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Blood on the tracks: hematopoietic stem cell-endothelial cell interactions in homing and engraftment

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Abstract

Cells of the hematopoietic system undergo rapid turnover. Each day, humans require the production of about one hundred billion new blood cells for proper function. Hematopoietic stem cells (HSCs) are rare cells that reside in specialized niches and are required throughout life to produce specific progenitor cells that will replenish all blood lineages. There is, however, an incomplete understanding of the molecular and physical properties that regulate HSC migration, homing, engraftment, and maintenance in the niche. Endothelial cells (ECs) are intimately associated with HSCs throughout the life of the stem cell, from the specialized endothelial cells that give rise to HSCs, to the perivascular niche endothelial cells that regulate HSC homeostasis. Recent studies have dissected the unique molecular and physical properties of the endothelial cells in the HSC vascular niche and their role in HSC biology, which may be manipulated to enhance hematopoietic stem cell transplantation therapies.

Keywords

Hematopoietic stem cell; Endothelial cell; Stem cell niche; Hematopoietic stem cell transplantation; Homing; Engraftment

Endothelial cells in development

As embryonic development progresses, the site and cellular makeup of the HSC niche changes, as does HSC cell cycle state, gene expression, and differentiation potential, reflecting the importance of HSC-niche interactions [1]. Blood development occurs in two waves—primitive and definitive hematopoiesis. During the primitive wave of hematopoiesis, blood cells, including erythrocytes necessary for oxygenating rapidly growing tissues, are produced in the yolk sac in mammals. Although this branch of hematopoiesis is transient, elegant cell tracing experiments in mice revealed that yolk sac-derived Runx1⁺ cells populate

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the adult bone marrow niche and contribute to adult multilineage hematopoiesis [2–4]. Definitive hematopoiesis begins when HSCs bud off from specialized hemogenic endothelial cells in the aorta-gonad mesonephros (AGM) (Fig. 1a) [5–8]. These HSCs can both self-renew and give rise to more specialized progenitors [1]. By mouse embryonic day 11 (E11), HSCs from the AGM can functionally and completely repopulate an irradiated host [9]. Sequential waves of hematopoiesis continue as HSCs migrate to, and colonize, the placenta, fetal liver, thymus, spleen, and finally, the bone marrow, the adult hematopoietic niche (Fig. 1a) [1].

The bone marrow houses multiple niches, made up of numerous cell types, which are the subject of a large body of research (reviewed in [10–12]). The perivascular niche is made up of multiple types of stromal cells, sympathetic nerves, megakaryocytes, and macrophages, among others. Critical to the makeup and identity of the perivascular niche are ECs, including sinusoidal vessels and arterioles. In the adult niche, HSCs are found adjacent to, or near, the endothelium [13, 14]. Rather than simply acting as a highway for HSC migration, ECs are important niche cells in their own right. Endothelial cells express angiocrine factors, which include growth factors, chemokines, and extracellular matrix (ECM) components that regulate HSC maintenance, self-renewal, differentiation, and regeneration after injury [11, 15–17]. With initial studies suggesting that ECs support multilineage hematopoiesis in vitro [18–21], it was subsequently shown that normal function of bone marrow endothelial cells is required for maintaining hematopoiesis in vivo [22]. Deleting cytokine receptor gp130 in ECs and HSCs led to a hypocellular marrow and anemia. The defects could not be attributed to a requirement of *gp130* in HSCs as *gp130*-deficient HSCs were able to reconstitute irradiated wild-type recipients. However, wild-type HSCs could not reconstitute hematopoiesis in *gp130*-deficient mice. This data demonstrates that niche factors expressed by endothelial cells are important for hematopoiesis in vivo [22]. ECs, along with stromal cells, are an important source of the two main niche factors—stem cell factor (SCF) and chemokine CXCL12 (also known as stromal derived factor-1). Together, stromal cell and EC expression of SCF and CXCL12 promotes HSC maintenance and localization in the perivascular niche [10, 17, 23, 24]. Although stromal cells express these factors at much higher levels, production of SCF and CXCL12 by ECs remains important for adequate niche function. Depleting either SCF or CXCL12 from ECs in the perivascular niche using endothelial specific receptor tyrosine kinase Tie2-Cre depletes stem cells in the bone marrow [17, 23–25].

The hematopoietic cell types and genetic pathways that control their regulation are highly conserved through vertebrate evolution [26]. The teleost zebrafish is a unique model for studying hematopoiesis. In zebrafish, hematopoiesis occurs through primitive and definitive waves, orthologues to mammalian transcription factors are expressed and regulate blood development, and large-scale genetic screens have identified zebrafish mutations that model known human diseases [26, 27]. In zebrafish, primitive hematopoiesis takes place in the intermediate cell mass, producing erythroid and myeloid cells [26]. As in mammals, definitive HSCs bud off from the AGM and subsequently migrate to and colonize other niches, where the hematopoietic stem and progenitor cell (HSPC) population expands [28, 29]. The zebrafish fetal niche, analogous to the fetal liver, is a vascularized plexus in the tail, known as the caudal hematopoietic tissue (CHT) [28]. After HSPCs double in the CHT, they

migrate to and colonize adult niche sites, the thymus and kidney marrow [28, 29]. The transparency of the zebrafish embryo allows for high-resolution time-lapse imaging of unperturbed HSPC birth, migration, proliferation, and engraftment, including complex cell-cell interactions between HSPCs and endothelial cells [7, 8, 28–31].

Hematopoietic stem cell-endothelial cell interactions from birth to the niche

Hematopoietic stem cells (HSCs) maintain a unique relationship with endothelial cells throughout life. Endothelial cells (ECs) make up the inner cellular lining of blood vessels and lymphatics. Rather than acting as a passive barrier, ECs are metabolically active and play important roles in HSC development, homeostasis, and regeneration [32]. Forming a complex network throughout the body, ECs are important for delivering oxygen and nutrients to tissues, serving as a conduit for blood cell trafficking, and playing a role in innate and adaptive immunity [32]. Far from being a homogenous population of cells, ECs exhibit structural, molecular, and functional heterogeneity between and within organs [32, 33]. The transcriptional regulation and physical properties of the local microenvironment that influence EC specialization are areas of active investigation.

Early in development, endothelial progenitors, or angioblasts, differentiate from the mesoderm. Vessels form *de novo* from the coalescence of angioblasts in a process known as vasculogenesis [27]. During angiogenesis, the vascular network is elaborated by the sprouting or elongation of existing vessels [27]. Even before the onset of circulation, ECs undergo specialization as the artery and vein are specified. While there are many factors that are expressed differentially between artery and vein ECs, sonic hedgehog, VEGF, and Notch signaling have been shown to be key players in specifying arterial identity [27]. Venous identity is promoted when the transcription factor COUP-TFII cell-autonomously represses Notch and suppresses arterial identity [34, 35]. This complex hierarchical signaling program that regulates arterial-venous identity requires spatial and temporal regulation of gene expression and a coordinated effort by multiple families of transcription factors [36, 37]. ETS transcription factors are critical to EC specification. ETS-binding motifs are present in all known EC promoter and enhancer regions, and multiple family members are expressed by ECs [38]. SOX transcription factors have also been shown to play a critical role in EC fate [39], as have GATA and FOX family members [37, 40]. While there is no marker that uniquely and specifically identifies all endothelial cells, in general, Tie2, vascular endothelial-cadherin (VE-cadherin), platelet endothelial cell adhesion molecule (PECAM), and vascular endothelial growth factor receptor (VEGFR-2) mark the endothelium [36]. Further research will reveal the genetic and transcriptional differences that specify subsets of ECs, and the molecular mechanisms that regulate HSC development, homeostasis, and regeneration.

Endothelial cell regulation of HSC homing and engraftment

Sequential waves of hematopoiesis require HSCs to migrate over long distances between niches. After entering circulation, HSCs must home to the next site of blood development [41]. ECs not only make up the inner lining of blood vessels through which HSC traffic but also play an important role in the initial steps of the homing process. ECs both direct HSCs

to the bone marrow and function as a molecular brake to slow cell migration under hemodynamic shear stress [42]. Homing receptors facilitate HSC tethering to, and rolling along, the endothelium [43]. Chemokine receptors then activate adhesion molecules and their ligands on stem cells and endothelium to facilitate firm adhesion and allow for endothelial transmigration (Fig. 1b) [42, 43]. HSCs actively enter the bone marrow, migrate through the extravascular space, and lodge in the marrow niche. Engrafted HSCs reside within the bone marrow niche and are able to self-renew and differentiate into all mature blood lineages [44].

The initial tethering and rolling of HSCs along the blood vessel wall occur under the physiological shear conditions of blood flow and are regulated by adhesion molecules known as selectins [42, 43]. Outside of the bone marrow, E- and P-selectins are upregulated on vascular endothelium to attract leukocytes to sites of injury and inflammation [45]. In contrast, marrow ECs constitutively express selectin, and loss of function studies revealed that homing to the marrow niche is greatly impaired upon E- and P-selectin deficiency [46–48]. Several selectin ligands are expressed on human CD34⁺ HSCs including PSGL-1 (P-selectin glycoprotein ligand-1) and HCELL (hematopoietic cell E-/L-selectin ligand) [49–51], which mediate the rolling and tethering of HSCs along bone marrow vasculature [52, 53]. The transition to firm adhesion is then triggered by CXCL12 [54], a key chemokine expressed by marrow ECs, which directs HSC homing and migration into the niche [55, 56]. Binding of CXCL12 to the CXCR4 receptor on HSCs activates integrins on the surface of HSCs and converts rolling along the vascular endothelium into a stable shear-resistant arrest [57]. The arrest is established through interactions between integrins VLA-4 and LFA-1, and their receptors expressed on the microvasculature, VCAM-1 and ICAM-1, respectively [47, 52, 57]. Loss of both integrin and selectin signaling further impairs HSC homing [58]. VLA-4 and LFA-1 also mediate transendothelial migration across the endothelium [57, 59, 60]. Once extravasated, HSCs transmigrate through the ECM to engraft in the niche. These studies highlight that ECs are much more than a passive border separating peripheral HSCs from the bone marrow niche. Rather, they play an active and crucial role in engraftment by directing migration to, and polarization across, the vascular endothelium into the niche.

Endothelial cells in the perivascular HSC niche

The bone marrow niche contains multiple types of ECs, which play critical roles in HSC regulation. Here, ECs support HSC proliferation and differentiation, homeostasis, and regeneration through production of angiocrine factors [19]. The majority of HSCs are found less than 10 μ M from the endothelium [61]. Distinct types of endothelial cells make up vessels with distinct characteristics, and ongoing research seeks to determine whether and how vessel heterogeneity differentially regulates HSCs (Fig. 2a). In the perivascular niche, arteries branch into small-diameter arterioles, which control the blood flow into the marrow [62, 63]. Arterioles are well oxygenated and have high blood flow velocity and shear rates. These vessels are surrounded by smooth muscle and express high levels of adhesion and tight junction molecules that lead to low permeability [64]. Arterioles are connected to highly branched sinusoidal microvessels, which form a complex network throughout the bone marrow [63, 65, 66]. The thin walls, discontinuous basement membrane, and fenestrations of sinusoidal vessels increase permeability and allow cells and soluble factors

to traffic between blood and the marrow [12, 67, 68]. Sinusoidal vessels are distributed evenly through the bone marrow, making up about 30% of the bone marrow volume, whereas arterioles are a rarer population (about 1%) and preferentially located near the bone [62].

Differences in vessel integrity, permeability, and oxygenation may create distinct HSC microenvironments (Fig. 2b). Arteriolar ECs express higher levels of VCAM-1, which has been associated with HSC retention [64, 69, 70], while sinusoidal ECs express higher levels of E-selectin [64], which is important for HSC homing and proliferation [71]. While multiple recent studies have reported that quiescent HSCs are localized near arterioles [62, 72, 73], several others have identified dormant HSC populations close to the sinusoidal endothelium, which is where the vast majority of HSCs reside [14, 61, 62, 74]. Differential vascular permeability between arterioles and sinusoids affects the degree to which HSCs are exposed to blood plasma components and reactive oxygen species (ROS) levels. Arterioles are less permeable and maintain a ROS^{low} environment, whereas leaky sinusoids increase exposure of perivascular HSCs to ROS [64]. High ROS levels promote HSC differentiation, exhaustion, and mobilization rather than maintaining HSC quiescence, self-renewal, and long-term repopulating potential [64, 75–78]. Recent work reports that arteriolar-associated HSCs have low intracellular ROS levels whereas both ROS^{low} and ROS^{high} HSCs are found near sinusoids [64]. Distinct stromal cells associate with arteriolar and sinusoidal vessels, which may help regulate HSC cycling states [62]. Future research is necessary to investigate whether endothelial subtypes truly create unique micro-environments within the perivascular bone marrow niche that have distinct properties in regulating HSC homeostasis.

Until recently, it has been hard to map out the microvasculature organization and flow due to the technical difficulties of imaging through opaque bone. HSC homing and HSC-niche localization have been successfully imaged directly in the perivascular endosteal niche in the calvarium of the skull, a functional site of hematopoiesis [65, 79]. Similar techniques were used to study the effect of blood flow on HSC homing in the unperturbed marrow in a living mouse [80]. These studies showed that while marrow arterioles exhibit flow velocities and shear stress similar to arterioles in other locations, the flow velocity and shear stress of marrow sinusoidal vessels are significantly lower than those in capillaries elsewhere [80]. It is possible that it is this lower shear stress that helps regulate HSC homing to the sinusoidal vessels in the marrow.

Real-time endogenous HSC-niche interactions can be exquisitely imaged in the living transparent zebrafish embryo. Lineage tracing of single cells in zebrafish embryos demonstrated that a bipotential hemangioblast can give rise to both ECs and hematopoietic cells [81]. The birth of definitive HSCs from an endogenous niche was first visualized using non-invasive live imaging in the zebrafish [7, 8]. A single EC in the ventral wall of the aorta in the AGM bends and is released into the sub-aortic space, where it then transforms into a hematopoietic cell (Fig. 1a) [7]. This study demonstrated that HSCs do not arise from an asymmetric division, but rather from an endothelial to hematopoietic transition. Recent studies have used high-resolution imaging to study the cellular interactions that occur between HSPCs and ECs to form the fetal niche [29]. In unperturbed embryos, Tamplin et al. [29] visualized multiple HSPC-endothelial cell interactions that culminate in HSPC

engraftment: HSPCs migrated through circulation to the CHT (the fetal niche), adhered to the endothelial cell wall, extravasated across the vessel, and lodged in the niche. Once lodged, nearby ECs remodeled around the HSPC to form a pocket in a novel behavior termed “endothelial cell cuddling.” Within this pocket, HSPCs were contacted by ECs, as well as stromal cells and fibroblasts, and underwent cell division. Further, they found that ECs similarly surround HSPCs in dissected mouse E11.5 fetal livers [29] (Fig. 1a). In mouse, HSCs maintain a close association with ECs in the adult niche [14, 62, 64]. A *HoxB5* reporter that labels long-term HSCs (LT-HSC) demonstrated that 94% of LT-HSCs directly adhere to the abluminal surface of ECs in the adult mouse bone marrow [13]. These studies demonstrate the important and diverse roles of ECs in HSC development and engraftment.

It has long been recognized that ECs exhibit dramatic structural heterogeneity between different tissues and organs [82]. Genetic studies have identified the main axes that regulate arterial-venous specification [27, 36]. It is still unknown, however, what factors regulate tissue-specific EC distinctions. Likely, a combination of transcriptional regulation and chemical and physical cues from the microenvironment influence tissue-specific EC identity and specialization. By combining tissue-specific EC purification and microarray profiling, Nolan et al. (2013) identified some of the molecular determinants that belie this diversity. They found that tissue-specific ECs differ by combinatorial expression of transcription factors, angiocrine growth factors, adhesion molecules, and chemokines [33]. Functional analysis of distinct populations of ECs and the molecules they express is hindered by the inability to mark or knockout specific subtypes of ECs, for example by using reporter constructs or Cre drivers. Continued research will uncover markers unique to specific subtypes of endothelial cells in individual tissues, which will help parse apart their specific roles in HSC development and maintenance.

Hematopoietic stem cell transplantation

HSCs are required throughout life to produce specific progenitor cells that will replenish all blood lineages and maintain hematopoietic homeostasis [1]. This balance requires exquisite control, and its disruption can lead to severe disorders and malignancies, such as leukemias and lymphomas [83]. Hematopoietic stem cell transplantation (HSCT) reconstitutes the blood system of patients who have congenital or acquired disorders of the hematopoietic system or hematologic malignancies [84]. Donor stem cells are injected into the veins of the recipient after which point they must migrate through circulation, exit at the correct location, home to the bone marrow, and engraft in the stem cell niche, a process that mimics normal development [85]. Once in the bone marrow, the transplanted HSCs can differentiate and self-renew to replace the hematopoietic cells of the patient and reconstitute their entire immune system [83]. During this process, patients are severely immunocompromised, which can lead to infection, anemia, or thrombocytopenia, and can increase the risk of transplant-related deaths [83]. Enhancing the processes that underlie homing and engraftment could improve outcomes of transplantation.

Improving homing through ex vivo HSC-endothelial cell co-culture

The first indications that bone marrow endothelial cells regulate HSC function came from the observation that these ECs exhibit adhesive affinity for HSCs and support proliferation and multilineage differentiation of both umbilical cord blood- and peripheral blood-derived HSCs through the release of cytokines [86]. Later co-culture studies with bone marrow sinusoidal ECs revealed that distinct angiocrine factors regulate HSC expansion and differentiation. Self-renewal is stimulated by Kit-ligand and Notch ligands, among others, whereas G-CSF, GM-CSF, IL1, and IL6 direct lineage-specific differentiation [16, 87, 88]. Serum-free co-culture conditions revealed that physiological levels of these angiocrine factors increase self-renewal of HSCs. Interestingly, direct contact between ECs and HSCs is essential for HSC self-renewal and differentiation in culture [16, 87, 88]. This might explain why ex vivo HSC cultures supplemented with only cytokines increases the total number of nucleated cells, but not necessarily the number of long-term engrafting progenitors. As such, it has been proposed that co-culture of HSCs with ECs is necessary to promote ex vivo expansion of HSCs. ECs from the brain, heart, and yolk sac have been shown to enhance expansion of human HSCs in vitro, which are capable of in vivo repopulation in mice and nonhuman primates [89–94].

Umbilical cord blood contains ECs that are highly proliferative, and human umbilical vein endothelial cells (HUVECs) can readily be expanded in culture [95]. HUVECs have regularly been used as an in vitro model of the endothelial niche [16, 87, 96]. Several preclinical studies confirmed that co-culturing HSCs in the presence of HUVECs increased progenitor populations and enhanced HSC engraftment and reconstitution in vivo [91, 92, 97–100]. In fact, HUVECs can be collected from the same cord that provides the cord blood-derived HSCs, eliminating the need to harvest ECs for ex vivo culture by biopsy. These studies illustrate the regulatory effect of ECs on HSCs and may provide an approach for improving HSCT.

Primary adult ECs obtained from bone marrow aspiration are low in number and require growth factor-enriched media for in vitro maintenance. Unfortunately, prolonged culture of bone marrow ECs in the presence of serum leads to loss of angiogenic potential. Growth factor deprivation, however, rapidly induces apoptosis, and therefore, angiocrine-competent ECs can only be maintained in serum-free conditions for very short periods of time. To address this issue, methods to culture vascular endothelial cells were established that maintain angiogenic state and provascular functions [101, 102]. Activation of Akt-PI3K-mTOR signaling in primary ECs, by introduction of the adenoviral E4ORF gene or constitutively active Akt, promotes survival of ECs in serum-free conditions and induces expression of angiocrine factors that supports expansion of murine HSCs. Co-activation of Akt and MAPK shifts the balance towards differentiation and is associated with pro-differentiation angiocrine factors such as IL6 [16]. These engineered EC-HSC co-cultures promote expansion of human and mouse HSPCs ex vivo and enhanced in vivo long-term multilineage engraftment and reconstitution [16, 87, 102–104]. E4ORF1⁺ ECs have been shown to enter a state of replicative senescence and have not demonstrated any malignant potential. A preclinical trial where co-cultured E4ORF1⁺ EC and HSC were transplanted into irradiated recipients demonstrated enhanced expansion and long-term hematopoietic

reconstitution in a nonhuman primate study [105]. This study revealed that E4ORF1⁺ EC-mediated HSC expansion may be safe and effective in human clinical transplantation and could possibly improve transplant outcomes.

Improving homing through modulation of chemokine and adhesion molecule interactions

Different strategies have been developed to increase homing and engraftment of HSCs upon transplantation. Not surprisingly, much research has focused on the CXCR4/CXCL12 signaling axis. Various studies have sought to prime CXCR4 receptors to CXCL12 gradients. Within the cell membrane, receptors can organize into specific subdomains known as lipid rafts. These domains play an important role in efficient interactions between signaling molecules and their cell surface receptors. Moreover, they orchestrate interactions with downstream effectors of signaling pathways, such as Rho and Rac [106]. Activation of these small GTPases results in cytoskeleton reorganization, an essential event for cell adhesion and migration [107]. Therefore, it was hypothesized that increasing CXCR4 localization in lipid rafts may improve HSCTs. Several ex vivo stimuli enhanced lipid raft formation and improved homing and engraftment efficiency. These stimuli, such as mild heat treatment of HSCs, are now proposed as priming strategies for clinical applications [108–110]. Homing can also be enhanced by increasing CXCR4 expression on HSCs. Platelet-derived microparticles (PMP) improve homing efficiency through receptor transfer. HSCs can interact with PMPs and incorporate part of the platelet membrane, hereby increasing their CXCR4 expression through receptor transfer [111, 112]. Because transgenic overexpression of CXCR4 may have negative long-term side effects, CXCR4 receptor expression has also been upregulated using small molecules. The small lipid mediator prostaglandin E2 (PGE2) increases CXCR4 expression and engraftment of human HSCs [113–116]. Ex vivo PGE2 treatment of HSCs exhibited safety and clinical potential in a phase I clinical trial [116], and a phase 2 trial is ongoing. Additionally, valproic acid, a histone deacetylase inhibitor, increased CXCR4 expression on HSCs and promoted migration towards a CXCL12 gradient [117, 118]. Homing may also be enhanced by preventing inactivation and/or degradation of CXCL12. CD26 is a peptidase expressed on HSCs that can inactivate CXCL12 before it interacts with CXCR4. Inhibition of CD26 increased HSC homing and engraftment in vivo and the CD26 inhibitor DPP-4 is currently in clinical trial for improving umbilical cord blood transplantation [117, 119, 120].

Several studies have modulated selectin signaling between HSCs and ECs to enhance homing and engraftment. Proper adhesion between selectins and their ligands requires fucosylation of selectin ligands. Ex vivo treatment of human cord blood-derived HSCs with fucosyltransferase successfully improves rolling and engraftment in the bone marrow of sub-lethally irradiated NOD/SCID mice [121]. Enhancing selectin-mediated adhesion holds great potential for improving umbilical cord blood transplantations. Selectin ligand PSGL-1 is expressed in a non-fucosylated form on cord blood HSCs [53]. As a result, HSC rolling, extravasation, and engraftment along the marrow endothelium are reduced. This could be augmented by fucosyltransferase treatment [121]. Insights gained from the cell-cell interactions and molecular signaling pathways that regulate HSC development, homing, and

engraftment can help inform studies to improve HSC expansion and hematopoietic stem cell transplantation.

Conclusion

Hematopoietic stem cells are critical for survival. The cells and signals that regulate HSC production, expansion, homing, engraftment, homeostasis, and regeneration are areas of active research. Improving our understanding of these processes could greatly improve our ability to expand HSCs ex vivo, which could advance HSCT. HSCs have a unique relationship with endothelial cells throughout their life. Recent studies have identified molecular and physical properties of bone marrow perivascular endothelial cells that are critical for HSC maintenance, localization, and regeneration. Continued efforts to further define these cells will improve our understanding of HSC development and transplantation.

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References

- Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*. 2008; 132:631–644. [PubMed: 18295580]
- Samokhvalov IM, Samokhvalova NI, Nishikawa SI. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature*. 2007; 446:1056–1061. [PubMed: 17377529]
- North TE, de Bruijn MFTR, Stacy T, Talebian L, Lind E, Robin C, Binder M, Dzierzak E, Speck NA. Runx1 expression marks long-term repopulating hematopoietic stem cells in the midgestation mouse embryo. *Immunity*. 2002; 16:661–672. [PubMed: 12049718]
- Göthert JR, Gustin SE, Hall MA, Green AR, Göttgens B, Izon DJ, Begley CG. In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis. *Blood*. 2005; 105:2724–2732. [PubMed: 15598809]
- Sanchez MJ, Holmes A, Miles C, Dzierzak E. Characterization of the first definitive hematopoietic stem cells in the AGM and liver of the mouse embryo. *Immunity*. 1996; 5:513–525. [PubMed: 8986712]
- Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*. 2010; 464:116–120. [PubMed: 20154729]
- Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. *Nature*. 2010; 464:112–115. [PubMed: 20154732]
- Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DYR, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*. 2010; 464:108–111. [PubMed: 20154733]
- Müller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E. Development of hematopoietic stem cell activity in the mouse embryo. *Immunity*. 1994; 1:291–301. [PubMed: 7889417]
- Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014; 505:327–334. [PubMed: 24429631]
- Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med*. 2014; 20:833–846. [PubMed: 25100529]
- Birbrair A, Frenette PS. Niche heterogeneity in the bone marrow. *Ann N Y Acad Sci*. 2016; 1370:82–96.

13. Chen JY, Miyanishi M, Wang SK, Yamazaki S, Sinha R, Kao KS, Seita J, Sahoo D, Nakauchi H, Weissman IL. Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche. *Nature*. 2016; 530:223–227. [PubMed: 26863982]
14. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005; 121:1109–1121. [PubMed: 15989959]
15. Rafii S, Butler JM, Ding BS. Angiocrine functions of organ-specific endothelial cells. *Nature*. 2016; 529:316–325. [PubMed: 26791722]
16. Kobayashi H, Butler JM, O'Donnell R, Kobayashi M, Ding BS, Bonner B, Chiu VK, Nolan DJ, Shido K, Benjamin L, et al. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. *Nat Cell Biol*. 2010; 12:1046–1056. [PubMed: 20972423]
17. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. 2012; 481:457–462. [PubMed: 22281595]
18. Ohneda O, Fennie C, Zheng Z, Donahue C, La H, Villacorta R, Cairns B, Lasky LA. Hematopoietic stem cell maintenance and differentiation are supported by embryonic aorta-gonad-mesonephros region-derived endothelium. *Blood*. 1998; 92:908–919. [PubMed: 9680359]
19. Rafii S, Shapiro F, Pettengell R, Ferris B, Nachman RL, Moore MA, Asch AS. Human bone marrow microvascular endothelial cells support long-term proliferation and differentiation of myeloid and megakaryocytic progenitors. *Blood*. 1995; 86:3353–3363. [PubMed: 7579438]
20. Lu LS, Wang SJ, Auerbach R. In vitro and in vivo differentiation into B cells, T cells, and myeloid cells of primitive yolk sac hematopoietic precursor cells expanded >100-fold by coculture with a clonal yolk sac endothelial cell line. *P Natl Acad Sci USA*. 1996; 93:14782–14787.
21. Li W, Johnson SA, Shelley WC, Ferkowicz M, Morrison P, Li Y, Yoder MC. Primary endothelial cells isolated from the yolk sac and para-aortic splanchnopleura support the expansion of adult marrow stem cells in vitro. *Blood*. 2003; 102:4345–4353. [PubMed: 12893772]
22. Yao L, Yokota T, Xia L, Kincade PW, McEver RP. Bone marrow dysfunction in mice lacking the cytokine receptor gp130 in endothelial cells. *Blood*. 2005; 106:4093–4101. [PubMed: 16118327]
23. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature*. 2013; 495:231–235. [PubMed: 23434755]
24. Greenbaum A, Hsu YMS, Day RB, Schuettelpelz LG, Christopher MJ, Borgerding JN, Nagasawa T, Link DC. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature*. 2013; 495:227–230. [PubMed: 23434756]
25. Kisanuki YY, Kisanuki YY, Hammer RE, Hammer RE, Miyazaki J, Miyazaki JI, Williams SC, Williams SC, Richardson JA, Richardson JA, et al. Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. *Dev Biol*. 2001; 230:230–242. [PubMed: 11161575]
26. de Jong JLO, Zon LI. Use of the zebrafish system to study primitive and definitive hematopoiesis. *Annu Rev Genet*. 2005; 39:481–501. [PubMed: 16285869]
27. Swift MR, Swift MR, Weinstein BM, Weinstein BM. Arterial-venous specification during development. *Circ Res*. 2009; 104:576–588. [PubMed: 19286613]
28. Murayama E, Kissa K, Zapata A, Mordélet E, Briolat V, Lin HF, Handin RI, Herbomel P. Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. *Immunity*. 2006; 25:963–975. [PubMed: 17157041]
29. Tamplin OJ, Durand EM, Carr LA, Childs SJ, Hagedorn EJ, Li P, Yzaguirre AD, Speck NA, Zon LI. Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. *Cell*. 2015; 160:241–252. [PubMed: 25594182]
30. Zhen F, Lan Y, Yan B, Zhang W, Wen Z. Hemogenic endothelium specification and hematopoietic stem cell maintenance employ distinct Scl isoforms. *Development*. 2013; 140:3977–3985. [PubMed: 24046317]
31. Kissa K, Kissa K, Murayama E, Murayama E, Zapata A, Zapata A, Cortés A, Cortes A, Perret E, Perret E, et al. Live imaging of emerging hematopoietic stem cells and early thymus colonization. *Blood*. 2007; 111:1147–1156. [PubMed: 17934068]
32. Aird WC, Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res*. 2007; 100:158–173. [PubMed: 17272818]

33. Nolan DJ, Ginsberg M, Israely E, Palikuqi B, Poulos MG, James D, Ding BS, Schachterle W, Liu Y, Rosenwaks Z, et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell*. 2013; 26:204–219. [PubMed: 23871589]
34. You LR, Lin FJ, Lee CT, DeMayo FJ, Tsai MJ, Tsai SY. Suppression of Notch signalling by the COUP-TFII transcription factor regulates vein identity. *Nature*. 2005; 435:98–104. [PubMed: 15875024]
35. Chen X, Qin J, Cheng CM, Tsai MJ, Tsai SY. COUP-TFII is a major regulator of cell cycle and Notch signaling pathways. *Mol Endocrinol*. 2012; 26:1268–1277. [PubMed: 22734039]
36. Corada M, Corada M, Morini MF, Morini MF, Dejana E, Dejana E. Signaling pathways in the specification of arteries and veins. *Arterioscl Throm Vas*. 2014; 34:2372–2377.
37. De Val S, Black BL. Transcriptional control of endothelial cell development. *Dev Cell*. 2009; 16:180–195. [PubMed: 19217421]
38. De Val S, De Val S. Key transcriptional regulators of early vascular development. *Arterioscl Throm Vas*. 2011; 31:1469–1475.
39. Francois M, Koopman P, Beltrame M. SoxF genes: key players in the development of the cardiovascular system. *Int J Biochem Cell B*. 2010; 42:445–448.
40. Dejana E, Dejana E, TADDEI A, Taddei A, Randi AM, RANDI A. Foxs and Ets in the transcriptional regulation of endothelial cell differentiation and angiogenesis. *BBA-Rev Cancer*. 2007; 1775:298–312.
41. Heazlewood SY, Oteiza A, Cao H, Nilsson SK. Analyzing hematopoietic stem cell homing, lodgment, and engraftment to better understand the bone marrow niche. *Ann N Y Acad Sci*. 2014; 1310:119–128. [PubMed: 24428368]
42. Sackstein R. Fulfilling Koch's postulates in glycoscience: HCELL, GPS and translational glycobiology. *Glycobiology*. 2016; 26:560–570. [PubMed: 26933169]
43. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007; 7:678–689. [PubMed: 17717539]
44. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol*. 2006; 6:93–106. [PubMed: 16491134]
45. Langer HF, Chavakis T. Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med*. 2009; 13:1211–1220. [PubMed: 19538472]
46. Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell*. 1996; 84:563–574. [PubMed: 8598043]
47. Schweitzer KM, Dräger AM, van der Valk P, Thijsen SF, Zevenbergen A, Theijssmeijer AP, van der Schoot CE, Langenhuijsen MM. Constitutive expression of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues. *Am J Pathol*. 1996; 148:165–175. [PubMed: 8546203]
48. Mazo IB, Andrian von UH. Adhesion and homing of blood-borne cells in bone marrow microvessels. *J Leukoc Biol*. 1999; 66:25–32. [PubMed: 10410986]
49. Dimitroff CJ, Lee JY, Rafii S, Fuhlbrigge RC, Sackstein R. Cd44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol*. 2001; 153:1277–1286. [PubMed: 11402070]
50. Merzaban JS, Burdick MM, Gadhoun SZ, Dagia NM, Chu JT, Fuhlbrigge RC, Sackstein R. Analysis of glycoprotein E-selectin ligands on human and mouse marrow cells enriched for hematopoietic stem/progenitor cells. *Blood*. 2011; 118:1774–1783. [PubMed: 21659548]
51. Burdick MM, Chu JT, Godar S, Sackstein R. HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells. *J Biol Chem*. 2006; 281:13899–13905. [PubMed: 16565092]
52. Katayama Y, Hidalgo A, Peired A, Frenette PS. Integrin alpha4beta7 and its counterreceptor MAdCAM-1 contribute to hematopoietic progenitor recruitment into bone marrow following transplantation. *Blood*. 2004; 104:2020–2026. [PubMed: 15161666]
53. Hidalgo A, Weiss LA, Frenette PS. Functional selectin ligands mediating human CD34(+) cell interactions with bone marrow endothelium are enhanced postnatally. *J Clin Invest*. 2002; 110:559–569. [PubMed: 12189250]

54. Peled A, Grabovsky V, Habler L, Sandbank J, Arenzana-Seisdedos F, Petit I, Ben-Hur H, Lapidot T, Alon R. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34+ cells on vascular endothelium under shear flow. *J Clin Invest.* 1999; 104:1199–1211. [PubMed: 10545519]
55. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science.* 1999; 283:845–848. [PubMed: 9933168]
56. Dar A, Kollet O, Lapidot T. Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Exp Hematol.* 2006; 34:967–975. [PubMed: 16863903]
57. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood.* 2000; 95:3289–3296. [PubMed: 10828007]
58. Frenette PS, Subbarao S, Mazo IB, Andrian von UH, Wagner DD. Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. *P Natl Acad Sci USA.* 1998; 95:14423–14428.
59. Imai K, Kobayashi M, Wang J, Shinobu N, Yoshida H, Hamada JI, Shindo M, Higashino F, Tanaka J, Asaka M, et al. Selective secretion of chemoattractants for haemopoietic progenitor cells by bone marrow endothelial cells: a possible role in homing of haemopoietic progenitor cells to bone marrow. *Brit J Haematol.* 1999; 106:905–911. [PubMed: 10519991]
60. Carstanjen D, Gross A, Kosova N, Fichtner I, Salama A. The alpha4beta1 and alpha5beta1 integrins mediate engraftment of granulocyte-colony-stimulating factor-mobilized human hematopoietic progenitor cells. *Transfusion.* 2005; 45:1192–1200. [PubMed: 15987366]
61. Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, Mahoney JE, Park SY, Lu J, Protopopov A, Silberstein LE. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. *Nat Cell Biol.* 2013; 15:1016–1016.
62. Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, Mizoguchi T, Wei Q, Lucas D, Ito K, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature.* 2013; 502:637–643. [PubMed: 24107994]
63. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature.* 2014; 507:323–328. [PubMed: 24646994]
64. Itkin T, Gur-Cohen S, Spencer JA, Schajnovitz A, Ramasamy SK, Kusumbe AP, Ledergor G, Jung Y, Milo I, Poulos MG, et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. *Nature.* 2016; 532:323–328. [PubMed: 27074509]
65. Lassailly F, Lassailly F, Foster K, Foster K, Lopez-Onieva L, Lopez-Onieva L, Currie E, Currie E, Bonnet D, Bonnet D. Multimodal imaging reveals structural and functional heterogeneity in different bone marrow compartments: functional implications on hematopoietic stem cells. *Blood.* 2013; 122:1730–1740. [PubMed: 23814020]
66. Li XM, Li XM, Hu Z, Hu Z, Jorgenson ML, Jorgenson ML, Slayton WB, Slayton WB. High levels of acetylated low-density lipoprotein uptake and low tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2 (Tie2) promoter activity distinguish sinusoids from other vessel types in murine bone marrow. *Circulation.* 2009; 120:1910–1918. [PubMed: 19858415]
67. Abboud CN. Human bone marrow microvascular endothelial cells: elusive cells with unique structural and functional properties. *Exp Hematol.* 1995; 23:1–3. [PubMed: 7995366]
68. Rafii S, Rafii S, Möhle R, Mohle R, Shapiro F, Shapiro F, Frey BM, Frey BM, Moore MA, Moore MAS. Regulation of hematopoiesis by microvascular endothelium. *Leukemia Lymphoma.* 2009; 27:375–386.
69. Ulyanova T, Scott LM, Priestley GV, Jiang Y, Nakamoto B, Koni PA, Papayannopoulou T. VCAM-1 expression in adult hematopoietic and nonhematopoietic cells is controlled by tissue-inductive signals and reflects their developmental origin. *Blood.* 2005; 106:86–94. [PubMed: 15769895]

70. Papayannopoulou T, Priestley GV, Nakamoto B, Zafiropoulos V, Scott LM. Molecular pathways in bone marrow homing: dominant role of alpha(4)beta(1) over beta(2)-integrins and selectins. *Blood*. 2001; 98:2403–2411. [PubMed: 11588037]
71. Winkler IG, Barbier V, Nowlan B, Jacobsen RN, Forristal CE, Patton JT, Magnani JL, Lévesque JP. Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. *Nat Med*. 2012; 18:1651–1657. [PubMed: 23086476]
72. Kunisaki Y, Frenette PS. Influences of vascular niches on hematopoietic stem cell fate. *Int J Hematol*. 2014; 99:699–705. [PubMed: 24756874]
73. Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A, Nakauchi H. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell*. 2011; 147:1146–1158. [PubMed: 22118468]
74. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity*. 2006; 25:977–988. [PubMed: 17174120]
75. Tesio M, Golan K, Corso S, Giordano S, Schajnovitz A, Vagima Y, Shvitiel S, Kalinkovich A, Caione L, Gammaitoni L, et al. Enhanced c-Met activity promotes G-CSF-induced mobilization of hematopoietic progenitor cells via ROS signaling. *Blood*. 2011; 117:419–428. [PubMed: 20585044]
76. Golan K, Vagima Y, Ludin A, Itkin T, Cohen-Gur S, Kalinkovich A, Kollet O, Kim C, Schajnovitz A, Ovadya Y, et al. S1P promotes murine progenitor cell egress and mobilization via S1P1-mediated ROS signaling and SDF-1 release. *Blood*. 2012; 119:2478–2488. [PubMed: 22279055]
77. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med*. 2006; 12:446–451. [PubMed: 16565722]
78. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell*. 2007; 1:101–112. [PubMed: 18371339]
79. Celso Lo C, Fleming HE, Wu JW, Zhao CX, Miake-Lye S, Fujisaki J, Côté D, Rowe DW, Lin CP, Scadden DT. Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature*. 2008; 457:92–96. [PubMed: 19052546]
80. Bixel MG, Kusumbe AP, Ramasamy SK, Sivaraj KK, Butz S, Vestweber D, Adams RH. Flow dynamics and HSPC homing in bone marrow microvessels. *Cell Rep*. 2017; 18:1804–1816. [PubMed: 28199850]
81. Vogeli KM, Jin SW, Martin GR, Stainier DYR. A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. *Nature*. 2006; 443:337–339. [PubMed: 16988712]
82. Florey. The endothelial cell. *Brit Med J*. 1966; 2:487–490. [PubMed: 5913081]
83. Tuthill M, Hatzimichael. Hematopoietic stem cell transplantation. *Stem Cells and Cloning: Advances and Applications*. 2010; 3:105–117. doi: [PubMed: 24198516]
84. Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, Szer J, Lipton J, Schwendener A, Gratwohl M, et al. Hematopoietic stem cell transplantation: a global perspective. *JAMA*. 2010; 303:1617–1624. [PubMed: 20424252]
85. Ratajczak MZ. A novel view of the adult bone marrow stem cell hierarchy and stem cell trafficking. *Leukemia*. 2014; 29:776–782. [PubMed: 25486871]
86. Rafii S, Shapiro F, Rimarachin J, Nachman RL, Ferris B, Weksler B, Moore MA, Asch AS. Isolation and characterization of human bone marrow microvascular endothelial cells: hematopoietic progenitor cell adhesion. *Blood*. 1994; 84:10–19. [PubMed: 7517203]
87. Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M, et al. Endothelial cells are essential for the self-renewal and re-population of Notch-dependent hematopoietic stem cells. *Cell Stem Cell*. 2010; 6:251–264. [PubMed: 20207228]
88. Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, Kopp HG, Shido K, Petit I, Yanger K, et al. Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell*. 2009; 4:263–274. [PubMed: 19265665]

89. Li W, Johnson SA, Shelley WC, Yoder MC. Hematopoietic stem cell repopulating ability can be maintained in vitro by some primary endothelial cells. *Exp Hematol.* 2004; 32:1226–1237. [PubMed: 15588947]
90. Chute JP, Muramoto GG, Fung J, Oxford C. 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. [PubMed: 15345596]
91. Brandt JE, Galy AH, Luens KM, Travis M, Young J, Tong J, Chen S, Davis TA, Lee KP, Chen BP, et al. Bone marrow repopulation by human marrow stem cells after long-term expansion culture on a porcine endothelial cell line. *Exp Hematol.* 1998; 26:950–961. [PubMed: 9728930]
92. Brandt JE, Bartholomew AM, Fortman JD, Nelson MC, Bruno E, Chen LM, Turian JV, Davis TA, Chute JP, Hoffman R. Ex vivo expansion of autologous bone marrow CD34(+) cells with porcine microvascular endothelial cells results in a graft capable of rescuing lethally irradiated baboons. *Blood.* 1999; 94:106–113. [PubMed: 10381503]
93. Chute JP, Saini AA, Kampen RL, Wells MR, Davis TA. A comparative study of the cell cycle status and primitive cell adhesion molecule profile of human CD34+ cells cultured in stroma-free versus porcine microvascular endothelial cell cultures. *Exp Hematol.* 1999; 27:370–379. [PubMed: 10029177]
94. Li N, Eljaafari A, Bensoussan D, Wang Y, Latger-Cannard V, Serrurier B, Boura C, Kennel A, Stoltz J, Feugier P. Human umbilical vein endothelial cells increase ex vivo expansion of human CD34(+) PBPC through IL-6 secretion. *Cytotherapy.* 2006; 8:335–342. [PubMed: 16923609]
95. Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest.* 1973; 52:2745–2756. [PubMed: 4355998]
96. Yildirim S, Boehmler AM, Kanz L, Möhle R. Expansion of cord blood CD34+ hematopoietic progenitor cells in coculture with autologous umbilical vein endothelial cells (HUVEC) is superior to cytokine-supplemented liquid culture. *Bone Marrow Transpl.* 2005; 36:71–79.
97. Salter AB, Meadows SK, Muramoto GG, Himburg H, Doan P, Daher P, Russell L, Chen B, Chao NJ, Chute JP. Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo. *Blood.* 2009; 113:2104–2107. [PubMed: 19141867]
98. Chute JP, Muramoto GG, Salter AB, Meadows SK, Rickman DW, Chen B, Himburg HA, Chao NJ. Transplantation of vascular endothelial cells mediates the hematopoietic recovery and survival of lethally irradiated mice. *Blood.* 2007; 109:2365–2372. [PubMed: 17095624]
99. Li B, Bailey AS, Jiang S, Liu B, Goldman DC, Fleming WH. Endothelial cells mediate the regeneration of hematopoietic stem cells. *Stem Cell Res.* 2010; 4:17–24. [PubMed: 19720572]
100. Raynaud CM, Butler JM, Halabi NM, Ahmad FS, Ahmed B, Rafii S, Rafii A. Endothelial cells provide a niche for placental hematopoietic stem/progenitor cell expansion through broad transcriptomic modification. *Stem Cell Res.* 2013; 11:1074–1090. [PubMed: 23978474]
101. Seandel M, Butler JM, Kobayashi H, Hooper AT, White IA, Zhang F, Vertes EL, Kobayashi M, Zhang Y, Shmelkov SV, et al. Generation of a functional and durable vascular niche by the adenoviral E4ORF1 gene. *P Natl Acad Sci USA.* 2008; 105:19288–19293.
102. Hadland BK, Varnum-Finney B, Poulos MG, Moon RT, Butler JM, Rafii S, Bernstein ID. Endothelium and NOTCH specify and amplify aorta-gonad-mesonephros-derived hematopoietic stem cells. *J Clin Invest.* 2015; 125:2032–2045. [PubMed: 25866967]
103. Poulos MG, Crowley MJP, Gutkin MC, Ramalingam P, Schachterle W, Thomas JL, Elemento O, Butler JM. Vascular platform to define hematopoietic stem cell factors and enhance regenerative hematopoiesis. *Stem Cell Reports.* 2015; 5:881–894. [PubMed: 26441307]
104. Butler JM, Gars EJ, James DJ, Nolan DJ, Scandura JM, Rafii S. Development of a vascular niche platform for expansion of repopulating human cord blood stem and progenitor cells. *Blood.* 2012; 120:1344–1347. [PubMed: 22709690]
105. Gori JL, Butler JM, Kunar B, Poulos MG, Ginsberg M, Nolan DJ, Norgaard ZK, Adair JE, Rafii S, Kiem HP. Endothelial cells promote expansion of long-term engrafting marrow hematopoietic stem and progenitor cells in primates. *Stem Cells Transl Med.* 2017; 6:864–876. [PubMed: 28297579]

106. Cancelas JA, Lee AW, Prabhakar R, Stringer KF, Zheng Y, Williams DA. Rac GTPases differentially integrate signals regulating hematopoietic stem cell localization. *Nat Med.* 2005; 11:886–891. [PubMed: 16025125]
107. Gu Y, Filippi MD, Cancelas JA, Siefiring JE, Williams EP, Jasti AC, Harris CE, Lee AW, Prabhakar R, Atkinson SJ, et al. Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases. *Science.* 2003; 302:445–449. [PubMed: 14564009]
108. Wu W, Kim CH, Liu R, Kucia M, Marlicz W, Greco N, Ratajczak J, Laughlin MJ, Ratajczak MZ. The bone marrow-expressed antimicrobial cationic peptide LL-37 enhances the responsiveness of hematopoietic stem progenitor cells to an SDF-1 gradient and accelerates their engraftment after transplantation. *Leukemia.* 2011; 26:736–745. [PubMed: 21931324]
109. Capitano ML, Hangoc G, Cooper S, Broxmeyer HE. Mild heat treatment primes human CD34(+) cord blood cells for migration toward SDF-1 α and enhances engraftment in an NSG mouse model. *Stem Cells.* 2015; 33:1975–1984. [PubMed: 25753525]
110. Wysoczynski M, Reza R, Ratajczak J, Kucia M, Shirvaikar N, Honczarenko M, Mills M, Wanzeck J, Janowska-Wieczorek A, Ratajczak MZ. Incorporation of CXCR4 into membrane lipid rafts primes homing-related responses of hematopoietic stem/progenitor cells to an SDF-1 gradient. *Blood.* 2005; 105:40–48. [PubMed: 15328152]
111. Moghaddam F, Oodi A, Nikougoftar Zarif M, Amani M, Amirizadeh N. Expression of CXCR4 in cord blood-derived CD133+ cells treated with platelet micro-particles. *Artif Cell Nanomed B.* 2016; 44:1702–1707.
112. Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reza R, Turner AR, Ratajczak J, Emerson SG, Kowalska MA, Ratajczak MZ. Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood.* 2001; 98:3143–3149. [PubMed: 11698303]
113. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature.* 2007; 447:1007–1011. [PubMed: 17581586]
114. Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood.* 2009; 113:5444–5455. [PubMed: 19324903]
115. Goessling W, Allen RS, Guan X, Jin P, Uchida N, Dovey M, Harris JM, Metzger ME, Bonifacio AC, Stroncek D, et al. Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell.* 2011; 8:445–458. [PubMed: 21474107]
116. Cutler C, Multani P, Robbins D, Kim HT, Le T, Hoggatt J, Pelus LM, Despons C, Chen YB, Reznar B, et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood.* 2013; 122:3074–3081. [PubMed: 23996087]
117. Broxmeyer HE, Pelus LM. Inhibition of DPP4/CD26 and dmPGE2 treatment enhances engraftment of mouse bone marrow hematopoietic stem cells. *Blood Cell Mol Dis.* 2014; 53:34–38.
118. Gul H, Marquez-Curtis LA, Jahroudi N, Lo J, Turner AR, Janowska-Wieczorek A. Valproic acid increases CXCR4 expression in hematopoietic stem/progenitor cells by chromatin remodeling. *Stem Cells Dev.* 2009; 18:831–838. [PubMed: 18847317]
119. Yoo E, Paganessi LA, Alikhan WA, Paganessi EA, Hughes F, Fung HC, Rich E, Seong CM, Christopherson KW. Loss of CD26 protease activity in recipient mice during hematopoietic stem cell transplantation results in improved transplant efficiency. *Transfusion.* 2013; 53:878–887. [PubMed: 22882234]
120. Christopherson KW, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science.* 2004; 305:1000–1003. [PubMed: 15310902]
121. Xia L, McDaniel JM, Yago T, Doeden A, McEver RP. Surface fucosylation of human cord blood cells augments binding to P-selectin and E-selectin and enhances engraftment in bone marrow. *Blood.* 2004; 104:3091–3096. [PubMed: 15280192]

Fig. 1.

Endothelial cell-HSC interactions during homing and engraftment. During development and transplantation HSCs must home to, and engraft in, the stem cell niche. **a** Definitive hematopoiesis is initiated when HSCs are specified from hemogenic endothelium in the aortagonad-mesonephros (AGM) region. HSCs bud from the ventral wall of the dorsal aorta, enter circulation, and home to the fetal liver. After extravasating into the fetal niche, ECs remodel around the HSC in a process termed “endothelial cell cuddling.” HSCs expand in the fetal niche and then home to, and engraft in, the adult niche in the bone marrow. HSCs engraft in complex and specialized niches consisting of multiple specialized cell types. These niches maintain HSCs and regulate their self-renewal and differentiation into all mature blood lineages. **b** HSCs undergo a series of stereotyped actions to home towards the niche, all of which depend on endothelial cells—rolling, arrest and firm adhesion, spreading, and extravasation. Rolling is initiated when E- and P-selectin receptors on ECs tether to their ligands on the HSC, serving as a molecular brake to slow HSC migration in circulation. Chemokine CXCL12 then activates VCAM and ICAM adhesion molecules, which promote firm adhesion of HSCs to vascular ECs. Finally, chemokines and adhesion molecules direct polarized migration across the endothelium and result in HSC extravasation

Fig. 2. Endothelial cells regulate HSC engraftment and maintenance in the adult bone marrow niche. The bone marrow niche consists of different types of blood vessels. **a** The vascular architecture and properties of bone marrow ECs differ between arteriolar and sinusoidal vessels. Arterioles, which have low permeability and high rates of blood flow, branch into sinusoids, which have high permeability and low rates of flow. Differences in arteriolar and vascular associated cells can be observed and may have an impact on the regulation of HSCs within these niches. Non-myelinating Schwann cells that maintain hibernating HSCs exclusively associate with arterioles, whereas megakaryocytes, that also establish a quiescent niche for HSCs localize near sinusoids. **b** The bone marrow perivascular niche contains numerous cell types including stromal cells, macrophages, megakaryocytes, osteoblasts and osteoclasts, sympathetic nerves, and endothelial cells. Endothelial cells play an important role in HSC maintenance, self-renewal, and differentiation, and are an important source of two key angiocrine factors, CXCL12 and SCF. Distinct subsets of endothelial cells, arterioles and sinusoids, may make up unique bone marrow stem cell niches with different properties. Arteriolar ECs have been associated with HSC retention, whereas sinusoidal ECs release factors that promote HSC proliferation, such as Kit-ligand and Notch ligands. Moreover, sinusoids are the exclusive site of HSC homing and trafficking. The difference in

ROS levels between arteriolar and sinusoidal microenvironments may affect HSC cell cycle state and metabolism. Thus far, quiescent HSCs have been observed both near arteriolar and sinusoidal vessels. Nonetheless, the unique physical and molecular properties of the sinusoidal and arteriolar microenvironments may affect HSC state

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