Regulating traffic in the hematopoietic stem cell niche

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Cells in multicellular organisms do not live in a vacuum. Coordinated responses of individual cells to challenges at the level of the organism are instructed by integrated prompts from their environment. This environmental signaling determines the cells' properties and behavior to a considerable degree. Tissue stem cells, in particular, are dependent on environmental signaling to rapidly process indicators of stress to exert their regenerative functions while restricting their proliferative potential during homeostasis. Studies in both invertebrate and vertebrate systems indicate that this is achieved in a specialized, anatomically defined, regulatory environment referred to as the 'stem cell niche'. The capacity of the niche to shape a cell's behavior has been powerfully illustrated in Drosophila, in which the niche can impose stem-cell like properties upon other cell types.[1]

In the hematopoietic system, the endosteal surface has been recognized as such an anatomically defined regulatory environment for tissue stem cells. Although sometimes referred to as the 'osteoblastic niche', the endosteal surface comprises a broader range of components. These include osteolineage cells at various developmental stages, vascular endothelium, osteoclasts, neurogenic cells, 'stromal' bone lining cells, CXCL12-expressing reticular cells and extracellular matrix proteins. Some of these components, including osteolineage cells, have been established in genetic models to have a regulatory role in determining hematopoietic stem cell (HSC) behavior, but it is likely that the orchestrated (direct and indirect) action of most cellular components as a 'functional unit' is required for all aspects of stem cell control under conditions of both homeostasis and stress.[2]

HSC are not statically positioned within their niches but are known to migrate and are found at distinct locations throughout the bone marrow, blood and peripheral tissues. While the physiological significance of their migratory behavior has remained relatively obscure in post-natal hematopoiesis, this capacity is exploited in stem cell mobilization to harvest these cells for regenerative purposes. Granulocyte colony-stimulating factor (G-CSF)-mediated mobilization has thus been used as a powerful means to obtain mechanistic insights into the process of HSC trafficking. Trafficking occurs through an intricate process of de-adhesion, chemo-attraction and lodging into a new location.[3] In diverse stem cells systems, including the hematopoietic system, the CXCL12-CXCR4 axis has emerged as a major determinant of these processes. Deficiency of the ligand or receptor results in embryonic death and impaired colonization of bone marrow by hematopoietic progenitors, including HSC, during ontogeny while conditional ablation of CXCR4 in hematopoietic cells results in loss of HSC from the marrow environment. CXCL12 gradients are established through the coordinated interaction of several degrading enzymes that can cleave the cytokine, its receptor or both.

In this issue of Haematologica, Staudt *et al.* provide additional insights into this process.[4] Through a series of elaborate and detailed biochemical analyses, the cysteine protease cathepsin X was indicated to be a novel molecular player in HSC migration and trafficking. The authors demonstrated that cathepsin X is expressed by and secreted from human stromal cell lines and primary 'osteoblastic' cells (defined as bone-derived colony-forming units-fibroblast (CFU-F) differentiated towards the osteoblastic lineage). The protease was detected on the cell surface of primary osteoblasts, predominantly in the immature form, but also in the mature form. The secreted protein retrieved from supernatants was proteolytically active, as demonstrated by the cleavage of fluorescent substrates. Exogenous mature cathepsin X and shRNA-mediated knock-down of the gene disrupted pre-existing adhesion of $CD34^+$ cord blood cells to primary osteoblastic cells *in vitro* which suggests a role of cathepsin X in proteolytic attenuation of adhesive interactions between HSC and the osteoblasts, although the proteolytic substrate remains to be identified. Furthermore, cathepsin X was capable of degrading both isoforms of CXCL12 (SDF1α and β) which impaired the chemotactic properties of SDF1α on $CD34^+$ cells.

While elucidation of the relevance of cathepsin X in HSC mobilization and trafficking will have to await (targeted) knockdown of the protein in genetic *in vivo* models, a working hypothesis of its functional role can be formulated on the basis of the authors' findings (Figure 1). In this model, cathepsin X promotes HSC trafficking from the endosteal niche through the degradation of CXCL12 and proteolytic interference with, yet to be specifically defined, HSC-osteoblastic interactions. It has previously been shown that other enzymes, including other cathepsins (K and G)[5] and matrix-metalloproteinase-9 (MMP9),[6] have similar capacity to cleave CXCL12 and receptor-ligand interactions. Cathepsin X thus represents the latest addition to a rapidly expanding dynamic network of chemoattractant-degrading proteases involved in HSC localization and trafficking towards, away and perhaps along the endosteal surface.

The significance of elucidating molecular determinants in this network is of obvious relevance to stem cell transplantation biology. The niche is increasingly recognized as a potential target to facilitate donor cell mobilization and engraftment after transplantation. Pharmacological manipulation of the niche size has been proven successful in pre-clinical models of stem cell mobilization,[7] and promoting migration of HSC from their niches through pharmacological stimulation of degrading proteases may be beneficial. In retrospect, the clinical success of G-CSF as a mobilizing agent relies, partly, on creating a proteolytic microenvironment facilitating de-adhesion and migration of HSC.[8] While exploiting our expanding knowledge in optimizing cell replacement strategies is of obvious importance, key questions remain.

Why do hematopoietic stem cells migrate during post-natal hematopoiesis?

The answer to this question remains largely speculative to date, but recent findings have begun to create a conceptional

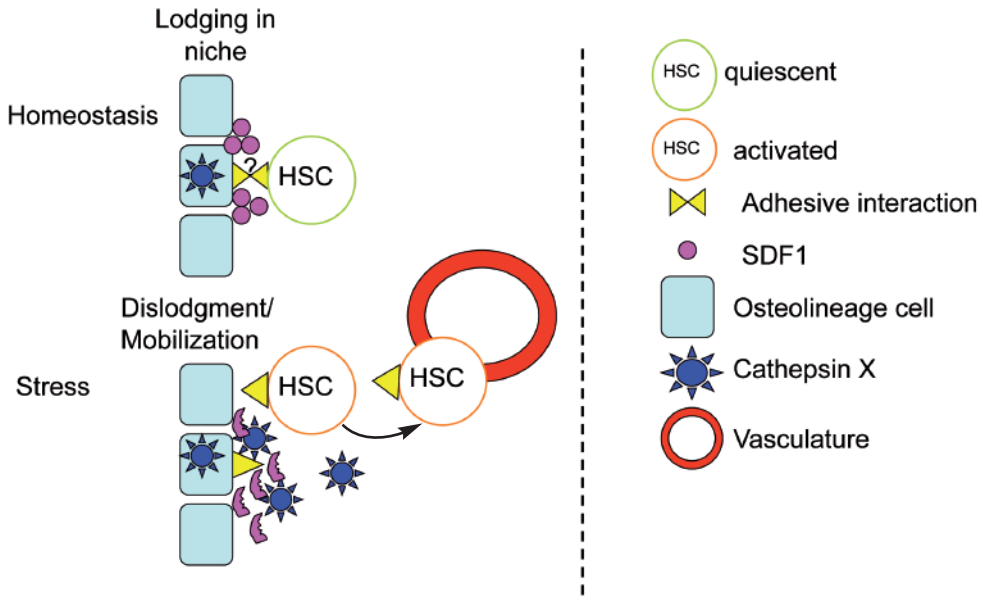

Figure 1. A speculative working model of the role of cathepsin X in hematopoietic stem/progenitor cell trafficking in the endosteal niche based on *in vitro* observations by Staudt *et al.* Cathepsin X is active in a network of degrading proteases promoting trafficking of HSC from the endosteal niche through cleavage of CXCL12 and ligand-receptor interactions at the endosteal surface. The nature of the adhesive interaction remains to be identified. Whether direct cell-cell contact is required for niche-induced signaling, as well established in invertebrates, remains controversial in the mammalian hematopoietic system.

framework in which HSC migration may have its place in stress response and immunosurveillance. Emerging evidence suggests that HSC trafficking between distinct niches in the bone marrow may be required for switching between homeostatic hematopoiesis and conditions of increased demand during hematopoietic stress.[9] HSC can reversibly acquire several states, including a dormant state (in which the cells are maintained long-term in the G0 stage of the cell cycle), a homeostatic state (in which the cells are occasionally cycling) and an injury-activated state (in which the cells are continuously cycling). HSC switch from dormancy to self-renewal under conditions of hematopoietic stress, such as bleeding and G-CSF mobilization. Similarly, activation of HSC cycling during chronic infection has recently been demonstrated.[10]

Given that several heterologous cell types can contribute to the HSC microenvironment and that functional heterogeneity may be present within each of them, it seems reasonable to propose that these distinct HSC states are associated with specific extrinsic signals that emanate from diverse and unique microenvironmental niches,[11] although this awaits experimental confirmation. HSC might migrate upon (neuro-adrenergic) stress signaling from a niche at the endosteal surface guarding its quiescence and longevity towards niches receptive or supportive of increased proliferation (Figure 1). The observation that mobilization strategies promote both HSC cycling and exit to the bloodstream supports the mechanistic link between cell cycle progression and interaction with the niche.

In addition to migration between bone marrow niches, it has long been recognized that HSC periodically enter the peripheral blood, where they are constitutively present.[12] Recent studies have further defined the circuit of hematopoietic stem/progenitor cell recirculation through peripheral tissues where they may provide a recruitable source of immune and inflammatory effector cells.[13] The observations point towards hematopoietic stem/progenitor cells having an immunosurveillance role, a view that may be supported by the observation that exit of HSC into the blood is governed by a circadian rhythm under adrenergic control and involving down-regulation of cxcl12.[14] Finally, it has been proposed that circulating HSC are required to occupy newly generated niches during (circadian) bone remodeling, both precluding the occupation of niches by non-stem cell types and providing a 'fitness' test for the recirculating stem cells.[15] Although a definitive answer to the question of why HSC migrate will have to await information from *in vivo* models in which this trafficking is blocked, the emerging data support a view in which HSC trafficking endows the organism with a adaptable system allowing it to meet the demands of supporting durable hematopoiesis and to be nimble enough to meet increased demand under conditions of hematopoietic stress. Deregulation of this process would be anticipated to result in mitigated responses to various hematopoietic challenges which may be reflected in the delayed hematopoietic recovery of MMP9$^{-/-}$ mice following 5-fluoruracil treatment.[6] Evolution, however, has likely provided the system with ample functional redundancy, as further documented by the work of Staudt *et al.* in this issue.

Can defective hematopoietic stem cell trafficking and localization play a role in hematopoietic disease?

As indicated, stem cell trafficking is a continuous, reversible process which involves de-adhesion and chemoattraction (where cathepsin X likely exerts its actions), but also re-attachment to the niche. Trafficking implies the eventual return of HSC to their (dormant) niches and there is evidence from murine models that failure of this to occur may result in hematopoietic abnormalities during homeostasis. Mice with a deficiency of functional receptors for key ligands determining lodgment at the endosteal surface (such as cxcl12,[16] calcium,[17] and stem cell factor[18]) display aberrant HSC localization away from the endosteal surface, which is associated with increased cycling and progressive loss due to replicative exhaustion and results in hypocellularity of the bone marrow. It has

been hypothesized that interference with niche-induced quiescence, either by altered niche signaling or detachment from the niche, may result in increased cycling of cells that have the inherent machinery of self-renewal, thus poising these cells ready for the acquisition of somatic mutations and malignant transformation. Recently experimental evidence introduced the concept of niche-induced oncogenesis in the hematopoietic system, showing that specific genetic abnormalities in osteoprogenitor cells can induce myelodysplasia and acute myeloid leukemia in mice.[19] Whether leukemic transformation in this system was associated with impaired trafficking or aberrant localization of HSC in their niche remains to be determined. It is noteworthy, however, that aberrant localization of hematopoietic stem/progenitor cells in human disease is not unprecedented, as atypical localization of immature progenitors is a common finding in myelodysplastic syndromes.[20] Indeed, recent findings in mouse models warrant reconsideration of a possible involvement of the dynamic interaction between HSC and their niche in the pathogenesis of certain hematopoietic diseases, including bone marrow failure syndromes and myelodysplastic syndromes. Studies such as the one published by Staudt *et al.* in this issue of the Journal, identifying determinants of this interaction, will greatly facilitate such investigations.

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Biological diversity and risk-adapted treatment of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the western world. Unlike other forms of leukemia, the proliferative component of CLL is relatively small, but recent evidence has pointed to the existence of a proliferative pool, which can be surprisingly sizeable.[1] There are several additional features of CLL that set it apart from other cancers. The most prominent pathogenic mechanisms include: (i) genomic aberrations targeting critical genes (e.g. miRNA, *TP53*, *ATM*); (ii) antigen drive and stereotyped B-cell receptors (BCR); and (iii) microenvironmental stimulation.[2,3] While the precise sequence of events is currently unclear, our growing understanding of CLL biology is enabling translation into clinical practice.

The papers by Marincevic *et al.*[4] and Giné *et al.*[5] in this issue of *Haematologica* touch on two important issues in