



## Concise Review: Paracrine Functions of Vascular Niche Cells in Regulating Hematopoietic Stem Cell Fate

JOSHUA P. SASINE,<sup>a</sup> KELLY T. YEO,<sup>a</sup> JOHN P. CHUTE<sup>a,b,c</sup>

**Key Words.** Hematopoietic stem cells • Endothelial cell • Bone marrow stromal cells • Self-renewal • Microenvironment

### ABSTRACT

The functions of endothelial cells (ECs) in regulating oxygen delivery, nutrient exchange, coagulation, and transit of inflammatory cells throughout the body are well-established. ECs have also been shown to regulate the maintenance and regeneration of organ-specific stem cells in mammals. In the hematopoietic system, hematopoietic stem cells (HSCs) are dependent on signals from the bone marrow (BM) vascular niche for their maintenance and regeneration after myelosuppressive injury. Recent studies have demonstrated the essential functions of BM ECs and perivascular stromal cells in regulating these processes. In the present study, we summarize the current understanding of the role of BM ECs and perivascular cells in regulating HSC maintenance and regeneration and highlight the contribution of newly discovered EC-derived paracrine factors that regulate HSC fate. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:482–489

### SIGNIFICANCE STATEMENT

Recent studies have shown that blood stem cells require signals from the bone marrow microenvironment or niche for their survival and regeneration. In the present study, the current understanding of the interactions between blood stem cells and niche cells is summarized and the potential for niche-derived secreted factors as therapeutic agents for regenerative medicine is highlighted.

### INTRODUCTION

Vascular endothelial cells (ECs) constitute the intimal lining of arteries, capillaries, and veins within the human vasculature and serve critical roles in the delivery of oxygen and nutrients, in the regulation of coagulation, and as the gatekeepers to inflammatory cells for entry into tissues [1–3]. During development, ECs are necessary for the formation of the hematopoietic system and the emergence of definitive hematopoietic stem cells (HSCs) [4–7]. In the past decade, discoveries from several laboratories have demonstrated a critical function for ECs in the adult HSC niche, such that ECs regulate HSC maintenance and regeneration after stress or injury [8–14], in concert with other microenvironmental cues. Exquisite microanatomical studies have confirmed that the most primitive HSCs reside in bone marrow (BM) vascular and perivascular niches [15, 16]. BM ECs regulate HSC fate, in part, via the elaboration of paracrine factors [17–19]. Commensurate with these observations, recent studies have suggested that ECs also regulate the self-renewal and regeneration of stem cells

in nonhematopoietic organs, including the liver, brain, lung, and skin [1, 20–29]. In the present study, we describe the hematopoietic-specific activities of BM ECs and highlight the potential for EC-derived paracrine mechanisms to be translated into regenerative therapies for patients.

### ENDOTHELIAL CELLS AND HEMATOPOIETIC DEVELOPMENT

Within the embryo, development of the hematopoietic system occurs in multiple locations at various stages, including the extraembryonic yolk sac, fetal liver, spleen, and, finally, adult BM. Evidence exists for two discrete anatomic origins of hematopoietic activity, one extraembryonic and one intraembryonic [30]. During the primitive streak stage, groups of mesodermal cells in the yolk sac form the extraembryonic blood islands (mouse gestational age E7.5). The peripheral cells differentiate into EC precursors (angioblasts), and the inner cells become primitive blood cells [31]. The two lineages are so closely related in time and space that this led to a hypothesis regarding a common precursor, the “hemangioblast.” Blast

<sup>a</sup>Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine, <sup>b</sup>Jonsson Comprehensive Cancer Center, and <sup>c</sup>Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, California, USA

Correspondence: John P. Chute, M.D., Division of Hematology/Oncology, Department of Medicine, University of California, Los Angeles, 545 OHRC Building, 617 Charles E. Young Drive South, Los Angeles, California 90095, USA.

Telephone: 310-206-3037; e-mail: jchute@mednet.ucla.edu  
Received May 31, 2016; accepted for publication August 4, 2016; published Online First on September 13, 2016.

©AlphaMed Press  
1066-5099/2016/\$20.00/0  
<http://dx.doi.org/10.5966/sctm.2016-0254>

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colony tracing studies later provided direct evidence for the existence of the hemangioblast [5].

Before 1975, it was widely accepted that definitive HSCs originated from the yolk sac blood islands. To evaluate this dogma, Françoise Dieterlen-Lièvre took advantage of the ability to distinguish chick and quail cell nuclei and grafted 2-day-old quail embryos on to chick yolk sacs of comparable developmental stages, before or shortly after the establishment of vascularization [32]. The chick cells never gave rise to adult hematopoiesis; thus, although it contributes to embryonic hematopoiesis, the source of definitive long-term HSCs was concluded to be intraembryonic.

The first definitive HSC known to be able to fully reconstitute the hematopoietic system on transplantation was identified in the aorto-gonad-mesonephros region (AGM) in mice and humans [5, 33–35]. Yet other studies have suggested that yolk sac cells can mature into definitive HSCs provided they are transplanted into a newborn and not an adult mouse [36]. In addition, a significant reservoir of HSCs can be found residing in the placenta during development [37, 38]. HSCs from the AGM are presumed to colonize the fetal liver, where they give rise to definitive hematopoietic precursors. Several seminal studies using lineage tracing in both mice and zebrafish have shown that definitive HSCs arise from hemogenic endothelium within the ventral aspect of the dorsal aorta at E10.5 [39–41]. *Runx1* is required for this process to occur [42], and mice lacking *Flk1*, a tyrosine kinase expressed on endothelial progenitor cells, fail to develop both vascular endothelium and blood islands during embryogenesis [43].

Later in development, HSCs reside in the fetal liver. Many questions regarding the fetal liver vascular niche remain, especially regarding the process of migration of HSCs from the fetal liver to the BM following birth. A recent study showed that nestin-positive NG2<sup>+</sup> pericytes associate with portal vessels, forming a fetal perivascular niche that promotes HSC expansion [7]. A rapid loss of HSCs in the postnatal liver is associated with dramatic postnatal changes in portal vessel circulation after closure of the umbilical inlet at birth. A corresponding loss of niche cells, including nestin-positive NG2<sup>+</sup> pericytes, also occurs, because the portal vessels undergo a transition from neuropilin-1<sup>+</sup>ephrin-B2<sup>+</sup> arteries to an EphB4<sup>+</sup> vein phenotype. This suggests that the changes in postnatal circulation and hemodynamics alter the liver vasculature and are associated with the loss of HSC-supporting cells. The HSCs then migrate to the BM wherein long-term hematopoiesis persists postnatally.

### CONCEPTUAL HISTORY OF THE HSC NICHE

In 1968, Wolf and Trentin directly implanted BM stroma into the spleens of irradiated recipients and showed that the proportions of outgrowing erythroid and myeloid colonies could be biased by their local environment, providing some evidence for microenvironmental influence of immature hematopoietic cell fate [44]. The existence of a niche for stem cells within the BM was formally proposed by Schofield in 1978 [45]. At that time, Becker et al. showed that HSCs likely existed based on their research using the colony-forming unit-spleen (CFU-S) assay [46]. Schofield noted that the putative CFU-S stem cells were less robust than cells of the BM at reconstituting hematopoiesis in irradiated animals and proposed the existence of a specialized location within the BM that maintained HSCs [45]. However, definitive evidence of an HSC population and an HSC niche was lacking, and many

questions regarding the factors that governed HSC differentiation and maintenance remained unanswered for decades. Some groups proposed stochastic mechanisms and others favored an “inductive” hypothesis in which the niche governed HSC fate. Over the subsequent decades, numerous studies clarified the critical role of BM microenvironment cells in regulating HSC fate.

### ANATOMY OF THE ADULT HSC NICHE

In addition to HSCs and their progeny, the BM is composed of a diverse array of cells with specialized functions. These include vascular ECs, perivascular cells, osteoblasts, sympathetic nerves, adipocytes, macrophages, and many subsets of stromal cells [1, 47, 48]. BM niche cells provide both positive and negative regulatory signals for HSCs. Adipocytes have been shown to negatively regulate HSC self-renewal in vivo [49]. Adipogenic differentiation of stromal cells also leads to increased adipocyte numbers in the BM, which, in turn, hampers hematopoietic recovery after injury [50]. Bone-degrading osteoclasts are dispensable for HSC maintenance in *op/op*, *Fos*-deficient, and *Rankl*-deficient mice, which lack osteoclasts [51, 52].

Most mammalian hematopoiesis occurs in the axial skeleton (so-called red marrow) in the flat bones, such as the pelvis, sternum, skull, ribs, vertebrae, and the metaphyseal and epiphyseal ends of long bones. Other BM, composed of higher fat content (“yellow marrow”), can be found in the hollow interior of the diaphyseal portion (shaft) of the long bones. Arteries enter through the bone cortex, terminate in the endosteum (the connective tissue lining the inner surface of compact bone), and branch in the metaphysis or diaphysis of long bones [53, 54]. Most of the branching arteries lie in the metaphysis, and the central diaphysis contains few, largely unbranched arteries [53]. Some distal arterioles terminate at capillaries in the endosteum, although most termination points are found in the metaphysis [53]. Near the bone, the arterioles open up and anastomose with a plexus of venous sinuses [53]. These venous sinuses drain via collecting venules that lead back centrally to the central longitudinal vein [53]. The arterioles associate more closely with the endosteal region and are ensheathed exclusively by rare NG2<sup>+</sup> (also known as CSPG4<sup>+</sup>), nestin<sup>bright</sup> pericytes, which are distinct from sinusoid-associated leptin receptor (*LepR*)<sup>+</sup> or Nestin<sup>dim</sup> perivascular cells [16]. Veins are located in the central diaphysis, where they connect to metaphyseal capillaries. The venous sinuses are thin-walled, consisting of a layer of flat ECs with little to no basement membrane and covered with pericytes and perivascular stromal cells, including C-X-C motif chemokine ligand 12 (CXCL12) abundant reticular cells (CAR cells) [55] and *Lepr*<sup>+</sup> cells [16, 56, 57]. The BM does not have lymphatic drainage [58], and all vessels are interspersed within a meshwork of trabecular bone. This anatomy establishes a circular pattern to the blood flow, from the center of the marrow cavity toward the periphery and back again.

Defining the anatomic location of HSCs in the adult BM as it relates to distinct niche cells has proved to be challenging owing to the difficulty in retaining histological integrity when sectioning bone, the number of markers necessary to identify HSCs, the limitations of microscopy, and the close proximity of bone and vascular elements throughout the BM. The discovery of a pattern of SLAM family receptor expression (CD150<sup>+</sup>CD244<sup>-</sup>CD48<sup>-</sup>) on HSCs catalyzed the ability of researchers to localize HSCs in the niche. Most SLAM<sup>+</sup> HSCs were found in that study to be associated with sinusoidal endothelium [59]. Previously, up to 10 markers

had been necessary to identify HSCs, and even then, the purity of the population as long-term HSCs rarely exceeded 20% [60].

It has also been shown that the local oxygen concentration and HSC metabolic state in the BM contributes to regional differences in HSC spatial localization [16, 61–66]. Quiescent HSCs have been shown to have lower levels of intracellular reactive oxygen species (ROS), and high ROS levels damage HSC self-renewal capacity and promote HSC exhaustion [61–65]. Quiescent HSCs with lower levels of ROS have been shown to reside adjacent to arteriolar blood vessels rather than sinusoids [16, 66], and conditional depletion of the ensheathing arteriolar pericytes induces HSC cycling, with ensuing reduced functional long-term repopulating ability [16]. However, if the metabolic state is ignored, this differential vascular localization no longer applies, and HSCs are found throughout arteriolar and sinusoidal niches [66]. Compared with arterioles, the more permeable BM sinusoids promote HSC activation. Furthermore, HSC exposure to blood plasma was shown to increase HSC ROS levels, augmenting their migration and differentiation and compromising their long-term repopulation abilities and survival [66]. In contrast, Acar et al. demonstrated that HSCs were localized primarily in the central BM away from bone surfaces, in the diaphysis relative to the metaphysis, and close to sinusoidal vessels [15]. In their study, HSCs were determined to be relatively distant from both arterioles and transition zone vessels and the location was independent of quiescence [15].

Given that HSCs are mobile and in constant low-level flux [67], the possibility exists that microscopic images capture a short-lived interaction of HSCs with neighboring vascular and perivascular niche cells. Using sequential high-resolution, three-dimensional imaging of the calvarium of mice over time [68, 69], primitive HSCs have been shown to traffic to BM vessels, where the chemokine CXCL12 and the glycoprotein E-selectin were rich. HSCs were shown to lodge in these areas for weeks, wherein they underwent self-renewal and expansion [68, 70].

### BM OSTEOLINEAGE CELLS

The concept of an endosteal niche for HSCs evolved from initial studies that indicated that BM stromal cells could maintain HSCs *ex vivo* [71], that hematopoietic stem/progenitors localized near endosteal margins [72], and that osteoblasts produce cytokines that support hematopoietic cells [73]. Calvi et al. showed that amplification of BM osteoblasts and trabecular bone content led to expansion of the HSC pool *in vivo*, and Zhang et al. suggested that amplification of N-cadherin<sup>+</sup>CD45<sup>-</sup> osteoblastic cells correlated with increased HSC numbers [74, 75]. Other studies also suggested that transplanted HSCs resided preferentially in the BM endosteal area in recipient mice [70, 76, 77].

Endochondral ossification, the process of bone formation through a cartilage intermediate, has also been shown to be required for HSC niche formation in mice [78]. A clonal, lineage-restricted common skeletal progenitor cell (bone, cartilage, stromal progenitor) has also been described that contributes CD105<sup>+</sup>, Thy1<sup>+</sup>, and 6C3<sup>+</sup> stromal cells capable of supporting hematopoietic stem and progenitor cells [79]. The maintenance of quiescent HSCs with preserved long-term repopulating capacity has also been suggested to be dependent on the presence of BM osteolineage cells [80, 81].

Recent studies have challenged whether BM osteoblasts are necessary for HSC maintenance. Several studies have found that most HSCs are not in contact with or in close approximation to BM

osteoblasts [59, 65, 68, 82]. Second, depleting osteoblasts via *Bgn* deficiency [83] or treatment with ganciclovir [84, 85] had no effect on HSC frequency. Third, increasing BM osteoblast numbers via strontium treatment had no acute effect on HSC frequency [86]. It is important to note that the observations of HSC localization and frequency could have been affected in these studies by differences in the criteria used to define the HSC phenotype. However, Ding et al. demonstrated that cell-specific deletion of stem cell factor (SCF) in BM osteoblasts had no effect on HSC maintenance as measured by competitive repopulation assays in mice [56, 57]. Greenbaum et al. also showed that deletion of CXCL12 in BM osteoblasts had no effect on HSC maintenance as measured via competitive repopulation assays [87]. Taken together, these data suggest that BM osteoblasts might provide signals that are sufficient to promote HSC expansion; however, it remains unclear whether BM osteolineage cell-derived signals are necessary for HSC maintenance or regeneration.

### BM ECs IN NORMAL HEMATOPOIESIS AND HSC REGENERATION

As early as 1961, it was observed that the recovery of hematopoiesis in rats after 10 Gy of total body irradiation (TBI) required the recovery of an intact vasculature [88]. In addition, extramedullary hematopoiesis is known to occur in patients in locations devoid of osteolineage cells (e.g., liver and spleen) [89], and ECs were known to create stem cell niches in other tissues, such as the brain [24]. Furthermore, given the essential role of ECs in hematopoietic development, investigators have explored the role of ECs in regulating adult hematopoiesis. As noted, HSCs reside in the adult BM in association with vascular and perivascular niche cells [15, 16, 59, 66]. In 1972, it was observed that hematopoietic regeneration was linked to vascular regeneration in areas of curretted BM in adult mice [90]. More recently, conditional deletion of the gene that encodes the gp130 cytokine receptor in ECs led to a reduction in HSC numbers and overall BM hypocellularity [91].

Ding et al. established the essential role of BM ECs in regulating the maintenance of the HSC pool via cell-specific deletion of SCF [56]. Ding and Morrison [57] and Greenbaum et al. [87] later demonstrated that deletion of *CXCL12* in BM ECs also impaired HSC maintenance in mice. In the same studies, Ding and Morrison demonstrated that deletion of *CXCL12* from LepR<sup>+</sup> perivascular stromal cells depleted HSCs [57]. In contrast, Greenbaum et al. showed that deletion of *CXCL12* from Prx1<sup>+</sup> mesenchymal progenitor cells markedly decreased HSC content [87]. Taken together, these studies confirmed a necessary role for BM ECs and BM perivascular stromal cells in maintaining the HSC pool in steady state.

Human ECs can promote and maintain HSCs in culture [8, 9, 92], and BM ECs promote long-term reconstituting HSC expansion in culture [2, 93]. The BM sinusoidal vasculature is radiosensitive but regenerates and reorganizes within 3–4 weeks after sublethal radiation exposure [10, 94]. Following radiation injury, coculture of irradiated HSCs with ECs can rescue HSCs with multilineage reconstituting capacity that are capable of radioprotecting lethally irradiated recipient mice after transplantation [8, 9]. Moreover, Chute et al. and Salter et al. demonstrated that systemic infusion of autologous or allogeneic murine ECs into lethally irradiated mice accelerated both BM vascular and hematopoietic regeneration and markedly improved survival, in the absence of transplanted hematopoietic cells [10, 94]. Salter et al. demonstrated that transplanted ECs do not engraft in the BM vasculature,

suggesting that the regenerative effects were mediated via indirect activities or elaboration of EC-derived soluble factors [94]. This is consistent with clinical studies that have shown that reconstitution of the BM vasculature arises primarily from host BM ECs rather than donor-derived ECs [95].

Several lines of evidence suggest that BM ECs have a necessary role in HSC regeneration [2, 13, 94]. Genetic deletion of *VEGFR2* or antibody-mediated blockade of VE-cadherin-mediated vasculogenesis was shown to disrupt BM vascular regeneration in irradiated mice and result in prolonged hematopoietic toxicity and delayed HSC regeneration [2, 13, 94]. Gain of function models have also shown that ECs promote HSC maintenance and regeneration. *Tie2Cre;Bak1<sup>-/-</sup>;Bax<sup>fl/fl</sup>* mice, which bear deletion of the intrinsic mediators of apoptosis, BAK and BAX, in *Tie2<sup>+</sup>* ECs, were shown to have radioprotection of the BM vasculature and the hematopoietic system compared with mice that retained *BAX* expression in *Tie2<sup>+</sup>* ECs [12]. Similarly, genetic activation of the Akt-mTOR pathway in primary human ECs augments the capacity to promote HSC self-renewal in culture, and mitogen-activated protein kinase (MAPK) activation favors HSC differentiation in coculture [96]. Most recently, the role of SCF-expressing ECs in promoting extramedullary hematopoiesis in the spleen was also demonstrated in murine models [97]. *Tcf21<sup>+</sup>* stromal cells were also shown to have a necessary role in maintaining splenic HSCs via CXCL12 secretion in the setting of extramedullary hematopoiesis in the same study [97].

### BM EC-DERIVED PARACRINE FACTORS

Although recent studies have confirmed the essential role of BM ECs in maintaining the HSC pool in steady state and regeneration, the precise mechanisms through which BM ECs regulate HSC maintenance and regeneration remain incompletely understood. Although cell-cell interactions are clearly important, our laboratory has focused on discovering and characterizing the paracrine factors that are produced by BM ECs that regulate HSC fate [92, 98]. Some of these factors have been elucidated and well characterized, such as SCF and CXCL12 [56, 57, 87]. Via an unbiased gene expression analysis of human ECs that supported human HSC expansion [8], Himburg et al. identified pleiotrophin (PTN), a heparin-binding growth factor, to be more than 25-fold overexpressed by ECs that promote HSC expansion [18, 99, 100]. Ex vivo treatment of murine or human HSCs with PTN caused a marked increase in long-term repopulating HSCs in culture [18], and systemic administration of PTN to irradiated mice caused a pronounced amplification of BM stem and progenitor cells in vivo [18]. The effects were mediated via inhibition of the protein receptor tyrosine phosphatase- $\zeta$  (PTP $\zeta$ ) expressed on HSCs [100]. Subsequently, Himburg et al. demonstrated that both BM ECs and CXCL12-abundant reticular cells express PTN in the HSC niche and that PTN regulates HSC homing to and retention in the BM vascular niche [99]. Mechanistically, PTN acts in a paracrine manner in the vascular niche, and binding of PTN with PTP $\zeta$  on HSCs has been shown to induce phosphorylation of anaplastic lymphoma kinase, yielding downstream activation of Ras/MEK/ERK signaling in the HSC pool [100].

Doan et al. reported that BM ECs in radioprotected *Tie2Cre;Bak1<sup>-/-</sup>;Bax<sup>fl/fl</sup>* mice secreted significantly increased concentrations of epidermal growth factor (EGF) and amphiregulin, another EGF receptor (EGFR) agonist, in the BM at steady state and early after myelosuppressive TBI [12]. These results suggested that EGF might be produced by *Tie2<sup>+</sup>* BM ECs and that EGFR signaling might

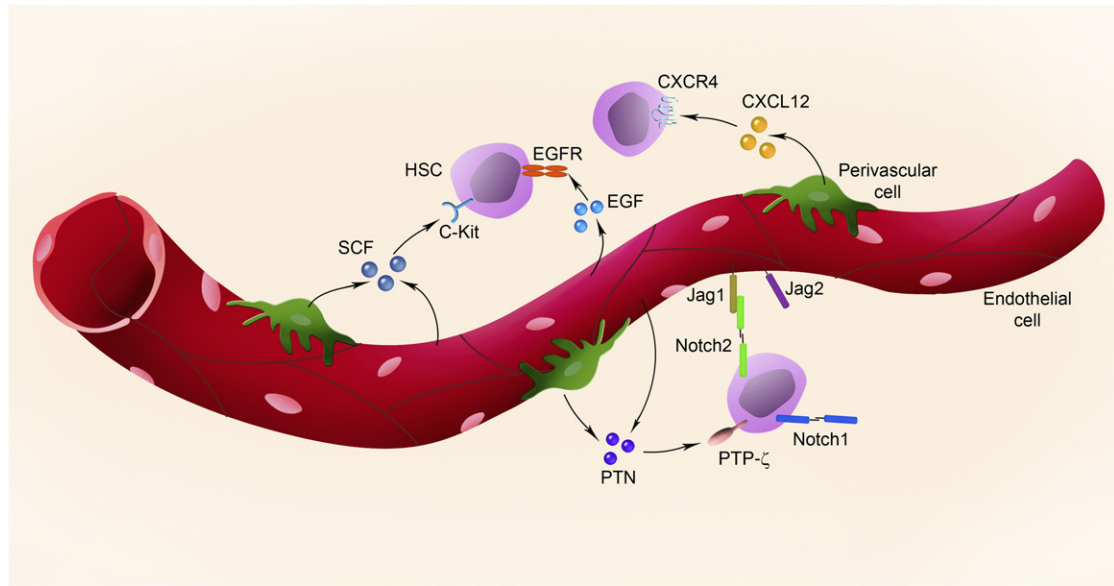
be functionally relevant to HSC regeneration. Subsequently, Doan et al. showed that systemic administration of EGF promoted HSC regeneration and improved survival in mice after lethal irradiation [101]. Mechanistically, EGF promoted HSC regeneration via repression of *PUMA*-mediated apoptosis in HSCs, leading to increased hematopoietic recovery and improved survival [101].

Poulos et al. reported that BM ECs also secrete Jagged-1, a Notch ligand, and conditional deletion of *Jagged-1* in BM ECs led to hematopoietic exhaustion and HSC depletion over time [17]. Hematopoietic regeneration after 650 cGy of TBI was also impaired in mice with EC-specific Jagged-1 deficiency [17]. Although previous studies have suggested that Notch signaling might be dispensable for normal hematopoiesis [102], the recent studies by Butler et al. and Poulos et al. have indicated that Jagged-1 signaling, mediated by BM ECs, is necessary for hematopoietic regeneration [2, 17]. A schematic representation of the vascular and perivascular-derived paracrine factors that regulate HSC fate is shown in Figure 1.

### PERIVASCULAR STROMAL CELLS

As noted, several recent studies have highlighted the importance of perivascular cells in regulating HSC fate. Anatomically, BM vessels are ensheathed by pericytes or adventitial reticular cells, including CXCL12-abundant reticular cells (CAR cells) [55], and other populations characterized by expression of nestin, myxovirus resistance-1 (Mx1), *Lepr*, the transcription factor paired related homeobox-1 (Prx1), and the transcription factor osterix [56, 87, 103, 104]. Current understanding indicates substantial overlap in the expression of several of these markers in BM perivascular cells. Pericytes represent structurally unique perivascular cells that express NG2 and surround BM arterioles, whereas *LepR<sup>+</sup>* perivascular stromal cells surround BM sinusoidal vessels [16, 105].

The functional role of mesenchymal stromal cells (MSCs) in the HSC niche was suggested by the observation that HSCs reside near nestin-expressing MSCs and that deletion of nestin-positive cells depleted BM HSC content [103]. Additionally, BM stromal cells with many hallmarks of MSCs express fibroblast activation protein (FAP) [106, 107], and ablation of FAP<sup>+</sup> cells led to BM hypocellularity and anemia [108, 109]. Deletion of the RhoGTPases, *Rac1* and *Rac2*, in nestin-positive BM cells was associated with decreased nestin-positive cells, increased trabecular bone, increased sinusoidal space, decreased arteriolar volume, and decreased HSCs in the BM [110]. Increased HSC cycling was observed after conditional deletion of NG2-expressing pericytes, which envelope BM arterioles, suggesting that HSC quiescence is maintained near BM arterioles and is mediated, at least in part, by arteriolar pericytes [16]. Ding et al. found that deletion of *SCF* or *CXCL12* from nestin-positive MSCs had no effect on HSC maintenance [56, 57] but that deletion of *CXCL12* from *LepR<sup>+</sup>* perivascular stromal cells caused a significant depletion of HSCs. Greenbaum et al. also demonstrated that deletion of *CXCL12* from nestin-negative, Prx1-positive mesenchymal cells yielded HSC depletion [87]. Taken together, these studies reveal an essential role for BM perivascular stromal cells in regulating HSC maintenance in vivo. In light of the recent study by Itkin et al., which demonstrated that less permeable arterial blood vessels maintain HSCs in a low ROS state and higher permeability sinusoidal vessels promote hematopoietic stem/progenitor cell activation [66], it will be important to determine which perivascular stromal cells are associated with these two functionally distinct HSC niches.



**Figure 1.** Paracrine factors in the bone marrow (BM) vascular niche. A schematic diagram of a BM vessel in longitudinal view and representation of several paracrine factors that are secreted by BM endothelial cells and perivascular cells. Abbreviations: CXCL12, C-X-C chemokine ligand 12; CXCR4, C-X-C chemokine receptor type 4; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HSC, hematopoietic stem cell; Jag1, Jagged-1; Jag2, Jagged-2; PTN, pleiotrophin; PTP- $\zeta$ , protein receptor tyrosine phosphatase- $\zeta$ ; SCF, stem cell factor.

The production of extracellular matrix proteins by BM stromal cells and the interaction of these molecules with HSCs have also recently come into focus. Deletion of the glycosyltransferase gene, *Ext1*, in *Mx1*<sup>+</sup> BM stromal cells was shown to cause a reduction in heparan sulfate production [111]. Decreased heparan sulfate levels promoted hematopoietic stem/progenitor cell mobilization and promoted donor hematopoietic cell engraftment in the absence of conditioning [111]. In contrast, deletion of protein tyrosine phosphatase  $\sigma$  (PTP $\sigma$ ), a receptor for heparan sulfate and chondroitin sulfate, was shown to substantially increase the BM repopulating capacity [112]. These results suggest the importance of the interaction of the proteoglycans with HSCs in the stromal cell niche and highlight the need for further study into these important mechanisms of HSC regulation.

#### ADDITIONAL PARACRINE MECHANISMS IN THE VASCULAR AND PERIVASCULAR NICHE

In the present review, we focused on the role of BM vascular and perivascular cells in regulating HSC fate. All the important HSC regulatory mechanisms could not be covered comprehensively in the present review, including the Wnt signaling pathway, the Notch pathway and the Tie2/angiopoietin pathway. The function of Wnt ligands in the hematopoietic niche has been previously reviewed in detail [113]. Several lines of evidence have suggested that Wnt pathway activation in HSCs and progenitors is under a careful balance among the effects of canonical Wnt ligands, non-canonical Wnt ligands, and Wnt antagonists and that the dosage of Wnt signals can mediate distinct effects on HSC self-renewal and differentiation [113, 114]. Angiopoietin-1 production by BM osteoblasts was shown by Arai et al. to regulate HSC quiescence via action on Tie2<sup>+</sup> HSCs [115]. More recently, deletion of angiopoietin-1 from HSCs or LepR<sup>+</sup> perivascular stromal cells was shown to accelerate hematopoietic and vascular recovery in mice while increasing vascular permeability [116]. These data

suggest that HSCs and perivascular cells elaborate soluble factors that regulate the vascular response to injury and hematopoietic regeneration.

Although it was not the focus of the present review, recent studies have suggested that corruption of BM niche cells might contribute to or promote the transformation of hematopoietic progenitor cells to malignancy [117–119]. Deletion of the microRNA processing endonuclease, *Dicer1*, in BM osteoprogenitor cells promoted myelodysplasia in mice [118]. In contrast, mutation in  $\beta$ -catenin in BM osteoblasts promoted acute myeloid leukemia (AML) development in mice [119]. It remains unclear whether mutations in BM ECs also contribute to the pathogenesis of myeloid or lymphoid neoplasms, and this is the subject of ongoing study. It has also been shown that AML cells release exosomes containing protein and RNAs that cause downregulation of SCF and CXCL12 in BM MSCs, thereby promoting normal HSC mobilization from the niche and clonal dominance by the AML clone [120, 121]. These data suggest that leukemic cells actively participate in subverting the normal HSC niche for the purpose of facilitating AML growth at the expense of normal hematopoiesis.

#### EXTRAMEDULLARY HEMATOPOIESIS IN VASCULAR NICHES

In the setting of myelofibrosis, chronic myelogenous leukemia, and other myelophthitic processes and stress conditions, extramedullary hematopoiesis can occur in humans in various organs, including the liver, spleen, lymph nodes, retroperitoneum, lungs, genitourinary system, skin, muscle, and rarely, the central nervous system [122, 123]. These clinical observations have suggested that these organs must contain an adequate microenvironment to sustain hematopoiesis or that migrating HSCs are capable of modulating the microenvironment in extramedullary sites to support hematopoiesis temporarily. Recently, Inra et al. demonstrated that splenic ECs and Tcf21<sup>+</sup> stromal cells in the spleen supported EMH via elaboration of SCF or CXCL12 [97].

Mendt and Cardier also showed that liver sinusoidal ECs “recruit” hematopoietic stem/progenitor cells via secretion of CXCL12 [124], and Wittig et al. showed that liver sinusoidal endothelial cells (LSECs) also support B lymphopoiesis [125]. These results are consistent with the established role of LSECs in promoting liver regeneration [126] and also are consistent with the recent demonstration that hematopoiesis in the fetal liver occurs in association with fetal portal vessels encompassed by nestin-positive NG2<sup>+</sup> pericytes and that such fetal liver hematopoiesis ceases with closure of the umbilical vein and the loss of nestin-positive NG2<sup>+</sup> pericytes as the portal vessels transition from the arterial to the venous phenotype [7].

#### OPPORTUNITIES FOR TRANSLATION

Elucidation of the mechanisms through which BM ECs, perivascular cells, and osteolineage cells regulate HSC self-renewal, regeneration, and malignant transformation will increase the potential for development of targeted therapeutic agents for the human hematopoietic system. Novel vascular niche-derived paracrine factors that regulate HSC fate are particularly exciting in this regard because the systemic administration of such factors could be readily developed for clinical testing. Several BM EC-derived soluble proteins described in the present study, such as PTN and EGF, and derivatives of these proteins, are in advanced

preclinical testing for investigational new drug evaluation. The ongoing discovery of novel mechanisms through which vascular, perivascular, and osteolineage cells regulate hematopoiesis, coupled with the explication of the cross-talk signals among BM ECs, perivascular cells, osteolineage cells, and other niche elements, will certainly yield many exciting tools for regenerative medicine.

#### ACKNOWLEDGMENTS

The authors acknowledge Zhongwei Cao (Cornell University) for her critical assistance with schematic figure design. This work was supported, in part, by funding from the California Institute for Regenerative Medicine Leadership Award, LA1-08014 (J.P.C.), National Heart, Lung, and Blood Institute (NIH) Grant HL-086998 (J.P.C.), and National Institute of Allergy and Infectious Diseases (NIH) Grant AI-107333 (J.P.C.).

#### AUTHOR CONTRIBUTIONS

J.P.S., K.T.Y., and J.P.C.: manuscript writing, final approval of manuscript.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

#### REFERENCES

- Rafii S, Butler JM, Ding BS. Angiocrine functions of organ-specific endothelial cells. *Nature* 2016;529:316–325.
- Butler JM, Nolan DJ, Vertes EL et al. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. *Cell Stem Cell* 2010;6:251–264.
- Nolan DJ, Ginsberg M, Israely E et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell* 2013;26:204–219.
- Choi KD, Vodyanik MA, Togarrati PP et al. Identification of the hemogenic endothelial progenitor and its direct precursor in human pluripotent stem cell differentiation cultures. *Cell Reports* 2012;2:553–567.
- Choi K, Kennedy M, Kazarov A et al. A common precursor for hematopoietic and endothelial cells. *Development* 1998;125:725–732.
- Mikkola HK, Orkin SH. The journey of developing hematopoietic stem cells. *Development* 2006;133:3733–3744.
- Khan JA, Mendelson A, Kunisaki Y et al. Fetal liver hematopoietic stem cell niches associate with portal vessels. *Science* 2016;351:176–180.
- Chute JP, Clark W, Saini A et al. Rescue of hematopoietic stem cells following high-dose radiation injury using ex vivo culture on endothelial monolayers. *Mil Med* 2002;167(suppl):74–77.
- Chute JP, Fung J, Muramoto G et al. Ex vivo culture rescues hematopoietic stem cells with long-term repopulating capacity following harvest from lethally irradiated mice. *Exp Hematol* 2004;32:308–317.
- Chute JP, Muramoto GG, Salter AB et al. Transplantation of vascular endothelial cells mediates the hematopoietic recovery and survival of lethally irradiated mice. *Blood* 2007;109:2365–2372.
- Chute JP, Saini AA, Chute DJ et al. Ex vivo culture with human brain endothelial cells increases the SCID-repopulating capacity of adult human bone marrow. *Blood* 2002;100:4433–4439.
- Doan PL, Russell JL, Himburg HA et al. Tie2(+) bone marrow endothelial cells regulate hematopoietic stem cell regeneration following radiation injury. *STEM CELLS* 2013;31:327–337.
- Hooper AT, Butler JM, Nolan DJ et al. Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell* 2009;4:263–274.
- Kopp HG, AVECILLA ST, Hooper AT et al. Tie2 activation contributes to hemangiogenic regeneration after myelosuppression. *Blood* 2005;106:505–513.
- Acar M, Kocherlakota KS, Murphy MM et al. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature* 2015;526:126–130.
- Kunisaki Y, Bruns I, Scheiermann C et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature* 2013;502:637–643.
- Poulos MG, Guo P, Kofler NM et al. Endothelial Jagged-1 is necessary for homeostatic and regenerative hematopoiesis. *Cell Reports* 2013;4:1022–1034.
- Himburg HA, Muramoto GG, Daher P et al. Pleiotrophin regulates the expansion and regeneration of hematopoietic stem cells. *Nat Med* 2010;16:475–482.
- Muramoto GG, Chen B, Cui X et al. Vascular endothelial cells produce soluble factors that mediate the recovery of human hematopoietic stem cells after radiation injury. *Biol Blood Marrow Transplant* 2006;12:530–540.
- Chen L, Tredget EE, Wu PY et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008;3:e1886.
- Traktuev DO, Prater DN, Merfeld-Clauss S et al. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res* 2009;104:1410–1420.
- Zannettino AC, Paton S, Arthur A et al. Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. *J Cell Physiol* 2008;214:413–421.
- Tang W, Zeve D, Suh JM et al. White fat progenitor cells reside in the adipose vasculature. *Science* 2008;322:583–586.
- Shen Q, Goderie SK, Jin L et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 2004;304:1338–1340.
- Leventhal C, Rafii S, Rafii D et al. Endothelial trophic support of neuronal production and recruitment from the adult mammalian subependyma. *Mol Cell Neurosci* 1999;13:450–464.
- Ramírez-Castillejo C, Sánchez-Sánchez F, Andreu-Agulló C et al. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 2006;9:331–339.
- Wang B, Zhao L, Fish M et al. Self-renewing diploid Axin2(+) cells fuel homeostatic renewal of the liver. *Nature* 2015;524:180–185.
- Ding BS, Nolan DJ, Guo P et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell* 2011;147:539–553.
- Ding BS, Gomi K, Rafii S et al. Endothelial MMP14 is required for endothelial-dependent

growth support of human airway basal cells. *J Cell Sci* 2015;128:2983–2988.

**30** Dzierzak E, Medvinsky A. Mouse embryonic hematopoiesis. *Trends Genet* 1995;11:359–366.

**31** Risau W. Embryonic angiogenesis factors. *Pharmacol Ther* 1991;51:371–376.

**32** Dieterlen-Lievre F. On the origin of haematopoietic stem cells in the avian embryo: An experimental approach. *J Embryol Exp Morphol* 1975;33:607–619.

**33** Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* 1996;86:897–906.

**34** Godin I, Dieterlen-Lievre F, Cumano A. Emergence of multipotent hemopoietic cells in the yolk sac and paraortic splanchnopleura in mouse embryos, beginning at 8.5 days postcoitus. *Proc Natl Acad Sci USA* 1995;92:773–777.

**35** Ivanovs A, Rybtsov S, Welch L et al. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. *J Exp Med* 2011;208:2417–2427.

**36** Yoder MC, Hiatt K, Mukherjee P. In vivo repopulating hematopoietic stem cells are present in the murine yolk sac at day 9.0 postcoitus. *Proc Natl Acad Sci USA* 1997;94:6776–6780.

**37** Medvinsky A, Rybtsov S, Taoudi S. Embryonic origin of the adult hematopoietic system: Advances and questions. *Development* 2011;138:1017–1031.

**38** Gekas C, Dieterlen-Lievre F, Orkin SH et al. The placenta is a niche for hematopoietic stem cells. *Dev Cell* 2005;8:365–375.

**39** Zovein AC, Hofmann JJ, Lynch M et al. Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell* 2008;3:625–636.

**40** Bertrand JY, Chi NC, Santoso B et al. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature* 2010;464:108–111.

**41** Eilken HM, Nishikawa S, Schroeder T. Continuous single-cell imaging of blood generation from haemogenic endothelium. *Nature* 2009;457:896–900.

**42** Chen MJ, Yokomizo T, Zeigler BM et al. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature* 2009;457:887–891.

**43** Shalaby F, Rossant J, Yamaguchi TP et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995;376:62–66.

**44** Wolf NS, Trentin JJ. Hemopoietic colony studies. V. Effect of hemopoietic organ stroma on differentiation of pluripotent stem cells. *J Exp Med* 1968;127:205–214.

**45** Schofield R. The relationship between the spleen colony-forming cell and the haematopoietic stem cell. *Blood Cells* 1978;4:7–25.

**46** Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 1963;197:452–454.

**47** Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med* 2014;20:833–846.

**48** Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature* 2014;505:327–334.

**49** Naveiras O, Nardi V, Wenzel PL et al. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature* 2009;460:259–263.

**50** Pietras EM, Lakshminarasimhan R, Techner JM et al. Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. *J Exp Med* 2014;211:245–262.

**51** Miyamoto K, Yoshida S, Kawasumi M et al. Osteoclasts are dispensable for hematopoietic stem cell maintenance and mobilization. *J Exp Med* 2011;208:2175–2181.

**52** Kollet O, Dar A, Shivtiel S et al. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat Med* 2006;12:657–664.

**53** Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* 2014;507:323–328.

**54** Trueta J, Harrison MH. The normal vascular anatomy of the femoral head in adult man. *J Bone Joint Surg Br* 1953;35:442–461.

**55** Krause DS, Scadden DT, Preffer FI. The hematopoietic stem cell niche—Home for friend and foe? *Cytometry B Clin Cytom* 2013;84:7–20.

**56** Ding L, Saunders TL, Enikolopov G et al. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* 2012;481:457–462.

**57** Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature* 2013;495:231–235.

**58** Munka V, Gregor A. Lymphatics and bone marrow. *Folia Morphol (Praha)* 1965;13:404–412.

**59** Kiel MJ, Yilmaz OH, Iwashita T et al. SLAMF family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 2005;121:1109–1121.

**60** Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1994;1:661–673.

**61** Ito K, Hirao A, Arai F et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med* 2006;12:446–451.

**62** Miyamoto K, Araki KY, Naka K et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 2007;1:101–112.

**63** Tesio M, Golan K, Corso S et al. Enhanced c-Met activity promotes G-CSF-induced mobilization of hematopoietic progenitor cells via ROS signaling. *Blood* 2011;117:419–428.

**64** Golan K, Vagima Y, Ludin A et al. S1P promotes murine progenitor cell egress and mobilization via S1P1-mediated ROS signaling and SDF-1 release. *Blood* 2012;119:2478–2488.

**65** Nombela-Arrieta C, Pivarnik G, Winkel B et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. *Nat Cell Biol* 2013;15:533–543.

**66** Itkin T, Gur-Cohen S, Spencer JA et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. *Nature* 2016;532:323–328.

**67** Abkowitz JL, Robinson AE, Kale S et al. Mobilization of hematopoietic stem cells during

homeostasis and after cytokine exposure. *Blood* 2003;102:1249–1253.

**68** Lo Celso C, Fleming HE, Wu JW et al. Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* 2009;457:92–96.

**69** Sipkins DA, Wei X, Wu JW et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* 2005;435:969–973.

**70** Xie Y, Yin T, Wiegraebe W et al. Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature* 2009;457:97–101.

**71** Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haematopoietic stem cells in vitro. *J Cell Physiol* 1977;91:335–344.

**72** Lord BI, Testa NG, Hendry JH. The relative spatial distributions of CFUs and CFUc in the normal mouse femur. *Blood* 1975;46:65–72.

**73** Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *J Exp Med* 1994;179:1677–1682.

**74** Calvi LM, Adams GB, Weibrecht KW et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003;425:841–846.

**75** Zhang J, Niu C, Ye L et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 2003;425:836–841.

**76** Nilsson SK, Johnston HM, Coverdale JA. Spatial localization of transplanted hemopoietic stem cells: Inferences for the localization of stem cell niches. *Blood* 2001;97:2293–2299.

**77** Wilson A, Murphy MJ, Oskarsson T et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev* 2004;18:2747–2763.

**78** Chan CK, Chen CC, Luppen CA et al. Endochondral ossification is required for haematopoietic stem-cell niche formation. *Nature* 2009;457:490–494.

**79** Chan CK, Lindau P, Jiang W et al. Clonal precursor of bone, cartilage, and hematopoietic niche stromal cells. *Proc Natl Acad Sci USA* 2013;110:12643–12648.

**80** Yoshihara H, Arai F, Hosokawa K et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell* 2007;1:685–697.

**81** Coşkun S, Chao H, Vasavada H et al. Development of the fetal bone marrow niche and regulation of HSC quiescence and homing ability by emerging osteolineage cells. *Cell Reports* 2014;9:581–590.

**82** Sugiyama T, Kohara H, Noda M et al. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 2006;25:977–988.

**83** Kiel MJ, Radice GL, Morrison SJ. Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. *Cell Stem Cell* 2007;1:204–217.

**84** Visnjic D, Kalajzic Z, Rowe DW et al. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. *Blood* 2004;103:3258–3264.

**85** Zhu J, Garrett R, Jung Y et al. Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells. *Blood* 2007;109:3706–3712.

- 86** Lymperti S, Horwood N, Marley S et al. Strontium can increase some osteoblasts without increasing hematopoietic stem cells. *Blood* 2008;111:1173–1181.
- 87** Greenbaum A, Hsu YM, Day RB et al. CXCL12 in early mesenchymal progenitors is required for hematopoietic stem-cell maintenance. *Nature* 2013;495:227–230.
- 88** Fliedner TM, Bond VP, Cronkite EP. Structural, cytologic and autoradiographic (H3-thymidine) changes in the bone marrow following total body irradiation. *Am J Pathol* 1961;38:599–623.
- 89** Taniguchi H, Toyoshima T, Fukao K et al. Presence of hematopoietic stem cells in the adult liver. *Nat Med* 1996;2:198–203.
- 90** Knospe WH, Gregory SA, Hussein SG et al. Origin and recovery of colony-forming units in locally curretted bone marrow of mice. *Blood* 1972;39:331–340.
- 91** Yao L, Yokota T, Xia L et al. Bone marrow dysfunction in mice lacking the cytokine receptor gp130 in endothelial cells. *Blood* 2005;106:4093–4101.
- 92** Chute JP, Muramoto GG, Dressman HK et al. Molecular profile and partial functional analysis of novel endothelial cell-derived growth factors that regulate hematopoiesis. *STEM CELLS* 2006;24:1315–1327.
- 93** Li W, Johnson SA, Shelley WC et al. Hematopoietic stem cell repopulating ability can be maintained in vitro by some primary endothelial cells. *Exp Hematol* 2004;32:1226–1237.
- 94** Salter AB, Meadows SK, Muramoto GG et al. Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo. *Blood* 2009;113:2104–2107.
- 95** Slayton WB, Li XM, Butler J et al. The role of the donor in the repair of the marrow vascular niche following hematopoietic stem cell transplant. *STEM CELLS* 2007;25:2945–2955.
- 96** Kobayashi H, Butler JM, O'Donnell R et al. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of hematopoietic stem cells. *Nat Cell Biol* 2010;12:1046–1056.
- 97** Inra CN, Zhou BO, Acar M et al. A perisinusoidal niche for extramedullary hematopoiesis in the spleen. *Nature* 2015;527:466–471.
- 98** Chute JP, Muramoto GG, Fung J et al. Soluble factors elaborated by human brain endothelial cells induce the concomitant expansion of purified human BM CD34+CD38- cells and SCID-repopulating cells. *Blood* 2005;105:576–583.
- 99** Himburg HA, Harris JR, Ito T et al. Pleiotrophin regulates the retention and self-renewal of hematopoietic stem cells in the bone marrow vascular niche. *Cell Reports* 2012;2:964–975.
- 100** Himburg HA, Yan X, Doan PL et al. Pleiotrophin mediates hematopoietic regeneration via activation of RAS. *J Clin Invest* 2014;124:4753–4758.
- 101** Doan PL, Himburg HA, Helms K et al. Epidermal growth factor regulates hematopoietic regeneration after radiation injury. *Nat Med* 2013;19:295–304.
- 102** Maillard I, Koch U, Dumortier A et al. Canonical notch signaling is dispensable for the maintenance of adult hematopoietic stem cells. *Cell Stem Cell* 2008;2:356–366.
- 103** Méndez-Ferrer S, Michurina TV, Ferraro F et al. Mesenchymal and hematopoietic stem cells form a unique bone marrow niche. *Nature* 2010;466:829–834.
- 104** Park D, Spencer JA, Koh BI et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell* 2012;10:259–272.
- 105** Ono N, Ono W, Mizoguchi T et al. Vasculature-associated cells expressing nestin in developing bones encompass early cells in the osteoblast and endothelial lineage. *Dev Cell* 2014;29:330–339.
- 106** Morikawa S, Mabuchi Y, Kubota Y et al. Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. *J Exp Med* 2009;206:2483–2496.
- 107** Pinho S, Lacombe J, Hanoun M et al. PDGFR $\alpha$  and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. *J Exp Med* 2013;210:1351–1367.
- 108** Tran E, Chinnasamy D, Yu Z et al. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J Exp Med* 2013;210:1125–1135.
- 109** Roberts EW, Deonaraine A, Jones JO et al. Depletion of stromal cells expressing fibroblast activation protein- $\alpha$  from skeletal muscle and bone marrow results in cachexia and anemia. *J Exp Med* 2013;210:1137–1151.
- 110** Ciuculescu MF, Park SY, Canty K et al. Perivascular deletion of murine Rac reverses the ratio of marrow arterioles and sinusoid vessels and alters hematopoiesis in vivo. *Blood* 2015;125:3105–3113.
- 111** Saez B, Ferraro F, Yusuf RZ et al. Inhibiting stromal cell heparan sulfate synthesis improves stem cell mobilization and enables engraftment without cytotoxic conditioning. *Blood* 2014;124:2937–2947.
- 112** Quarmyne M, Doan PL, Himburg HA et al. Protein tyrosine phosphatase- $\sigma$  regulates hematopoietic stem cell-repopulating capacity. *J Clin Invest* 2015;125:177–182.
- 113** Malhotra S, Kincade PW. Wnt-related molecules and signaling pathway equilibrium in hematopoiesis. *Cell Stem Cell* 2009;4:27–36.
- 114** Luis TC, Nabar BA, Roozen PP et al. Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion. *Cell Stem Cell* 2011;9:345–356.
- 115** Arai F, Hirao A, Ohmura M et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* 2004;118:149–161.
- 116** Zhou BO, Ding L, Morrison SJ. Hematopoietic stem and progenitor cells regulate the regeneration of their niche by secreting angiopoietin-1. *eLife* 2015;4:e05521.
- 117** Walkley CR, Olsen GH, Dworkin S et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell* 2007;129:1097–1110.
- 118** Raaijmakers MH, Mukherjee S, Guo S et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature* 2010;464:852–857.
- 119** Kode A, Manavalan JS, Mosialou I et al. Leukaemogenesis induced by an activating  $\beta$ -catenin mutation in osteoblasts. *Nature* 2014;506:240–244.
- 120** Huan J, Hornick NI, Goloviznina NA et al. Coordinate regulation of residual bone marrow function by paracrine trafficking of AML exosomes. *Leukemia* 2015;29:2285–2295.
- 121** Huan J, Hornick NI, Shurtleff MJ et al. RNA trafficking by acute myelogenous leukemia exosomes. *Cancer Res* 2013;73:918–929.
- 122** Chunduri S, Gaitonde S, Ciurea SO et al. Pulmonary extramedullary hematopoiesis in patients with myelofibrosis undergoing allogeneic stem cell transplantation. *Haematologica* 2008;93:1593–1595.
- 123** Koch CA, Li CY, Mesa RA et al. Non-hepatosplenic extramedullary hematopoiesis: Associated diseases, pathology, clinical course, and treatment. *Mayo Clin Proc* 2003;78:1223–1233.
- 124** Mendt M, Cardier JE. Stromal-derived factor-1 and its receptor, CXCR4, are constitutively expressed by mouse liver sinusoidal endothelial cells: Implications for the regulation of hematopoietic cell migration to the liver during extramedullary hematopoiesis. *Stem Cells Dev* 2012;21:2142–2151.
- 125** Wittig O, Paez-Cortez J, Cardier JE. Liver sinusoidal endothelial cells promote B lymphopoiesis from primitive hematopoietic cells. *Stem Cells Dev* 2010;19:341–350.
- 126** Ding BS, Nolan DJ, Butler JM et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature* 2010;468:310–315.