Advances in Stem Cells and their Niches

VOLUME 1

Hematopoietic Stem Cell Niche

Edited by

Dr Dominique Bonnet

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DOMINIQUE BONNET
The Francis Crick Institute
London, United Kingdom

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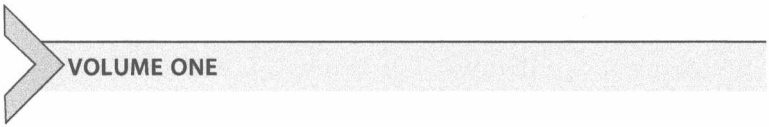

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CONTRIBUTORS

P. Charbord
Sorbonne Universités, UPMC Univ Paris 06, IBPS, CNRS UMR7622, Inserm U 1156, Laboratoire de Biologie du Développement, Paris, France

D. Duarte
Imperial College London; The Francis Crick Institute, London, United Kingdom

C. Durand
Sorbonne Universités, UPMC Univ Paris 06, IBPS, CNRS UMR7622, Inserm U 1156, Laboratoire de Biologie du Développement, Paris, France

A. García-García
Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge; National Health Service Blood and Transplant, Cambridge, United Kingdom; Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

K. Golan
Weizmann Institute of Science, Rehovot, Israel

L. Han
The University of Texas MD Anderson Cancer Center, Houston, TX, United States

E. Khatib-Massalha
Weizmann Institute of Science, Rehovot, Israel

O. Kollet
Weizmann Institute of Science, Rehovot, Israel

M. Konopleva
The University of Texas MD Anderson Cancer Center, Houston, TX, United States

D.S. Krause
Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany

A. Kumari
Weizmann Institute of Science, Rehovot, Israel

T. Lapidot
Weizmann Institute of Science, Rehovot, Israel

L. Li
Stowers Institute for Medical Research, Kansas City, MO; University of Kansas Medical Center, Kansas City, KS, United States

C. Lo Celso
Imperial College London; The Francis Crick Institute, London, United Kingdom

S. Méndez-Ferrer
Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge; National Health Service Blood and Transplant, Cambridge, United Kingdom; Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

G. Stik
Sorbonne Universités, UPMC Univ Paris 06, IBPS, CNRS UMR7622, Inserm U 1156, Laboratoire de Biologie du Développement, Paris, France

D. Verma
Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany

M. Zhao
Zhongshan School of Medicine; Key Laboratory for Stem Cells and Tissue Engineering, Ministry of Education, Sun Yat-Sen University, Guangzhou, Guangdong, China

B.O. Zhou
State Key Laboratory of Cell Biology, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai, China

PREFACE

It is essential to understand how stem cells interact with their microenvironment, the so-called stem cell niche to establish and maintain their function.

In this new series "Advances in Stem Cells and their niches" each volume will focus on a specific organ looking at how the niche components could regulate stem cells in both normal and disease conditions.

The first volume is dedicated to the *Hematopoietic Stem Cell Niche*. It contains seven contributions aiming at providing the latest reviews on our current understanding of the role of the hematopoietic stem cell (HSC) niche in the regulation of both normal and malignant hematopoiesis as well as new technological advances in in vivo imaging of HSC in their niche as well as new development on how to improve homing or mobilization of HSC in and out of the bone marrow niche.

In the first contribution, Dr. Stik et al. discuss our current knowledge on the cellular and molecular identity of the HSC niches during ontogeny and explore the molecular requirements for the HSC-supportive capacity of the niche cells and their cross talk with HSCs.

In the second chapter, Dr. Zhou et al. discuss the origin of the HSC niche concept, and examine the complexity of the bone marrow environment by describing the different players being involved. In the third chapter, Dr. García-García and Méndez-Ferrer discuss our current knowledge on the interplay between the nervous and the hematopoietic systems, with particular emphasis on the regulation of HSCs.

The next chapter by Duarte and Celso describes the use of intravital imaging to visualize and follow the dynamic behavior of HSC in their bone marrow niche allowing direct live observation of HSC–niche interactions in vivo. The following chapter by Kumari et al. addresses the mechanisms responsible for the homing and mobilization of the HSC.

The next chapter by Han and Konopleva discusses the recent insights into the role of altered niche function in the setting of myeloid malignancies, and the last chapter by Verma and Krause addresses the potential approaches to restore normal hematopoiesis through targeting of malignant niches.

This book should thus be of interest for academic researchers, research scientists, and graduate students in universities, industry, and government.

D. Bonnet
The Francis Crick Institute, London, United Kingdom

CONTENTS

CHAPTER ONE

Stroma Cell Niche Regulation During HSC Development

G. Stik*,[1], P. Charbord*, C. Durand*,[2]

*Sorbonne Universités, UPMC Univ Paris 06, IBPS, CNRS UMR7622, Inserm U 1156, Laboratoire de Biologie du Développement, Paris, France

[2]Corresponding author: e-mail address: charles.durand@upmc.fr

Contents

1. INTRODUCTION

Hematopoietic stem cells (HSCs) constitute a rare population of cells at the foundation of the adult hematopoietic system. Through their ability to self-renew and to differentiate, HSCs are responsible for the continuous production of mature blood cells throughout life. HSCs thus represent a unique model to study stem cell biology and have also major implications in the field of regenerative medicine (Wagers, 2012). Hematopoietic stem and progenitor cells (HSPCs) reside in close association with stromal cells that provide a supportive microenvironment (also called niche) for HSPCs. In the adult, HSPCs are located in the bone marrow (BM). The combination of transgenic mouse models with imaging and functional assays revealed that HSPCs are closely associated with blood vessels (arterioles and sinusoids) in contact with mesenchymal stem/stromal cells (MSCs), endothelial cells, and pericytes (Ding & Morrison, 2013; Ding, Saunders, Enikolopov, &

[1] Present address: Centre for Genomic Regulation (CRG), C/Dr. Aiguader, 88, PRBB Building, 08003 Barcelona, Spain.

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Morrison, 2012; Greenbaum et al., 2013; Kiel, Yilmaz, Iwashita, Terhorst, & Morrison, 2005; Kunisaki et al., 2013; Mendez-Ferrer et al., 2010). The BM HSPC niche appears to be highly complex, since Schwann cells, osteoblasts, osteoclasts, megakaryocytes, and macrophages have all been found to play an active role in the regulation of HSPCs (Mendez-Ferrer, Scadden, & Sanchez-Aguilera, 2015; Morrison & Scadden, 2014). At the molecular level, niche cells are known to regulate HSPC biology through the production extrinsic factors (such as growth factors and morphogens) and the regulation of cell adhesion and extracellular matrix remodeling cues. On top of these noncell autonomous mechanisms, systemic factors involving for instance the sympathetic nervous system and the circadian clock have also been shown to play an important role (Mendez-Ferrer, Lucas, Battista, & Frenette, 2008).

Importantly, HSCs are not produced in the BM, but early during embryonic life. The first adult-type HSCs are autonomously generated in the aorta-gonad-mesonephros (AGM) region at midgestation (Fig. 1; Cumano, Dieterlen-Lievre, & Godin, 1996; Medvinsky & Dzierzak, 1996; Muller, Medvinsky, Strouboulis, Grosveld, & Dzierzak, 1994). The hematopoietic activity in the AGM is characterized by the production of intraaortic hematopoietic clusters (IAHCs) closely attached to the ventral side of the dorsal aorta (Cumano & Godin, 2007; Dzierzak & Speck, 2008; Medvinsky, Rybtsov, & Taoudi, 2011). In the mouse, IAHCs are also observed on the dorsal side of the aorta. Interestingly, if both dorsal and ventral IAHCs harbor clonogenic potential, the HSC repopulating activity is preferentially assigned to ventral IAHCs (Souilhol et al., 2016; Taoudi & Medvinsky, 2007). Taken together, these observations demonstrate that the AGM hematopoietic activity is polarized along the dorsoventral axis and that the embryonic tissues underneath the dorsal aorta may serve as an important niche for the first emerging HSCs. The process of endothelial to hematopoietic transition by which HSPCs are produced from hemogenic endothelial cells lining the floor of the dorsal aorta is well conserved during evolution and has been documented in multiple vertebrate models (de Bruijn et al., 2002; Jaffredo, Gautier, Eichmann, & Dieterlen-Lievre, 1998; Oberlin, Tavian, Blazsek, & Peault, 2002). Moreover, these findings have been recently confirmed through in vivo intravital live imaging in the zebrafish and mouse embryos (Bertrand et al., 2010; Boisset et al., 2010; Kissa & Herbomel, 2010). Following their emergence in the dorsal aorta, HSPCs then colonize the fetal liver (FL) where they massively amplify. By the end of gestation, HSPCs migrate to the BM where they will be

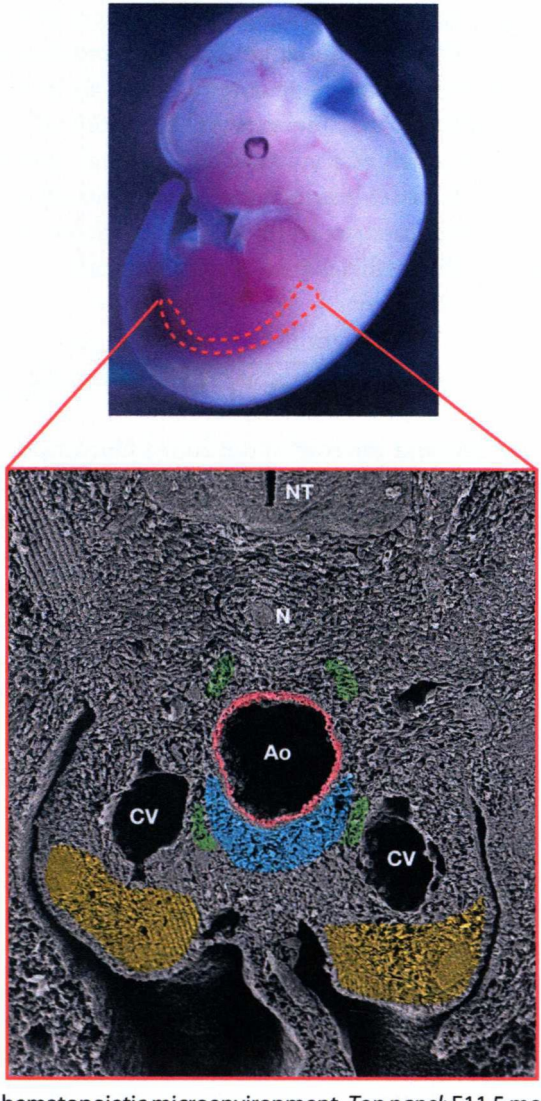

Fig. 1 The AGM hematopoietic microenvironment. *Top panel*: E11.5 mouse embryo. The AGM is indicated with a *red-dotted line*. *Bottom panel*: Electron micrograph showing different cellular compartments of the AGM HSPC microenvironment: endothelial cells (*red*), vascular smooth muscle cells, and subaortic mesenchyme (*blue*), sympathetic nervous system (*green*), and urogenital ridges (*yellow*). *Ao*, dorsal aorta; *CV*, cardinal vein; *NT*, neural tube; *N*, notochord. *Courtesy of Thierry Jaffredo.*

maintained throughout life. Thus, during their ontogeny, HSPCs are found in different niches that successively support their emergence, amplification, and maintenance. As emphasized by recent studies on the reprogramming of somatic cells to HSPCs (Batta, Florkowska, Kouskoff, & Lacaud, 2014; Pereira et al., 2013; Riddell et al., 2014; Sandler et al., 2014), identifying the similarities and differences between these distinct developmental HSPC niches is then of critical importance for our basic understanding of HSPC biology and also for their efficient ex vivo amplification and production for clinical purposes.

2. THE AGM HEMATOPOIETIC MICROENVIRONMENT

To better understand the role of the AGM hematopoietic microenvironment, a large panel of stromal cell lines was established not only from the subcompartments of the AGM, the Ao and its surrounding mesenchyme, and the urogenital ridges (UG) but also from the embryonic liver (EL) and the gastrointestinal tract of midgestation mouse embryos (Oostendorp, Medvinsky, et al., 2002). Coculture experiments with embryonic or adult HSPCs followed by colony forming cell or in vivo transplantation assays revealed that some embryonic stromal lines (including UG26-1B6 or EL08-1D2) provide a potent support for HSPCs, whereas other lines are less efficient in maintaining HSPCs ex vivo (Buckley et al., 2011; Oostendorp, Harvey, et al., 2002; Oostendorp et al., 2005). Interestingly, some of these stromal lines have also been shown to support the hematopoietic differentiation of mouse and human embryonic stem cells (Krassowska et al., 2006; Ledran et al., 2008). These AGM stromal cells have a mesenchymal origin and exhibit a myofibroblastic morphology. They are committed to the vascular smooth muscle cell (VSMC) differentiation pathway (Charbord et al., 2002) but can still give rise to other mesenchymal derivatives (such as osteoblasts and adipocytes) when cultured under appropriate in vitro conditions (Durand, Robin, & Dzierzak, 2006). The existence in the AGM of primary mesenchymal progenitors with adipogenic, osteogenic, and chondrogenic potentials has been documented at time of HSC production in the AGM (Mendes, Robin, & Dzierzak, 2005). Mesenchymal progenitor cells are then detected in the FL, neonatal, and adult BM, suggesting a developmental association between HSPCs and primitive mesenchymal cells. Existence of mesenchymal stem/progenitor cells in the AGM was also shown in human embryo (Wang et al., 2008). In vivo, the process of

HSPC production in the AGM occurs in a very short developmental window and is finely influenced by the surrounding embryonic tissues. Understanding how the AGM microenvironment is shaped in the developing embryo and how the adjacent tissues of mesodermal, endodermal, and ectodermal origins cooperate to initiate and control AGM HSPC production thus constitutes a major challenge in the field (Fig. 1). Experimental embryology approaches combined with gene expression profiling and functional studies have started to address this important question. In the chick embryo, preventing by ligature the migration and the installation of the subaortic mesenchyme was shown to inhibit the production of IAHCs and the expression of *Runx1* encoding a major transcription factor involved in the establishment of definitive hematopoiesis (Richard et al., 2013). In the mouse, explant cultures of early E10 AGM alone or associated with dorsal (neural tube) or ventral (gut) tissues followed by in vivo transplantation revealed that ventral tissue induce and increase HSC activity whereas dorsal tissues have a negative effect (Peeters et al., 2009). More recently, by taking advantage of an ex vivo culture system based on dissociation and reaggregation of embryonic tissues (Taoudi et al., 2008), it was shown that AGM HSC activity is strongly influenced by signals emanating from the ventral and dorsal domains of the Ao as well as from the adjacent UG (Souilhol et al., 2016). In the zebrafish, experimental evidence suggested that the somites together with the noncanonical Wnt signaling play an important role in the specification of HSCs, possibly via the recruitment of VSMC precursors (Clements et al., 2011).

At the molecular level, few cytokines, such as IL-3, Tpo, and Scf (Petit-Cocault, Volle-Challier, Fleury, Peault, & Souyri, 2007; Robin et al., 2006; Souilhol et al., 2016); the cell cycle regulator p57Kip2 (Mascarenhas, Parker, Dzierzak, & Ottersbach, 2009); and multiple signaling pathways, including Notch (Burns, Traver, Mayhall, Shepard, & Zon, 2005; Kumano et al., 2003; Robert-Moreno, Espinosa, de la Pompa, & Bigas, 2005; Robert-Moreno et al., 2008; Yoon et al., 2008), Hedgehog (Peeters et al., 2009; Souilhol et al., 2016; Wilkinson et al., 2009), bone morphogenetic protein (Bmp) (Crisan et al., 2015; Durand et al., 2007; Marshall, Kinnon, & Thrasher, 2000; Souilhol et al., 2016; Wilkinson et al., 2009), transforming growth factor (Monteiro et al., 2016), fibroblast growth factor (Lee et al., 2014; Pouget et al., 2014), and insulin growth factor (Mascarenhas et al., 2009), have all been shown to be active in the AGM and to play a role in the process of HSPC specification, maturation, or maintenance (Clements & Traver, 2013). How these pathways

are regulated in time and space and how are they eventually interconnected is also a critical issue. For example, studies in the zebrafish revealed a functional link between the Shh, Vegfa, and Notch signaling (Lawson, Vogel, & Weinstein, 2002). Shh is produced by dorsal tissues (the floor plate, the notochord, and the hypochord) and regulates the expression of Vegfa in the somites. Vegfa in turn induces Notch receptor expression in aortic endothelial cells. Bmp4 is also an interesting regulator since its expression at the mRNA and protein levels is preferentially located in the subaortic mesenchyme (Durand et al., 2007; Marshall et al., 2000; Pimanda et al., 2007; Souilhol et al., 2016; Wilkinson et al., 2009) and together with the Scl transcriptional network the Bmp/Smad signaling was shown to regulate Runx1 activity (Pimanda et al., 2007). Using a Bmp responsive element green fluorescent protein transgenic mouse line, it was recently reported that all E11 HSCs were in the GFP^+ fraction (Crisan et al., 2015). Functional studies in the zebrafish and mouse embryos suggested that the Bmp pathway is required for the emergence and maintenance of HSCs (Durand et al., 2007; Wilkinson et al., 2009). However, a recent study revealed that the Bmp signaling is downregulated during the maturation of HSCs (Souilhol et al., 2016), suggesting a complex role for the Bmp pathway in the production, maturation, and maintenance of AGM HSCs. Interestingly, loss- and gain-of-function experiments in the zebrafish indicated that the Fgf signaling negatively regulates the formation of HSCs in the dorsal aorta by repressing Bmp activity in the subaortic mesenchyme (Pouget et al., 2014).

On top of these local signals, systemic factors have recently emerged as critical regulators of AGM HSPCs. Among them, the blood flow and associated mechanical forces (Adamo et al., 2009; Diaz et al., 2015; North et al., 2009), the inflammatory pathway (Espin-Palazon et al., 2014; Li et al., 2014; Sawamiphak, Kontarakis, & Stainier, 2014), the sympathetic nervous system via the production of catecholamines (Fitch et al., 2012), and the central nervous system via stress-responsive glucocorticoid receptor signaling (Kwan et al., 2016) play an important role in the biology of AGM HSPCs. Collectively, these observations strongly support that the specification and subsequent maturation of HSPCs in the dorsal aorta is a complex event controlled in time and space by cell autonomous and noncell autonomous mechanisms. These latters operate both locally in the AGM and also at the systemic level to connect the development of the definitive hematopoietic system with other embryonic tissues and with the needs of the developing embryo.

3. THE FL AND PLACENTA HEMATOPOIETIC MICROENVIRONMENTS

Several tissues harbor HSC activity in the embryo including the AGM, umbilical and vitelline vessels, yolk sac, and placenta (Dzierzak & Speck, 2008; Medvinsky et al., 2011). Following their emergence, HSCs then massively amplify in the FL and in the placenta. So far, very little is known regarding the niches that support HSC expansion. As for the AGM, the establishment of a large collection of stromal lines was instrumental for exploring the role of the FL hematopoietic microenvironment (Moore, Ema, & Lemischka, 1997). Moreover, the comparison of the gene expression profiles of FL stromal lines with differing capacity to support HSPCs ex vivo leads to a better understanding of the molecular signature of the HSPC niche and to the identification of novel HSPC regulators (Charbord et al., 2014; Hackney et al., 2002). More recently, by taking advantage of transgenic mice, $Nestin^{+}NG2^{+}$ pericytes were shown to be associated with portal vessels in the E14.5 FL and to promote HSC expansion (Khan et al., 2016). Interestingly, BM and FL $Nestin^{+}NG2^{+}$ cells exhibit similar gene expression, although FL cells express preferentially genes involved in cell cycle and metabolism, suggesting that HSCs and their niche cells may actively proliferate in the FL (Khan et al., 2016). Based on a coculture system, it has been reported that DLK^{+} FL hepatic progenitors expressing the α-fetoprotein and albumin as well as hematopoietic cytokines such as Scf, Cxcl12 and Tpo support, and expand HSPCs in vitro (Chou, Flygare, & Lodish, 2013). These observations thus suggest that hepatic progenitors may also constitute an important constituent of the HSPC niche in the FL (Chou & Lodish, 2010).

The placenta was recently shown to harbor hematopoietic progenitors (Alvarez-Silva, Belo-Diabangouaya, Salaun, & Dieterlen-Lievre, 2003) and stem cells with long-term, high-level, multilineage hematopoietic repopulating potential (Gekas, Dieterlen-Lievre, Orkin, & Mikkola, 2005; Ottersbach & Dzierzak, 2005). Importantly, HSC activity in the placenta starts to be detected at E10.5–E11, dramatically increases between E11.5–E12.5 and then declines from E13.5, whereas the pool of HSCs continues to expand in the FL (Gekas et al., 2005). By utilizing Ly6A-GFP transgenic mice, where GFP expression is under the control of the regulatory elements of the HSC marker Sca-1, Ottersbach, and Dzierzak reported that all HSCs in the placenta (as in the AGM and adult BM) are GFP^{+} and are

associated with the vasculature of the placenta (Ottersbach & Dzierzak, 2005). In human, HSPCs are also detected in the placenta. However, in contrast to the transient hematopoietic potential harbored by the murine placenta, HSCs with the ability to repopulate the hematopoietic system of NOD-SCID recipients are still present in the full term human placenta (Robin et al., 2009). In the same study, stromal lines were generated from various developmental stages (3–38 weeks of gestation) and characterized for cell morphology, cell surface phenotype, and hematopoietic supporting capacity. Some of these stromal lines exhibit osteogenic, adipogenic, and eventually endothelial differentiation potential when cultured in vitro under appropriate conditions and express the pericyte markers NG2 and CD146. Coculture experiments with $CD34^+$ cord blood cells revealed that the placental stromal lines support the expansion of $CD34^+$ cells and hematopoietic progenitors. Together with the identification of pericytes stained for CD146, NG2 and smooth alpha actin and associated with endothelial cells on placental cryosections, these results suggest that perivascular cells may play an important role in the HSC niche in the placenta (Robin et al., 2009).

4. EXTRACTING THE MOLECULAR CORE OF THE HSPC NICHE

Although important extrinsic regulators (such as cytokines, morphogens, and components of the extracellular matrix) have been identified and shown to be active in the HSC niche throughout development, a comprehensive and integrated understanding of the molecular framework of the HSPC niche still remained elusive. To address this critical question, we have recently designed a systems biology approach based on the comparison of the gene expression profiling of murine stromal lines established from the AGM, FL, and adult BM and differing in their capacity support HSPCs ex vivo (Charbord et al., 2014). Our working hypothesis was that important genes for the stromal function should be conserved in at least two out of the three potent supportive lines generated from the developmental hematopoietic tissues. By combining bioinformatics and statistical analyses, we identified a set of 481 mRNAs and 17 microRNAs that was representative and predictive of the ability of stromal cells (including stromal lines and primary stromal cells) to support HSPCs. Using Weighted Gene Correlation Network Analysis (WGCNA) (Langfelder & Horvath, 2008), we showed that the mRNA gene set is organized into a network containing subnetworks or modules positively and negatively correlated to the factor "HSPC support"