Current Concepts in Bone Marrow Microenvironmental Regulation of Hematopoietic Stem and Progenitor Cells

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Introduction

Within the microanatomy of mammalian bone, hematopoietic stem and progenitor cells (HSPCs) interact with numerous cell types and molecules. Throughout development as well as in adulthood, the local microenvironment of HSPCs plays an instructive role in stem cell fate. Here we will discuss recent reports on these regulatory interactions. While a comprehensive report on all the cellular components of the HSC niche is beyond the scope of this concise review, we have specifically chosen to highlight topics with therapeutic relevance, where clinical translation can be envisioned within the near future. A comprehensive summary of cellular components of the niche identified so far is included (TABLE).

Anatomical Location of HSCs in Mammals

Hematopoietic stem cells (HSC) originate developmentally from the dorsal aortic endothelium of the aorta-gonad-mesonephros (AGM) region (reviewed in [1,2]). From the AGM HSCs migrate to the fetal liver. At time of formation of the bone cavities, the HSCs begin to migrate to the endosteum, forming the bone marrow (BM), where this migration is completed after birth[3]. There, the HSCs and their immediate progeny reside in specialized niches for the remainder of the life of the individual. Over the past decade, attention has focused on osteolineage cells based on the observation that HSPCs preferentially localize near the endosteum[4][5–9], however the persistence of a vascular HSC niche[10] and the conserved roles of many signals for both developmental and adult HSPC regulation suggests certain cellular and molecular factors are indispensable to the multiple cell fates of HSPCs biology, as will be reviewed below.

Cellular Components of the HSC Niche

Osteoblastic cells

The importance of the endosteal region of the bone marrow as an anatomical location for immature hematopoietic cells was appreciated very early[8]. However, many cell types exist in close proximity within this region. Our laboratory and others utilized genetic strategies resulting in activation of the osteolineage cell pool to provide initial in vivo evidence of phenotypic and functional HSPC expansion through targeted activation of the osteoblastic cell lineage[4, 11]. Osteoblastic activation in these models was accomplished either through a transgenic mouse model of constitutively active parathyroid hormone (PTH) signaling driven by the 2.3 kilobase fragment of the alpha1(I) collagen gene promoter or through models of intermittent systemic PTH treatment[12]. Intermittent PTH treatment is bone anabolic (rev in[13]). Overstimulation by PTH, as occurs in patients with parathyroid

adenomas, results in increased mobilization of phenotypically-defined HSPCs and endothelial progenitor cells [14,15]. Concurrent to the initial discovery that PTH expanded HSPCs through their microenvironment, Zhang et al. found that conditional inactivation of BMP receptor IA, which also resulted in greater trabecular bone and expansion of OBs, produced a similar increase in HSPCs [4].

Further studies have confirmed the central role of osteoblastic lineage cells in HSPC regulation. Ablation of OBs resulted in 3- to 10- fold reductions in HSPC numbers with corresponding defects in hematopoietic progenitor populations, reduced BM cellularity and induction of splenic extramedullary hematopoiesis [16], indicating that the osteoblastic population is essential for HSPC residence in the BM. However, expansion of osteoblastic cells alone is not sufficient to increase HSCs, since with some modalities of osteoblastic expansion, HSCs are actually decreased[17], just as general disruption of OBs is not necessarily sufficient to impair HSPCs [18]. Moreover, mode of activation of the osteoblast is critical. For example, in a mouse model of osteoblastic activation characterized by massive increases in trabecular bone there were reduced HSPCs, lineage-specific defects in megakaryocyte and erythrocyte development and impaired hematopoietic recovery from BM injury[19]. These unexpected effects of manipulation of the osteolineage cell pool suggested that specific stages of osteoblastic differentiation may be capable of greater HSPC support and that multiple osteolineage cells are likely to play roles in HSPC regulation [20]. In particular, mesenchymal stem cells (MSCs), the multipotent stromal cells that give rise to the osteogenic lineage, have been implicated as active components of the HSPC niche. The cotransplantation of MSCs with HSPCs improves donor engraftment [21] and enhances HSPC self-renewal [22]. Nestin+ MSCs express high homeostatic levels of HSPC maintenance genes, and are expanded by bone anabolic PTH regimen. Nestin+ MSC depletion resulted in impaired BM HSPC function and a shift to splenic hematopoiesis[23]. Consistent with these results, Runx2, a transcription factor most highly expressed at early stages of osteoblast differentiation, appears to identify an osteoblastic population with greater HSPC maintenance and enhancement potential [24,25], further supporting the concept that immature osteolineage cells are critical to HSPC regulation. Recently, studies examining more differentiated osteoblastic cells have provided additional support to the concept that it is the immature osteolineage cell population which more importantly supports and regulates HSCs. When terminally differentiated osteoblastic cells or osteocytes are activated by constitutive PTH signaling, HSPC frequency and function are unchanged in spite of increased osteoblastic cells and trabecular bone [26]. Together these reports demonstrate heterogeneity in the osteoblastic cell pool with respect to its HSPC-supportive properties, which appear to be dependent at least in part on osteoblast differentiation stage. In spite of the considerable complexity of the osteolineage population with respect to HSC support and regulation, since osteoblastic cells are activated by anabolic bone treatment, the potential for niche stimulation through is real. In fact, PTH is currently routinely used for its anabolic action in osteoporosis[27], and is well tolerated in patients even at high doses[15], therefore its use for HSC expansion in patients is likely to be imminent. One additional clinical application is highlighted by our recent report demonstrating that leukemia disrupts the osteoblastic microenvironment[28]. This finding may result in targeting the niche as an adjunct strategy in the treatment of leukemia or potentially other malignancies that affect the bone and marrow.

Osteoclastic cells

Osteoclasts (OCL), the bone resorbing cells, are an obligate partner of osteolineage cells. Therefore, it is not surprising that this cell population may also participate in HSC regulation. The role of OCL in HSC regulation that has emerged is complex and depends highly on the experimental model selected by the investigators, as we review next. OCL

activity is required for BM cavity formation and establishment of BM HSC niches [29]. Moreover, properly-formed, non-osteopetrotic BM cavities have been reported to be necessary for HSPC mobilization [30]. G-CSF administration in humans and in mice increases OCL activity[31,32], an effect that was initially found to play a critical role in HSPC mobilization[33]. Active, bone resorbing OCLs produce matrix metalloproteinase 9 and cathepsin K, which proteolytically inactivate SDF-1/CXCL12, releasing HSPC from the BM [34,35,36]. However, the role of OCL in HSC maintenance remains controversial. Miyamoto and colleagues [37] investigated the HSPC-regulatory role of OCL by assessing hematopoietic recovery following injury and G-CSF-induced HSPC mobilization in the context of genetically impaired OCL differentiation or pharmacologic OCL inhibition. OCL and/or a properly-formed BM cavity conferred resistance to hematologic stress, as OCL-deficient mice succumbed to death 8–10 days following 5-FU administration while control mice survived. In healthy mice, HSPC mobilization increased in the absence of OCL and a normal BM cavity. Conversely, when serial G-CSF was administered to mice with increased osteoclastogenesis due to Osteoprotegerin (OPG)-deficiency, HSPC mobilization was reduced. Histologic analysis suggested that, while the microanatomy of OCL-deficient bone is severely disrupted, functional HSPC niches are maintained. In contradiction to a previous report by Kollet et al.[33], these findings imply that neither normal BM cavities nor OCL are required for maintenance and mobilization of functional HSPCs and that in fact they may negatively regulate hematopoiesis. This discrepancy may result from biphasic or BM cavity-specific effects of OCL on HSPC mobilization. Since superior G-CSF-induced HSPC mobilization was achieved with pharmacologic OCL inhibition, this work points to OCLs as therapeutic targets in HSPC mobilization strategies. Multiple pharmacologic strategies are currently employed to inhibit OCLs for the treatment of osteoporosis, therefore OCLs remain a very feasible therapeutic target for HSC niche manipulation.

Endosteal monocytes and macrophages

In addition to OCLs, other cells of the monocyte-macrophage lineage have been implicated in HSPC regulation. Depletion of endosteal monocytes or macrophages occurs during G-CSF-induced HSPC mobilization and can induce HSPC mobilization independent of G-CSF administration [38, 39]. Reduced endosteal monocyte-macrophage populations coincide with reduced levels of HSPC-active factors in the niche, providing a potential mechanism of how these myeloid cells function in HSPC regulation [40]. Therefore, monocytes and macrophages participate in HSPC mobilization in response to G-CSF and are capable of modulating the endosteal HSPC niche. Recently, a sub-population of αSMA+ BM macrophages HSC-supportive capabilities was also identified[41]. Together these data suggest that macrophages/monocytes contribute importantly to HSC regulation.

Vasculature and perivascular stromal cells

From the time embryonic HSCs bud off from the hemogenic endothelium of the dorsal aorta[1,2], HSPCs and blood vessels interact closely with one another. BM blood vessels are dynamically positioned at the interface of peripheral blood (PB), hematopoietic marrow space and the endosteum. Indeed, the marrow vasculature plays critical roles in HSPC trafficking between bone and bloodstream[42] as well as in developmental bone growth [43] and in adult bone remodeling [44]. Based on the proximity of BM sinusoids to HSPCs [10] and the functional interactions between blood vessels, trabecular bone [45] and OCLs [46], the vasculature is actively being studied as an HSPC niche as well as a potential therapeutic target for hematologic and/or orthopedic diseases. Blocking endothelial cells (ECs) or BM angiogenic signaling can be detrimental to the engraftment of transplanted HSPCs [47, 48] and coculture of HSPCs with immortalized ECs promotes HSPC expansion [49]. Several vascular-derived HSPC-active factors have been found in the BM microenvironment, as we discuss below. Contrary to the reports of vascular regulation of HSPCs, Soki et al. reported that

vascular involvement is not required for HSPC expansion [50]. With conflicting reports on the role of the vascular HSPC niche, further studies are necessary to identify direct effects of the BM vasculature on the maintenance and activation of HSPCs.

Based on the observation that HSPCs preferentially localize near BM sinusoids [10], reports have implicated perivascular stromal cells as a niche for HSPCs [51, 52]. As was the case with osteoblastic cells, it is likely that the perivascular stromal population is heterogeneous, comprising other candidate HSPC niche entities including some Nestin+ mesenchymal cells [23] and CXCL-12 (SDF-1)-abundant reticular cells (CAR cells) [51]. An unexpected component of this cell pool was identified using genetic models of targeted Stem cell factor (SCF) or KitL deletion, molecules long noted for their roles in HSPC regulation [5354, 55]. Among deletion from hematopoietic, osteoblastic, Nestin-expressing stromal, endothelial or Leptin receptor (Lepr)-expressing perivascular stromal cell types, HSPC frequency and function were only impaired by the absence of SCF from Tie2+ endothelial and Lepr+ perivascular cells. These perivascular, Scf-expressing stromal cells lacked *Nestin* expression but expressed high levels of *Cxcl12*, *alkaline phosphatase*, *Vcam1*, *Pdgfra* and *Pdgfrb* as compared to whole BM, confirming their mesenchymal and stromal cell composition. These data indicate there is an ongoing endothelial niche-derived SCF requirement to ensure normal hematopoiesis and that SCF from Lepr+ perivascular cells is necessary for HSPC maintenance in adult BM.

In spite of the cellular complexity of the vascular/perivascular niche, numerous antiangiogenic pharmacologic tools approved for patient use exist, therefore as the role of endothelial and perivascular cells is experimentally clarified, there is hope for rapid translation of these strategies to the clinic in order to manipulate HSC pools through the microenvironment.

Molecular components of the HSC niche

N-cadherin

Cadherins, calcium-dependent homotypic adhesion molecules that form adherens junctions, play essential roles in fate specification of germline stem cells [56]. Several reports implicated N-cadherin (N-CAD) in osteoblastic-HSPC regulation [4–6]. Yet other works contradicted this conclusion (rev in [57]). It has been shown that *Ncad* expression increases with age in HSPCs and in endosteal stromal cells [58], representing a possible explanation for the inconsistencies among various N-CAD studies. In response to this controversy, our lab rigorously examined the bone and hematopoietic phenotypes of *Ncad* deletion from mature osteoblastic cells in both young and aged mice[12]. Loss of N-CAD corresponded to an age-dependent decrease in mineralized bone, suggestive of N-CAD involvement in terminal osteoblastic maturation, but no change in HSPC number or function at any age examined and no change in recovery from radiation-induced BM injury in spite of speculation that the N-CAD+ osteoblastic population, which expands rapidly in response to injury [59], may be involved in HSPC recovery. Since no effect of N-CAD loss from mature OBs was seen in HSPC maintenance at steady state or after injury, we wanted to assess whether N-CAD was required for HSPC expansion during osteoblastic niche activation by PTH. Without osteoblastic N-CAD however, PTH was still able to expand trabecular bone and increase phenotypically-defined long and short term HSPCs, thereby demonstrating N-CAD does not play a role in HSPC regulation. These results are consistent with a companion report in which *Ncad* was deleted from immature OBs using an osterix-driven cre recombinase model and no hematopoietic effects were observed [60]. Notably, there is evidence that *Ncad* expression colocalizes with factors that do affect HSPCs. *Ncad* expression was found to increase up to 8.5 fold following stimulation with dimethyl prostaglandin E2 (dmPGE2) a synthetic analog of the inflammatory mediator prostaglandin E2 known to have effects on HSPC survival and

proliferation. Furthermore, N-CAD was reported to identify osteoprogenitor cell sources of HSPC-active noncanonical Wnt ligands and inhibitors of canonical Wnt signaling at homeostasis [61, 62] (both of which will be discussed below). Therefore, modulation of NCAD is a potential strategy for HSC niche expansion.

Wnt/β catenin signaling

Wnt signaling occurs via 19 different, glycosylated secreted ligands that can be bound by frizzled receptors and low density lipoprotein co-receptors to elicit either β-catenin (canonical) or non-β-catenin, JNK or PKC-mediated (noncanonical) effects [63]. Evidence for a dose and developmental stage-specific role of Wnt signaling in embryonic HSPC generation and adult HSPC maintenance has emerged. The self-renewal capacity of HSPCs is impaired by loss of β-catenin-mediated canonical Wnt signaling in HSPCs, by treatment with the canonical Wnt inhibitor DKK1 or by loss of the canonical Wnt ligand Wnt3a in HSPCs [64–66]. While β/γ-catenin in the BM is dispensable for HSPC self-renewal[67, 68], activation of Wnt/β-catenin can increase HSPC activity, eventually leading to stem cell burn out [69–72]. In these models, Wnt/β-catenin activity was enforced through genetic manipulations, however a more transient model of pharmacologic β-catenin stabilization showed no such impairment of HSPC self-renewal capacity [73]. Recent work by Ruiz-Herguida et al. interrogates the distribution and hematopoietic role of Wnt/β-catenin signaling in the developing mouse embryo. In this study, active, nuclear β-catenin in non-hematopoietic ECs is located adjacent to the emerging HSC clusters. This endothelial niche must express β-catenin in order for HSCs to emerge [63]. In the adult, T cell-derived Wnt10b is required for PTH induced niche activation and expansion of short-term repopulating HSPCs (ST-HSPCs)[74]. Moreover, EC-derived Wnt10b has been implicated in HSPC activation and recovery following cyclophosphamide and G-CSF-induced hematologic stress [75], emphasizing the importance of canonical Wnt signaling from various niche cell types in regulating HSPCs during development and hematopoietic activation.

Sugimura et al. recently set out to examine the effects of noncanonical Wnt signaling on HSPC regulation in vivo [62]. In their report they demonstrate by RNA seq data that at homeostasis, expression of canonical Wnt ligands is low compared to expression of noncanonical ligands and inhibitors of Wnt signaling in the BM microenvironment. N-CAD + OBs expressed the highest level of Wnt inhibitors and noncanonical ligands (Wnt6 and Wnt16). To better understand how noncanonical Wnt signaling affects HSPC regulation, the authors focused on two noncanonical Wnt components that were found to be differentially expressed among HSPC subsets. The cadherin adhesion molecule Flamingo (Fmi) that facilitates noncanonical Wnt signaling [76] and the Wnt receptor Frizzled8 (Fz8) have been found to be more highly expressed in phenotypically-defined quiescent HSPCs as compared to populations enriched for multipotent hematopoietic progenitors (MPPs) or ST-HSPCs [62, 77]. Disruption of noncanonical Wnt signaling through Fmi and/or Fz8 knockout mouse models decreased frequencies and absolute numbers of HSPCs, with greater proportions of the remaining Flt3- LSKs cycling as compared to wild type cells and a reduced frequency of the quiescent HSPCs in contact with N-CAD+ osteoblastic cells [62]. This thorough, in vivo examination of a previously underexplored component of the BM microenvironment establishes a role for noncanonical Wnt signaling in maintaining HSPC quiescence at the endosteum in contrast to the apparent HSPC-activating role of canonical Wnt signals from the vasculature.

Again following discoveries in the bone field for the treatment of osteoporosis, a clinical strategy for modulation of Wnt signaling in the bone microenvironment includes sclerostin inhibition, which has already proven successful in initial clinical trials[78]. Further, anti-DKK strategies are available for the treatment of certain hematopoietic malignancies[79, 80]. Therefore, modulation of Wnt signaling is a potential strategy for niche regulation.

VEGF

As the two major microenvironments within bone, osteoblasts and vascular ECs are in close physical and regulatory association with one another. Direct coupling between ECs and OBs has been reported [81] and VEGF is a key mediator of this regulatory interaction[82, 83]. VEGFR2, the main receptor through which VEGF-A signals in the BM, is expressed by OBs, OCLs and chondrocytes as well as BM ECs[84], evidencing the diverse roles of VEGF signaling in the BM microenvironment. In fact, the full bone anabolic effect of PTH in rats requires VEGF-mediated vascular remodeling characterized by the relocation of microvessels closer to sites of new bone formation[83]. VEGF can couple osteogenesis and angiogenesis by stimulating survival and proliferation of ECs, OB lineage commitment of osteoprogenitors and production of the OCL formation inhibitor OPG from OBs [84]. Furthermore, VEGF is critical for maturation of the vascular endothelial HSPC niche and has been reported to potently stimulate HSPC proliferation[85] and to be induced by HSPC-activating dmPGE2 treatment[61]. In the setting of BM injury, inhibition of VEGF signaling severely compromises hematopoietic recovery[48], potentially due to the loss of VEGF-induced Notch ligand expression that would otherwise prevent HSPC exhaustion during repopulation [47]. Conversely, conditional over-expression of osteoblastic VEGF has been reported to dysregulate OB-EC coupling, resulting in increased microvascular density and greater HSPC mobilization[84]. In a study by Maes et al., VEGF overexpression altered BM OCL frequencies and increased hematopoietic progenitors in PB with a corresponding 25% rate of occurrence of extramedullary splenic hematopoiesis.

VEGF is strongly induced by hypoxia, therefore the direct role of hypoxia on HSPC regulation has also been explored. It has been established that the most quiescent HSPCs reside in the least perfused niches and are better preserved ex vivo in hypoxia[86–89]. Although their niches tend to be hypoxic, HSPCs are also capable of maintaining intracellular hypoxia and stabilizing HIF1α protein [90]. In a murine model of inducible *Hif1a* deletion, HSPCs were less quiescent and fared worse during BM transplantation and other physiologic stresses [90]. Emerging data suggest a role for HIF signaling in the osteoblastic HSPC niche as well. A recent report by Rankin et al. overstabilized HIF-1 and -2 in osteoblastic lineage cells and saw expansion of HSPCs, with selective increases in the erythroid progenitors in the BM and spleen, greater VEGF and Erythropoietin (EPO) production in bone, reduced EPO production in the kidney and protection from stress-induced anemia [91]. The bone architecture was also affected by osteoblastic HIF-1 and -2 overstabilization exhibiting increased bone mass with concomitant expansion of the BM vasculature [43, 91]. The authors demonstrate that elevated red blood cell counts and hematocrit are EPO dependent but no other niche factors were interrogated thus further investigation is necessary to uncover the mechanism by which osteoblastic HIF activation increases HSPCs in the BM.

Over the years VEGF and its receptors have been targeted as antiangiogenic therapies for cancer and other pathologies, therefore these tools could be promptly refocused for niche manipulation.

PGE2/dmPGE2

Although HSPCs are routinely used in transplantation to treat hematopoietic malignancies or BM failure, the number of HSPCs that can be harvested remains a potential limiting factor, especially when stem cells are harvested from cord blood (CB). Thus far we have discussed in vivo regulation of HSPCs, however, the ex vivo expansion of HSPCs would be of great clinical use to improve transplant efficacy from limiting stem cell sources. Cells of the BM microenvironment produce the inflammatory mediator PGE2, an arachidonic acid derivative that has been shown to increase HSPC numbers in vivo and in vitro [92]. In fact, the ex vivo

treatment of murine BM with the synthetic PGE2 analog 16,16-dimethyl PGE2 (dmPGE2) prior to transplantation increased the frequency of repopulating HSPCs and improved engraftment in irradiated recipients [92, 93]. Work in our laboratory demonstrated that in vivo PGE2 treatment increased short term repopulating HSPCs and MPPs in the BM, even in the absence of injury [94]. Moreover, we have shown that dmPGE2 ameliorates sublethal irradiation-induced damage to the HSPC pool by inhibiting apoptosis while preserving long term function [95]. dmPGE2-mediated enhancement of hematopoietic recovery is likely to occur both through direct effects on HSPCs as well as changes to the microenvironment. Specifically, dmPGE2 expanded αSMA+ BM macrophages and induced microenvironmental COX2 activity to further support HSPCs and augment the pharmacologic PGE2 stimulation, respectively. Recently, Goessling et al. stimulated human CB (hCB) specimens with dmPGE2 to assess whether this could be a feasible strategy for expanding HSPCs in vitro prior to transplant [61]. As expected from murine results, dmPGE2 treatment of either whole hCB or CD34+ hCB decreased apoptosis while increasing proliferation and hematopoietic colony formation of CD34+ cells, indicating a direct effect of dmPGE2 on hCB HSPC regulation. To test the durability and appropriate behavior of dmPGE2 stimulated cells over time, competitive autologous transplants of dmPGE2 treated mobilized PB stem cells from rhesus macaques were performed. dmPGE2-treated cells displayed normal PB reconstitution for up to one year following the transplant, further illustrating the safety and clinical utility of dmPGE2 for HSPC expansion. Mechanistically, dmPGE2 stimulated cAMP-mediated Wnt signaling, leading to HSPC expression of pro-proliferative, anti-apoptotic genes [96] and in the upregulation or maintenance of gene programs associated with developmental HSPC emergence from hemogenic endothelium suggesting that dmPGE2 may have a rejuvenating effect on hCB stem cells.

Conclusion

Numerous elements of the BM microenvironment intersect to appropriately regulate HSPC fate choices. While osteolineage cells, particularly immature osteoblastic cells, regulate the HSPC pool size, OBs are in close physical and regulatory interaction with many other cell types, including OCLs and the BM vasculature, which is emerging as an essential component of HSPC maintenance and hematopoietic recovery from injury. With multiple niches and a variety of HSPC-active factors, the BM microenvironment provides many potential therapeutic targets for HSPC expansion in the setting of recovery from myeloablation, modulating HSPC behavior in vivo or by recapitulating the niche ex vivo. Moreover, to the extent that malignancies disrupt the normal HSPC niche, the identification of niche components could also be exploited for treatment of malignancy or prevention of its recurrence.

Acknowledgments

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Table

NICHE CELL TYPE:	DETAILS OF MODEL(S):	EFFECTS:
Osteoblastic cells	• Stimulation of PTH1R in mice	• Increased BM HSPCs in BM. Induction of HSPC- active factors [11,12,23,97]
	• Hyperparathyroidism in humans	• Increased mobilization of HSPCs and endothelial progenitors [15,98]
Osteoclasts	• Inhibition of OCL	• Altered baseline HSPC mobilization, reduced HSPC homing and poorer recovery from hematologic injury [29,33]
	• Genetic activation of OCL	• Less efficient HSPC mobilization [37]
Perivascular stromal cells	• Immunofluorescent studies of the BM	• HSPCs located near vascular-associated reticular cells and endosteal cells [51,23,52]
	• Conditional deletion of membrane-bound SCF from BM cell types	• Impaired HSPC maintenance without SCF in Tie2+ endothelial or Lepr+ perivascular cells [53]
	• Depletion of Nestin+ perivascular cells	• Reduction of BM HSPCs [23]
Endothelial cells	• Pharmacologic inhibition of VEGFR2 during recovery from BM injury	• Mice succumb to BM failure [51,52]
	• Conditional over-expression of VEGF-A by osteolineage cells	• Increased circulating hematopoietic progenitors, correlated with extramedullary splenic hematopoiesis in 25% of mice [84]
Endosteal monocytes and macrophages	• Depletion of monocyte/macrophage or specifically macrophage populations.	• Reduction in HSPC-active factors in the BM and induction of HSPC mobilization [39,40]
Sympathetic nervous system neurons	• Inhibition of adrenergic neurotransmission	• Lack of HSPC mobilization in response to G-CSF [30]
	• Pharmacologic stimulation of β2 adrenergic receptors	• Enhanced HSPC mobilization [30]
Glial (nonmyelinating Schwann) cells	• Immunofluorescent examination of HSPC niche factors and cell types	• GFAP+ glial cells were in contact with HSPCs and produce TGF-β that appears to regulate HSPC dormancy [99]
	• Denervation of sympathetic nerves to reduce BM glial cells	• HSPC loss [99]
T cells	• PTH treatment of mice lacking T cell PTH1R or Wnt10b	• PTH fails to expand short term HSPCs in the absence of T cells [100]