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Review Article

The hematopoietic stem cell niche: What's so special about bone?

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ABSTRACT

Hematopoietic stem cells (HSCs) require a supportive microenvironment to regulate their function and produce sufficient hematopoietic cells over the lifetime of an individual. With the exception of fish, all vertebrates, including mammals, maintain HSCs in a complex niche within the bone marrow. Several bone specific cellular populations have been implicated as components of the HSC niche and are part of a complex network that regulates HSC functions. However, the full extent of interactions within the HSC niche, and the role of individual cell populations remain to be fully elucidated. Further, it is not clear why fish are the exception, and what advantage is gained by housing HSCs in the bone marrow. To gain a better understanding of hematopoiesis and the mechanisms that drive hematopoietic disease processes a clearer picture of the complex HSC regulatory interactions in the bone marrow microenvironment is required.

1. Introduction to the niche

Hematopoietic stem cells (HSCs) reside at the apex of hematopoiesis and the first identification of an HSC-like hematopoietic progenitor cell derived from the murine bone marrow was provided by Till and McCulloch in 1961 [1]. It was subsequently demonstrated that murine hematopoietic progenitor cells thus identified could be maintained in tissue culture ex vivo only in the presence of an adherent population of bone marrow cells [2], and the concept of a stem cell niche was first posited by Schofield in 1978 [3]. The HSC niche was proposed as a possible explanation for the disparate regenerative capacity of HSCs with unlimited self-renewal derived from the bone marrow, and those with limited self-renewal that are derived from nodular colonies in the spleen following bone marrow transplantation [1,3]. The concept of the HSC niche has since been widely studied and multiple cellular and molecular components have been experimentally determined, giving rise to a more modern, yet still incomplete, understanding of a complex network within the bone marrow that regulates the fate of HSCs, and hematopoietic homeostasis. But why are HSCs maintained in the bone marrow? Fish maintain HSCs in a niche within the kidney that is considered to provide largely equivalent support to HSCs as the bone marrow in land dwelling vertebrates [4]. However, there are multiple bone specific cellular populations that are a part of the HSC niche network in the bone marrow.

2. Osteoblastic cells

Following Schofield's initial hypothesis, it would take more than 20 years for the conclusive identification of the first cellular components of the HSC niche, osteoblastic cells. Osteoblasts are responsible for forming bone, and, as such, reside primarily at the endosteal surface where immature hematopoietic cells were previously found to be preferentially located [5]. In 1996 Taichman et al. demonstrated via ex vivo experiments that osteoblastic cells provided the necessary support to maintain immature human hematopoietic cells in long-term cultures providing the first evidence of a specific population of cells that was a part of the HSC niche within the bone marrow [6]. Building on this observation 2 laboratories developed genetically altered mouse models to investigate the in vivo role of osteoblasts in regulation of hematopoiesis. Zhang et al. developed a murine model in which *BMPr1a* is conditionally deleted upon administration of PolyI:C. Deletion of *BMPr1a* resulted in an increased population of N-Cadherin expressing osteoblastic cells on the endosteal surface of bone. They further observed that phenotypic and functional HSCs were increased proportionally to, and were found in close proximity with, N-Cadherin expressing osteoblastic cells [7]. Calvi et al. simultaneously developed a murine model that expressed a constitutively active parathyroid hormone receptor (caPTH1R) specifically in osteoblastic cells by controlling expression with a 2.3 kb fragment of the α1 collagen promoter. This model demonstrated an increase in bone mass, osteoblastic cells, and a concurrent increase in phenotypic and functional HSCs [8]. These

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simultaneous publications provide the first clear evidence of an individual population of cells, osteoblastic cells, as a component of the HSC niche within the bone marrow. Additionally, pharmacologic treatment of mice with parathyroid hormone both activates osteoblastic cells leading to an anabolic bone response, and increases HSCs [9]. The converse experiment has also been performed, in which osteoblastic cells were specifically ablated in the bone marrow leading to loss of HSC function [10]. Though the evidence is clear that signals originating from osteoblastic cells can alter HSC fate, the role of osteoblasts as direct regulators of HSC function during homeostatic hematopoiesis is still in question. For example, the caPTH1R can be expressed in osteocytes, terminally differentiated osteoblastic cells that are entombed in the bone matrix. Activation of PTH signaling in osteocytes then results in osteoblastic expansion and activation leading to an increase in bone mass [11]. However, no increase in bone marrow HSCs was observed suggesting that increased osteoblastic cell number and activity is not sufficient to increase HSCs [12]. Additionally, N-Cadherin expressing osteoblasts have been proposed as a critical member of the HSC niche [7,13], however, conditional deletion of N-Cadherin from osteoblastic cells does not alter bone marrow support for HSCs [14].

Therefore, while activation of osteoblasts under certain conditions, including PTH receptor signaling, can increase numbers of HSCs, and loss of osteoblastic cells can decrease HSC function, homeostatic HSC maintenance is likely supported by additional cell populations in the bone marrow including the osteoblastic precursors, mesenchymal stem cells (MSCs).

3. Perivascular MSCs

Following the initial identification of the osteoblast as a key component of the HSC niche, the complexity inherent in the regulation of HSCs in the bone marrow has begun to be elucidated. Multiple cellular populations are now considered as a part of the HSC niche network including the mesenchymal stem cell (MSC). The MSC in the bone marrow is a multipotent cell that can give rise to at least 3 mature populations: adipocytes, chondrocytes, and osteoblasts [15]. Perivascular MSCs that reside in the bone marrow and are associated with sinusoidal vascular structures were initially described as CXCL12 abundant reticular (CAR) cells [16]. These CAR cells were shown to colocalize with hematopoietic progenitor cells in the bone marrow, and the receptor for the chemokine CXCL12, CXCR4 was demonstrated to be critical for the maintenance of a functional population of HSCs in the bone marrow of mice [16]. The identification of CAR cells as a likely regulator of HSC maintenance was followed by further support for perivascular MSCs as a central component of the HSC niche. Genetically altered mice allowed the identification of multiple cellular populations, all functionally capable of multilineage differentiation, and all supportive of HSCs in the marrow. This suggests that MSCs are not a homogeneous population of cells, but rather, that there are several cellular populations in the bone marrow that perform the functions of MSCs, and that one of those functions is the support of HSCs.

Of the various MSC populations identified one of the first that was demonstrated as necessary for HSC support was perivascular cells that express GFP under the control of the Nestin promoter [17]. In the bone marrow Nestin-GFP-positive cells were perivascular and closely associated with HSCs. In vitro, sorted Nestin-GFP-positive cells were capable of adipogenic, osteogenic, and chondrogenic differentiation potential indicating that they were a population that contained MSC activity. Ablation of Nestin-GFP-positive cells resulted in the loss HSCs and HSC function from the bone marrow as well as a concomitant increase in HSC numbers in the spleen. Thus Nestin-GFP-positive cells represent a population of MSCs that regulate HSC function and maintenance in the bone marrow [17].

A non-overlapping population of perivascular cells that has been identified as a component of the HSC niche is leptin receptor (Lepr) positive stromal cells [18]. This population of cells has been suggested as an MSC population based on gene expression analysis and when stem cell factor (SCF) was genetically lost in Lepr-positive cells HSC numbers in the bone marrow were decreased. However, conditional deletion of SCF from osteoblastic, hematopoietic or Nestin-GFP-positive cells did not affect HSC numbers highlighting that SCF expression is required for the maintenance of HSCs only on specific populations of cells [18]. This further highlights the heterogeneous nature of the MSC populations in the bone marrow as SCF is expressed by Nestin-GFP-positive cells [17], but its expression is not required for HSC maintenance.

Further evidence for both the heterogeneity of MSC populations, and their central role in the HSC niche was provided by the lab of Dan Link. Prx-1 is a marker of MSCs previously described as specific to the limb bud during development [19]. Using a Prx-1-cre expressing genetic construct CXCL12 was deleted from MSCs in the bone marrow resulting in a dramatic loss of HSCs [20]. Similar to what was observed with depletion of SCF [18], depletion of CXCL12 from osteoblastic cells did not result in loss of HSCs or HSC function in the bone marrow.

Taken together these studies demonstrate the importance of perivascular MSC populations to the regulation of HSCs in the bone marrow. Additionally, though osteoblastic cells were the first population identified as a component of the HSC niche, their role in homeostatic maintenance of HSCs is not clear as the expression of important niche factors such as CXCL12 and SCF is not required in osteoblastic cells for the support of HSCs.

4. Bone marrow macrophages

Macrophages that reside in the bone marrow highlight the complex interactions that take place within the HSC niche. It is well established that HSCs migrate out of the bone marrow to circulate in the peripheral blood in numbers that are regulated by circadian mechanisms [21]. Cytokines and chemokines, including G-CSF and CXCL12, are critical to the regulation of HSC release from the bone marrow [22,23]. Osteoblastic cells appear to play a role in the release of HSCs from the bone marrow into circulation, as osteoblast numbers, and expression of CXCL12, are lost during G-CSF mediated HSC mobilization [24,25]. However, osteoblastic cells do not express the G-CSF receptor [25,26], while bone marrow macrophages do [27]. Further, ablation of macrophages had a similar effect on osteoblast numbers and expression of CXCL12 as well as mobilization of HSCs from the bone marrow following G-CSF administration [28]. Additional studies have confirmed that macrophages in the bone marrow promote the expression of CXCL12 on MSCs as well as osteoblastic cells, leading to the retention of HSCs within the niche [29]. Therefore, macrophages play an important role in the regulation of HSCs, largely through interactions with other cells within the HSC niche, namely MSCs and osteoblasts.

5. Bone marrow endothelial cells

Endothelial cells that comprise the vasculature of the bone marrow are also a part of the complex HSC niche network. Early evidence demonstrated that endothelial cells support the self-renewal and expansion of HSCs in in vitro co-cultures through the expression of Notch ligands and subsequent activation of Notch signaling in HSCs [30]. In vivo analysis of endothelial cells leads to the same conclusion as the in vitro studies, that activation of Notch signaling in HSCs maintains self-renewal and expansion. However, the role of perivascular MSCs cannot be definitively excluded [30]. The depletion of SCF from endothelial cell populations through the use of endothelial specific expression of Cre recombinase with the Tie2 promoter also leads to a loss of HSCs similarly to perivascular Lepr positive cells [18]. Additionally the combined loss of SCF from both Lepr positive and endothelial cells leads to a much more dramatic loss of HSCs in the bone marrow than either population alone. This suggests that SCF produced by both endothelial cells and perivascular Lepr positive cells contributes to HSC maintenance.

As with MSC populations, endothelial cell populations exhibit considerable heterogeneity and differ in their support for HSCs. Endothelial cells that express E-selectin represent a subset of endothelial cells that localize close to the endosteal surface, and through E-selectin, promote the proliferation of HSCs [31]. The barrier function provided by arterial endothelial cells also promotes the maintenance of an HSC population in the bone marrow, while leaky sinusoidal vasculature promotes the migration of HSCs out of the bone marrow [32]. Loss of arteriolar integrity either through genetic or pharmacological means leads to loss of functional HSCs in the bone marrow, and loss of perivascular MSCs as defined by their expression of Sca-1 and PDGFR-α [32,33]. These reports highlight a complex network of cellular populations required to regulate HSC maintenance and function.

6. Sympathetic nervous system

Sympathetic nervous system (SNS) neurons are found throughout the bone marrow and regulate the function of osteoblastic cells, as well as osteocytes to regulate HSC mobilization out of the bone marrow in response to granulocyte-colony stimulating factor (G-CSF) administration as ablation of sympathetic enervation of bone marrow through genetic, or pharmacologic means results in loss of HSC mobilization in response to G-CSF [26,34]. HSCs are also mobilized out of the bone marrow in a circadian fashion with photic cues regulating the level of HSCs in the bloodstream [21]. This circadian regulation is also controlled by the SNS that initiates fluctuations in the expression of CXCL12 [21]. Neurons are not the only cells of the SNS that represent cellular components of the HSC niche in the bone marrow. The glial population of non-myelinating Schwann cells sheaths the axons of SNS neurons and maintain HSC quiescence through TGF-β signaling [35]. These studies highlight that the bone marrow SNS regulates HSCs directly through non-myelinating Schwann cells as well as indirectly through neuronal control of bone specific populations such as osteoblasts and osteocytes. These studies also help to further our growing understanding of the cellular network that regulates HSC function and its complexity.

7. Why the bone? the fish exception

Research into this area has revealed a complex network of cellular populations that includes many bone specific cell types. The question remains however, why the bone? The only vertebrates known to not have a hematopoietic bone marrow are fish, as the bone marrow niche for hematopoiesis and HSCs appears to have evolved around the same time as amphibians. Hematopoiesis in the frog *Rana temporaria* was the focus of a study performed in Russia at Moscow State University [36]. In this study hematopoiesis in the larval stage takes place in the spleen, liver, and pronephros. Following metamorphosis erythropoiesis and granulopoiesis has migrated to the bone marrow and erythropoiesis in the liver is lost. Thus it appears that during the aquatic life cycle of *Rana temporaria* hematopoiesis occurs primarily in the pronephros similar to fish such as the popular vertebrate animal model system *Danio rerio* or zebrafish [37], while in the semiaquatic/terrestrial phase of the life cycle of *Rana temporaria* hematopoiesis has largely shifted to the bone marrow [36].

Is there an advantage to hosting hematopoiesis in the bone marrow for terrestrial vertebrates as compared to fish? It is possible rather, that the evolution of the bone marrow HSC niche is coincidental with emergence from water onto land, as the development of specialized hematopoietic tissues and organs was an ongoing evolutionary process prior to the emergence from water [38]. For example the thymus is the site of T-cell maturation in mammals, and also in jawed fish [39]. However, it is absent from the earliest vertebrates in the class cyclostomata, that includes lampreys and hagfish [39]. The spleen, another important hematopoietic organ in mammals is also present in all fish except for those in the class cyclostomata [40,41]. Therefore, perhaps the evolution of terrestrial vertebrates and a bone marrow HSC niche are not dependent on one another, but separate events that happen to have occurred in the same phylogenic period.

However, it is also possible that the adaptation to life on land resulted in evolutionary pressure that and subsequent relocation of the HSC niche from the kidney to the bone marrow. One such pressure could be a greater need for skeletal remodeling in response to increased load bearing [42]. In mammals osteocytes are responsible for responding to stress and strain on bones in order to repair normally occurring microfractures [43]. In many fish species however, bone is acellular, lacking osteocytes to sense stress, meaning these bones are incapable of bone remodeling [44,45]. This suggests that fish species do not require bone remodeling to the same extent as mammals. Since osteoclasts that resorb bone are an essential component of bone remodeling and are derived from HSCs [42], it is conceivable that maintaining a ready source of osteoclasts in the bone marrow through relocation of the HSC niche is evolutionarily advantageous to land dwelling vertebrates.

Osteoclastic activity could be advantageous for a separate reason as well, accessing calcium stored in the skeleton. Fish do not have a parathyroid gland and do not respond to parathyroid hormone (PTH) [46]. And while bone is not the only target organ of PTH it is well accepted that the skeleton of fish is not a significant contributor to calcium homeostasis [47]. The reason for this may be that environmental calcium ions dissolved in water serve as a calcium store for fish but not for terrestrial vertebrates, necessitating the use of the skeleton as a calcium store in the latter [48]. Interestingly, the parathyroid gland evolved in amphibians around the same phylogenic period as the bone marrow HSC niche, and dominates calcium regulation in more evolved terrestrial amphibians [48]. Further, parathyroid hormone indirectly regulates HSCs through interactions with the bone marrow microenvironment in mammals [8,14]. This further suggests a possible driving force for the development of the bone marrow HSC niche that does not involve improved regulation of HSCs, but rather, improved regulation of the skeletal health, and calcium homeostasis.

If we are to consider pressures unique to dwelling on land that would result in a bone marrow HSC niche being superior to a kidney HSC niche radiation exposure is one possibility. The hematopoietic system is one of the most sensitive tissues to radiation exposure [49]. Water is a relatively dense material that provides radiation shielding against ionizing radiation, as evidenced by its use for that purpose in storing spent nuclear fuel in pools. Therefore, compared to land-dwelling vertebrates aquatic vertebrates would receive a lower dose of radiation following radiation exposure. Ionizing radiation that can penetrate beyond the skin is limited to gamma rays and X-rays. Shielding for these types of radiation provided by a given material is determined by that material's density, atomic number, and thickness, a higher value for each of those parameters providing greater shielding [50]. Bone has the highest density of body tissues and contains large amounts of calcium, an element that has a higher atomic number, 20, than the other major elements that make up most tissues, namely Carbon, Hydrogen, Oxygen and Nitrogen with respective atomic numbers of 6, 1, 8 and 7. Therefore, bone will absorb significantly more radiation than other body tissues, a property that is used routinely for medical X-ray imaging. This also provides some relative radiation protection for the bone marrow, which may have proven advantageous to early land dwelling vertebrates.

Ultimately, why the kidney was abandoned for the bone marrow as the primary niche for HSC maintenance is an open question. However, a complex cellular network, including many bone specific cell types, has evolved in the bone marrow to regulate HSC function and provide a steady source of hematopoietic cells throughout the lifetime of an individual (Fig. 1). The composition and interconnectedness of this complex microenvironment is still being revealed and continuing research into this area will undoubtedly improve our understanding of HSC regulation within their niche.

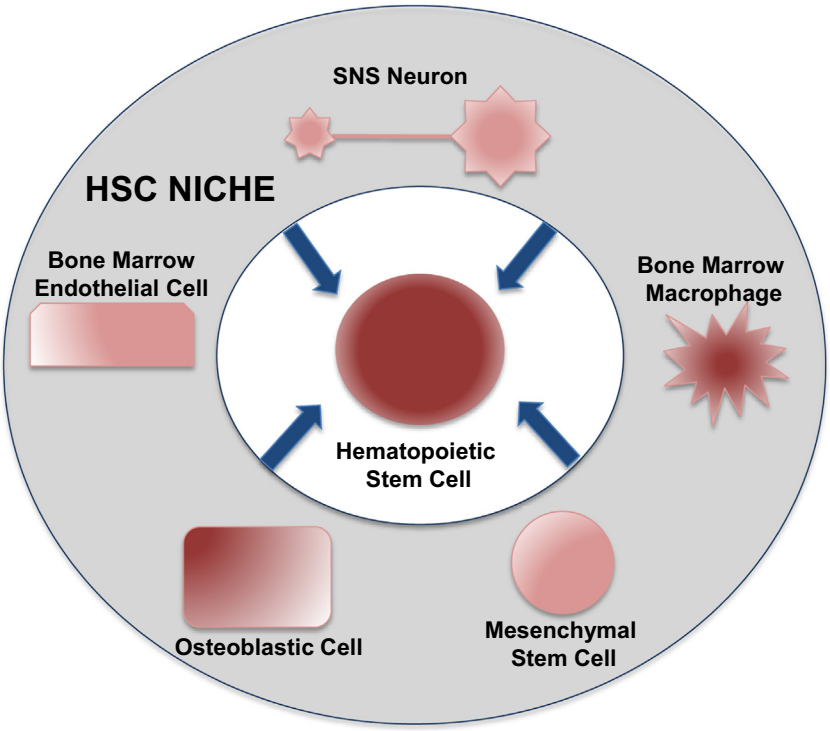

Fig. 1. The hematopoietic stem cell niche in the bone marrow is comprised of multiple cell types that act cooperatively in a complex, interactive network to regulate hematopoietic stem cell fate decisions.

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