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The functional interplay between systemic cancer and the hematopoietic stem cell niche

Amber J. Giles^{a,*}, Christopher D. Chien^b, Caitlin M. Reid^a, Terry J. Fry^b, Deric M. Park^a, Rosandra N. Kaplan^{b,1}, and Mark R. Gilbert^{a,1}

^aNeuro-Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

^bPediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Hematopoietic cells are increasingly recognized as playing key roles in tumor growth and metastatic progression. Although many studies have focused on the functional interaction of hematopoietic cells with tumor cells, few have examined the regulation of hematopoiesis by the hematopoietic stem cell (HSC) niche in the setting of cancer. Hematopoiesis occurs primarily in the bone marrow, and processes including expansion, mobilization, and differentiation of hematopoietic progenitors are tightly regulated by the specialized stem cell niche. Loss of niche components or the ability of stem cells to localize to the stem cell niche relieves HSCs of the restrictions imposed under normal homeostasis. In this review, we discuss how tumor-derived factors and therapeutic interventions disrupt structural and regulatory properties of the stem cell niche, resulting in niche invasion by hematopoietic malignancies, extramedullary hematopoiesis, myeloid skewing by peripheral tissue microenvironments, and lymphopenia. The key regulatory roles played by the bone marrow niche in hematopoiesis has implications for therapy-related toxicity and the successful development of immune-based therapies for cancer.

Keywords

Stem cell niche; Hematopoiesis; Microenvironment; Cancer

* Corresponding author at: 37 Convent Dr., Bldg. 37 Rm. 1142B, Bethesda, MD 20892. Tel: 301-402-8448. Fax: 301-480-4743. amber.giles@nih.gov.

¹Co-senior authors

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1. Introduction

Hematopoietic stem cells (HSCs) respond to cues from distant stress such as infection and wounding by entering a proliferative phase that ceases upon resolution of the injury (Wilson, et al., 2008). Tumors can elicit a wounding response, and patients with breast cancer whose tumors express a “wounding response signature” have markedly diminished overall and metastasis-free survival (Chang, et al., 2005). Yet, unlike a wound, tumors do not “heal,” and activated HSCs cannot return to a quiescent state. Here, we discuss how hematologic malignancies as well as distant solid tumors utilize the bone marrow’s hematopoietic stem cell niche to foster tumor growth and suppress anti-tumor immunity.

The bone marrow microenvironment is a coordinated signaling network that supports HSCs and hematopoiesis over the lifespan of the organism. Although more than one HSC niche has been defined in the bone marrow, all have the commonality to maintain the unique features of HSCs over time. The bone marrow is a cellularly diverse and dynamic structure that includes HSCs, stromal cells, vascular cells, osteolineage cells, neuronal cells, and the extracellular matrix in which these cells reside. HSC niches are formed from these elements and function to regulate hematopoiesis and maintain HSC quiescence. HSCs that cannot home to the niche lose quiescence and are unable to maintain stemness (Sugiyama, Kohara, Noda, & Nagasawa, 2006). Because the niche dynamically regulates the balance of stem cell quiescence, renewal, differentiation, and mobilization/homing, a change in stem cell activity reflects a change in the niche microenvironment. However, a better understanding of how tumors “remove the brakes” controlling HSC quiescence is needed. Determining how a tumor influences the regulatory HSC niche could help to minimize tumor-associated immune suppression thereby enhancing anti-tumor immune therapies.

2. Structural and Regulatory Properties of the HSC Niche

Osteolineage cells and mesenchymal stem cells

In mice, hematopoiesis is supported in all bones, including the femur and tibia. In contrast, the major sites of hematopoiesis in adult humans are located within the axial skeleton such as the skull, sternum, ribs, spine, sacrum, and pelvis (Ellis, 1961; Hayman, et al., 2011). HSCs are part of the soft tissue of bone that contains hematopoietic cells, blood vessels, and neurons. Soft tissue is distributed throughout interconnecting bone rods or plates called trabeculae. This matrix provides a structural foundation for soft tissue, and the opposing actions of osteoblasts and osteoclasts shape and dynamically regulate the size of the bone matrix. Trabeculae are covered with a cellular layer termed the endosteum that consists of osteoblasts, osteoprogenitor cells, and osteoclasts. These osteolineage cells create the “osteoblastic niche” and primitive stem cells are located proximal to the endosteal region (Grassinger, Haylock, Williams, Olsen, & Nilsson, 2010; Lo Celso, et al., 2009).

Increasing osteoblast number with parathyroid hormone (PTH) permits HSC expansion through Notch activation (Calvi, et al., 2003). Mice with constitutively activated osteoblasts due to a transgenic parathyroid hormone receptor have significantly increased trabecular bone area (Calvi, et al., 2001). Expanding the available HSC niche in this model likewise increases the number of long-term repopulating HSCs (Calvi, et al., 2003; J. Zhang, et al.,

2003). Expansion of the trabecular bone is accompanied by increased blood vessels such that multiple niches are created by the bone-building action of osteoblasts.

Osteolineage cells can develop from mesenchymal stem cells (MSCs), which are defined by their ability to differentiate into osteoblastic, adipocytic, and chondrocytic lineages. MSCs can be identified in mice through *Nestin* promoter-driven GFP expression, and these develop into Col2.3⁺ osteoblasts (Mendez-Ferrer, et al., 2010). Depletion of nestin-expressing cells leads to a selective reduction in long-term repopulating HSCs, linking MSCs to the endosteum and HSC maintenance. Nestin-expressing cells are found distributed around vascular structures, associated with nerve fibers, and adjacent to bone. This distribution permits MSCs to contribute to both the osteoblastic and vascular niches.

The vascular system

Blood vessels in the bone marrow differ by location, structure, and their functional relationship with HSCs. Long-term HSCs (LT-HSC) are associated with arterioles whereas venous sinusoids provide HSCs rapid access to the bloodstream during mobilization. Arterial vessels enter the bone marrow and branch into smaller arterioles that are located near the endosteum. Layers of smooth muscle, pericytes, and non-myelinating Schwann cells surround arterioles, and quiescent HSCs are associated with this vascular niche (Kunisaki, et al., 2013). Recent work in mice demonstrates that highly enriched LT-HSCs, defined as highly expressing *Hoxb5*, are localized to the abluminal surface of VE-cadherin-expressing endothelial cells (Chen, et al., 2016). This proximity facilitates signaling of membrane-bound and soluble factors from endothelial cells to HSCs.

Endothelial cells produce an array of factors, termed angiocrine factors, that support and regulate HSC activity (reviewed in (Rafii, Butler, & Ding, 2016)). Notch signaling provided through direct cell contact is required to maintain HSC repopulation capacity following *in vitro* co-culture with endothelial cells (Butler, et al., 2010). The Notch pathway also promotes arterial formation by increasing endothelial and perivascular cell numbers. Blood vessel loss caused by ageing can be reversed through activation of Notch signaling in mice, and restoration of the vascular niche is accompanied by increased HSC frequency (Kusumbe, et al., 2016). Further, the activation state of endothelial cells determines their ability to maintain HSC stemness versus inducing proliferation and differentiation (Kobayashi, et al., 2010).

In addition to protein factors, the endosteal-vascular niche provides an hypoxic microenvironment to promote quiescence of stem cells (Itkin, et al., 2016). At low oxygen tensions, cells activate adaptive transcriptional programs mediated largely by the hypoxia-inducible factor-1 α (HIF-1 α). Because molecular oxygen is a key component required for the proteasomal degradation of HIF-1 α , in hypoxic conditions, HIF-1 α is retained and activates signals that promote survival and alter energy metabolism. Direct *in vivo* measurement studies have shown the local oxygen tension in murine bone marrow to range from 31.7 to 9.9 mm Hg (4.2% to 1.3%), and oxygen levels drop as blood vessels traverse from cortical bone to the bone marrow (Spencer, et al., 2014). Hypoxic microenvironment prevents HSC differentiation and maintains cell cycle quiescence by stabilization and strict regulation of HIF-1 α (Takubo, et al., 2010). *In situ* imaging demonstrates that

hematopoietic stem and progenitor cells maintain high expression of HIF-1 α throughout the bone marrow microenvironment (Nombela-Arrieta, et al., 2013). Recent work demonstrates that even transient exposure to ambient air can have a striking negative impact on HSC numbers recovered during collection and processing of bone marrow and cord blood (Broxmeyer, O'Leary, Huang, & Mantel, 2015; Mantel, et al., 2015). Both the integrity of blood vessels and the presence of densely-populated, respiring cells in the bone marrow are needed to maintain hypoxia in the HSC niche.

Sinusoids are fed from capillaries branching from arterioles and are radially arranged around a central sinus that drains blood out of the bone marrow. The bone marrow sinusoids are created from a layer of endothelial cells and covered with discontinuous pericytes and basal lamina. Fenestrated sinusoids permit HSC and leukocyte mobilization (Itkin, et al., 2016). Because direct contact with the HSC niche is required for LT-HSC maintenance, extended periods of mobilization, as is seen in patients with cancer, reduces the stemness potential of HSCs and promotes differentiation to mature hematopoietic cells.

External Innervation of the bone marrow

Myelinated and non-myelinated nerve fibers are distributed throughout the bone marrow (K. Yamazaki & Allen, 1990). Nerve fibers generally run alongside arterioles, yet subsets are found in the sinus wall and the hematopoietic parenchymal tissue. The autonomic nervous system (ANS) influences both the stem cell niche and migration of hematopoietic cells into peripheral tissues. The sympathetic nervous system (SNS) regulates circadian rhythm of HSC mobilization (Mendez-Ferrer, Lucas, Battista, & Frenette, 2008). Circadian oscillations of HSC numbers in peripheral blood are directed by rhythmic secretion of noradrenaline that decreases CXCL12 expression in bone marrow stromal cells. CXCL12 is an essential chemokine for HSC localization to the niche and signals by binding CXCR4 on hematopoietic cells. Decreased CXCL12 levels release CXCR4-expressing HSCs from the niche, permitting cycling, mobilization, and differentiation.

Non-myelinating Schwann cells ensheath sympathetic nerves and promote stem cell quiescence. Surgical denervation of sympathetic nerves to the femur in a murine model reduces HSC numbers (but not total BM numbers) in comparison to the contralateral femur (S. Yamazaki, et al., 2011). Together, these studies demonstrate a role for the nervous system in the HSC niche by regulating HSC mobilization and quiescence according to circadian rhythm.

Hematopoietic cells in the hematopoietic stem cell niche

Hematopoietic cells are a highly migratory component of the stem cell niche, permitting the bone marrow microenvironment to communicate on a systemic level. Small numbers of HSCs regularly enter circulation and transit through peripheral tissues (Wright, Wagers, Gulati, Johnson, & Weissman, 2001). It is estimated that 1–5% of the total pool of HSCs enters the circulation each day, leaving vacant niche spaces for recirculating HSCs to home back to the bone marrow (Bhattacharya, et al., 2009). In a murine parabiosis model, partner-derived HSCs were found in spleen, lung, peripheral blood, liver, kidney, and bone marrow of the conjoined mouse, demonstrating the HSCs not only enter circulation but migrate

through peripheral tissues (Massberg, et al., 2007). Multiple factors, including growth factors, chemokines, and adhesion molecules can impact HSC activity. Interestingly, many factors that affect HSCs do not act directly upon HSCs themselves but instead influence niche components (reviewed in (Thomas, Liu, & Link, 2002)).

Neutrophils are abundant, short-lived, highly migratory myeloid cells that are sensitive to tissue damage or infection. Neutrophil production is dependent on granulocyte colony stimulating factor (G-CSF). G-CSFR-deficient mice have diminished numbers of neutrophils, hematopoietic progenitors, and increased sensitivity of neutrophils to apoptosis (Liu, Wu, Wesselschmidt, Kornaga, & Link, 1996). Similar to HSCs, neutrophil release from the bone marrow is tightly regulated. Under homeostatic conditions, bone marrow-derived neutrophils follow circadian rhythm of emigration from and homing back to the bone marrow that mirrors HSC migration (Casanova-Acebes, et al., 2013). Aged neutrophils express low levels of CD62L and high levels of CXCR4 that increases homing to the bone marrow. Within the bone marrow, CD169⁺ macrophages engulf dying neutrophils. Following neutrophil engulfment, these CD169⁺ macrophages, in turn, inhibit CXCL12 production by niche stromal cells, thereby releasing HSCs from the niche. Conversely, in the absence of neutrophil engulfment, CD169⁺ macrophages promote HSC retention by increasing CXCL12 production from nestin⁺ MSCs. Depletion of CD169⁺ macrophages reduces the ability of BM stromal cells to produce CXCL12, resulting in HSC mobilization and linking the circadian rhythm of neutrophil migration to niche regulation of HSC mobilization (Chow, et al., 2011).

Cytotoxic CD8 T cells (CTLs) responding to infection home to the bone marrow where they stimulate myelopoiesis by acting upon MSCs (Schurch, Riether, & Ochsenbein, 2014). MSCs respond to interferon gamma secreted from CTLs and, in turn, produce IL-6. IL-6 production then acts on hematopoiesis to promote myelopoiesis. In this manner, CTLs responding to an infection in a peripheral organ can indirectly stimulate innate immune production within the bone marrow by communicating with the HSC niche.

3. Modulation of the HSC niche by hematopoietic malignancy

Since hematopoietic malignancies arise from the bone marrow, it is not surprising that they are affected by the bone marrow microenvironment. Numerous murine models and correlative human studies support the concept that the BM niche can actively participate in leukemic initiation and progression (reviewed in (Boulais & Frenette, 2015; Calvi & Link, 2015; Krause & Scadden, 2015; Schepers, Campbell, & Passegue, 2015)). The converse is also true; hematologic malignancies can alter the stromal compartments of the bone marrow, such as the HSC niche, to the detriment of the HSC and the benefit of blood diseases (reviewed in (Duhrsen & Hossfeld, 1996)). The predominant body of work in this area has been examined in the setting of leukemia, though it has been reported that HSC function is impaired in multiple myeloma patients (Bruns, et al., 2012). In this section of the review we will focus on work describing how blood cancers affect HSC niche components and the mechanisms utilized to induce these changes.

Chemokines and growth factors

As described previously, CXCL12 has been identified across many studies to be a critical HSC niche factor. Diagnosis using bone marrow and peripheral blood serum from patients with pediatric acute lymphoblastic leukemia (ALL) demonstrated that CXCL12 was significantly lower than samples from normal donors (van den Berk, et al., 2014). Advances in intravital microscopy have allowed for the visualization of geographic location of both niche and leukemia cells in their native bone marrow environment. One particular study characterized ALL cells with HSC niche factors stem cell factor (SCF) and CXCL12 (Colmone, et al., 2008). By imaging calvaria in mice injected with NALM-6 ALL, they showed that ALL recruited CD34 HSCs from normal HSC niches into the leukemic areas of the bone through the upregulation of SCF and downregulation of CXCL12. CD34 cells in ALL bearing mice also were impaired in their ability to mobilize in response to G-CSF; suggesting that normal HSC regulation had been completely disrupted by leukemia.

Acute myeloid leukemia (AML) has been shown to induce CXCL12 deficiencies in HSC niche cells. An MLL-AF9 AML model induced changes in nestin⁺ CD51⁺ PDGFR α ⁺ mesenchymal stem and progenitor cells such as lowering expression of HSC retention and maintenance genes CXCL12, SCF, Vcam1, and Angpt1. As a result, the frequency of LT-HSCs decreased and HSCs were mobilized from the bone marrow to the periphery (Hanoun, et al., 2014). Another study observed decreased CXCL12 in AML patient BM plasma as well as cell culture supernatants of AML patient-isolated MSCs despite the fact that the MSC contained elevated intracellular CXCL12 relative to normal donor MSCs (Ge, Hou, Liu, Zhu, & Liu, 2011). In the context of a chronic myelogenous leukemia (CML) model, CXCL12 downregulation in the BM was seen concurrently with an upregulation of macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , interleukin-1 α (IL-1 α), IL-1 β , tumor necrosis factor- α (TNF- α), IL-6, and G-CSF suggesting a shift of the normal HSC niche one that favors the development of leukemia (B. Zhang, et al., 2012). This was confirmed by the loss of LT-HSC in the BM with a simultaneous gain of LT-HSC in the spleen in leukemic mice, which is consistent with the finding of decreased CXCL12 in the BM.

Other factors released in leukemic bone marrow also contribute to the changing of the HSC niche. G-CSF and granulocyte macrophage-colony stimulating factor (GM-CSF) were inducible from endothelial cells cultured in medium conditioned by patient-derived AML. This effect was inhibited by IL-1 blockade implying that IL-1 secreted by AML cells regulates the HSC niche alteration (Griffin, et al., 1987). Placental growth factor (PIGF) was identified as a new bone marrow stromal cell factor that can be induced by BCR/Abl positive chronic myelogenous leukemia (CML) (Schmidt, et al., 2011). Interestingly, this upregulation was dependent on NF- κ B and direct interaction of the AML with stromal cells unlike other models where only paracrine factors mediated the effects. These studies suggest a role for inflammatory related cytokines in the progression of leukemia.

Niche cell population alterations

Reports show that the balance and function of bone marrow stromal cells is altered in the context of leukemia. In a Notch-induced T cell ALL model, bone marrow-derived MSCs

isolated from T cell ALL-bearing mice have decreased expression of hematopoietic stem and progenitor cell (HSPC) niche-associated genes and impaired functionality of Lineage⁻Sca-1⁺cKit⁺ (LSK) stem cells after short term co-culture (Lim, et al., 2016). More specifically, there are examples of changes in bone marrow stromal cells in the osteoblastic, neural, and endothelial compartments. In both CML and AML, there is an increased number of osteoblastic cells in the bone marrow. CML induced by BCR/Abl caused bone marrow stromal cells to skew to osteoblastic cells (OBC) (Schepers, et al., 2013). Interestingly, wild-type mice transplanted with BCR/Abl cells that developed into CML demonstrated an increase in OBC indicating that the shift to osteoblastic lineage cells was independent of leukemic transgene expression and rather due to a leukemia-induced mechanism. This OBC expansion was driven by MIP-1 α , thrombopoietin (TPO), and direct leukemia:OBC cell contact. These OBC were functionally compromised in their ability to support HSC expansion *in vitro* and *in vivo*, further supporting the concept that leukemic cells hijack normal bone marrow stromal cells to establish a new bone marrow microenvironment that is favorable to leukemia cells. This skewing to the osteoblastic lineage of MSC was also seen in an MLL-AF9 AML model (Hanoun, et al., 2014). However, in a separate BCR/Abl driven murine AML model there was a decrease in osteoblast lineage cells with a decrease in bone mineral density and an increase in bone remodeling osteoclasts (Frisch, et al., 2012). Despite these differences the mouse AML model showed significant upregulation of MIP-1 α in both the mouse model and AML patient samples suggesting MIP-1 α as an important mediator of the inhibitory effect on OBC.

In contrast to myeloid leukemia studies, mice with Notch-induced T cell ALL displayed a decrement of OBC numbers concurrently with loss of known the HSC niche factor CXCL12 and decreased numbers of LSK cells in engraftment studies (W. Wang, et al., 2016). T cell ALL cells were shown to compete with HSPC for perivascular niches, and the decrease in OBC was reversible when Notch inhibitors were administered to the model indicating that the effects observed were driven by the leukemia.

Neurons that constitute the nerves that innervate the bone marrow are also transformed during the disease pathogenesis of hematopoietic malignancies. A myeloproliferative neoplasm model (MPN) driven by Janus Kinase 2 V617F (JAK2V617F) showed that sympathetic nerve fibers, Schwann cells and nestin⁺ MSCs were consistently reduced in the BM of JAK2V617F MPN patients and mice (Arranz, et al., 2014). Treatment with β 3 adrenergic agonists restored nestin⁺ MSCs and decreased leukemia stem cells in the bone marrow. Because this model utilized HSCs that expressed the mutant JAK2, it is unclear what would happen to a normal HSC in the context of MPN. Similar results were obtained in an AML model where Adr β 3 expression was decreased in MSPCs and Adr β 2 inhibition lead to increased leukemic infiltration in the bone marrow (Hanoun, et al., 2014).

Lastly, endothelial cells are manipulated by blood cancers as observed in patient samples from ALL, CLL, AML, and CML (Aguayo, et al., 2000; Perez-Atayde, et al., 1997). These patients had significantly higher numbers of microvesicles per field in leukemic areas of the bone marrow with concomitant detection of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and TNF- α in the plasma and urine of patients. More recent studies have tried to

mechanistically tie the clinical observation of increased angiogenic factors as being related to the leukemia. Primary ALL patient samples when transplanted into immunocompromised mice showed evidence of neoangiogenesis, and BM plasma from ALL patients which expressed increased levels of VEGF and bFGF relative to normal control BM samples induced neovascularization in matrigel plugs (Veiga, Costa, Sallan, Nadler, & Cardoso, 2006). Co-culture of AML patient samples with either human lung or dermal microvascular endothelial cells caused increased proliferation of endothelial cells in the majority of cases tested and was associated with pro-angiogenic CXCL8 and inversely correlated with anti-angiogenic factors CXCL9, CXCL10, and CXCL11 (Hatfield, et al., 2009). Though specific effects on HSCs were not shown in these studies it is likely that changes in bone marrow vascularity would induce changes HSC niches.

Tumor-derived exosomes

Examples of leukemia-secreted exosomes come from CML and AML cells. Exosomes from the CML cell line LAMA84 induce interleukin-8 (IL-8) expression from HS5 human bone marrow stromal cells. This was shown to be a CML survival factor, suggesting that CML microvesicles could aid in the creation of bone marrow niches favorable to leukemia growth (Corrado, et al., 2014). Further work using LAMA84-derived exosomes showed that they contain miR126 which targets the HSC niche factors CXCL12 and VCAM1 (Taverna, et al., 2014). The major phenotype determined with these studies was that leukemic cell progression was likely enhanced by modulating HSC niche components. How these CML driven changes affect the niche and function of HSCs is yet to be determined. AML cell lines such as HEL, HL-60, U937, and MOLM-14 as well as primary AML samples were shown to secrete exosomes which were incorporated into OP9 murine bone marrow stromal cells (Huan, et al., 2013). These exosomes had unique RNA and microRNA profiles and were likely the mechanism whereby they exert their effects. A subsequent study demonstrated that AML microvesicles impacted HSCs via direct and indirect effects (Huan, et al., 2015). AML exosomes caused downregulation of CXCR4 and c-Kit in HSCs and CXCL12 and SCF in OP9 stromal cells. AML bearing mice were also shown to have fewer colony forming cells from the bone marrow suggesting a loss of HSCs.

4. Modulation of the HSC niche by solid tumors

It is well-established that solid tumors accumulate immune-suppressive hematopoietic lineages at the primary tumor site and future metastatic sites. This hematopoietic response relies upon increased HSC cycling to supply immature progenitors (Balducci & Hardy, 1992). Elevated levels of circulating hematopoietic stem and progenitor cells correlate with higher tumor stage and decreased progression-free survival (Giles, et al., 2016; Wu, et al., 2014). One mechanism by which these circulating hematopoietic progenitors can promote metastatic progression is by developing into myeloid lineages that foster an immune suppressive microenvironment at future metastatic sites (Giles, et al., 2016). Cancer cells orchestrate this hematopoietic response through expression of surface proteins, secretion of chemokines and growth factors, and exosome shedding. These factors can have direct effects on local tissue stroma and be carried through circulation to mediate effects at distant sites. As with hematopoietic malignancies, these factors can influence hematopoiesis directly by

acting upon HSCs and hematopoietic progenitors or indirectly by signaling to components of the HSC niche.

Chemokines and Growth factors

G-CSF is pharmacologically used to mobilize HSCs, and it is commonly produced by tumor cells. Although HSCs express G-CSFR, receptor expression on HSCs is not necessary for their mobilization by G-CSF. By using a series of bone marrow chimera transplants, it was found that G-CSF acts in *trans* on hematopoietic cells to mediate hematopoietic stem and progenitor cell mobilization (Liu, Poursine-Laurent, & Link, 2000). Rather than directly influencing homing, G-CSF disrupts the endosteum by depleting bone marrow macrophages (Winkler, et al., 2010) and by inhibiting proliferation of *Nes*-GFP⁺ cells and other stromal lineages (Mendez-Ferrer, et al., 2010). G-CSF also significantly decreases transcription of key HSC-modulating factors that regulate HSC homing and survival, including Cxcl12, Scl, Angpt1, Il7, and Vcam1 in *Nes*-GFP⁺ cells.

Osteopontin (OPN) production by osteoblasts is associated with negative regulation of HSC numbers under homeostasis (Stier, et al., 2005). However, secreted osteopontin (OPN) is found in the plasma of patients with various tumors and is associated with increased metastatic risk (Ng, et al., 2015; Thoms, et al., 2012). Interestingly, OPN does not appear to influence the primary tumor size or ability to incorporate bone marrow-derived cells. Instead, OPN influences the growth of distant metastatic lesions by activating the bone marrow (Fremder, et al., 2014; McAllister, et al., 2008). OPN mediates macrophage migration, activation, and survival (Lund, et al., 2013; Weber, et al., 2002). In this context, it is possible that OPN indirectly affects HSC proliferation in the setting of cancer by inducing macrophage chemotaxis and cytokine production in the HSC niche.

VEGF is often produced by solid tumors and promotes angiogenesis. Interestingly, production of VEGF by tumor cells results in a loss of sinusoidal bone marrow vasculature area (O'Donnell, et al., 2016). Perturbations in bone marrow sinusoids could reduce HSC niche area and promote mobilization of HSCs from the bone marrow. In this manner, tumor-mediated HSC proliferation and mobilization would result from a reduction in the available vascular niche.

IL-8 levels are elevated in certain types of cancer, such as breast cancer and colorectal cancer (Ning, et al., 2011). Administration of IL-8 to mice and non-human primates mobilizes hematopoietic progenitor cells within 5 minutes (Laterveer, Lindley, Hamilton, Willemze, & Fibbe, 1995; Laterveer, et al., 1996). However, HSCs do not express the IL-8 receptor (J. Wang, et al., 1996). Neutrophils respond to IL-8 by releasing matrix metalloproteinases (MMPs), which cleave extracellular matrix molecules (van der Sluijs, Baert, & Ploemacher, 1994). Neutralizing MMP-9 antibodies are sufficient to block IL-8-mediated HSC mobilization in rhesus monkeys (Pruijt, et al., 1999). Anti-GR1 antibody-mediated neutropenia was sufficient to block IL-8 induced hematopoietic stem and progenitor cell mobilization in mice; yet MMP-9 deficient mice mobilized hematopoietic stem and progenitor cells to a similar extent as wild-type controls (Pruijt, et al., 2002). This suggests that compensatory signaling pathways exist in the HSC niche to regulate HSPC mobilization in response to tumor-derived factors such as IL-8.

Tumor-derived exosomes

Exosomes are a type of small, secreted membrane vesicle (30–100nm) that are endocytic in origin and secreted by many cell types (Thery, Zitvogel, & Amigorena, 2002). Exosomes are increasingly recognized as a means of cell-to-cell communication during normal physiology and response to injury. Exosomes laterally transfer information, including protein, DNA, mRNA, and microRNA, between different cell types (Kahlert, et al., 2014; Valadi, et al., 2007). Tumor cells release high quantities of exosomes, and patients with tumors have a larger quantity of exosomes in circulation than healthy controls. Circulating exosomes can promote tumor growth and metastatic progression by acting upon hematopoietic cells in the bone marrow (Peinado, et al., 2012). Intriguingly, lethally irradiated mice reconstituted with tumor exosome-educated bone marrow have greater number of bone marrow-derived cells in the primary tumor and greater metastatic burden. This suggests that tumor exosomes mediate direct, long-term changes in bone marrow progenitors. The depth of tumor exosome penetration into the HSC stem hierarchy could determine whether increased metastasis is due to tumor exosome-mediated changes in hematopoietic cycling or lineage differentiation programs.

Tumor-derived exosomes have been recently shown to mediate production of inflammatory cytokines from MSC, including IL-6, IL-8, and MCP-1 *in vitro* (X. Li, et al., 2016). This suggests a role for exosomes in indirectly influencing the HSC by acting upon MSCs. In order to better understand the biology and possible therapeutic targeting, it would be important to dissect the role of tumor-derived exosomes on MSCs in the bone marrow niche from the impact on HSCs and hematopoiesis.

5. Considerations of anti-cancer therapy on HSC niche

Anti-cancer treatment regimens often involve cytotoxic chemotherapeutic drugs and radiation therapy that can result in irreversible chronic bone marrow damage and lymphopenia. Ionizing radiation induces long-term bone marrow injury through direct effects on HSCs and the HSC niche. MSCs and blood vessels in the bone marrow are destroyed by free radicals, and HSCs are no longer found in these regions of the bone marrow (Cao, et al., 2011). Radiation and chemotherapy cause bone marrow to lose the hypoxic microenvironments that are essential for niches to maintain HSC quiescence, possibly due to the loss of oxygen-consuming hematopoietic cells (Spencer, et al., 2014). Venous sinusoids depend upon the structural support provided by hematopoietic cells, making these structures particularly sensitive to hypocellularity resulting from cytotoxic agents and radiation. Regeneration of venous sinusoids through VEGFR2 activation is essential for HSC engraftment and hematopoietic reconstitution following irradiation in mice (Hooper, et al., 2009).

The chemotherapeutic drugs cisplatin and vincristine each reduce the density of SNS fibers in the bone marrow, resulting in impaired hematopoietic regeneration (Lucas, et al., 2013). This study also demonstrated the neuro-protective ability of 4-methylcatechol to shield the bone marrow from chemotherapy and accelerate bone marrow regeneration after transplantation. This highlights the potential to protect and modulate the HSC niche in the setting of anti-cancer treatment.

G-CSF is used to expand and mobilize HSCs in the bone marrow. It is administered concurrently with dose-escalating chemotherapy or following chemotherapy to maintain circulating white blood cell numbers. However, G-CSF disrupts osteolineage cells in the HSC niche that function to maintain HSC quiescence and prevent stem cell exhaustion. Therefore, the hematopoietic recovery achieved by G-CSF following chemotherapy may be short-lived (van Os, Robinson, Sheridan, & Mauch, 2000). Further, concurrent chemotherapy and G-CSF significantly impairs autologous stem cell transplant engraftment, suggesting that the long-term effects of G-CSF treatment on the HSC niche should be considered for patients with cancer (Freedman, et al., 1997). Administration of PTH, previously shown to expand the osteoblastic niche area, is capable of maintaining HSC cell numbers in mice treated with both G-CSF and cyclophosphamide (Adams, et al., 2007; S. Li, et al., 2015), suggesting a means to protect HSC niche integrity during chemotherapeutic treatment.

6. Conclusions

HSCs, the source of all hematopoietic lineages, are tightly regulated by the HSC niche. Cancer induces profound changes in the hematopoietic profile stemming from deregulated HSC niche components. This leads to loss of HSC quiescence along with increased cycling and mobilization. Residency time of hematopoietic cells in peripheral tissues is prolonged, disrupting the normal circadian rhythm migratory feedback loop and further prompting the HSC niche to increase hematopoietic output. This leads to accumulation of immune suppressive hematopoietic lineages in the tumor microenvironment and future metastatic sites. These events combine to result in the overwhelming increase in immune suppressive hematopoietic subsets that promote tumor growth and metastatic progression.

Niche fitness plays an important role in patient health and immune function. As immune therapy progresses, the hematopoietic stem cell niche will likely emerge as an important mediator of the hematopoietic response. Many questions remain regarding the relative number of HSC niches and how these communicate with local and distant tissue microenvironments, such as the pre-metastatic niche. As our understanding of the key signaling molecules and pathways in these environments expands it will provide insights into timing and type of therapy or therapeutic strategies that would limit the harmful impact of therapies on this essential microenvironment.

Abbreviations

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
BM	bone marrow
CML	chronic myelogenous leukemia
G-CSF	granulocyte colony stimulating factor
HIF-1α	hypoxia-inducible factor -1 α

HSC	hematopoietic stem cell
IL	Interleukin
LT-HSC	long-term hematopoietic stem cell
MSC	mesenchymal stem cell
OBC	osteoblastic cells
OPN	osteopontin
PTH	parathyroid hormone
VEGF	vascular endothelial growth factor

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Figure 1. Structural and regulatory properties of the HSC niche

The endosteum is formed from osteoblasts, osteoprogenitor cells, and osteoclasts. This creates the “osteoblastic niche” and quiescent HSCs are found proximal to this region. Arterial vessels surrounded by a thin layer of smooth muscle cells and nestin-expressing perivascular MSCs enter the bone and branch into smaller arterioles that run adjacent to the endosteum. Non-myelinating Schwann cells ensheath sympathetic nerve fibers that run alongside arterioles, innervating smooth muscle cells and nestin-expressing perivascular MSCs. Highly enriched HSCs are localized to the abluminal surface of VE-cadherin-expressing endothelial cells. Arterioles drain into sinusoids that contain wide intercellular clefts that allow for cell migration. Oxygen levels decrease as blood vessels travel deeper into bone. CD169⁺ macrophages contribute to HSC retention by promoting CXCL12 production from MSCs. However, upon engulfing aged neutrophils that home back to the bone marrow, CXCL12 production is inhibited, permitting HSC mobilization and contributing to circadian oscillations of HSCs. Interferon gamma-expressing cytotoxic CD8⁺ T cells stimulate myelopoiesis by inducing MSCs to secrete IL-6, linking adaptive and innate immune responses.

Table 1

Growth Factors	Source	Effect on bone marrow	Reference
VEGF	Tumor	Reduction of bone marrow sinusoidal vessels	O'Donnell, et al., 2016
Notch ligand	Osteoblasts; Endothelial cells	ALL competes with HSCs for ligand, reducing HSC repopulation potential	W. Wang, et al., 2016
Cytokines	Source	Effect on bone marrow	Reference
IL-1	AML	Increases expression of G-CSF and GM-CSF from endothelial cells	Griffin, et al., 1987
IL-6	MSCs; endothelial cells	Increased expression due to CML or tumor-derived exosomes promotes myeloid lineage differentiation.	X. Li, et al., 2016
IL-8/CXCL-8	bone marrow stromal cells; tumor	Increased expression due to tumor-derived exosomes results in HSC mobilization	X. Li, et al., 2016; Corrado, et al., 2014
CXCL12	bone marrow stromal cells; endothelial cells	Reduced expression impairs HSC homing to niche.	B. Zhang, et al., 2012; Hanoun, et al., 2014
Kit ligand/SCF	nestin-expressing mesenchymal stem and progenitor cells	Decreased expression results in loss of HSC repopulation potential	Hanoun, et al., 2014; Huan, et al., 2015
MIP-1	Bone marrow macrophages	Increased expression due to CML	B. Zhang, et al., 2012
G-CSF	nestin-expressing mesenchymal stem and progenitor cells; tumor cells	Decreases expression of HSC homing and survival factors	Mendez-Ferrer, et al., 2010
Other	Source	Effect on bone marrow	Reference
Adrenergic signaling	Sympathetic nerve fibers; Schwann cells; nestin-expressing MSCs	Sympathetic neuropathy results in skewing of perivascular mesenchymal stem and progenitor cells to immature osteoblasts.	Hanoun, et al., 2014
Angiopoietin-1	Osteoblasts; nestin-expressing MSCs	Reduced expression impairs HSC quiescence	Hanoun, et al., 2014
Osteopontin	Osteoblasts; secreted by tumor	OPN from tumor activates bone marrow and enhances metastatic potential of tumors.	McAllister, et al., 2008; Lund, et al. 2013