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Regulation of stem cell migration and development by chemokines, adhesion molecules and proteolytic enzymes

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Blood forming stem cells are a rare population of quiescent cells within the bone marrow. These progenitors continuously replenish maturing myeloid and lymphoid blood cells, by extensive proliferation and differentiation throughout life while maintaining a small pool of undifferentiated stem cells. Hematopoietic cells are motile and while most progenitors reside in the bone marrow, extremely low levels can be monitored migrating via the blood circulation during steady state homeostasis. These low levels are dramatically increased in response to various stress signals which are elicited in alarm situations caused by bleeding, organ injury and inflammation due to infection, documenting that stem cells are part of the bone marrow reservoir of maturing immune cells (leukocytes) which migrate and participate in host defense and repair processes. In addition to multilineage blood cell differentiation, hematopoietic stem cells are pluripotent and can also fuse and develop into liver hepatocytes, heart muscle and endothelial cells as well as neuronal cells. Stem cell recruitment into the circulation in response to stress signals is also a clinical procedure which is induced by repetitive daily stimulations with cytokines such as G-CSF, alone or after DNA damaging chemotherapy treatment to cancer patients, a process which is termed mobilization and is used to harvest high levels of stem and progenitor cells for clinical transplantations. Stem cells are transplanted by infusion into the blood circulation of the recipients, which were previously conditioned with total body irradiation. The transplanted progenitor cells migrate in the circulation and home into the host bone marrow (BM), leading to their durable engraftment and repopulation, i.e. continues high level multi lineage differentiation of maturing blood cells of all lineages which in turn are released back into the circulation while maintaining a small pool of undifferentiated stem cells in the host bone marrow. However, the mechanisms, which mediate and regulate these migratory and developmental (homing, retention, proliferation, differentiation, release and mobilization) processes, are not fully understood.

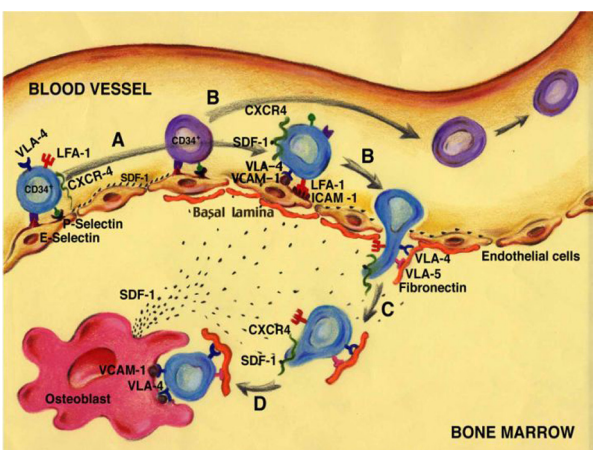

Fig. 1 *SDF-1/CXCR4 interactions regulate human CD34 stem cell homing and repopulation.*
CD34/CXCR4 + (blue) repopulate while CXCR4 – (purple) do not. in vitro prestimulation with IL-6 and SCF induces CXCR4 expression, converting purple CXCR4 – into blue CXCR4 +, NOD/SCID repopulating stem cells.

Chemokines are small signaling molecules which are part of the cytokine family and are best known for their ability to attract leukocytes to cites of inflammation. During late embryonic development hematopoietic stem cells migrate via the circulation from the fetal liver, home to and durably repopulate the bone marrow, shifting hematopoiesis to this tissue. Knockout experiments demonstrated that murine embryos which lack the chemokine Stromal Derived Factor one (SDF-1) or its receptor CXCR4 have multiple defects which are lethal, including lack of bone marrow seeding by hematopoietic stem cells migrating from the fetal liver. These

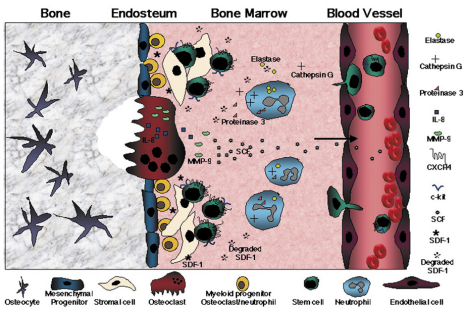

Fig. 2 *Osteoclast osteoblast interactions regulate stem cell migration and development.*

results reveal a central role for SDF-1/CXCR4 interactions in stem cell migration and development during murine embryogenesis. In contrast to pro-inflammatory chemokines, SDF-1 is constitutively expressed in steady state homeostasis in many organs, including adult human and murine bone marrow endothelium and the stem cell rich endosteum region. Multiple stromal cell types: bone forming osteoblasts, adipocytes, fibrobalsts and endothelial cells produce this chemokine. The seven transmembrane G coupled receptor CXCR4 is expressed by many cell types which include neuronal, epithelial and endothelial cells as well as by a wide variety of lymphoid and myeloid, mature and immature hematopoietic cells, primitive human $CD34^+/CD38^-$ and adult murine $Sca\text{-}1^+/ckit^+/lin^-$ stem cells. Furthermore, SDF-1 is the only known chemokine with the potential to attract high levels of stem cells, suggesting a major role for SDF-1/CXCR4 interactions in regulation of leukocyte trafficking and stem cell homing and retention within the bone marrow microenvironment. Human and murine SDF-1 are cross reactive, enabling murine SDF-1 in the preclinical small animal model of immune deficient NOD/SCID mice transplanted with immature human $CD34^+$ stem and progenitor cells to signal via cell surface human CXCR4. We have demonstrated that SDF-1/CXCR4 interactions are essential for homing to the murine bone marrow and high level multi lineage repopulation in NOD/SCID mice transplanted with enriched human $CD34^+$ stem cells. Murine BM endothelial SDF-1 mediated activation of the adhesion machinery: LFA-1, VLA-4, VLA-5 and CD44 on immature human $CD34^+$ /CXCR4+ cells, which are essential for homing and repopulation. This functional model was also used to identify leukemic stem cells obtained from patients with lymphoid Pre-B ALL or myeloid AML leukemias. Both normal and leukemic human stem cells are dependent on SDF-1/CXCR4 interactions for their migration and development. *in vivo* DNA damage induced by total body irradiation or chemotherapy drugs such as Cyclophosphamide increase the levels of SDF-1 in the bone marrow since this chemokine is also a survival factor for hematopoietic stem cells and also for malignant progenitors. Increase in SDF-1 levels in the bone marrow also improves human stem cell homing and repopulation in transplanted NOD/SCID mice, suggesting manipulation of this ligand and/or its receptor in order to navigate cells *in vivo*. Lastly, recent results demonstrate involvement of SDF-1/CXCR4 interactions also in G-CSF induced mobilization of hematopoietic stem and progenitor cells in which secreted proteolytic enzymes such as elastase and MMP2/9 which degrade SDF-1 in the bone marrow leading to increased surface CXCR4 expression on hematopoietic progenitors as part of the mobilization process which essentially breaks the adhesion and retention bonds between the hematopoietic progenitors to the bone marrow microenvironment. Taken together, these results reveal mechanistic insights of stem cell biology and document the major roles for SDF-1/CXCR4 interactions in regulation of human stem cell migration and development.

Selected Publications

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Lapidot T. Petit I & Kollet O. (2003) Current understanding of factors influencing stem cell mobilization. In: Hematology 419-424, Education session on Stem Cell Mobilization.

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