson, A. M.; Bradaschia-Correa, V.; Lee, S.; Leclerc, K.; Patel, K. S.; Muinos Lopez, E.; Litwa, H. P.; Neibart, S. S.; Kadiyala, M.; Wong, M. Z.; Mizrahi, M. M.; Yim, N. L.; Ramme, A. J.; Egol, K. A.; Leucht, P. Age-related inflammation triggers skeletal stem/progenitor cell dysfunction. *Proc Natl Acad Sci U S A*. **2019**, *116*, 6995-7004.
123. Oh, J.; Lee, Y. D.; Wagers, A. J. Stem cell aging: mechanisms, regulators and therapeutic opportunities. *Nat Med*. **2014**, *20*, 870-880.
124. Ransom, R. C.; Hunter, D. J.; Hyman, S.; Singh, G.; Ransom, S. C.; Shen, E. Z.; Perez, K. C.; Gillette, M.; Li, J.; Liu, B.; Brunski, J. B.; Helms, J. A. Axin2-expressing cells execute regeneration after skeletal injury. *Sci Rep*. **2016**, *6*, 36524.
125. Tevlin, R.; Seo, E. Y.; Marecic, O.; McArdle, A.; Tong, X.; Zimdahl, B.; Malkovskiy, A.; Sinha, R.; Gulati, G.; Li, X.; Wearda, T.; Morganti, R.; Lopez, M.; Ransom, R. C.; Duldulao, C. R.; Rodrigues, M.; Nguyen, A.; Januszyk, M.; Maan, Z.; Paik, K.; Yapa, K. S.; Rajadas, J.; Wan, D. C.; Gurtner, G. C.; Snyder, M.; Beachy, P. A.; Yang, F.; Goodman, S. B.; Weissman, I. L.; Chan, C. K.; Longaker, M. T. Pharmacological rescue of diabetic skeletal stem cell niches. *Sci Transl Med*. **2017**, *9*, eaag2809.
126. Rosen, V. BMP2 signaling in bone development and repair. *Cytokine Growth Factor Rev*. **2009**, *20*, 475-480.
127. Lowery, J. W.; Pazin, D.; Intini, G.; Kokabu, S.; Chappuis, V.; Capelo, L. P.; Rosen, V. The role of BMP2 signaling in the skeleton. *Crit Rev Eukaryot Gene Expr*. **2011**, *21*, 177-185.
128. Salazar, V. S.; Capelo, L. P.; Cantù, C.; Zimmerli, D.; Gosalia, N.; Pregizer, S.; Cox, K.; Ohte, S.; Feigenson, M.; Gamer, L.; Nyman, J. S.; Carey, D. J.; Economides, A.; Basler, K.; Rosen, V. Reactivation of a developmental Bmp2 signaling center is required for therapeutic control of the murine periosteal niche. *Elife*. **2019**, *8*, e42386.
129. Liu, Y.; Tian, H.; Hu, Y.; Cao, Y.; Song, H.; Lan, S.; Dai, Z.; Chen, W.; Zhang, Y.; Shao, Z.; Liu, Y.; Tong, W. Mechanosensitive Piezo1 is crucial for periosteal stem cell-mediated fracture healing. *Int J Biol Sci*. **2022**, *18*, 3961-3980.
130. Gao, B.; Deng, R.; Chai, Y.; Chen, H.; Hu, B.; Wang, X.; Zhu, S.; Cao, Y.; Ni, S.; Wan, M.; Yang, L.; Luo, Z.; Cao, X. Macrophage-lineage TRAP+ cells recruit periosteum-derived cells for periosteal osteogenesis and regeneration. *J Clin Invest*. **2019**, *129*, 2578-2594.
131. Gan, Y.; He, J.; Zhu, J.; Xu, Z.; Wang, Z.; Yan, J.; Hu, O.; Bai, Z.; Chen, L.; Xie, Y.; Jin, M.; Huang, S.; Liu, B.; Liu, P. Spatially defined single-cell transcriptional profiling characterizes diverse chondrocyte subtypes and nucleus pulposus progenitors in human intervertebral discs. *Bone Res*. **2021**, *9*, 37.

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