

The role of novel and known extracellular matrix and adhesion molecules in the homeostatic and regenerative bone marrow microenvironment

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Keywords: haematopoietic stem cells, extracellular matrix, bone marrow niche, BIGH3, integrins, periostin, bone marrow regeneration, adhesion molecules, matrix proteins, differentiation

Maintenance of haematopoietic stem cells and differentiation of committed progenitors occurs in highly specialized niches. The interactions of haematopoietic stem and progenitor cells (HSPCs) with cells, growth factors and extracellular matrix (ECM) components of the bone marrow (BM) microenvironment control homeostasis of HSPCs. We only start to understand the complexity of the haematopoietic niche(s) that comprises endosteal, arterial, sinusoidal, mesenchymal and neuronal components. These distinct niches produce a broad range of soluble factors and adhesion molecules that modulate HSPC fate during normal hematopoiesis and BM regeneration. Adhesive interactions between HSPCs and the microenvironment will influence their localization and differentiation potential. In this review we highlight the current understanding of the functional role of ECM- and adhesion (regulating) molecules in the haematopoietic niche during homeostatic and regenerative hematopoiesis. This knowledge may lead to the improvement of current cellular therapies and more efficient development of future cellular products.

Haematopoietic Stem Cells: Introduction

Billions of mature blood cells are produced daily from a limited number of haematopoietic stem cells (HSCs) in the bone marrow (BM) via a hierarchical differentiation process called hematopoiesis.^{1–4} The number of HSCs is tightly regulated by intrinsic and extrinsic signals to balance the maintenance of the HSC population and the differentiation of HSCs into haematopoietic progenitors that supply the life-long daily need for mature blood cells.⁵ Regulation of this balance also controls haematopoietic regeneration after, for instance, radiation or chemotherapy. The majority of HSCs is

present in a quiescent state. In mice, HSCs divide on average once per 32 days, but a subpopulation of HSCs divides only 5 times during the lifespan of a mouse, although these cells are reversibly activated upon massive haematopoietic cell loss.^{6,7} Depending on intrinsic and microenvironmental cues, HSC proliferation results in self-renewal or differentiation. HSC differentiation yields HSCs with limited self-renewal capacities or multipotent progenitors (MPPs) that lack self-renewal capacities and subsequently produce lineage-restricted progenitors.^{5,8}

The ability of environmental cues to affect stem cell fate is a concept from embryology that extends to the adult organism. Haematopoietic stem and progenitor cells (HSPCs) interact with neighboring cells, matrix components and supportive factors in the BM, which define a niche that is critical for the homeostatic maintenance of the HSC population.⁹ Adhesive interactions between HSPCs and their microenvironment influence their self-renewal, proliferation and differentiation fate. However, it is not completely elucidated which adhesive molecules are involved in these processes, and how they exert these effects.

This review provides an overview of the features of haematopoietic stem cells and the BM niche, with emphasis on extracellular matrix (ECM), cellular adhesion molecules and other environmental cues that affect stem cell fate. First we focus on the ECM- and adhesion molecules being involved in the homeostatic situation. The second part focuses on adhesion molecules that are differentially regulated during BM regeneration. Comparative array studies of HSPCs revealed many adhesion molecules that were differentially regulated upon bone marrow injury¹⁰ and/or between HSPC differentiation stages.¹¹ In particular we focus on the ECM protein BIGH3 (transforming-growth-factor- β inducible gene h3), the adhesion molecules CD164 (Endolyn), CD166 (activated leukocyte cell adhesion molecule; ALCAM), Embigin and Biglycan and their role in the homeostatic and regenerative BM environment.

Haematopoietic stem cells: HSPC phenotypes

Great progress has been made in the functional and phenotypic characterization of adult HSPCs. Long-term human HSCs (LT-HSC) are defined by their ability to repopulate BM upon transplantation in sub-lethally irradiated non-obese diabetic severe combined immune deficiency (NOD-SCID) mice, and

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Submitted: 04/08/2014; Revised: 06/14/2014; Accepted: 06/30/2014

<http://dx.doi.org/10.4161/19336918.2014.968501>

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provide a life-long (>16 weeks) multi-lineage haematopoietic reconstitution, reviewed in Schroeder et al.¹² Strict definitions of LT-HSCs even require multi-lineage repopulation of secondary transplanted mice. Transplantation of short-term HSCs (ST-HSC) in sub-lethally irradiated NOD-SCID mice results in short-term reconstitution of the mice by all mature haematopoietic lineages, but only for a limited time course due to their limited self-renewal capacity. *In vitro* assays like cobble stone area forming cell (CAFC) assays are developed and calibrated to compare the *in vitro* stem cell capacity of cell populations with the *in vivo* repopulation capacities.¹³

These functional features are correlated with the expression of cell surface markers that enables prospective isolation of specific populations of HS(P)Cs. HSC functional activity is detected in human CD34⁺CD38⁻ BM cells,¹⁴ and is more enriched in the population of protein tyrosine phosphatase CD45RA negative and thymus-antigen (Thy1; CD90) positive CD34⁺CD38⁻ cells (CD34⁺CD38⁻CD45RA⁻CD90⁺).¹⁵ Additional selection for Laminin-binding Integrin- α 6 (ITGA6/CD49f) positive cells allowed isolation of a very pure human HSC population, as long-term multi-lineage repopulation was obtained in 28% of the injected mice, by single cell intrafemoral injection into female NOD-SCID- interleukin 2 receptor gamma chain (IL2Rg) null mice. The ITGA6⁻ cells within the HSPC population showed only short-term multi-lineage reconstitution of the BM,^{16,17} suggesting a major role for ITGA6 in the regulation of LT-HSCs. The most primitive, but extremely rare human HSCs, are CD34⁻ and lineage negative, tyrosine protein kinase Kit (c-Kit; CD117) negative and Fms-like Tyrosine Kinase-3 negative (Lin⁻c-Kit⁻Flt3⁻) and the reconstitution capacities upon intrafemoral single cell injections are comparable to those of ITGA6⁺ HSCs.^{18,19}

Murine HSCs are isolated as Flt3⁻, CD34⁻, c-Kit-ligand positive, stem-cell-antigen-1 positive (Ly6A; Sca1) and lineage negative (Flt3⁻CD34⁻KitL⁺Sca1⁺Lin⁻)²⁰ or, alternatively, as signaling-lymphocytic-activation-molecule (SLAMF1; CD150) positive, SLAMF2 (CD48) negative, Integrin- α 2B (CD41) negative and KitL⁺Sca1⁺Lin⁻ (CD150⁺CD48⁻CD41⁻KitL⁺Sca1⁺Lin⁻) cells.²¹ Single cell transplantations of these populations showed in 20–33% of the injected mice a multi-lineage repopulation, reviewed in Challen & Goodell.²² Heterogeneity still exists within these purified populations, indicated by intrinsically distinct lineage-bias or self-renewal capacities within these populations dependent on e.g. Integrin- α 2 (ITGA2) and CD150 expression.^{12,21-24} In addition, environmental cues are necessary for the maintenance of self-renewal, support of survival, proliferation and lineage instruction.^{25,26}

Haematopoietic stem cells: *in vitro* cell cultures of HSPCs

In vitro HSPC expansion has long relied on co-culture of HSCs with stromal or endothelial cells, which provide signals that suppress HSPC differentiation.²⁷ Nowadays, it is possible to expand HSPCs using cytokine cocktails in the absence of stromal cells. Expansion of murine HSPCs in feeder-free cell cultures was achieved by use of the Lodish and Zhang cocktail,²⁸ resulting in a 30-fold net HSPC expansion.

Cytokine cocktails containing stem cell factor (SCF), Flt3-ligand (Flt3L) and thrombopoietin (TPO) occasionally supplemented with Interleukin-6 (IL-6), IL-11, IL-3 or granulocyte-macrophage-cell-stimulating-factor (GM-CSF) have been extensively studied in HSC expansion in co-cultures and feeder-free-cultures, reviewed in Sauvageau et al.²⁹ Haematopoietic regulators that specify HSPCs during ontogeny are also able to increase *in vitro* HSPC expansion. Addition of wingless-type-MMTV-integration-site-3A (Wnt3A) or Wnt5A³⁰⁻³³ or an immobilized form of Notch^{34,35} can induce HSPC expansion *in vitro*. Other promising developmental regulators that improve *in vitro* HSPC expansion include soluble Sonic Hedgehog, the endothelium produced insulin-like-growth factor 2 (IGF2), IGF binding protein (IGFBP2), angiopoietin-like proteins (Angpl1) and the novel microenvironmental factor Pleiothrophin, that is involved in the coagulation cascade.³⁶ A group of small chemical molecules also includes potent inducers of HSPC expansion: the copper chelator tetraethylenepentamine (TEPA) attenuates HSPC differentiation, resulting in expansion of early progenitors.³⁷ Other promising chemical compounds are the histone deacetylase inhibitor Valproic Acid, the Wnt-agonist BIO, prostaglandin E2 (PGE2) that may inhibit apoptosis of HSPCs, and the TPO-agonist NR-101, reviewed in Walasek et al.³⁸ Despite all progress in HSPC expansion protocols, it is still impossible to maintain LT-HSCs in culture. HS(P)C fate determination is tightly regulated by extrinsic signals, including soluble molecules and complex outside-in-signaling networks.

Haematopoietic stem cells: normal life cycle of HSPCs

BM microenvironmental signals are needed to keep HSPCs localized in the BM, whereas other signals mobilize HSPCs into the circulation and *vice versa* mobilized HSPCs home back to the BM upon specific cues. These processes depend on directed HSPC migration, which involves signaling in response to chemo-tactic stimuli, polarization of signaling complexes, cytoskeletal remodeling and polarization of adhesive structures.^{7,39} Chemokines bind G-coupled receptors on HSPCs, which triggers signaling cascades leading to directed motility. The most important chemokine for HSPCs is CXCL12 (also named stromal-derived-factor-1; SDF1). It is produced by BM-cells and bound by the chemokine receptors CXCR4 and CXCR7 on HSPCs.^{40,41} In CXCL12 or CXCR4 deficient embryonic mice, haematopoietic precursors fail to shift from the fetal liver into the fetal BM.^{42,43} In addition, CXCL12 is important for HSPC homeostasis, since conditional deletion of CXCR4 in adulthood reduced HSC numbers in the BM.⁴⁴ Both, CXCL12 and CXCR4 are essential for HSPC homing to and egress from the BM, and relocalization into different BM-microenvironments,^{7,37,45} emphasizing that CXCL12 is required for normal BM-niche function.

Therapeutic mobilization of human HSPCs into the circulation is routinely obtained by administration of Granulocyte-colony-stimulating-factor (G-CSF) or G-CSF combined with cyclophosphamide.⁴⁶ or other variants of chemotherapy. This inhibits osteoprogenitor proliferation, thereby reducing the

CXCL12 concentrations in the BM and allowing HSPC egress.⁴⁷⁻⁴⁹ In addition, G-CSF stimulates neutrophils to release proteases to further cleave CXCR4,^{45,50} although non-proteolytic mechanisms seem to be dominant, as protease deficient mice show normal G-CSF induced HSPC mobilization.⁵¹

Similarly, injection of GRObeta, the ligand of the chemokine-receptor CXCR2, mobilizes HSPCs from the BM into the circulation in mice, indicating an essential role of CXCR2 in maintaining the HSPC population localized in the BM-niche.⁵² In addition to chemotactic proteins, gradients of the bioactive lipids sphingosine-1-phosphate and ceramide-1-phosphate induce HSPC migration, thereby cross-talking with chemokine-induced migration.⁵³⁻⁵⁵ Furthermore, complement proteins (C1q, C3a, C5a), hyaluronic acid and pharmacological agents such as valproic acid can be used to induce HSPC mobilization, reviewed in Marquez-Curtis et al.⁵⁶ Interestingly, the soluble SLIT proteins are expressed by bone marrow (BM) stromal cells and function as chemorepellents. Their cognate ROBO receptors are expressed on HSPCs and previous work in our department demonstrated that pretreatment of human HSPCs with SLIT3 increased homing to the bone marrow of NOD-SCID mice.⁵⁷ Together, this emphasizes the complex regulation of HSPC maintenance in the BM-niche.

Bone Marrow Niches

Bone marrow niches: introduction

The BM microenvironment forms a specialized niche for HSPC maintenance. A specific HSPC niche was first proposed in 1978 by Schofield et al.³⁸ The adult BM consists of endosteal osteoblasts, lining the calcified bone matrix, endothelial cells, forming the arterial and venal vasculature, reticular cells, smooth muscle cells, adipocytes, macrophages, stromal cells and sparse sympathetic nerves. These cells together shape into a structural network embedded in viscous ECM, thus forming a scaffold with which HSPCs can interact.

It has been proposed that HSCs are localized mainly in 2 different types of bone marrow niches, namely the endosteal niche, which is close to the bone and lined by osteoblasts,^{58,59} and the vascular niche, which includes sinusoids and their endothelial cells.²¹ Yet, this separation of 2 distinct niches might not be as strict as originally suggested, since vasculature is also found close to the endosteum. It has recently become clear that endothelial cells play a crucial role in the maintenance of self-renewal HSCs, among others through the production of stem cell factor⁶⁰ and E-selectin.⁶¹ The HSC supporting function of the vascular bone marrow niche is further enhanced by a population of perivascular, Nestin-expressing stromal cells.⁶² Moreover, a distinction between arteriolar and sinusoidal vasculature became evident, revealing that small arterioles support long-term repopulating HSCs, while sinusoids harbour less potent repopulating HSPCs.⁶³

The contribution of distinct cellular compartments to BM homeostasis was revealed by studies that use targeted deletion of growth factors or receptors. In addition, genetic mutagenesis and

biochemical studies have established the role of distinct ECM proteins in BM homeostasis. A closer view at recent insights regarding the actual composition of the BM niche and the contribution of cellular and ECM components to BM homeostasis is provided here.

Bone marrow niches: endosteal niche

HSPCs reside preferentially in the spongy bone in the metaphyse of the long bones, rather than in the diaphyse.⁶⁴ Within the spongy bone HSPCs preferentially localize to the endosteal surfaces of osteoids, where bone matrix, osteoblasts, osteoclasts, and marrow meet.^{65,66} Continuous remodeling of trabecular bone by osteoclasts liberates cytokines that are immobilized by proteoglycans and support HSC maintenance.⁶⁷ This scenario is supported by studies, showing that HSCs express a calcium-sensing receptor (CaR) and preferentially home to and are retained at calcium-enriched regions where trabecular bone is being remodeled.⁶⁸ Osteoblasts can expand long-term culture-initiating cells *in vitro*, and are shown to regulate the number of HSCs *in vivo*.^{58,69-71} Osteoblast-specific activation of the parathyroid hormone (PTH) pathway induces expansion of osteoblasts and results in increased HSPC numbers, through enhanced Notch-ligand expression by the osteoblasts.⁵⁸ In addition, PTH treatment improved the engraftment and survival after BM transplantation.⁵⁸ However, activation of the PTH pathway in osteocytes did expand osteoblasts and trabecular bone, but not HSPCs,⁵⁸ indicating that osteoblast expansion itself is not sufficient for HSPC expansion. Similarly, multiplication of osteoblasts by the agent strontium did not increase HSPC numbers, suggesting that only certain subtypes of osteoblasts regulate HSPC numbers,^{48,72} which may include Nestin+ peritrabecular cells.⁵⁸

Osteoblasts support HSPC maintenance by secretion of factors like CXCL12, osteopontin, angiopoietin-1, TPO and SCF.^{48,73-75} In addition, osteoblasts function in HSPC retention in the BM. G-CSF reduces osteoblast numbers and lowers CXCL12 expression that results in mobilization of HSPCs into the blood.^{76,77,45} Similarly, reduction of collagen-embedded osteocytes inhibited the osteoblast activity and impaired the G-CSF-induced HSPC mobilization.⁴⁸ This again illustrates that osteoblasts (indirectly) mediate the HSPC-BM-niche, by direct contact and secreted factors.⁵¹

Bone marrow niches: vascular niche

Endothelial cells function as bi-directional gate keepers of the blood-BM-barrier and endothelial permeability is tightly regulated.⁷⁸ Treatment of mice with fibroblast-growth-factor-2 (FGF-2) decreased the BM endothelial permeability and inhibited the HSPC homing and egress, via inhibition of the Nitric Oxygen (NO) content, matrix-metallo-proteinase-9 (MMP9) activity and vascular-endothelial-cadherin (VE-Cadherin) shedding.⁷⁸

Endothelial cells contribute to the vascular niche, and promote HSPC proliferation, as opposed to the endosteal niche that promotes quiescence.^{79,80} However, controversy was raised, because the marrow is highly vascularized and endothelial cells

are abundantly present in close proximity of the endosteum.^{61,81} Moreover, endothelial cells are enriched in the endosteal BM region compared to central BM.⁶¹ The model is now further refined by the recent proposal of 2 functional different vascular niches: the cycling sinusoidal niche and the quiescent arteriolar niche.⁶³ Arteriolar endothelium is surrounded by Nestin-GFP-bright, chondroitin sulfate proteoglycan 4 (CSPG4/NG2)-positive, α -smooth-muscle-actin-positive, leptin receptor (LepR)-negative pericytes and is innervated by sympathetic nerves, whereas sinusoids were negative for these markers and positive for the perivascular marker LepR. Arteriolar pericytes are more quiescent than sinusoidal pericytes, moreover, quiescent HSCs were over-represented in the proximity of arterioles, and HSCs in these regions were protected from cytostatics. Sinusoidal endothelium harbored cycling HSC. Upon G-CSF treatment HS(P)Cs are relocated away from the arteriolar niche.⁶³

This illustrates that the arteriolar vascular niche, residing close to the endosteum, contributes to the quiescence of HSCs, whereas a dynamic balance exists between arteriolar and sinusoidal HSCs.

Bone marrow niches: stromal subpopulations function as niche

Mesenchymal stromal cells can differentiate into osteoblasts, adipocytes and chondrocytes. It was demonstrated that murine Nestin⁺ stromal cells constitute an essential HSC niche component. Nestin⁺ stromal cells contain all the bone-marrow colony-forming-unit fibroblastic (stromal stem cell) activity from the flushed femur and were propagated as non-adherent 'mesospheres' that can self-renew and expand in serial transplantations.⁶² Moreover, a major subpopulation (75%) of human stromal cells that co-express platelet-derived-growth-factor-receptor (PDGFR α) and Integrin- α V (ITGAV/CD51) are Nestin⁺ cells, that form functional 'mesospheres' and provide HSPC support.⁸²

Based on expression of the low-affinity-nerve-growth-factor-receptor (CD271) and the melanoma-cell-adhesion-molecule (CD146), our group recently identified 2 new human BM stromal subpopulations, that show enrichment for stromal stem cells, the colony-forming-unit fibroblast (CFU-F).⁸³ The main subset in adult BM was CD271⁺CD146⁻, whereas the CD271⁺CD146⁺ population was dominant in pediatric and fetal BM.⁸³ The CD271⁺CD146⁻ fraction localized to the endosteal niches.⁸⁴ The endosteal niche harbors quiescent HSCs and the increase of the CD271⁺CD146⁻ stromal cells with age corresponds to the increase in long-term HSCs in aged murine BM.^{85,86} Interestingly, our preliminary data suggest that there is a difference in haematopoietic support between stromal subsets, and that the CD271⁺CD146⁻ stromal fraction may give better long term HSC support (Maijenburg *et al*, manuscript in preparation).

BM niche cells maintain HSPCs by direct cell-cell contacts and synthesis of soluble factors such as CXCL12 and SCF, as mentioned above. The contribution to haematopoietic support of the CXCL12 and SCF synthesis by stromal stem cells and differentiated stromal cell populations is revealed by cell-type-specific conditional deletion of these factors.^{60,87-89} CXCL12

synthesis in osteoblasts (Collagen 2.3⁺) was dispensable for HSC maintenance, however, it reduced the number of B-lymphoid progenitors.^{87,88} These data suggest that the endosteal niche is accumulating the early lymphoid progenitors. The function of the osteogenic lineage was further revealed by conditional deletion of CXCL12 in osteoprogenitors (Osterix⁺) that show induced mobilization of HSPCs into blood and spleen.⁸⁷ Conditional deletion of CXCL12 in sinusoidal pericytes (LepR⁺) did not influence HSPC numbers in the BM, but also induced HSPC mobilization into blood and spleen.⁸⁷ Remarkably, the synthesis of SCF by these perivascular (LepR⁺) cells was essential for HSC maintenance.⁶⁰ Modest defects in HSC numbers and function were revealed upon endothelial specific (endothelial tyrosine kinase/Tie2⁺ cells) CXCL12 deletion.^{87,88} Conditional deletion of CXCL12 in a broad range of (Peroxiredoxin1; PRX1⁺) stromal cells, including all Nestin⁺ cells and a fraction of the osteoprogenitors, showed a dramatic reduction in BM HSPCs and displayed recruitment of HSPCs into the spleen.⁸⁸ Remarkably, CXCL12 expression in Nestin⁺ pericytes, which include mesenchymal stem cells, is dispensable for HSC maintenance and retention.⁸⁷ Deletion of Nestin⁺ cells, however, induced HSPC mobilization into the spleen.⁶² These data indicate that perivascular stromal (stem) cells, osteoprogenitors and endothelial cells contribute to HS(P)C maintenance and retention.

Bone marrow niches: neuronal regulation of the BM niche

There is accumulating evidence that sympathetic nerve signaling is involved in HSPC maintenance in the BM, as reviewed by others.⁹⁰ Initial experiments were performed in mice with disrupted sympathetic nerve conduction (ceramide galactosyltransferase-deficient mice). The egress of HSPCs upon G-CSF injection was completely prevented in these mice.⁴⁷ Norepinephrine seems the responsible factor, as administration of a β_2 -adrenergic agonist enhanced the mobilization of HSPCs upon G-CSF treatment in control mice. In addition, dopamine deficient mice retained normal CXCL12 levels and osteoblast numbers upon G-CSF treatment, while HSPC mobilization was impaired.⁴⁷ Together, these data suggest that G-CSF-induced HSPC mobilization depends on the activation of sympathetic neuronal signals. G-CSF treatment activates sympathetic signals, which reduce osteoblast numbers and mobilize HSPCs.⁴⁷

Remarkably, also monocytes and/or macrophages are involved in the BM niche network.⁹¹⁻⁹³ Deletion of BM macrophages reduced CXCL12 expression in Nestin⁺ pericytes and mobilized HSPCs into the blood.⁹¹ Therefore, macrophages retain HSPCs in the BM. This physiological mobilization of HSPCs upon depletion of BM macrophages was reduced, but still present, in mice that were deficient in the sympathetic β_3 -receptors.⁹¹ This indicates that BM macrophages and sympathetic signaling act via distinct pathways, and fulfill antagonistic functions. In addition, depletion of BM macrophages also enhanced G-CSF-induced HSPC mobilization.⁹¹ Together, BM macrophages and sympathetic signals balance both the physiologically and the G-CSF-induced HSPC egress.

Bone marrow niches: perfusion

Low oxygen levels are proposed to contribute to HSC maintenance in the endosteal niche. However, the vasculature penetrates the complete BM up to a distance corresponding to a few cell diameters. This suggests that perfusion rates are not expected to be highly discriminative between BM anatomical sides.⁸¹ However, perfusion of the BM vasculature with the small fluorescent molecule Hoechst, showed that cells that were less exposed to the perfused Hoechst harbour the most HSC capacity, indicating that long-term HSCs reside in less perfused niches.⁹⁴ Moreover, the anatomical locations of the less perfused niches are not necessarily at the endosteum. Recently, direct high-resolution measurement of oxygen tensions in the BM revealed that Nestin⁺ vessels, locating closer to the endosteum, contain higher oxygen tensions, compared to Nestin⁻ vessels.⁹⁵ Similarly, both vascular and extravascular oxygen tensions are lower at sites near the central sinusoid (>40 μm away from the bone surface) compared to sites within 20 μm of the bone surface.⁹⁵ This suggests that vascular oxygen levels drop when the vessels penetrate deeper into the BM due to the high BM-cellularity and oxygen demand.

In addition, analyses of the hypoxic signaling molecules in HSPCs reveal their metabolic state. Upon knock-out of hypoxia-inducible factor-1 α (HIF1A) in mice, HSPC cell cycle quiescence was completely lost.⁹⁶ At homeostatic conditions, HIF1A knockout mice show normal phenotypic HSPC numbers and haematopoietic compartments, but upon BM stress the HSPCs get exhausted.⁹⁶ In line with this, the HIF1A target *Cripto* is specifically expressed in immature HSPCs. *Cripto* is essential for HSPCs localization in the endosteal niche, and for maintenance of repopulating capacities of HSPCs in culture.^{97,98} It remains unclear why HSPCs are found preferentially in close proximity of microvessels in the endosteal zone by imaging cytometry, while a hypoxic profile, including HIF1A expression, was confirmed.⁸¹ Therefore, the hypoxic state of HSPCs might also be regulated by stem cell-specific mechanisms.^{81,99,100} Low oxygen levels are necessary but not sufficient for HSPC quiescence.¹⁰¹ In conclusion, HSPCs are characterized by a hypoxic state, but the mutual contributions of the microenvironment and the stem cell-specific mechanisms are to be elucidated.

Bone marrow niches: soluble factors

Soluble factors involved in HSPC mobilization and homing are discussed above. Various other niche-derived soluble factors regulate HSC maintenance. Angiopoietin and its receptor Tie2, highly produced by endothelial cells, are required for the maintenance of quiescent HSCs.¹⁰² The glycoprotein osteopontin, highly expressed in the endosteal niche, was required to control HSC numbers.^{103,104} Free calcium ions, released by osteoclastic activity in the endosteal niche, are required as well, since depletion of the calcium-sensing receptor reduced the number of HSCs in the BM.¹⁰⁵ HSPC maintenance is also regulated by SCF and TPO that are proposed to function in the vascular and the osteoblastic niche, respectively.^{75,106,107} Furthermore, stromal derived transforming-growth-factor- β (TGF- β) is a strong inhibitor of HSPC proliferation. Active TGF- β specifically

inhibits the proliferation of early multipotent HSPCs, whereas it enhances the proliferation of later progenitors.¹⁰⁸⁻¹¹² TGF- β also skews progenitor lineage choice, as myeloid differentiation is stimulated by TGF- β , while lymphoid differentiation is inhibited.²² In conclusion, a wide range of niche-derived chemokines and other soluble factors mediate the interaction between HSPCs and the BM niches.

Extracellular Matrix Molecules in the Bone Marrow

Extracellular matrix molecules: introduction

The BM extracellular matrix is produced by niche cells and interacts with matrix-associated molecules, including immobilized secreted proteins and membrane-proteins with large extracellular domains.^{113,114} The most abundantly present ECM proteins in the BM are fibronectin, the collagens I-XI, laminin, tenascin, thrombospondin and elastin.¹¹⁵⁻¹¹⁹ Important for ECM integrity are also the proteoglycans with their large glycosaminoglycan (GAG) sidechains, including hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate and heparin.¹²⁰ Other ECM (associated) molecules are the soluble or membrane bound glycoproteins of the sialomucin family, including CD34, glycosylation dependent cell adhesion molecule1 (GlyCAM1), mucosal vascular addressin CAM1 (MadCAM1), platelet-selectin glycoprotein ligand (PSGL1/CD162), sialophorin (SPN/CD43), CD45RA and CD164. Essential mediators of the cell-cell and cell-matrix interactions in the BM are integrins and selectins,¹²¹⁻¹²³ whereas membrane bound receptors of the immunoglobulin superfamily, including intercellular adhesion molecule-1 (ICAM1; CD54), vascular cell adhesion molecule-1 (VCAM1; CD105) and CD166, also shape the bone marrow niche.¹²⁴

Matrix biophysical properties

Recently, the importance of mechanic features of the BM matrix in HSC fate decision has been highlighted.^{125,126} Murine BM cultured on tropo-elastin-coated plates is enriched for HSCs, but this was not observed when the density of the tropo-elastin-coating was increased by crosslinking.¹²⁷ This indicates that substrate biophysical properties regulate HSC fate, although it does not clarify whether this is a direct effect.

The direct impact of matrix density on HSPCs was analyzed by culturing HSCs on collagen-coated polyacrylamide (PA) substrates with variable stiffness. The viability, spreading and adhesion of HSCs were increased with increasing collagen concentrations on PA substrates, although colony forming capacities and *in vivo* repopulating capacities were not modulated.¹²⁵ Similarly, presentation of ECM molecules in nanostructure patterns with variable interligand spacing modulated HSPC adhesion, although colony forming capacities were not affected.¹²⁸

These studies illustrate that matrix density and the presentation of specific adhesive ligands contribute to the HSPC niche in the BM. The evaluation of semisolid hydrogels and other artificial BM niches with or without the addition of cellular and

molecular factors will reveal insight in HSPC niche requirements.¹²⁹⁻¹³⁵

Proteoglycans, collagens and fibronectin

Proteoglycans are essential for maintenance of long-term culture-initiating cells in *in vitro* cultures, via interaction with IL3 and other growth factors.¹³⁶ An abundant proteoglycan in the BM is perlecan (heparan sulfate proteoglycan; HSPG).¹³⁷ Perlecan interacts with other ECM molecules, thereby stabilizing for example Collagen-X that is formed by hypertrophic chondrocytes at the chondro-osseous junction of the spongy bone. Mutations in perlecan or collagen-X indirectly attenuate hematopoiesis via disruption of the endochondral ossification processes, resulting in marrow aplasia and lymphopenia.^{67,138} In addition, loss of function mutations in the proteoglycan glypican-3 perturbed hematopoiesis.¹³⁹ Heparan sulfate modification of proteoglycans is also an essential element in the BM, critical for the establishment of the HSPC niche.^{67,140,141} In addition, hyaluronic acid, the non-sulfated proteoglycan, is required for *in vitro* hematopoiesis and enhances hematopoiesis in the bone marrow after 5-fluorouracil (5-FU) treatment.¹⁴² Together, this illustrates that proteoglycans and collagens are essential for HSPC maintenance.

In addition, specific inhibition of collagen secretion in the BM (by non-toxic levels of Cis-4-hydroxyproline) reduces hematopoiesis and the number of haematopoietic progenitors.¹⁴³ Direct adhesion of HSPCs to fibronectin attenuates HSPC proliferation and differentiation, which is mediated by integrins.¹⁴⁴ In addition, the abundant ECM protein tenascin-C supports the regeneration of haematopoietic cells after myeloablation.¹⁴⁵ Therefore, adhesion of HSPCs to fibronectin and other ECM molecules in the BM, contributes to the BM niche and regulates HSPC maintenance.

Extracellular matrix and integrins

HSPCs can interact with other cells and matrix molecules via the transmembrane glycoprotein family of integrins. Integrins are heterodimeric proteins, composed of an α - and a β -subunit, that are selected from 16 α -subunits and 8 β -subunits. HSPCs express Integrin- α 4 β 1 (VLA4; ITGA4 and ITGB1), - α 5 β 1 (VLA5; ITGA5 and ITGB1), - α L β 2 (LFA1; ITGAL and ITGB2) and - α M β 2 (MAC-1; ITGAM and ITGB2) and 10% of the BM HSPCs expresses Integrin- β 3 (ITGB3).¹⁴⁶⁻¹⁴⁸ Recently, human LT-HSCs are specifically identified by expression of ITGA6^{16,17} (see paragraph 1.4).

Integrin-ligand binding induces “outside-in” signaling, that regulate cell differentiation.¹⁴⁹ On the other hand, cellular processes regulate integrin-dependent adhesion through “inside-out” signaling, including clustering of integrins and conformation changes of integrins, which increases the affinity for their ligands (integrin activation).¹⁵⁰

Blocking of β 2-integrins inhibits adhesion of HSPCs to bone-marrow stroma *in vitro*, without inducing mobilization of stem cells into the blood *in vivo*.¹⁵¹ Antibodies against VLA4, however, do induce mobilization.^{152,153} Treatment of human HSPCs with antibodies against ITGB1 also prevented engraftment of HSPCs in mice and sheep.^{154,155} ITGB1 associates with the

potassium ion channel upon HSPC activation, which is essential for engraftment in the BM niche¹⁵⁶⁻¹⁵⁸ and also is suggested to sustain leukemic cells in the BM-niche.¹⁵⁹

In conclusion, integrin signaling is essential for a broad range of HSPC functions, including the reconstitution of the BM-niche and HSPC differentiation.

Extracellular matrix and selectins

Another protein family that plays a crucial role in cell-cell and cell-matrix interactions in the specific BM microenvironments is the selectin family. Selectins are transmembrane proteins and the family members endothelial (E-), leucocyte (L-) and platelet (P-) selectin share closely related extracellular domains, whereas their transmembrane- and intracellular domains are not conserved. Selectins bind sialylated, fucosylated or sulfated carbohydrate moieties on glycoproteins and proteoglycans. E-selectin ligands expressed in HSPCs include the CD34 antigen, PSGL-1, the HSPC-specific modified CD44 antigen HCELL (haematopoietic cell E-/L-selectin ligand), E-selectin ligand-1 (ESL-1; GLG1) and glycosphingolipids.^{61,160} Other E-selectin ligands include CD43, endoglycan (a CD34-family member) and Basigin (CD147).¹⁶¹

E-selectin is exclusively expressed on endothelial cells, with high expression in small sinusoids of the BM.^{162,163} E-selectin mRNA expression was enriched in the endosteal region of the BM compared to central BM. Accordingly, E-selectin-expressing endothelial cells were enriched in the endosteal BM region compared to central BM. It is specifically co-expressed with CXCL12 in BM sinusoids and form targets of HSPC homing after transplantations.¹⁶²

E-selectin mediates adhesion of HSPCs to BM endothelial cells¹⁶⁴ and promotes HSC proliferation and maturation.⁶¹ Knock-down of E-selectin or treatment of mice with an E-selectin antagonists enhanced HSC quiescence and self-renewal, which was partially mediated by interaction of PSGL-1 and HCELL on HSPCs with endothelial E-selectin.⁶¹ This indicates that the vasculature of the endosteal BM region actively induce HSC proliferation and maturation.⁶¹ Thus, in addition to the endosteal arteriolar niche that supports HSPC quiescence,⁶⁰ an sinusoidal niche exists that activates HSPCs and is characterized by E-selectin expression.

P-selectin, however, is diffusely expressed throughout the BM vasculature and also expressed in endothelial cells of large blood vessels.¹⁶² L-selectin is expressed on leucocytes and mediates adhesion and migration of HSPCs on lymphatic endothelial cells.¹⁶⁵ Both P- and L-Selectin bind ECM molecules, including the chondroitin-sulfated versican¹⁶⁶ and the heparin-sulfated collagen-18 and collagen-15.¹⁶⁷ Thus, selectins mediate cell-cell and cell-matrix interactions, and E-selectin specifies microdomains in the sinusoidal BM niche that contributes to activation and proliferation of HSPCs.

Extracellular matrix and homeostatic factors

HSPC homeostasis is also regulated by structural blood plasma proteins that are responsible for fibrinolysis during the dissolution of thrombotic clots. Fibrin clots are dissolved by

plasmin, an enzyme that is activated when its zymogen, plasminogen (PLG), is cleaved by urokinase PLG activator (uPA) or tissue PLG activator (tPA). The uPA protein is the main ligand for the uPA receptor (uPAR) that is GPI-anchored at the surface of HSPCs, thereby converting cell surface PLG into active Plasmin. PLG and uPAR are both required for G-CSF-induced HSPC mobilization, because the G-CSF-induced mobilization was impaired in PLG- or uPAR knock-out mice.^{168,169} Conversely, genetic loss of plasmin inhibitor increased HSPC mobilization.¹⁷⁰ Plasmin stimulates the HSPC mobilization by its proteolytic activity toward collagen, fibrin, laminin and fibronectin. In addition, it activates other proteases, as matrix metalloproteases (MMP3/9/12/13), elastase and cathepsin B.^{171,172} In conclusion, coagulation factors are essential regulators of HSPC mobilization and retention through processing of extracellular matrix proteins.

Adhesion Molecules in Regenerative Bone Marrow

Molecular signatures of regenerative bone marrow: introduction

Comparative array studies of HSCs revealed many proteins that are involved in cell-cell and cell-matrix interactions and are differentially regulated during BM regeneration compared to steady state conditions.^{10,11,173} In addition, many adhesion proteins were differentially regulated in HSCs during development.¹⁷⁴ For this review we focus on cell adhesion molecules that are differentially expressed during regeneration, in particular Biglycan,^{10,11} Embigin,¹¹ CD164,^{10,11} CD166¹⁰ and BIGH3.^{10,175} Some targets upregulated in murine regenerative BM were also overrepresented among targets upregulated during murine HSC differentiation (short-term HSCs versus long-term HSCs) (Table 1). In addition, targets downregulated during regenerative BM conditions are overrepresented among targets downregulated during murine HSC development (Fetal-liver-HSCs vs. BM-HSCs). These trends imply a strong correlation between regulation of adhesion molecules in HSPCs during regeneration, differentiation and development. We compared the

mRNA expression of CD48, CD164, CD166, BIGH3, Embigin and Biglycan in HSPCs derived from human BM, cord blood and mobilized peripheral blood (MPB) (Fig. 1; Table 1, right column). These proteins are differentially expressed during regeneration, and some of them are also differentially expressed during development^{10,174} (Table 1) and/or in HSPCs derived from adult BM compared to MPB-derived HSPCs. However, the direction of this correlation is not consistent. This implies that, during the process of BM regeneration and HSPC mobilization, distinct gene expression profiles are found in HS(P)Cs that persistently remain in the BM and HSPCs that are mobilized into the blood. These variations may represent HSPC populations originating from distinct BM niches.

BIGH3 and its paralogue periostin in regenerative bone marrow

Analysis of murine BM-derived HSPCs in homeostatic and regenerative conditions identified the extracellular matrix protein (TGF-Beta Induced Gene-H3) BIGH3, also named TGF- β induced gene (TGFB1), being one of the top most upregulated proteins one day after G-CSF, cyclophosphamide or combined G-CSF and cyclophosphamide treatment.¹⁷⁵ Similarly, BIGH3 is upregulated in murine BM HSPCs after 5FU treatment.¹⁰ BIGH3 expression is also enhanced during fetal development, showing increased expression in murine fetal BM HSPCs compared to fetal liver HSPCs.¹⁷⁴ This suggests a role for BIGH3 in HSPC-niche interactions during homeostasis and regeneration of the BM HSPC population.

In addition, BIGH3 is the top-one most differentially expressed gene comparing BM-derived mesenchymal stromal cells to HSPCs,¹⁷⁶ indicating the abundant production of (soluble) BIGH3 in the stromal niche. Similarly, BIGH3 is higher expressed in CB-derived stromal cells compared to CB-derived total mononuclear cells.¹⁷⁷ BIGH3 is involved in osteogenic differentiation, being upregulated in the first 3 d of stromal to osteogenic differentiation,¹⁷⁸ while being downregulated in osteoblast terminal differentiation.¹⁷⁸⁻¹⁸⁰

BIGH3 is a secreted protein detected in various human tissues.^{181,182} The 68-kDa protein consists of 4 fasciclin 1 domains

Table 1. Differential expression of adhesion molecules in HSPCs during BM regeneration^{10,175}, HSPC differentiation¹¹ and development^{10,174} The analyzed mRNA targets are in the first column. The second column depicts the change in mRNA in murine BM HSPCs after 5-fluorouracil (5FU) treatment, G-CSF treatment or combined G-CSF and cyclophosphamide (Cy) treatment.^{5,10,17} The second column depicts how the mRNA expression in short-term HSPCs relates to that in long-term HSPCs in the murine BM.¹¹ The third column represents the difference in mRNA expression between HSPCs isolated from the fetal-liver or the fetal-BM.^{4,10,17} The last column indicates the differential mRNA expression of adhesion molecules as determined on different sources of human CD34⁺ cells as depicted in Figure 1. Arrows in green boxes mean increased mRNA expression, arrows in red boxes mean decreased mRNA expression. NS = not significant

	Regeneration		Differentiation	Development	Source
	Post 5FU ¹⁰	Post Cy/G ¹⁷⁵	ST vs LT ¹¹	FL-HSC vs BM ^{10,174}	MPB vs BM
BIGH3	↑		NS	↓	↓
Embigin	↑		↑	NS	↑
CD48	↑		↑	↑	NS
Biglycan	↓		↓	NS	NS
CD164	↓		↑	↓	NS
CD166	↓		NS	↓	↑

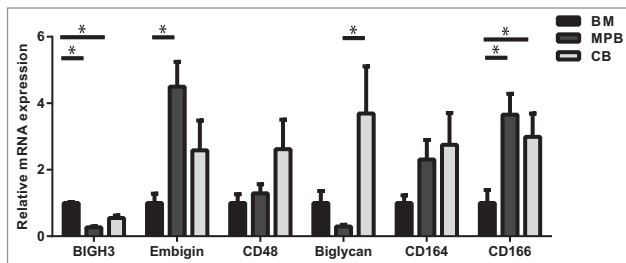


Figure 1. Relative mRNA expression of adhesion molecules in HSPCs from BM, cord blood (CB) and mobilized peripheral blood (MPB). CD34+ cells were isolated using a MACS cell separation kit from Miltenyi Biotec. Cells were lysed and mRNA was isolated using a Qiagen mRNA isolation kit. c-DNA was prepared and the mRNA was quantified using a StepOne-Plus Taqman machine. The mRNA expression was calculated relative to the GUS mRNA expression in each sample, by use of the $2^{-\Delta\text{Ct}}$ value. Subsequently, the expression of each gene was depicted relative to its expression in BM HSPCs. Statistics were performed using a Student T-test. $n \geq 5$ for MPB; $n \geq 4$ for BM; $n \geq 5$ for CB; bars represent mean \pm SEM. * means $P > 0.05$.

and a C-terminal arginyl-glycyl-aspartic acid (RGD) sequence, both of which can mediate the interaction of BIGH3 with integrins.¹⁸³ Whereas the role of BIGH3 in cell-ECM interactions is extensively studied,¹⁸³ less is known about its role in the haematopoietic BM microenvironment.

HSPCs increase the BIGH3 expression upon adherence to mesenchymal stromal cells compared to non-adhered or fibronectin-adhered HSPCs.¹⁸⁴ We recently showed that CD34+ HSPCs adhere to BIGH3 in an integrin-dependent manner.¹⁸⁵ Expression of BIGH3 in HSPCs inhibited their adhesion and CXCL12-induced migration capacities. The balance between the small GTPases ras-related-C3-botulinum-toxin-substrate-1 (RAC1) and Ras-homolog-A (RHOA) was affected upon BIGH3 expression, as indicated by inhibition of the phorbol-myristate-acetate (PMA)-induced activation of RAC1.¹⁸⁵ Therefore, we propose that BIGH3 secretion may prepare the HSPCs for BM egress, as BIGH3 shifts the RAC1/RHOA balance and modulates HSPC adhesion to its niche, via blockage of integrins and other adhesion molecules.

BIGH3 was originally identified as a TGF- β responsive gene in lung adenocarcinoma cells.¹⁸¹ TGF- β itself is a potent inhibitor of the growth of primitive haematopoietic stem cells¹⁸⁶⁻¹⁸⁸ and skews HSCs toward a myelocytic HSC subtype.²² This raises the question whether BIGH3 has similar effects on hematopoiesis. We showed that BIGH3 is strongly upregulated in HSPCs in response to TGF- β (Klamer et al, manuscript submitted for publication). Reduced BIGH3 levels in HSPCs enhanced the ability of mesenchymal stromal cells to maintain HSCs, which may be explained by reduced cell cycle progression and proliferation upon reduced BIGH3 levels. Therefore, regulation of the expression of *BIGH3* in HSPCs modulates their fate (Klamer et al, manuscript submitted for publication).

Considering the role of coagulation factors in HSPC mobilization and niche-interactions, it is of interest that BIGH3 is cleaved by plasmin¹⁸⁹ and contains a vitamin-K-dependent gamma-carboxylation site, only prevalent in proteins that are

involved in coagulation pathways.¹⁹⁰ Moreover, BIGH3 stimulates thrombus formation by activation of platelets, suggesting that it contributes to stabilization of blood clots, whereas its proteolysis by plasmin may contribute to the resolution of clots and to HSPC homeostasis.¹⁹¹

Remarkably, transgenic mice that abundantly express mutant BIGH3 (containing the R555W mutation) under control of an ubiquitous promoter, show lymphoid follicular hyperplasia of the spleen and increased spleen size,¹⁹² which may indicate deregulated hematopoiesis and/or lymphopoiesis by aberrant BIGH3 expression.

The ECM protein Periostin is the only paralogue of BIGH3 within the human genome and their encoding sequences are 48% homolog. Periostin is expressed in tissue that stands biomechanical forces and it enhances collagen cross-linking.¹⁹³ However, its expression in HSPCs is not described to change upon BM regeneration.^{10,173} Nonetheless, Periostin modifies the BM microenvironment, by enhanced bone formation at the periosteum.^{194,195} In addition, Periostin is highly expressed in the BM fibroblast cell line OP9, that supports hematopoiesis in the presence of TPO, whereas it is not expressed in fibroblasts that fail to support hematopoiesis, suggesting a role for Periostin in HSPC maintenance and expansion.¹⁹⁶ On the other hand, BM expression levels of Periostin correlate with the grade of BM fibrosis in myelofibrotic pathologies and moderate levels of Periostin were proposed to support the clonal expansion of haematopoietic cells in myelofibrosis by interaction with integrins and remodeling of the BM ECM.¹⁹⁷ Interestingly, recent studies reveal that Periostin enhances axon outgrowth of embryonic derived glial cells after spinal tract injury.¹⁹⁸ Therefore, Periostin may be important in HSPC-niche interactions by remodeling of the periosteum and the BM ECM and possible by nerve development, shaping both the neuronal and osteoblastic BM niche.

In conclusion, BIGH3 is a relatively novel ECM factor in the BM niche and its expression in HSPCs may contribute to BM homeostasis by altering HSPC-niche interactions and the differentiation status of HSPCs (as depicted in Fig. 2).

Embigin, CD164, CD166 and biglycan in regenerative bone marrow

One of the other genes upregulated in HSPCs during BM regeneration encodes Embigin or gp70.¹⁰ Embigin was originally isolated from teratocarcinoma cells and is a transmembrane glycoprotein member of the immunoglobulin superfamily.¹⁹⁹ Embigin enhances integrin-dependent cell-substrate adhesion.^{199,200} Embigin is required for proper plasma membrane expression of monocarboxylate transporters (MT1-4) that exchange lactate for protons during anaerobic metabolic activity.²⁰¹ Basigin fulfills similar functions, but shows higher affinity for MT1 and MT4, while Embigin is associated with MT2.²⁰² These complexes provide neurons with lactate as energy source and prevent acidification of the intracellular milieu.²⁰³ Embigin mRNA was highly upregulated in denervated muscle cells compared to innervated muscles²⁰⁴ and is involved in neuromuscular synapse formation. In haematopoietic cells, Embigin is highly expressed in HSPCs, upregulated in activated T-cells, and

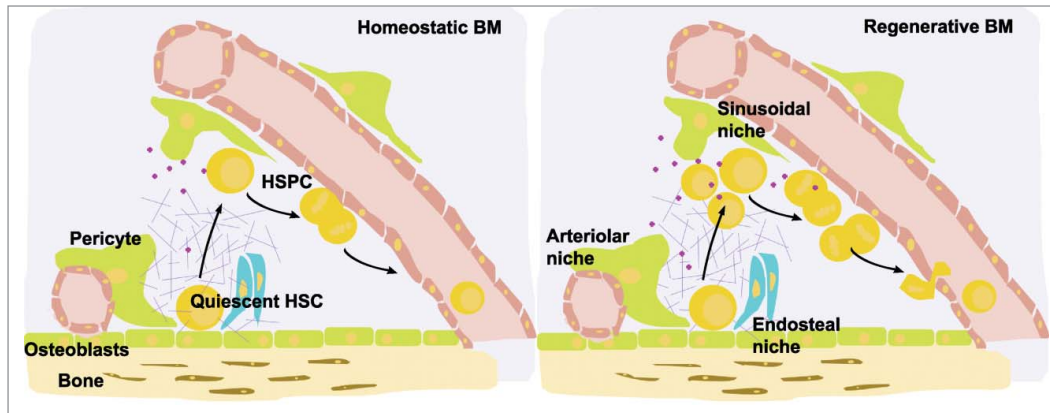


Figure 2. Model of the endosteal, arteriolar and vascular bone marrow niches. The arteriolar niche localizes close to the endosteum and harbours mostly quiescent HSCs, whereas the sinusoidal niches are distributed throughout the BM and harbour mostly HSPCs that are in cell cycle. During haematopoietic regeneration after myeloablation, HSPCs proliferate and are mostly localized in sinusoidal niches. A range of ECM and matrix-associated molecules are differentially expressed upon BM regeneration. BIGH3 (purple dots) expression in HSPCs is upregulated in regenerative BM, suggesting its expression is dominant in sinusoidal HSPCs. BIGH3 expression is higher in BM-derived HSPCs compared to MPB-derived HSPCs, indicating that its expression is down-regulated upon transmigration into the blood. Adapted by permission from Macmillan Publishers, Ltd, <http://www.nature.com/nri/posters>, 2007 volume 7 no. 6 (T. Graf and A. Trumpp)

specifically repressed upon B-cells maturation.²⁰⁵ It is upregulated in short-term HSPCs compared to long-term HSCs,¹¹ and we found it higher expressed in MPB- compared to BM-derived HSPCs (Fig. 1). The upregulation of Embigin in activated HSPCs and T-cells and its neuronal function hint toward a role of Embigin in the neuronal regulation of hematopoiesis.

CD164, CD166 and Biglycan are down-regulated upon BM regeneration. CD164, also named Endolyn, is a member of the sialomucin family. It is expressed on human CD34⁺ haematopoietic progenitors and BM stromal and endothelial cells.²⁰⁶ Early HSPCs also express CD164, indicated by co-expression of early haematopoietic markers: prominin-1⁺ (CD133⁺), CD38⁻, CD117⁺, CD90⁺ and (FLT3; CD135⁺).^{207,208} CD164 mediates HSPC adhesion to stromal and endothelial cells via homotypic interactions and inhibits HSPC proliferation.²⁰⁶ CD164 is absent in most mature blood cells, although the sialyl-independent epitopes are present in monocytes and weakly present in granulocytes.²⁰⁷ CD164 is required for CXCR4-mediated HSPC migration and takes part in the membrane migration complex together with CXCR4 and integrins.²⁰⁹ Moreover, in various tumors upregulation of CD164 is associated with bone metastasis.^{210,211} Remarkably, the expression of CD164 is increased in HSPCs from patients with idiopathic myelofibrosis, a condition that is characterized by BM fibrosis and mobilization of HSPCs into the peripheral blood.²¹² Thus, CD164 is an adhesion molecule on early and more differentiated HSPCs as well as BM niche cells, and modulates HSPC adhesion, migration and differentiation.

Activated leukocyte cell adhesion molecule (ALCAM), also named CD166, is a transmembrane glycoprotein of the immunoglobulin superfamily and was originally isolated from CD34⁺ BM HSPCs.²¹³ CD166 mediates homotypic cell-cell adhesion and binds the T-cell antigen CD6.^{214,215} Immature osteoblasts express CD166 and CD166-negative osteoblasts fail to support HSPCs.²¹⁶ CD166 knockout mice show normal hematopoiesis

upon birth, but their HSCs show reduced long-term function.²¹⁷ CD166-deficient adult mice show decreased blood leukocyte numbers and increased phenotypic long-term HSCs. Upon transplantation, however, these HSCs display myeloid bias and express age-associated genes, indicating enhanced aging of the haematopoietic system in the absence of CD166.²¹⁷ CD166 expression is required for effective (bone) metastasis of various tumors, and its expression is enhanced upon active TGF- β release.^{218,219} In various tumors, cancer stem cells can be identified by high membrane expression of CD166.^{220,221} These studies show that CD166 regulates self-renewal in HSCs and tumor cells, and its expression in the endosteal niche and HSPCs is essential for proper hematopoiesis.

Biglycan is downregulated upon BM injury and is higher expressed in LT-HSCs compared to ST-HSCs. Biglycan is a small proteoglycan and binds other ECM molecules, such as collagen, fibronectin, Decorin, fibromodulin and BIGH3.²²² Furthermore, Biglycan binds TGF- β , thereby blocking its activity.²²³ Murine knock-down of Biglycan resulted in decreased bone mass²²⁴ and a reduced production of BM stromal cells and osteoprogenitors.²²⁴ Biglycan induces nitric oxide synthases, thereby protecting cells from hypoxia and reoxygenation injury.²²⁵ Biglycan expression correlates with the malignancy of colorectal and gastric cancer.^{226,227} Biglycan is high expressed in tumor endothelial cells and is required for endothelial cell migration.^{228,229} Biglycan, like Embigin, is also involved in the formation of the neuromuscular synapse.²³⁰ Thus, Biglycan is a major regulator of stromal, osteogenic and possibly neuronal BM niche cells, and thereby facilitates hematopoiesis, reflected by its higher expression in LT-HSCs.¹¹

In conclusion, differential expression of various ECM- and adhesion molecules in HSPCs upon BM regeneration is an integral process within the complex regulatory network that controls hematopoiesis by HSPC-niche interactions.

Conclusions

The decision of HSPCs to sustain the HS(P)C population in the BM-niche or to differentiate and proliferate, depends on a complex network of HSPC-niche interactions and cellular signaling pathways. The HSPC niche consist of an endosteal, arterial, sinusoidal, mesenchymal and neuronal component and a broad range of soluble factors, ECM- and adhesion molecules modulate the HSPC fate during homeostasis and BM regeneration. A range of ECM and adhesion molecules is differentially regulated upon BM regeneration and includes novel factors, like BIGH3, that modulate HSPC-niche interactions.

Further analysis of the described ECM and adhesion molecules would reveal their exact role in the bone marrow niche. Importantly, manipulation of their expression in HSPCs, the *ex vivo* microenvironment or the recipient microenvironment would improve the generation of cellular products. Procedures to either induce HSC self-renewal (relevant for cord blood transplantation) or enhanced HSC differentiation/proliferation

(generation of mature haematopoietic cells) might benefit. Thus, better understanding of the functions of extracellular matrix and adhesion might lead to improvement of current cellular therapies and efficient development of future cellular products.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We faithfully thank CE van der Schoot and M von Lindern for critical reading of the manuscript.

Funding

SK is supported by PPO-C (grant 06-005). CV is supported by the Landsteiner Foundation for Blood Transfusion Research (LSBR Fellowship, #1101F).

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