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PERSPECTIVES

Hematopoietic-Osteoblastic Interactions in the Hematopoietic Stem Cell Niche

Laura M. Calvi

University of Rochester School of Medicine and Dentistry, Rochester, New York, USA

Abstract

Hematopoietic stem cells (HSCs), rare primitive cells capable of reconstituting all blood cell lineages, are the only stem cells currently routinely used for therapeutic ends. Clinical experience has shown that HSC number is an important limiting factor in treatment success. Strategies to expand HSCs are of great clinical appeal since they would improve therapeutic use of these cells in stem cell transplantation and in conditions of bone marrow failure. To survive throughout the life of an individual, HSCs must balance self-renewal and differentiation. This essential regulation of stem cells has been postulated to be determined at least in part by the environment, or niche, in which these cells reside. The concept of a niche, which was hypothesized in the 1970s for HSCs and their regulation, has since been demonstrated for other stem cell systems, such as in the *Drosophila melanogaster* gonad. However, the niche harboring and regulating HSCs, likely the best characterized stem cells to date, has been difficult to define. This review focuses on our current understanding of the recently characterized pivotal role of osteoblastic cells in HSC control by the niche. *BoneKEy-Osteovision*. 2006 May;3(5):10-18.

Keywords: Stem cell niche; Osteoblast; Parathyroid hormone; Hematopoiesis; Notch; Jagged1; Self-renewal

Introduction

Stem cells are defined as rare cells with extensive proliferative potential, the ability to give rise to one or more differentiated cell types, and unlimited self-renewal. During mammalian embryonal development, only the fertilized egg and the early cleavage blastomere are totipotent stem cells, capable of generating all cell types. Later in differentiation, the inner cell mass of the blastocyst is composed of stem cells that are pluripotent, or capable of generating all the cell types found in the embryo and adult (1). In addition to the embryonic stem cells described above, adult stem cells have been hypothesized and identified as rare cells with the ability to indefinitely self-renew. These cells are defined as multipotent, as they are capable of regenerating several but not all cell types present in the adult. Stem cells have to be poised continuously between self-renewal and commitment to differentiation in order to be able to exist throughout the life of an individual, and at the same time function as a reservoir which can be called upon at times of stress (2). Since a number of pathologies are caused by destruction or degeneration of tissues and organs, the ability to understand and control adult stem cells at will has enormous therapeutic potential. This stem cell control could be intrinsic to stem cells and studies have begun to define a set of genes that define “stemness” (3-5). Alternatively or in addition, external regulatory mechanisms could determine stem cell fate decisions (6). Although the existence of a microenvironment or niche was postulated as early as the 1970s (7), the niche

hypothesis was verified *in vivo* only recently, through work on *D. melanogaster* ovarian and testis stem cells (8-10). Interestingly, the demonstration of a HSC niche has been elusive, although much evidence pointed to the bone endosteal surface as an important harbor for the most primitive hematopoietic cells during adult life. Only recently, our laboratory and others have demonstrated that osteoblastic cells are a regulatory component of the HSC niche, and have begun to elucidate the cellular and molecular mechanisms mediating osteoblastic-hematopoietic interactions.

HSCs and Self-Renewal

HSCs are the best understood stem cells. Surface markers identify oligopotent precursor and differentiated progeny as well as the most primitive cells, allowing for the prospective isolation of HSCs, which has been accomplished in both mice and humans (11-15). These cells can also be quantified through functional assays (16;17), which rely on stromal/HSC interactions and identify limiting dilution long-term culture initiating-cells (LTC-ICs). In addition, the ability of these cells to give rise to the full hematopoietic repertoire is demonstrated and can be quantified through competitive transplantation (18). Finally, not only are HSCs well characterized, but they were the first stem cells to be utilized therapeutically, and they are currently routinely relied upon in bone marrow transplantation for the treatment of hematologic malignancies and bone marrow failure states (19). In this setting, clinical experience has shown that HSC number is an important limiting factor in treatment success (20;21).

A defining characteristic of stem cells is their ability to self renew indefinitely, allowing not only for the preservation of the stem cell pool but also for the generation of an unlimited number of more differentiated progenitors and differentiated progeny. Self-renewal, a characteristic of both long-term and short-term HSCs (22) is therefore essential for primitive stem cells to persist for the lifetime of an individual. A number of signaling pathways have been identified that appear to be important for HSC self-renewal, including Sonic hedgehog (23), Wnt (24-26) and Notch (27-30). In all of these pathways, interaction of a receptor on the surface of the HSCs with either a secreted or a cell-bound ligand leads to physiologic activation of the pathway and self-renewal rather then differentiation. In addition to these pathways, a number of genetic regulatory pathways have been identified that regulate HSC self-renewal, such as HOXB4 (31), Bmi-1 (32), NF-Ya (33) and PTEN (34). Interestingly, some of these pathways are important not only for HSCs but also for other stem cell systems.

The HSC Niche

The concept that key control of stem cell fate and self-renewal may not be intrinsic to the stem cell itself but instead could be conferred to the cells by the microenviroment is not a new one. Schofield initially proposed in 1978 that, in order to explain the limited expansion of HSCs in transplanted animals, it would be necessary to envision a specialized location, or niche, in which the stem cells would be located and that would regulate their ability to either self-renew or differentiate (7). Such a niche would be a custom microenvironment, composed of specialized cells capable of supporting stem cells. Stem cells would be physically anchored to support cells, which should be able to provide the factors necessary for fate determination and self-renewal. While the niche hypothesis was proposed for HSCs, proof of its existence was demonstrated initially in the *D. melanogaster* ovary and testis (8-10). In the *D. melanogaster* ovary, somatic cap cells anchor germline stem cells through adherens junctions, stimulate the reception of an essential BMP signal and are capable of reprogramming cells to become stem cells (8;35;36). Identification of a HSC niche had however remained elusive, in part because of the complex organization of the bone marrow space, and in part because of the difficulty in identifying and labeling HSCs *in vivo*.

In spite of the difficulty in physically identifying the HSC niche, mounting evidence had suggested that such a niche would exist. If the hypothesis of a niche as a physical space were true for HSCs, some architectural organization of the HSCs and their progeny would be expected, as was seen in the *D. melanogaster* gonads. In fact, in the 1970s much evidence already pointed to such spatial organization: proliferation and differentiation of primitive hematopoietic cells were shown to be regulated differently depending on cell location within the bone marrow cavity, with more primitive hematopoietic progenitor cells in close proximity to the endosteal surface, while more differentiated cells were seen in the center of the bone marrow space (37-40). More recent studies confirmed these findings, and showed that infused primitive hematopoietic cells also tend to preferentially home to the endosteal surface (41;42).

The ability to assess physiologic exogenous cues has been partly limited by poor definition of stromal constituents of the HSC niche. Stromal cell components have been thought to include fibroblasts, adipocytes and endothelial cells, as well as cells of the osteoblastic lineage. However, in light of the architectural organization of the bone marrow reviewed above, cells on the endosteal surface, and particularly cells of the osteoblastic lineage, would be natural candidates as HSC niche cells. In fact, a number of studies have suggested that osteoblastic cells may play a role in the regulation of the hematopoietic system. Osteoblastic cells can support both terminal granulomatopoiesis and HSC survival *in vitro* (43). They can also stimulate intermediate progenitors (CFU-Cs) and more primitive cells (LTC-ICs) (44), expand HSCs 2-4 fold *in vitro* (45), and produce high levels of factors important for HSC support (43). In addition, osteoblastic cells engraft during bone marrow transplantation, and their co-transplantation with HSCs can increase engraftment rate (46;47).

Osteoblasts Are A Regulatory Component of the HSC Niche

Only recently have osteoblastic cells been shown *in vivo* to be a regulatory component of the HSC niche (48;49) through the use of genetically altered animal models. Specific expansion and/or activation of osteoblastic cells resulted in a specific increase in HSC frequency (48;49), while osteoblastic destruction resulted in loss of HSCs (50). In the work by Zhang *et al*., mice with conditional inactivation of the BMP receptor IA (BMPRIA) were found to have a characteristic bone phenotype, with additional trabecular bony structures along the femoral endosteal surface (49). In these mice, the osteoblastic cell pool is expanded, and HSCs are phenotypically and functionally increased (49). The investigators demonstrate that the HSC phenotype of the transgenic mice with conditional inactivation of the BMPRIA is mediated by the microenvironment, given the pattern of expression of the BMPRIA, which is not expressed in HSCs, and given the results of reciprocal bone marrow transplant experiments. They then show that HSCs are attached to an N-cadherin positive subset of osteoblastic cells. Although visualization of labeled HSCs in the intact bone by long-term Brd-U retention remains a controversial topic, expansion of osteoblastic cells was responsible for the HSC increase, supporting the hypothesis that osteoblastic cells are one component of the HSC niche.

Parathyroid Hormone Modulates the HSC Niche

In a study simultaneous to the work by Zhang *et al*., we explored the important relationship between osteoblasts and HSCs by studying a genetically altered mouse model in which osteoblast-specific expression of an activated PTH1R is targeted by the 2.3 kb fragment of the α1(I) collagen promoter (Col1-caPTH1R mice) (51). The mutant PTH1R used in Col1-caPTH1R mice causes ligand-independent cAMP accumulation, without inositol phosphate production (52;53). The bones of Col1-caPTH1R mice demonstrated brisk trabecular bone formation, increased trabeculae and trabecular osteoblastic cells.

Osteoblastic cells expressing the constitutively active PTH1R were increased in number, produced high levels of the Notch ligand, Jagged1, and supported an increase in the number of HSCs, identified by flow cytometric analysis, functional assays and competitive transplantation, with evidence of Notch1 activation in stem cells *in vivo*. PTH treatment both *in vivo* and *in vitro* could reproduce the HSC expansion. Interestingly, when myeloablated mice were transplanted with limiting numbers of bone marrow mononuclear cells (BMMC), PTH treatment dramatically improved survival and bone marrow morphology compared to controls (48).

These studies indicate that osteoblastic cells represent a regulatory component of the bone marrow microenvironment that exerts an effect on HSCs through Notch signaling. It remains unclear whether PTH1R activation results in increased levels of expression of Jagged1 in all or a particular subpopulation of osteoblastic cells, whether Jagged1 is necessary for PTH-dependent HSC expansion, and whether PTH can directly alter the HSC niche through Notch signaling.

Novel Regulators of HSC-Osteoblastic Interactions

Since these initial reports, a number of studies have confirmed the pivotal role of osteoblastic cells in HSC control, and have started to explore the cellular and molecular mechanisms regulating the HSC niche. Consistent with the importance of N-cadherin in mediating HSC-osteoblastic interactions, Angiopoietin and Tie-2 were recently shown to regulate HSC quiescence at least partially through osteoblastic N-cadherin (54). In addition, c-Myc-deficient HSCs have impaired differentiation, increased N-cadherin expression and are localized to the osteoblastic niche, while c-Myc overexpression in HSCs diminishes N-cadherin with loss of HSC self-renewal (55), once again suggesting the importance of homotypic N-cadherin adhesion for HSC regulation. However, the role and expression of N-cadherin in osteoblastic cells is currently poorly understood (56), and additional studies are required to determine whether this adhesion molecule is essential for HSC-osteoblastic interactions, and whether it specifies a subpopulation of osteoblastic cells.

A number of studies now support the concept that a specific subpopulation of osteoblastic cells may be important for HSC support. Gata2-directed GFP fluorescence marked HSCs in mice, and identified HSCs as solitary cells bound to a very small fraction of endosteal osteoblastic cells (57). It would follow, therefore, that in addition to N-cadherin, Angiopoietin and potentially Jagged1, other osteoblastic characteristics/functions would be necessary to regulate HSC support. Two independent reports identified the osteoblastic secreted matrix protein osteopontin as another osteoblastic-dependent regulatory component of the HSC niche capable of negatively regulating HSC self-renewal (58;59). In a surprising recent report, adrenergic signaling was found to modify osteoblastic function, decreasing osteoblastic CXCL12 production and releasing HSCs from the niche (60).

Future Directions

The usual strategy for identification of HSCs by flow cytometric analysis has been based on the exclusion of surface antigens for differentiated hematopoietic cells (corresponding to the lineage or lin designation). This approach is obviously not feasible for immunohistochemical analysis, and has limited our ability to identify and characterize the HSC niche *in vivo*. However, recent work has shown that a small number of antigens are sufficient for HSC identification, and that these antigens can be exploited for immunohistochemical analysis of the niche. In particular, Tie-2, the receptor for Angiopoietin, has been show to identify a subpopulation of HSCs (54). More recently, the SLAM family receptors CD150, CD244, and CD48 have been shown to identify HSCs and discriminate them from more mature hematopoietic progenitors not only by flow cytometric analysis, but also by

immunofluorescence (61). Finally, a recent study identified endothelial protein C receptor (EPCR or CD201) as a single HSC marker which can be utilized alone to isolate HSCs from the bone marrow as a nearly homogeneous population (62). These more defined HSC identifiers afford us the opportunity to identify more precisely and unambiguously novel patterns of contact between hematopoietic progenitors and osteoblastic cells.

While osteoblastic cells have been recently identified as cellular participants in the HSC niche, we are only beginning to define the molecular and cellular mechanisms mediating this very specific hematopoietic-osteoblastic interaction. The data reviewed here suggest that it may be possible to define the complexity of the niche and better understand the systems directing stem cell behaviour, with important potential implications for the treatment of disease states. Future challenges of this nascent field include definition of the osteoblastic cell necessary for HSC support, including its differentiation stage and adhesion molecules. It will be important to better understand the mechanisms by which PTH improves osteoblastic-HSC interaction and to assess whether osteoblastic Jagged1 expression and/or activation of a specific Notch receptor are necessary to achieve PTH-dependent HSC expansion. Twenty-five years after the niche hypothesis was proposed for HSCs, we are only beginning to shed light on the complex interactions between osteoblasts and primitive hematopoietic cells.

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