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Bioengineering Hematopoietic Stem Cell Niche toward Regenerative Medicine☆

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ABSTRACT

The scope of this chapter is to introduce the current consensus of hematopoietic stem cell (HSC) niche biology to bioengineering field so that can apply to regenerative medicine. A decade of research has been addressing "what is HSC niche", then next step is "how it advances medicine". The demand to improve HSC transplantation has advanced the methodology to expand HSC *in vitro*. Still precise modeling of bone marrow (BM) is demanded by bioengineering HSC niche *in vitro*. Better understanding of HSC niche is essential toward this progress. Now it would be the time to apply the knowledge of HSC niche field to the venue of bioengineering, so that a promising new approach to regenerative medicine might appear. This chapter describes the current consensus of niche that endothelial cell and perivascular mesenchymal stromal cell maintain HSC, expansion of cord blood HSC by small molecules, bioengineering efforts to model HSC niche by microfluidics chip, organoids, and breakthroughs to induce HSC from heterologous types of cells.

Contents

1. Introduction

Stem cell niche is the microenvironment which supports stem cells, facilitated by soluble factors and adhesion molecules leading subsequent signal transduction [1].

Hematopoietic stem cells (HSCs) are functional unit of hematopoiesis to facilitate self-renewal and multilineage contribution. Being pushed by clinical demands of HSCs for bone marrow (BM) transplantation, understanding HSC niche is a significant focus of stem cell biology. The research of HSC niche goes back where Dexter had cultured hematopoietic cells with BM stromal cells for months with particular batch of serum [2]. Stromal cells in BM support hematopoietic cells, but the particular cell type and factors remained unknown. Soon after, Schofield proposed the concept 'niche' that stem cells are in association with other cells determining fate of stem cells [3]. However, the characterization of niche cells and its factors needed to wait till mouse transgenic begins to play a major role in medical biology. Through a decade of study, the consensus of HSC niche has been almost built up [4].

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The better understanding of HSC niche would benefit clinical application of HSCs in regenerative medicine. *In vitro* expansion of HSCs, a functional unit of BM transplantation, is highly demanded due to low supply of donor cells in clinics. Recent efforts identified several small molecules to successfully maintain and expand HSCs *in vitro* [5]. These small molecules are promising to advance the efficacy of BM transplantation. However more benefit might come from by looking at close association of HSCs with BM stromal cells and applying the mimetics of *the Mother Nature* to maintain HSCs in BM [6]. Thus, intensive needs be made on mimicking HSC niche *in vitro* by bioengineering approach. Engineering an artificial bone marrow that reconstitutes natural marrow structure and function could be a powerful platform to study hematopoiesis and test new therapeutics.

This chapter summarizes the current consensus of HSC niche field, then discusses how the insight from the field would move bioengineering society to advance regenerative medicine.

2. Part1. Current Consensus of HSC Niche

2.1. Early reports to identify HSC niche and controversy

In 2003, Scadden and Li independently reported that osteoblasts (OBs) support HSCs by close adjacent [7,8]. Soon after, Morrison reported CD150, a new marker of HSCs, and by using this revealed that HSCs are actually closely associated with endothelial cells in sinusoids, rather than OBs [9]. Since then, the controversy that which is HSC niche, 'endothelial cells or OBs', last for several years.

Indeed, a report said that OBs express ANGPT1 and maintain HSC [10]. On contrary, endothelial cells had been implicated to regulate self-renewal and proliferation of HSCs mediated by VEGF and NOTCH signaling [11,12]. In addition, mesenchymal stromal cells in the perivascular region associate with and presumably maintain HSC [13,14]. These arguments based on close association of HSCs with heterologous cells and assuming that these cells express factors to maintain HSCs. However, lacking direct test of 'niche'-specific deletion of factors, it had been difficult to judge which cells are niche.

2.2. Why the controversy came and how to solve this?

There are several points to consider about the controversy of HSC niche. Some preceding studies based on correlation of immunostaining and systemic knockout mouse model, because niche-specific Cre models were not available at that time. For example, if a factor is expressed in certain niche cells, and systemic knockout of the factor reduced HSCs, thus assuming that the factor maintains HSCs in the niche. The caveat is that it is not sure if the factor derived from the niche maintains HSCs, or other cells do so. Indeed, heterologous cells express the same factors, for example, SCF and SDF1 [15,16]. The next issue is how to label HSCs by immnostaining. The combination of CD150 + CD48-CD41-Lineage- is a standard marker to detect HSCs in bone marrow section [17,18]. However, there could be an argument that very quiescent HSCs, the most potent population, might be missing and more precise marker is required. BrdU-label-retaining population was referred as quiescent HSCs in several studies, but inherent challenge of this system is lack of functional supports since cells should be fixed prior to the analysis. Indeed, BrdU-label-retaining population does not reflect HSC exactly [19]. On other hand, H2B-GFP label-retention system well correlates with functionality of HSCs. H2B-GFP does not affect cell-cycle whereas BrdU does, that again explains why later would not reflect HSCs [20,21]. But H2B-GFP system demands over 100 days of chasing, thus it is not 'user-friendly' system and lack feasibility to be repeated in multiple labs. Recently reported *Fgd5-ZsGreen* reporter mouse model may be noteworthy that is specific to HSCs, and ZsGreen enables enough strong fluorescence for imaging [22]. As *Fgd5* gene is also expressed in vessels, it might help imaging purpose by combining with AcLDL-labeling of vessels so that only HSCs could be green. Another brand-new mouse model is *alpha-Catulin-GFP* reporter that could better reflects HSCs, though still vasculartures are also green [23]. HSC-specific report mouse models are still awaited.

2.3. Systematic reductionist approach revisits previous reports

Correlation took HSCs everywhere, but logical approaches finally gets them to HSC niche. Along with the substantial increase of availability of Cre mouse models, it has become possible to delete each factor in specific cell types in BM. The first comprehensive analysis was done by Morrison deleting SCF in each hematopoietic cells, endothelial cells, mesenchymal stromal cells and OBs [15]. The frequency of HSCs was reduced only when SCF was deleted in either endothelial cells or mesenchymal stromal cells. The deletion of SCF in OBs and its progenitors did not affect HSCs. These results provided the evidence that endothelial cells and mesenchymal stromal cells are a fundamental source of functional SCF to maintain HSCs. Though messenger of *Scf* can be detected in OBs, it is not functional. This paradox was solved by looking at isoforms of SCF. Membrane-bound form of SCF, known to maintain HSCs, was actually expressed in endothelial cells and mesenchymal stromal cells, but not in OBs.

The similar strategy was conducted in SDF1 and ANGPT1 [16,24,25]. SDF1 is one of the most pronounced factors derived from mesenchymal stromal cells to maintain HSCs [13]. Though ANGPT1 was implicated to maintain HSC in OB niche, the actual role was not tested *in vivo* [10]. HSCs were reduced when SDF1 was deleted in endothelial cells or mesenchymal stromal cells, but less or not at all affected in other cells. On contrary to a previous report, ANGPT1 was expressed not only in OBs but also in hematopoietic cells and mesenchymal stromal cells. Indeed expression of ANGPT1 in BM is not exclusive to OBs in other literature [26–28]. Actually, ANGPT1 derived from hematopoietic and perivascular cells did not support HSC maintenance, but actually facilitated HSC regeneration under stress. The take home message of these studies is that systematic reductionist approach by niche-specific Cre models is necessary to define HSC niche.

The controversy over HSC niche lasted for almost decade. There is not just down side but also an example where scientists in opposing side can make collaborative efforts to solve conflicting issue. In the model of OB niche, it had been believed that N-cadherin maintains HSCs with direct association with OBs [8]. Though this is a tempting model and a similar case exists in Drosophila stem cells [29], there were conflicting reports if the gene is expressed in HSCs or not [30,31]. Thus, the two teams repeated the experiments in each lab, then reported the results to the journal *Cell Stem Cell* where original observations have been published [32]. The messenger and protein expression of N-cadherin in HSCs was not detected, if expressed, it should be extremely low close to the threshold of detection of RT-PCR. More importantly, by conditionally deleting N-cadherin in HSCs, no hematopoietic defect was revealed [33]. Later, similar observations were repeated in conditional deletion of N-cadherin in OBs, and osteoprogenitors that there is no role of this gene in either HSCs or niche side [34,35]. This example tells us that collaborative efforts between multiple teams solve scientific conflicts.

2.4. Current consensus and remained issues

The systematic reductionist approach by niche-specific Cre models has now more precisely determined the components that regulate HSCs (Table 1). Endothelial cells and mesenchymal stromal cells maintain HSCs though SCF and SDF1 [15,16,24]. The early studies that implied effects on HSC maintenance by genetic manipulation in OBs actually reflected indirect effects rather than proving the existence of an OB niche. Genetic deletion of OBs primarily showed reduction in lymphoid committed progenitors, but not in HSCs [36]. OBs may supports lymphoid committed progenitors, and probably late-onset hematopoietic defect as a preceding study showed [37]. Taken that,