Interactions between hematopoietic stem cells and their niche

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Abstract

Hematopoietic stem cells (HSCs) provide all blood cells throughout our lives. HSCs reside in a specialised microenvironment in the bone marrow (BM) referred to as the niche, where they are kept in a quiescent state for the majority of time. In recent years considerable research into the composition of and signalling in the niche has been performed, yielding an increased understanding of niche regulation and HSC homeostasis. Many non-hematopoietic niche cells, such as: mesenchymal stem cells, endothelial cells and HSCs descendants, such as: megakaryocytes, regulatory T-cells and macrophages support niche regulation. Other niche constituents, being the extracellular matrix and the sympathetic nervous system also assist in HSC regulation. The niche is a highly vascularised complex micro-environment and scientist hypothesize that there are many perivascular niches, each with distinct functions. Even though many regulatory aspects are elucidated, plenty of niche cells and factors remain undiscovered. In this essay, the existing knowledge regarding niche composition and regulation during homeostasis will be outlined and future directions will be discussed.

Abbreviations: AECs, arteriolar ECs; ANG1, angiopoietin 1; BM, bone marrow; CAR; CXCL12 abundant reticular stromal cells; CLP, common lymphoid progenitors, CMP; common myeloid progenitor; CXCL4, CXC-chemokine receptor type 4; CXCL12, CXC-chemokine ligand 12; ECM, extracellular matrix; ECs, endothelial cells; EVs, extracellular vesicles; FGF1, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GMP, granulocyte–macrophage progenitor; HA, hyaluronic acid; HSCs, hematopoietic stem cells; HSPCs, hematopoietic stem and progenitor cells; LMPP, lymphoid-primed multipotent progenitor; MEP, megakaryocyte–erythroid progenitor; MiRNAs, microRNAs; MK, megakaryocyte; MPP, metalloproteinase; MPP, multipotent progenitors; MSCs, perivascular mesenchymal stem cells; MSPCs, mesenchymal stem and progenitor cells; MV, microvesicles; NK, natural killer cell; OPN, osteopontin; OSM, oncostatin M; PTN, pleiotrophin; ROS, reactive oxygen species; SCF, stem cell factor; SECs, sinusoidal ECs; SDF-1, stromal cell-derived factor-1; SNS, sympathetic nervous system; THPO, thrombopoietin; Tregs, regulatory T-cells; VCAM, vascular cell adhesion molecule 1

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Introduction

Us humans depend our whole lives on the ability HSCs to differentiate into all mature blood cells. HSCs are undifferentiated cells defined by two key characteristics; multipotency and the ability to self-renew. The differentiation of (unspecialised) HSCs into specialised cells is called hematopoiesis, and occurs in the BM in adults. Previously, hematopoiesis was depicted as quite a straight-forward process in which HSCs differentiate into multipotent progenitors (MPPs) which differentiate either towards common myeloid progenitors (CMPs) or common lymphoid progenitors (CLPs), to eventually develop into specialised blood and immune cells. However, recent findings have shown that this is an oversimplification, and myeloid and lymphoid potential can be determined further down the differentiation tree than previously thought, at lymphoid-primed multipotent progenitor (LMPP) and multi lymphoid progenitor (MLP) levels[1,2,3]. Furthermore, recent studies conducting single cell assays have shown that cell-fate decisions are made upon single-cell levels, and that the HSC and progenitor pools are vastly heterogenous[4], where lineages potential is sometimes already primed in MPPs[5]. Showing that hematopoiesis is a very complex and dynamic system. Figure 1 shows current interpretations of the hematopoietic hierarchy[6], in Figure 1A dashed lines indicate newly discovered lineages of differentiation. In Figure 1B the differentiation routes are presented as a continuum rather than a set pathway.

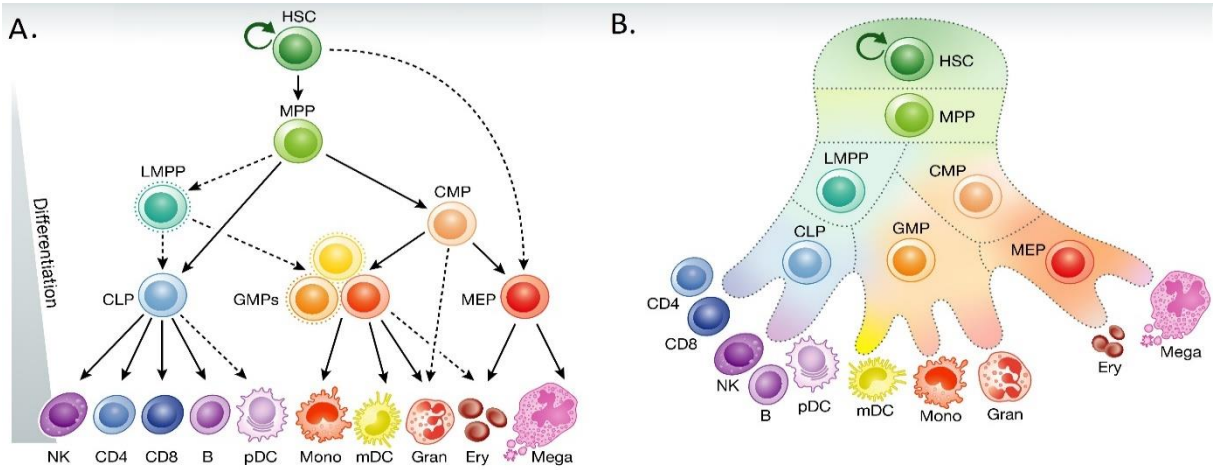

Figure 1: hematopoiesis hierarchy shown by two interpretations. Figure adapted from Bao et al (2019) mono, monocyte; gran, granulocyte; ery, erythroid; mega, megakaryocyte; CD4, CD4+ T cell; CD8, CD8+ T cell; B, B cell; NK, natural killer cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; GMP, granulocyte–macrophage progenitor; MEP, megakaryocyte–erythroid progenitor

HSCs are utilized in stem cell therapies, where depleted or damaged BM is replaced by HLA-matched autologous or allogenic HSCs through transplantation. BM or HSC transplantation is performed to treat malignant and non-malignant blood- and immune diseases such as leukemia and sickle cell disease[7]. In allogenic transplantation, the donor BM is intravenously administered where ideally the donor HSCs will reach and engraft in the recipients BM due to a process called 'homing'. During homing, the chemokine stromal cell-derived factor-1 (SDF-1 also referred to as CXCL12) activates C-X-C chemokine receptor type 4+ (CXCR4) cells which are arrested at the BM extracellular matrix and form vascularization into the BM. SDF-1 and macrophage inflammatory protein-1 in turn attract and initiate binding of CD34+ HSCs[8,9] to the VLA-4/5 extracellular matrix (ECM) receptors, from where HSCs relocate from the bloodstream to the BM. Transplanted progenitors do not reach the BM and are depleted within the first 2-3 weeks after transplantation[1]. HSC functionality is tested through transplantation assays, where prospective HSCs are transplanted in a suitable host followed by cellular

output measurements. With single cell transplantation assays, lineage output and HSC bias and heterogeneity can be analysed[10].

Expanding HSCs in vitro has been shown to be a challenge as the synthetic environment differs so much from the BM. This is an obstacle when limited suitable HSCs are available for transplantation. A way to improve in vitro expansion and thus HSC therapy, is gaining an understanding of the in vivo environment in which HSCs are located and the factors and cells that contribute to HSC expansion and proliferation. HSCs reside in the bone marrow in a specialised environment called 'niche'. The HSC niche contains a vast number of non-hematopoietic cells which support HSCs, these niche cells contribute extensively to HSC regulation such as homeostasis, quiescence and proliferation. The last ~15 years substantial research has focussed on investigating the composition and regulation of the HSC niche, which has given a big leap in understanding these processes. In this paper we will focus on how the niche and its constituents contribute to HSC regulation, we will address the non-hematopoietic cell types and several extrinsic factors that contribute to maintaining a stable micro-environment.

Regulation of the HSC microenvironment by extrinsic factors

The BM is a complex micro-environment where HSCs are tightly regulated through cell-intrinsic and extrinsic stimuli and cell-to-cell interactions[11]. In the last decade the anatomy of the HSC niche and non-hematopoietic cells residing in the HSC niche have been mostly resolved. We know now that the HSC niche is highly vascularized, with small blood-capillaries called sinusoids that are evenly distributed[12] and arterioles which show a higher density in the endosteal region of the BM[13]. Furthermore, the BM is innervated by sympathetic[14] and sensory nerves[15,16]. Due to the heterogenic nature of hematopoietic stem and progenitor cells (HSPCs) pools, the current idea is that there are several distinct 'niches' within the BM where specific HSC events take place. Quiescent HSCs are thought to reside and be maintained in the osteoblast rich endosteal niche[17], and HSCs migrate towards a sinusoidal rich region called the vascular niche where differentiation and proliferation takes place[18].

Non-hematopoietic niche cells

Most of the non-hematopoietic niche cells have been proposed to contribute to HSC regulation directly or indirectly. Table 1 outlines the relevant functions of all non-hematopoietic niche cells that have thus far been implicated to aid in niche regulation, and Table 2 shows the respective niche factors and their functions.

Osteoblasts

Osteoblasts were the first cell types to be investigated as candidate niche cells, given that in early research HSPCs were mostly found near the osteoblast rich endosteal niche in the BM[19]. Furthermore, osteoblasts produce several molecules that are linked to HSC regulation such as stem cell factor (SCF), CXC-chemokine ligand 12 (CXCL12), angiopoietin 1 (ANG1)[20], osteopontin (OPN), tyrosine kinase with immunoglobulin and EGF homology domains 2 (TIE2)[20] and thrombopoietin (THPO). However, upon investigation, osteoblasts do not seem to be the main source of these factors (except for OPN) and hematopoiesis and HSC maintenance is unaffected by osteoblast depletion[21,22]. Hepatocytes, which do not reside in the BM niche, seem to be the main source of HSC maintenance factor THPO[23]. OPN deficiency leads to an increased stem cell pool size and addition of OPN ex vivo increased stem cell apoptosis[24]. This suggests that osteoblasts are not directly involved HSCs differentiation and proliferation but are involved in regulating SC pool size and quiescence.

Mesenchymal stem cells

Another stem cell population that resides in the BM are mesenchymal stem cells (MSCs), these are stromal cells that can differentiate into osteocytes, chondrocytes and adipocytes. Furthermore, a sub-population of MSCs referred to as pericytes (perivascular cells) displaying stem-cell like qualities also reside in the BM, these cells are essential to vasculature maintenance and development and niche maintenance. Pericytes are viewed as a highly heterogenous population of MSCs, since they carry tissue dependent cell-markers but are able to differentiate into the same lineages as MSCs. There are several distinct subpopulations of MSCs/pericytes that contribute in unique ways to niche homeostasis. A relatively small perivascular sub-population marked by the expression of GFP under the Nestin promotor referred to as *Nes*-GFP+ cells, were found associated with the sympathetic nervous system (SNS) nerves and HSCs[25]. These cells express higher doses of SCF, CLXCL12, ANG1, IL-7, vascular cell adhesion molecule 1 (VCAM1) and OPN in comparison to other BM stromal cells. Furthermore, depletion of *Nes*-GFP+ cells cause migration of HSCs out of the BM, showing the involvement of these cells HSC maintenance and homing. Moreover, expression of maintenance genes by *Nes*-GFP+ MSCs (except OPN) are significantly downregulated in response to granulocyte colony-stimulating factor (G-CSF) or β_3-AR agonist[25], hinting the involvement of these cells in SNS dependent HSC homing. Moreover, HSCs are found in high concentrations in perivascular regions in the BM near stromal cells expressing high doses of CXCL12[26]. CXCL12 abundant reticular (CAR) stromal cells are the major CXCL12 producing cells in the BM, CAR cells overlap with *Nes*-GFP+ stromal cells and are found around sinusoids, these cells also produce SCF and IL-7 and are involved in B-lymphocyte and HSC maintenance[27]. Finally, two stromal cell sub-types, arteriolar NG2+ and sinusoidal LepR+, were discovered that reside in separate perivascular niches that are hypothesized to contribute in unique ways to HSC regulation[28]. Studies have shown that deletion of CXCL12 from arteriolar NG2+ cells, but not from LepR+ cells, caused HSCs reduction and shifting placement of HSCs in the BM. But SCF deletion in LepR+ cells, but not from NG2+ cells, led to reduced niche HSCs, proving that LepR+ cells are a critical source of SCF and pleiotrophin (PTN)[29]. Furthermore, studies have shown that quiescent cells reside near arterioles and NG2+ stromal cells[13], and when HSCs enter the cell cycle they distribute towards sinusoids. However, we must remember that sinusoids are very densely present throughout the BM, thus HSCs distribution around sinusoids could be random, and whether NG2+ really control HSC quiescence is still under debate. Further investigation must be concluded to gain insight into this very heterogenous cell population, and the ways perivascular cells aid in HSC regulation.

Adipocytes

Adipocytes are present in adult BM, adipocyte levels increase upon ageing and their number correlates inversely with haematopoietic activity[30,31]. Upon chemotherapy or irradiation stromal cells are depleted and adipocyte levels rise in the BM, several studies have shown negative effects of adipocytes on HSC proliferation, population and engraftment, suggesting that adipocytes are negative regulators of HSCs[32,33]. Additionally, increased HSC engraftment is found in irradiated 'fatless' mice[34,32]. In contrast, SCF producing adipocytes have been discovered, these cells differentiate from a LepR+ subpopulation called *Adipoq*-Cre/ER+ cells and aid in hematopoietic regeneration after irradiation[35,36].

Endothelial cells and the vasculature

The bone marrow is a highly vascularised microenvironment and 80% of HSCs are found associated with sinusoids, 10% with arterioles and 10% with transition zone vessels[28,37,29]. Blood vessels are lined with endothelial cells (ECs), and these ECs are in turn ensheathed with many other supporting niche cells such as the previously mentioned: Lepr+, CAR, *Nes*-GFP+ and NG2+ cells. Together, the vasculature ECs and stromal cells provide an essential part of the BM, ensuring entrance and exit of oxygen, waste molecules, HSCs, growth factors and other essential molecules. ECs have been shown to express many niche factors such as: CXCL12, SCF, PTN, E-selectin, gp130 and notch ligands. Most

SCF secreted by BM ECs is secreted by arteriolar ECs (AECs), deletion of *Scf* from AECs, but not sinusoidal ECs (SECs) resulted in a decrease of functional HSCs and depletion of HSCs from the niche[38,39]. Another difference between the endosteal and vascular niche is the permeability of the vasculature. Arterial vessels are less permeable and HSCs in the endosteal region are maintained in lower reactive oxygen species (ROS) resulting in increased quiescence. Whilst sinusoidal vessels have higher permeability and display higher ROS, which stimulates HSC activation, differentiation and migration[40]. These results indicating differential contribution of the vascular (SECs rich) and endosteal (AECs rich) niche to HSCs maintenance. PTN has been shown to support the expansion of HSCs in vitro and administration of PTN in vivo leads to increased HSPC expansion[41]. Moreover, HSC maintenance through PTN is a collective effort of stromal cells and ECs, where *Lepr+* stromal cells are the main source of PTN during normal homeostasis, but PTN secretion by ECs seems to be required for HSC regeneration after irradiation[42]. In the BM, E-selectin is expressed solely by ECs where it induces proliferation in vivo, *E-selectin*$^{-}$ mouse models show increased HSC quiescence[43]. Gp130 is a receptor subunit expressed by many cells, gp130 transduces signals from several cytokines (IL-6, IL-11) and Oncostatin-M. Deletion of *Gp130* in ECs and HSCs in mice resulted in disturbed hematopoiesis, suggesting a major role for this receptor in maintaining normal hematopoiesis[44]. Even though BM ECs only contribute slightly to the total SCF and CXCL12 levels in the BM, deletion of these factors from ECs do result in reduced HSC numbers, indicating that ECs contribute to HSC maintenance and quiescence[22].

HSC descendants: Megakaryocytes, macrophages and regulatory T-cells

Finally, HSC descendants can also regulate HSCs by feedback mechanisms. Megakaryocytes (MKs) reside in the BM close to the vasculature, as these cells produce platelets which need to be released into the bloodstream[45,46]. MKs and HSCs have been associated with sinusoids, and reside in close proximity with each other[47,48]. MKs have also been found to influence HSC quiescence through expression of soluble factors, such as CXCL4 and TGF-β1. MKs are the main source of CXCL4 in the BM, and injection of CXCL4 in mice resulted in reduced HSCs due to increased quiescence[49]. Furthermore, depletion of CXCL4 in mice lead to increased HSC proliferation[49]. MK ablation leads to reduced active TGF-β1 levels in the BM and reduced SMAD2/3 signalling in HSCs, leading to increased proliferation and HSC activation, whilst TGF-β1 injection in mice restored quiescence[48,50,51]. In contrast, during stress caused by chemotherapy MKs release fibroblast growth factor 1 (FGF1), which stimulates HSC expansion and mobilisation, hereby stimulating BM recovery[52]. MKs seem to be dynamic regulators of the HSC niche, through CXCL4, TGF-β1 and FGF1 signalling. Phagocytic cells have also been implied to aid in HSC retention and quiescence in the BM. HSC retention is promoted especially by CD169+ macrophages, possibly through the secretion of oncostatin M (OSM), which induces CXCL12 expression in *Nestin+* MSCs[53,54]. An important factor in HSC mobilisation is G-CSF, upon G-CSF signalling macrophages and osteoblasts in the BM are depleted, lowering CXCL12 signalling, leading to attenuated anchoring of CXCL4 HSC receptors to stromal cells, and releasing HSCs into the bloodstream[55]. However, osteoblasts do not carry the G-CSF receptor. Studies have shown that G-CSF induced HSC mobilisation is sufficiently regulated through G-CSF receptors residing on monocytic cells[56,57]. Moreover, regulatory T-cells (Tregs) are believed to be beneficial during allogeneic HSC engraftment after transplantation[58,59]. T-cells produce the cytokine IL-10 and hereby provides the HSC niche with an immune privilege[58]. Furthermore, a Treg subpopulation expressing CD150 promotes quiescence by lowering oxidative stress through generating adenosine, and after transplantation CD150^{high} Tregs promote HSC allo-engraftment through adenosine production[60].

The sympathetic nervous system

Sympathetic nerves innervate bone and BM[61], where they regulate circulation of HSCs in the blood-stream through release of adrenergic signals, which target CXCL12 expression in the BM[62,63]. Noradrenaline is released by the SNS in a circadian manner and is transduced by the adrenergic receptor β3 (ADRβ3) expressed by MSPCs and *Nes*-GFP+ cells[62,25]. Transduction results in downregulation of the Sp1 transcription factor, which causes rapid decreased expression of CXCL12. CXCL12 regulates HSC retention in the BM[64] and low levels of this chemokine cause immediate release of HSCs from the BM into the bloodstream via sinusoids[28]. CXCL12 levels are reduced upon treatment with G-CSF, resulting in rapid migration of HSCs from the BM. G-CSF suppresses osteoblast expression, and studies show that adrenergic signalling suppresses G-CSF induced mobilisation[56]. HSC retention depends critically on the indirect regulation of the SNS, as sympathetic nerves are in direct contact with *Nes*-GFP+ cells[26]. Furthermore, a structural and regulatory network consisting of sympathetic nerves and *Nes*-NG2+ perivascular stromal cells wrapped around arterioles referred to as the 'neuro-reticular complex' was found in mice[65]. Ablation of SNS nerves and loss of ADRβ3 signalling in the BM causes rapid migration of HSCs into the bloodstream, and leads to premature aging of HSCs in mice[66]. Furthermore, non-myelinating Schwann cells that sheathe sympathetic and sensory nerves, have been found to contribute to HSC dormancy by regulating the activation of latent TGF-β, hence regulating TGFβ/SMAD signalling[67]. TGFβ signalling maintains quiescence in vitro[68,69]. Additionally, nerve ablation causes glial death and causes rapid loss of HSCs[67].It is unknown if sympathetic nerves can directly influence HSC proliferation, but it is clear they regulate HSC retention indirectly through adrenergic signalling[70].

***Table 1**: Non-hematopoietic niche cells and their function*

Cell type	Main niche factor expression	Function	Location	Cell markers
Adipocytes		Negative regulators	BM, levels increase upon ageing	
Adipoq-Cre/ER cells	SCF	Regeneration after radiation		
CD169+ Macrophages	OSM	HSC retention by inducing CXCL12 expression in *Nes*-GFP+ MSCs		CD169
CXCL12 abundant reticular (CAR) stromal cells	CXCL12, SCF, IL-7	Maintenance	Near endosteum	Nestin
Endothelial cells	VCAM1, SCF, gp130, CXCL12, Pleiotrophin, Notch ligands, E-selectin	Retention, maintenance, proliferation	Lining of blood vessels	
LepR+ perivascular cells	SCF	Maintenance,	Near sinusoids	Lepr
Macrophages	OSM, DARC, TGFβ	Retention		
Megakaryocytes	CXCL4, TGFβ1, FGF1	Quiescence, retention, expansion/BM recovery (FGF1)		
Nes-GFP+ Perivascular cells	SCF, CXCL12, ANGPT1, OPN, IL-7, VCAM1	Maintenance, retention, homing	Associated with nerves from the SNS and HSCs	Nestin

NG2+ perivascular cells	CXCL12	Retention, maintenance, quiescence	Near arterioles	NG2
Nonmeyelinating Schwann cells	TGFβ, SMAD	Quiescence	Wrapped around sympathetic and sensory nerves	GFAP
Osteoblasts	OPN	Negative regulators of stem cell pool size, quiescence	Endosteal region	
Regulatory T-cells	IL-10, CDC39, adenosine	Quiescence (adenosine, CD39), immune advantage (IL-10, adenosine)		
Sympathetic nervous system (SNS) nerves	Noradrenaline	Regulation HSCs trafficking in bloodstream by adrenergic signals	Innervate BM	

Table 2: *Niche derived soluble factors and their function*

Factor	**Abbreviation**	**Function**
Adrenergic signals/noradrenaline		Act directly on β2-adrenergic receptor (ADRβ2) expressing cells, promoting migration or engraftment
Angiopoietin 1	ANG1	Indirect, regulates niche regeneration after radiation
CXC chemokine receptor type 4	CXCL4	Quiescence, retention
CXC-chemokine ligand 12	CXCL12 (SDF1)	Retention, maintenance, quiescence
Duffy antigen receptor for chemokines	DARC	Quiescence, through TGFβ-SMAD3 signalling
Fibroblast growth factor 1	FGF1	Expansion, mobilisation
Glycoprotein 130	Gp130	Maintaining normal hematopoiesis
Granulocyte colony stimulating factor	G-CSF	Decreases CXCL4/ CXCL12 levels in BM, leading to mobilisation of HSPCs from the BM
Interleukin 10	IL-10	Indirect, immune privilege
Jagged-1	Jag1	Proliferation
Jagged-2	Jag2	Supports expansion of ST-HSCs through expression of Notch1 and Notch2 receptors
Notch ligands		Proliferation
Oncostatin M	OSM	Indirect, may mediate crosstalk between Nes-GFP+ MSCs and macrophages
Osteopontin	OPN	Negative regulator of SC-pool size, quiescence
Pleiotrophin	PTN	Expansion, maintenance
Stem cell factor	SCF (KIT ligand)	Maintenance
Thrombopoietin	THPO	Maintenance
Transforming growth factor-β	TGFβ	Quiescence
Vascular cell adhesion molecule 1	VCAM1	Retention
Wnt signalling		Regulation HSC self-renewal

The extracellular matrix

As we know now HSCs are influenced by a lot of external factors, such as non-hematopoietic niche cells, the sympathetic nervous system, and many niche-derived factors. However it is important to remember that the BM and niche cells are connected and placed in a certain way because of the existence of the extracellular matrix. The ECM is not only a skeleton that forms the tissue, it also instructs and regulates metabolism and development of cells and functions as a substrate for cell migration[71]. This 3D arranged complex consists of various molecules such as structural proteins and proteoglycans, which are interlinked with stromal cells, the vasculature and niche cells. In recent years the ECM has got much attention in tissue engineering and regenerative medicine, and could be of interest in improving ex vivo HSC expansion and BM reconstruction. The ECM is very complex and not yet completely defined, however there is a certain understanding of ECM molecules that contribute to niche function (Table 3). The glycoprotein laminin, was identified as a major component of the BM ECM in mice and men, where they are found near HSCs and surrounding sinusoids[72,73]. Laminins exist in many isoforms, and regulate cell functions by adhesion to integrin and nonintegrin receptors, where they are presumed to regulate the BM niche in differential ways[74,75]. A study performed in *Lama4*$^{-/-}$ mice found reduced HSPC cycling and recirculation between blood and BM, due to increased retention and quiescent phenotype of HSCs in the niche, leading to reduced hematopoietic potential in *Lama4*$^{-/-}$ mice[76]. Another study found that ex vivo culturing of HSCs in fibronectin and laminin coated plates resulted in improved HSC expansion and marrow engrafibility[77]. Moreover, hyaluronic acid (HA) is essential for HSC retention in the BM, where it is mostly found in the endosteal[78]. HA is highly expressed by osteoblasts and HSCs[78], HA binds to the CD44 receptor expressed by HSCs[79], CD44 can be cleaved by metalloproteinase (MMP). MMP was found to be increased upon G-CSF treatment, resulting in increased CD44 cleavage and dislodgement of HSCs from the niche[80]. Moreover, the CD44 receptor has an important role in HSC connection to the niche since CD44 also binds to collagen, tenascin-C, laminins and osteopontin. Stromal cells are also important ECM producers as they secrete several ECM molecules such as: type I-IV collagen, tenascin and laminin[81,82]. Tenascin-C deletion in mice resulted in failed hematopoiesis after BM ablation, showing that this molecule is important in hematopoietic regeneration[83]. It is beyond the scope of this essay to address all the ECM molecules in the HSC niche, this has been reviewed extensively elsewhere[84]. However, with increasing knowledge about the exact functions of these ECM molecules it makes a promising venue to explore in regards to expanding HSCs ex vivo and even increase homing and engraftment after transplantation. Decellularized BM scaffolds could be used to increase HSC culturing and favourable ECM molecules could be upregulated through therapy post-transplantation.

Table 3: Extracellular matrix molecules and their proposed function[84]

ECM molecule	**Function (proposed)**
Collagen (I, II, III, IV, X)	Structural, cell adhesion, chemotaxis, migration
Decorin	Regulates function of growth factors
Fibronectin	Regulates hematopoiesis
Hyaluronic Acid	Involved in extravasation, engraftment, inflammatory response, structural integrity
Laminin	Cell proliferation, migration, regulating hematopoiesis
MMPs	Degradation of ECM, release of SCF, regulation of CXCL12, release of ECM bound cytokines
Nidogen	Involved in formation and maintenance of basement membrane, bone formation and growth factor distribution
OPN/tcOpn	Negative regulator of HSCs, quiescence[85]
Tenascin-C	Involved in cell proliferation

Thrombospondin	Homeostasis
Vitronectin	Cell migration and attachment

Extracellular vesicles and microRNA signalling

Extracellular vesicles are a heterogenous group of bilayer membrane structures (EVs, i.e. exosomes and microvesicles) that are secreted by most eukaryotic cells. These EV subtypes, exosomes and microvesicles (MVs), have different characteristics and mechanisms of biogenesis. Exosomes are small homogenous membrane particles originating from the endocytic pathway and are 40-120 nm in size. MVs originate from the plasma membrane, have a more heterogenous content and are 50-1000 nm in size. Both MVs and exosomes contain similar molecules, however it is unclear if they have a varying function. Previously, EVs where thought to be insignificant and used by apoptotic cells to discard non-functional cellular components. But recently it was found that healthy cells also secrete EVs, depending on their origin and biogenesis, EVs can contain biologically active molecules such as proteins, soluble factors, and microRNAs (miRNAs). There are implications that EVs mediate intercellular communication, as they are able to stably traffic molecules due to their small size and target specific cell-types through surface receptors. EVs are promising therapeutic agents, as they are safer to use compared to their parent cells due to low immunogenicity and no tumorgenicity since they are unable to multiply[86]. However, isolation and characterisation methods should be standardized and optimised in order to safely use EVs as therapeutic agents due to high heterogeneity. Important EV content are miRNAs, these small non-coding RNAs play an important role in regulating gene expression, miRNAs are found intra- and extracellular. miRNAs are secreted by cells via EVs and are able to mediate in cell-to-cell communication, once taken up by the target cells miRNAs presumably target the recipients mRNA, changing their expression profile.

So far, mainly EVs secreted by MSCs have been implicated to aid in niche regulation. MSCs are major mediators in HSC regulation, and these cells secrete MVs both during normal hematopoiesis and hematological malignancies. Furthermore, MSC-EVs are promising tools to be used to improve HSC transplantation and in vivo HSC expansion. Research found that MSC-EVs improve viability, decrease differentiation and increase homing of umbilical cord blood(UCB)-CD34+ cells after transplantation due to mediating gene expression in CD34+ cells via specific miRNAs[87]. Additionally, MSC-EVs seem to support MSC function during culturing and support CD34+ expansion in vivo[88]. Moreover, EVs derived from human umbilical cord-derived MSCs are suggested to prevent graft-versus-host disease in mice through modulation of the immune response upon allogenic-HSC transplantation[89]. Furthermore, MSC-EVs act on endothelial cells by transferring proangiogenic miRNAs such as miR424, miR30c and miR30b. Also, MSC-EVs exert immunomodulatory effects through secretion of miR143.

A recent study found that secretion of microRNA-126 (miR126) containing EVs in the BM is stimulated by G-CSF. Furthermore they found that miR126 reduces the expression of vascular cell adhesion molecule 1 (VCAM1) in HSCs, ECs and stromal cells. VCAM1 is a critical component of HSC retention in the BM, implying a role for miR126 in mediating HSC mobilisation[90]. ECs have been shown to secrete EVs carrying miR126, yielding a pro-angiogenic effect[91].

Finally, HSC descendants also release EVs targeting HSCs, resulting in steered HSC differentiation. Megakaryocyte derived EVs target HSCs and bind to the surface of HSCs through CD54 (ICAM-1), CD11b, CD18 and CD43 markers, upon internalisation, HSPC differentiation is skewed towards functional megakaryocytes possibly through RNA-mediated signalling[92]. Moreover, in response to hypoxia erythroleukemia cells release miR486 containing EVs that target HSPCs and increase erythroid differentiation[93,94]. Finally, HSCs may modulate their own stemness through VPS33B mediated secretion of EVs carrying stemness factors such as thrombopoietin and angiopoietin-like protein 2 and

3[95,]. Even though there are implications as to how niche derived EVs contribute to homeostasis and malignancy, it remains largely unknown how exactly EVs are involved in these processes and what the cargo is of these constructs.

Future directions

Major advances have been made the last decade in decoding the BM microenvironment and many key regulators and their molecular functions have been identified. Still, many cellular components involved in niche regulation are still illusive, as for the molecular functions of niche derived factors.

Modern imaging techniques have led to a better understanding of the location of HSCs and non-hematopoietic niche cells and led to the identification of perivascular niches. However, proposed is that the BM consists of many more perivascular niches that regulate the differentiation of specific HSCs descendants. Tagging of specific protein markers on perivascular stromal cells have led to the functional identification of many sub-populations such as: Lepr+, *Nes*-GFP+, NG2+ and CAR cells, but many more stromal cells remain to be classified. The functions of these cells are mainly analysed using knock-out mouse models. However, given the complex nature of the niche, other cells can adopt the knocked out functions in these models, findings should thus be carefully interpreted. We must bear in mind that niche regulation is an interplay between many cells and signalling pathways. With new technologies such as CRISPR-Cas9 multiple genes can be knocked-out which can help identifying connected pathways. Furthermore, multi-fluorescent tagging can help in following more lineages in vivo. Also, single cell analysis can be performed to analyse heterogenic cell populations, in perivascular cells but also HSCs. In addition, mouse models with a better representation of the human BM can be engineered, as to gain more accurate results. Ultimately, System biology approaches such as proteomics and metabolomics can help resolve the precise working of the BM microenvironment.

Regulatory signals coming from the BM microenvironment are very context dependent and complicated, since there is a vast cooperative network of cells and signals. Thus it is highly likely that HSCs react differentially towards in vivo vs in vitro stimulus, complicating in vitro HSC culturing. The ECM is a fundamental factor in HSC regulation, thus creating a similar environment in vivo and in vitro could benefit HSC expansion considerable. This environment can be simulated using 3D dynamic culturing techniques, biochemical hydrogels which imitate vascularised organs have already been developed. Also, co-culturing of HSCs with other niche cells could improve in vitro expansion. EVs could also be employed for both in vitro culturing and as ‘helping agents’ during transplantation therapy. Considering that EVs have been shown decrease post-transplantation immune reactions such as graft-vs-host disease. However, there is still a large gap of knowledge regarding the exact content and function of EVs. Considering the large heterogeneity amongst EVs, in depth analysis of their cargo must be performed. Finally, engraftment and homing after transplantation could be improved by upregulating beneficial cell and ECM factors such as CXCL12, ANG1, VLA4/5 and HA.

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