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PERSPECTIVES

Regulatory Interactions in the Bone Marrow Microenvironment

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Abstract

Hematopoietic stem cells (HSCs) are the immature, pluripotent cells from which all myeloid and lymphoid cell types originate. As stem cells, HSCs are capable of two very different fate choices: self-renewal, ensuring they will persist throughout the lifetime of an organism, and differentiation to mature progeny. Therapeutic applications of HSCs include their routine use in stem cell transplantation to treat hematopoietic malignancies or bone marrow failure. Research and clinical experience have provided tools for the immunophenotypic identification and functional analysis of HSCs and there is increasing evidence suggesting that HSC regulation is greatly influenced by signals from their niches in the bone marrow. Although they represent one of the most rigorously studied stem cell types, still more remains to be known about how HSCs are regulated and respond to stress conditions. *IBMS BoneKEy*. 2011 February;8(2):96-111.

Introduction

The study of stem cell biology experienced a paradigm shift with the 1978 work of Schofield, who presented the idea of hematopoietic stem cells (HSCs) in close regulatory association with their environment. This was in opposition to the previously held belief that HSCs merely passed through transient environments as circulating cells. To account for variations in HSC response to different cytotoxic treatments and to explain the limited HSC activity he observed following certain transplantation scenarios, Schofield reasoned that HSC fate decisions were not solely cell-autonomous in nature. He proposed that HSCs were in fact fixed-tissue cells, with behaviors and responses that were, at least in part, dictated by their local environment (1). Studies using *Drosophila melanogaster* later provided *in vivo* proof of this hypothesis, demonstrating the existence of niches in the *Drosophila* ovary and testis to which germ-line stem cells are anchored and from which they receive regulatory cues (2;3).

Research conducted over the past decade has contributed greatly to our understanding of the structural, cellular and molecular aspects of the HSC niche. Two microenvironments are usually discussed when describing HSC niches in the bone marrow (BM): the endosteum or bone surface and the vasculature, though it is important to keep in mind the close physical proximity of these structures within the microanatomy of the bone. In this *Perspective* we begin by discussing these two microenvironments' diverse cellular constituents, structural attributes and responses to external stimuli including evidence for their distinct HSC regulatory functions. Then we briefly review some of the key soluble and cell-bound factors specific to one or shared by both of these two microenvironments. Finally, we conclude with examples of niche components capable of participating in hematopoietic malignancy and bone metastasis.

Principal Cellular Components of the HSC Niche

Endosteum

With the knowledge that normal adult hematopoiesis in mammals resides in bone, early efforts to characterize the HSC niche focused on cells found at the endosteum – cells of the osteoblastic lineage. *In vitro* experiments using human osteoblasts (OBs) revealed HSC-supportive properties, such as the secretion of the hematopoietic growth factors granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and leukemia inhibitory factor, and the ability to sustain and expand hematopoietic stem and progenitor cells (HSPCs) 2-4-fold (4-8). In an *in vivo* setting, El-Badri *et al.* found that when OBs were transplanted with HSCs, donor engraftment improved (9). Subsequent reports have shown that osteoblastic cells can affect numerous aspects of HSC biology, including quiescence (10-12), mobilization (13), and even the expansion or restriction of HSC numbers (14;15). Osteoblastic associations with other cell types can also modify HSC behavior. OB-derived RANKL activates osteoclastic bone resorption, which has been shown to impact HSC mobilization (16). Hematopoietic impairment seen in graft-versus-host disease was recently found to occur through targeted destruction of OB niche cells by donor T cells (17). Several studies have also indicated that osteoblastic cells translate systemic cues directly to HSCs. Examples of such a function include the emerging role of the sympathetic nervous system in modulating OB activity and signaling to control HSCs, as occurs in G-CSF-induced HSPC mobilization (18), and the evidence of osteoblastic susceptibility to systemic factors that can change the niche, thereby affecting HSC behavior (19).

Our laboratory and others provided the initial *in vivo* evidence suggesting that osteoblastic activation in mice can affect the HSC pool. Both a transgenic mouse model of constitutively active parathyroid hormone (PTH) signaling driven by the 2.3 kilobase fragment of the α1(I) collagen gene promoter and models of intermittent systemic PTH treatment result in expansion of phenotypic and functional HSCs, in addition to increasing trabecular bone and bone lining cells (20-22). Concurrently, Zhang *et al.* found that conditional inactivation of BMP receptor IA, which also results in greater trabecular bone and expansion of osteoblasts, elicits a similar increase in functional and phenotypic HSCs (23). This work also included immunofluorescent images of Brd-U-labeled HSCs interacting closely with N-cadherin-expressing osteoblasts, implicating N-cadherin as a potential HSC-regulating factor. Because HSCs lack both PTH1R and BMPRIA and because reciprocal transplantation schemes (in which genetically altered HSCs are placed in normal microenvironments) do not recapitulate these phenotypes, it can be concluded that OBs modulate HSC behavior or at least initiate changes in the BM microenvironment that expand HSCs. Further genetic evidence also supported the central role of osteoblastic cells in HSC regulation, this time in a model of OB loss using transgenic mice expressing the herpes virus thymidine kinase gene under the control of a 2.3 kilobase fragment of the rat α1(I) collagen promoter. In this system, ganciclovir treatment conditionally ablated developing OBs. The ablated, ganciclovir-treated animals not only experienced bone loss due to disrupted bone formation but also demonstrated a 3- to 10-fold decrease in HSC numbers with coordinate defects in hematopoietic progenitor populations, reduced overall marrow cellularity and an accompanying shift towards extramedullary hematopoiesis in the liver and spleen, indicating that OB dysfunction renders the BM inhospitable to HSCs (24).

Taken together, these models of osteoblastic manipulation and the dramatic effects they exert on HSCs demonstrate a regulatory role for osteoblastic cells in HSC maintenance. However, general expansion of OBs *per se* is not sufficient to expand HSCs (25), just as general disruption of osteoblastic function is not sufficient to impair HSCs (26). These data strongly

suggest that only a subpopulation of osteoblastic cells is capable of HSC regulation, however, the specific osteoblastic differentiation stage responsible for HSC support remains unknown. Emerging data suggest that mesenchymal stem cells (MSCs), the multipotent stromal cells from which cells of the osteogenic lineage develop, may be active components of the HSC niche, as co-transplantation of MSCs with HSCs has been shown to improve donor engraftment (27) and increase self-renewal of co-transplanted HSCs (28). A recent report by Méndez-Ferrer *et al.* identified a population of predominantly perivascular, CD45- (non-hematopoietic) Nestin+ MSCs that express high levels of HSC maintenance genes at steady state but downregulate these genes following administration of the HSC-mobilizing agent G-CSF (29). Furthermore, depletion of Nestin+ MSCs resulted in impaired BM HSC activity as evidenced by the *in vitro* long-term culture-initiating cell (LTC-IC) assay, an approximately 50% reduction in phenotypically-defined HSCs and a shift in hematopoiesis to the spleen. In addition, the authors showed that a bone-anabolic, HSC-expanding regimen of *in vivo* PTH administration selectively induced proliferation of Nestin+ MSCs, resulting in twice as many Nestin+ MSCs at the end of treatment. In contrast to these studies, immunofluorescent data have demonstrated HSCs residing close to osteocalcin+ OBs, potentially revealing a functional role for this osteoblastic population in HSC maintenance (23). As techniques for isolating OBs have improved, additional reports have demonstrated a role for immature OBs in HSC regulation (30) while still others have found HSC-supportive properties in cells at various stages of osteoblastic differentiation (31;32). Later, in our discussion of malignancy in the niche, we will review another distinct population of osterix+ osteoprogenitors that, if dysfunctional, appears capable of provoking malignant genetic changes in HSPCs (33). Future studies will continue to improve our understanding of which osteoblastic populations or OB-mediated mechanisms are most relevant to HSC maintenance.

Over the past decade *in vivo* studies have attempted to visualize the localization of immature hematopoietic cells within the BM microenvironment. Immunofluorescent analysis revealed hierarchical patterns of HSPC distribution, in which the most immature progenitors engrafted closest to the interior bone surface while the maturing precursor populations radiated inwards towards the longitudinal central axis of the marrow (34). As imaging techniques improved, these results were enhanced by the use of high-resolution confocal and two-photon video microscopy in mouse calvaria to observe HSPC migration and engraftment in real-time. These videos again revealed differentiation status-dependent lodging in the marrow (35). Remarkably, this work as well as the analysis of transverse sections of femoral BM found that HSPCs engrafted significantly closer to the endosteum in irradiated hosts (36). Confocal microscopy and real-time imaging have also revealed a very close association of OBs and endothelial cells at the endosteum (35;37), again emphasizing the intimate relationship between niche components.

Vasculature

Data suggest that HSCs possess the ability to migrate rapidly through the circulation, trafficking between marrow interstitium to the bloodstream and back to marrow (38). Of course it is the BM blood vessels, particularly the sinusoids, which form the interface between peripheral blood and hematopoietic marrow space; therefore, it is not surprising that endothelial cells (ECs) may play a regulatory role in the BM microenvironment. Tissue sections stained for endothelial-specific MECA-32 antigen and HSC subset-specific SLAM family receptor expression illustrate the association of HSCs with sinusoidal endothelial cells in homeostatic BM and spleen (39). To exclude the possibility that these HSCs may be relocating to the vasculature just as they begin cycling, cell cycle analysis of the tissue sections was performed and revealed that less than 4% of HSCs are in S/G2/M, leaving the majority of the population in G0/G1. Because these observations are made in a healthy, unperturbed system,

perivascular positioning of HSCs is likely to reflect a real functional relationship between the two cell types as opposed to a transient location during HSC mobilization (39). Additional support for such a relationship between HSCs and the BM sinusoidal endothelium is found in examples of other stem cell microenvironments, such as the adult neural stem cell niche in which stem cells reside on or near vessels of the subventricular zone, poised to receive soluble regulatory signals from vascular endothelial cells (40).

BM endothelial function not only appears to be an important component of homeostatic HSC maintenance and trafficking but it is also critical to recovery from hematopoietic injury. Conditioning radiation or chemotherapeutic regimens are used to myeloablate host BM and prepare patients for BM transplants and although such preparations are necessary, the vascular side effects may be severe. *In vitro* studies have shown that even sublethal doses of radiation damage BM sinusoids, stimulating EC apoptosis (41;42). The chemotherapeutic agent 5-fluorouracil (5-FU) or total body irradiation results in marked dilation and eventual fusion of BM sinusoids (43). Immunohistochemical staining for DNA damage, apoptotic and cell cycle markers demonstrated that irradiation predominantly causes limited, non-apoptotic sinusoidal EC loss and extreme vessel dilation that must be countered by DNA repair mechanisms, proliferation of remaining ECs and support from surrounding hematopoietic cells (44). While it appears that sinusoidal integrity requires physical support from hematopoietic cells of the BM interstitium, the reciprocal relationship also exists. Hooper *et al*. blocked vascular endothelial growth factor A (VEGF-A) signaling through pharmacologic VEGFR2 inhibition or conditional VEGFR2 deletion in mice exposed to radiation and subsequent BM cell transplantation. Not only did VEGFR2 inhibition block sinusoidal repair but it also resulted in failure to reconstitute the hematopoietic system, suggesting proper sinusoidal function is necessary for HSPC engraftment (45).

Adipocytes

In addition to OBs, osteoclasts and endothelial cells, the BM is also home to an abundant number of MSC-derived adipocytes; however, their role in hematopoiesis is still controversial. Examination of adipocytic cytokines revealed that through their production of TNF-α and adiponectin, adipocytes can stimulate self-renewal of primitive HSCs while inhibiting proliferation of hematopoietic progenitors (46;47). In contrast, hematopoietic analysis of mice that do not develop adipocytes suggested instead that adipocytes suppress hematopoiesis, physiologically and following myeloablation (48).

Molecular Components of the Niche

Notch signaling is an evolutionarily conserved cell fate specification pathway known to function in a variety of cell types and stem cell fate decisions. Expression of Jagged1, a Notch ligand, by human BM stromal cells was previously found to inhibit differentiation of murine myeloblast-like cells *in vitro* (49). Furthermore, activation of the Notch receptor in hematopoietic cells is capable of suppressing transcriptional activity – inhibiting HSC differentiation and promoting primitive self-renewal (50). Experiments in our laboratory demonstrated that PTH induced *in vivo* Jagged1 expression by trabecular and spindle-shaped OBs, but not by osteocytes or periosteal osteoblastic cells. *In vitro* PTH stimulated Jagged1 expression in rat osteosarcoma UMR106 cells, through an adenylate cyclase, protein kinase A pathway (51), providing a direct mechanism for PTH-dependent activation of osteoblastic cells initiating stimulation of the Notch signaling pathway. HSC expansion in a transgenic mouse model of osteoblastic activation of PTH receptor signaling was found to coincide with greater Notch activation in HSCs and was abolished by inactivation of the Notch-processing enzyme γ secretase, thereby demonstrating the involvement of Notch signaling in this phenotype (22). Emerging lines of data also suggest a role for endothelial expression of Notch ligands

in proliferation of immature hematopoietic cells at the expense of their differentiation (52). Further investigation of the specific role of Notch in HSC regulation by the BM microenvironment is necessary as Notch signaling has also been found to be nonessential in homeostatic hematopoiesis (53;54).

Several sources have provided evidence for the involvement of N-cadherin (N-CAD), a calcium-dependent homotypic adhesion molecule that forms adherens junctions, in OB-HSC regulation (10;23;55). These findings, however, have been called into question by reports of low N-CAD expression among HSC subsets, with the highest N-CAD-expressing hematopoietic cells not representing stem cells at all and by other results that found undetectable levels of N-CAD expression in populations containing HSC activity (56;57). One recent account, however, using N-CAD shRNA found that decreasing N-CAD caused greater cell cycling and deficient HSPC adhesion to the endosteum following transplantation (58). Given the controversy surrounding N-CAD, further studies are necessary to clarify the role of this adhesion molecule.

Annexin A2 (AnxA2), another calcium-dependent binding protein expressed by osteoblasts and BM endothelial cells, has been postulated to play a role in HSC engraftment in the BM. When AnxA2 is inhibited, fewer BM HSCs are observed and *in vitro* binding assays demonstrate that AnxA2-deficient OBs have reduced HSC adhesion ability (59). Interestingly, hypoxia and VEGF were recently found to induce AnxA2 expression by OBs and ECs through a Src and MEK kinase-dependent pathway (60). These data imply that heightened AnxA2 expression in hypoxic niches of the endosteal BM may support HSC lodging and retention.

The receptor tyrosine kinase Tie2 is found exclusively on ECs and primitive HSCs (61-63). *In vitro* studies revealed that HSCs stimulated with the Tie2 ligand angiopoietin 1 (Ang-1) had a survival advantage over control cultures (64). Subsequent *in vivo* studies by Arai *et al.* (10) detected hematopoietic Tie2 expression confined to a quiescent subset of HSCs, the majority of which adhered tightly to the endosteum. Immunofluorescent colocalization of Ang-1 and the results of Western blot analysis of OB cell lysate confirmed that OBs are indeed a principal source of Ang-1 in BM. Co-culture experiments demonstrated that Tie2+ HSCs grown with Ang-1-expressing OP9 stromal cells downregulated chemokine (C-X-C motif) receptor 4 (CXCR4), α4-integrin and VEGFR2 but upregulated adhesive β1-integrin expression. Importantly, *ex vivo* Ang-1 treatment of Tie2+ HSCs conferred a quiescent, non-proliferative phenotype to these cells when they were transplanted. The authors also demonstrated a protective effect when animals pretreated with exogenous Ang-1 were challenged with 5-FU or lethal doses of radiation, as shown by their increased survival rates. A subsequent study proposed an explanation for this protective effect, showing that the homeostatic level of Tie2 expression by the BM endothelium is low but can be upregulated following exposure to chemotherapeutic agents (65). Inhibiting Tie2 signaling resulted in impaired neoangiogenesis of the BM vasculature and negatively affected hematopoietic recovery, thus evidencing a role for Ang-1/Tie2-mediated vascular regeneration in hematopoietic recovery from myelosuppressive injury (65). Together these studies provide multiple roles for Ang-1/Tie2 signaling in the maintenance of HSCs directly by osteoblastic cells and indirectly by the BM vasculature.

Soluble and Non-cellular Components of the Niche

The chemokine stromal cell-derived factor-1 (SDF-1, also known as CXCL12), which activates CXCR4, is known to regulate HSPC trafficking between the BM and peripheral circulation (66-67). In the BM, OBs and ECs produce SDF-1 (68) and both hematopoietic and stromal cells express CXCR4 (69). CXCR4 on stromal and endothelial cells facilitates the internalization and release of SDF-1, allowing translocation of the chemokine across blood-BM barriers

(69). On hematopoietic cells, CXCR4 does not mediate SDF-1 transcytosis as it does on stromal cells and ECs (69), but rather it has been shown to function in HSC maintenance – both at steady state and during hematologic stress situations such as exposure to cytotoxic agents or in response to HSPC-mobilizing strategies (70). Sugiyama *et al.* (70) conditionally inactivated CXCR4 in mice and observed a significant reduction in BM HSC populations, a defect they attributed to impaired HSC response to sinusoidal reticular cell-derived SDF-1. Not only can OBs and stromal cells modulate their expression of CXCR4 and SDF-1 to affect HSCs, but there is also evidence that neutrophil- and osteoclast-derived matrix-metalloproteinase-9 (MMP-9) and cathepsin K (CTK) proteolytically inactivate BM SDF-1, causing HSC release from the BM (16;71-73). The dipeptidylpeptidase IV CD26 also has the capacity to proteolytically inactivate SDF-1 and moreover, inhibition of CD26 on HSPCs has been found to improve homing and engraftment of transplanted cells (74;75). Subsequent studies have shown that disrupting SDF-1/CXCR4 signaling through the use of the CXCR4 antagonist AMD3100 was sufficient to mobilize functional HSCs in mice and humans, as early as 4 hours following administration (67; 76), and thus AMD3100 has been adopted clinically as a method of peripheral blood HSC mobilization for BM transplantation. Another commonly used clinical strategy for mobilizing HSCs – administration of the cytokine G-CSF – has been found to act, at least in part, through downregulation of SDF-1 in the BM (71;72;77). These data clearly indicate that SDF-1/CXCR4 signaling between osteoblastic, osteoclastic, endothelial and hematopoietic cells represents an important intersection of many HSC regulatory mechanisms in the BM.

Osteoblasts are also capable of producing the inflammatory mediator prostaglandin E_2 (PGE2) (78), an arachidonic acid derivative that has been shown to affect HSC regulation. *Ex vivo* stimulation of PGE2 production in zebrafish BM cells expanded HSCs (79). In a mammalian system, the *ex vivo* treatment of murine BM with the synthetic analog 16,16-dimethyl PGE2 prior to transplantation resulted in increased frequency of repopulating HSCs and improved engraftment in irradiated recipients (79;80). Most recently, we have demonstrated that *in vivo* PGE2 treatment increases short-term HSCs and multipotent progenitors in the BM, even in the absence of injury (81).

HSC niches within the BM are subject to the dynamic physiology of bone, notably the maturation of cells of the osteoblastic lineage to form bone and the concurrent osteoclast-mediated resorption of bone and release of calcium ions. HSCs possess calcium-sensing receptors (CaR) and demonstrate calcium-dependent localization in the BM (82). In fact, HSCs lacking the CaR show impaired homing to the BM, especially non-myeloablated BM in which CaR-deficient cells must compete with wild-type HSCs for niches or niche factors (82). Similarly, studies in which calcium homeostasis is disrupted by deletion of the vitamin D receptor find the resultant BM unable to retain HSCs, which are instead found specifically in the spleen (83;84). These phenotypes may be explained by direct effects of calcium on HSC function through the CaR, or if HSCs preferentially home to regions of extracellular calcium release as has been suggested, they may undergo altered calcium-dependent adhesive interactions at the endosteum.

BM hypoxia, a condition most severe in poorly perfused regions of the endosteum, has recently been shown to correlate with HSC activity and to possibly exert regulatory control over stem cells through transcriptional actions of HIF-1α in HSCs. It has been established that the most quiescent HSCs reside in the least perfused niches, thought to be enriched for locally secreted factors (85-88). In hypoxia, HIF-1α protein that would otherwise undergo prolyl hydroxylation and subsequent degradation is stabilized and transcriptionally activates target genes (89;90). HIF-1α in HSCs may be further stabilized by molecules found in the niche, including thrombopoietin and stem cell factor, even in normoxia (91;92). In a study by Takubo *et al.*, HSCs were shown to express HIF-1α *in vivo* and in a murine

model of inducible HIF-1α deletion, HSCs demonstrated reduced quiescence during BM transplantation and other physiologic stresses. These data were in agreement with previous *in vitro* results showing enhanced HSC quiescence under hypoxic culture conditions (93;94). Such an effect is reasonable given the hypothesis that lower oxidative stress makes HSCs less likely to differentiate (95). Genetic analysis revealed that transplanted HIF-1α-deficient HSCs had elevated levels of Ink4a, a gene associated with stem cell senescence (96). In the absence of HIF-1α, genetic changes and accumulation of reactive oxygen species (97) provide potential explanations for HSC senescence. The authors concluded that stable expression of HIF-1α at the endosteum confers anti-senescent properties to HSCs, potentially via suppression of Ink4a.

Malignancy in the BM Microenvironment

Metastases to bone are a frequent consequence of certain types of cancer that usually signify worsening survival outcomes (98;99). Because selective metastases originate from the seeding of mobilized cancer cells, the well-perfused regions of red marrow may provide an ideal site for development of metastasis (100). However, it has been suggested that it is not merely the degree of blood perfusion that determines metastatic frequency (101); rather it is the local environment cancer cells encounter. As a location of hematopoietic cell trafficking and engraftment, the microenvironment of the BM is not only uniquely accessible to circulating cancer cells but it possesses structural and molecular components that engage with cancer cells to allow the growth of metastases. In fact, many of the same cellular mechanisms employed by HSPCs to home to the BM can be used by malignant cells to lodge within bone. Prostate cancer (PCa) metastases are a well characterized example of this. Taichman *et al.* have demonstrated that PCa cells, through their expression of CXCR4 and CXCR7, respond to SDF-1 produced by OBs and BM ECs (68) with greater invasiveness, expression of proangiogenic molecules including IL-8 and VEGF and stimulation of anti-apoptotic AKT signaling pathways (102-105). In this way, physiologic signals involved in HSC guidance and retention offer a hospitable environment for metastatic cancer cells as well.

In addition to being an appropriate “soil” for the seeding of tumor cells, there is emerging evidence that dysfunction within the bone can create a malignant microenvironment capable of initiating neoplastic changes in immature hematopoietic cells. One such study observed myeloproliferative disorders when the gene for cell cycle regulator retinoblastoma was globally deleted but then found that conditional inactivation of the gene specifically in HSCs or in the BM microenvironment was not sufficient to recapitulate the myeloproliferative phenotype (106). The same phenotype was only achieved when retinoblastoma was deleted from both HSCs and the microenvironment. Moreover, retinoic acid receptor γ deficiency in the BM microenvironment has been found to be sufficient for the development of myeloproliferative disorders, however, this phenotype is not initiated by the hematopoietic cells themselves but arises when wild-type hematopoietic cells are transplanted into a retinoic acid receptor γ-deficient microenvironment (107). In another example, Raaijmakers *et al.* manipulated osterix+ osteoprogenitor cells in the niche by deleting a gene essential for RNA processing and microRNA biogenesis, *Dicer1* (33). Mice with the conditional *Dicer1* deletion experienced myelodysplastic BM changes, peripheral blood cytopenia and extramedullary hematopoiesis in the spleen. Transplantation strategies revealed that myelodysplasia was not initiated by hematopoietic BM cells from osteoprogenitor-specific *Dicer1*(-/-) animals given a normal BM microenvironment but that the mutant *Dicer1*(-/-) microenvironment was sufficient to induce myelodysplastic hematopoiesis in wild-type BM cells. Collectively, these data demonstrate the susceptibility of otherwise healthy hematopoietic cells to malignant cues from their niche.

Concluding Remarks

HSCs are exposed to a host of cell types and signals within the BM microenvironment. Recent findings clearly demonstrate the instructive role osteoblasts play in HSC regulation; however, the complexity of the niche and the changes in HSC activity that can occur when other elements of the BM are altered makes it likely that proper stem cell function relies on the coalescence of many different and dynamic factors. Although we typically think of HSCs as being reactive to their microenvironment, there is also some evidence that HSCs actively shape their niche according to external stimuli (108;109). Together these mechanisms define an exquisitely controlled, yet delicate, system of HSC maintenance. This complex area of investigation has benefited from contribution from the hematopoiesis and stem cell fields, bone and mineral metabolism, vascular biology, bioengineering as well as the tumor microenvironment research community, and it is likely that interactions bridging these disciplines will be most productive in defining the characteristics and therapeutic potential of both benign and malignant niches. Particularly, emerging data on endothelial interaction with HSCs through Notch signaling, the evidence for HSCs actively shaping their niche and the concept of malignant niches reveal exciting areas of research that will likely advance our understanding of regulatory interactions in the HSC niche. Improving our understanding of the ways in which HSC behavior is specified will continue to provide novel targets for disease treatments, both for hematologic malignancies and cancer metastases to bone.

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