Functional Heterogeneity of BMSC Subpopulations in Physiology

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Diverse cell surface antigens and complex gene expression of bone marrow mesenchymal stem cells (BMSCs) have indicated that BMSCs represent heterogeneous populations, and the natural characteristics of BMSCs make it difficult to identify the specific subpopulations in pathological processes which are often obscured by bulk analysis of the total BMSCs. Meanwhile, the therapeutic effect of total BMSCs is often less effective partly due to their heterogeneity. Therefore, understanding the functional heterogeneity of the BMSC subpopulations under different physiological and pathological conditions could have major ramifications for global health.

functional heterogeneity of BMSCs | physiological status | pathological-related BMSC subpopulation

1. Development

1.1. Embryonic Development

Diverse bone marrow mesenchymal stem cells (BMSCs) subpopulations are involved in the embryonic period [1][2][3][4][5][6][7]. CD105 positive BMSC subpopulations can be detected as early as embryo day 13 (E13) [1], which supports hematopoietic generation by endochondral ossification in fetal mice [1][8]. Simultaneously, another finding has reported that E13.5 Grem1-Cre traces almost the entire embryonic mesenchyme and primary spongiosis, which overlaps with metaphyseal anatomical localization of adulthood Grem1-expressing cells, suggesting the subpopulation has chondrogenic potential [9]. Moreover, 6C3-supportive stromal cells and leptin receptor $(LepR)^+$ cells in the long bone of E17.5 mice co-emerge with HSCs, underscoring the supporting hematopoietic niche of these two subpopulations [1][10]. In addition to different time points of embryonic development, BMSCs from differential bone regions also exhibited heterogeneity. For example, Maruyama et al. proposed that axis inhibition protein 2 positive ($Axin2^+$) subpopulations are restricted to the midline of craniofacial sutures, mainly traced cranial skeletal stem cells (SSCs) throughout all stages of development in mice crania, but which are virtually absent in long bones [11][12][13], while Glioma-associated oncogene 1(Gli1)-expressing cells share characteristics with long bone BMSCs and are abundantly present along with the whole craniofacial sutures, but both subpopulations are thought to promote growth and regeneration [13][14]. The Sox9-expressing BMSC subpopulation is involved in initial cartilage templates formation, and tracing the subpopulation from early embryonic stages found that it marked osteochondrogenic, adipogenic, and stromal cells like CAR (CXCL12-abundant reticular) cells of adult bone marrow [15]. Similarly, Osterix $(Osx)^+$ cells reside in bone marrow stroma during fetal development, and further form perivascular, osteogenic, and adipogenic cells [4][16]. paired related homeobox 1 (Prx1) is highly expressed by limb bud mesenchymal progenitors during long bone development [17], and genetic lineage tracing by Prx1-Cre labels

all skeletal lineage cells in long bones, including skeletal stem/progenitor cells (SSPCs), osteoblasts, osteocytes, chondrocytes, and adipocytes [18].

Early studies of BMSC subpopulations on embryonic bone development mainly focused on mice. Most recently, Yue and his colleagues explored spatiotemporal ontogeny of human embryonic limb and long bones during early skeletogenesis through single-cell RNA sequencing (scRNA-seq) [19]. They revealed distinguished heterogeneity of mesenchyme cells within different human limb bud regions, like mesenchyme and epithelium, as well as aligning them along the proximal–distal and anterior–posterior axes [19]. Moreover, they proposed that osteo-chondrogenic populations first appeared in the core limb bud mesenchyme, which derived multiple populations of stem/progenitor cells in embryonic long bones through endochondral ossification [19]. Importantly, a perichondrial embryonic skeletal stem/progenitor cell (eSSPC) population marked by adhesion molecule 1 ($CADM1^+$) and podoplanin ($PDPN^+$) was identified, which could self-renew and give rise to the osteochondral lineage cells, but not adipocytes or hematopoietic stroma. Interestingly, neural crest-derived cells with similar phenotypic markers with limb buds-derived eSSPC were also found in the sagittal suture of human embryonic calvaria [19].

1.2. Adulthood

From postnatal to adulthood, there are still various bone-resident BMSC subpopulations to maintain bone homeostasis. While the essential role of BMSCs in the postnatal development of the skeleton has been generally established, identifying pure BMSC subpopulations remains very important.

Mouse BMSCs

Mouse BMSCs have been prospectively identified in the perivascular [20][21], while the populations usually lack expression of hematopoietic and endothelial markers but have positive expression of platelet-derived growth factor receptor (PDGFR) α [22][23][24]. Later, Morikawa and Omatsu identified two distinctive subpopulations of $PDGFR\alpha^+$ BMSCs, $PDGFR\alpha^+$ $Sca\text{-}1^+$ $CD45^-$ $Ter119^-$ BMSCs and $PDGFR\alpha^+$ $Sca\text{-}1^-$ $CD45^-$ $Ter119^-$ BMSCs [22][23]. The former one resides primarily around arterioles but does not express the HSC niche factor Cxcl12, while the latter one resides primarily around sinusoids and expresses high levels of Cxcl12 to support hematopoiesis [22][23]. Similarly, another study found that Nestin-GFP^+ BMSCs are heterogeneous, including both Nestin-GFP^{high} cells that localize mainly around arterioles and Nestin-GFP^{low} cells that localize mainly around sinusoids; both subpopulations can osteogenesis but Nestin-GFP^{low} cells secret more Cxcl12 [25][26]. Additionally, a previous study of Yue showed that LepR also marks SSPCs which localize in the perivascular region of the adult bone marrow [10] and promote osteogenesis [27], supporting the hematopoietic microenvironment by secreting high levels of Cxcl12 [10][28][29][30]. It has to be noticed that $LepR^+$ BMSCs overlap with Nestin-GFP^{low} cells [25][26], Cxcl12-abundant reticular (CAR) cells [29], and $Osterix^+$ BMSCs [24]. In contrast to $LepR^+$ cells, myxovirus resistance 1(Mx-1)-Cre cells overlap with Nestin-GFP, PDGFRα, Sca1, and CD105 BMSCs and give rise to most of the osteoblasts formed in adult bone marrow, but Mx-1 also robustly labels hematopoietic cells, meaning that this marker could not be specific to selection of osteogenic progenitors population [24]. Moreover, Prx1 also is another important BMSC marker and overlaps with $LepR^+$ stromal cells as well as $LepR^-$ osteoblasts and chondrocytes within the bone marrow of limb bones, but not in the axial skeleton [31]. Most recently, Shu et al. found that $Acan^+$ BMSCs and

LepR$^+$ BMSCs controlled bone formation before and after adolescence, respectively, and Acan$^+$ BMSCs mediate bone lengthening, while LepR$^+$ BMSCs regulate bone thickening [32]. In addition to the heterogeneity of BMSCs at different bone development stages, the heterogeneity of BMSCs from different bone regions has also received increasing attention. A recent publication proposes parathyroid hormone-related protein (PTHrP) as a label for chondrocytes of the resting zone in the growth plate of long bones which descend from a PTHrP$^+$ SSCs [33]. Other findings also revealed that both Grem1$^+$ cells and Gli1$^+$ cells mainly reside in metaphyseal areas, Grem$^+$ cells are non-adipogenic, while Gli1$^+$ cells contribute to osteochondrogenesis and adipogenesis in vivo [34][35]. Later evidence suggested that Gli1 is also seen in many mature bone cell types (like, Osx, Col1) not uniquely marking an homogeneous BMSC population [35]. Recently, Sivaraj et al. characterized the heterogeneity of BMSCs during skeletal development. They identified that BMSCs from metaphysis and diaphysis have distinct properties, and the subpopulation of metaphyseal MSCs (mpMSCs) has multi-lineage differentiation potential to give rise to bone cells and LepR$^+$ BMSCs, and transcription factors of platelet-derived growth factor B (PDGF-B) and Jun-B control BMSC osteogenesis [36]. Aside from growth plate and metaphyseal areas, many MSCs also reside along the periosteum, where they can quickly get “activated” upon injury and facilitate proper fracture healing [37].

Human BMSCs

Taking advantage of flow cytometry and scRNA-seq application, research on the heterogeneity of human BMSC subpopulations has also made good progress. In earlier studies, CD45 negative non-hematopoietic fibroblast colony-forming cells have been confirmed, and copious markers, including CD105, CD140a, CD73, CD90, STRO-1, CD271, and CD44 [38][39][40][41]. Another study reported that the CD146$^+$ MSC subpopulation resides around sinusoidal blood vessels in the ossicles in the human bone marrow which can differentiate into osteogenic, chondrogenic, and adipogenic cells in culture and give rise to the bone upon transplantation in vivo, express HSC niche factors, and form bony ossicles that become invested with hematopoietic bone marrow [8][20]. Subsequent studies identified CD271$^+$ PDGFRα^{low} and the stromal cell antigen 1(STRO-1$^+$) turned out to most efficiently select for perivascular residing SSC-like cells that are also able to maintain human HSCs for an extended time in culture [42][43]. Groundbreaking experiments by Chan et al. have transplanted human single cells, termed bone cartilage stromal progenitor (BCSPs; CD45$^-$ Ter119$^-$ Tie2$^-$ Thy1$^-$ 6C3$^-$ CD51$^+$), to a renal capsule of mice, and confirmed the CD45$^-$ Ter119$^-$ Tie2$^-$ Thy1$^-$ 6C3$^-$ CD51$^+$ BCSPs were bone fide stem cells in vivo [1]. Currently, Chan et al. identified a highly purified human skeletal stem cell (hSSC) distinct from the reported CD146-positive SSCs; and found in fetal and all adult stages throughout different skeletal sites but specifically enriched in the hypertrophic zones of the growth plate labeled by PDPN, CD73, CD164 and lacking expression of CD235, CD45, CD146, Tie2, and CD31 [44], and the hSSC populations can give rise to osteoprogenitor cell types, never producing fat [45][46]. Moreover, Liu et al. have analyzed the BMSC heterogeneity of one healthy subject hip through sc-RNAseq, and they divided the BMSCs into three subpopulations [47]. Subpopulation A was characterized by the high expression of fibroblast growth factor receptor 2 (FGFR2) which was involved in osteogenesis; Subpopulation B expressed higher levels of fibroblast growth factor 5 (FGF5) which could increase osteogenic differentiation of MSCs; and Subpopulation C was characterized by high expression of plasminogen activator tissue type (PLAT) and vascular adhesion molecule 1(VCAM1) which promoted angiogenesis [47]. Similarly, Zhang et al. have proposed umbilical cord mesenchymal stem cells (UC-MSCs) having two subpopulations via sc-RNAseq [48].

Group 1 MSCs are enriched in the expression of genes in immune response/regulatory activities (e.g., *TNFα*, *IL17*, *TLR*, *TGFβ*, infection, *NOD*, *NF-κB*, and *PGE* pathways), muscle cell proliferation and differentiation, stemness, and oxidative stress while group 2 MSCs are enriched with gene expression in extracellular matrix production, bone, and cartilage growth as well glucose metabolism [48].

In sum, human BMSC subpopulations possess more complex cell surface markers, exhibit specific bone regions distributions and different differentiation potential, and the subpopulations from the hip and umbilical cord show distinctive functional heterogeneity. In detail, human BMSCs express copious markers, including CD105, CD140a, CD73, CD90, STRO-1, CD271, and CD44 et al., which exhibited variable CFU-F ability and multi-lineage differentiation potential. $CD146^+$, $CD271^+$ $PDGFR\alpha^{low}$, and $STRO\text{-}1^+$ BMSC subpopulations contribute to HSC niche stability. $CD45^-$ $Ter119^-$ $Tie2^-$ $Thy1^-$ $6C3^-$ $CD51^+$ labels BCSPs; while $PDPN^+$ $CD73^+$ $CD164^+$ $CD235^-$ $CD45^-$ $CD146^-$ $Tie2^-$ $CD31^-$ labels BMSCs, residing in hypertrophic zones of the growth plate, and these subpopulations just give rise to osteoprogenitor cell types, never producing fat. In addition, BMSCs of the healthy subject can be divided into $FGFR2^+$; $FGF5^+$ BMSCs and $PLAT^+$ $VCAM1^+$ BMSCs promote osteogenesis and angiogenesis, respectively. Similarly, UC-MSCs also can be divided into immune response/regulatory activities related to MSC group 1 and bone and cartilage growth-related MSC group 2. Importantly, human BMSC subpopulations showed distinctive markers with mouse BMSCs.

1.3. Aging

Both the number and function of BMSCs decline dramatically with aging. Thus, it is very important to identify exact BMSC subpopulations that dismiss or increase during aging. Recent studies have been devoted to identifying some elderly BMSC subpopulations. Liu et al. have reported that a $Sca1^+$ BMSC subpopulation from aged mice exhibited lower paracrine support for retinas than a $Sca1^+$ BMSC subpopulation from young mice [49], and injecting young $Sca\text{-}1^+$ BMSCs into 18-month-old mice through the tail vein can increase brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), FGF2, and insulin-like growth factor 1 (IGF-1) expression, downregulation of the apoptotic protein Bax with upregulation of the antiapoptotic protein Bcl2 to attenuate aging-related retinal degeneration ultimately [49]. Other studies have indicated impaired osteogenic potential of $Prx1^+$ SSCs in aging mice because the loss of the peroxlsome proliferator-activated receptor-g coactivator-1 a (PGC-1α), an expression in aging $Prx1^+$ SSCs, and overexpression of PGC-1α in SSCs reversed the unbalance of SSC osteo-adipogenic differentiation during aging [50]. Wang et al. have confirmed that alpha-ketoglutarate (αKG) can rejuvenate the osteogenic capacity of $LepR^+$ BMSCs and ameliorate age-related osteoporosis by decreasing the accumulations of H3K9me3 and H3K27me3, and subsequently upregulates BMP signaling and Nanog expression [51]. Recently, a study by Yue revealed that $LepR^+$ BMSCs were decreased with aging and enhanced adipogenesis in bone marrow, and downregulated HSC-supportive Kitl and Cxcl12 expression derived from $LepR^+$ $Notch3^+$ BMSCs [52]. Zhong et al. have found that the great expansion of $LepR^+$ marrow adipogenic lineage precursors (MALPs) in 16-month-old mice might explain why there is more adipose tissue accumulation with aging [53].

In addition to aged mice, the study of Zhu et al. recently investigated the heterogeneity of BMSCs ($CD29^+$ $CD44^+$ $CD90^+$ $CD105^+$ $CD34^-$ $CD45^-$ $HLA\text{-}DR^-$) from an 85-year-old human by sc-RNAseq [54]. They divided the BMSCs populations into three clusters according to the expression of functional genes [54]. Cluster 1 enriched the expression of cell self-renewal including cell division cycle-associated 5 (*CDCA5*) [55] and V-Myb avian myeloblastosis viral oncogene homolog-like 2 (*MYBL2*) [56], the cell metaphase–anaphase transition-related regulator family with sequence similarity 64 (*FAM64A*) [57], the integrity of the human centromere DNA repeats related to centromere protein A (*CENP-A*) [58], cell proliferation-related progestin and adipoQ receptor family member 4 (*PAQR4*) [59], DNA replication and repair related histone chaperone anti-silencing function 1B (*Asf1b*) [60], and chromatin assembly factor-1 (*CAF-1*) [61] and high mobility group protein 2 antibody (*HMGB2*) [62]. Cluster 2 is high expression multidirectional differentiation of BMSCs, including chondrogenesis-related transglutaminase 2 (*TGM2*) [63] and *COL11A1* [64], osteogenesis-related nuclear factor of activated T-cells (*NEAT1*) [65] and Type V collagen [66]. Cluster 3 enriched the expression of secretory factors to participate in immune regulation and damage repair including microbial killing and innate immunity-related *Cyba* [67], regulation of immunity and inflammation-related tissue inhibitor of metalloproteinase-1 (*TIMP-1*) [68] and annexin A1 (*ANXA1*) [69], cell migration and wound repair-related lumican (LUM) [70], and dermatopontin (*DPT*) [71], regulating cytokine secretion-related endoplasmic reticulum resident protein 44 (*ERp44*) [72], and protein import into endoplasmic reticulum-related heat shock protein family A, member 5 (*HSPA5*) [73].

Taken together, specific BMSC subpopulations exhibit changed numbers and impaired osteogenesis, and enhanced adipogenesis during aging. For example, $Sca1^+$, $Prx1^+$, $LepR^+$, $LepR^+$ $Notch3^+$ BMSCs of aging mice were all declined and exhibited impaired paracrine support for retinas and HSC niche, while $LepR^+$ MALPs were increased in aging mice and contributed to adipogenesis. Moreover, elderly human BMSC populations can be divided three populations, including self-renewal, multidirectional differentiation, and immune regulation and damage repair.

2. Environmental Stresses

Apart from the different development stage, the heterogeneous BMSCs are also important for bone to react to different environmental stresses.

2.1. Loading

Mechanical loading stimulates bone formation while BMSC subpopulations can contribute to this process. Many recent studies have focused on which specific BMSC subpopulations sense the signal of mechanical loading to osteogenesis. Cabahug-Zuckerman et al. have reported that mice $Sca\text{-}1^+$ $Prx1^+$ subpopulations participate in load-induced periosteal bone formation [74][75]. Mice without periosteal $Prx1^+$ MSC exhibited mechanically impaired response capacity and declined bone formation and mineralization, accompanied by lower levels of osteogenic markers expression [76]. The research of Zannit has proposed that the Osx^+ BMSC subpopulations also can be activated and contribute to loading-induced periosteal bone formation in both male and female mice [77]. Leucht et al. have reported that stromal cell-derived factor-1 (SDF-1, also known as CXCL12) was expressed in marrow

cells, participating in load-induced bone formation [78], and subsequent studies found peri-sinusoids CAR cells [22] [23]. The most recent work of Shen has reported that peri-arteriolar $LepR^+$ $osteolectin^+$ subpopulations can sense mechanical stimulation by mechanosensitive ion channel PIEZO1 to osteogenesis [79]. They observed that the number and osteogenic potential of $LepR^+$ $osteolectin^+$ BMSC subpopulations were increased when the mice were under voluntary running, while it would be the opposite when the mice were under hindlimb unloading. It has to be stressed that $LepR^+$ $osteolectin^+$ BMSC subpopulations just give rise to bone, not fat [79].

In sum, many specific BMSC subpopulations of mice are sensitive to loading-induced bone formation. In detail, periosteal $Sca\text{-}1^+$ $Prx1^+$, Osx^+ BMSCs, peri-sinusoids $CXCL12^+$ BMSCs, and peri-arteriolar $LepR^+$ $osteolectin^+$ BMSCs can sense mechanical loading to participate in bone formation.

2.2. Microgravity

Studies of astronauts in spaceflight and animals exposed to simulated microgravity revealed that exposure to microgravity induces trabecular bone loss and increased adipogenesis in the bone marrow [80][81]. As opposite as mechanical loading stimulates bone formation, it remains to be shown if there are also specific BMSC subpopulations to sense the microgravity to induce bone loss and promote adipogenesis. The earlier finding showed that mice subjected to hindlimb unloading showed a declining number of the Sca^+ $CD90.2^+$ BMSC subpopulations [82]. Other research has identified Lin^- $LepR^+$ SSCs under mechanical unloading are more quiescent and exhibit lower bone anabolic and neurogenic pathways [83]. Shen et al. have reported that the number and osteogenic potential of peri-arteriolar $LepR^+$ $osteolectin^+$ BMSCs were impaired when the mice were under hindlimb unloading [79].

All in all, Sca^+ $CD90.2^+$, Lin^- $LepR^+$, and $LepR^+$ $osteolectin^+$ BMSC subpopulations each showed decreased number and osteogenic potential under hindlimb unloading in mice.

2.3. Hypoxia

Bone marrow is a naturally hypoxic environment. Imaging analysis of BMSCs by cell surface markers found that these cells are located in regions where oxygen tension ranges from 4 to 1% [20][22]. How do the heterogenous BMSC subpopulations adapt physiologic oxygen tensions in the bone marrow to proliferation or differentiation? The previous evidence indicated that the number of $CD13^+$ $CD29^+$ $CD44^+$ $CD73^+$ $CD90^+$ $CD105^+$ $CD151^+$ $CD34^-$ BMSCs colonies developed was higher under hypoxia (3% O2) [84]. The recent study of Guo showed that $PDGFR\alpha^+$ and $LepR^+$ BMSC subpopulations exhibited increased proliferation under 1% O2, while a 10-fold reduction in $CD45^+$ hematopoietic cells [85]. In addition to proliferation, hypoxia also can influence specific BMSC subpopulations differentiation. For example, low oxygen tension stabilizes hypoxia-inducible factor alpha (HIFα) in $SP7^+$ BMSCs (also known as Osx^+ BMSCs) to stimulate osteogenic differentiation through direct activation of key glycolysis enzymes like pyruvate dehydrogenase kinase 1 (PDK1) [86]. Moreover, Nick et al. have reported that primary mouse skeletal stem cells ($7AAD^-$ $CD45^-$ $Ter119^-$ $Tie2^-$ $CD51^+$ $CD105^-$ $CD90.2^-$ $CD249^-$ $CD200^+$) under 2% O2 were prone to

chondrogenic differentiation via upregulating the expression of Sox9 while inhibiting fatty acids β-oxidation level [87].

Overall, BMSC subpopulations exhibit increased proliferation and enhanced osteo-chondrogenesis under hypoxia. $CD13^+$ $CD29^+$ $CD44^+$ $CD73^+$ $CD90^+$ $CD105^+$ $CD151^+$ $CD34^-$, $PDGFR\alpha^+$, and $LepR^+$ BMSC subpopulations exhibited increased proliferation, while $SP7^+$ and $7AAD^-$ $CD45^-$ $Ter119^-$ $Tie2^-$ $CD51^+$ $CD105^-$ $CD90.2^-$ $CD249^-$ $CD200^+$ BMSC subpopulations were prone to osteogenic and chondrogenic differentiation via upregulating glycolysis or fatty acids β-oxidation, respectively.

2.4. Irradiation

Irradiation is a common method to eliminate the resident bone marrow hematopoietic stem before transplanted cells reconstitute hematopoiesis [88]. Recent efforts have found that irradiation can also alter BMSC subpopulations. For example, irradiation can lead to some BMSC subpopulations loss including $LepR^+$ and $Nestin^+$ subpopulations [88]. In contrast, a subpopulation expressing Ecto-5′-nucleotidase (CD73) was retained in mice after irradiation conditioning and a specific $CD73^+$ $NGFR^{high}$ BMSC subpopulation contributes to HSPC engraftment and acute hematopoietic recovery via express various hematopoiesis supporting related factors like SCF, SDF1, Kit-L, Osteopontin, interleukin (IL)-3, IL-6, IL-7, and tumor necrosis factor alpha (TNF-α) et al. [88]. Zhong et al. has found that peri-arteriolar $LepR^+$ MALPs express high myofibroblast genes (such as *Myl9*, *Col9a1*, *Col10a1*, et al.) to participate in bone marrow repair after radiation damage [53]. Recently, Yue's group has revealed that osteogenesis subpopulations of $LepR^+$ BMSCs were dramatically expanded in irradiation-conditioning mice, and the subpopulation-specific express transcriptional factor Npdc1 and Hoxb2 [52]. Importantly, the research of Xiang has studied the response of human bone marrow stromal cells to irradiation in vitro [89]. They found that human BMSCs labeled by $CD73^+$ $CD90^+$ $CD105^+$ $CD14^-$ $CD34^-$ $CD45^-$ HLA-DR^- showed senescent and impaired immunomodulation capacity after irradiation [89].

Taken together, irradiation perturbates the number, osteogenic capacity, and immunomodulation capacity of specific BMSC subpopulations. $LepR^+$, $Nestin^+$ BMSCs were lost after irradiation, $CD73^+$ $NGFR^{high}$, $LepR^+$ MALPs, $LepR^+$ BMSCs with high expression of Npdc1/Hoxb2 were expanded and contribute hematopoietic and bone marrow repair. Additionally, $CD73^+$ $CD90^+$ $CD105^+$ $CD14^-$ $CD34^-$ $CD45^-$ HLA-DR^- human BMSCs exhibited senescent impaired immunomodulation capacity after irradiation.

2.5. PTH

Parathyroid hormone (PTH) is a potent bone anabolic hormone [90]. The heterogeneous BMSC subpopulations have been recognized in PTH-induced bone formation. There was a study that identified quiescent $LepR^+$ BMSCs located in healing sockets tissue of teeth [91]. The subpopulations can be activated by tooth extraction and contribute to extraction socket healing and alveolar bone regeneration of extraction sockets via response to PTH/PTH1R signaling [91]. Similarly, Yang et al. have used mouse genetic lineage tracing, which indicated that iPTH treatment increased the number of $LepR^+$ BMSCs and the capacity of differentiating into type I collagen

(Col1)$^+$ mature osteoblasts [92], accompanied by increasing the expression levels of osteogenic markers *SP7/Osx* and *Col1* while decreasing the expression of adipogenic markers *Cebpb*, *Pparg*, and *Zfp467* [92]. They further found iPTH treatment can also inhibit 5-fluorouracil- or ovariectomy (OVX)-induced adipogenesis of LepR$^+$ BMSCs and promote osteogenesis in bone marrow, even under adipocyte-induced conditions [92]. A further mechanism of PTH-induced LepR$^+$ BMSCs osteogenesis may be due to promoting osteolectin expression [93]. Another different insight proposed that iPTH induced bone formation of LepR$^+$ BMSCs by increasing numbers of type H endothelial cells (which are labeled by Edm^{high}/CD31^{high}) and mobilizing LepR$^+$ cells from these vessels close to the bone surface [94]. In addition, other studies have found that LepR$^+$ Runx2-GFP^{low} exhibited enhanced Runx2 expression and more multilayered structures formation near the bone surface after PTH administration, and multilayered cells express Osterix and Type I collagen α, ultimately leading to the generation of mature osteoblasts [95].

In sum, PTH treatment can increase the number of LepR$^+$ and LepR$^+$ Runx2-GFP^{low} BMSC subpopulations and skewed their lineage differentiation toward osteoblasts may by promoting osteolectin expression or increasing numbers of type H endothelial cells to make sure LepR$^+$ cells transfer to the bone surface.

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