Review

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Regulation of hematopoietic stem cells by bone marrow stromal cells

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Hematopoietic stem cells (HSCs) reside in specialized microenvironments (niches) in the bone marrow. The stem cell niche is thought to provide signals that support key HSC properties, including self-renewal capacity and long-term multilineage repopulation ability. The stromal cells that comprise the stem cell niche and the signals that they generate that support HSC function are the subjects of intense investigation. Here, we review the complex and diverse stromal cell populations that reside in the bone marrow and examine their contribution to HSC maintenance. We highlight recent data suggesting that perivascular chemokine CXC ligand (CXCL)12-expressing mesenchymal progenitors and endothelial cells are key cellular components of the stem cell niche in the bone marrow.

The HSC niche

Hematopoiesis is the process by which all mature blood cells are produced. It must balance enormous production needs (>500 billion blood cells are produced every day) with the need to regulate precisely the number of each blood cell type in the circulation. In vertebrates, the vast majority of hematopoiesis occurs in the bone marrow and is derived from a limited number of HSCs that are multipotent and capable of extensive self-renewal. In mammals, it is estimated that there are ~10 000 HSCs, of which, in humans, ~1000 contribute hematopoiesis at any given time [1]. Prospective identification of HSCs using cell surface markers and flow cytometry is best described for murine HSCs. c-Kit$^+$ Sca-1$^+$ lineage$^-$ (KSL) CD150$^+$ CD48$^-$ cells [2] and CD34$^-$ Flk2$^-$ KSL cells [3] represent the two most commonly used murine HSCs phenotypes; each containing ~50% HSCs based on long-term repopulating assays.

Key properties of HSCs are multipotency, self-renewal capacity, and quiescence. The bone marrow microenvironment appears to be uniquely adapted to support these and other HSC properties. As first proposed by Schofield in 1978 [4], the concept of a stem cell niche in the bone marrow has gained widespread popularity. In this model, specialized niche cells in the bone marrow are physically associated with HSCs and provide specific signals that help maintain their function. In this review, we highlight recent studies defining the bone marrow stromal cells that comprise the stem cell niche and the signals that they generate that contribute to HSC maintenance.

Anatomy of the bone marrow

The bone marrow is the major site of hematopoiesis and bone formation in most vertebrates. Thus, in addition to containing hematopoietic cells, the bone marrow contains cells that contribute to bone homeostasis, including mesenchymal stem cells (MSCs; also called skeletal stem cells), osteoprogenitors, osteoblasts, osteocytes, and chondrocytes (Figure 1). To add further complexity, other stromal cell populations that reside in the bone marrow may regulate hematopoiesis, including neuronal cells, glial cells, and adipocytes. Recent advances in imaging technologies have greatly advanced our understanding of the organization of the bone marrow. It is clear that the bone marrow in both long bones (e.g., femur) and flat bones (e.g., calvaria) is highly vascular [5,6]. In long bones, central longitudinal arteries give rise to radial arteries that in turn branch into arterioles near the endosteum (Figure 2) [5]. The transition from Sca-1$^+$ arterioles to Sca-1$^-$ venous endothelium occurs in close proximity to the endosteum. Venous sinusoids extend back towards the central cavity where they coalesce into a large central sinus. Of note, the venous sinusoids, which contain numerous fenestra, are thought to be the major sites of leukocyte egress from the bone marrow into the circulation [7]. Osteoblasts and bone lining cells form a layer between mineralized bone and the bone marrow. Spindle-shaped N-cadherin$^+$ osteoblasts (SNO cells), which may represent immature osteoblasts, also are localized near the endosteum [8]. There is a rich network of stromal cells interspersed between islands of hematopoietic cells and include MSCs, CXCL12-abundant reticular cells, and adipocytes.

The location of HSCs in the bone marrow is controversial. Initial studies using labeled HSC-enriched cell populations transplanted into recipients suggested a mostly endosteal location for HSCs [9–11]. This is consistent with data showing that long-term bromodeoxyuridine (BrdU)-retaining cells (presumed to be HSCs) are preferentially localized next to SNO cells at the endosteum [8]. By contrast, using a rigorous definition of HSCs (CD150$^+$ CD48$^-$ CD41$^-$ lineage$^-$ cells), Kiel *et al.* showed that the majority of HSCs are in contact with sinusoidal endothelium at bone-distant sites

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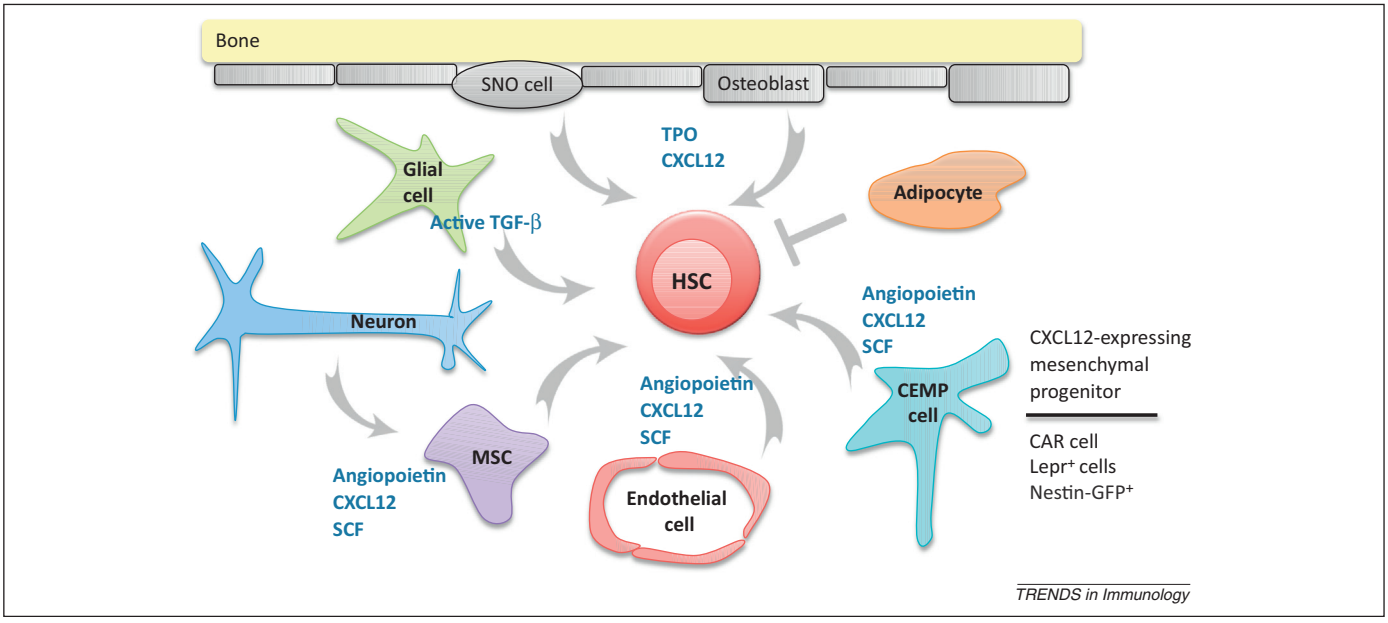

Figure 1. Distinct stromal cell populations in the bone marrow contribute to hematopoietic stem cell (HSC) maintenance. A complex and diverse group of stromal cells in the bone marrow have been implicated in HSC maintenance. Endothelial cells, mesenchymal stem cells (MSCs), and chemokine CXC ligand (CXCL)12-expressing mesenchymal progenitors (CEMP cells) are perivascular stromal cells that produce several factors that support HSCs, including CXCL12, angiopoietin, and stem cell factor (SCF). CEMP cells have been identified as CXCL12-abundant reticular (CAR) cells, leptin receptor$^+$ stromal (Lepr$^+$) cells, and Nestin-GFP$^+$ cells; these stromal cell populations likely overlap considerably. Osteoblasts and spindle-shaped N-cadherin$^+$ osteoblast (SNO cells) produce several factors that support HSCs, including thrombopoietin (TPO) and CXCL12. Sympathetic neurons indirectly regulate HSCs by targeting CXCL12 expression. Finally, glial cells, through production of active transforming growth factor (TGF)-β, and adipocytes regulate HSCs.

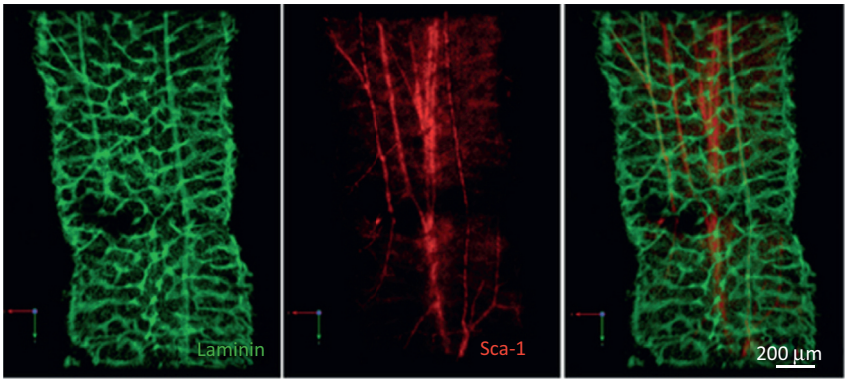

Figure 2. The bone marrow is highly vascularized. Confocal microscopic images of the femoral diaphysis stained with the panvasculature marker laminin (green) and the arterial specific marker, Sca-1 (red). A Sca-1$^+$ central artery runs through the central marrow. The central artery branches off to smaller arterioles towards the endosteum. Reproduced, with permission, from [5].

[2]. This is consistent with studies showing that most HSCs are in direct contact with perivascular CXCL12-abundant reticular (CAR) cells [12] and nestin-GFP$^+$ stromal cells [13]. Interestingly, a recent study suggested that both HSCs and lineage-committed hematopoietic progenitor cells (HPCs) were localized near endothelium with a preference for the endosteal region [5]. Collectively, these data suggest that the majority of HCSs are perivascular and enriched in the highly vascular endosteal region.

Hypoxia and the stem cell niche

The prevailing view is that HSCs are maintained in a hypoxic niche in the bone marrow. As noted, HSCs are localized to the endosteal region, which previous studies characterized as having low perfusion and relative hypoxia [14–16]. Indeed, HSCs with lower cellular levels of reactive oxygen species have higher self-renewal potential [17]. Moreover, disruption of hypoxia-inducible factor (HIF)-1α results in loss of HSC quiescence and repopulating activity [18], whereas stabilization of HIF-1α induces HSC quiescence and enhances repopulating activity [18,19]. However, as noted in the previous section, the endosteal region is actually highly vascularized and most HSCs are perivascular [5]; thus, HSCs are likely to be relatively well oxygenated. Interestingly, hematopoietic stem/progenitor cells (HSPCs) display a hypoxic profile (defined by strong retention of pimonidazole and expression of HIF-1α) regardless of their location in the bone marrow [5]. Indeed, even HSPCs in the peripheral circulation display a hypoxic profile. Thus, intrinsic differences in