Published in final edited form as:

Exp Hematol. 2012 September ; 40(9): 685–694. doi:10.1016/j.exphem.2012.05.004.

Getting Blood from Bone: An Emerging Understanding of the Role That Osteoblasts Play In Regulating Hematopoietic Stem Cells Within Their Niche

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Abstract

Blood and bone are dynamic tissues that are continuously renewed through out life. Early observations based upon the proximity of bone and hematopoietic progenitor populations in marrow suggested that interactions between skeletal and hematopoietic elements are likely to be crucial in the development and function of each system. As a result of these morphologic observations, several groups have demonstrated that the osteoblasts play an important role in hematopoiesis by serving as a specific local microenvironment, or “niche”, for hematopoietic stem cells (HSC). Significant new developments in this area of active investigation have emerged since our last examination of this area in 2005. Here we discuss these new insights into the function and morphology of the HSC niche with a particular focus on cells of the osteoblastic lineage.

Introduction

During the 3rd week of gestation, hematopoiesis is first initiated in the yolk sac. By the fifth week of development, primitive hematopoietic cells may be detected in aorta-gonad-metanephros region, liver and spleen [1]. By the 11th week of gestation, hematopoietic activity begins to shift towards the bone marrow such that by the time of birth, the marrow remains the major location for hematopoiesis in humans [1]. Marrow-derived hematopoietic stem cells (HSCs), which are the top of the hematopoietic hierarchy, have the potential to differentiate into all mature blood cells while maintaining the capacity for self-renewal. To provide a constant supply of mature blood elements, HSCs must maintain a balance between differentiation and self-renewal. The site that regulates self-renewal, proliferation and differentiation of HSCs has been termed either the HSC microenvironment or the ‘HSC niche’ [2]. Within the HSC niche, HSCs are likely to be exposed to both support/maintenance/renewal and growth signals. Most evidence indicates that combinations of cell intrinsic and extrinsic factors influence the decision of HSCs to self-renew or differentiate.

In the early 1970’s, studies examining the interactions between bone marrow stromal cells and HSCs provided clues that osteoblasts may play a central role in hematopoiesis [3-5]. In

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1994, we hypothesized that osteoblasts play an important role in hematopoiesis in human system and demonstrated that human osteoblasts support maintenance of HSCs and expansion of progenitors *in vitro* [6]. In late 2003 and early 2004, several reports demonstrated that HSCs were closely localized to osteoblasts based upon morphology and the expression of N-cadherin and Ang-1 using murine models [7-9]. In addition, the essential roles of the osteoblastic niche in hematopoiesis were also confirmed by depleting the osteoblastic niche with ganciclovir in Col2.3Δ-TK transgenic mice [10, 11].

More recently, sinusoidal endothelium [12, 13], CXCL12 highly expressing reticular cells [14], adipocytes [15], and mesenchymal stem cells [16, 17] have been described as components of the HSC niche. However, what the function of these cells are, and how they control primitive hematopoiesis are poorly understood and remain controversial. In fact, only scant evidence exists as to what are the support/maintenance/renewal and growth signals produced by these cells that regulate HSC function. Recent controversy centers on where the niche is itself – is it located close to the endosteal surfaces or near vascular surfaces? Likely both endosteal and vascular components participate in the generation of the HSC niche. Yet how the functions of these two HSCs niches are synchronized is not clear. In this review, we will discuss the current understanding as to the various cells that participate in the function of the HSC niche, with a particular focus on what is known regarding the osteoblastic HSC niche.

HSC homing to the HSC niche

Beginning in the 11th week of fetal life, HSCs begin to migrate from the liver and spleen into the bone marrow where the majority of hematopoietic activity occurs after birth [1]. Later, under basal conditions, the majority of HSCs reside in the marrow. However a small but significant number of HSCs can be found in the circulation. The number of circulating HSCs may increase dramatically during infection or in response to recombinant growth factors. It is likely that these HSCs play a significant role in regulating and coordinating HSC activities across the organism where hematopoietic activities are widely dispersed.

Yet the abilities of circulating and donor HSCs to establish hematopoietic activities during transplantation are dependent on their ability to home and lodge in the marrow. The chemokine stromal derived factor-1 (**SDF-1/CXCL12**) is perhaps the best characterized molecule that regulates HSC homing. Two receptors CXCR4 and CXCR7 are known to bind CXCL12 and both CXCR4 and CXCR7 are expressed by HSCs [18]. Considerably more is known regarding the role that CXCR4 plays in regulating HSC homing and engraftment [19, 20], where competitive inhibitors have entered therapeutic trials as HSC mobilization agents [21]. Several cell types have been identified as sources of CXCL12 in normal marrow. Osteoblasts appear to be a major source of CXCL12 [22] where synthesis is regulated by several inflammatory stimuli, DNA damaging agents and hormones. Moreover, osteoblastic production of CXCL12 appears to be regulated by circadian rhythms [23, 24]. Importantly, CXCL12 production appears to be an early feature of the osteoblastic lineage, while CXCL12 production is down regulated upon maturation and matrix synthesis [20, 25].

Vascular endothelial cells represent other cellular source of CXCL12 in the marrow [26]. Less is understood regarding regulation and production of CXCL12 by these cells. However, a unique function for CXCR4 expressed on marrow endothelial cells has been described. Endothelial CXCR4 internalizes circulating CXCL12 to translocate the protein into the bone marrow [27]. By this mechanism, CXCL12 has been demonstrated to increase the homing of transplanted human CD34⁺ hematopoietic progenitors into the bone marrow. The transporter function of CXCR4 appears to be cell type specific where endothelial and other stromal cells share in the capacity, but not of hematopoietic cells. Whether osteoblasts share

in this capacity has not been determined and what the physiologic significance of such a mechanism to place CXCL12 in the bone matrix is uncertain, but CXCL12 could serve as a mechanism to localize osteoclasts into areas requiring active bone turnover [28].

Additional molecular regulators of HSC homing have also been recently identified in addition to CXCL12. For example, under basal conditions, the normal physiologic oxygen tension in marrow is hypoxic particularly at the level of the endosteal/osteoblastic niche. One thought suggests that a low O₂ environment is necessary for the ability of HSCs to maintain their stemness [29]. In addition, hypoxia induces both CXCL12 secretion [30] and CXCR4 expression [31]. Together, these observations suggest that hypoxia may be involved in the recruitment of HSCs to the niche through the regulation of the CXCR4/CXCL12 axis. Just as low oxygen levels are thought to increase CXCL12, degradation of CXCL12 is also likely to play a major role in regulating HSC levels in the marrow. In particular, a report suggesting that CD26 plays a major role in degrading CXCL12 is of considerable interest. As blocking CD26 activity lead to enhanced engraftment of HSCs into marrow niches [32], although this has recently been called into question [33].

Over the last several years, CXCL12-independent mechanisms of HSC homing have also been identified. For example, expression of the calcium-sensing receptor (**CaR**) and the guanine-nucleotide-binding protein stimulatory α subunit (**G α_s**) expressed by HSCs plays a crucial role in their homing to osteoblastic niche [34, 35]. CaRs are expressed mostly by HSC in the marrow and are thought to be related to the localization of HSCs to the endosteal surfaces [34]. Consequently, HSCs derived from *CaR*^{-/-} mice fail to engraft into wild-type marrow [34]. Likewise, HSCs from *G α_s* ^{-/-} mice have lost the ability of homing to the marrow [35]. In fact, when *G α_s* was conditional deleted in HSCs after engraftment to wild-type bone marrow microenvironment, the migratory ability of *G α_s* ^{-/-} HSCs in response to granulocyte colony-stimulating factor (**G-CSF**) treatment was impaired compare to *G α_s* ^{+/+} HSCs [35]. In contrast, when *G α_s* activity in HSCs was stimulated using cholera toxin (a compound known to constitutively activate *G α_s* by preventing GTP hydrolysis from the ADP-ribose-*G α_s* -GTP complex), the ability to engraft and home to the marrow is enhanced [35].

As maintaining chemokine gradients are critical for HSC homing, occupancy of established niches also appears to regulate the ability of transplanted HSCs to engraft into the niche. Without available or “vacant niches”, exogenous (or donor) HSCs are not able to lodge in the HSC niche occupied by endogenous (or recipient) HSCs. Thus, pre-treatment with the CXCR4 antagonist AMD3100 prior to transplantation facilitates stem cell engraftment [36, 37]. Likewise, antibody to c-kit (ACK2) which is expressed by HSCs has been shown to increase the level of circulating HSCs by opening up the HSC niches to prepare niche space for donor HSCs to engraft into the marrow of recipient animals [38]. Clearly, these observations along with studies on how to prevent CXCL12 degradation offer significant hope as either pre-conditioning or HSC mobilization regimes during stem cell transplantation.

Adhesion interactions between HSC and HSC niche

Direct cell-to-cell and/or cell-to-extra cellular matrix contacts between HSCs and their niche are thought to play a crucial role in the regulation of HSC fate, although what adhesion molecules maintain HSCs in the marrow remains unclear [20]. Mouse models reveal that adhesion interactions between HSCs and the osteoblastic niche are partially regulated by Tie2/Ang-1 and N-cadherin/N-cadherin interactions [8, 9]. However, controversies as to the roles of N-cadherin in the early hematopoiesis have also been appreciated [39]. Earlier work by Zhang *et al* in 2003 suggested that N-cadherin expressing “spindle shape osteoblasts” are

critical components of the HSC niche, and approximately 10% of HSCs express N-cadherin [9]. Homotypic binding between HSCs and their niche via N-cadherin appeared to be of great significance [9]. Nonetheless, recent studies have revealed that primitive HSCs express low or no N-cadherin on their surface [40, 41], whereas mature blood cells express high levels of N-cadherin [41]. A more recent study using N-cadherin knock-out animals failed to demonstrate the effects of N-cadherin on HSC maintenance [42]. Moreover, although the N-cadherin in osteoblasts was conditionally deleted using Cre/lox system, the function of HSCs, the engraftment capacity of wild-type cells, and the responses to the G-CSF treatment in those animals are not impaired [43]. Thus the role of N-cadherin homodimers regulating HSC interactions within the niche remains unclear. N-cadherin may however regulate niche interactions of either more mature progenitor cells or via heterodimers with other receptor molecules expressed by HSCs.

The $\alpha_4\beta_1$ integrin, or very late antigen-4 (**VLA-4**), is also widely known as one of the adhesion molecules involved in HSCs fate. Vascular cell adhesion molecule-1 (**VCAM-1**), a ligand for VLA-4, is known to be expressed by stromal reticular cells [44], endothelial cells lining bone marrow sinusoids [44] and osteoblasts in the marrow [10]. When serial transplantation assays were performed using VLA-4 conditional deleted HSCs, the HSC homing and self-renewal are diminished [45]. Strikingly, more hematopoietic progenitor cells (**HPCs**) were found by colony-forming-unit assays in the peripheral blood recovered from *VLA-4*^{-/-} chimeras compared with the animals transplanted with wild-type marrow [45]. Intriguingly, one-third of the animals survived when *VLA-4*^{-/-} peripheral blood cells were transplanted into wild-type animals, whereas no animals survived when transplanted with wild-type peripheral blood [45].

Another molecule of great interest to our group that is involved in the HSC homing and binding to the niche is annexin II (**Anxa2**). Moreover, it has been demonstrated that Anxa2 expressed by osteoblastic niche serves as an anchor for CXCL12 to support the HSCs homing to the niche [46]. Anxa2 is also known to regulate osteoclastic activity [47] and participate in plasminogen activation, cell adhesion and tumor metastasis and invasion [48-51]. High levels of Anxa2 are expressed by the marrow osteoblasts and endothelial cells and subsequently Anxa2 is involved in the adhesion between HSCs and osteoblasts [52]. By blocking Anxa2 using monoclonal antibodies and competing peptides, both short- and long-term HSC repopulation are impaired [52]. Importantly, less HSCs are found in the *Anxa2*^{-/-} mice [52]. In prostate cancer [51] and multiple myeloma [53], the receptor for Anxa2 is identified and Anxa2/its receptor interaction has been known as a regulator of tumor growth in the marrow. Although further study will be needed, these data suggest that HSCs also interact with the niche through the Anxa2/Anxa2 receptor axis.

HSC mobilization from HSC niche

HSCs continue to circulate between the marrow and the bloodstream throughout life to maintain homeostasis. Under steady-state conditions, few HSCs are circulating in the peripheral blood to respond to the stress conditions such as injury and inflammation. G-CSF and granulocyte/macrophage colony-stimulating factor (**GM-CSF**) have been largely known as HSC mobilizing agents during hematopoietic stem cell transplantation [54]. As well as the hematopoietic growth factors, chemotherapeutic agents (cyclophosphamide), the blockade of cytokine-receptor interactions (CXCL12/CXCR4; CXCL2/CXCR2), and the degradation of adhesive connections (VLA-4/VCAM-1; CD44/HA) have been also utilized to mobilize HSCs from the marrow [54]. Interestingly, both CXCL2/CXCR2 [55] and CD44/HA [56] regulate the mobilization of HSCs by synergizing with the CXCL12/CXCR4 axis. One potential mechanism for mobilization induced by G-CSF is the activation of protease activities, including neutrophil serine proteases, metalloproteinases (**MMPs**) and

CD26 [54], which results in the proteolytic cleavage or degradation of CXCL12 in the marrow. G-CSF may also induce mobilization by decreasing CXCL12 levels by reducing the production and the number of osteoblastic cells capable of producing CXCL12 [22, 57]. The ability to suppress CXCL12 production following the suppression of osteoblastic differentiation or numbers appears to be a common pathway for agents that mobilize HSCs including Flt3L and SCF [57]. Recent studies also revealed that G-CSF reduces monocytic cells/macrophages, leading to the inhibition of the osteoblast formation and the HSC mobilization, suggesting that monocytic cells/macrophages are involved in the osteoblastic niche formation and HSC mobilization [58-60]. On the other hand, osteoclasts have been directly involved in G-CSF-induced mobilization by activating CXCL12 degradation in conjunction with increased levels of MMP9 and cathepsin K [28]. However, it was also demonstrated that osteoclasts might not be involved in HSC mobilization [61].

AMD3100 has been approved as a HSC mobilizing agent [62]. The mechanism whereby AMD3100 stimulates mobilization appears to differ from G-CSF and other agents which reduce CXCL12 expression directly or indirectly by blocking CXCL12 interactions with its receptors. Moreover, cells mobilized by AMD3100 including CD34+ cells appear to be different than G-CSF-mobilized CD34+ cells in that they are at a different stage of the cell cycle (G1 phase vs. G0 phase) [63]. In fact, partially due to enhanced levels of CXCR4 and VLA-4, AMD3100-mobilized CD34+ cells are more potent in their ability to home when compared to G-CSF-mobilized cells [63, 64]. In addition, G-CSF suppresses the macrophage and osteoblastic niche formation to mobilize HSCs, while AMD3100 does not appear to do so [65]. Further evidence that the mode of action differs comes from studies that demonstrate that when AMD3100 and G-CSF are administered together, enhanced mobilization is seen compared to either agent alone [21]. What is more, the combination of VLA-4 blockade with AMD3100 and/or G-CSF mobilized HSC dramatically better than either of these agents alone [66, 67]. Thus several mechanisms may regulate HSC mobilization such that combination of agents may prove to be superior and may yield different profiles of HSC and progenitors when engraftment is examined.

Circadian rhythm and the HSC niche

Among the more interesting findings in the past several years are observations that suggest HSC numbers in the peripheral blood is controlled by the central nervous system. In fact, CXCL12 expression by osteoblasts is regulated by signals generated locally in marrow from the sympathetic nervous system (SNS) [23]. Moreover, the number of HSC mobilized in response to cytokine stimulation depends upon the circadian rhythms [24, 68]. Reductions in CXCL12 levels are due in part to reduced Sp1 transcription factor levels because of noradrenaline secretion by sympathetic nerves in the marrow [24]. Part of the mechanism involved in the circadian oscillations of HSCs for Murine and human bone marrow CD34+ cells is dependent on the expression of the clock genes *Per1*, *Per2*, *Bmal1*, *Cry1*, *Cry2*, *Clock* and *Rev-erb α* [69-71]. The clock gene expression in murine whole bone marrow cells show circadian rhythmic expression, but most of clock gene expression in murine HSCs is not circadian rhythmic [70]. Yet subtle differences exist between murine and human HSCs where all but *Clock* shows circadian rhythms [71]. As a result, the pattern of HSC mobilization in humans is opposite to what is found in mice suggesting considerable heterogeneity between the two species [68]. At present it remains less clear whether circadian rhythms also play a major role in engraftment [72].

Circadian oscillations also appear to affect the HSC niche through actions mediated by osteoclastic bone remodeling. One molecule thought to play a major role is leptin. Leptin is known to regulate bone formation by binding to its receptor located in hypothalamus rather than acting directly on osteoblasts [73-77]. Leptin-dependent SNS promotes osteoblast

proliferation through transcriptional activation of AP-1. This leads to up-regulation of *c-myc* and *cyclin D1* [73]. Conversely, leptin-dependent SNS prevents osteoblast proliferation through the clock genes probably by down-regulating *c-myc* and *cyclin D1* [73]. Neuromedin U is also thought to be another downstream target of leptin signaling to inhibit osteoblast proliferation [75]. In addition, SNS induces bone loss by enhancing osteoclastic activity and osteoclast formation [76, 77]. Leptin-dependent SNS regulates bone resorption by inducing RANKL expression of osteoblasts [77]. For this sympathetic function, phosphorylation of transcription factor ATF4 in osteoblasts expressing β 2-adrenergic receptors is indispensable [77]. The bone remodeling is controlled by not only the balance between the osteoblastogenesis and the osteoclastogenesis but also the osteoblastic differentiation from mesenchymal stem cells (MSCs). It has been recently demonstrated that MSCs express the clock genes, and that the potential roles of MSC in bone metabolism and HSC biology as HSC niche in response to circadian mechanisms [78]. Taken together, circadian clocks play an important role in the HSC physiology directly and indirectly by stimulating osteoblastic niche components and by targeting CXCL12 levels produced in the niche.

Signaling pathways and the HSC niche

During the last several years a number of signaling molecules including Wnt [9, 79-81], Notch [7, 82], Hedgehog [83, 84] and bone morphogenic proteins (BMPs) [9] have been implicated in HSC proliferation and quiescence. Recent work also suggests that Tie2 (expressed by HSC) and Ang-1 (expressed by osteoblast) regulate HSC quiescence in the marrow [8]. Likewise, osteopontin, a matrix glycoprotein expressed by osteoblasts has been shown to inhibit HSCs entering into replicative cycles [85, 86]. Accumulating evidence also suggests that hyper activation of mTOR signaling pathways that enhances lineage commitment of HSCs may ultimately lead to HSC exhaustion and effect which may be reversed by rapamycin [87-89].

It has also recently been appreciated that thrombopoietin (THPO) and its receptor Mpl participate in the maintenance of HSC quiescence [90, 91]. Morphologic studies demonstrated that Mpl expressing HSCs were located close to THPO expressing osteoblastic niche cells in the marrow [90]. When animals are treated with neutralizing anti-Mpl antibody, the numbers of quiescent HSCs are reduced [90]. In addition, the number of HSCs in *Thpo* knockout mice are less than wild-type mice and the cell cycle of *Thpo*^{-/-} HSCs is accelerated [91]. Part of the proposed mechanisms for these observations is that cyclin-dependent kinase inhibitors *p57^{Kip2}* and *p19^{INK4D}* are down-regulated during THPO/Mpl signaling [90, 91]. Importantly, *Thpo* deficiency on both HSCs and niche cells results in limited engraftment after transplantation [91]. Moreover, neutralizing anti-Mpl antibody is able to open the HSC niche to facilitate the ability of transplanted-HSCs to engraft into “vacant niche” without irradiation [90]. Alternatively, the adaptor protein Lnk known as an inhibitor of THPO/Mpl signaling pathway [92], negatively regulates self-renewal and quiescence of HSCs [93]. HSCs recovered from *Lnk*^{-/-} animals enter into a quiescent state relative to HSCs derived from wild-type animals, whereas HSCs from *Mpl*^{-/-} animals lost a quiescent state [93]. However, when animals are generated with a double-knock out of both Mpl and Lnk, the HSC phenotype resembles wild-type HSCs in terms of quiescence [93]. It was also revealed that JAK2 was involved in regulating the balance between Lnk and Mpl in response to THPO [93]. Thus, the signaling axis THPO/Mpl/JAK2/Lnk may be one of the major pathways regulating self-renewal and quiescence of HSCs [93].

Regulation of the niche by HSCs

Crosstalk between HSCs and the HSC niche is thought to be important for hematopoiesis. However, the mechanisms of this process are largely unknown. The BMP signaling pathway

is a good model for exploring the interactions between HSC and HSC niche, as BMPs affect both HSCs and niche cells. For example, BMP4 is thought to be essential for mesoderm commitment of embryonic stem cells to the hematopoietic lineage during embryogenesis [94, 95], suggesting that BMPs play important roles in deciding HSC fate. However, it has been demonstrated that the enhancement of the spindle shape N-cadherin expressing osteoblastic HSC niche size results in an increase in the HSC numbers in BMP receptor type IA knock down animals [9]. In addition, BMP4 deficient animals decrease the number of HSCs and the engraftment of normal HSCs is impaired within BMP4 deficient microenvironment [96]. These data suggest that the effect of BMPs effect on the HSC microenvironment is also relevant to how BMPs regulate hematopoiesis.

An additional mechanism whereby HSCs participate in regulation of the niche has come from work by our group where it was demonstrated that HSC are able to directly regulate bone formation [97]. Part of the mechanisms appears to be the expression of BMP2 and BMP6 by HSCs in response to signals that are received following acute bleeding or other marrow damaging agents [97]. In addition, erythropoietin (**Epo**) supports BMP production in HSCs through JAK2/STAT3 signaling pathway [98]. Importantly, HSCs-recovered from stressed-animals induced osteoblastic differentiation from bone marrow stromal cells *in vitro* and *in vivo* significantly more than those from control animals [97]. Yet HSCs recovered from aged animals and under conditions of experimentally induced osteoporosis, HSCs are less efficient in generating a BMP response following acute blood loss [97]. These findings suggest that HSCs do not only rest passively in their niche but also directly participate in development of an osteoblastic niche [97]. However, it is less clear if BMP production by HSCs regulates endothelial niche expansion.

Deeply quiescent HSCs

Hypoxia is thought to contribute to the ability of HSC to become quiescence (or dormant) and self-renewal [99]. It has been also demonstrated that oxidative stress reverses HSC dormancy [100, 101]. In the marrow, the osteoblastic niche is thought to represent a lower O₂ environment compared with the vascular niche [29]. For this reason, it has been hypothesized that the osteoblastic niche maintains quiescence and self-renewal of HSCs, while the vascular niche may regulate the proliferation and differentiation of HSCs [29]. In other words, HSCs reside near the osteoblastic nice (**dormant HSCs**) are in slow-dividing state and maintain the cell compartment in the marrow, whereas HSCs near the vascular niche (**active HSCs**) are in cell-cycling state and are ready to egress from the marrow in response to stress including infection, injury or chemotherapy. It has also been recently revealed that not only active HSCs respond to stress to maintain homeostasis but so do dormant HSCs [102]. The switch from an actively cycling cell to dormancy and back to an active state appears to be quite rapid as would be required in order to respond to physiologic and non-physiologic stressors on the hematopoietic system [102]. These data support the idea that osteoblastic niche and vascular niche, including perivascular cells [13] and nestin-expressing mesenchymal stem cells [17] that are located near the endothelial cells, cooperate with each other in constructing and maintaining the HSC niche.

Future Challenges

Several lines of evidence has demonstrated that cells of the osteoblastic lineage participates in the development of the HSC niche in murine [7-9, 12, 24, 52, 68] and human models [6] (Figure 1). More recent findings have expanded these observations suggesting that the central nervous system is involved in the regulation of HSC movement in and out of the niche with direct effects on osteoblast function [24, 68] (Figure 1). Yet the precise location of the HSC niche and whether it is endosteal-osteoblastic or endothelial-vascular or both

remain unclear. It has recently been suggested that endochondral ossification is essential for osteoblastic niche formation [103], and that pre-osteoblasts may play an important role in normal hematopoiesis [104]. However, which cell in the osteoblastic lineage is directly responsible for establishing/participating in the niche, and what is its relationship to other cells in the marrow are unclear. Recent advances in *in vivo* real-time imaging have begun to address these issues [105-107]. However until actual markers emerge that denote the “HSC” niche as the niche this area is likely to serve as a source for future controversy.

Just as important are observations that not all niches are the same. Several investigators have suggested that niches that regulate HSC quiescence in marrow may differ in either location or quality from niches that participate in HSC maturation events (Figure 1). For example, adipocytes negatively regulate hematopoiesis in the marrow [15, 108] by up-regulating neuropilin-1 [108] and nestin positive MSCs are involved in the HSC niche formation [17]. Adding further complexity to our understanding is that HSCs probably migrate between niches at low levels that can fluctuate in response to physiologic stimuli and in response to circadian rhythms. Nor is it known how or when the endosteal-osteoblastic or endothelial-vascular niches, or any other HSC niches, cross-talk to one another. It also remains uncertain whether there are times or conditions in which one niche takes priority over the other. If so, what are the signals that participate in setting priority?

Stem cell transplantation is the treatment of choice where restoration of normal hematopoiesis is required including several blood neoplasms, immune deficiencies and during the treatment of solid tumors following chemotherapy [109]. Because of limited number of HSCs and the potential for delayed engraftment [110], it is critical to develop improved techniques for *ex vivo* HSC expansion while maintaining HSC stemness. However, most of the *ex vivo* HSC expansion protocols lead to exhaustion of the critical target cells [110]. Interestingly, it has been appreciated that the niche itself may be targetable - such that manipulating the microenvironment for HSCs (e.g. expanding HSC niche by PTH treatment) may support HSC engraftment during transplantation [111]. Just as critical is the necessity for a greater understanding of the HSC-niche interactions to facilitate targeting of the region to improve mobilization of HSCs out of the niche and to facilitate enhancements in engrafting capabilities.

Adding another layer of complexity to our understanding of the niche is the notion that hematologic malignancies [112, 113] and solid tumor [114] target the HSC niche. Intriguingly, metastatic prostate cancer cells compete for occupancy of the endosteal niche with HSCs [114]. Once there, the occult malignant cells may become dormant, parasitizing the molecular signals that regulate HSCs dormancy. Under these conditions, tumor cells may be able to escape immune surveillance mechanisms by resembling HSCs in the niche and evade conventional radiation and/or chemotherapy that target rapidly dividing cells. Moreover, the effects of the bone marrow microenvironment on the development of malignancy have been revealed. Myeloproliferative syndromes (MPS) develop in the animals when the retinoic acid receptor γ (RAR γ) [115] or retinoblastoma protein (RB) [116] was deleted from the bone marrow microenvironment. However, RAR γ - or RB-deficient HSCs function normally in the wild-type microenvironment [115, 116]. Indeed, whether malignancies create their own specific niche or use normal HSC niche as their niche remains an open question. A recent study by Raaijmakers et al has begun to address this question [104]. Disfunctional osteoprogenitor cells were established by deleting *Dicer1* which lead to secondary hematopoietic malignancies including myelodysplastic syndrome, myeloid sarcoma and acute myelogenous leukemia [104]. Strikingly, Raaijmakers et al also provide the linkage between their findings and human disease (e.g. Shwachman–Diamond–Bodian syndrome) [104]. Although further study is clearly needed, this intriguing concept

could suggest that the HSC niche serve as a new potential target therapy for malignancies [111, 113].

Over the past five years it is clear that a variety of the bone marrow components are intricately intertwined and support many aspects of hematopoiesis in a collaborative fashion. Osteoblasts have been shown to be a major compartment of HSC niche (Table 1), although clearly not the only cell that has 'niche' capacity. Although our knowledge of HSC niche has made dramatically progress, critical questions are still unanswered since our previous review [20]. i) Does only one single component of the marrow cells serve as HSC niche? - Which is the HSC niche (e.g. osteoblasts vs endothelial cells)? ii) If there are several different HSC niche components, how do they interact? iii) How does the HSC niche balance self-renewal and the differentiation of HSCs? iv) Is the HSC niche involved in tumorigenesis? - Are the normal HSC niche and leukemia/cancer niche different? Although further studies will be needed, these questions have begun to be addressed. If successful, the HSC niche could be a potential target during the bone marrow transplantation as well as the leukemia/cancer treatment.

Acknowledgments

This work was directly supported by a Pediatric Oncology Research Fellowship (Y.S.), the Department of Defense (Y.S. and R.S.T.) and National Institutes of health (DK082481 R.S.T., DE020721 R.S.T., 1RC1DE020721 R.S.T., CA141426 R.S.T., CA093900 R.S.T. and CA163124, Y.S. and R.S.T.), and the Prostate Cancer Foundation (Y.S. and R.S.T.).

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Figure 1. The model of ecological hematopoietic stem cell niche

Hematopoietic stem cell (HSC) homing, maintenance and differentiation depend upon their specific microenvironment (or niche). In the bone marrow there are at least two major HSC niches. The vascular niche, which is a gateway for HSCs between the periphery and marrow that likely facilitates the proliferation and differentiation of HSCs, while the osteoblastic niche likely maintains self-renewal and quiescence of HSCs. Osteoclasts are also known to participate in niche function partially by regulating HSC mobilization. In addition, there are growing indications that other types of cells including fibroblasts, CXCL12-expressing reticular cells, nestin-expressing mesenchymal stem cell (MSC) and adipocytes, also serve as the HSC niche in the marrow. Homing of stem cells is mainly regulated through CXCL12 expressed by the HSC niche. Interestingly, circadian rhythm is involved in these relationships by regulating CXCL12 expression in the marrow. As such, variety of the marrow cells may dynamically affect each other and participate in hematopoiesis.

Table 1
Milestones of the osteoblastic niche

Year	Authors	Major discoveries associated with osteoblastic niche	Reference
1972	Patt HM et al.	Osteogenesis and hematopoiesis	3
1975	Lord BI et al.	Endosteal surface and hematopoiesis	4
1978	Gong JK et al.	Endosteal surface and hematopoiesis	5
1978	Schofield R	Stem cell niche concept	2
1994	Taichman RS & Emerson SG	Human osteoblast and hematopoiesis	6
2003	Calvi LM et al.	PTH	7
	Zhang J et al.	BMP	9
2004	Arai F et al.	Tie2/Ang1	8
	Visnjic D et al.	Osteoblast KO mouse	11
2005	Nilsson SK et al.	Osteopontin	86
	Stier S et al.	Osteopontin	85
2006	Adams GB et al.	Calcium-sensing receptor	34
	Kollet O et al.	Osteoclast	28
2007	Zhu J et al.	B-cell	10
	Qian H et al.	Thrombopoietin	91
	Yoshihara T et al.	Thrombopoietin	90
	Walkley CR et al.	Myeloproliferative syndrome and retinoic acid receptor γ	115
	Walkley CR et al.	Myeloproliferative syndrome and retinoblastoma protein	116
2008	Mendez-Ferrer S et al.	Circadian oscillations	24
2009	Adams GB et al.	Guanine-nucleotide-binding protein stimulatory α subunit	35
	Lo Celso C et al.	<i>In vivo</i> real-time imaging	105
	Xie Y et al.	<i>In vivo</i> real-time imaging	106
2010	Raaijmakers MH et al.	Leukemogenesis	104
	Winkler IG et al.	Macrophage and HSC mobilization	58
2011	Christopher MJ et al.	Monocytic cells and HSC mobilization	59
	Chow A et al.	Macrophage and HSC mobilization	60
	Shiozawa Y et al.	Niche competition between HSC and metastatic prostate cancer	114