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COMMENTARIES

Neural Control of Hematopoietic Stem Cell Mobilization via Osteoblasts

Shu Takeda[1] and Millan S. Patel[2]

[1]Tokyo Medical and Dental University, Tokyo, Japan
[2]University of British Columbia, Vancouver, British Columbia, Canada

Commentary on: Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell*. 2006 Jan 27;124(2):407-421.

Until recently, a large quantity of bone marrow was needed to harvest hematopoietic stem cells (HSCs) for bone marrow transplantation therapy. With the use of granulocyte-colony-stimulating factor (G-CSF), a potent factor to mobilize HSCs, these cells are now commonly obtained from peripheral blood. HSCs reside in the bone marrow in two microenvironments, or niches, one associated with endothelial cells and the other associated with osteoblasts lining the endosteum. Recently, multiple lines of evidence have demonstrated a direct role for osteoblasts as critical regulators of the HSC niche (1;2). However, the role of osteoblasts and the molecular mechanism underlying the effect of G-CSF on the mobilization of HSCs remains to be elucidated. In a recent issue of *Cell* (3), Katayama *et al*. addressed the mechanism of G-CSF-mediated HSC mobilization and beautifully demonstrated that the mobilization of HSCs from bone marrow is regulated by the sympathetic nervous system (SNS) via osteoblasts.

Perhaps because they were elucidating a novel pathway, the authors came to their conclusion by a rather circuitous route. Starting six years previously with the arcane observation that a sulfated polymer from seaweed could rapidly mobilize HSCs (4), the Frenette group looked at mice deficient in the synthesis of an analogous molecule, sulfatide (UDP-galactose ceramide galactosyltransferase (Cgt)-deficient mice). As expected, Cgt-deficient mice exhibited a reduction in HSC mobilization by G-CSF; however, this defect was not rescued by the seaweed polymer. This suggests that Cgt is an obligate member of this pathway and that Cgt or its products play an important role in HSC mobilization. Further analysis of the response of Cgt-deficient mice to G-CSF demonstrated dysregulation of the osteoblast cytokine CXCL12 (also known as stromal cell-derived factor-1), a key protein that mediates homing of HSCs to the endosteal niche. To determine the mechanism linking G-CSF to CXCL12, the authors focused on two additional phenotypes of Cgt-deficient mice, abnormal nerve conduction and an abnormally flattened osteoblast appearance. These observations led the authors to consider the role of neural control of osteoblast function in their mice.

Several recent studies have demonstrated that the SNS is an important regulator of osteoblastic activity. First, mutant mice lacking either dopamine-β-hydroxylase (DBH), the enzyme responsible for norepinephrine synthesis, or the adrenergic-β2 receptor, have high bone mass phenotypes (5;6). Consistent with this, pharmacological blockade of the SNS by adrenergic β-blockers also increases bone mass. Therefore, the authors determined whether SNS regulation of osteoblast function could play a role in G-CSF-mediated HSC mobilization. Indeed, G-CSF treatment of wild-type mice led to flattened osteoblasts with shorter projections into the

underlying bone. To demonstrate the key role of the SNS in this pathway, the authors used three mouse models of diminished sympathetic activity: mice chemically sympathectomized by 6-OHDA, mice deficient in DBH and mice treated with β-blockers. Each model was resistant to G-CSF's effect on HSC mobilization and was resistant to the regulation of bone CXCL12 by G-CSF. Importantly, the chemically and genetically sympathectomized mice showed no osteoblast flattening *in vivo* with G-CSF treatment. That this is an SNS-mediated effect is supported by our own observations that chronic intracerebroventricular (ICV) infusion of leptin, a well established activator of the SNS, results in a similar flattening of osteoblasts *in vivo*. Moreover, DBH-deficient mice treated with a β2-selective agonist showed partial rescue of the HSC mobilization phenotype. These results clearly demonstrate that G-CSF requires the SNS for maximal stimulation of HSC mobilization from bone marrow and raise the notion of a neural regulation of stem cells.

The authors went on to refine the G-CSF-SNS-osteoblast pathway significantly, demonstrating that only peripherally, but not centrally (ICV), administered G-CSF was able to mobilize HSCs. Furthermore, G-CSF was able to stimulate norepinephrine depletion in the bone, but not in the heart, suggesting tissue-specific specialization of the SNS response to G-CSF. G-CSF stimulation of bone norepinephrine depletion was rapid, occurring within 3 hours. Finally, Cgt-deficient mice were tied into this pathway since their tissues had a longer half-life of norepinephrine, which was thought to indicate decreased sympathetic signaling. This last point is somewhat weak since the mobilization phenotype of Cgt-deficient mice was not rescued by a β2-selective agonist. Furthermore, based on the inability of β2-selective agonist monotherapy to mobilize HSCs in wild-type mice, the authors concluded that the SNS was important, but not sufficient, for G-CSF-mediated mobilization.

Mobilization of HSCs takes 3 to 4 days after G-CSF administration, whereas sympathetic activity is enhanced within 3 hours by G-CSF. This discrepancy suggests the participation of other molecules or may simply reflect the time required to modulate osteoblast cell biology. Neither Cgt nor the G-CSF receptor is expressed in osteoblasts, supporting the notion that this pathway acts indirectly, using the SNS and possibly other mediators. This question could be directly addressed by analysing mice deficient for G-CSF receptors only in sympathetic neurons. In addition, the sizeable number of well-characterized mouse models of osteoblast dysfunction provides a rich resource for evaluating the ability of other signaling pathways to regulate G-CSF-mediated HSC mobilization at the level of the osteoblast.

The work by Katayama *et al*. illustrates the power of one specific type of translational research, where human regulatory pathways are molecularly dissected in the mouse. CXCL12 receptor antagonists are already being tested in clinical trials and appear to act synergistically with G-CSF to mobilize HSCs (7). It remains to be seen whether modulation of β-adrenergic signaling can further enhance HSC mobilization in the clinical setting. This is an area of major need for patients undergoing potentially curative bone marrow transplantation.

Conflict of Interest: The authors report that no conflicts of interest exist.

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