How do stem cells find their way home?

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Running head: Stem cell homing

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Abstract
Migration of hematopoietic stem cells through the blood, across the endothelial vasculature to different organs and to their BM niches, requires active navigation, a process termed homing. Homing is a rapid process and is the first and essential step in clinical stem cell transplantation. Similarly, homing is required for seeding of the fetal BM by hematopoietic progenitors during development. Homing has physiological roles in adult BM homeostasis, which are amplified during stress-induced recruitment of leukocytes from the BM reservoir and during stem cell mobilization, as part of host defense and repair. Homing is thought to be a coordinated, multi step process, which involves signaling by SDF-1 and SCF, activation of LFA-1, VLA-4/5 and CD44, cytoskeleton rearrangement, MT1-MMP activation and secretion of MMP2/9. Rolling and firm adhesion of progenitors to endothelial cells in small marrow sinusoids under blood flow is followed by trans-endothelial migration across the physical endothelium/ECM barrier. Stem cells finalize their homing uniquely, by selective access and anchorage to their specialized niches in the extravascular space of the endosteum region and in peri-arterial sites. This review is focused on mechanisms and key regulators of human stem cell homing to the BM in experimental animal models and clinical transplantation protocols.
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Introduction

The hallmark of hematopoietic stem cells (HSC) is their migration and repopulation potential (self-renewal and multilineage differentiation capacities). Progenitors isolated from the murine embryo, which lack directional migration and homing in transplanted adult mice, are non-definitive, cannot engraft the bone marrow (BM) and fail to rescue lethally irradiated recipients. These progenitors require in vitro stimulation with stromal cells, which convert them into functional, BM repopulating stem cells endowed with migration and homing potential\(^1\). Interestingly, migration of mobilized human CD34+ cells towards a gradient of the chemokine stromal derived factor-1 (SDF-1) in vitro correlates with their in vivo repopulation potential in autologous transplanted patients and in immune deficient NOD/SCID mice\(^2-4\).

Most clinical and experimental stem cell transplantation protocols utilize intra-venues (i.v.) injections of recipients previously conditioned with DNA damaging agents such as total body irradiation (TBI) and/or chemotherapy. These agents induce massive cell death of cycling hematopoietic cells in the bone marrow, spleen and blood and damage the physiological BM endothelium barrier. Tissue damage, leads to a dramatic increase in the levels of secreted chemokines, cytokines and proteolytic enzymes in many organs as part of the regeneration and repair process, which have profound impacts on stem cell migration and repopulation. DNA damage and stress also lead to proliferation and mobilization of quiescent stem cells from the BM to the circulation (reviewed in\(^5\)). For example, cyclophosphamide is used clinically to induce stem cell mobilization and to destroy malignant cells prior to stem cell transplantation. Yet, DNA damage aimed at eliminating malignant cells in patients also increases secretion of survival, migration and angiogenic factors such as SDF-1. This chemokine can increase CXCR4+ malignant stem cell survival, proliferation, invasion and metastasis, leading to the spreading of tumors in a broad range of cancers including breast, prostate, and brain tumors as well as leukemias\(^6-9\). Interactions between stem cells and their BM microenvironment, which are broken during cell mobilization, should be re-established to enable efficient BM homing and moreover, retention. In genetically marked parabiotic mice with a shared circulation, G-CSF induced mobilization also dramatically increases the levels of stem cell
engraftment in the partner bone marrow\textsuperscript{10}. Thus, stem cell mobilization, homing and repopulation are sequential events with physiological roles.

Finally, there is lack of clarity with regards to overlapping definitions of homing to the bone marrow, engraftment and repopulation. While homing is essential for successful engraftment and repopulation, it is not sufficient. In order to clarify the differences between these processes, we provide our definitions.

Our definitions are:

**Homing:** The first and a fairly rapid process (measured in hours and no longer than 1-2 days) in which circulating hematopoietic cells actively cross the Blood/BM endothelium barrier and lodge at least transiently in the BM compartment by activation of adhesion interactions prior to their proliferation. Aging neutrophils express high CXCR4 levels and home to the BM prior to their apoptotic cell death\textsuperscript{11}. Stem cells also home to other organs (especially in response to stress signals, transmitted in response to alarm situations such as TBI) and can first migrate to the spleen prior to BM homing\textsuperscript{12,13}.

**Engraftment and Repopulation:** Short-term engraftment (ranging from weeks to a few months) is initiated by differentiating progenitors. Durable long-term multi-lineage engraftment (months in mice, and years in patients) is carried out by stem cells following their unique homing to their specialized niches\textsuperscript{14}. Hematopoietic stem cells require the BM microenvironment, which regulates their migration, proliferation and differentiation in order to maintain active hematopoiesis throughout life\textsuperscript{15,16}.

**Similarities:** The presence of donor type hematopoietic cells in the BM is the outcome of both homing and engraftment/repopulation. Both processes are partially regulated by common mechanisms of migration and adhesion (e.g. SDF-1/CXCR4 signaling, VLA-4/VCAM-1 and CD44/hyaluronic acid (HA) interactions).

**Differences:** Homing is a rapid process, which can lead to transient retention and does not require cell division while engraftment does. Many cell types including long-term repopulating, human CD34+/CD38- stem cells, short-term repopulating CD34+/CD38+ progenitors and mature, specialized T cells and neutrophils can home to the BM, but only stem cells initiate long-term repopulation. Murine BM homing cells, which successfully adhere to the recipient stroma are viable, most probably due to protection from
apoptosis\textsuperscript{17}. Lineage- immature cells demonstrate stronger adhesion properties (30 fold) than Lineage+ cells\textsuperscript{18}. Homing does not require host pre-conditioning with DNA damage while durable engraftment with high levels of repopulation by transplanted stem cells is largely dependent on this procedure and the time interval between TBI and transplantation. For example, significantly increased survival rates of lethally irradiated mice transplanted 24 and 48 hours post TBI with graded doses of syngeneic BM cells were documented compared to mice transplanted immediately after TBI\textsuperscript{19}. Of interest this time frame is in correlation with increased secretion of SDF-1 and stem cell factor (SCF) by the irradiated murine marrow and spleen\textsuperscript{20,21}, suggesting that these and other factors influence repopulation levels of transplanted cells as documented for human CD34+/CD38-/low progenitors in NOD/SCID mice transplanted immediately after TBI, or 24 and 48 hours later\textsuperscript{20}.

**The BM architecture, DNA damage, homing and repopulation.**

The BM endothelium is the first anchoring site for homing cells, presenting adhesion molecules and stimulating chemokines. Human and mouse BM share common structures but species-dependent differences can also be observed. The small blood vessels in both human and murine BM, the sinusoids in which trans-endothelial migration is thought to take place, are composed of specialized cell structures, which regulate cell trafficking (Fig. 1a,c and\textsuperscript{22}). TBI and Cyclophosphamide disrupt the physical BM endothelium barrier allowing erythrocyte passage from the circulation to the extravascular hematopoietic space of treated, but not control mice, despite their discontinuous BM endothelium with small gaps\textsuperscript{23-25}. We documented disruption of necrotic irradiated endothelium, which requires repair in the BM of conditioned patients (Fig. 1d). G-CSF induced mobilization increases the permeability and creates larger gaps between murine BM endothelial cells\textsuperscript{26}. The chemokine SDF-1 is expressed by immature human osteoblasts in the endosteum region\textsuperscript{20}. This chemokine and its receptor CXCR4, constitutively expressed by human and murine BM endothelium\textsuperscript{20,27}, are essential for stem cell seeding of the murine BM during fetal development and for definitive repopulation in adult transplanted mice (reviewed in\textsuperscript{5}). However, murine CXCR4 knockout (KO) cells can home to the BM, and the reduced retention of CXCR4(KO)
stem cells in the BM of primary but not serially transplanted recipients can be corrected by increasing the cell dose\textsuperscript{28-30}. SDF-1/CXCR4 expression is regulated by Hypoxia Inducible Factor one (HIF-1) and since the BM is partially hypoxic\textsuperscript{27}, this pair is functionally expressed by many cell types including immature and maturing hematopoietic cells, stromal cells lining the stem cell rich endosteum region and human Stro1+ mesenchymal progenitors\textsuperscript{20,31}. TBI induces increased SDF-1 and SCF secretion within 24-48 hours in the murine BM and spleen\textsuperscript{5,20,21}. SDF-1 secreted to the circulation from the injured murine liver can functionally pass the endothelium barrier in a CXCR4 dependent manner into the BM and recruit hematopoietic progenitors to the circulation\textsuperscript{13}. In addition i.v. injected human SDF-1 increased homing of human mobilized and cord blood (CB) CD34+ enriched cells to the BM and spleen of non-irradiated B2mnull NOD/SCID mice\textsuperscript{32}. Interestingly, SDF-1 expressing endothelial cells in the murine BM are the first anchoring cells for transplanted murine stem cells, as revealed by intravital microscopy\textsuperscript{33}. Other organs also induce HIF-1 activation and a shift in SDF-1 expression by endothelial cells in the liver\textsuperscript{13}, heart\textsuperscript{34} and brain\textsuperscript{35} during stress. These changes together with the secretion of other chemokines, cytokines and proteolytic enzymes influence stem cell homing and repopulation in opposite directions. Increased homing of transplanted progenitors to the BM, similar levels of stem cell retention but significantly decreased donor derived repopulation levels (which require transplantation of very high cell doses) are documented in non-irradiated mice compared with TBI pre-conditioned recipients. The latter require much lower cell doses for donor cell repopulation (discussed in\textsuperscript{36-38}). These results suggest that stem cell homing to other organs and mobilization induced by DNA damage, reduce direct homing to and/or early retention of progenitors within the BM of irradiated recipients 1-2 days post TBI. However, durable repopulation by transplanted stem cells is enhanced in recipients pre-conditioned with TBI, which eliminate host stem cells and hematopoiesis. In addition, stress signals transmitted following conditioning with DNA damage as part of the repair process can also influence definitive stem cells in a time dependent manner by enhancing their migration, proliferation and differentiation, and the time interval of transplantation post TBI has major impacts on repopulation and survival\textsuperscript{19,20}. 
Human and mouse BM have also species-specific structure which needs to be considered when alternative clinical procedures (intra-venous vs. intra-osseous transplantation) are applied. Intra-osseous transplantation of human CD34+ and CD34-enriched CB progenitors in immune deficient mice resulted in much higher seeding efficiencies of both short and long term repopulating cells^39-42 compared to intra-venous transplantation. In contrast, no significant differences were observed in hematopoietic recovery of human individuals undergoing BM transplantation with both procedures^43,44. This may be attributed to structural and size related differences between murine bones (smaller with compact bone marrow) as opposed to human bones (less compact BM containing many fat cells, bigger blood vessels and higher blood volume, Fig.2), which may require implantation of stem cells with an anchoring matrix needed to retain the progenitors in the injected bone.

**Homing of human stem cells in preclinical animal models**

The biology of human progenitor cell homing has been studied in two major preclinical animal models. The pre-immune sheep model, developed by Zanjani et al, utilizes pre-immune sheep fetuses which are implanted with immature human CD34+ and CD34- cells without pre-conditioning, allowing short and long-term stem cell engraftment in both primary and serially transplanted secondary sheep recipients. In this model, immature human BM CD34+ enriched cells transplanted to sheep fetuses can be detected 24 and 48 hours later in the recipient BM^45. The second model is based on immune deficient mice pre-conditioned with sublethal TBI. SCID mice transplantation with normal and leukemic human progenitor cells developed by the group of Dick et al, facilitate high levels of multi-lineage (both myeloid and lymphoid) human hematopoiesis in the murine BM^46, and identified leukemic CD34+/CD38- stem cells (SLIC) obtained from AML patients^47. Later, NOD/SCID mice with additional reduced immunity were created by Shultz et al. These mice were used to identify primitive, normal human CD34+/CD38- SCID repopulating cells (SRC)^48, CD34-/CD38- SRC^49 and SLIC from different AML FAB subtypes which can initiate the disease in serially transplanted mice^50. B2mnull NOD/SCID mice have further reduced innate immunity due to the lack of NK activity. Definitive, SRC function of high level multi-lineage engraftment in
primary NOD/SCID mice and serially transplanted B2mnull NOD/SCID mice is dependent on human CXCR4 signaling by murine SDF-1 which is cross-reactive and SLIC function is also CXCR4 dependent. CD34+/CD38+ short term repopulating cells (STRC), which are enriched in mobilized peripheral blood (MPB) were identified by the group of Eaves et al using B2mnull NOD/SCID mice or by inactivation of NK cell activity in NOD/SCID mice by other groups. Mechanisms of human stem cell homing were mainly characterized in immune deficient NOD/SCID and B2mnull NOD/SCID mice, however the xenogeneic-barrier dependent limitations of these models, may explain the low frequency of homing progenitors. Nevertheless, a positive correlation between engraftment in patients and NOD/SCID and B2mnull NOD/SCID mice was demonstrated.

**Homing is a rapid process:** Murine progenitors and human CD34+ cells transplanted i.v are rapidly cleared from the recipient circulation, within minutes. Human CD34+ progenitors home to different organs including spleen, liver and lungs and may home later from these organs to the BM as previously shown for murine cells. Of interest, radiolabeled BM CD34+ cells transplanted to patients with acute myocardial infarction rapidly homed to the liver and spleen, (but were not detected in the BM) within a few hours. In transplanted B2mnull NOD/SCID mice, we could detect homing of human CB CD34+ cells to the BM and spleen already 2 hours after infusion, which is twice faster than homing of the same cell population in NOD/SCID mice.

**Origin dependent properties of CD34+ cells:** Neonate, umbilical CB derived CD34+ cells differ from adult BM or MPB CD34+ derived cells in their BM homing (which is reduced) and SRC repopulation properties (which are increased) in transplanted NOD/SCID mice. CB CD34+ cells demonstrate reduced rolling on BM endothelium, which is a prerequisite step for transendothelial extravasation and successful homing, in comparison with adult CD34+ cells. Partial expression of a nonfunctional form of the P-selectin ligand PSGL-1 on the surface of some CB CD34+ progenitors enriched with the primitive CD34+/CD38- subset, mediates this reduced function. Of interest, children transplanted with a 13 times higher cell dose of BM cells, gained faster neutrophil and platelet recovery, however, the levels of progenitors in the BM of children one year post transplantation with CB were significantly higher. These data imply for different
repopulation potential, which may result from different homing, proliferation and differentiation properties of transplanted stem cells and suggest that CB progenitors which have successfully homed to and are retained in the host BM do not fully respond to differentiating signals and are biased to maintaining their immature state.

Progenitor subsets: Different cell subpopulations may vary in their homing properties. Total nucleated cells from human CB, BM, PB and CB CD34+ enriched cells transplanted to lethally irradiated NOD/SCID mice, demonstrate similar seeding efficiencies in the murine BM and spleen within 24 hours61. However, despite similar homing efficiencies 3 hours after transplantation of enriched CD34+ cells (co-transplanted with irradiated mature cells) derived from the above mentioned sources, they display different repopulation rates53. We have demonstrated that enriched CB CD34+/CD38- cells, home successfully to the murine BM and spleen while sorted CD34+/CD38+ maturing progenitor cells, which lack long term repopulation capacity have poor homing capabilities unless prestimulated with cytokines such as SCF in vitro or G-CSF introduced for mobilization of adult progenitors in vivo62 or co-transplanted with mature mononuclear cells53,54. In contrast to CD34+ progenitors, sorted Lin-/CD34-/CD38- cells have very low engraftment levels in vivo and rare colony formation in vitro49. These cells express low levels of membrane CXCR462 and have negative regulators of CXCR4 signaling63. While these cells display enhanced random migration, they poorly migrate towards a gradient of SDF-1 in vitro, explaining their extremely reduced homing and repopulation49,62. Bypassing the mechanical blood/BM barrier by direct injection into the bone, enabled the development of CD34+ cells in the murine bone injected with human CB CD34- cells, and also their migration in vivo to non-injected bones40. This transplantation approach revealed that CD34- primitive SRC/stem cells have higher migration, proliferation and secondary transplantation potential64. Interestingly, when Lin-/CD34- cells were pre-cultured with CD34+ cells, their repopulation potential as well as their development into CD34+ cells are increased65. Alternatively, in vitro incubation of CD34- progenitors with stromal cells and their stimulation with cytokines, induce CD34+ expression, colony formation and definitive SRC repopulation66.
The accessory effect: Non-repopulating maturing and mature cells facilitate the repopulation potential of cotransplanted CD34+ progenitors\(^{54,67}\). Co-transplantation of CD34+/CD38+ maturing progenitors with CD3-depleted CB cells, facilitated their homing and transient engraftment\(^{53}\). We found that prestimulation of CB CD34+ cells with SCF induces surface CXCR4 expression\(^{4}\) and the appearance of cycling G1 CD34+/CD38+ cells with increased production of the proteolytic enzyme matrix metalloproteinase 9 (MMP-9), SDF-1-directed migration and homing to the BM and spleen of transplanted NOD/SCID. More importantly, these G1 CD34+ cells actively support and enhance SDF-1 induced migration, homing, and BM repopulation capacity of co-transplanted, quiescent G0 CD34+ cells which include all the primitive CD38-/low cells\(^{68}\). Possible mechanisms to explain the accessory effect exerted by maturing cells are cytokine secretion\(^{69}\) or production of proteolytic enzymes such as MMP-9\(^{68}\) which enhance CXCR4 expression, cell motility and SDF-1-directed migration of progenitor cells\(^{13}\). Other cell types, such as CD8+ T cells\(^{70}\) were also shown to augment the homing of CD34+ cells in co-transplantation studies. A prolonged accessory effect is apparently exerted by co-transplanted mesenchymal cells which home to the murine lungs and augment engraftment but not homing of human CB CD34+ cells\(^{71}\).

SDF-1/CXCR4 interactions

SDF-1/CXCR4 interactions and signaling have been implicated as a principal axis regulating retention, migration and mobilization of hematopoietic stem cells during steady state homeostasis and injury (reviewed in\(^{5}\)). We have shown that enriched human CD34+ SRC/stem cells homing to the BM require functional interactions between human CXCR4 and murine SDF-1, which is presented on the vasculature endothelium and various BM stromal cells. Repopulation by SRC\(^{4,20,62}\) and SLIC function (both homing and development) are also CXCR4 dependent\(^{8,9}\).

Intracellular and surface expression: CXCR4 expression is very dynamic. Cytokine stimulation with SCF+IL-6 or HGF, upregulates CXCR4 expression by human CD34+ cells within 24-48 hours, leading to their increased SDF-1 mediated migration, in vivo homing and repopulation\(^{4,13,62}\). Similarly, SCF+IL-6 stimulation increases CXCR4 expression by Sca-1+/Lin- murine progenitors\(^{72}\) and membrane bound SCF is essential
for murine stem cell homing to the endosteum region\textsuperscript{73}. Overexpression of this receptor by human CB and MPB CD34+ cells by lenti-viral infection, led to increased cell motility and SDF-1 induced migration, higher proliferation rates in vitro and moreover, increased SRC repopulation\textsuperscript{74,75}. Interestingly, CXCR4 overexpressing CD34+ cells demonstrate increased homing to the spleen but not to the BM of NOD/SCID recipients\textsuperscript{74}, suggesting that human cells home first to the more permissive tissue, the spleen, and only later to the murine BM\textsuperscript{58,59}. High doses of SDF-1 induce desensitization, receptor internalization, cell survival and quiescence, while low doses promote cell motility, proliferation and migration (reviewed in\textsuperscript{54}). Low dose stimulation with SDF-1 or anti CXCR4 Ab both increase migration to this ligand and repopulation by MPB CD34+ cells, but not BM CD34+ cells\textsuperscript{3}. A single injection of human SDF-1 to non-irradiated B2mnull NOD/SCID mice followed by CD34+ cell transplantation increased homing of MPB and CB CD34+ cells to the murine BM and spleen. The injected ligand can cross the BM endothelium in a CXCR4 dependent manner to the endosteum region conveying messages from peripheral organs and increasing homing of human progenitors including primitive CD34+/CD38-/low cells\textsuperscript{32}. Sorted CB CD34+ cells, which do not express CXCR4 are less motile and home at a much slower rate compared to their CXCR4+ counterparts. These CD34+/CXCR4- cells harbor low levels of intra-cellular CXCR4. Nevertheless, membrane expression of this receptor is induced in vivo upon transplantation, mediating their delayed homing and reduced repopulation. In vitro pre-stimulation (24-48hr.) with cytokines can also convert these cells into definitive SRC with high surface and intra-cellular CXCR4 expression and SDF-1 induced migration\textsuperscript{76}. In order to block new surface CXCR4 expression from the large intra-cellular pool of CD34+ cells, cell transplantation with excess amounts of unbound anti CXCR4 Ab is required\textsuperscript{9,62,76}. The low levels of engraftment obtained with high doses of human CD34+/CXCR4+ and CXCR4- sorted cells, both treated with neutralizing Ab which were washed before transplantation\textsuperscript{77}, most probably reflect the dynamic expression of this receptor. CXCR4 can also be functionally transferred from platelet microparticles to BM CD34+ cells and augment their SDF-1 mediated migration, adhesion, homing and engraftment potential\textsuperscript{78}. 
**CXCR4 co-localization in lipid rafts:** Gene knockout studies in mice demonstrate the essential role of Rac1 in BM homing of murine stem/progenitor cells. Interestingly, Rac1 co-localization with CXCR4 in lipid rafts, is suggested to potentiate human BM CD34+ cell migration and repopulation as previously shown for human T cells. However SDF-1-mediated chemotaxis and in vivo homing can be augmented by other mechanisms, as shown by stimulation with the complement component C3a or other leukopheresis products from mobilized patients which increased incorporation of CXCR4 into membrane lipid rafts of CD34+ cells and their homing.

Homing and repopulation by CD34+ cells can also be manipulated by enhancing the production of BM SDF-1 via application of DNA-damage inducing agents such as TBI or chemotherapy or hypoxia that has been shown to regulate SDF-1 and CXCR4 expression by HIF-1α. Incubation of CD34+/CD38- BM cells in hypoxic conditions, led to HIF-1α stabilization and increased the frequency of SRC, suggesting that cultured cells acquire functional CXCR4 facilitating higher proliferation and homing capacities. In addition, CD8+ T cells, which improved the migration, BM homing and engraftment of CD34+ cells also reduced FAK phosphorylation in CD34+ cells cultured with them in response to SDF-1 stimulation.

**CXCR4 signaling and regulation of homing:** An important component of the CXCR4 signaling pathway is atypical PKCζ, which translocates to the cell membrane upon SDF-1 stimulation. We revealed that SDF-1-induced chemotaxis, polarization, MMP-9 secretion and adhesion of human CD34+ cells are PKCζ-dependent. Interestingly, while a PKCζ inhibitor prevented BM repopulation and in vitro migration to SDF-1 by human CD34+ cells, their homing was not affected, suggesting a role for PKCζ in BM seeding and retention of SRC. The broad range PKC inhibitor, chelerythrine chloride, totally abrogates homing of both human CD34+ cells and murine progenitors to the murine BM. In addition, PKCζ mediates functional elevation of CXCR4 expressed on immature human CD34+ cells and MMP2/9 secretion, in response to cAMP stimulation. cAMP-treated CD34+ cells gain increased SDF-1 induced transendothelial migration, adhesion to stromal cells and enhanced homing to the BM. Pertussis toxin (PTX), known to inhibit signaling of Gαi protein coupled receptors such as CXCR4, blocks in vitro migration of human CD34+ cells towards a gradient of SDF-1,
however their in vitro SDF-1 mediated adhesion and in vivo homing were contrary enhanced when 1 hour incubation with PTX was applied. Similarly, PTX treatment of murine progenitors reduced their colonization of the murine spleen but not the BM. Interestingly, co-stimulation of murine fetal liver and BM cells with PTX and SCF for 24 hr., significantly reduced their homing. This study suggests that longer PTX incubations than previously performed are needed to block homing of murine stem cells. However, in our original experiments with human CD34+ cells, when incubations with PTX were prolonged for 24 hours, no inhibition of homing was documented. Alternatively, short PTX treatment for 1 hr. which was preceded by 24 hours stimulation with SCF, significantly reduced homing levels by human CB CD34+ cells (unpublished data) suggesting that co-stimulation with cytokines such as SCF and longer SCF stimulation with short PTX treatment, block homing of human CB CD34+ progenitors since their motility is influenced by cytokine signaling which induce cell polarization. Of interest, dissemination of leukemic murine cells to the liver and spleen but not the BM were blocked by PTX, while inhibition of Gq11 signaling prevented both liver, spleen dissemination and BM colonization. Interactions mediated by the Rac, Rho and Cdc42 pathway are also implicated in stem cell migration. The lysophospholipid S1P and its specific receptors S1PRs, a novel class of G-protein coupled receptors, interact via the Rho family proteins, and are implicated in cell migration. The lysophospholipid S1P and its specific receptors S1PRs, a novel class of G-protein coupled receptors, interact via the Rho family proteins, and are implicated in cell migration. In the mouse system, S1P synergizes with SDF-1 to augment its chemotactic effect on primitive hematopoietic cells. Activation of S1PRs expressed by human MPB CD34+/CD38- cells, increase their CXCR4 signaling, transendothelial migration and in vivo homing without affecting CXCR4 expression, however increased engraftment was not statistically significant. Knocking out Rac-1 significantly reduced SDF-1 migration and abolished the homing of murine stem cells, while cells deleted of both Rac-1 and Rac-2 alleles, highly express CXCR4, fail to migrate to SDF-1 and are massively mobilized to the circulation.

The homing machinery – cell adhesion molecules: Adhesion molecules (selectins and integrins) mediate rolling and adhesion of homing cells to the blood vessel wall prior to their extravasation and seeding the BM. A subset of CB CD34 cells, which are enriched in primitive CD38- cells, demonstrate reduced rolling and repopulation due to partial expression of a nonfunctional form of the P-selectin ligand PSGL-1.
Neutralizing the integrin VLA-4, blocks homing of human CD34+ cells to the BM of fetal sheep\textsuperscript{45}. While VLA-4, VLA-5 and LFA-1 participate in the homing of CD34+ cells to the BM and spleen of NOD/SCID mice\textsuperscript{62}, VLA-4 and VLA-5\textsuperscript{94} but to a much lesser extent LFA-1\textsuperscript{95}, are essential for BM repopulation by these cells. We found that SDF-1 is involved in the regulation of CD34+ cell adhesion, during their homing to the BM. Stimulation by SDF-1 induces transendothelial migration\textsuperscript{95}, activates cell surface integrins and mediates firm arrest of CD34+ progenitors under shear flow\textsuperscript{96}. SDF-1 synergizes with other molecules to potentiate CD34+ cell adhesion and motility. Thus, we have shown that the adhesion molecule CD44 expressed by human CD34+ cells and its ligand, hyaluronic acid (HA) expressed in BM sinusoid endothelium and the endosteum region are essential for homing and repopulation which are both SDF-1 dependent\textsuperscript{97}. Of interest, inhibition of CD44 vetoed CD34+ cell activation and protrusion formation by SDF-1 suggesting that combined CD44 ligands, HA and osteopontin (binding different sites of CD44) expressed in the stem cell niche, prevent SDF-1 induced migration and secretion of MMP-2/9 by CXCR4+ progenitor cells. Interestingly, human CD34+ cells express HA (preferentially the CD38- subset\textsuperscript{98}), and a unique isoform of CD44, which is not expressed by mature cells, shown to mediate rolling on endothelial E/L selectin ligand under flow\textsuperscript{99}. Preliminary results demonstrate an essential role for the CD45 phosphatase, which negatively regulates adhesion interactions in homing and repopulation by human CD34+ cells. Neutralization of this receptor impaired migration to SDF-1, BM homing and repopulation of NOD/SCID mice\textsuperscript{100}.

\textit{Degrading enzymes:} Proteolytic enzymes are widely expressed by various cell types during steady state and injury conditions, regulating hematopoietic cell migration and tissue localization. SDF-1 and other factors induce the secretion of matrix metalloproteinases MMP-2 and MMP-9. These enzymes are secreted by circulating but not BM CD34+ cells, facilitating their in vitro migration towards SDF-1\textsuperscript{101}. Freshly isolated CB CD34+ cells do not express MMP-2/9\textsuperscript{68,102}, however upon incubation with MMP-2/9 rich conditioned media, these cells gain increased CXCR4 expression and SDF-1 directed migration\textsuperscript{13}. In accordance, MMP-2/9 inhibition reduces homing of human CB CD34+ cells. Similarly, incubation with SCF induces expression of MMP-2/9 and moreover increased homing and repopulation of NOD/SCID mice\textsuperscript{68,102}. Preliminary
results document that the cell surface proteolytic enzyme membrane type 1 matrix metalloproteinase (MT1-MMP) is expressed by human CD34+ progenitors, is activated by SDF-1 and G-CSF and is needed for SDF-1 induced migration in vitro and for in vivo homing and mobilization\textsuperscript{103,104}. Interestingly, proteolytic enzymes also negatively regulate cell migration. N-terminal cleavage of SDF-1 associated with its chemotactic inactivation, was demonstrated by several degrading enzymes including neutrophil elastase, cathepsin G\textsuperscript{105-107}, cathepsin K\textsuperscript{108} and MMP-2/9\textsuperscript{109}, and C-terminal cleavage was attributed to carboxypeptidase N\textsuperscript{110}. Neutrophil elastase and cathepsin G also cleave CXCR4 on human T cells and CD34+ cells which can also affect their motility\textsuperscript{107,111}. These studies demonstrate that proteolytic enzymes are involved in both turning on and off the locomotion machinery of human CD34+ cells, thus mediating stem cell recruitment and organ localization. CD26, a cell surface peptidase, is expressed on the surface of different hematopoietic subsets, including normal BM CD34+ cells\textsuperscript{112} and a subfraction of CB CD34+ cells which are mainly CXCR4 expressing cells\textsuperscript{113}. CD26 inhibition, enhanced the migratory response of CB CD34+ treated cells to SDF-1\textsuperscript{114} supporting a previous finding that CD26 cleaves and inactivates many ligands including SDF-1\textsuperscript{115}. Murine Sca-1+/Lin- stem cells obtained either from CD26 KO mice or wild type cells treated with a CD26 inhibitor, demonstrate increased (~30%) homing to the BM of wild type mice preconditioned with TBI\textsuperscript{72}. Higher levels of functional SDF-1 documented in the circulation of CD26 KO mice, suggest that CD26 is mostly responsible for inactivation of this ligand in the circulation\textsuperscript{116}.

**A short cut to the BM: Intra Osseous stem cell transplantation**

In an attempt to improve the efficiencies of BM homing, seeding and repopulation, transplanted cells were injected directly into the BM cavity of immune deficient NOD/SCID and normal mice (intra osseous (i.o.), intra bone marrow (IBM) or intra femur (IF) transplantation). Studies with enriched human progenitors demonstrate superior seeding efficiencies when the endothelial and ECM barriers are bypassed. IBM injections yielded a 15 fold higher frequency of SRC in sorted CB CD34+/CD38- cells in comparison to conventional i.v. injection. Increased engraftment and improved abilities of BM cells obtained from primary recipients to repopulate secondary transplanted
recipients were also documented. Blocking simultaneously the integrins VLA-4/5, which have been implicated in the homing and repopulation processes, profoundly inhibit engraftment of human progenitor cells injected intra osseously. Interestingly, CXCR4 neutralization similarly abolishes engraftment by human CD34+ enriched cells administrated in both protocols, demonstrating an essential role for this receptor not only in SRC homing to the BM but also in BM seeding and colonization. Thus, this method enables to dissect different steps of BM repopulation. CB derived Lin-CD34- primitive cells display rare and low repopulation potential when transplanted via the tail vein, as opposed to high SRC activity when transplanted intra osseously, despite low expression of CXCR4 and reduced migration to SDF-1. These progenitors acquire in vivo expression of the CD34+ marker demonstrating slower differentiation and reconstitution kinetics but higher absolute numbers of CD45+ and immature human CD34+ cells in comparison to human cells initially expressing CD34. Applying intrafemoral injection of CB CD34+CD38-/-lowCD36- purified cells, Dick and colleagues could identify a new short-term SRC subset which can migrate to other bones, colonizing them with myeloid and erythroid cells. Taken together, this method enables the revealing of repopulating human stem cell subsets that can hardly be detected when injected i.v. Of interest, when total CB MNC were transplanted, increased engraftment including a dramatic increase in immature human CD34+ cells were documented transiently only one month post i.o. transplantation compared to i.v. transplantation, but not 2 and 3 months post transplantation.

Studies with primitive murine BM cells demonstrate similar results. IBM injection of BM MNC led to a 1.4 fold increase in donor type cells in the recipient BM, while their levels in the peripheral blood were lower (1.3 fold) in comparison with i.v. injection, 3 months post transplantation. IF injection of murine Lin- cells supported increased homing after 20 hours both to the non-injected femur and spleen of non-ablated mice, in comparison to i.v. transplantation. However, effective bypass of the endothelial and ECM barriers is not sufficient for improved long term BM reconstitution. TBI preconditioning, which is widely used for clinical transplantation, led to egress of some Lin- cells transplanted immediately after TBI, from the injected femur to the circulation of ablated mice only (within 20 hours), suggesting that implanted progenitors are...
mobilized or not retained well, due to the disruptive BM endothelium. Despite reduced IF homing in irradiated recipients, donor cell proliferation and repopulation rates are increased compared to non-irradiated mice. However, retention of undifferentiated donor stem cells in the BM of non-conditioned recipients is evident by transplantation into lethally irradiated secondary recipients\(^3^6\), implying that TBI is further needed to reduce competition by host stem cells, and to provide signals which enhance BM reconstitution in a time dependent manner\(^1^9\). IBMT of marked cells without preconditioning in primates also led to low levels of engraftment in the circulation (<0.1%), despite much higher levels in the implanted bone (up to 30%)\(^1^1^8\).

Finally, to prevent stress and severe damage to the BM structure associated with intra femur injection, and to ensure cell introduction to the femur blood vessels rather than to the BM cavity, Scadden and colleagues have examined homing properties of murine BM Lin- cells injected to the femoral artery. While homing to the BM and spleen was significantly increased by femoral artery versus tail vein injection in mice, no differences were found in long-term donor cell repopulation\(^1^1^9\), suggesting that BM repopulation is not enhanced by this procedure and that stress and damage due to the i.o implantation procedure also contribute to the repopulation levels. These results together with the need for anchoring i.o. transplanted human progenitors with a matrix to prevent their release to the circulation may explain the lack of improvement in human i.o. transplantation compared to conventional i.v. transplants and suggest modified i.o. protocols.

**Clinical relevance and concluding remarks:**

Key experiments by Veormans et al, showing a correlation between spontaneous and SDF-1 mediated migration in vitro of CD34+ cells and hematopoietic neutrophil and platelet recovery in patients transplanted with autologous CD34+ enriched MPB cells, emphasize the importance of definitive human stem cell motility and repopulation in clinical protocols\(^2\). Similar results revealed a correlation between CXCR4 expression and repopulation with allogeneic CB transplantation and the rate of platelet recovery in allogeneic matched transplantations with CD34 enriched cells\(^1^2^0,1^2^1\). The levels of SDF-1 induced migration of human CD34+ cells also correlate with repopulation in NOD/SCID
mice\textsuperscript{3,4} and moreover, human SRC function (including platelet and CFU-Mk) in transplanted B2mnull NOD/SCID mice correlate with platelet recovery in autologous transplanted patients\textsuperscript{55}. Mobilizing agents affect not only the levels of cell egress but also have an impact on CXCR4 function and the potential of mobilized cells to home back to the recipient BM\textsuperscript{80}. Human CD34+ cells from donors mobilized with both G-CSF and the CXCR4 inhibitor AMD3100, express higher levels of VLA-4, significantly migrate better to SDF-1 (7 fold) and engraft to much higher levels (8 fold) in transplanted NOD/SCID recipients, compared to cells obtained from donors treated with G-CSF alone\textsuperscript{122}. Similarly, Rhesus CD34+ enriched cells mobilized with AMD3100 have higher VLA-4 and CXCR4 expression, SDF-1 induced migration in vitro and in vivo BM repopulation in autologous primate transplantation compared to G-CSF mobilized cells\textsuperscript{123}.

This review has surveyed similarities between human progenitor cell motility, homing to the BM (Fig. 3) and repopulation. Stem cell migration and repopulation are sequential events, which are largely overlapped in their regulation. However differences between homing and repopulation and the critical role of DNA damage and stress in successful repopulation, but not BM homing (which is reduced) are also described. Future directions should be aimed at a better understanding of the factors involved in homing and repopulation. Specific modifications of host factors such as SDF-1, SCF and MMP-2/9 levels in the recipient BM, as well as further characterization of the human stem cell niche, including manipulation of these niches (e.g. i.o. transplantation with an anchoring matrix directly to the niche) may lead to improved in vivo navigation and repopulation potential of definitive stem cells. In parallel, donor factors such as increased CXCR4 expression and signaling by a wide variety of agonists, will add to host manipulations in order to develop improved transplantation protocols.

**Figure Legends:**

Fig 1. *Disruption of human and murine BM sinusoids post irradiation and chemotherapy*. Transmission electron microscope images of murine (a, b) and human (c, d) BM sinusoids. Elongated endothelial cells (EC) form the luminal layer of the sinusoid, surrounded by discontinuous cytoplasmic protrusions of reticular adventitial (RAC) cells. Erythrocytes (E), platelets (P) and nucleated hematopoietic cell (H) are seen within the
sinusoid lumen (L). The continuous murine endothelial cell layer is damaged post irradiation, resulting in gap formation due to rupture of the EC cytoplasm (b, arrow). Tight junctions between adjacent EC (c inset, arrow) are frequently seen in normal human sinusoids (c). Chemotherapy and irradiation cause significant narrowing of EC cytoplasm (d, arrow) together with rupture of the cell membrane and EC necrotic appearance (d).

Fig 2. BM architecture. Light microscopy images of murine (a, c) and human (b, d) BM sections stained with hematoxylin and eosin. Extensive invasions of spongy bones (trabeculae, T) and many fat cells (adipocytes, A), residing between the hematopoietic niches, characterize human BM (b) and distinguish it from the murine BM (a). Murine (c) and human (d) BM contains many small blood vessels (sinusoids, S), which are the site where progenitors home to the BM and where maturing cells and erythrocytes egress to the circulation (b). A monolayer of immature osteoblasts, which lines the bone and the trabeculae, defines the endosteum region (arrowheads, c-d), wherein hematopoietic stem cells reside.

Fig 3. Stem cell homing to the endosteum. Homing of human CD34+/CXCR4+ stem cells from the blood circulation via the murine BM sinusoids to the endosteum. Key molecules involved in this process, reported in recent studies (in addition to P/E selectins and SDF-1 activated LFA-1, VLA-4, VLA-5, and their ligands (Ref. 95 Fig.6 and Refs. 62, 96) which were omitted for simplicity) are illustrated.

Note: We apologize to those whose excellent work could not be cited given the space limitations.
Figure 2
Figure 3
References


73. Driessen RL, Johnston HM, Nilsson SK. Membrane-bound stem cell factor is a key regulator in the initial lodgment of stem cells within the endosteal marrow region. Exp Hematol. 2003;31:1284-1291.
95. Peled A, Kollet O, Ponomaryov T, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in


How do stem cells find their way home?

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