

Mechanism of Stem Cell Migration and Immune Development

Regulation of the bone marrow reservoir of immature and maturing leukocytes as part of host defense and repair during homeostasis and alarm situations.

The Role of Stromal Cells, Cytokines, Chemokines, Adhesion Molecules and Proteolytic Enzymes in Normal and Leukemic Human Hematopoiesis:

Hematopoietic stem cells are a rare population within the bone marrow which actively maintain continuous production of all mature blood cell-lineages throughout life, including major components of the immune system such as T and B lymphocytes, myeloid neutrophils and monocytes, while maintaining a small pool of undifferentiated stem and progenitor cells.

During development, or in experimental and clinical transplantations, stem cells migrate through the blood circulation and home to the bone marrow, repopulating it with immature and maturing blood cells, which in turn are released into the circulation. We have previously developed a preclinical in vivo model that identifies normal and leukemic human CD34/low stem cells based on their functional ability to initiate multi-lineage (both lymphoid and myeloid) hematopoiesis in transplanted immune deficient NOD/SCID and NOD/SCID/B2mnull mice.

The process of hematopoietic stem cell homing, bone marrow repopulation and release back to the circulation which are crucial for stem cell function and for development of the immune system, is not well understood. Our major goal is to determine the molecular mechanisms

which govern stem cell migration, proliferation and differentiation and to identify the interplay between bone remodeling via osteoclast/osteoblast interactions and regulation of the stem cell niche and hematopoiesis. The unique roles of stromal cells, hematopoietic cells, chemokines, cytokines, adhesion molecules and proteolytic enzymes which regulate these processes and their relationship are currently investigated.

Our research is focused on interactions between the bone marrow reservoir of leukocytes and peripheral organs during steady state homeostasis as well as during alarm situations in which immature and maturing leukocytes are recruited from the bone marrow to the circulation as part of host defense and repair mechanisms. In particular, the chemokine stromal derived factor one (SDF-1), which is produced by many cell types and also by bone marrow stromal and endothelial cells, is the only powerful chemoattractant for immature human and murine stem cells which express its receptor CXCR4, is extensively studied. This chemokine is highly preserved throughout evolution since human and mouse SDF-1 are cross-reactive and differ in one amino acid only. SDF-1 also serves as a survival factor for stem and progenitor cells, and both the ligand and its receptor are regulated by HIF-1 which is activated by hypoxia. Our results reveal a partial overlap between regulation of stem cell homing, and release with stem cell proliferation and differentiation. In addition both similarities and differences exist between regulation of normal and leukemic human stem cell migration and development.

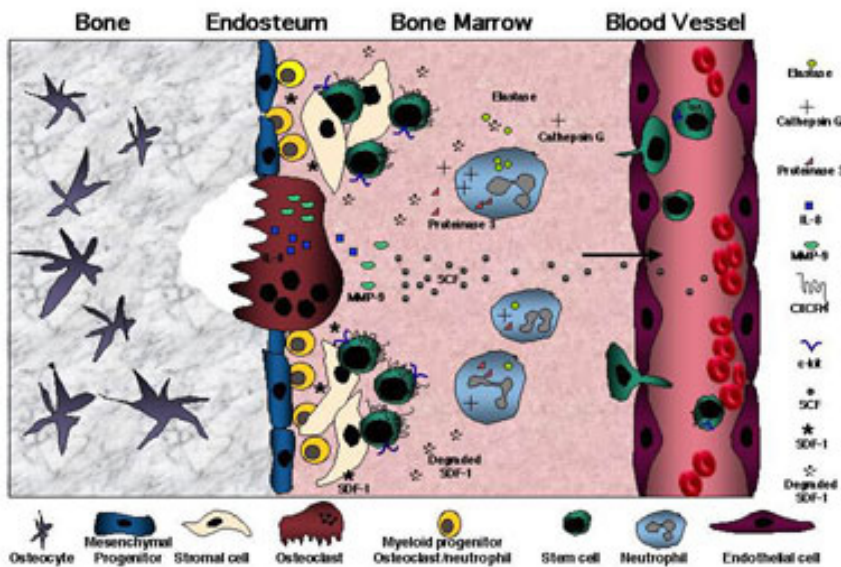


Fig. 1 A model for stress-induced stem cell mobilization (Hematology 2003;419). DNA-damaging chemotherapy and inflammatory cytokines such as G-CSF induce a transient increase in SDF-1 levels within the BM as part of the alarm situation. G-CSF triggers neutrophils to proliferate and release proteases (elastase, cathepsin G, and proteinase 3). Concurrently, proliferation and activation of osteoclasts, which release the mobilizing chemokine IL-8 and secrete MMP-9 in response to SDF-1 stimulation, take place. The massive inflammatory proteolytic enzyme activity leads to degradation of stem cell anchorage and retention signals (VCAM-1 and SDF-1), inactivation of G-CSF, and remodeling of the BM extracellular matrix. MMP-9 mediates shedding of membrane-bound SCF, which together with proteinase 3 induces progenitor cell proliferation and CXCR4 upregulation, followed by partial inactivation of CXCR4 and c-kit by the proteolytic machinery. These sequential events, which are repeated and intensified after each cycle of G-CSF stimulation, orchestrate the egress of progenitors from the BM into the circulation.

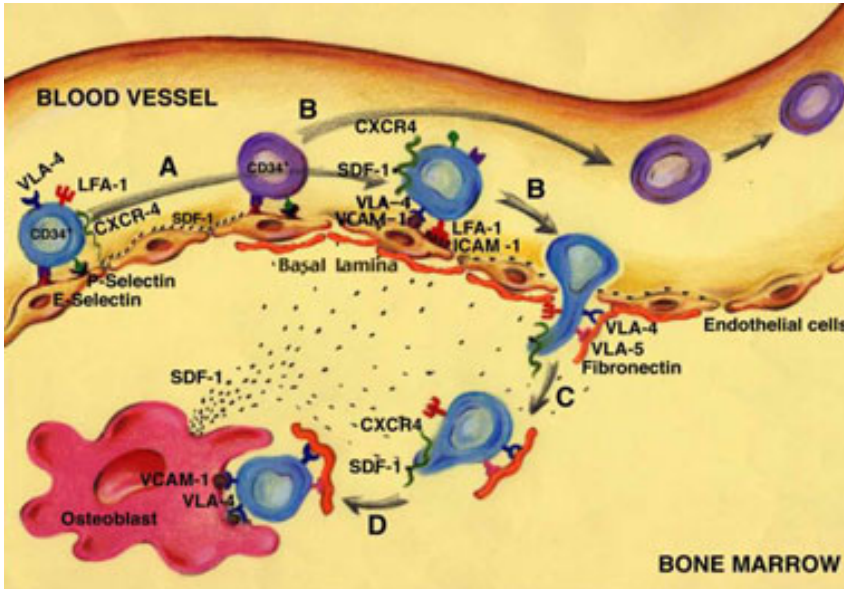


Fig. 2 SDF-1/CXCR4 interactions and other regulators of stem cell homing (Blood 2000; 95:3289). (a) Stem cell rolling interactions on constitutively expressed endothelial E and P selectins. Following rolling, CXCR4 stem cells (blue cells) are activated by SDF-1, which is secreted from bone marrow endothelial cells and triggers LFA-1/ICAM-1 and VLA-4/VCAM-1 interactions, supporting firm adhesion to endothelial cells. (b) Cells that do not express sufficient levels of CXCR4 (purple) will detach from the endothelial layer and return to the blood stream. (c) The arrested human CXCR4 stem cells, in response to SDF-1, will extravasate and migrate through the underlying basal lamina ECM using VLA-4 and VLA-5 integrin receptors to FN. (d) Migrating stem cells will eventually reach the "stem cell niches," which consist of stromal cells that present the proper set of adhesion molecules (e.g. VCAM-1 and FN), SDF-1, and growth stimulatory factors.

Selected Publications

- Spiegel, A. & Lapidot, T. et al (2007). Catecholaminergic neurotransmitters regulate migration and repopulation of immature human CD34+ cells through Wnt signaling. *Nat Immunol.* 8(10):1123-31.
- Kalinkovich, A. & Lapidot, T. et al. (2006). Functional CXCR4-expressing microparticles and SDF-1 correlate with circulating acute myelogenous leukemia cells. *Cancer Res.*, 15;66(22):11013-20.
- Kollet O. & Lapidot T. et al (2006). Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat Med.*, Jun;12(6):657-664.
- *News & Views by Louise E Purton & David T Scadden (2006). Osteoclasts eat stem cells out of house and home. *Nat Med.*, Jun;12(6):610-611.
- Goichberg, P. & Lapidot, T. et al. (2005). cAMP-induced PKC ζ activation increases functional CXCR4 expression on human CD34+ hematopoietic progenitors. *Blood* [Epub ahead of print].
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- Petit, I. & Lapidot, T. et al. (2005). Atypical PKC- ζ regulates SDF-1-mediated migration and development of human CD34+ progenitor cells. *J Clin Invest* 115:168-176.
- Tavor, S. & Lapidot, T. et al. (2004). CXCR4 regulates migration and development of human AML stem cells in transplanted NOD/SCID mice. *Cancer Res* 64:2817-24.
- Avigdor, A. & Lapidot, T. et al. (2004). CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to the bone marrow. *Blood* 103(8):2981-9.
- Kahn, J. & Lapidot, T. et al. (2004). Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. *Blood* 103(8):2942-9.
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- Petit, I. & Lapidot, T. et al. (2002). G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat. Immunol.* 3(7):687-94.
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- Ponomaryov, T. & Lapidot, T. et al.(2000). Induction of the Chemokine Stromal-Derived-Factor-1 Following DNA Damage Improves Human Stem Cell Function. *J. Clinical Investigations* 106:133-9.
- Peled, A. & Lapidot, T. et al. (2000). The chemokine SDF-1 activates the integrins LFA-1, VLA-4 and VLA-5 on immature human CD34+ cells: role in transendothelial/stromal migration and engraftment of NOD/SCID Mice. *Blood* 95,11:3289-96.
- Peled, A. & Lapidot, T. et al.(1999). Dependence of Human Stem Cell Engraftment and Repopulation of NOD/SCID Mice on CXCR4. *Science* 283:845-848.

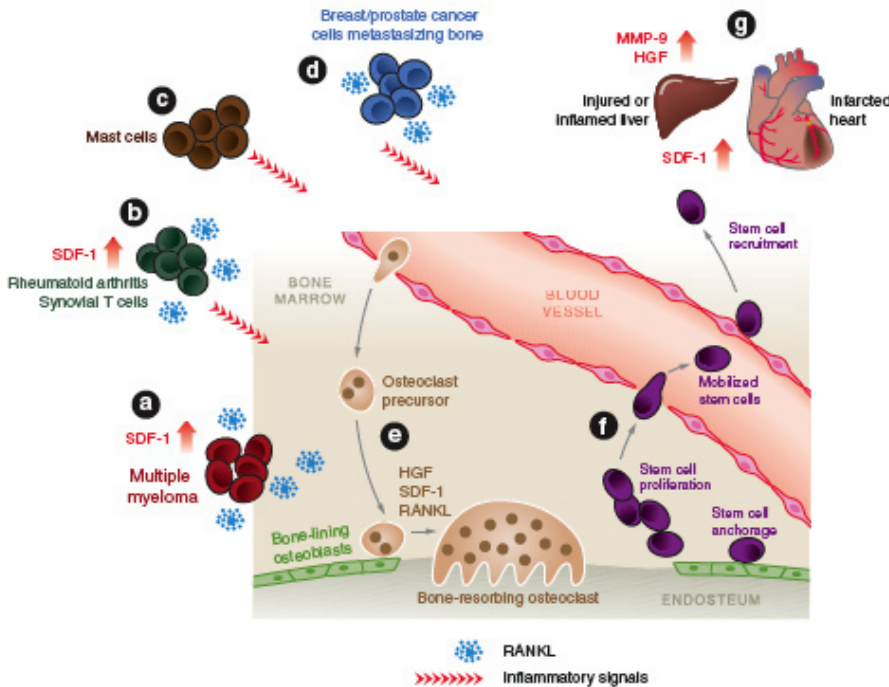


Fig. 3 Bone-resorbing osteoclasts induce stem cell mobilization in response to inflammatory and other stress signals.

Inflammatory signals, RANKL expression, and SDF-1 production by (a) MM cells, (b) synovial T cells of RA patients, (c) mast cells, or (d) breast and prostate cancer cells metastasizing the bone increase recruitment of osteoclast progenitors to the bone and their activation (e). Proteolytic enzymes secreted by bone-resorbing osteoclasts temporarily alter the endosteal stem cell niche and induce proliferation and mobilization of hematopoietic stem and progenitor cells (f) and their recruitment to high SDF-1-expressing injured tissues or organs (g) as part of host defense and repair mechanisms.

Invited Reviews

- Kollet, O. & Lapidot, T. et al. (2006). The Multiple Roles of Osteoclasts in Host Defense: Bone Remodeling and Hematopoietic Stem Cell Mobilization. *Annu. Rev. Immunol.*, Oct 16. [Epub ahead of print].
- Dar A, Kollet O & Lapidot T. (2006) Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Exp. Hematol.* 30(9):973-81.
- Lapidot T., Dar A., & Kollet O. (2005) How do stem cells find their way home? *Blood* 34(8):967-75.
- Lapidot, T. Petit I. & Kollet O. (2003) Current understanding of factors influencing stem cell mobilization. In: *Hematology , Hematology (Am Soc Hematol Educ Program)*,419-37.
- Lapidot T, Kollet O. (2002) The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2mnull mice. *Leukemia* 16:1992-2003.
- Lapidot T, Petit I. (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp. Hematol.* 30(9):973-81.

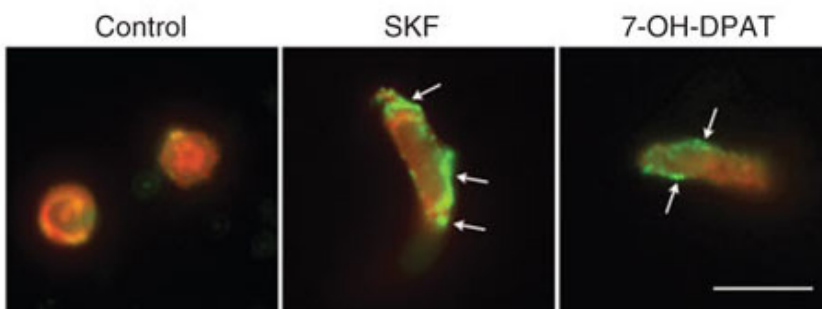


Fig. 4 Dopamine receptor agonists increase the polarization and motility of CD34 cells. (*Nat Immunol.* 2007 10:1123-31) Microscopy of cells immunolabeled for Dopamine receptor-5 (green) and stained for polymerized actin (red). Arrows indicate clustering of DR5 in polarized cells. (Scale bar, 10 μ m).