Making sense of hematopoietic stem cell niches

Philip E. Boulais¹,³ and Paul S. Frenette¹-³*

Departments of ¹Cell Biology and ²Medicine, ³The Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx, New York, USA

Running title: Dissecting HSC niches

*To whom correspondence should be addressed

Paul S. Frenette MD
Albert Einstein College of Medicine
1301 Morris Park Avenue,
Bronx, NY 10461
Tel: 718.678.1255
paul.frenette@einstein.yu.edu
Abstract

The hematopoietic stem cell (HSC) niche commonly refers to the pairing of hematopoietic and mesenchymal cell populations regulating HSC self-renewal, differentiation and proliferation. Anatomical localization of the niche is a dynamic unit from the developmental stage, allowing proliferating HSCs to expand before they reach the bone marrow where they adopt a quiescent phenotype that protects HSCs’ integrity and functions. Recent studies have sought to clarify the complexity behind the HSC niche by assessing the contributions of specific cell populations in HSC maintenance. In particular, perivascular microenvironments in the bone marrow confer distinct vascular niches, regulating HSC quiescence and the supply of lineage-committed progenitors. Here, we review recent data on the cellular constituents and molecular mechanisms involved in the communication between HSCs and putative niches.
The origins of hematopoiesis

The hematopoietic system supplies our body with >100 billion mature blood cells every day that carry out functions such as oxygen transport, immunity and tissue remodeling. Hematopoietic stem cells (HSCs), located at the top of the hematopoietic hierarchy, are responsible for replenishing our pool of blood cells throughout life. Early work by James Till and Ernest McCulloch provided evidence that single bone marrow cells could give rise to multilineage progenitors and could undergo at least short-term self-renewal. These studies paved the way to the conceptual hierarchy in HSC differentiation and their role in the maintenance of hematopoietic homeostasis. Whether, and if so how, HSCs could autonomously modulate their function or be influenced by extrinsic factors, however, has remained poorly understood until recently. In the adult stage, most HSCs are found in a quiescent state which protects them from genotoxic insults and ensure their long-term repopulating ability. HSC state and function must be finely tuned in order to protect their self-renewal capacity and prevent their exhaustion which is crucial for blood system homeostasis. Differences in spatial localization of colony-forming unit spleen (CFU-S) within rodent long bones is associated with discrete proliferative state which suggests that specific microenvironments within the bone marrow can regulate HSPC state and function. Bone marrow stromal cells promote ex vivo proliferation and differentiation of HSPCs in long-term cultures, supporting the notion that microenvironmental cues may influence HSC fate and modulate hematopoiesis. This idea is crystalized by the “niche hypothesis”, where the niche forms a regulatory unit limiting HSC entry into cell cycle, thereby protecting them from exhaustion or errors in DNA replication. Therefore,
identification of molecular cues regulating HSC fate will improve our knowledge on the regulation of hematopoiesis in health and disease.

During development, HSCs traffic between niches in order to establish hematopoiesis. Primitive hematopoiesis takes place in the yolk sac around embryonic day (E)7.0 where immature precursors give rise to erythrocytes to supply oxygen to the developing embryo\(^\text{10}\). The presence of the first “definitive” HSC known to be able to fully reconstitute the hematopoietic system upon transplantation is found in the aorta-gonad-mesonephros (AGM) in mice and humans\(^\text{11,12}\). However, other studies have suggested that yolk sac cells from E9.0-E10.0 can mature into definitive HSCs when transplanted into a newborn rather than adult mouse\(^\text{13,14}\). In addition, the placenta represents a significant reservoir of HSCs during development\(^\text{15,16}\). Once the vasculature is developed, HSCs migrate to the fetal liver around E12.0 where they expand and differentiate\(^\text{10}\). Fetal liver HSCs are actively cycling, by contrast to their bone marrow counterparts, and can also outcompete adult bone marrow HSCs when transplanted into irradiated recipients\(^\text{17}\). During HSC expansion in the fetal liver, chondrocytes and osteoblasts are produced within mesenchymal condensations to create cartilage and bone\(^\text{10}\). Skeletal remodeling is associated with bone vascularization which allows homing of HSCs and colonization of the fetal bone marrow by E17.5\(^\text{10}\). This process is mediated through CXCL12 production by bone marrow stromal cells which attracts HSCs expressing the CXCR4 receptor\(^\text{18}\) and specific adhesion molecules expressed on bone marrow endothelium\(^\text{19,20}\).
A shelter between blood and bone

Knowledge on the identities and functions of HSC niches has markedly improved in the past few years (Figure 1). While the association of progenitor activity with the endosteum has been appreciated for several decades\textsuperscript{7}, a direct role for osteoblasts in HSC maintenance is suggested by experiments showing that cultured osteoblasts are capable of expanding hematopoietic progenitors \textit{in vitro}\textsuperscript{21,22}, leading to studies revealing that the genetic or pharmacological manipulation of osteoblast numbers correlates with HSC counts in the bone marrow\textsuperscript{23,24}. In addition, imaging of the transplanted lineage negative progenitor fraction of bone marrow cells shows that progenitors are preferentially distributed along the endosteal region\textsuperscript{25}. Osteoblasts were proposed to support HSC function by forming direct interactions via N-cadherin-mediated adhesion\textsuperscript{24}, although this idea has been highly controversial. Functional studies using conditional knockout of N-cadherin (\textit{Cdh2}) in hematopoietic and stromal cells\textsuperscript{26}, osteoprogenitors\textsuperscript{27} and osteoblasts\textsuperscript{28} do not reveal any change in HSC numbers, although overexpression of N-cadherin have been reported to alter HSC numbers\textsuperscript{29}. Activated osteoblasts can produce Osteopontin that limits HSC expansion\textsuperscript{30} as well as Angiopoietin-1 and Thrombopoietin that bind the Tie2 and MPL receptors, respectively, and contribute to HSC quiescence\textsuperscript{6,31,32}. Bone resorption and the subsequent release of calcium by osteoclasts also promote HSC maintenance and localization to the endosteal region\textsuperscript{33,34}. By contrast, the SLAM cell surface markers CD150 and CD48 have identified HSCs in proximity of sinusoidal blood vessels\textsuperscript{35}. Using a knockin mouse strain expressing GFP driven in the \textit{Cxcl12} locus, a chemokine critical for the maintenance and quiescence of HSCs, perivascular cells known as CXCL12-abundant
reticular (CAR) cells are reported to contact HSCs mainly near sinusoids in endosteal and non-endosteal marrow\textsuperscript{36}. \textit{In vivo} imaging of the bone marrow in the calvarium reveals that specific sinusoidal domains expressing both CXCL12 and E-selectin are targeted for homing and engraftment of normal and leukemic HSCs\textsuperscript{37}. Innervation by the sympathetic nervous system (SNS) regulates HSC mobilization through circadian release of noradrenaline that modulates CXCL12 expression in the bone marrow\textsuperscript{38,39}. In addition, sympathetic nerves play a critical role in bone marrow regeneration where ablation of adrenergic innervation in the bone marrow impairs HSC recovery after chemotherapy\textsuperscript{40}. Physical association between nerves and bone marrow vasculature also supports the importance of a vascular niche for HSCs. Recent imaging studies of the bone marrow did not reveal a significant association between osteoblasts and HSCs\textsuperscript{41,42}. These data and the selective deletion of critical niche factors in osteoblasts (see below), argue that osteoblasts do not directly contribute to HSC maintenance.

Although the endosteal region has been suggested to be enriched in HSCs, its boundary may not be as narrow as initially suggested\textsuperscript{43}. The endosteum is highly vascularized by the presence of sinusoids with CAR cells\textsuperscript{36} and also with arterioles that often run along the endosteal area\textsuperscript{42}, both of which are associated with HSCs. After transplantation in irradiated mice, HSCs home preferentially to endosteal surfaces of the trabecular bone region but randomly distribute in non-irradiated recipient\textsuperscript{44,45}. HSCs undergo expansion following bone marrow damage in the endosteal region where osteoblasts and blood vessels are in close proximity\textsuperscript{44,45}. Lethal irradiation is known to disrupt the sinusoidal network which may account for the relocalization of HSCs to the endosteum\textsuperscript{46}. Since the endosteum is vascularized with arteriolar vessels which are
more resistant to genotoxic insults, it is likely that vascular niches may differentially contribute to bone marrow regeneration.

**Perivascular niches dictate HSC fate**

By tracking the origins of HSCs, studies have linked HSC activity to vascular development\(^\text{15}\). The endothelial layers of the dorsal aorta along with the vitelline and umbilical arteries have been proposed as primary sites for the emergence of definitive HSCs\(^\text{11,47}\). Defective angiogenesis in AML1-deficient mice that lack definitive hematopoiesis can be rescued by the presence of HSCs which suggests that they can play a role in shaping a vascular network during development\(^\text{48}\). Using Runx1-lacZ and CD41 marker to mark HSCs, HSCs were shown to be generated in the placental vasculature of mouse embryos prior to liver colonization\(^\text{49}\). Stromal cell lines derived from human placenta can support the expansion of HSPCs and express pericytic markers\(^\text{50}\). Transcriptome analyses have revealed organ-specific molecular signatures involved in the HSPC-supportive activity of stromal cells\(^\text{51}\). The association among HSCs, perivascular, and endothelial cells suggests that these cell populations can indeed coexist in an environment that promotes their maintenance and expansion. Although the mesenchymal stem and progenitor cell (MSPC) activity was described decades ago in colony-forming unit fibroblasts (CFU-F)\(^\text{52}\), the lack of unique cell surface markers as well as the disparity between lineage tracing models and isolation methods, have hampered their characterization. Perivascular human CD146\(^+\) CD45\(^-\) MSPCs residing in the bone marrow contain virtually all the CFU-F activity and are capable of reconstituting a heterotopic bone marrow niche, suggesting that MSPCs and their progeny contribute to the development of the HSC niche and regulate hematopoiesis\(^\text{53}\). A mouse fetal bone
CD51+CD105+CD90−CD45−Tie2− progenitor cell population is able to reconstitute the HSC niche by forming donor-derived ectopic bone through endochondral ossification creating a marrow cavity with host-derived vasculature and HSCs\textsuperscript{54}. Other studies have found that PDGFR\textalpha+Sca-1+CD45−Ter119− (P\textalpha S) markers identify MSPCs capable of differentiating into osteoblasts, reticular cells and adipocytes \textit{in vivo}\textsuperscript{55}. Transgenic mice expressing green fluorescent protein (GFP) under the control of the promoter and second intronic enhancer of Nestin (Nes-GFP)\textsuperscript{56}, an intermediate filament highly expressed in the brain, allows the prospective identification of perivascular mesenchymal stem cells that are significantly associated with HSCs in the bone marrow. Stem cell activity of these Nes-GFP\textsuperscript{+} cells is suggested by the fact that this fraction contains all the CFU-F activity in the bone marrow and is able to form clonal spheres that can self-renew, multi-differentiate at the clonal level into the major mesenchymal lineages, and generate hematopoietic activity \textit{in vivo} upon serial transplantation\textsuperscript{57}. Nes-GFP\textsuperscript{+} cells express endogenous Nestin\textsuperscript{57,58} and are also tightly associated with sympathetic nerves that regulate the expression of CXCL12 in a circadian fashion\textsuperscript{39,57}. In further support for a role of sympathetic nerve fibers in regulating the HSC niche, nonmyelinating Schwann cells expressing glial fibrillary acidic protein (GFAP) have been reported to activate transforming growth factor-\textbeta1 (TGF-\textbeta1) promoting HSC quiescence\textsuperscript{59}. Although GFAP\textsuperscript{+} glial cells are described in close proximity to HSCs, only about a quarter of CD150+CD48−CD41−Lin− HSCs appears in direct contact with them while 97% of HSCs were directly associated with CAR cells\textsuperscript{96} and 60% with Nes-GFP\textsuperscript{+} MSPCs\textsuperscript{57}. However, association of HSCs with these various stromal cells would require further statistical analyses to determine the
exact significance of these relationships. Further refinement of cell surface markers identifying MSPCs population demonstrates that PDGFRα+CD51+CD45−Ter119−CD31− stromal cells are enriched for HSC maintenance genes, formed clonal multipotent self-renewing mesenpheres in culture and are able to reconstitute a bone marrow niche heterotopically. These PDGFRα+CD51+ stromal cells largely overlap (~75%) with Nes-GFP+ MSPCs and contain the majority of the CFU-F activity in both adult mice and human fetal bone marrow. They also represent a small subset of CD146+ human skeletal stem cells enriched in MSPC and HSC niche activities. Sca-1 is not highly expressed in bone marrow PDGFRα+CD51+ cells which may suggest they are distinct stromal cell populations from the previously identified PaS MSPCs. Indeed, the latter were isolated from crushed bone with discarded bone marrow, whereas studies with Nes-GFP+ cells have been carried out with flushed bone marrow. Bones appear to contain a higher concentration of CFU-F (~10-fold greater than BM) and bone-derived mesenchymal progenitors express Sca-1 whereas the population of bone marrow mesenchymal progenitors (based on Nes-GFP) appears mostly negative for Sca-1.

Endothelial cells lining sinusoidal blood vessels in the bone marrow may also contribute to HSC regulation. Disruption of VEGFR2 and VE-cadherin-dependent angiogenic signaling pathways using monoclonal antibodies shows that sinusoidal endothelial cells (SECs) expand the HSC pool, support self-renewal and prevent exhaustion of HSCs in both serum-free coculture assays and in vivo through Notch signaling. Endothelial-specific deletion of Jagged-1 by VE-cadherin-cre reveals that SECs are directly involved in HSC self-renewal for homeostatic and regenerative hematopoiesis while the stromal and perivascular cell compartments, including
PDGFRα⁺CD51⁺ MSPCs, are not altered in numbers or in their ability to generate CFU-F°. In contrast, CD31hi Endomucinhi endothelial cells were proposed to mediate neo-angiogenesis in bone through Notch signaling pathways and influence perivascular osteoprogenitor levels. Therefore, the activation state of endothelial cell may play a crucial role in secreting factors instructing HSPC fate. Conditional knockdown of SCF in endothelial and perivascular cells using Tie2-cre and leptin receptor (LepR)-cre mice, respectively, lead to reductions in HSC frequency in the bone marrow and spleen. These functional analyses are consistent with the expression pattern of GFP knocked into the Scf locus which shows that SCF is largely expressed by perivascular cells and mostly around sinusoids, but also near venules and arterioles. The authors have also suggested that hematopoietic cells (Vav1-cre), osteoblasts (Col2.3-cre) and Nestin⁺ cells (Nestin-cre, Nestin-creER) do not represent important contributors of SCF in the bone marrow niche although the recombination efficiency of Scf deletion in Nestin⁺ cells and osteoblasts was not provided to confirm efficient gene deletion in these cells.

Recent studies have also determined the contribution of different niche populations in CXCL12 production. Targeted Cxcl12 deletion in osteoblasts reveals no HSC and myeloprogenitor phenotype, although the authors have noted lower reconstitution of B and T cells in irradiated mice as well as lower numbers of lymphoid progenitors in the bone marrow using Col2.3-cre mice. Conditional deletion in osteoprogenitors using Osterix (Osx)-cre results in hematopoietic progenitor mobilization to the blood and spleen and reduced B lymphoid progenitors. These results are in accordance with studies showing that CXCL12 is involved in B-cell maintenance and that depletion of osteoprogenitors or CAR cells leads to reduction in B...
lymphoid progenitor cells. Endothelial-specific deletion (Tie2-cre) of CXCL12 reduces HSC frequency while hematopoietic progenitors and lineage reconstitution levels after transplantation are unaffected. Deletion of CXCL12 in perivascular stromal cells using LepR-cre yields no change in HSC and progenitor number as well as in reconstitution level but shows an increase in mobilization to blood and spleen. Hematopoietic cells (Vav-cre) and Nestin+ MSPCs (Nes-cre) also induce no phenotype upon CXCL12 deletion although whether Cre-mediated deletion occurred in Nestin+ cells was not demonstrated, thus limiting the conclusions that can be reached on the contribution of Nes-GFP+ cells in CXCL12 secretion. By contrast, using transcription factor paired related homeobox-1 (Prx1)-cre that recombines mostly in osteoblasts and bone marrow stromal cells, a clear reduction in HSC and lymphoid progenitors with an increase in mobilization can be observed. Prx1-cre induces recombination in mesenchymal progenitors, which adds further support to the critical role of MSPCs in forming an HSC niche.

One of the proposed views of the niche was that the “endosteal niche” might provide a hypoxic environment maintaining HSCs a quiescent state while the “vascular niche” allowed HSCs to proliferate and differentiate in an environment where oxygen was more available. Expression of E-selectin, exclusively found on endothelial cells, promotes HSC proliferation whereas E-selectin antagonists promote HSC quiescence and self-renewal. Most of the bone marrow space is occupied by sinusoids but the endosteal region is also highly vascularized and perfused by arterioles that further subdivide in arterial capillaries ultimately connecting with sinusoids. Using three-dimensional bone marrow imaging, Nes-GFP+ cells with different GFP expression levels
discriminate between arterioles (Nes-GFP\textsuperscript{bright}) and sinusoids (Nes-GFP\textsuperscript{dim}) allowing to determine by computational simulations that quiescent HSCs preferentially associate with arterioles and that proliferative HSCs move away from arterioles\textsuperscript{42}. Nes-GFP\textsuperscript{bright} arterioles are themselves quiescent and their integrity is preserved following treatment with 5-fluorouracil (5-FU), suggesting that they may provide a shelter for quiescent HSCs following genotoxic insults\textsuperscript{42}. Furthermore, the pericyte marker NG2 appears to largely label arteriole-associated Nes-GFP\textsuperscript{+} cells whereas LepR labels preferentially Nes-GFP\textsuperscript{dim} associated sinusoids\textsuperscript{42}. Depletion of NG2\textsuperscript{+} cells also induces HSC cycling and distribution away from arterioles\textsuperscript{42}. HSC distribution between proliferative (sinusoids) and quiescent (arterioles) niches may represent specific milieux regulating distinct HSC pools.

Consistent with the idea that HSC quiescence is associated with a hypoxic niche\textsuperscript{76,77}, quiescent HSCs are enriched in stabilized transcription factor hypoxia-inducible factor-1\textalpha (HIF-1\textalpha) allowing their maintenance and resistance to stress in hypoxic conditions\textsuperscript{78,79}. Live \textit{in vivo} imaging of oxygen concentration using phosphorescence lifetime sensing nanoprobes provides evidence that the most hypoxic region is located within less than 40 \textmu m from the bone towards the perisinusoidal region while there is a modest increase in oxygen concentration inside 20 \textmu m of the bone in the endosteal region\textsuperscript{80}. Interestingly, higher oxygen tension is found around Nes-GFP\textsuperscript{+} vessels\textsuperscript{80}, which harbor quiescent HSCs\textsuperscript{42}. The possibility remains open that the oxygen tension may in fact be much lower just outside the relatively thick-walled arterioles. Other studies have revealed that hypoxia, as sensed by pimomidazole staining, appears HSC autonomous which raises the possibility that the “hypoxia” factors may in fact be
regulated through different cues\textsuperscript{41}. Indeed, the microenvironment could also influence the hypoxic and metabolic profile of HSCs since SCF\textsuperscript{81} and Thrombopoietin\textsuperscript{82} are known to increase HIF-1\textalpha levels which could alter the distribution of “hypoxic” HSCs. In addition, P\alphaS MSPCs exhibit a hypoxic profile and are enriched in HIF factors that inhibit HSPC expansion and differentiation\textsuperscript{83}. Therefore, studies in the near future should be able to confirm the existence and the role of a hypoxic niche and its overlap with quiescent and proliferative niches.

**Hierarchy and crosstalk in the HSC niche**

In the adult bone marrow, MSPCs are responsible for the generation of mesenchymal lineage tissues such as bone, fat and cartilage as well as bone marrow stromal cells that constitute an environment supporting HSCs and hematopoiesis (Figure 2). Although the stromal cell composition of the bone marrow is thought to be as complex as its hematopoietic counterpart, the hierarchical relationship among stromal cells and their influence on the niche remains unclear. The myxovirus resistance-1 (Mx-1) induces Cre-mediated recombination in osteogenic progenitors maintaining most of the osteoblast pool at steady state and after tissue stress, while Osx-cre\textsuperscript{ERT2} only induces a transient source of osteoblasts in adult mice\textsuperscript{84}. Interestingly, when labeling is induced perinatally, long-lived bone marrow Nes-GFP\textsuperscript{+} LepR\textsuperscript{+} stromal cells capable to contribute to tissue regeneration after injury are marked by Osx\textsuperscript{85}. Nes-GFP\textsuperscript{+} LepR\textsuperscript{+} stromal cells indeed appear to contain MSPC activity in the adult bone marrow\textsuperscript{85,86}. The observation that Osx, which is a transcription factor thought to be specific for the osteolineage, marks the stroma during development was independently reported by other groups\textsuperscript{87,88}. Interestingly, labeling of Osx\textsuperscript{+} cells during the fetal stage also marked
postnatally bone marrow stromal cells but these cells were short-lived and replaced by definitive MSPCs during the late fetal and neonatal period\textsuperscript{85}. These studies open the interesting possibility of the presence of a “primitive” and “definitive” bone marrow stroma, both marked by Osx during ontogeny, whose differential characteristics and functions remain to be defined.

Although much of the focus about bone marrow stroma has been on the HSC niche, emerging data suggest that subsets of stromal cells also contribute to the maintenance of hematopoietic progenitor populations (Figure 3A). For example, conditional deletion of CXCL12 in both osteoblasts and perivascular stromal cells results in a decrease of common lymphoid progenitors while its deletion in osteoprogenitors is associated with a decrease in B-lymphoid-committed progenitors\textsuperscript{67,68}. This is consistent with studies showing that depletion of CAR cells, known to be tightly associated with pre-pro B cells\textsuperscript{69}, reduces both common lymphoid progenitors and pro-B cell levels\textsuperscript{70}. It is thus possible that the endosteal region might represent a suitable microenvironment for the maintenance of lymphoid progenitors. In addition, macrophages constitute the central unit in erythroblastic islands thereby providing a niche for the maturation of erythropoietic cells in the steady state, after hemolytic or myeloablative stress and during malignant hematopoiesis\textsuperscript{89,90}.

The progeny of HSPCs supplies our body with mature blood cells which may also constitute important feedback regulators of the HSC niche (Figure 3B). CD169\textsuperscript{+} macrophages promote HSC retention through the regulation of CXCL12 production in the bone marrow and by inducing down-regulation of HSC maintenance genes in Nes-GFP\textsuperscript{+} MSPCs\textsuperscript{91}. Granulocyte colony-stimulating factor (G-CSF) treatment leads to a loss
of monocyte and macrophage populations and functions which is associated with HSC egress to the blood\textsuperscript{92,93}. Regulatory T cells may also provide an immune privilege site that protects allogeneic HSCs in the niche after transplantation\textsuperscript{94}. Neutrophil depletion is reported to increase CAR cell population and CXCL12 levels in the bone marrow while their clearance by macrophages promoted HSPC mobilization\textsuperscript{95}. In addition, recent studies show that megakaryocytes localize specifically with HSCs promoting their quiescence through the production of CXCL4 (also known as platelet factor-4, PF4)\textsuperscript{96} and TGF-β\textsuperscript{1}\textsuperscript{97}. FGF1 production by megakaryocytes can also promote HSC expansion under stress\textsuperscript{97}. Heterogeneity among HSCs suggests that specific molecular cues inside the niche will instruct HSC fate at different levels depending on the targeted subpopulation\textsuperscript{98}.

**Niche of hematopoietic malignancies**

The importance of identifying and characterizing specific cell populations in the hematopoietic and stromal hierarchy is underscored by their involvement in myeloid neoplasms (Table 1). Interestingly, alteration of the microenvironment, for example by the deficiency in retinoic acid receptor, appears sufficient to induce a myeloproliferative syndrome\textsuperscript{99}. Simultaneous deletion of retinoblastoma protein in myeloid cells and the stromal compartment can also enhance myeloproliferative diseases\textsuperscript{100}. In addition, the deletion of Dicer1 in osteoprogenitors (using Osx-cre) but not mature osteoblasts (Osteocalcin-cre) promotes a myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), indicating that alterations in specific stromal cell populations can induce a hematopoietic malignancy\textsuperscript{101}. Overexpression of β-catenin in osteoblasts (driven by Col1-cre) can also induce MDS/AML via increased Jagged-1 expression and
Notch signaling\textsuperscript{102}. Treatment with an inhibitor of gut serotonin restores osteoblast number in leukemic mice and suppresses ALL and AML progression\textsuperscript{103}. Since osteoblasts have a high turnover, it is likely that alterations at the MSPC levels, rather than the osteoblast, are driving niche-induced hematopoietic malignancies. The expression of Osx-cre in MSPCs\textsuperscript{85} and the continuous overexpression of $\beta$-catenin in osteoblasts\textsuperscript{102}—a situation unlikely to occur naturally—are also consistent with this idea, although formal demonstration awaits. Distinct signals in the microenvironment may also differentially alter malignant transformation, as suggested by the expression of a constitutively active PTH receptor in osteoblasts which inhibits chronic myelogenous leukemia (CML)-like myeloproliferation while enhancing MLL-AF9-driven AML\textsuperscript{104}. MSPC differentiation towards the osteoblastic lineage in BCR-ABL CML results in increased mature osteoblast numbers\textsuperscript{105} in contrast to BCR-ABL CML blast crisis\textsuperscript{106}. Similar expansion of MSPCs has recently been reported for AML which destroys sympathetic nerves in bone marrow and spleen that are infiltrated with AML. The loss of adrenergic activity in the bone marrow environment leads to the proliferation of Nestin$^+$ cells primed to differentiate into the osteoblastic lineage. Blockade of the $\beta_2$-adrenergic receptor enhanced AML infiltration whereas a $\beta_2$-adrenergic agonist reduces disease activity\textsuperscript{107}. Interestingly, a similar effect of JAK2$^{V617F}$-driven myeloproliferative syndrome is observed on bone marrow innervation but the disease, by contrast, leads to the depletion of Nestin$^+$ cells and this could be rescued by $\beta_3$-adrenergic agonists\textsuperscript{108}. Further analyses are needed to dissect the influence of cancer on the bone marrow microenvironment to find the common abnormalities and the cancer type-specific abnormalities.
Concluding remarks

Many, but not all, discrepancies in the literature about the osteoblastic versus the vascular niche can be explained by unfaithful fate-mapping of “osteoblast” promoters that are in fact expressed throughout the mesenchymal lineage. Uneven recombination efficiencies of Cre recombinase among cell types and transgenic mouse strains also represent meaningful sources of variability. Further understanding the complexity of the HSC niche will be achieved by targeting specific niche cells to shed light on contributions of overlapping cell populations. Recent studies have established a major role for the vasculature in HSC maintenance and subsets of microenvironments have emerged from these studies. Feedback mechanisms and crosstalks by mature hematopoietic cells have also become known. Although the interplay between the hematopoietic and non-hematopoietic compartments is likely more complex than we can even imagine at the moment, common principles will guide the design of new approaches to tackle hematologic diseases. Hematologic malignancies represent an area that will likely to benefit from niche-targeted therapies against cancer-mediated attacks on the healthy microenvironment. For example, therapies preserving innervation, differentiation or loss of healthy niche cells in myeloid neoplasms may maintain normal HSC function while preventing the establishment of a cancer-promoting niche. New insights from HSC niches in the extremes of life (development and aging) will allow the identification of new cellular and molecular players involved in the regulation of hematopoiesis. This knowledge will one day be exploited to engineer ex vivo niches for HSC expansion, and to allow the discovery of novel pharmacological approaches for blood diseases.
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Authorship

P.E.B. and P.S.F. wrote and revised the manuscript.

Conflict of interest

The authors declare no competing financial interests.


Figure Legends

Figure 1. The adult bone marrow HSC niche. The vasculature has emerged as a key structure for the maintenance of HSCs in the bone marrow. Dormant HSCs are found around arterioles where factors such as CXCL12 and SCF secreted by perivascular, endothelial, Schwann, and sympathetic neuronal cells promote their maintenance. Less quiescent or activated HSCs are located near sinusoidal niches which are likely diverse in their influence for self-renewal, proliferation and differentiation. Hematopoietic cells such as macrophages or megakaryocytes are examples of HSC-derived progeny that can feedback to the niche to influence HSC migration or proliferation. CAR, CXCL12-abundant reticular cell; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; LepR, leptin receptor; Nes, Nestin; SCF, stem cell factor; TGFβ, transforming growth factor-beta.

Figure 2. Hierarchy of the stromal niche cells. MSPC populations are found in both bone and bone marrow compartments and contribute to the generation of mesenchymal lineage cells that generate bone, fat and cartilage tissues. The relationship between bone-derived MSPCs and marrow MSPCs remains unclear. Stromal cells are also thought to derive from MSPCs, but the nature or origin of most stromal cells is still obscure. Self-renewing mesenchymal cells and their progeny can be prospectively isolated using specific surface markers and lineage tracing approaches with transgenic labeling to decipher the origin of niche cells. Full arrows indicate that a cell population that can give rise to another while dashed lines indicate a relationship between cell populations that remains to be clarified.
Figure 3. Progenitor niches and influence of differentiated progeny. A) Microenvironment promoting progenitors. Stromal cells labeled by Prx1-cre or Osx-cre and expressing CXCL12 (CAR cells) have been shown to regulate both lymphoid progenitor maturation and myeloid progenitor retention. Depletion of CXCL12 in Osx-cre-targeted stromal cells depletes B-lymphoid progenitor cells and induces the mobilization myeloid progenitors from the bone marrow. Macrophages also constitute the central unit of the erythroblastic island allowing erythroblast to mature and generate red blood cells (RBC). B) Regulation by HSC progeny. Macrophages promote HSC retention in the bone marrow by regulating CXCL12 production from Nes-GFP⁺ perivascular cells. Macrophage-mediated clearance of neutrophils inhibits the retention signals allowing HSC mobilization. Regulatory T cells (T_{reg}) enable an immune privilege site, protecting HSCs against rejection following allogeneic transplantation. Megakaryocytes localize with HSCs and promote their quiescence.
Table 1. The HSC niche in acute and chronic myeloid neoplasms

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<td>CML</td>
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<td>BCR-ABL CML</td>
<td>↑CFU-F size</td>
<td>↑</td>
<td>-</td>
<td>NA</td>
<td>(LinCD45⁻CD31⁻Sca-1⁻)</td>
<td>NA</td>
<td>↑TPO</td>
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<tr>
<td></td>
<td>↑Osteoprecursors (CD51⁺Sca1⁻)</td>
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<td></td>
<td>↓Niche Factors</td>
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<tr>
<td>JAK2-V617F MPN</td>
<td>↓Nes-GFP⁺ cells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>↓GFAP⁺ Schwann cells</td>
<td>↑IL-1β</td>
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<td>↑IL-1R</td>
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AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm; TRAP, tartrate-resistant acid phosphatase; Th, tyrosine hydroxylase; NA, not available; CFU-OB, colony-forming unit osteoblasts; TPO, thrombopoietin; IL-1R, interleukin 1 receptor; GFAP, Glial fibrillary acidic protein.
Figure 3

A

Bone

Maturation Maintenance

CXCL12

Lymphoid progenitor

Prx1- and Osx-cre targeted CAR cell

CXCL12

Myeloid progenitor

Retention

Erythroblast

Erythroblastic island

Blood vessel

RBC

Macrophage

Osteoblast

B

Bone

Clearance

Nes-GFP+ perivascular cell

Retention

Immune privilege

Quiescence

CXCL12

Macrophage

TGF-β1

IL-10

Neutrophil

Immune privilege

Bone

Osteoblast

Megakaryocyte
Making sense of hematopoietic stem cell niches

Philip E. Boulais and Paul S. Frenette