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Hematopoietic Stem Cell and Its Bone Marrow Niche

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Abstract

Stem cells do not thrive without their niche. The bone marrow microenvironment is where hematopoietic stem cells maintain their cell state while receiving physiological input to modify their activity in response to changing physiological demands. The complexity of the bone marrow microenvironment is being unraveled and indicates that multiple different cell types contribute to the regulation of stem and progenitor cells. Further, it is becoming evident that the bone marrow represents a composite of niches with different components and different functional roles in hematopoiesis. It is now evident that alterations in specific stromal cells that comprise the bone marrow microenvironment can contribute to hematologic pathology. In this chapter, we will review the history of the niche concept, evolving information about its components and how niche dysfunction may contribute to disease.

1. INTRODUCTION

In the human body, the softest organ of all, the blood, is ironically encapsulated by the hardest—the skeleton. The bone marrow (BM) microenvironment with its specialized anatomy and interconnected vasculature provides a sanctuary where hematopoietic stem cells (HSCs) reside, are maintained, and differentiate into multiple blood lineages. The bone marrow niche is a critical microenvironment that regulates many stem cell activities including self-renewal, mobilization, engraftment, and lineage differentiation. The importance of the hematopoietic niche is highlighted by evidence, showing that mutations of the nonhematopoietic cells of the marrow microenvironment are sufficient to cause hematopoietic neoplasia. This chapter will provide an overview of the niche concept, the functional and anatomical relationships of cells within the bone marrow, and summarize the recent literature of the hematopoietic niche in blood diseases.

2. EVOLUTION OF THE STEM CELL NICHE CONCEPT

Formulation of the niche hypothesis indirectly relates to the first experimental demonstration of tissue stem cells. Exploring how ionizing radiation affects mammalian cells, Till and McCulloch laid out the first experimental demonstration of the “self-renewing unit” in the

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hematopoietic system (Till & McCulloch, 1961). They accomplished this by irradiating mice with a dose that would kill the animals within 30 days if the mice did not receive a transplant of fresh cells. Transplantation of donor bone marrow cells conferred radioprotection. Not only did the donor cells reconstitute the bone marrow of the recipients, but they also gave rise to nodules in the spleen. Using chromosome breaks as durable genetic markers of individual transplanted cells, Till, McCulloch, and colleagues elegantly demonstrated that these spleen nodules were myeloid, erythroid, and lymphoid cell containing colonies derived from single BM donor cells. They proposed that these “self-renewing units” must be the primitive cell source that were capable of giving rise to multiple lineages and regenerated the whole hematopoietic system, and therefore they hypothesized these cells to be stem cells (Worton, McCulloch, & Till, 1969). Although it was not known until much later that these cells were not stem cells but progenitors, this break-through experiment laid the experimental groundwork that led to the discovery of the long-term repopulating HSC many years later.

Following the study of Till and McCulloch, Schofield was puzzled by the fact that transplantation of bone marrow cells derived from either young or old wild-type mice into W/W^v mice (having a c-kit mutation) was able to reconstitute hematopoiesis indefinitely. However, cells that formed colonies in the spleen upon transplantation, the ones that Till and McCulloch defined as stem cells and named colony-forming units-spleen cells (CFU-Ss), could not reconstitute W/W^v mice and had a limited serial passage capacity. Schofield hypothesized that CFU-Ss were not stem cells but reflected a different cell state due to the spleen in which they resided. Around that time, Dexter and colleagues published a landmark paper describing the requirement of a bone marrow stromal feeder layer in order to sustain primitive hematopoietic cells in ex vivo cultures (Dexter, Allen, & Lajtha, 1977). Driven by his own experimental observation and the findings of his laboratory neighbor and colleague, Dexter, Schofield articulated the stem cell niche concept in 1978. He concluded that stem cells needed to reside in the bone marrow to retain their stemness. Once they left the niche, they could become CFU-Ss, but at the expense of their immortality (Fig. 1). He proposed that when these cells reoccupied the niche, they could regain their stemness (Schofield, 1978). Schofield’s proposal presented the basic concepts of a stem cell niche: (1) a defined anatomical site, (2) a location where stem cells could be maintained and reproduce, (3) a place where stem cell differentiation was inhibited, and (4) a defined space that limited the number of stem cells. He had no experimental evidence to prove these new concepts and was challenged by McCulloch and others, but he was correct.

3. LOCATION MATTERS

Schofield proposed that the bone marrow microenvironment was special, providing an HSC niche. His concept was well supported by comparative biology where it is clear that land animals with bones have hematopoiesis in those bones. Therefore, the quest for defining HSC niches reasonably began with assessment of how bone cells influenced blood cell production. This was first shown by two studies. One where osteoblastic cells were constitutively expressing an activated parathyroid hormone receptor in the mouse. The animals had an increase in hematopoietic stem and progenitor cell (HSPC) (Calvi et al., 2003). The other where the bone morphogenic protein receptor type IA (BMPRIA) was

conditionally depleted in hematopoietic cells using the Mx1-Cre promotor (Zhang et al., 2003). In both studies, they observed increased number of osteolineage cells, correlated with increased number of HSPCs (Lineage^{Lo}Sca⁺c-Kit⁺ cells). When interpreting these data, one must be mindful that at the time when these studies were conducted, Lineage^{Lo}Sca⁺c-Kit⁺ (LKS) cells were used to immunophenotypically define HSCs. We now know that within the LKS population, progenitor cells represent the major population, while HSCs only constitute a minority. Second, in these studies, hematopoietic reconstitution of transplanted hematopoietic cells was tracked up to 12 weeks. It is now known that progenitors contribute to the hematopoietic system up to 12 weeks, and therefore to assess the function of long-term HSCs, a longer period of reconstitution is needed. Nonetheless, these studies were the first in vivo demonstration that a specific microenvironmental cell could modulate HSPC, thereby validated Schofield's niche concept.

Pursuit of more specific definition of niche components remains a main driving force in the field. In addition to selected genetic changes in cell populations to assess the role of specific cells and specific genes in the HSPC niche, imaging experiments provided important information. They have defined certain principles. Under homeostatic conditions, HSCs reside in close proximity to blood vessels. Following radiation conditioning, bone marrow microvessels are disrupted and transplanted HSCs engraft in periendosteal sites.

Identification of transplanted cell localization was conducted by a number of laboratories. In vivo monitoring of HSPC after transplant over time has been particularly useful for fully defining that infused cells arrive at particular CXCL12 abundant positions in the microvasculature (Sipkins et al., 2005). The cells then either remain there or migrate to periendosteal position with more immature HSPCs in more proximate relationship to osteoblastic cells and more actively cycling cells at a greater distance (Lo Celso et al., 2009). These events occur within minutes to hours of HSPC infusion in the mouse conditioned with lethal irradiation.

Under homeostatic conditions, similar live animal in vivo imaging has not been possible due to lack of HSPC-specific fluorescent tags. Immunohistochemistry has revealed, however, that CD150⁺, CD48⁻, and CD41⁻ (SLAM markers) HSCs localize adjacent to microvascular sinusoids and are scattered diffusely throughout the marrow (Kiel, Yilmaz, Iwashita, Terhorst, & Morrison, 2005). Further, other studies reported that HSCs were close to cells expressing high amounts of CXCL12, also known as the CXCL12-abundant reticular (CAR) cells. These cells surround the sinusoidal endothelial cells located near the endosteum (Sugiyama, Kohara, Noda, & Nagasawa, 2006).

Laser scanning cytometry then enabled imaging of HSCs throughout the longitude of the long bones in mice. Early application of this technology revealed that HSPCs preferentially localize in endosteal zones (Nombela-Arrieta et al., 2013), where the majority of cells closely interact with sinusoidal and nonsinusoidal BM microvessels. Combined whole-mount confocal immunofluorescence imaging and computational modeling have been used to demonstrate a three-dimensional association in the mouse BM among vascular structures, stromal cells, and HSCs. Quiescent HSCs were found to associate with small arterioles preferentially found in the periendosteal bone marrow (Kunisaki et al., 2013). These

arterioles are ensheathed by NG2⁺ pericytes, distinct from the sinusoid-associated leptin receptor (LEPR⁺) cells. Interestingly, when HSCs enter into cell cycle, they were reported to redistribute from NG2⁺ periarteriolar niches to LEPR⁺ perisinusoidal sites. Depletion of NG2⁺ cells caused HSC cycling and reduced functional long-term repopulating HSCs in the BM, implicating that the arteriolar NG2⁺ cells control HSC quiescence. However, the debate continues as where precisely HSCs reside and whether there are specific locations that provide quiescence signals.

Other issues are more clear based on recent studies using probes detecting elemental oxygen. The marrow space is hypoxic with progressively decreasing O₂ levels from endosteum to central sinus (Spencer et al., 2014). Further, timelapse and knockout mouse models have revealed that HSCs are highly dynamic and transit between different niches upon stimulation. They are mobile, regularly entering and exiting the circulation (Wright, Wagers, Gulati, Johnson, & Weissman, 2001). While much prior work has provided single snapshots of HSC within the BM microenvironment, it is likely that we will soon be able to acquire a dynamic view of HSC interactions with regulatory partners in the BM under homeostatic and, ultimately, disease conditions.

However, one should be mindful that most in vivo data are obtained through mouse models. There are major differences that exist between rodents and humans with regard to location of hematopoiesis. For instance, in mice, all bones support hematopoiesis, and the long bones are the sites in which the BM hematopoiesis is studied. In adult humans, the axial skeleton (the cranium, sternum, ribs, vertebra, and ilium) is the major site of blood cell production, and the red marrow in the long bones is replaced by hematopoietically inactive yellow marrow between 5 and 7 years of age, with the exception of the proximal regions of the long bones (Kricun, 1985). Second, the distinction between bone marrow under homeostasis vs postconditioning must be emphasized. Total body irradiation or myeloablative cytotoxic reagents are necessary in a transplant setting to permit HSC engraftment. However, these interventions are known to markedly disrupt the marrow environment. In particular, they destroy sinusoidal vessels and lead to marked hemorrhage within the marrow space. Therefore, the architecture and cellular composition of the marrow microenvironment are very different postconditioning compared with homeostasis. Third, the niche is a dynamic entity. Many of the experimental approaches focused on a static moment of cell–cell interaction may not be reflective of changes that occur over time or under different physiological contexts. This is more problematic if molecular modification of cells is driven by constitutively active Cre recombinases, as these models almost certainly associate with compensatory changes and changes in additional cell types than those putatively expressing the Cre at the point of study.

4. CELLULAR PARTICIPANTS IN THE BONE MARROW MICROENVIRONMENT

A number of cell populations have been examined for their roles in the HSPC bone marrow niche.

4.1 Osteolineage Cells

Early HSCs and bone marrow stromal cell coculture experiments suggested that osteoblasts provide support for primitive hematopoietic cells. The first in vivo demonstration of osteolineage cell requirement for hematopoiesis came from two groups simultaneously using different mouse models. One group conditionally deleted the *BMPRIA* in hematopoietic cells using the *Mx1-Cre* promotor (Zhang et al., 2003), while another group used a 2.3 kb promoter of collagen *I α* (*Col(I) α 2.3*) to target osteoblast-specific expression of the parathyroid hormone protein (PTH) or PTH-related protein receptor (Calvi et al., 2003). While these studies illustrate genetic modification of osteolineage cells alters HSPC number and downstream hematopoiesis, it was recently shown that certain osteolineage subtypes (including the *Osx*⁺ and *Ocn*⁺ cells) are partially dispensable (50–70% cell ablation) in adult BM without compromising LT-HSC maintenance under homeostasis (Yu et al., 2015). Nevertheless, different osteolineage cells play distinct roles in several important hematopoietic processes.

First, mature osteolineage cells appear to be important in granulocyte colony-stimulating factor (G-CSF)-mediated HSPC mobilization. In studies where osteocalcin (*Ocn*)-expressing cells were depleted, G-CSF induced mobilization was markedly compromised (Ferraro et al., 2011). Further, different osteolineage subtypes create distinct lymphopoietic niches. Specifically, it was described that the deletion of early osteolineage cells (including the osterix-expressing (*Osx*⁺) and *Col(I) α 2.3*-expressing osteolineage cell populations) or of *CXCL12* expression from these cells impacted B progenitor cell maturation (Greenbaum et al., 2013; Visnjic et al., 2004; Zhu et al., 2007) and reduced mature B and T cell numbers (Ding & Morrison, 2013). Targeted deletion of mature osteocalcin-expressing (*Ocn*⁺) osteolineage cells resulted in a loss of T lineage cells. These animals have decreased T competent common lymphoid progenitors (CLPs) with a minimal effect on B cell biased CLP (*Ly6D*⁺) (Yu et al., 2015) due to a defective generation of C–C chemokine receptor type 7 dependent thymic-seeding progenitor cells. The T cell lymphopenic effect seen in the *Ocn*⁺ cell-depleted animals could be recapitulated by selective deletion of the Notch ligand, delta-like protein 4 expression in *Ocn*⁺ cells, or of its receptor and downstream signaling molecules in primitive hematopoietic cells. Another study using genetic mouse model to achieve endogenous depletion of osteocytes demonstrated systemic disruption of metabolism and similar loss of T cells (Sato et al., 2013). Altogether, these studies show that early- and late-stage osteolineage cells play very different roles in supporting B and T lymphopoiesis. While *Osx*⁺ osteoprogenitors have a more pronounced role in the maturation of B cell progenitors, *Ocn*⁺ cells modestly alter B cell production while distinctly affecting T cell specification. These data redefine bone cells as important immune participants that regulate specific production of cells of the adaptive immune system. The emerging model is one of a highly interrelated system with “intermediate” populations of both skeleton and blood having very specific interactions (Fig. 2). Whether these interactions are perturbed is of particular relevance in settings where specific subsets of cells are deficient as in particular blood disorders, or most problematically in the lack of T cell generation postallogeic bone marrow transplantation. Future directions will involve exploring these heterologous cell interactions in malignant processes such as lymphoma, leukemia, and bone metastatic processes.

4.2 Perivascular Cells

The frequent localization of HSCs near blood vessels led to the hypothesis that perivascular regions may promote HSC maintenance. Mesenchymal stromal cells (MSCs) are cells that localize adjacent to blood vessels in bone marrow and are thought to be primitive cells capable of differentiation into osteolineage cells, chondrocytes, and adipocytes. Different markers have been used to define MSCs, including CD146 (Sacchetti et al., 2007) and CD271 (Matsuoka et al., 2015) in humans, CXCL12-GFP (Sugiyama et al., 2006), Nestin-GFP (Mendez-Ferrer et al., 2010), LEPR (Ding, Saunders, Enikolopov, & Morrison, 2012), Prx-1-Cre (Greenbaum et al., 2013), and Mx-1-Cre (Park et al., 2012). These cells are capable of differentiation into osteolineage cells in mice and express factors that promote HSC maintenance.

CAR cells adjacent to sinusoids were first shown to colocalize with HSCs by immunohistochemistry on bone marrow sections (Sugiyama et al., 2006). Ablation of these CAR cells resulted in severe impairment of adipogenic and osteogenic capacity of bone marrow cells, leading to reduced production of cytokines SCF (stem cell factor) and CXCL12 in the bone marrow, and eventually decreased cycling lymphoid and erythroid progenitors and HSCs (Omatsu et al., 2010). Using a different marker to identify MSCs, Frenette and colleagues found that cells that express a Nestin-GFP transgene similarly localize around blood vessels throughout the bone marrow, express high levels of SCF and CXCL12, and are indispensable for HSC maintenance (Mendez-Ferrer et al., 2010). Fibroblast activation protein (FAP) is expressed by stromal cells with many MSC characteristics, including expression of SCF, CXCL12, PDGFR α , Nestin, Sca-1, and CD51 (Morikawa et al., 2009; Pinho et al., 2013). Genetic depletion of FAP⁺ cells led to reduction of osteolineage cells, impaired B lymphopoiesis and erythropoiesis, and eventually cachexia and anemia. Despite our incomplete characterization of the heterogeneity of the different mesenchymal stem cell subtypes, their relationships, and the respective markers to identify these cells, these studies provide strong evidence that mesenchymal cells are critical components that maintain HSPCs in the BM niche.

4.3 Endothelial Cells

Endothelial cells are known to secrete specific paracrine growth factors, cytokines (Kobayashi et al., 2010), and adhesion molecules such as E-selectin, P-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (Mazo et al., 1998; Rafii, Mohle, Shapiro, Frey, & Moore, 1997) to regulate the homeostasis of HSPCs. For instance, bone marrow sinusoidal endothelial cells had been shown to express Notch ligands to promote HSPC expansion in culture (Butler et al., 2010). E-selectin has been suggested to be exclusively expressed by endothelial cells in the bone marrow and E-selectin blockade in mice improved HSC survival upon chemotherapeutic agents or irradiation treatment (Winkler et al., 2012). One pathway by which endothelial cells balance the rate of proliferation vs differentiation of HSPCs is possibly through recruiting mTOR and activating AKT signaling. Selective activation of AKT in the endothelial cells of adult mice increased the number of colony-forming units in the spleen and CD34⁺Flt3⁺ LKS HSPCs with LT-HSC activity in the bone marrow and accelerated hematopoietic recovery (Kobayashi et al., 2010).

Another approach to study the niche cells was to examine which cell populations were the key sources of factors that promote HSC maintenance in vivo. Conditional deletion of the SCF from mesenchymal stem cells (LEPR-expressing) or endothelial cells (Tie2-expressing) depleted endogenous HSCs (Ding et al., 2012). Likewise, conditional deletion of CXCL12 from Tie2⁺ endothelial cells reduced HSC number without inducing stem cell mobilization (Greenbaum et al., 2013).

Therefore, endothelial cell is another regulatory component of the HSC niche and appear to have pleotropic effect on HSC properties, although these studies did not address whether the effect was direct or indirect in vivo.

4.4 Adipocytes

In mouse and human, the percentage of adipocytic cells within the bone marrow, the so-called “fatty marrow,” gradually increases as the organism ages, but the function of HSC decreases inversely. This observation led scientists to question whether BM adipocytes influence hematopoiesis. In a mouse model of lipoatrophy, which was genetically incapable of forming adipocytes, and in another model that inhibits adipogenesis by treating mice with the peroxisome proliferator-activated receptor gamma (PPAR γ) inhibitor bisphenol-A-diglycidyl-ether, one group demonstrated that bone marrow engraftment by HSCs was accelerated in the fatless mice or in the PPAR γ inhibitor-treated mice compared to wild type or untreated controls (Naveiras et al., 2009). These data implicate adipocytes as negative regulator of hematopoiesis, although the resultant alteration in marrow space or the number of other stromal cells in those mouse models could have indirectly contributed to this result.

4.5 Macrophages

Macrophage was recently found to be another player in the niche mediating HSC mobilization through signaling the MSCs. In studying the mobilization of HSCs into the bloodstream in response to G-CSF, scientists found that there was concomitant loss of macrophages and reduced bone formation (Winkler et al., 2010). In vivo depletion of macrophages, in either macrophage Fas-induced apoptosis (Mafia) transgenic mice or by administration of clodronate-loaded liposomes into wild-type mice, led to marked reduction of endosteal osteoblasts and HSC mobilization into blood, mimicking the phenotype observed during G-CSF administration. Specifically, it is the CD169⁺ macrophage but not the Gr-1^{hi} monocyte that promotes the retention of HSCs in the endosteal niche. CD169⁺ macrophage secretes oncostatin M (OSM), which in turn induces Nestin⁺ cells and possibly other MSCs to express CXCL12 via a mitogen-activated protein kinase kinase-p38-signal transducer and activator of a transcription 3-dependent pathway (Albiero et al., 2015; Chow et al., 2011). CXCL12 engagement with CXCR4 receptor on HSC cell surface is crucial for HSC retention in the BM niche. In mice and patients with diabetes, there were increased CD169⁺ macrophages and elevated OSM in the BM, and reduced HSC mobilization. OSM neutralization (Albiero et al., 2015) and CXCR4 antagonist (Chow et al., 2011) in diabetic mice have been shown to improve G-CSF-induced HSC mobilization, suggesting that targeting CD169⁺ macrophages or antagonizing OSM may be a strategy to restore niche function, particularly in diabetic settings.

Interestingly, CD169⁺ macrophage also seems to be a participant that supports late erythroid lineage development. Specific depletion of CD169⁺ macrophages in mice reduced the number of erythroblasts in the BM but did not lead to overt anemia under homeostatic conditions. However, in the case of hemolytic anemia, CD169⁺ macrophage depletion led to myeloablation, impaired erythropoietic recovery, and eventually acute blood loss. In contrast, in a JAK2(V617F)-driven mouse model of polycythemia vera, macrophage deletion normalized the erythroid compartment, suggesting that erythropoiesis in polycythemia vera is mediated through macrophages in the BM microenvironment.

Altogether, these studies show that the macrophage is an indispensable cellular participant needed for HSC retention in the BM, through signaling other bone marrow stromal cells, and it also serves as a niche cell that supports erythropoiesis through a yet unknown pathway. The macrophage studies are excellent examples illustrating the concept of one cell type playing multiple roles in supporting different hematopoietic cell processes in the BM, through very different mechanisms.

5. THE SYMPATHETIC NERVOUS SYSTEM

Sensory and autonomic innervation of the bone marrow not only regulates physiological homeostatic trafficking of hematopoietic cells but also enables them to respond to acute stress signals. HSCs and progenitors circulate in the bloodstream under homeostatic conditions, exhibiting robust circadian fluctuations in antiphase with the expression of the chemokine CXCL12 by stromal cells in the BM niche. Sympathetic tone delivered by nerves in the bone marrow is transmitted to stromal cells through the beta(3)-adrenergic receptor, leading to reduced Sp1 transcription factor and the rapid downregulation of CXCL12 expressed by these stromal cells (Mendez-Ferrer, Lucas, Battista, & Frenette, 2008). As such, the cyclical circadian release of HSCs into the bloodstream and the concomitant reduction of CXCL12 in the BM microenvironment are maintained by core genes of the molecular clock through noradrenaline secretion from the sympathetic nervous system (SNS).

The concept that signals from the SNS are critical for HSPC egress from the bone marrow was further illustrated by several studies using complementary mouse models targeting this pathway. In one study, nonmyelinating Schwann cells were proven to be responsible for maintaining HSC hibernation through inducing TGF- β production from stromal cells. Mice with autonomic nerve denervation, reduced number of TGF- β -producing cells, or TGF- β type II receptor-deficient HSCs all exhibited reduced level of Smad activation in HSCs, loss of HSCs from BM, and impaired long-term repopulation activity (Yamazaki et al., 2011). In a different study, UDP-galactose ceramide:galactosyltransferase-deficient (Cgt^{-/-}) mice exhibited aberrant nerve conduction and displayed no HSPC egress from the BM following G-CSF or fucoidan administration (Katayama et al., 2006). Pharmacological or genetic ablation of adrenergic neurotransmission in mice indicates that norepinephrine (NE) signaling controls G-CSF-induced osteolineage cell suppression, CXCL12 downregulation from stromal cells, and HSPC mobilization. In contrast, administration of a β (2) adrenergic agonist enhances mobilization in both NE-deficient and control mice, suggesting that the SNS is responsible for the attraction of stem cells to their niche. It was found in later studies

that the cells that relay the adrenergic signals from the SNS to HSPCs appear to be nestin⁺ MSCs. First, nestin⁺ cells display a number of genes that regulate HSPC mobilization, including CXCL12, Kitl, Angpt1, IL7, and Vcam-1, and the expression of these genes decreases upon G-CSF stimulation or $\beta(3)$ adrenoceptor activation. Second, purified HSCs were found to home near nestin⁺ MSCs in the bone marrow of lethally irradiated mice. Third, administration of parathyroid hormone, an agent that favors HSPC engraftment in the BM (Adams et al., 2007), doubles the number of HSC-associated nestin⁺ MSCs and their osteoblastic differentiation. Most importantly, in vivo nestin⁺ cell depletion significantly reduces BM homing of HSPCs (Mendez-Ferrer et al., 2010).

It is known that some diabetic patients fail to achieve sufficient CD34⁺ HSPCs upon G-CSF stimulation. Using mouse models of type 1 (streptozotocin-induced) and type 2 (db/db) diabetes, scientists found that HSPCs that were unable to egress upon G-CSF treatment and localized aberrantly in the BM niche with altered pattern of catecholaminergic nerve termini (Ferraro et al., 2011). This altered sympathetic innervation was associated with decreased sensitization of beta-adrenergic receptor in nestin-GFP⁺ cells, which was in turn unable to downmodulate CXCL12 in response to G-CSF treatment. The HPSC mobilization defect in diabetic mouse models can be rescued by pharmacological inhibition of the interaction of CXCL12 with its receptor CXCR4 using the receptor antagonist AMD3100.

In summary, these studies described an unprecedented pathway of mesenchymal cell control of stem cell trafficking—through the SNS. Sympathetic nerve fiber was an indispensable component in relaying signals from the niche cells to the HSCs and regulates stem cell trafficking during hibernation, circadian oscillation, and G-CSF-induced mobilization. These data offer new intervention opportunities to overcome poor HPSC mobilization in clinical and disease settings including diabetes.

6. HSC MOBILIZATION FROM THE BM NICHE

HSCs for HSC transplantation (HSCT) can be derived from BM, peripheral blood, or umbilical cord blood. Harvesting adult HSCs from peripheral blood is relatively easy and much less painful than from the BM, but the frequency of HSCs in the peripheral blood is low. Mobilization of HSCs from the BM niche has thus been explored as a mechanism to enhance HSC frequency in the donor's peripheral blood for the purpose of HSCT.

G-CSF was first described as an agent that causes HSC mobilization in 1988 (Socinski et al., 1988) and has since been the mainstay in the clinic for HSC mobilization, yet its mechanisms of action are still not fully understood today. Given that HSCs themselves do not express the G-CSF receptor but rather cells of the myelomonocytic series, including macrophages and osteomacs, express the receptor, it was hypothesized that mobilization through G-CSF is indirect and several mechanisms have been proposed. Increasing evidence suggests that one of the pathways involves activation of osteomacs and macrophages, which in turn regulates neighboring stromal cells to decrease SDF-1 production (Albiero et al., 2015; Chow et al., 2011). Reduced stromal SDF-1 leads to attenuated anchoring of HSCs to the BM stroma through the CXCR4 receptor expressed on HSCs. Additional pathways involve degradation of VCAM-1, fibronectin, and OPN, leading to reduced cellular adhesion

of HSC to stromal cells through very late antigen 4 (VLA-4) adhesion receptor (Bonig & Papayannopoulou, 2012; Levesque, Takamatsu, Nilsson, Haylock, & Simmons, 2001). Recently, two independent studies show that G-CSF-induced mobilization signal is relayed through the SNS, as pharmacological intervention or sympathectomy with β -blockers results in impaired HSC mobilization in mice (Ferraro et al., 2011; Katayama et al., 2006). Therefore, the mechanism of G-CSF-induced HSC mobilization is indirect, multifactorial, and likely involves multiple stromal components.

SDF-1 (CXCL12) is a CXC chemokine, secreted by various BM stromal cells, including CAR, nestin⁺ cells, osteolineage cells, and endothelial cells. The interaction of SDF-1 with its receptor CXCR4 on HSC plays a pivotal role in HSC retention to the niche, as treatment of human cells with a CXCR4 antibody prevented their engraftment in a xenotransplantation model (Peled et al., 1999), and treatment of mice with neutralizing anti-SDF-1 or anti-CXCR4 antibodies inhibited HSC mobilization (Petit et al., 2002). These discoveries led to the development of plerixafor (AMD3100), a US Food and Drug Administration (FDA) approved CXCR4 mobilizing agent, that disrupts the SDF-1/CXCR4 axis in a synergistic way to G-CSF.

The β 1 integrin, VLA-4, is expressed by HSCs and facilitates their adhesion on BM stroma through interaction with vascular cell adhesion molecule 1 (VCAM-1), fibronectin, and OPN. Studies in mice, primates, and humans have shown that administration of the anti-VLA-4 monoclonal antibody (natalizumab) or blockade of its ligands leads to a potent mobilization effect (Bonig & Papayannopoulou, 2012; Bonig & Papayannopoulou, 2013; Zohren et al., 2008). The disruption of the VLA-4/VCAM-1 axis, however, is dependent upon induction by G-CSF and CXCR4 inhibitors.

The interaction between CD44 on HSC and hyaluronic acid, a component of the extracellular matrix protein, is essential for the retention of HSC in the BM. The most hyaluronic acid-rich regions of the bone marrow are the endosteum and the sinusoidal endothelium. CD44 cleavage on HSC membrane is associated with mobilization and is dependent on the abundance of its proteolytic enzyme, metalloproteinase (MMP). G-CSF administration has been documented to increase MMP on CD34⁺ cells, resulting in cleavage of CD44 on HSC cell surface and HSC egress from the BM (Avigdor et al., 2004; Vagima et al., 2009). In addition, homing of HSC is impaired upon administration of anti-CD44 antibodies in mice, highlighting the importance of CD44 in HSC retention at the niche.

Heparan sulfate proteoglycans (HSPGs) are thought to serve as extracellular binding partners for secreted signaling molecules. In hematopoiesis, HSPG has been theorized a potential role in bone marrow compartmentalization, by forming matrices that retain the right cytokines and/or morphogens to the vicinity of HSPCs. It has been hypothesized that gradients of cytokine and morphogen maintained by interaction with locally secreted matrix proteins are essential in sustaining the HSPC niche. Indeed, conditional deletion of the *Ext1* gene, a glycosyltransferase essential for the synthesis of heparin sulfate, in Mx1⁺ BM stromal cells affected HSPC localization and retention in the BM, in part by modulating VCAM-1 (Saez et al., 2014). This mechanistic pathway was further confirmed by the data that showed pharmacologic inhibition of endogenous heparin sulfate enhanced the

mobilization efficacy of G-CSF, including in the setting of mobilization resistance in a murine diabetes model. HSPCs mobilized by heparin sulfate inhibition were shown to have improved reconstitution ability in primary and secondary transplanted mice compared to G-CSF-mobilized HSPCs. Finally, HSPCs engrafted efficiently in the BM of *Ext1*-deficient mice without cytotoxic conditioning. These findings suggest that targeting heparin sulfate or the enzyme, *Ext1*, may provide novel means to mobilize HSCs of improved quality or to achieve noncytotoxic conditioning, both are of critical value for clinical HSC transplantation.

7. NICHE OF HEMATOPOIETIC MALIGNANCIES

In analogy to normal HSCs, cancer-initiating cells also require a proper niche to thrive and expand. Recent discoveries of the niche's critical involvement in the induction and development of hematologic neoplasia further stressed the importance of defining and characterizing the stromal subpopulations that support these pathologic processes.

It is well documented that in diseased and aged conditions, not only the hematopoietic cells exhibit genetic alterations, but massive reorganization of the BM niche also takes place. These alterations within the BM microenvironment include osteoporosis in aged individuals and increased vascularization in hematopoietic neoplasms and leukemias. In the case of multiple myeloma, it is well established that myeloma patients experience acute bone lesions. There was activation of osteoclastogenesis and suppression of osteoblastic activity due to an unbalanced RANK/OPG ratio, and dysregulation of a variety of inflammatory cytokines such as MIP-1 α , MIP-1 β , IL-3, IL-6, IL-1 β , TNF- α , HGF, VEGF, OPN, and SDF-1 α in the myeloma niche (Reagan, Liaw, Rosen, & Ghobrial, 2015). While these were thought to be evidence of disease induced changes in the microenvironment, it was not known until the first two reports that showed genetic mutation in the BM niche itself can induce irreversible intrinsic changes in the hematopoietic cells. In these studies, mice deficient for the retinoblastoma protein (RB) (Walkley, Shea, Sims, Purton, & Orkin, 2007a) or the retinoic acid receptor gamma (RAR γ) (Walkley et al., 2007b) developed myelodysplasia. Unexpectedly, transplant studies revealed that the disease was not intrinsic to the hematopoietic cells themselves because BM from wild-type mice transplanted into an RB^{-/-} or RAR γ ^{-/-} recipient mice developed the myeloproliferative disease, suggesting for the first time that mutation in the BM microenvironment can confer neoplastic development of hematopoietic cells. Following these studies, our laboratory discovered that deletion of the RNase III endonuclease, Dicer1, in BM osteoprogenitor cells but not in mature osteoblasts resulted in myelodysplasia and predisposition to acute myelogenous leukemia (AML) (Raaijmakers et al., 2010). Interestingly, mice with osteoprogenitor-specific Dicer1 deletion had reduced expression of Sbds, the gene mutated in Shwachman–Bodian–Diamond syndrome, corroborating with a human disease condition characterized by bone marrow failure and leukemia predisposition. Deletion of Sbds in mouse osteoprogenitors further confirmed the development of myelodysplasia, proving the perturbation of specific subsets of mesenchymal cells can disorient the differentiation, proliferation, and apoptosis of parenchymal hematopoietic cells. Confirming the concept of niche-induced leukemia, another group found that activating mutation of β -catenin in osteoblasts led to the development of AML with cell autonomous disease progression (Kode et al., 2014), likely

through elevated Notch signaling in hematopoietic progenitor cells. Collectively, these in vivo mouse studies indicate that mutation in the niche can be the sole cause of hematopoietic disorders, and that primary stromal cell dysfunction can result in secondary hematologic pathologies.

It appears that the BM niche not only capable of inducing leukemia, but during disease state, it remodels itself to further support the growth of specific leukemias. One of the factors secreted by osteoblasts that differentially regulate the development of chronic myelogenous leukemia (CML) and AML is transforming growth factor beta 1 (TGF- β 1). Osteoblast-specific activation of the PTH receptor attenuated BCR-ABL1-induced CML-like myeloproliferative neoplasia while enhanced MLL-AF9-induced AML in mouse models, possibly through opposing effects of increased TGF- β 1 on the respective leukemia initiating cells (Krause et al., 2013). This study illustrates the distinct niche requirement for CML and AML and suggests that niche modulation may have very specific outcome depending on the type of the disease.

Aberrant or malignant hematopoietic cells can also confer signals to the stromal cells to initiate niche remodeling that reinforces leukemic growth. In the context of myeloproliferative neoplasia, leukemic blasts make direct contact with MSCs and produce TPO and CCL3 to stimulate MSCs to overproduce functionally altered osteolineage cells (Scheepers et al., 2013). These myeloproliferative neoplasia-expanded osteolineage cells have altered gene expression profile and accumulate in the BM cavity as myelofibrotic cells. They exhibit impaired ability to support normal HSCs, yet produce inflammatory signals that effectively support leukemic development. In a BCR/ABL-induced model of CML, leukemic cells produce proinflammatory cytokines to alter the microenvironment such that it favors disease development (Welner et al., 2015). Interestingly, the normal bystander cells acquired gene expression profiles resembling their malignant counterparts. IL-6 is a strong candidate responsible for most of these changes. IL-6 produced by CML cells induces normal hematopoietic progenitors to suppress lymphoid lineage differentiation but expand myeloid lineage cells (Reynaud et al., 2011; Welner et al., 2015), a phenomenon highly reinforcing disease progression. Mice deficient of IL-6 receptor on hematopoietic cells or treated with anti-IL-6 neutralizing antibody rescued the skewed lymphoid and myeloid cell ratio and attenuated the CML (Welner et al., 2015), suggesting that the differentiation bias caused by IL-6 is reversible and blockade of IL-6 may be a potential mechanism to treat CML.

As mentioned in the earlier section, SNS regulates normal HSC retention in the niche by delivering sympathetic tone to stromal cells in the bone marrow through the β (3)-adrenergic receptor, causing a downregulation of CXCL12 expressed by stromal cells and attenuated engagement of CXCR4 receptor on HSCs. The end result is reduced lodgment of HSCs in the BM microenvironment. SNS also participates in AML development, although through a different mechanism. In a mouse model of SNS neuropathy, BM infiltration with MLL-AF9 AML cells was significantly enhanced. Development of AML was found to disrupt SNS nerves, decrease the number of HSC-maintaining NG2⁺ periaarteriolar niche cells, reduce production of cytokines that support normal HSC maintenance, and prime nestin⁺ niche cells for osteoblastic differentiation. But most importantly, transplantation of MLL-AF9 leukemic

cells into mice treated with adrenergic receptor $\beta 2$ but not $\beta 3$ antagonist, or mice deficient of adrenergic receptor $\beta 2$, significantly augmented leukemic cell engraftment and number in the bone marrow in comparison to control mice. These studies indicate that malignant cells are capable of reorganizing its surrounding niche architecture and supportive cellular neighbors to transform the normal HSC niche into a cancer-supporting niche. The current developing concept is that there is a coevolution that exists between cancer cells and the niche, and that there are dynamic interactions between the two. This implies that the BM environment adapts according to the different stages of the disease. These data stress the importance of considering the corresponding changes that occur in the niche while targeting cancer. Cutting off environmental support may improve the efficacy to eradicate cancer cells.

8. PERSPECTIVES

Decades of experimentation have validated the importance of the niche in stem cell regulation and revealed some fundamental insights about the cellular and molecular participants involved. Studies of mutated BM niches have revealed unprecedented information on niche-induced and niche-maintained hematologic neoplasia and leukemias. Yet the complete list of the stromal participants in the normal and leukemic hematopoietic niches remains incomplete and many outstanding questions regarding the biological nature of HSC under homeostasis and in aberrant conditions are unanswered. Here, we will provide our perspectives on some future directions.

Dissecting the complexity of the niche components within the BM remains a high order priority. To date, our knowledge of the regulatory cell types that exist in the bone marrow “stroma” is limited to a few that can be targeted using existing cell markers, and the list of niche participants is far from complete. Single-cell sequencing and single-cell proteomic technologies will enable the study of cellular heterogeneity in the stroma independent of any existing knowledge about these cells.

HSCs in the fetal liver expand on a daily basis but maintained at a steady level under homeostasis in the bone marrow. Differences in the cellular components of these two sites may provide insights to achieve HSC expansion for transplantation therapies. Much can be learned from parallel stem cell systems in different tissues or even species. Comparison of stromal components among different organs from the adult may inform us about shared characteristics or functions among these niches. As aforementioned, the physiological site, the cellular, and the molecular components of the BM niche in mouse and human do not directly translate. Comparing homologous niches between human and rodent may answer why certain leukemias develop in one species but not the other, and also aid our effort in translating therapeutic molecular targets from mice to human.

New technologies will enable us to pinpoint stem cell engagement with particular cellular components in the niche with increased precision and study their dynamic interactions over time. For instance, we can now achieve a highly precise, short-range cell perturbation using laser capture (unpublished). Gene expression can be manipulated in single cells in vivo using nanowave or fluorescent protein conversion laser technologies. Multifluorescent transgenic mouse models will allow simultaneous labeling and tracing of multiple stromal

subtypes in an animal. Conventional gene knockout studies using homologous recombination enable the knockout of one gene at a time. However, if the gene is involved in a compensatory network, this strategy may not create a visible phenotype. The emerging CRISPR/Cas9 technology can disrupt multiple genes simultaneously in a cell or in a mouse, allowing the study of multifactorial pathways that will only manifest a phenotype when multiple members of the gene network are perturbed.

Recent literature reporting niche involvement in myelodysplasia and leukemias only revealed the tip of the iceberg. How the niche contributes to hematologic malignancies or bone marrow diseases remains largely unexplored. Is there competition of anatomical space or niche resources between normal and malignant cells? Is there a hierarchy of components in the niche that confers cancer cell survival? Can specific intervention of these niche interactions improve therapeutic outcome?

The promises of unraveling niche contribution to normal and disease physiology are warranted with new tools at hand. It is anticipated that with better understanding of what specific subsets of hematopoietic cells are governing by what stromal elements, the ability to engineer a particular hematopoietic outcome will become feasible. Further, gaining a “systems” level understanding of the hematopoietic bone marrow may provide useful paradigms for studying other tissue niches with the long-term goal of ultimately manipulating better regenerative processes in contexts of disease.

ABBREVIATIONS

AML	acute myelogenous leukemia
BM	bone marrow
BMPRIA	bone morphogenic protein receptor type IA
CAR	CXCL12-abundant reticular
CFU-S	colony-forming unit-spleen cell
CLP	common lymphoid progenitor
CML	chronic myelogenous leukemia
Col(I)α2.3	2.3 kb promoter of collagen Ia
CXCL12	CXC chemokine ligand (CXCL) 12
CXCR4	receptor for CXC chemokine ligand (CXCL) 12
FAP	fibroblast activation protein
FDA	US Food and Drug Administration
G-CSF	granulocyte colony-stimulating factor
HSC	hematopoietic stem cell

HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem and progenitor cell
HSPG	heparan sulfate proteoglycan
LEPR	leptin receptor
LKS	Lineage ^{Lo} Sca ⁺ c-Kit ⁺
MMP	metalloproteinase
NE	norepinephrine
Ocn	osteocalcin
OSM	oncostatin M
Osx	osterix
PPARγ	peroxisome proliferator-activated receptor gamma
PTH	parathyroid hormone
RARγ	retinoic acid receptor gamma
RB	retinoblastoma protein
SCF	stem cell factor
SDF-1	stromal cell-derived factor 1
SLAM markers	Lineage, c-Kit, Sca, CD150, CD48, CD41
SNS	sympathetic nervous system
TGF-β1	transforming growth factor beta 1
VCAM-1	vascular cell adhesion molecule 1
VLA-4	very late antigen 4

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Fig. 1.

The hypothetical view of the stem cell niche from R. Schofield. The stem cell can become the daughter cell, CFU-S, once the stem cell leaves its niche. But if the stem cell finds and reoccupies the niche, it will itself return to its stem cell state. *Adopted from Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells, 4, 7–25.*

Fig. 2.

The hematopoietic stem cell niche. Extrinsic signals including growth factors, cytokines, morphogens, extracellular matrix proteins, and adhesion molecules regulate the behavior of hematopoietic stem cells (HSCs) and protect them from exhaustion, although few have been pinpointed to derive from a specific cell source. Recent advances in flow cytometry, transgenic mouse models, and intravital microscopy have revolutionized our understanding of the BM niche and how it controls various stem cell behaviors including self-renewal, proliferation, differentiation, lineage commitment, and mobilization. Enlisted in the diagram are cellular and molecular participants that have been experimentally shown to impact different HSC activities through promoter-specific Cre deletion or overexpression. But the diagram is far from complete and represents an active area of ongoing investigation.