Tsvee Lapidot

Loya Abel
Abraham Avigdor
Ayelet Dar
Inna Goichberg
Joy Kahn
Orit Kollet
Isabelle Petit
Tanya Ponomaryov
Sarit Samira
Asaf Spiegel

Human stem cell migration and development: Regulation by cytokines, chemokines and adhesion molecules

Department of Immunology

Tel. 972 8 934 2481 **Fax.** 972 8 934 4141
E-mail: tsvee.lapidot@weizmann.ac.il

Recently we discovered that homing and repopulation by human stem cells in transplanted immune deficient mice, is dependent on SDF-1/CXCR4 interactions.

Oscillation of CXCR4 on human stem cells

CXCR4$^-$ sorted stem cells contain internal CXCR4, which can oscillate and express on the cell surface. New CXCR4 receptors are functional and mediate low SDF-1 dependent repopulation in transplanted NOD/SCID mice. Homing of CXCR4$^-$ cells to the murine BM is CXCR-dependent and reduced compared to CXCR4$^+$ cells. In conclusion, cell surface CXCR4 expression on human stem cells is a dynamic process, which is regulated by their environment (i.e. cytokines, chemokines, and stromal cells).

Role of SDF-1/CXCR4 interactions in G-CSF induced stem cell mobilization

We observed involvement of SDF-1/CXCR4 interactions within the BM following G-CSF administration as part of stem cell mobilization. Proteolytic enzymes such as elastase released by neutrophils in response to G-CSF, degrade BM SDF-1. In parallel, the levels of CXCR4 expression increased prior to stem cell mobilization. Each G-CSF stimulation increased the expression of SDF-1 mRNA by osteoblasts, induced transient elevations of bone marrow SDF-1 levels leading to transient decreases of CXCR4 expression, followed by a profound decrease of SDF-1 levels and CXCR4 upregulation. Co-administration of neutralizing anti-CXCR4 or anti SDF-1 Ab significantly reduced both human and murine mobilization in G-CSF treated mice, demonstrating a major role for SDF-1/CXCR4 interactions in stem cell mobilization.

Migration of leukemic stem cells

SDF-1 also plays a role in migration of leukemic cells. Different homing patterns as well as SDF-1 signaling pathways were found comparing malignant human Pre-B ALL cells with normal cells. Our data, reveals new insights into SDF-1-induced signaling pathways utilized by normal and leukemic cells.

CXCR4 knockout mice

CXCR4$^{-/-}$ knockout embryos die in utero due to multiple defects, which include impaired bone marrow hematopoiesis. However, fetal liver cells from these embryos can home/repopulate the BM of wild type mice. CXCR4$^{-/-}$ cells interact with SDF-1 via another receptor which partially compensates for the absence of CXCR4, suggesting an additional pathway for SDF-1 signaling in mice.

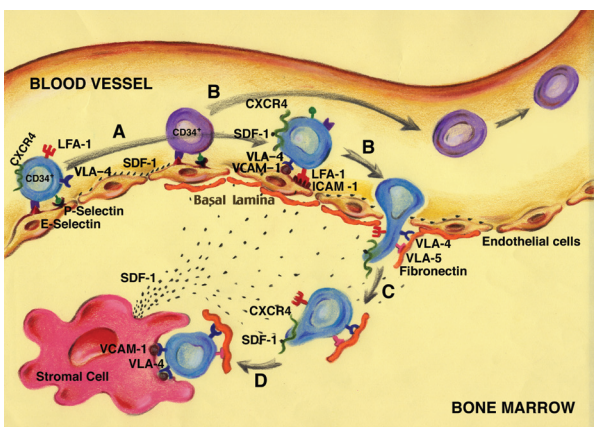

Fig. 1 *Circulating cells role on the endothelium of the blood vessel. CXCR4$^+$ stem cells are activated by SDF-1 presented by endothelial cells, which support firm adhesion. Arrested cells extravasate and home to the bone marrow.*

Regulation of SDF-1 expression

SDF-1 is produced mainly by immature bone forming osteoblasts and is highly expressed by BM endothelial cells. DNA damaging agents (such as irradiation or cytotoxic drugs) increase SDF-1 secretion which augments the levels of transplanted stem cells. The mechanism of SDF-1 regulation and cell cycle status of SDF-1 producing cells are currently investigated.

Stem cells / blood vessel wall interactions

Expression of SDF-1 on the surface of endothelial cells within blood vessels is crucial for human stem cell arrest under shear flow, an essential step for transendothelial migration from the circulation into the BM. In addition, SDF-1 activates the major adhesion molecules: CD44, LFA-1, VLA-4 and VLA-5

on migrating human stem cells as part of the multi step process of homing and transendothelial migration. Currently, we are utilizing immunohistochemistry and electron microscopy to visualize CXCR4 expression on migrating stem cells and SDF-1 localization on endothelial cells of the small blood vessel-wall.

CXCR4/SDF-1 signaling

Signal transduction pathways, triggered by SDF-1/CXCR4 are investigated. Activation of PI3K, but not MAPK, is required for motility of immature human cells. The atypical PKC zeta isoform is essential for migration. Moreover, activation of PKC zeta by SDF-1 is PI3K dependent.

Lentiviral vectors for gene transfer

We established that soluble IL-6R/IL6 chimera is an important factor for maintenance and expansion of human stem cells. The effect of overexpression and constitutive secretion of IL-6R/IL6 chimera on stem cell potential is examined. Lentiviral vectors are also used to target mesenchymal stem cells into the bone marrow. Overexpression of human CXCR4 on mesenchymal stem cells improved their homing into the murine bone marrow in a CXCR4-dependent manner whereas control cells demonstrated no homing capacity.

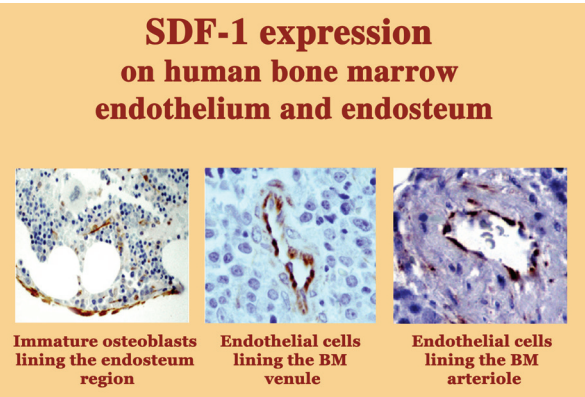

Fig. 2 *SDF-1 immunoreactivity in human bone marrow sections: immature bone marrow osteoblasts lining the endosteum region and stromal cells (left), venule (middle) and arteriole (right) endothelial cells.*

Human T cell development

TNF pretreatment of transplanted cells, induced human T cell development. Our in vivo and in vitro methodologies offer useful tools for studying the development of human T lymphocytes from stem cells.

In conclusion, we demonstrate that SDF-1/CXCR4 interactions have key roles in the homing, repopulation and mobilization of human stem cells. Our data suggest manipulation of SDF-1/CXCR4 interactions to improve the outcome of clinical stem cell transplantation.

Selected Publications

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