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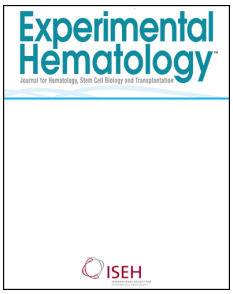

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Perspective

The Evolving View of the Hematopoietic Stem Cell Niche

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Abstract

Hematopoietic stem cells (HSC) reside in specialized microenvironments known as niches. The niche is essential to support HSC function and to maintain a correct balance between self-renewal and differentiation. Recent advances in defining different mesenchymal and endothelial bone marrow cell populations as well as hematopoietic stem and progenitor cells greatly enhanced our understanding of these niches and of the molecular mechanisms by which they regulate HSC function. In addition to the role in maintaining HSCs homeostasis, the niche has also been implicated in the pathogenesis of blood disorders including hematological malignancies. Characterizing the extrinsic regulators and the cellular context in which the niches interact with HSCs will be crucial to define new strategies to enhance blood regeneration. Furthermore, a better understanding of the role of the niche in leukemia development will open new possibilities for the treatment of these disorders, by using therapies aiming to specifically target the leukemic niche. To update on recent findings on this topic, the International Society for Experimental Hematology (ISEH) organized a webinar, presented by Prof. Sean J. Morrison and Dr. Simón Méndez-Ferrer and moderated by Dr. Cristina Lo Celso, entitled “The evolving view of the hematopoietic stem cell niche”, which we summarize here.

Introduction

Continuous production of blood cells is sustained throughout life by a rare population of hematopoietic stem cells (HSC), which reside in specialized niches mainly in bone marrow (BM) [1]. The existence of specific niches or microenvironments promoting HSC maintenance was initially proposed by Schofield in 1978 [2]. Already at that time Schofield suggested that stem cells are in close association with other tissue-resident cells that prevent stem cell differentiation, while ensuring its continuous proliferation. However, only more recently advances have been made to determine the location of HSCs and start unrevealing the molecular mechanisms by which they are regulated by the niche. For this, the finding that SLAM family markers could be used to define HSCs was a crucial step, by allowing the identification and purification of these cells to a higher purity level and with a simple combination of markers, compatible with microscopy analysis [3].

The BM is a complex network of endothelial cells (including sinusoids, arterioles and transition zone vessels) and mesenchymal stromal cells (comprising mesenchymal stem and progenitor cells, as well as osteolineage cells, chondrocytes and adipocytes) [4-14]. Traditionally, two main niches have been proposed based on proximity to bone: the endosteal niche in the immediate vicinity of bone lining osteoblasts [12], and the vascular niche at a greater distance from bone and with a more central localization within BM [3]. However, this dichotomous view of the niche has been challenged by the fact that the endosteal region is also highly vascularized [5]. In addition, several studies have expanded the number of cell types contributing to the stem cell niche, including different endothelial and mesenchymal lineage cell types [3,6,10,15] and also other hematopoietic cells such as megakaryocytes [16,17] and macrophages[18], as well as cells of the sympathetic nervous system [13,19] (Fig 1). Of note, phenotypically defined HSCs have been found in close proximity to different putative niche cell types, suggesting that different niches supporting different subsets of HSCs may exist.

Here we highlight recent advances in the characterization of the HSC niche by summarizing the webinar “The evolving view of the hematopoietic stem cell niche”, organized by the International Society for Experimental Hematology (ISEH), which was presented on June 14th, 2016 by Prof. Sean J. Morrison and Dr. Simón Méndez-Ferrer and moderated by Dr. Cristina Lo Celso.

Sean Morrison: Niche(s) for hematopoietic and osteogenic stem cells in adult bone marrow

The first session, presented by Dr. Sean Morrison, highlighted some of the recent developments in defining novel markers of a sinusoidal niche, and the genetic tools designed to elucidate the key cells contributing to stem cell maintenance. Earlier work by the Morrison group identified HSCs using the “SLAM code” and provided a method to characterize and prospectively isolate stem cells using a combination of the expression levels of CD150 (SlamF1) CD48, and CD41.

Using this combination, they found that long-term HSCs (CD150 high/CD48 low/CD41low) reside in close proximity to the sinusoidal endothelium, though some were also found in the endosteum [3]. To further define the niche environment, Dr. Morrison's group developed genetic tools to examine the location of critical niche factors, and to explore the effects of conditionally deleting these factors. They selected genes previously implicated as niche factors important for HSC maintenance, Scf and Cxcl12 [12,20,21], together with a proposed osteoblast-specific niche factor, Angpt1 [22], and generated knock-in reporter mice and systematically examined the expression of these factors in the bone marrow.

Rare SCF positive cells reside largely near sinusoids, with some SCF+ cells also localizing near arterioles and venuoles. Additionally, the cells expressing the highest SCF levels were perivascular stromal cells, while SCF was present only at low levels in the endothelial cells. Further characterization of perivascular stromal cells expressing high levels of SCF using gene expression profiling identified that these cells also express mesenchymal stem/progenitor cell markers (*Cxcl12*, *Vcam* and *Pdgfra-b*) in addition to full-length leptin receptor (*Lepr*) [23].

Lepr+ cells are found primarily near the sinusoids, but are also present near small diameter arterioles [24]. To establish if these cell populations contained niche cells for HSCs, the Morrison group generated a series of genetic models. They generated mice with a floxed Scf allele and crossed them to mice with various tissue-specific, inducible and constitutive Cre-recombinases (like *Lepr*-Cre). Global deletion of *Scf* in young adult mice in *Ubc-creER;Scf*$^{fl/fl}$ mice led to a significant reduction in bone marrow HSC numbers. This loss of HSC maintenance was only reproduced in mice lacking *Scf* expression in *Lepr*+ perivascular cells or *Tie2*+ endothelial cells[25], whereas no HSC phenotype was seen when *Scf* was deleted specifically in osteoblasts, hematopoietic cells, periarteriolar stroma (Nestin-Cre, Nestin-Cre^{ER}, or *Ng2-cre*^{ER}, or megakaryocytes. The loss of HSCs due to deletion of *Scf* in *Lepr* + cells was specific to the adult mice, as no significant change in HSC number was seen in the liver of newborn mice, highlighting the different niche environments of HSCs during development compared to adulthood. They found similar expression patterns of *Cxcl12*, another known niche factor, with the majority of *Cxcl12* expression localizing to perivascular *Lepr*+ and endothelial cells. No significant effect was seen on the bone marrow HSCs in any of the other tissue-specific deletion models tested. Conditional deletion of *Scf* in both endothelial and LepR+ cells (using Tie2-Cre; Lepr-Cre crossed to floxed alleles) led to significant alterations of the stem cell compartment- including loss of HSC number (assayed by both immunophenotype and transplant experiments). These mice also have truncated life spans, and die of hematopoietic failure due to loss of quiescent HSCs.

Dr. Morrison notes that there may be other critical niche cells that are not expressing *Scf* and *Cxcl12*, and that there is also a minor fraction of *Scf*- or *Cxcl12*-expressing cells that are not Lepr+ or endothelial cells. To explore if

angiopoietin (*Anptl1*) might mark some of these niche cells they also systematically knocked out *Anptl1* ubiquitously or in a tissue-specific manner, but were unable to detect an effect on bone marrow HSCs.

To approach the question of the HSC niche from a different angle, the Morrison group wanted to directly visualize the location of the HSCs in the bone marrow. They optimized bone-clearing protocols, which replace low refractive components within tissue with agents that have higher refractive indexes [26], ultimately leading to see-through bones. Using this technique, the group was able to perform high-resolution confocal microscopy to identify stem cell locations. As confocal microscopy has limited fluorescence channels compared to flow cytometry, the Morrison group identified another HSC marker, α-catulin. *α-catulin* is a relatively un-characterized gene with robust expression largely restricted to HSCs and megakaryocyte progenitors in the bone marrow [27] and CD45-Tie2+ endothelial cells in neonatal bone marrow [15,26,27]. By examining the localization of a-catulin/ckit+ cells, Acar *et al* demonstrated that the vast majority of these cells were immediately adjacent to the sinusoidal blood vessels and also close to endothelial cells and *Lepr*+ cells [26]. Though the vast majority of the HSCs were found near sinusoidal blood vessels, there were also small numbers of HSCs localized to both arterioles and near the transition zone.

Finally, Dr. Morrison also addressed how other cell types that have been implicated in the HSC niche, such as skeletal stem cells, fit into this model. He presented data in support of skeletal stem cells in the HSC niche, and further that these cells are present in the heterogenous Lepr+ population.

Simon Mendez-Ferrer: The niche in health and disease

It has become increasingly evident that the niche harboring HSCs consists of a complex assembly of multiple niche components existing in close proximity to each other. Importantly, changes in cellular composition of the niche, in the cytokine/growth factor milieu, or systemic factors produced in response to local changes may not only affect the HSCs, but also impact on the other cellular components of the microenvironment. Dr. Simon Mendez-Ferrer opened his part of the webinar by stressing that it remains to be determined how modulating one niche component will in turn impact on the function and HSC supportive capacity of the other cell types within the bone marrow microenvironment. He also raised the issue that studying the HSC niche and its composition is further complicated by the fact that deleting single molecules in single niche cells is not always robust. This can be due to redundancy and compensation from other niche cell types producing the same niche factor, or inefficient recombination. In line with this, the strongest effects on HSCs have been demonstrated in studies using constitutive Cre-lines affecting multiple cell types, such as Prx1-Cre and Lepr-Cre [24,25,28,29].

In addition to the early defined niche cells such as osteoblasts, endothelial and perivascular cells, Dr. Mendez-Ferrer and colleagues have identified Nestin-GFP expressing bone marrow mesenchymal stem cells (BMSCs) to constitute an

essential HSC niche component [6]. Nestin-GFP+ BMSCs are innervated by the sympathetic nervous system, express high levels of HSC maintenance genes, and localize with perivascular distribution in very close proximity to HSCs. Furthermore, Nestin-GFP+ BMSCs have been shown to be important for HSCs homing, and are predicted to regulate HSC traffic under homeostasis [6,19]. In a recent study, the group of Dr. Mendez-Ferrer demonstrated that some Nestin-GFP+ BMSCs that provide HSC-supportive function arise from the neural crest, and contribute to establishing the HSC niche during prenatal development by secreting Cxcl12 [30]. Depletion of Nestin+ cells at E15.5 greatly reduced the migration of HSCs from the fetal liver to the fetal bone marrow [30]. Furthermore, conditional deletion of Cxcl12 in Nestin+ cells during the first post-natal week resulted in 30% reduction of bone marrow HSPCs as measured by long-term competitive repopulation assays [30]. In contrast, Cxcl12 deletion in adult Nestin+ cells does not significantly affect BM HSC numbers [24], probably due to reduced recombination efficiency in adult BMSCs and/or compensation by other cell types that also produce Cxcl12. The fact that combined Cxcl12 deletion in multiple cell types (i.e. those targeted by *Prx1-cre*, or combined *Lepr-cre* and *Tie2-cre*) was needed to observe a robust HSC phenotype supports this possibility. However, stage-dependent differences might be also influenced by the fact that there are different waves of BMSCs during ontogeny which appear to exhibit different functions ([30] and summarized in [31]).

Despite different groups stressing the importance of their own favorite niche cell types, there is actually more consensus than discrepancies in the field. Endothelial and bone marrow stromal cells (BMSCs) (traced using Nestin, Lepr and Prx1) constitute important sources of HSC niche factors. However, MSCs are heterogeneous with regards to origin, markers and function, and future work is needed to dissect the specific subpopulations of niche cells involved, their interaction and their specialized functions in HSC regulation. Furthermore, the rapid development of single cell techniques has revealed a previously unrecognizable heterogeneity in the HSC pool. Hence, it is possible that differently primed HSCs or different HSC states require different niches. Alternatively, different niches may imprint distinct HSC states.

It has previously been recognized that malignant hematopoiesis is associated with an abnormal microenvironment. However, only in recent years did it become apparent that malignant cells and their surrounding niche cells can affect each other, and that this bi-directional interaction play a functional role in initiation and development of disease [32-39]. Myeloproliferative neoplasms (MPNs) are diseases caused by mutations in the HSC compartment. Most MPN patients have a common acquired mutation of Janus kinase 2 (JAK2) gene in HSCs [40,41], resulting in constitutive kinase activity and uncontrolled cell expansion. In a recent study Arranz et al. demonstrated that both sympathetic nerve fibers and Nestin-GFP+ MSCs were consistently reduced in the bone marrow of MPN patients and in mice expressing the human JAK2(V617F) mutation in the HSCs [32]. Importantly, in vivo depletion of Nestin+ cells accelerated MPN progression, whereas administration of neuroprotective or sympathicomimetic drugs improved the myelofibrosis associated with this disease [32]. This suggests that mutated

HSC damage their own niche in MPN and can initiate disease only after overcoming niche control. A better understanding of how the niche is modified in different hematological disease states could lead to the ability to protect and/or treat the niche, and hence be of therapeutic importance.

Concluding remarks:

Collectively the work presented here highlights the essential role of the HSC niche in regulating blood homeostasis and in the development of hematological malignancies. This knowledge will be of particular relevance in the regenerative medicine field to enhance blood production in patients with impaired hematopoiesis, for the ex vivo production/expansion of HSC and mature blood cells, and to generate new therapeutics for the treatment of leukemia.

The full webinar is available online at http://iseh.site-ym.com

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References

1 Orkin, S. H. & Zon, L. I. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* **132**, 631-644 (2008).

2 Schofield, R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7-25 (1978).

3 Kiel, M. J., Yilmaz, O. H., Iwashita, T., Terhorst, C. & Morrison, S. J. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121 (2005).

4 Nilsson, S. K., Johnston, H. M. & Coverdale, J. A. Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* **97**, 2293-2299 (2001).

5 Lo Celso, C. *et al.* Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* **457**, 92-96, doi:10.1038/nature07434 (2009).

6 Mendez-Ferrer, S. *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* **466**, 829-834, doi:10.1038/nature09262 (2010).

7 Sugiyama, T., Kohara, H., Noda, M. & Nagasawa, T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* **25**, 977-988, doi:10.1016/j.immuni.2006.10.016 (2006).

8 Omatsu, Y. *et al.* The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. *Immunity* **33**, 387-399, doi:10.1016/j.immuni.2010.08.017 (2010).

9 Taichman, R. S. & Emerson, S. G. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *The Journal of experimental medicine* **179**, 1677-1682 (1994).

10 Kunisaki, Y. *et al.* Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature* **502**, 637-643, doi:10.1038/nature12612 (2013).

11 Zhang, J. *et al.* Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836-841, doi:10.1038/nature02041 (2003).

12 Calvi, L. M. *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841-846 (2003).

13 Katayama, Y. *et al.* Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* **124**, 407-421, doi:10.1016/j.cell.2005.10.041 (2006).

14 Yamazaki, S. *et al.* Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell* **147**, 1146-1158, doi:10.1016/j.cell.2011.09.053 (2011).

15 Chen, J. Y. *et al.* Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche. *Nature* **530**, 223-227, doi:10.1038/nature16943 (2016).

16 Bruns, I. *et al.* Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. *Nature medicine* **20**, 1315-1320, doi:10.1038/nm.3707 (2014).

17 Zhao, M. *et al.* Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. *Nature medicine* **20**, 1321-1326, doi:10.1038/nm.3706 (2014).

18 Winkler, I. G. *et al.* Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. *Blood* **116**, 4815-4828, doi:10.1182/blood-2009-11-253534 (2010).

19 Mendez-Ferrer, S., Lucas, D., Battista, M. & Frenette, P. S. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature* **452**, 442-447, doi:10.1038/nature06685 (2008).

20 McCulloch, E. A., Siminovitch, L., Till, J. E., Russell, E. S. & Bernstein, S. E. The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl-Sld. *Blood* **26**, 399-410 (1965).

21 Barker, J. E. Sl/Sld hematopoietic progenitors are deficient in situ. *Experimental hematology* **22**, 174-177 (1994).

22 Arai, F. *et al.* Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**, 149-161, doi:10.1016/j.cell.2004.07.004 (2004).

23 Zhou, B. O., Yue, R., Murphy, M. M., Peyer, J. G. & Morrison, S. J. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell stem cell* **15**, 154-168, doi:10.1016/j.stem.2014.06.008 (2014).

24 Ding, L. & Morrison, S. J. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature* **495**, 231-235, doi:10.1038/nature11885 (2013).

25 Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. J. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* **481**, 457-462, doi:10.1038/nature10783 (2012).

26 Acar, M. *et al.* Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature* **526**, 126-130, doi:10.1038/nature15250 (2015).

27 Seita, J. *et al.* Gene Expression Commons: an open platform for absolute gene expression profiling. *PloS one* **7**, e40321, doi:10.1371/journal.pone.0040321 (2012).

28 Greenbaum, A. *et al.* CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature* **495**, 227-230, doi:10.1038/nature11926 (2013).

29 Mendez-Ferrer, S., Scadden, D. T. & Sanchez-Aguilera, A. Bone marrow stem cells: current and emerging concepts. *Annals of the New York Academy of Sciences* **1335**, 32-44, doi:10.1111/nyas.12641 (2015).

30 Isern, J. *et al.* The neural crest is a source of mesenchymal stem cells with specialized hematopoietic stem cell niche function. *eLife* **3**, e03696, doi:10.7554/eLife.03696 (2014).

31 Kfoury, Y. & Scadden, D. T. Mesenchymal cell contributions to the stem cell niche. *Cell stem cell* **16**, 239-253, doi:10.1016/j.stem.2015.02.019 (2015).

32 Arranz, L. *et al.* Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature* **512**, 78-81, doi:10.1038/nature13383 (2014).

33 Raaijmakers, M. H. *et al.* Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature* **464**, 852-857, doi:10.1038/nature08851 (2010).

34 Walkley, C. R. *et al.* A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell* **129**, 1097-1110, doi:10.1016/j.cell.2007.05.014 (2007).

35 Walkley, C. R., Shea, J. M., Sims, N. A., Purton, L. E. & Orkin, S. H. Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell* **129**, 1081-1095, doi:10.1016/j.cell.2007.03.055 (2007).

36 Pitt, L. A. *et al.* CXCL12-Producing Vascular Endothelial Niches Control Acute T Cell Leukemia Maintenance. *Cancer cell* **27**, 755-768, doi:10.1016/j.ccell.2015.05.002 (2015).

37 Medyouf, H. *et al.* Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell stem cell* **14**, 824-837, doi:10.1016/j.stem.2014.02.014 (2014).

38 Schepers, K. *et al.* Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell stem cell* **13**, 285-299, doi:10.1016/j.stem.2013.06.009 (2013).

39 Hanoun, M. *et al.* Acute myelogenous leukemia-induced sympathetic neuropathy promotes malignancy in an altered hematopoietic stem cell niche. *Cell stem cell* **15**, 365-375, doi:10.1016/j.stem.2014.06.020 (2014).

40 Baxter, E. J. *et al.* Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **365**, 1054-1061, doi:10.1016/S0140-6736(05)71142-9 (2005).

41 Kralovics, R. *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *The New England journal of medicine* **352**, 1779-1790, doi:10.1056/NEJMoa051113 (2005).

Figure Legend:

Figure 1. Schematic representation of the bone marrow HSC niche. HSCs have been prospectively observed in different BM anatomical locations and in close proximity to distinct candidate niche cell types.