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Hematopoietic niche and bone meet

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Abstract

Purpose of review—To provide an overview of the hematopoietic stem cell (HSC) niche in the bone marrow. In addition to highlighting recent advances in the field, we will also discuss components of the niche that may contribute to the development of cancer, or cancer metastases to the bone.

Recent findings—Much progress has been very recently made in the understanding of the cellular and molecular interactions in the HSC microenvironment. These recent findings point out the extraordinary complexity of the HSC microenvironment. Emerging data also suggest convergence of signals important for HSC and for leukemia or metastatic disease support.

Summary—The HSC niche comprises complex interactions between multiple cell types and molecules requiring cell-cell signaling as well as local secretion. These components can be thought of as therapeutic targets not only for HSC expansion, but also to modify behavior of hematopoietic malignancies and cancer metastases to the bone.

Keywords

hematopoietic stem cells; niche; notch

Introduction

The hematopoietic stem cell (HSC) microenvironment or niche regulates critical HSC fate decisions. Over the last year, the complexity of the niche, with its cellular and noncellular components, has continued to come into focus. Moreover, emerging evidence begins to suggest involvement of the niche in leukemia stem cells and also in bone metastatic disease derived from solid tumors such as cancers of the breast.

Cellular components of the hematopoietic stem cell niche

Schofield [1] first hypothesized that the microenvironment may provide regulatory signals to HSCs. However, the cellular components of the bone marrow microenvironment were not identified for another 20 years. Human bone-forming cells, or osteoblasts, produce important hematopoietic cytokines (granulocyte colony-stimulating factor or G-CSF, granulocyte macrophage colony-stimulating factor, and leukemia inhibitory factor) [2,3], can support HSCs *in vitro* and have the ability to maintain long-term culture initiating cells (LTC-ICs), primitive self-renewing hematopoietic cells [4]. Osteoblasts also improve engraftment during an allogeneic transplant when coinjected with HSCs [5]. To add to these early data, labeled HSCs transplanted *in vivo* preferentially engraft near the endosteum, where osteoblasts are also found [6]. Genetic data from our laboratory demonstrate that mice expressing constitutively active

parathyroid hormone (PTH) and parathyroid-related peptide receptor (PTH1R) on osteoblasts (Col1-caPTH1R mice) display an increase in osteoblast number, trabecular bone volume, and HSC number. These data establish that targeted activation of osteoblastic cells alone is sufficient to modify HSC behavior. PTH treatment also expanded HSC numbers *in vivo*. As the PTH1R is not expressed in HSCs [7•], these data also implicate a microenvironmentally mediated effect [8]. Further *in-vitro* coculture experiments demonstrated that cell-cell contact is required for PTH to expand HSCs. Taken together, these data strongly suggest that osteoblasts are key regulators of HSCs, and that close contact of osteoblasts and HSCs is required for this effect. Conditional inactivation of bone morphogenic protein receptor 1A (BMPR1A), which is also not expressed in HSCs, again resulted in an increase in both osteoblasts and HSCs [9]. The same study suggested that HSCs are in contact with spindle-shaped N-CAD-positive osteoblasts on the endosteal surface. In a different *in-vitro* model, induced osteoblast deficiency caused a loss of hematopoietic progenitors in the marrow and a transfer of hematopoiesis to the spleen [10]. These data taken together with the earlier *in-vitro* work strongly implicate osteoblasts as a major component of what has been termed the endosteal niche (Fig. 1).

More recent data have suggested that the niche provided by osteoblasts maintains HSCs in a quiescent state. A recent study demonstrated that the typical procedure used to flush the marrow from the bone leaves behind a significant population of HSCs adhered tightly to the endosteum [11•]. Endosteum-adhered HSCs isolated by grinding the bone and enzymatically digesting the bone fragments have increased proliferative and long-term engraftment potential as compared with HSCs flushed from the central marrow, suggesting that the endosteal microenvironment provides quiescence signals.

Endothelial cells are also proposed to be important in the HSC microenvironment. *In-vivo* and tissue section imaging localizes HSCs next to endothelial cells [12,13]. Also, endothelial cells secrete soluble factors that can expand human primitive hematopoietic cells *ex vivo* [14]. However, endothelial cells have not yet been shown to be a necessary regulatory component of the HSC microenvironment *in vivo*.

Osteoclasts, specialized bone resorbing cells of hematopoietic origin, have also been proposed as components of the HSC microenvironment. Activation of osteoclasts increases the mobilization of hematopoietic progenitors into the circulation [15], and inhibition of osteoclastic activity by strontium, which in fact increases bone and osteoblasts, leads to delayed hematopoietic recovery following bone marrow transplantation in mice [16•].

Neurons of the sympathetic nervous system (SNS) were also recently implicated in HSC regulation. Mice lacking ceramide galactosyltransferase cannot produce the myelin sheath component galactocerebrosides, and have defects in the mobilization response of HSCs to G-CSF. Normally, in response to G-CSF, neurons of the SNS signal to osteoblasts, which downregulate chemokine (C-X-C motif) ligand 12 (CXCL12) resulting in a subsequent mobilization of HSCs out of the marrow [17]. Moreover, chemical lesioning of the SNS with 6-hydroxydopamine (6OHDA) reduces the circadian rhythm controlled release of HSCs into the circulation. Similarly, sympathectomy of the tibiae in mice altered CXCL12 expression locally, but not in the contralateral sham-operated tibiae of the same mice [18•], suggesting that local CXCL12 expression is controlled by neural innervation.

Adipocytes are very abundant in the bone marrow, but it is not known if they provide HSCs regulation. Recent data suggest that adipocyte products can affect HSC behavior. Specifically, the adipokine adiponectin increases murine HSC proliferation whereas maintaining HSCs undifferentiated *in vitro*. In addition, deletion of the adiponectin receptor AdipoR1 reduces HSCs reconstitution potential [19•]. However, though adiponectin expression was originally

identified in adipocytes [20], it has more recently been demonstrated to also be a product of osteoblasts [21]. Whether one or both cell types are responsible for adiponectin-mediated regulation of HSCs remains to be seen.

Molecular components of the niche

Regulation of cell fate decisions is an important function of the HSC niche. Our laboratory and others have provided evidence for the Notch signaling pathway's involvement in this regulatory function. Notch signaling influences cell fate decisions in many systems and is very important during development [22,23]. In HSCs, Notch signaling is important in the regulation of self-renewal [24-29]. Specifically, the Notch ligand Jagged1 (Jag1) is sufficient for stroma-dependent expansion of human HSCs [30]. Components of Notch signaling are present on multiple cell types in the bone marrow, in fact the importance of osteoblastic and osteoclastic Notch signaling has been demonstrated by our own laboratory and others [31-39]. Notch signaling was implicated in the osteoblastic-dependent increase of HSCs in the Col1-caPTH1R mice as there was an increase in the activated form of Notch1 in HSCs, and γ -secretase inhibition attenuated the observed increase in HSCs [8]. Intermittent PTH treatment of mice increases expression of Jag1 in trabecular and endosteal spindle-shaped osteoblasts and in osteoblastic UMR106 cells through the adenylate cyclase and protein kinase A (AC/PKA) pathway [39]. Recent data challenge the importance of canonical Notch signaling in HSC maintenance [40•]. However, whether Notch signaling is required for osteoblastic HSC expansion is not known.

To allow for cell-cell interactions within the niche, HSCs must be physically attached to at least some niche components through cell adhesion mechanisms. A number of mechanisms have been implicated in HSC homing to and maintenance in the niche, such as the binding of CD44 and integrin family proteins to osteopontin, and the binding of integrins to fibronectin and vascular cell adhesion molecule 1 (VCAM1) [41-48]. Integrin binding involves heterodimers of integrin family proteins on the cell surface, which bind to extracellular ligands such as fibronectin. Signaling through integrins protects HSCs from apoptosis, maintaining HSCs in a more quiescent state [49,50]. Binding of HSCs to fibronectin through the integrin molecules very late antigen-4 (VLA-4) and VLA-5 maintains undifferentiated HSCs [51•]. Further, the retention of LTC-IC capability in human hematopoietic progenitor cells (HPCs) appears to be mediated by β -integrins [52•].

N-CAD, a member of the cadherin family of adhesion molecules is another molecule implicated in HSC regulation. Osteoblasts comprising the HSC niche were shown to express N-CAD that dimerized with N-CAD on the surface of HSCs [53,54] to form an adherens junction [9]. However, there is controversy over whether N-CAD is expressed on HSCs or not [55•,56•].

Annexin II (Anxa2) is a regulator of cell motility and a recently identified novel modulator of the HSC niche expressed by endosteal osteoblasts as well as marrow endothelium. Anxa2 inhibitors impair HSC homing and engraftment, and there is loss of HSCs in the bone marrow of Anxa2-deficient mice [57••].

An additional signaling pathway that promotes the maintenance of HSCs in the marrow is the receptor tyrosine kinase Tie2 and its ligand angiopoietin-1 (Ang-1). Tie2-positive cells in the fetal liver contain the long-term repopulating capacity of HSCs [58], and mice with a combined Tie1 and Tie2 deletion are unable to maintain HSCs in the adult marrow [59]. Osteoblasts produce Ang-1 [60] and the interaction between Tie2 expressed on HSCs, and Ang-1 results in increased quiescence, stronger adhesion to bone and greater protection from injury [53]. Tie2 and Ang-1 signaling also increases expression of β 1 Integrins as well as N-CAD [61].

Soluble components of the niche

Stromal cell-derived factor-1 (SDF1) or CXCL12 is a member of the CXC motif family of chemokines and is produced by osteoblasts and endothelial cells in the bone marrow [62]. Binding of CXCL12 to its receptor chemokine (C-X-C motif) receptor 4 (CXCR4) is important in promoting HSC homing to and retention in the bone marrow [15,63-67]. PTH regulates CXCL12 in osteoblasts [8,68] and mice lacking CXCL12 have fetal hematopoietic defects [69,70]. CXCL12 also plays a role in promoting HSC quiescence, as adult mice with an induced deletion of CXCR4 have a higher proportion of HSCs exiting G₀ and entering the cell cycle. Interestingly, knock-in studies identify a subset of cells in the marrow with abundant CXCL12 expression (named CAR, or CXCL12 abundant reticular cells). These cells are described as 'reticular' as they form a network in the marrow with long processes extending from the cell bodies. HSCs are closely associated with CAR cells, which are surrounded by endothelial cells, potentially identifying another cellular component of the HSC niche. Interestingly, CAR cells also express a high level of Jag1 [66]. Additional recent data support a central role of CXCL12 in HSC homing and retention in the bone marrow [18•,71•]. Although CXCL12 binds both CXCR4 and chemokine (C-X-C motif) receptor 7 (CXCR7) *in vivo*, data suggest that CXCR4 is the required receptor for CXCL12 in hematopoiesis [72•]. The question remains whether CXCL12 is directly responsible for promoting HSCs quiescence or whether CXCL12 directs HSCs to the niche where subsequent interactions promote quiescence.

Prostaglandin E₂ (PGE₂) is a soluble factor locally produced by osteoblasts [73] recently shown to be important in HSC regulation. PGE₂ is an arachidonic acid derivative and is an important mediator of inflammation. Treatment of zebrafish with PGE₂-related chemicals expands HSCs. Murine bone marrow treated *ex vivo* with 16,16-dimethyl-PGE₂ and subsequently transplanted into irradiated recipients increased the number of spleen colony-forming units three-fold at 12 days after transplant [74•]. Interestingly, PTH is also one of the main stimulators of PGE₂ in osteoblasts [75], suggesting PGE₂ as a potential mediator of the PTH-dependent HSC increase.

Wnt signaling regulates cell fate in many mammalian systems, with the Wnt ligand signaling through its receptor frizzled and a coreceptor low-density lipoprotein receptor-related protein (LRP). Canonical Wnt signaling activates β -catenin, which signals to the nucleus. Wnt signaling increases HSC self-renewal with overexpression of activated β -catenin expanding the pool of HSCs in long-term cultures by both phenotype and function [76,77]. Also, *in-vivo* treatment with Wnt5A conditioned medium in mice increases the engraftment of human repopulating hematopoietic cells [78]. Osteoblasts have recently been implicated in Wnt-dependent HSC regulation, similarly to the action of osteoblasts in modulating the differentiation state of mesenchymal stem cells through Wnt signaling [79]. Osteoblastic overexpression of Dickkopf1 (Dkk1), an inhibitor of canonical Wnt signaling, inhibits Wnt signaling in HSCs, causing a defect in bone marrow repopulation after transplantation. As Dkk1 is endogenously expressed by osteoblasts, this suggests a means for osteoblasts to regulate Wnt signaling in HSCs [80•]. Recent data also suggest that noncanonical Wnt signaling regulates HSC fates [81•].

Interestingly, HSCs express the calcium-sensing receptor (CaR) and mice lacking CaR have defects in HSC homing to the endosteal niche [82]. Bone has a higher extracellular calcium ion concentration than other tissues [83] suggesting that HSCs may use the CaR to migrate up a calcium ion gradient and properly home to the marrow.

The hematopoietic stem cell niche and cancer

If the niche is mainly responsible for HSC regulation, then disruption of the microenvironment should lead to dysregulation of HSC fate. Several lines of emerging data support this hypothesis. Retinoblastoma was recently shown to regulate interactions between HSC and the

microenvironment. Conditional inactivation of retinoblastoma in either HSCs or the microenvironment alone is not sufficient to cause myeloproliferation or a loss of HSCs from the bone marrow. However, deletion of the retinoblastoma gene in both the microenvironment and hematopoietic cells leads to myeloproliferation and loss of HSCs from the bone marrow [84••]. A myeloproliferative syndrome also develops when the retinoic acid receptor γ (RAR γ) is removed from the bone marrow microenvironment [85••].

Several components of the HSC niche have also been implicated in malignancies either as a niche for cancer stem cells or as fertile ground for the development of bone metastases. The $\beta 1$ integrins, whose involvement in the HSC niche we discussed earlier, have been shown to protect cells from undergoing apoptosis induced by serum starvation [86]. $\beta 1$ integrin is expressed in malignant cells and integrin-mediated adhesion of these cells can provide cell adhesion-mediated drug resistance (CAM-DR) [87-90,91•].

In addition to suggesting potential therapeutic targets, the presence of ligands that can adhere to niche components in cancer cells raises the question of whether malignant cells can occupy the same niche as HSCs. Homing signals for HSC may also home leukemia; leukemia cells express CXCR4 and have a transendothelial migration in response to CXCL12 just as normal HSCs [92,93]. Although the malignant niche for leukemia has yet to be identified, one recent study localized acute myelogenous leukemia (AML) stem cells displaying chemotherapy resistance to the bone marrow endosteum [94••]. Moreover, β -catenin conditional knockout in the hematopoietic system had impaired induction of chronic myelogenous leukemia (CML) in a breakpoint cluster region (BCR) and c-abl oncogene 1, receptor tyrosine kinase (ABL) model of leukemogenesis, owing to a deficiency of self-renewal in the CML stem cell population, suggesting that Wnt signaling is important not only in normal hematopoiesis but also in hematologic malignancies [95••].

One pathway that has been implicated in maintaining the quiescence of HSCs is the phosphatidylinositol-3-OH kinase (PI(3)K)-Akt pathway. Loss of phosphatase and tensin homologue (PTEN), a repressor of the PI(3)K-Akt pathway, causes HSC activation resulting in an initial expansion of HSCs and an eventual decline over the long term. Another effect of PTEN deletion is the rapid development of leukemia [96•,97,98].

If it is possible for malignant cells to take advantage of the antiapoptotic properties of the HSC niche, then it may be possible to disrupt these interactions and sensitize these cells to chemotherapeutics. In fact, targeting adhesion-mediating molecules such as CD44 can eradicate human AML stem cells [99]. Inhibiting $\beta 1$ integrins can overcome CAM-DR to sensitize AML cells to Ara-C [100•]. $\alpha_v\beta_3$ integrin is a mediator of breast cancer metastasis to the bone, and specific inhibition of its binding can significantly reduce bone colonization of cancer cells [101•].

Conclusion

The HSC microenvironment is critical in regulating the biology of hematopoiesis. The many exciting advances in the field of the HSC niche in the last year have improved our understanding of the many cellular and molecular interactions involved in the niche. Furthering our knowledge of the mechanisms of HSC regulation by the niche cannot only improve our ability to expand HSCs without losing their regenerative potential, but also may point to novel strategies for the treatment of hematologic malignancies and cancer metastases to bone.

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Figure 1. Proposed molecular mechanisms for osteoblastic regulation of hematopoietic stem cell differentiation and self-renewal

Osteoblasts, the niche cells in this model, express the parathyroid (PTH) and parathyroid-related peptide receptor (PTH1R). PTH signaling through PTH1R activates the phospholipase C (PLC) and adenylate cyclase (AC) and protein kinase A (PKA) pathways. PKA activation stimulates the upregulation of Jagged 1 (JAG1), which signals through Notch receptors on adjacent cells, for example, hematopoietic stem cells (HSCs), in which the Notch intracellular domain (NICD) is cleaved by γ -secretase and translocates to the nucleus in which it acts as a transcription factor promoting self-renewal. Bone morphogenetic protein (BMP) binds to BMP receptors A and B on osteoblasts and promotes quiescence in HSCs. Wnt is produced and secreted by osteoblasts. Wnt's receptor on HSC is frizzled in conjunction with the coreceptor lipoprotein receptor-related protein (LRP) 5. A signaling pathway following Wnt binding of the receptor culminates in β -catenin translocating to the nucleus and regulating genes involved in self-renewal. Osteoblasts also produce Dickkopf1 (Dkk1) an inhibitor of Wnt signaling. The receptor tyrosine kinase TIE2 is expressed on HSCs and its ligand angiopoietin 1 (ANG-1) is expressed by osteoblasts. TIE2 signaling upregulates adhesion molecules and promotes HSC maintenance in the marrow. N-cadherin (N-CAD) is expressed on osteoblasts and on some HSCs. N-CAD expressed on adjacent cells dimerizes to form adherens junctions. β 1-Integrins bind to fibronectin and vascular cell adhesion molecule 1 (VCAM1) expressed by osteoblasts to immobilize HSCs and also promote quiescence and protect from apoptosis. BMPR1A, bone morphogenetic protein receptor 1A; BMPR1B, bone morphogenetic protein receptor 1B.