Prospects

Niche Extracellular Matrix Components and their Influence on HSC¹

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¹ This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:[10.1002/jcb.25905](https://doi.org/10.1002/jcb.25905)

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ABSTRACT

Maintenance of hematopoietic stem cells (HSC) takes place in a highly specialized microenvironment within the bone marrow. Technological improvements, especially in the field of *in vivo* imaging, have helped unravel the complexity of the niche microenvironment and have completely changed the classical concept from what was previously believed to be a static supportive platform, to a dynamic microenvironment tightly regulating HSC homeostasis through the complex interplay between diverse cell types, secreted factors, extracellular matrix molecules and the expression of different transmembrane receptors. To add to the complexity, non-protein based metabolites have also been recognized as a component of the bone marrow niche. The objective of this review is to discuss the current understanding on how the different extracellular matrix components of the niche regulate HSC fate, both during embryonic development and in adulthood. Special attention will be provided to the description of non-protein metabolites, such as lipids and metal ions, which contribute to the regulation of HSC behavior.

KEY WORDS

Hematopoietic Stem Cells, Extracellular Matrix Molecules, Niche, Microenvironment, Megakaryocytes, Lipids, Metal ions, Integrins

INTRODUCTION

Hematopoietic stem cells (HSC) reside in a specialized bone marrow (BM) microenvironment called the “niche” [Schofield, 1978]. It is now evident that the niche is composed of a combination of cell types as well as non-cellular components with different functional roles, all of which support HSC. The

immunophenotypical characterization of HSC has greatly evolved over multiple decades. Initially characterized as LSK (Lineage^{Lo} Sca⁺c-Kit⁺) [Spangrude et al., 1988], the HSC population was however known to be heterogeneous. The discovery of new selection markers led to the characterization of a more purified HSC population: HSC were recognized to be in the CD34⁻ fraction of LSK (LSK CD34⁻) [Osawa et al., 1996] and more recently, the HSC population was purified based on the expression of the SLAM proteins CD150 and CD48 [Kiel et al., 2005]. HSC are now commonly defined as LSK SLAM (Lineage^{Lo} Sca⁺c-Kit⁺ CD150⁺ CD48⁺). Throughout this review, the term HSC will encompass the LSK, LSK CD34⁻ and LSK SLAM phenotypes.

The microenvironment in which HSC reside provides unique cues and greatly influences HSC behavior, with HSC, which were phenotypically identical for the eight antigens used for their isolation, but residing in discrete BM regions (endosteal and central BM regions) having different biological properties [Grassinger et al., 2010]. The ability of HSC to home and engraft is one of their essential characteristics as the maintenance of HSC homeostasis and hematopoietic reconstitution potential is dependent on this ability. Moreover, accumulating evidence has demonstrated that HSC homing is directed by a variety of surface receptors [De Grandis et al., 2016]. Importantly, it has been shown that endosteally located HSC have enhanced homing capacity and increased proliferation potential compared to their central counterparts [Grassinger et al., 2010], suggesting that endosteal HSC may exhibit differential expression of extracellular matrix (ECM) receptors that enable them to home more efficiently.

The interplay between these components and the ECM provides a structural scaffold that contributes to the maintenance of HSC homeostasis. The importance of the cellular components of the niche is evident as alterations of non-hematopoietic cells are sufficient to induce hematopoietic disorders such as myeloproliferative diseases and leukemia (reviewed by [Yao and Link, 2016]). In fact, from what was

believed to be an inert supportive scaffold, the ECM became an essential player in HSC behavior regulation either through direct ligand interactions providing instructive signals or *via* indirect means through sequestration of growth factors and cytokines leading to their inactivation [Ahmed and Ffrench-Constant, 2016; Schonherr and Hausser, 2000]. The HSC/ECM interaction is a two way system, whereby ECM influences homeostatic maintenance of the HSC population but stem cells themselves are also able to remodel the niche in response to the signals they receive from it [Gattazzo et al., 2014].

This review provides an overview of the current understanding of the complex and dynamic microenvironmental components involved in the regulation of both embryonic and adult HSC behavior. In the first part, we discuss the soluble factors secreted by the different cells constituting the BM niche and the influence they have on stem cell localization and fate decision, both during development and in adulthood. We also discuss the different surface proteins and molecules expressed by HSC that allow them to directly bind to ECM components. These molecules account for the coupling of cell-cell and cell-matrix adhesion resulting in the activation of a variety of signaling pathways and allowing the cells to integrate signals from their microenvironment. In the second part, we concentrate on the ECM molecules that form the supportive scaffold which mediates a diverse range of cellular processes. Finally, we discuss the role of non-protein based metabolites in the BM niche, including lipids and metal ions which form an underappreciated component of the HSC niche.

SOLUBLE FACTORS AND SURFACE PROTEINS

SOLUBLE FACTORS

A number of soluble factors have been demonstrated to play pivotal roles in HSC regulation and their functions have been extensively reviewed [Mendelson and Frenette, 2014; Metcalf, 2008]. In this part of the review, we focus on recent findings concerning well-known soluble factors, further demonstrating

their importance, as well as more recently discovered soluble modulators of HSC. We will also address the importance of fibrinolytic and coagulation factors in HSC maintenance and mobilization, which is a newly appreciated area in HSC regulation.

Stromal cell derived factor-1 (SDF-1) is a key regulator in HSC maintenance, proliferation and migration. Its role has been well documented and recently reviewed by the field [Chotinantakul and Leeanansaksiri, 2012; Klamer and Voermans, 2014; Morrison and Scadden, 2014]. In the adult BM, SDF-1 is produced by many different types of cells, including perivascular mesenchymal stromal cells (MSC) [Ding and Morrison, 2013; Greenbaum et al., 2013], endothelial cells [Dar et al., 2005], as well as osteoblasts [Sugiyama et al., 2006]. SDF-1 influences HSC migratory behavior towards the BM niche mainly through chemotactic interaction with its receptor CXCR4, triggering a variety of cellular responses modulating HSC proliferation, differentiation and survival (reviewed by [Nagasawa, 2015]).

The role of SDF-1 in fetal hematopoiesis has also been extensively investigated and reviewed by the field [Chotinantakul and Leeanansaksiri, 2012; Klamer and Voermans, 2014; Morrison and Scadden, 2014]. The SDF-1/CXCR4 interaction has been reported to be critical for fetal BM colonization (reviewed by [Cao et al., 2013]). Moreover, recent studies revealed other niche molecules involved in fetal hematopoietic regulation *via* the SDF-1/CXCR4 axis. For example, in the absence of CD44, SDF-1 mRNA expression is up-regulated [Olofsson et al., 2014] and its protein expression is accumulated in the fetal liver [Cao et al., 2016b], inducing HSC to remain in the fetal liver microenvironment [Cao et al., 2016b]. Whilst numerous other molecules have been identified as important HSC regulators *in vitro*, but only show modest or no effect on HSC *in vivo*, SDF-1 is one of the few distinctive molecules that is indispensable for hematopoiesis, as

SDF-1^{-/-} mutant mice die prenatally and have severe defects in B cell lymphopoiesis and fetal BM myelopoiesis [Nagasawa et al., 1996; Rossi et al., 2012].

Although SDF-1 is the canonical ligand for CXCR4 dependent interactions in HSC biology, several other underappreciated modulators of the CXCR4/SDF-1 axis have recently been uncovered. In particular, cleavage fragments of the third component of complement cascade, β 2-defensin and the antimicrobial peptide cathelicidin (LL-37) have been shown to “prime” or enhance the responsiveness of CXCR4⁺ HSC to SDF-1 gradients (Reviewed in [Ratajczak et al., 2012]). More recently, a naturally occurring, serum albumin cleavage product termed “EPI-X4” was identified to be an endogenous antagonist and inverse agonist of CXCR4 and found to effectively mobilize murine HSC [Zirafi et al., 2016; Zirafi et al., 2015]. While all the components required for EPI-X4 production are abundant throughout the body, the presence of EPI-X4 in BM is yet to be confirmed. Furthermore, whether naturally occurring EPI-X4 can regulate HSC maintenance *in situ* is unknown, so it remains to be seen whether SDF-1 is the sole ligand involved in endogenous CXCR4 signaling [Zirafi et al., 2015].

Beside SDF-1, a wide range of niche-derived soluble growth factors, such as transforming growth factor-1 (TGF- β 1) [Blank and Karlsson, 2015] and stem cell factor (SCF) [Lennartsson and Ronnstrand, 2012], also mediate the interaction between HSC and components of the BM niche and their roles in HSC survival, proliferation and differentiation have long been recognized [Mendelson and Frenette, 2014]. Recently, new sources of growth factors have been identified and newly defined HSC regulators have been revealed to partially function *via* growth factor signaling pathways. For example, several groups have demonstrated the importance

megakaryocytes play in maintaining HSC quiescence through the release of different soluble growth factors. Megakaryocytes, being a source of thrombopoietin (TPO), play a key role in this process [Nakamura-Ishizu et al., 2014; Qian et al., 2007]. They showed that not only did higher ploidy (more mature) megakaryocytes contain more TPO transcript, but when BM was specifically depleted of megakaryocytes, a decrease in intra-BM TPO concentration drove HSC into cycle and this ultimately resulted in a decrease in HSC numbers within the BM [Nakamura-Ishizu et al., 2014]. Megakaryocyte and platelet released platelet factor-4 (PF4/CXCL4) also induce HSC quiescence [Bruns et al., 2014] as does transforming growth factor-1 (TGF- β 1), with the latter working *via* SMAD signaling [Gekas and Graf, 2013; Zhao et al., 2014]. In contrast, we published that insulin-like growth factor-1 and insulin-like growth factor binding protein-3 released by megakaryocytes increase HSC proliferation *in vitro* [Heazlewood et al., 2013]. Following hematopoietic challenges such as chemotherapy or irradiation therapy, basic fibroblastic growth factor, fibroblastic growth factor-1 and platelet-derived growth factor- β (PDGF- β) released from megakaryocytes can regulate both HSC proliferation as well as homing/engraftment; this can occur either *via* direct mechanisms or indirectly by the regulation of osteoblast numbers and function [Dominici et al., 2009; Zhao et al., 2014].

Furthermore, growth factors are also involved in neural regulation of hematopoiesis. The sympathetic nervous system (SNS) represents a critical regulatory component of the BM niche and its role in the regulation of HSC in both homeostasis and under stress conditions has been extensively investigated in the past decade (reviewed in [Hanoun et al., 2015]). Non-myelinating Schwann cells, which are wrapped around sympathetic nerves, have been shown to localize closely with HSC, maintaining their dormancy through the activation of TGF- β 1/SMAD

signaling [Yamazaki et al., 2011]. The interplay between the hematopoietic system and the SNS also extends to the early stages of development as the SNS has been implicated in the emergence of fetal HSC from the aorta-gonads-mesonephros (AGM) *via* the production of catecholamines [Fitch et al., 2012].

In addition, growth factors are also downstream target genes of HIF-1 α , which is the major regulator of transcription in response to hypoxia. Fetal HSC are generated and matured in the low oxygen microenvironment of the uterus and evidence suggests quiescent adult HSC predominantly localize in a low oxygen region within the BM (reviewed by [Imanirad and Dzierzak, 2013; Zhang and Sadek, 2014]). In these hypoxic microenvironments, HIF-1 α expression is increased and leads to the up-regulation of a large number of growth factors known to be important regulators of HSC behavior, such as vascular endothelial growth factor (VEGF), insulin-like growth factor-2, insulin-like growth factor binding proteins and erythropoietin (reviewed by [Imanirad and Dzierzak, 2013; Zhang and Sadek, 2014]). The study of the *Vegfa*(δ/δ) mouse model, in which the hypoxia response element (HRE) of the *Vegfa* promoter is mutated, demonstrated the importance of the hypoxia-VEGFA axis in the regulation of HSC. Indeed, the mutation of the HRE induced a significant decrease of VEGFA expression and led to a functional defect of HSC in competitive transplantation experiments [Rehn et al., 2011].

Recently, factors involved in fibrinolysis have also been revealed to play broad roles in HSC quiescence and mobilization. For example, the proteolytic enzyme plasmin, mainly known for its fibrinolytic action, has been shown to augment HSC mobilization post G-CSF administration, possibly through the cleavage of the interactions between HSC and their microenvironment

[Tjwa et al., 2008]. Moreover, it has recently been shown that some fibrinolysis associated factors can also play an indirect role in HSC regulation by creating a more supportive microenvironment. For example, tissue plasminogen activator (tPA) has been demonstrated to induce the expansion of $CD45^{+}TER119^{-}Sca-1^{+}PDGFR\alpha^{+}$ mesenchymal stromal cells (P α S MSCs) in BM [Dhahri et al., 2016]. Mechanistically, tPA induces the release of kit ligand from P α S MSCs which activates c-kit⁺ endothelial cells to secrete platelet derived growth factor-BB and fibroblastic growth factor-2. As a positive feedback, PDGFR α expression is upregulated in P α S MSCs ultimately leading to cell expansion [Dhahri et al., 2016].

Coagulation related components have also been implicated in the regulation of HSC behavior. The importance of the blood clotting protein FVIII in hematopoiesis was highlighted by the analysis of HSC in FVIII knockout mice. This study revealed a reduced long-term hematopoietic repopulating activity and an increased susceptibility to G-CSF stimulation [Aronovich et al., 2013]. More recently, other coagulation related proteins have been associated with the retention of HSC in the BM or their mobilization *via* two different protease activated receptor 1 (PAR1) mediated pathways [Gur-Cohen et al., 2016]. The anticoagulant endothelial protein C receptor (EPCR), which is expressed by HSC throughout development and in adulthood, plays a pivotal role in these mechanisms as its cleavage by thrombin or protease activated protein (aPC) results in the activation of different pathways. Indeed, thrombin-EPCR-PAR1 signaling promotes rapid HSC mobilization induced by EPCR shedding and nitric oxide production (NO), while the aPC-EPCR-PAR1 signaling supports the retention of HSC in the BM by limiting NO production [Gur-Cohen et al., 2016]. Collectively, these studies show that the homeostasis between

fibrinolysis and coagulation is also an important factor in the regulation of HSC quiescence and mobilization mechanisms.

Finally, several lines of evidence suggest that the BM microenvironment and the process of aging are closely linked to the regulation of HSC [Snoeck, 2013]. The antiaging hormone *klotho* exists both in a membrane bound form and a soluble form that is secreted into blood, urine and cerebrospinal fluid after cleavage of the extracellular domain [Chen et al., 2007]. A defect in the *Klotho* gene in mice results in a syndrome of premature aging, including growth retardation and vascular calcification [Kuro-o et al., 1997]. Interestingly, this antiaging hormone has recently emerged as a new potential regulator of hematopoiesis [Vadakke Madathil et al., 2014]. *Klotho* deficiency has been shown to result in a significant increase in erythropoiesis and a decrease in HSC numbers. Similar hematopoietic abnormalities were also observed in the fetal liver suggesting a novel role for the antiaging hormone *klotho* in the regulation of prenatal and postnatal hematopoiesis. [Vadakke Madathil et al., 2014].

SURFACE PROTEINS

HSC express a large repertoire of receptors, which directly mediate their interaction with the ECM as well as with cellular components of the BM niche. These specific interactions are involved in the regulation of key biological processes such as homing, engraftment and mobilization.

A number of different cell adhesion molecules (cadherins, selectins, immunoglobulin superfamily, mucin-like family, CD44 family members as well as integrins) expressed by HSC have been identified and extensively reviewed [De Grandis et al., 2016]. However, the role of the endothelial cell-selective adhesion molecule (ESAM) in HSC regulation was explored more recently. ESAM was originally identified

as being an endothelial lineage-related antigen, however it has been shown to be highly expressed by HSC both during embryonic development and in the adult [Hirata et al., 2001; Ooi et al., 2009; Yokota et al., 2009]. ESAM expression levels have been shown to be a powerful indicator of HSC cell cycle status [Sudo et al., 2012]. Cell cycle regulation is crucial to avoid HSC exhaustion or mutation, maintaining the HSC pool throughout life. Although HSC divide at an extremely low rate during homeostasis, they are rapidly activated to proliferate in response to BM injury. ESAM expression was shown to mirror the shift of HSC between quiescence and proliferation with ESAM^{hi} HSC dividing actively post 5-Fluorouracil (5-FU) administration [Sudo et al., 2012]. Moreover, it was shown that ESAM^{hi} HSC of 5-FU treated BM can reconstitute long-term hematopoiesis, suggesting that ESAM^{hi} HSC retain their engrafting capacity even after entering cell cycle [Sudo et al., 2012]. The functional importance of ESAM for HSC reconstituting activity was emphasized by the finding that ESAM-KO mice suffer from severe BM suppression after 5-FU administration due to a delayed erythroid recovery [Ishibashi et al., 2016].

Another cell adhesion molecule, junctional adhesion molecule (JAM-A), has recently been shown to regulate HSC behavior by enhancing their retention in the BM niche [Chang et al., 2016]. JAM-A is a cell adhesion molecule which is also highly expressed by HSC both during embryonic development and in adulthood [Sugano et al., 2008]. Although none of the JAM family of molecules has been previously shown to interact with CXCR4, it was demonstrated that not only does JAM-A co-associate with and regulate the function of CXCR4 on HSC, but is also involved in the adhesion of HSC to BM endothelial cells thereby enhancing HSC retention in the BM niche [Chang et al., 2016].

Cell adhesion molecules are also involved in the regulation of the stem cells throughout life. It is well documented that HSC migrate from the yolk sac to the AGM region before moving to the fetal liver and finally to the BM in a process called homing. Chemotaxis is an important player in

embryonic cell trafficking [Ciriza et al., 2013]. Indeed the production of SCF by fetal liver cells and later by BM cells generates chemoattractive signals for HSC [Chou and Lodish, 2010]. In addition, BM cells continuously produce SDF-1 which contributes in directing circulating HSC towards the BM niche. Integrin mediated adhesion is also crucial during embryonic development [Christensen et al., 2004]. For example, β_1 integrin has been shown to be required for HSC fetal liver colonization [Potocnik et al., 2000].

Collectively, evidence suggests that different surface proteins/molecules expressed by HSC are of great importance in the regulation of their behavior. Despite the fact that a great number of interactions between HSC and the ECM have been identified so far, the identification of new ones remains a topic of continuous interest.

EXTRACELLULAR MATRIX MOLECULES

It has long been appreciated that ECM molecules such as fibronectin, laminin, collagen, osteopontin (OPN), hyaluronan (HA) and tenascin C (TNC) are important components of the BM niche; providing structural integrity when these non-cellular components combine to produce the intricate meshwork or basement membrane to which cells can bind and be regulated. This section gives an overview of how these components do more than merely provide structural support but can also directly and indirectly interact with and regulate HSC. The biophysical properties of ECM components, such as mechanotransduction, stiffness and engineering artificial HSC niches using these components to provide 3D structure support for cell growth has been well reviewed elsewhere [Ahmed and Ffrench-Constant, 2016; Frantz et al., 2010; Gattazzo et al., 2014].

Research has shown that knockout mice deficient in fibronectin, collagen IV and some laminin chains/isoforms are embryonically lethal [George et al., 1993; Miner et al., 1998; Miner et al., 2004; Poschl et al., 2004; Watt and Hodivala, 1994]. All these proteins, have been shown to be important in HSC adhesion and proliferation in human and mouse BM *via* integrin binding [Gu et al., 1999; Klein et al., 1995; Siler et al., 2000]. Recently, it has been shown that cultured megakaryocytes are a source of fibronectin, collagen and laminin [Malara et al., 2014]. In turn, these components increased HSC proliferation and promoted megakaryocyte formation *in vitro*; and *in vivo*, megakaryocyte expression of these components was up-regulated during BM reconstitution following 5-fluorouracil induced myelosuppression [Malara et al., 2014]. Furthermore, megakaryocytes, which have been shown to influence HSC biology both directly and indirectly, are a source of thrombin, which has been shown to promote fibronectin secretion by human BM mesenchymal stem cells *via* PAR-1 and PAR-2 mediated ERK signaling [Chen et al., 2014; Heazlewood et al., 2013; Storan et al., 2015]. Megakaryocyte released thrombin has also been demonstrated to cleave full-length osteopontin [Storan et al., 2015].

OPN is a phosphorylated glycoprotein that is extensively expressed by many different cell types. Within the BM, the major source of OPN is cells of the osteoblastic lineage, which produce OPN during bone formation [Franzen and Heinegard, 1985]. In adult BM, the expression of OPN is restricted to the bone/BM interface where HSC with higher homing efficiency and greater proliferative potential reside [Grassinger et al., 2009; Grassinger et al., 2010]. The dominant form of OPN in human and murine BM is thrombin-cleaved OPN (tcOPN), to which integrins $\alpha_4\beta_1$ and $\alpha_9\beta_1$ expressed on HSC can bind [Grassinger et al., 2009]. TcOPN has been demonstrated to be critical in the attraction, retention and negative regulation of HSC

proliferation and differentiation within the endosteal region [Grassinger et al., 2009; Nilsson et al., 2005; Stier et al., 2005] and recently we demonstrated that integrins $\alpha_4\beta_1$ and $\alpha_9\beta_1$ on HSC within the endosteal BM region are endogenously activated; and are therefore in a more ligand receptive state than their central BM counterpart [Cao et al., 2016a]. Moreover, as previously mentioned, tcOPN is produced locally within the BM, with megakaryocytes releasing all the required factors to cleave full length OPN [Storan et al., 2015]. Therefore, in addition to this ECM component providing structural support for HSC, it can also directly affect HSC biology. OPN is expressed during embryogenesis. In fact, it has been demonstrated that fetal liver stromal cells show a 2-fold increase in the number of OPN expressing cells compared to their adult BM counterparts [Martin and Bhatia, 2005], suggesting that OPN is also a key regulator of fetal hematopoietic development.

The ECM molecule HA is known to be an important component of the BM niche, but has also been shown to be synthesized by HSC [Nilsson et al., 2003]. This proteoglycan has been identified as a negative regulator of HSC proliferation, inhibiting myeloid differentiation as well as reducing HSC homing to the endosteal BM region [Ellis et al., 2011; Nilsson et al., 2003]. The importance of HA in HSC homing and hematopoiesis was further examined using hyaluronan synthase (*Has1-3*) knockout mice [Goncharova et al., 2012], which confirmed previous reports. More recently, the role of HA has also been revealed in megakaryocyte maturation and proplatelet formation [Petrey et al., 2016]. Similar to HA, the glycoprotein TNC was shown to affect HSC migration, homing, distribution, adhesion, lineage commitment as well as reconstitution potential [Ellis et al., 2013; Nakamura-Ishizu et al., 2012]. Both groups provided evidence that the relationship between HSC and TNC is *via* the integrin α_9 expressed on HSC

[Grassinger et al., 2009]. In the BM niche, TNC, like the other ECM molecules discussed in this section, is not merely a structural element, but can also directly and indirectly regulate HSC biology.

Recently another ECM molecule, Perisostin (Postn) has been described as being involved in the regulation of HSC proliferation [Khurana et al., 2016]. Postn is a secreted ECM initially discovered in a mouse osteoblastic cell line [Horiuchi et al., 1999]. The regulation of HSC proliferation occurs through the direct interaction between Postn and the integrin $\alpha\text{v}\beta\text{3}$ (Itgavb3). The Postn-Itgavb3 interaction results in an increased expression of p27Kip1 caused by Postn-mediated inhibition of the FAK/PI3K/Akt pathway, which has previously been shown to induce HSC quiescence [Cheng et al., 2000]. The importance of the Postn-Itgavb3 interaction in the regulation of HSC has been emphasized by the study of Postn^{-/-} mice in which the numbers of HSC were increased with a higher proliferative capacity compared to WT HSC, while their engraftment ability was decreased [Khurana et al., 2016]. The authors demonstrated that it was the loss of the Postn-Itgavb3 interaction that was responsible for the increased HSC proliferation because of the deactivation of the FAK/PI3K/AKT pathway responsible for regulating p27Kip1 expression [Khurana et al., 2016].

NON-PROTEIN METABOLITES:

LIPIDS

While ECM molecules, cytokines and chemokines have well-established roles as regulators of HSC function, various non-protein based metabolites such as lipids have been described to regulate HSC behavior (reviewed in [Hoggatt et al., 2009]). In addition to OPN and SDF-1 as

described above, osteoblasts are also known to produce the arachidonic acid derived prostaglandin E₂ (PGE₂), which is a member of the eicosanoid family of lipids and a potent stimulator of bone resorption through activation of osteoclasts *via* the RANK-RANKL axis [Inada et al., 2006]. HSC were subsequently shown to express all receptors for PGE₂ (EP1-4) [Hoggatt et al., 2009] and exogenous treatment with PGE₂ was demonstrated to directly stimulate HSC expansion, improve HSC engraftment in irradiated recipients and expedite hematopoietic recovery after irradiation [Goessling et al., 2009; North et al., 2007; Porter et al., 2013]. Knockdown or inhibition of cyclo-oxygenases 1 and 2 (Cox1/Cox2) or PGE₂ synthase, the enzymes required for PGE₂ synthesis, was also shown to reduce HSC numbers, thus implicating PGE₂ as a regulator of HSC homeostasis [North et al., 2007]. Of note, PGE₂ was found to mediate its effects through activation of Wnt signaling pathways [Goessling et al., 2009], a mechanism which has previously been implicated in normal HSC homeostasis and maintenance of HSC population renewal [Reya et al., 2003]. Thus, it is plausible that PGE₂ produced by osteoblasts confers HSC maintenance and survival in endosteal BM and is a key component of the microenvironment. Interestingly, prostaglandin I₂ (PGI₂) has also recently been demonstrated to be preferentially synthesized in endosteal BM where it acts to protect HSC from irradiation stress, although no effect on HSC number or function was observed [Tay et al., 2016]. Together, these reports suggest different prostaglandins may exert differential regulatory effects on HSC in the BM microenvironment.

PGE₂ is also a critical regulator of HSC development during embryogenesis [North et al., 2007] as inhibition or knockdown of PGE₂ synthase, Cox1/Cox2 as well as the PGE receptors (Ptger11-Ptger41) significantly diminishes HSC formation in the AGM [North et al., 2007]. Consistent

with these results, knockdown of Cyp2aa9, a member of the CYP2 family of cytochrome P450 enzymes (Cyp2aa9) in zebrafish was also shown to impair embryonic HSC development as a result of reduced PGE₂ production and Wnt activation [Chen et al., 2016a]. It was noted that Cyp2aa9 is likely to function in the enzymatic step just preceding PGE₂ formation, as treatment of Cyp2aa9 mutants with PGE₂ metabolic precursors could not rescue the mutant phenotype [Chen et al., 2016b]. Nevertheless, the role of CYP2 in mammalian HSC biology has yet to be verified and further investigations are necessary to validate its role in PGE₂ mediated regulation of HSC in the BM niche.

In addition to PGE₂, other biologically active lipids derived from cytochrome P450 dependent arachidonic acid metabolism have also been described to modulate HSC function. In particular, the fatty acid diol dihydroxyeicosatrienoic acid (DHET), which is generated from its respective fatty acid epoxide epoxyeicosatrienoic acid (EET) by the soluble epoxide hydrolase (sEH) has been shown to stimulate the canonical Wnt signaling cascade in HSC [Fromel et al., 2012]. Knocking out sEH in mice results in impaired G-CSF dependent HSC mobilization as well as reduced HSC proliferation potential, which could be partially rescued by treatment with the sEH product DHET [Fromel et al., 2012]. Both fatty acid epoxides and their dihydroxy counterparts are increased in response to G-CSF treatment, suggesting a role of these lipids in HSC mobilization. Intriguingly, PGE₂ has also been previously implicated in HSC mobilization as inhibition of endogenous PGE₂ with non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to enhance G-CSF mediated HSC mobilization through reduction of BM OPN [Hoggatt et al., 2013]. It is possible that sEH as well as the arachidonic acid derived fatty acid diols, such as DHET described above, may also be involved in NSAID assisted HSC mobilization as inhibition

of the Cox pathway by NSAIDs shifts arachidonic acid metabolism towards other pathways including the cytochrome P450 pathways that can lead to EET and DHET (Reviewed in [Norwood et al., 2010]).

Another lipid that acts directly on HSC is sphingosine 1-phosphate (S1P), which exerts its effects through the receptor S1P₁ and functions as a potent chemoattractant with roles in HSC trafficking, homing and retention within BM [Ratajczak et al., 2010]. Under steady state, low S1P levels in BM are maintained by sphingosine lyase, which degrades S1P [Ratajczak et al., 2010]. Notably, steady state levels of S1P in peripheral blood (PB) is sufficient to generate a chemoattractive concentration gradient directed from BM to PB, which facilitates HSC mobilization [Golan et al., 2012]. In addition, while mice deficient in S1P (Sphk1^{-/-} mice) did not have impaired hematological homeostasis, impaired homing and engraftment of WT or CXCR4-deficient HSC was observed [Ratajczak et al., 2010], which collectively shows S1P expressed in BM plays an important role in HSC homing and mobilization but is not involved in HSC maintenance [Ratajczak et al., 2010]. Taken together, accumulating evidence shows that naturally occurring lipids represent an important family of niche molecules that have a variety of roles in HSC regulation.

METAL IONS

The BM niche has been thoroughly characterized at the tissue, cellular and protein level since the concept of a “niche” was established, but an underappreciated component is the various metal ions that are contained within the microenvironment. Bone is the primary storage site of many mineral ions such as calcium, magnesium, manganese, zinc and copper [Saltman and Strause,

1993]. Notably, while bone resorption by osteoclasts is known to degrade endosteal components such as OPN, SDF-1 and SCF, which are known mediators of HSC retention within the BM [Kollet et al., 2006], concomitant release of calcium ions is also observed, which leads to the formation of concentration gradients that radiate from the endosteal surface into the central medullary cavity [Cao et al., 2016a]. Indeed, the greater quantity of extracellular calcium in the endosteal BM has been proposed to facilitate HSC localization and engraftment in this region through the calcium-sensing receptor (CaR) that is expressed by HSC [Adams et al., 2006]. Further support for the role of bone calcium homeostasis in HSC maintenance was demonstrated through perturbation of vitamin D/vitamin D receptor (VDR) dependent signaling. Specifically, VDR knockout mice exhibited impaired HSC mobilization with G-CSF [Kawamori et al., 2010] as well as increased HSC numbers in the spleen [Jeanson and Scadden, 2010], which could be reversed with dietary calcium supplementation, suggesting VDR signaling and bone calcium homeostasis is important for HSC trafficking and localization in the niche. More recently, biologically active vitamin D3 was shown to directly stimulate zebrafish and human cord blood HSC expansion in a VDR-dependent but calcium independent manner [Cortes et al., 2016]. Although the role of direct VDR signaling in BM HSC has yet to be determined, these studies suggest that VDR signaling in the BM niche may be both HSC intrinsic and extrinsic and may not be strictly dependent on calcium homeostasis.

While calcium is the most recognized metal ion in the BM niche, magnesium and manganese ions have also been identified to be elevated in the endosteal region [Cao et al., 2016a], which along with calcium, are known modulators of integrin activity [Pepinsky et al., 2002]. In particular, the greater calcium, magnesium and manganese content in the endosteal region has

been shown to differentially prime/activate $\alpha_4\beta_1$ and $\alpha_9\beta_1$ integrins that are expressed by endosteal HSC [Cao et al., 2016a] as demonstrated by binding of a divalent-cation dependent $\alpha_4\beta_1/\alpha_9\beta_1$ integrin probe [Cao et al., 2014]. Although the physiological role for differential integrin activity on BM HSC has not been determined, it is possible that the greater homing ability of endosteal HSC, compared to central HSC which are phenotypically identical for the eight markers used to isolate them, is due in part to the enhanced integrin activity of HSC in this region [Grassinger et al., 2010]. Indeed, the well-known roles that integrins play in HSC retention, migration and homing [Sahin and Buitenhuis, 2012] suggest differential divalent cation dependent regulation of integrin function near bone may contribute to improved endosteal HSC homing.

Another intriguing thought is that the maintenance of metal ion gradients in BM may also be facilitated by binding to ECM. For example, OPN expression is restricted to the endosteal region within BM [Nilsson et al., 2005] and owing to its highly acidic nature, has previously been reported to potently bind the divalent metal cations Ca^{2+} and Mg^{2+} [Klaning et al., 2014]. Thus, it's possible that Ca^{2+} , Mg^{2+} and perhaps also Mn^{2+} gradients in BM are maintained through binding to ECM such as OPN, which may facilitate cation-dependent integrin function on HSC.

Equally important to the role metal ions play within the niche are the metal ion channels that are expressed by HSC. HSC express various ion channels including the P2 family of purinergic receptors (reviewed in [Feng et al., 2015]) and various K^+ channels [Park et al., 2011]. Specifically, HSC express both inwardly rectifying K^+ channels (K_{ir}) [Shirihai et al., 1998] and hERG K^+ channels [Pillozzi et al., 2002], which have been implicated in cytokine induced HSC

proliferation and differentiation. Notably, hERG K^+ channels have also been shown to regulate Ca^{2+} ion channels (Reviewed in [Bose et al., 2015]) and can form physical complexes with β_1 integrins, thereby regulating HSC cell adhesion and migration [Cherubini et al., 2005]. With consideration of the plethora of bulk and trace metals present in the body and BM, the different biological processes these metal ions modulate as well as the various ion channels/transporters that have now been described, further investigations into the role metal ions play in HSC biology is necessary to attain a full picture of how the BM niche regulates HSC.

CONCLUSION

ECM is a critical component of the BM microenvironment which not only provides structural integrity in the BM niche but these molecules are also crucial regulators of HSC behavior. The multiple modes by which they operate and the number of different elements involved render the ECM indispensable in the maintenance of hematopoiesis. This review has provided an overview of stem cell niche ECM components and how the dynamic orchestration of different signals allows the regulation of HSC behavior, both during embryonic development and in adulthood. We have also reviewed the contribution of non-protein based metabolites such as lipids and metal ions in the regulation of HSC behavior, which have only recently been integrated into the concept of the bone marrow HSC niche. These more recently identified niche elements warrant further investigation and with improved technology in metabolite analysis and spectroscopy based imaging techniques (e.g. X-ray fluorescence microscopy), studying low abundant trace metabolites and metal ions in BM will become feasible. Further work aimed at interrogating the role of various BM ECM components will advance our understanding of the fine balance required for maintaining HSC homeostasis.

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Figure 1:

Illustration of different soluble factors, surface proteins, ECM molecules and gradients of metal ions involved in the regulation of HSC behavior in the endosteal (upper panel) and central (lower panel) bone marrow.

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Illustration of different soluble factors, surface proteins, ECM molecules and gradients of metal ions involved in the regulation of HSC behavior in the endosteal (upper panel) and central (lower panel) bone marrow.

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Title:

Niche Extracellular Matrix Components and Their Influence on HSC

Date:

2017-08-01

Citation:

Domingues, M. J., Cao, H., Heazlewood, S. Y., Cao, B. & Nilsson, S. K. (2017). Niche Extracellular Matrix Components and Their Influence on HSC. JOURNAL OF CELLULAR BIOCHEMISTRY, 118 (8), pp.1984-1993. <https://doi.org/10.1002/jcb.25905>.

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