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The bone marrow niche for haematopoietic stem cells

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Preface

Niches are local tissue microenvironments that maintain and regulate stem cells. Haematopoiesis provides a paradigm for understanding mammalian stem cells and their niches, yet the haematopoietic stem cell (HSC) niche remains incompletely defined and beset by competing models. Here we review progress in elucidating the location and cellular components of the HSC niche in the bone marrow. The niche is perivascular, created partly by mesenchymal stromal cells and endothelial cells and often, but not always, located near trabecular bone. Outstanding questions concern the cellular complexity of the niche, the role of the endosteum, and functional heterogeneity among perivascular microenvironments.

Introduction

HSC niches are present in diverse tissues throughout development beginning in the aorta-gonad-mesonephros (AGM) region and the yolk sac, followed by the placenta, fetal liver, spleen, and bone marrow[1]. Postnatally, the bone marrow is the primary site of HSC maintenance and haematopoiesis but in response to haematopoietic stress the niche can shift to extramedullary sites. Defining niche components and how they work in concert to regulate haematopoiesis offers the opportunity to improve regeneration following injury or HSC transplantation and to understand how disordered niche function may contribute to disease. In this review we focus on the nature of the HSC niche in bone marrow because that has been the subject of most of the recent research and controversies.

Historic context

Following Darwin, there was much emphasis on defining hierarchical evolutionary relationships among organisms. Morphologic similarities were used to construct ancestral trees that connected complex multicellular organisms to an original monocellular "stem celle"[2]. Lineage relationships were formulated and Ernst Haeckel proposed that cell organization in a developing organism was the recapitulation of events in the evolution of the species, with cells deriving from a "stem celle" equivalent[3]. Thirty years later, Artur

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Pappenheim proposed a less grand and more accurate formulation based on improved ability to visualize cell morphology - that cells of the blood were related to one another, with mature cell types descending from a single cell type in a “unified view of haematopoiesis”[4]. In so doing, he articulated the hypothesis of tissue stem cells. This concept took approximately half a century to define experimentally through the inspired work of Till and McCulloch who showed that single cells could indeed yield multilineage descendants while preserving the multipotency of the mother cell[5–7]. They gave substance to the idea of a stem cell and gave us methods to define the cardinal properties of those cells, self-renewal and differentiation.

Till and McCulloch based much of their work on an in vivo spleen colony-forming assay (CFU-S) now known to measure mainly multipotent progenitors rather than long-term self-renewing haematopoietic stem cells (HSCs)[8,9]. The imprecise nature of that assay contributed to Ray Schofield’s formulation of the niche hypothesis in 1978. Recognizing that the putative CFU-S stem cells were less robust than cells of the bone marrow at reconstituting haematopoiesis in irradiated animals, he proposed that a specialized bone marrow niche preserved the reconstituting ability of stem cells[10]. His colleagues at the University of Manchester concurrently sought to define what made bone marrow a nurturing context for HSCs and Michael Dexter showed that largely mesenchymal ‘stromal’ cell cultures could maintain primitive haematopoietic cells ex vivo [11]. Further, Brian Lord progressively reamed long bone marrow cavities and showed that primitive cells tended to localize toward the endosteal margins, leading to the hypothesis that bone might regulate haematopoiesis (Fig. 1) [12].

These early studies were followed by in vitro evidence that osteoblasts differentiated in culture from human marrow stromal cells could produce haematopoietic cytokines and support primitive haematopoietic cells in culture [13]. This fostered the idea that bone cells might create the HSC niche but it was essential to move to engineered mouse strains to test the hypothesis in vivo. Two studies followed, including a mouse model in which a promoter restricted in activity to osteoblastic cells was used to drive expression of a constitutively active parathyroid hormone receptor [14]. Along similar lines, Linheng Li’s laboratory used a promoter since shown to be restricted in bone marrow stroma to primitive and mature osteolineage cells[15] to delete the *BMPr1a* gene[16]. In both models, the number of endosteal osteoblasts and the number of primitive haematopoietic cells (scored as stem cells given the measures in use at the time) increased. These data provided the first evidence of specific heterologous cells regulating mammalian stem cells in vivo, though it remained unclear whether the regulation was direct or indirect. This demonstrated that the niche was experimentally tractable, prompting a series of studies that have since refined our understanding of the complexity of the bone marrow microenvironment.

Studies of the niche have now more precisely determined the components that regulate HSCs, and to some extent other haematopoietic progenitors, in the bone marrow. Like any interactive system there are complex regulatory relationships among cells in the bone marrow. A perturbation in one cell type that leads to an effect in another cell type does not necessarily require the interaction between the cells to be direct. The data now suggest that the early studies that observed effects on HSC frequency as a consequence of genetic

manipulation in osteoblastic cells reflected indirect effects rather than the existence of an osteoblastic niche. Indeed, expression of constitutively active parathyroid hormone receptor in osteoblasts [14] likely causes widespread changes in many cell types the bone marrow, including in the vasculature. Current data suggest there are specialized niches for distinct types of haematopoietic stem and progenitor cells and that each niche may be created by multiple cell types that contribute to the niches in unique as well as redundant ways[17]. Indeed, there is heterogeneity among HSCs themselves[18–20], raising the possibility of cellularly distinct niches for distinct subpopulations of HSCs. This review will focus on the current data and the unanswered questions.

Mapping the marrow space

A niche is defined by anatomy and function[21] - a local tissue microenvironment that directly maintains and regulates a particular kind of stem cell or progenitor [22]. Determining what cells neighbor HSCs and regulate HSC maintenance has been complicated by the difficulty in retaining histologic integrity when sectioning bone as well as the complexity of immunostaining methods necessary to identify HSCs.

The identification of markers that reliably identify HSCs in vivo was an important step in defining the niche [22]. Despite the ability to isolate HSCs by flow cytometry for decades [23], the identification of HSCs within tissues remained a challenge because the combination of immunofluorescent markers used to isolate HSCs by flow cytometry was too complex for microscopy. Consequently, markers of poor specificity were often used. For example, “HSCs” have been localized in the bone marrow using BrdU or H2B-GFP label retention as a marker [24,25]. Although there is a subset of HSCs that preferentially retains H2B-GFP and BrdU, these markers by themselves have very poor specificity - the vast majority of bone marrow cells that retain these labels are not HSCs [18,19,26].

By combining positive staining for CD150 and negative staining for CD48 and CD41, HSCs could finally be highly purified using a simple two-color stain [27]. All serially transplantable HSCs in young adult mice are contained within the $CD150^{+}CD48^{-}CD41^{-/low}$ population of bone marrow cells, including the most quiescent HSCs[26–29]. This made it possible to localize HSCs in sections through haematopoietic tissues using markers validated to give high purity. Most $CD150^{+}CD48^{-}CD41^{-}Lineage^{-}$ cells in the bone marrow and spleen localize adjacent to sinusoid vessels and nearly all are within 5 cell diameters of a sinusoid (Fig. 2)[27,30]. Indeed, HSCs are five times more likely than other haematopoietic cells to be immediately adjacent to a sinusoid [30]. HSCs are distributed throughout the bone marrow, with less than 20% within 10μm of the endosteum[27,30–32]. Nonetheless, most HSCs are found in the trabecular region of bone marrow, suggesting that HSCs, or their niche, may be directly or indirectly regulated by factors present near bone surfaces.

The frequent localization of HSCs adjacent to blood vessels suggested that HSCs might be maintained in a perivascular niche by endothelial or perivascular cells[27,33]. Yet, HSC are mobile, regularly entering and exiting the circulation [34]. This raised the possibility that the cells observed near vessels were in transit, perhaps delayed in entering or exiting the

circulation by the process of migrating through vascular barriers. This issue could not be resolved by histologic analysis that captures a single moment in time.

The sequential imaging of mice with high resolution assessed the three dimensional position of cells in the calvarium over time [32,35]. These studies indicated that primitive haematopoietic cells trafficked to specific microdomains of marrow blood vessels where CXCL12 and E-selectin were abundant, then remained in these positions for weeks, generating new cells as indicated by partitioning of a cytosolic dye. When HSCs were visualized after transplantation into irradiated mice they preferentially localized near the endosteum, consistent with that region being particularly relevant for HSC maintenance [32,36]. However, it was subsequently learned that irradiation disrupts sinusoids in the bone marrow [37] raising the possibility that the only blood vessels preserved after irradiation are the arteriolar vessels near the endosteum. Therefore, the peri-endosteal localization of HSCs in these experiments may have reflected, in part, the destruction of sinusoidal niches by irradiation. Overall, the localization data emphasized the possibility of a perivascular niche. How could this be resolved with the historical data suggesting that the endosteum and osteoblasts were niche participants?

Osteoblasts: more harbinger than host

While osteoblastic cells were the first cell population shown to influence haematopoietic stem/progenitor cell frequency when perturbed in vivo [14,16], several lines of evidence raised concerns that the effect may not be direct. First, in vivo imaging studies using validated markers or labeled stem cells found few HSCs in contact with osteoblastic cells [27,33,38,31,32]. Second, studies that depleted osteoblasts by *biglycan* deficiency[30] or treatment with diphtheria toxin [39,40](Scadden unpublished) or that increased osteoblasts by strontium treatment[41] had no acute effect on HSC frequency. The studies in which osteoblasts were conditionally deleted by diphtheria toxin were particularly compelling as they showed acute depletion of lymphoid progenitors but not HSCs[39,40](Scadden unpublished). Third, genetic modification of primitive osteolineage cells had an effect on HSC proliferation and differentiation, but the same modification in mature osteoblasts did not[42]. Finally, a key adhesion molecule thought to mediate osteoblast-HSC interaction, N-cadherin, was called into question.

N-cadherin+ HSCs were proposed to adhere to N-cadherin+ osteoblasts by homophilic adhesion[16,36], promoting HSC maintenance[43–45]; however, these studies did not test whether *N-cadherin* deletion affected HSC function. The levels of N-cadherin staining in HSCs were difficult to distinguish from background fluorescence and depended upon anti-N-cadherin antibodies that gave non-specific staining in some haematopoietic cells[46]. Other studies failed to detect N-cadherin expression by HSCs using gene expression profiling [27,47](https://gexc.stanford.edu/model/3/gene/Cdh2)[48] quantitative RT-PCR, flow cytometry with multiple anti-N-cadherin antibodies, western blot, or *N-cadherin:LacZ* genetrap mice [18,28,30,38]. Conditional deletion of *N-cadherin* from HSCs or from osteoblast lineage cells had no effect on HSC frequency, HSC function, or haematopoiesis [38,49,50]. Collectively, these data undermined the notion of an N-cadherin+ ‘osteoblastic’ niche.

Is there any role for osteoblasts or osteolineage cells in HSC regulation? Several lines of evidence suggest that this possibility remains viable but not as initially envisioned. First, higher numbers of HSCs reside in the trabecular rich metaphysis[31,51]. This may simply reflect other components of bone marrow co-localizing with bony surfaces; however, conditional deletion of *osterix* results in chondrocytes without osteoblastic differentiation, increasing blood vessels and mesenchymal progenitors in the bone marrow but virtually eliminating haematopoiesis in the metaphysis[52]. These data argue that the presence of mature or maturing osteolineage cells in regions with abundant endosteum is critical for haematopoiesis. Indeed, mesenchymal progenitors capable of forming bone are sufficient to create bony ossicles that become invested by host vasculature and HSCs [53,54]. This suggests that bone or bone-forming progenitors can promote the formation or maintenance of HSC niches (for example by recruiting vasculature to the bone marrow) even if they do not directly promote HSC maintenance.

Transplanted haematopoietic stem/progenitor cells preferentially localize to blood vessels in endosteal regions even without prior cytotoxic conditioning [55]. Within the endosteal region, transplanted HSCs position themselves closer to the endosteal surface than progenitor cells [32]. These may again reflect indirect effects of bone forming osteolineage cells as bone turnover results in high local concentrations of ionic calcium and the calcium sensing receptor promotes bone marrow engraftment by HSCs during development or after transplantation [56]. Osteolineage cells also elaborate cytokines and extracellular matrix proteins that may influence a wide range of cell types, some of which may directly regulate HSC function. This is exemplified by Parathyroid Hormone Receptor (PTHr) activation, which induces expression of multiple regulatory molecules by osteoblasts (such as IL-6, RANKL, and Jagged1) that can influence other cells in the bone marrow, including the vasculature [14,57]. Also, osteoblastic expression of transgenes encoding the Wnt antagonists, Dkk1 and Wif1, depletes HSCs[58,59]. Finally, the depletion of osteocalcin expressing cells (osteoblasts or osteocytes) in vivo resulted in an inability to mobilize at least short-term repopulating cells to the blood using G-CSF[60,61] despite osteoblasts having little expression of CXCL12[17,33,62], a key molecule that regulates HSC mobilization. In aggregate, these data indicate that the endosteal region is important for haematopoiesis, but the mature osteolineage cells likely have an indirect role in modulating HSCs. Rather, these cells appear to be more important in directly regulating restricted progenitors, a topic discussed below.

It is important to therefore re-focus attention on the endosteum as a regulatory region and not on the osteoblasts themselves (Fig. 3). The endosteum has a diverse group of cells and anatomic elements including a rich endowment of arteriolar and sinusoidal blood vessels (Fig. 1)[31,32]. The cells include endothelial cells as well as mesenchymal cells with osteolineage potential. These mesenchymal cells reside perivascularly but traffic to the endosteal surface to differentiate to osteoblasts. Undifferentiated mesenchymal cells around blood vessels may promote HSC maintenance throughout the bone marrow but the mesenchymal cells around vessels in the endosteal region may differ from those distant from endosteal surfaces.

Perivascular regulators of HSCs

Given the localization of HSCs near blood vessels, it was critical to define the stromal cells surrounding the vessels and to test whether they promote HSC maintenance. Attention focused on the mesenchymal cells that surround blood vessels throughout the bone marrow. While mesenchymal stroma are likely to be heterogeneous, and the precise relationships between cells expressing various markers remain to be defined, perivascular mesenchymal cells that express CD146 in humans[54] and *Cxcl12*-GFP[33], *Nestin*-GFP[63], full length Leptin receptor[64], *Prx-1*-Cre[62], Osterix-Cre[62], and inducible *Mx-1*-Cre[15] in mice all generate osteoblastic cells and all express factors that promote HSC maintenance. CXCL12-abundant 'reticular' (CAR) cells adjacent to sinusoids were first shown to co-localize with HSCs throughout the bone marrow [33]. Ablation of *Cxcl12*-expressing bone marrow cells depletes HSCs as well as well as severely impairing the adipogenic and osteogenic capacity of bone marrow cells[65]. Human CD146+ skeletal stem cells also localize adjacent to sinusoids in the bone marrow and synthesize high levels of the HSC niche factors Stem Cell Factor (SCF) and CXCL12[54].

This possibility that mesenchymal stem/stromal cells (MSCs) are part of the HSC niche was further supported by Frenette and colleagues who found that MSCs in the bone marrow express a *Nestin*-GFP transgene and localize around blood vessels throughout the bone marrow[63]. HSCs commonly localize adjacent to *Nestin*-GFP+ cells and the *Nestin*-GFP+ cells express high levels of *Scf* and *Cxcl12*. Moreover, Fibroblast Activation Protein (FAP) is expressed by bone marrow stromal cells with many characteristics of MSCs, including *Cxcl12*, *Scf*, PDGFRα and Sca-1 expression [66,67], and ablation of these FAP+ cells leads to bone marrow hypocellularity, anemia, and depletion of osteogenic cells [68,69]. These studies provided strong evidence that MSCs are one component of a perivascular niche for HSCs.

Endothelial cells also contribute to the perivascular HSC niche[27]. The earliest functional evidence supporting this possibility was the observation that conditional deletion of the *gp130* cytokine receptor in endothelial cells led to bone marrow hypocellularity and a reduction in HSC numbers[70]. Inhibition of VEGFR2 signaling in irradiated mice using a blocking antibody impaired the regeneration of sinusoidal endothelial cells and prevented the recovery of LSK stem/progenitor cells as well as spleen colony-forming cells (CFU-S)[37]. Endothelial cells can promote HSC maintenance in culture[71] and bone marrow sinusoidal endothelial cells promote long-term reconstituting HSC expansion in culture[72,73]. E-selectin has been suggested to be exclusively expressed by endothelial cells in the bone marrow and *E-selectin* deficiency renders HSCs more quiescent and resistant to irradiation[74]. These studies suggested that endothelial cells are one component of the HSC niche, but did not address whether they directly or indirectly regulate HSC maintenance in vivo.

To formally identify the niche cells, studies examined which cell populations were the key sources of factors that promote HSC maintenance in vivo. For example, SCF is non-cell-autonomously required for HSC maintenance in vivo [75–79]. Differential splicing and proteolytic cleavage yield membrane-bound and soluble forms of SCF. HSCs are depleted in *Sl/Sl*^{d} mutant mice[80], which express soluble SCF but not the membrane-bound form,

indicating that the membrane-bound form is necessary for HSC maintenance[81]. Importantly, mice with a mixture of wild-type and *Sl/Sl*^{d} stromal cells only exhibit normal haematopoiesis in the immediate vicinity of the wild-type cells, demonstrating that SCF acts locally in creating the niche[82]. Since HSCs need cell-cell contact with the cells that synthesize SCF, the niche could be localized by identifying the key sources of SCF for HSC maintenance.

Analysis of the SCF expression pattern in *Scf*^{gfp} knock-in mice revealed that *Scf* is expressed perivascularly, primarily around sinusoids throughout the bone marrow[64]. Leptin Receptor+ (Lepr) perivascular stromal cells expressed the highest levels of *Scf* and endothelial cells expressed lower levels. Gene expression profiling suggested that these Lepr-expressing perivascular cells were mesenchymal. *Scf*-GFP expression could not be detected in osteoblasts or in haematopoietic cells. Conditional deletion of *Scf* from perivascular stromal cells (*Lepr*-Cre), or endothelial cells (*Tie2*-Cre) depleted HSCs[64]. However, deletion of *Scf* from haematopoietic cells (*Vav1*-Cre), osteoblastic cells (*Col2.3*-Cre), and Nestin-expressing perivascular stromal cells (*Nestin*-Cre and *Nestin*-CreER) did not affect HSC frequency[64]. These results proved there is a perivascular niche for HSCs in which endothelial cells and mesenchymal cells promote HSC maintenance by synthesizing SCF (Fig. 3).

It has been proposed that the endosteal region and its osteoblastic cells provide a unique zone for the maintenance of quiescent HSCs. However, when *Scf* was conditionally deleted from both endothelial cells and perivascular mesenchymal cells in *Lepr-Cre; Tie2-Cre; Scf*$^{fl/-}$ mice, 85% of all long-term multilineage reconstituting cells, including all serially transplantable HSCs and all HSCs in the most quiescent subpopulation, were eliminated[20]. Therefore, even the most primitive and quiescent HSCs are maintained by a perivascular niche. Whether there are functionally distinct perivascular niches in different regions of the bone marrow, such as in the endosteal region, remains an open question.

Are other key niche factors also synthesized primarily by perivascular cells? CXCL12 is a chemokine that is required for HSC maintenance and HSC retention in the bone marrow[33,83–86]. Global deletion of *Cxcl12*, or the gene that encodes its receptor, *Cxcr4*, depletes HSCs from the bone marrow[33,83,87]. CXCL12 is primarily expressed by perivascular mesenchymal stromal cells (CAR cells, *Nestin*-GFP, *Lepr*-Cre, or *Prx-1*-Cre expressing cells), with 100-fold lower levels of expression in endothelial cells and 1000-fold lower levels in osteoblasts[17,33,62,88,89]. Conditional deletion of *Cxcl12* from perivascular mesenchymal cells using *Prx1*-Cre and *Lepr*-Cre depleted and mobilized HSCs, respectively [17,62]. HSCs were depleted but not mobilized when *Cxcl12* was conditionally deleted from endothelial cells (*Tie2*-Cre)[17,62]. HSC frequency and bone marrow retention were not affected when *Cxcl12* was conditionally deleted from osteoblasts or their progenitors (*Col2.3*-Cre and *Osx*-Cre), haematopoietic cells (Vav1-Cre), or *Nestin*-Cre-expressing stromal cells[17,62]. These data confirmed that HSCs reside in a perivascular niche in which mesenchymal stromal cells and endothelial cells each synthesize multiple factors that promote HSC maintenance and localization.

While conditional deletion of *Scf* and *Cxcl12* with *Nestin*-Cre and *Nestin*-CreER did not have any effect on HSC frequency[17,64], *Nestin*-GFP+ perivascular cells are almost certainly part of the HSC niche[63]. Each of these Nestin alleles are transgenes with different expression patterns in the bone marrow[64]. *Nestin*-Cre appears not to be expressed in the bone marrow and *Nestin*-CreER exhibits very limited perivascular expression that does not resemble the *Scf*-GFP, *Cxcl12*-DsRed, *Nestin*-GFP, or *Nestin*-Cherry expression patterns[64]. However, *Nestin*-GFP expression strongly overlaps with *LepR*-Cre expression by perivascular cells throughout the bone marrow[64,67,90]. Thus, it is likely that *Nestin*-GFP+ perivascular MSCs are a component of the HSC niche even though *Nestin*-Cre mediated deletion of *Scf* or *Cxcl12* did not deplete HSCs. Going forward, it will be useful to identify other Cre alleles that are specifically expressed in *Nestin*-GFP+ cells to compare their function to other perivascular stromal cells.

Complexity of the perivascular HSC niche

Endothelial cells and mesenchymal stromal cells are not the only cell types that regulate the perivascular HSC niche (Fig. 3). The sympathetic nervous system regulates CXCL12 expression and HSC retention in the bone marrow [91,92]. It appears that this is accomplished by sympathetic nerve fibers that synapse upon perivascular cells around a subset of blood vessels in the bone marrow, conferring circadian regulation of CXCL12 expression and HSC mobilization. Circadian oscillation in the clearance of aged neutrophils by macrophages in the bone marrow also contributes to these circadian changes in CXCL12 expression and HSC circulation[93]. Consistent with this, macrophages modulate CXCL12 expression by *Nestin*-GFP+ cells and HSC retention in the bone marrow [94,95]. Non-myelinating Schwann cells appear to regulate the niche by regulating TGFβ activation and potentially by secreting other factors [96]. Osteoclasts, or osteoclast activity at the endosteum may also influence HSC maintenance and bone marrow retention [56,97,98]. Many different cell types are likely to directly or indirectly regulate the perivascular HSC niche.

Given the complexity of cell types implicated in the regulation of HSCs, there is no singular niche cell. Rather, the niche integrates the function of multiple participants. It is important to bear in mind that niche composition and niche function may change under different physiological conditions or in response to stress. It is also important to note that many of the Cre recombinase alleles used so far to study niche cells were active during development. While this was necessary to achieve efficient gene deletion (temporally regulated CreER alleles tend to give much lower levels of recombination) and no abnormalities in development were noted, indirect effects on surrounding cell types and compensatory changes cannot be excluded. Although endothelial cells and perivascular mesenchymal cells express SCF and CXCL12, conditional deletion of these factors from these cell types may have direct and indirect effects on HSCs.

There may also be long-range signals circulating through the blood that regulate HSC/niche function, perhaps integrating stem cell activity with overall physiology[99]. These may include hormones that signal reproductive or nutritional status, or even haematopoietic cytokines. For example, Thrombopoietin is required for HSC maintenance [100–103]. The major sites of Thrombopoietin synthesis are in the liver and kidney, though it is also

synthesized at lower levels by bone marrow stroma[104,105]. Conditional deletion experiments will be required to determine the physiologically important source(s) of Thrombopoietin for HSC maintenance.

There may also be functionally distinct perivascular environments in the bone marrow based on vessel type. Most studies of perivascular niches in the bone marrow have focused on sinusoids because they are the most abundant blood vessels in the bone marrow and most HSCs, MSCs, *Scf*-expressing cells, and *Cxcl12*-expressing cells are in close proximity to them [17,27,33,54,64]. However, other kinds of blood vessels, such as arterioles, may play an important role in HSC maintenance. Indeed, a recent study suggests that NG2+ but Lepr negative mesenchymal cells that surround arterioles in the bone marrow are important for the maintenance of quiescent HSCs[106]. This conclusion was based on the observation that HSCs were depleted and driven into cycle when *NG2*-CreER+ cells were ablated by treatment with diphtheria toxin. However, these data would appear to conflict with the prior observation that quiescent HSCs are eliminated from the bone marrow when *Scf* is conditionally deleted using *Tie2*-Cre and *Lepr*-Cre, which recombine in endothelial cells and mesenchymal cells that are primarily around sinusoids throughout the bone marrow [20]. It will thus be interesting to determine whether HSCs are depleted when *Scf* is conditionally deleted using *NG2*-CreER or whether *NG2*-CreER is expressed by cells other than periarteriolar cells in the bone marrow. Similarly, it will be important to assess whether *Lepr*-expressing perivascular cells contribute to arteriolar niches in the bone marrow. In the end, perivascular niches associated with both sinusoids and arterioles may regulate HSC maintenance and quiescence in the bone marrow. Dissecting the diversity in perivascular environments will require Cre alleles that are specifically expressed within distinct perivascular domains to map their functions.

Evidence has been presented that HSCs reside within relatively hypoxic domains within the bone marrow [31,107]. This has been based partly on staining with pimonidazole[31].. Pimonidazole stained HSCs often reside adjacent to sinusoids in the bone marrow and are found next to cells that do not stain with pimonidazole[31]. This suggests that pimonidazole staining does not reflect ambient oxygen or that it is cell-autonomously determined, rather than reflecting a hypoxic environment. Pimonidazole responds to reducing intermediates and may reflect more about the metabolic state of cells than ambient oxygen levels.

The dependence of HSC maintenance upon HIF-1α has also been interpreted to suggest that HSCs are maintained in a hypoxic niche[108]. However, a number of factors other than hypoxia regulate HIF-1α expression. A recent imaging study using a nanoprobe specifically reflective of ambient oxygen found that oxygen tension was lowest around sinusoids and highest near the endosteum (personal communication, Charles Lin). The entire marrow space was much reduced in oxygen compared with vessels entering the marrow, a feature largely lost when haematopoiesis was ablated by cytotoxic drugs. It is therefore likely that consumption of oxygen during haematopoiesis renders the marrow hypoxic but that no distinct hypoxic region exists at the endosteum.

Distinct haematopoietic progenitors have distinct niches

HSCs reside within a specialized niche that is distinct from the niches that nurture other haematopoietic progenitors. For example, while osteoblasts do not directly regulate HSC maintenance, they do regulate some B-lineage progenitors. Cultures enriched for osteoblasts support B lymphopoiesis and ablation of osteoblasts in adult mice acutely depletes common lymphoid progenitors (CLPs) [40,109](Scadden, unpublished data). Deletion of *Gs-alpha* in osteoblastic cells, which is necessary for PTHr signaling, markedly depleted pro- and pre-B cells in a manner that could be rescued with IL-7[110]. Approximately 30% of IL7R+Lineage- bone marrow cells, which are enriched for early lymphoid progenitors, localize immediately adjacent to bone lining cells at the endosteum[17]. Conditional deletion of CXCL12, a factor that promotes the proliferation and maintenance of B lineage progenitors[84,111] and CLPs[112], in osteoblastic cells depleted CLPs and certain other early lymphoid progenitors from the bone marrow without any effect on HSCs[17]. Therefore, some early lymphoid progenitors depend upon an osteoblastic niche that is cellularly and functionally distinct from the perivascular niche that maintains HSCs.

Other lineage-restricted niches may exist as well. For example, macrophages appear to be critical for erythroid maturation and macrophage depletion reduces normal and malignant erythropoiesis[113]. Other cellular components of the erythropoiesis niche will have to be identified to understand the relationship between this niche and HSC and lymphoid progenitor niches.

The approach of conditionally deleting specific niche factors from candidate niche cells and then examining the consequences for stem/progenitor cell maintenance in vivo offers the opportunity to map the niches for each stem cell and restricted progenitor in the haematopoietic system, limited only by the precision of the Cre alleles that are available.

Novel niche factors

In contrast to stem cells in a number of other tissues, HSCs cannot be sustainably expanded in culture. This has impeded the ability to safely and effectively transplant HSCs in certain clinical contexts, such as during gene therapy, in which it would be useful to expand transfected HSCs in culture and then verify the quality of the transfected HSCs prior to transplantation. One possibility is that the inability to expand HSCs in culture reflects the existence of yet-unidentified growth factors that are synthesized by the niche in vivo.

Some HSC niche factors have only recently been discovered. Addition of Pleiotrophin to culture promotes HSC maintenance [114] and *Pleiotrophin* deficiency is associated with HSC depletion and impaired haematopoietic regeneration after myelosuppression [115]. Pleiotrophin is synthesized by sinusoidal endothelial cells and *Cxcl12*-expressing perivascular stromal cells and acts non-cell-autonomously to promote HSC function[115]. Robo4, a Slit receptor expressed by HSCs and endothelial cells, regulates HSC localization in the bone marrow [116,117]. The *Slit2* ligand is restricted to MSCs and possibly other osteoblast lineage cells. This suggests that Pleiotrophin and Robo4/Slit2 are important elements of the perivascular niche. Tenascin-C[118], osteopontin[119,120], and non-canonical Wnts[25] have also been reported to positively or negatively influence HSC numbers in the

bone marrow and are among a number of factors that bear further characterization in terms of cellular source or role with respect to the niche.

Perspective

Ten years of experimentation has validated the niche concept and resolved some first order questions about the molecular and cellular nature of the HSC niche in the bone marrow. The 'parts' list remains incomplete, but with the pace of current work it is likely that additional components will be defined and ambiguity about overlapping cell populations resolved over the next several years. This will make it possible to compare anatomically and developmentally distinct HSC niches that have different functions. HSCs expand in number daily within the fetal liver but are sustained at nearly constant levels in the bone marrow, at least in the absence of injury. How components of these niches compare may inform methods for achieving HSC expansion. Similarly, comparing homologous niches among species, such as long-lived humans versus short-lived mice, may provide insight into mechanisms for preserving the integrity of haematopoiesis under stress or in response to aging. Finally, comparing niches among tissues will assess whether the mesenchymal and endothelial populations in brain, gut, and skin share characteristics and functions with those defined in the bone marrow. Do diverse adult tissues consistently have perivascular niches for stem cell maintenance? Do regenerative tissues have niches with common mechanisms for preserving self-renewal? Are there common components that can be engineered into niches ex vivo?

With the detail now emerging in our understanding of the bone marrow niche, a number of second order questions can be addressed. Niche cells can increasingly be genetically tagged or modified, enabling both quantification and molecular manipulation. Coupled with high resolution real-time imaging and well-validated methods to measure haematopoiesis, it is becoming possible to systematically elucidate how the niche responds to stresses or physiological changes to mediate changes at the stem cell and tissue levels. When stressed by infection, myeloablation, or neoplasia, what niche components change in number or function to modify haematopoiesis? Is there a hierarchy of niche components that determine these responses? Can such information enable predictive algorithms that guide specific interventions to achieve desired outcomes?

Another set of questions concerns the manner in which the niche participates in diseases of stem cell failure, such as aplastic anemia or neoplasia. The niche may be hostile to normal progenitors in those disease states and, with neoplasia, undergo a facultative response to support altered haematopoiesis[121]. Can changes in the niche be a primary but non-cell-autonomous driver of neoplasia in humans as has been suggested by animal models[42,122,123]? The potential for unraveling how the microenvironment participates in normal and disease physiology is at hand and promises new approaches to haematologic disorders.

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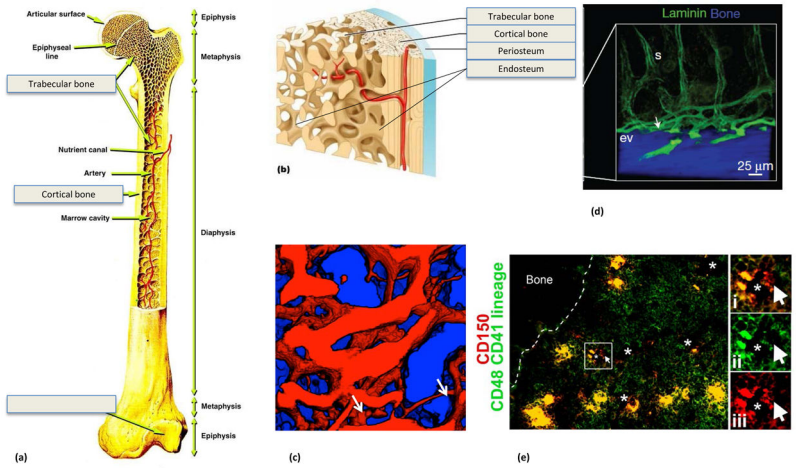

Figure 1. Bone marrow anatomy

Haematopoietic stem cells (HSCs) reside primarily within bone marrow during adulthood. Bone marrow is a complex organ containing many different haematopoietic and non-haematopoietic cell types. Bone marrow is surrounded by a shell of vascularized and innervated bone. a. Minute projections of bone (trabeculae) are found throughout the metaphysis such that many cells in this region are close to bone surface. b. The interface of bone and bone marrow is known as the endosteum, which is covered by bone-lining cells that include bone-forming osteoblasts and bone-resorbing osteoclasts. Arteries carry oxygen, nutrients, and growth factors into the bone marrow, before feeding into sinusoids, which coalesce as a central sinus to form the venous circulation. Sinusoids are specialized venuoles that form a reticular network of fenestrated vessels that allow cells to pass in and out of circulation. There is a particularly rich supply of arterioles as well as sinusoids near the endosteum. c. 3-D reconstructed photomicrograph from the marrow looking toward the endosteal surface (blue) from 50 μm below the surface, revealing the rich network of vessels (red) (courtesy of Charles Lin, Joel Spencer and Juwell Wu). Smaller arteriolar vessels (white arrows) become larger sinusoidal vessels. The field of view is 350μm × 350μm. d. A cross-sectional view of blood vessels that run along the endosteal surface (ev) and that transition (white arrow) into sinusoids (s) that then course toward the central sinus (from ref[31]). e. The bone marrow is cellularly complex with $CD150^{+}CD48^{-}CD41^{-}Lineage^{-}$ HSCs (arrow) residing in close contact with not only vascular and perivascular cells (*, sinusoid lumens) but also megakaryocytes (large yellow cells) and other haematopoietic cells (image from ref[124]).

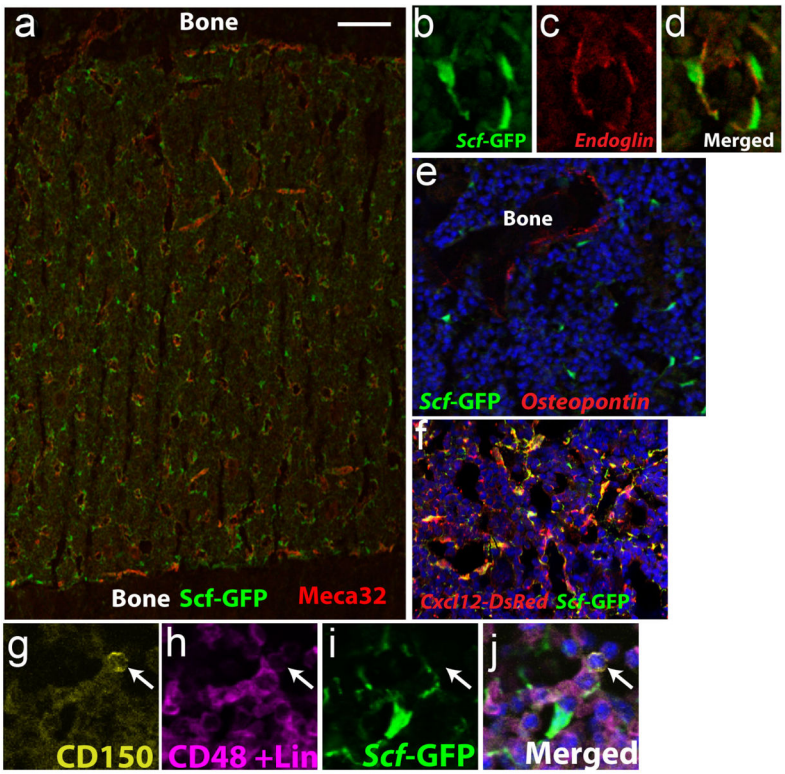

Figure 2. HSCs and their niche cells surround sinusoids throughout the bone marrow

a. Sections through the bone marrow of *Scf*$^{gfp/+}$ mice show that HSC niche cells (green) include mesenchymal stromal cells and endothelial cells that surround sinusoids and potentially other blood vessels throughout the bone marrow[64]. b–d. High magnification shows that *Scf*-GFP overlaps with the endothelial marker endoglin but also extends beyond the endoglin on the abluminal side of the sinusoids, indicating expression by mesenchymal stromal cells. e. *Scf*-GFP is not expressed by osteopontin+ bone lining cells around trabecular bone, but is expressed by some nearby perivascular cells. f. *Cxcl12*-DsRed exhibits a similar expression pattern, primarily by perivascular mesenchymal cells and endothelial cells around sinusoids throughout the bone marrow, in a pattern that strongly overlaps with *Scf*-GFP in Cxcl12$^{DsRed/+}$; Scf$^{gfp/+}$ mice[17]. g–j, Cells that are CD150+ (g) and CD48 and Lineage marker negative (h) are usually found immediately adjacent to *Scf*-GFP+ perivascular cells (i) in the bone marrow (see j for merge). Images are from references[17,64].

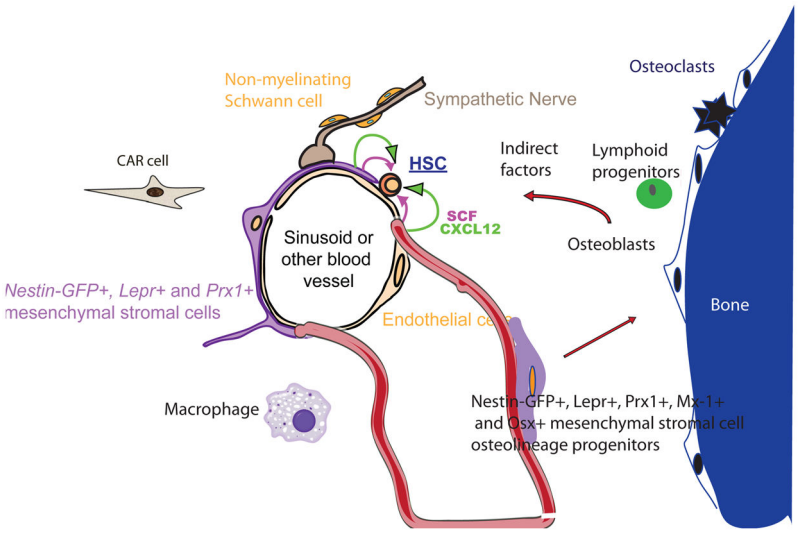

Figure 3. HSCs and restricted haematopoietic progenitors occupy distinct niches in the bone marrow

a. HSCs are found mainly adjacent to sinusoids throughout the bone marrow[27,30,31,33], where endothelial cells and mesenchymal stromal cells promote HSC maintenance by producing SCF[64], CXCL12[17,33,62], and likely other factors. Similar cells may also promote HSC maintenance around other types of blood vessels, such as arterioles. The mesenchymal stromal cells can be identified based on their expression of *Lepr*-Cre[64], *Prx1*-Cre[62], *Cxcl12*-GFP[33], or *Nestin*-GFP transgene[63] in mice and similar cells are likely to be identified by CD146 expression in humans[54]. These perivascular stromal cells, which likely include Cxcl12-abundant Reticular (CAR) cells[33], are fated to form bone in vivo, express Mx-1-Cre and overlap with CD45/Ter119^{-}PDGFRα $^{+}$Sca-1^{+} stromal cells that are highly enriched for MSCs in culture[66]. b. It is likely that other cells also contribute to this niche, likely including cells near bone surfaces in trabecular rich areas. Other cell types that regulate HSC niches include sympathetic nerves[91,92], non-myelinating Schwann cells (which are also Nestin+)[96], macrophages[95], osteoclasts[97], extracellular matrix [119,120], and calcium[56]. Osteoblasts do not directly promote HSC maintenance but do promote the maintenance and perhaps the differentiation of certain lymphoid progenitors by secreting Cxcl12 and likely other factors[13,17,39,40]. Early lineage committed progenitors thus reside in an endosteal niche that is spatially and cellularly distinct from HSCs.