Megakaryocytes in the hematopoietic stem cell niche

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Mesenchymal stromal cells are key components of hematopoietic stem cell (HSC) niches in the bone marrow. Two studies now show that hematopoietic-derived megakaryocytes also contribute to the HSC niche, regulating HSC quiescence and function.

HSCs are multipotent progenitors with extensive self-renewal capacity that reconstitute all hematopoietic lineages. As with other stem cell populations, HSC proliferation is tightly regulated, with the majority of HSCs being maintained in a nonproliferating quiescent state. This characteristic is closely linked to long-term repopulating potential, and unrestrained HSC proliferation can lead to bone marrow failure through stem cell exhaustion.

HSC quiescence is maintained by signals generated by stromal cells in localized microenvironments in the bone marrow termed stem cell niches. Murine bone marrow is highly vascularized, with large central arteries branching into progressively smaller arterioles that eventually transition into venous sinusoids near the bone (endosteal) surface (**Fig. 1**). Current data suggest that most HSCs are perivascular and localize preferentially to endosteal regions in the bone marrow[1,2]. This region also contains a complex network of stromal cells that have been implicated in HSC maintenance, including bone-lining osteoblasts, endothelial cells (both arteriolar and venous), pericytes, chemokine (C-X-C motif) ligand 12 (CXCL12)-abundant reticular cells, sympathetic nerves and Schwann cells. A recent study showed that quiescent HSCs localize preferentially to small arterioles near the endosteum, suggesting that distinct niches may exist for quiescent and proliferating HSCs[1]. Factors produced by stromal cells that have been implicated in HSC quiescence include angiopoietin[3], CXCL12 (refs. 4,5), kit ligand[6] and transforming growth factor-β (TGF-β)[7].

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Previous studies have suggested that megakaryocytes, the hematopoietic cells in the bone marrow that are responsible for platelet production, may regulate HSCs indirectly. Megakaryocytes are intimately associated with bone marrow sinusoidal endothelium, extending cytoplasmic protrusions into the sinusoids to produce platelets. After transplantation into irradiated mice, HSCs lodge preferentially near megakaryocytes[8], and inhibition of megakaryocytes impairs HSC engraftment[9]. Megakaryocytes are required for osteoblast expansion after irradiation, suggesting that megakaryocytes regulate HSC engraftment indirectly by expanding osteoblastic niches[9]. In this issue of *Nature Medicine*, Bruns *et al.*[10] and Zhao *et al.*[11] independently show that megakaryocytes regulate HSC proliferation directly.

Both groups found that ~20% of cells expressing HSC surface markers are located directly adjacent to megakaryocytes in mice. Bruns *et al.*[10] also used a computational approach to supplement their standard HSC immunohistochemistry by running simulations of HSC placement on bone marrow images. This analysis confirmed the nonrandom localization of HSCs near megakaryocytes. Both groups also used a mouse model in which cells expressing *Cxcl4* (also called *Pf4*), a chemokine that is highly expressed in megakaryocytes, are selectively ablated. Loss of megakaryocytes resulted in increased HSC cycling and an increase in HSC numbers. Although the

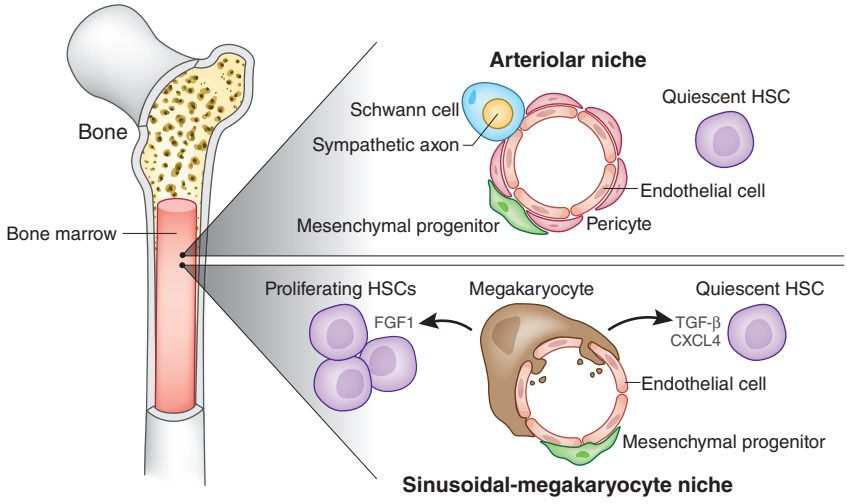

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Figure 1 The majority of HSCs are perivascular, localizing to either arteriolar or sinusoidal niches, which consist of a number of different nonhematopoietic stromal cell types. Bruns *et al.*[10] and Zhao *et al.*[11] have now shown that hematopoietic megakaryocytes are also an important component of the HSC niche. Megakaryocyte-derived CXCL4 and TGF-β contribute to the maintenance of HSC quiescence, whereas production of FGF1 by megakaryocytes in response to myeloablative stress enhances HSC recovery by stimulating HSC proliferation. The megakaryocyte-HSC interaction is independent of arterioles, suggesting that megakaryocytes form part of the sinusoidal niche.

magnitude of the increase in HSCs varied between the studies because of differences in the phenotypic markers used, both groups showed that the numbers of HSCs with long-term repopulating activity were increased after megakaryocyte ablation. These data provide strong evidence that under basal conditions, megakaryocytes restrain HSC proliferation. However, whether loss of HSC quiescence after megakaryocyte ablation is associated with a loss of HSC self-renewal capacity was not specifically addressed by either group.

Although both groups reported increased HSC proliferation and numbers after megakaryocyte ablation, they each proposed a distinct molecular mechanism by which megakaryocytes regulate HSC quiescence. Bruns *et al.*[10] focused on CXCL4, as this chemokine is highly expressed in megakaryocytes and has been implicated previously in hematopoietic cell proliferation. They found that CXCL4 treatment reduced the proliferation of HSCs *in vitro*, and systemic injection of CXCL4 in mice resulted in a modest reduction in HSC numbers and decreased engraftment after transplant. Conversely, HSC number, proliferation and repopulating activity were increased in *Cxcl4*-deficient mice. These data suggest that CXCL4 negatively regulates HSC proliferation. Interestingly, Bruns *et al.*[10] show that the localization of HSCs to megakaryocytes, but not arterioles, is reduced in *Cxcl4*-deficient mice, suggesting that the arteriolar niche is spatially distinct from the megakaryocyte-sinusoid niche.

A prior study suggested that TGF-β is produced by nonmyelinating Schwann cells and contributes to HSC quiescence[7]. Using RNA sequencing (RNA-seq) to screen for candidate molecules, Zhao *et al.*[11] show that megakaryocytes are also an important source of TGF-β in the bone marrow. Indeed, HSCs that are located near megakaryocytes displayed signs of activation by TGF-β (reflected in phosphorylation of SMAD2/3), and conditional deletion of *Tgfb1* in megakaryocytes resulted in increased HSC proliferation. They also show that injection of TGF-β restored HSC quiescence in megakaryocyte-depleted mice.

Zhao *et al.*[11] also found that fibroblast growth factor 1 (FGF1) production by megakaryocytes induces HSC proliferation and enhances HSC recovery after myeloablative stress. In addition, they found that FGF1 is highly expressed in megakaryocytes, and megakaryocyte numbers and FGF1 production increased during hematopoietic stress induced by chemotherapy treatment. HSCs in megakaryocyte-depleted mice showed defective expansion after chemotherapy treatment that was rescued by FGF1 injection. Thus, megakaryocytes produce factors that both negatively (CXCL4 and TGF-β) and positively (FGF1) regulate HSC proliferation.

These studies provide important new insights into components of the HSC niche. First, they suggest a feedback loop in which a terminally differentiated progeny (megakaryocytes) regulates HSC activity directly. Second, they suggest that in addition to the arteriolar niche[1], quiescent HSCs also localize to a sinusoidal-megakaryocyte niche (**Fig. 1**). Whether HCSs in these two niches have distinct properties or functions is an important unanswered question. Of note, as a substantial fraction of HSCs are not directly adjacent to megakaryocytes, it is possible that a distinct nonmegakaryocyte sinusoidal niche also exists. In addition, the studies identified several new factors that regulate HSC quiescence and proliferation, namely CXCL4 and FGF1, and they show that megakaryocytes contribute to the pool of TGF-β1, which is known to regulate HSC quiescence[7]. Although both CXCL4 and TGF-β1 promote HSC quiescence, it is unknown whether these signaling pathways synergize or are redundant and whether they regulate distinct or overlapping HSC pools. Nonetheless, these proteins, along with previously identified modulators of HSC niches such as CXCL12, TGF-β and angiopoietin, represent targets to pharmacologically modulate HSC proliferation. This finding might be particularly useful in enhancing HSC recovery after myeloablative therapy or in the setting of stem cell transplantation. CXCL4 appears to act selectively on HSCs in the megakaryocyte niche, as Bruns *et al.*[10] found that HSC localization to the arteriolar niche was unaffected in *Cxcl4*$^{-/-}$ mice. Whether other stem cell factors such as angiopoietin and TGF-β selectively regulate HSC proliferation in the other niches is unknown.

These studies also raise a number of questions about hematological disorders in which megakaryocyte numbers are altered. Pernicious anemia, immune thrombocytopenia, reactive thrombocytosis, essential thrombocythemia and primary myelofibrosis are all associated with increased megakaryocyte mass. Does this increased megakaryocyte mass alter HSC dynamics? Primary myelofibrosis and, to a lesser extent, essential thrombocythemia carry a risk of progression to acute myeloid leukemia. Could megakaryocyte-derived factors regulating HSC cycling contribute to malignant transformation in these disorders? Myelodysplastic syndrome is associated with increased HSC cycling and aberrant megakaryopoiesis; the current studies suggest a possible causal relationship between these processes. In addition, cancer chemotherapy is often associated with a decrease in megakaryocytes. The study by Zhao *et al.*[11] suggests that loss of megakaryocytes after chemotherapy may limit HSC proliferation, as megakaryocyte-ablated mice showed decreased HSC regeneration after chemotherapy. Thus, strategies to increase megakaryocyte recovery after myeloablative therapy may also augment HSC recovery. Further research dissecting the mechanisms by which megakaryocytes regulate HSCs may provide opportunities for translation in hematopoietic disorders with altered levels of megakaryocytes.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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