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Molecular mechanisms underlying adhesion and migration of hematopoietic stem cells

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Hematopoietic stem cell transplantation is the most powerful treatment modality for a large number of hematopoietic malignancies, including leukemia. Successful hematopoietic recovery after transplantation depends on homing of hematopoietic stem cells to the bone marrow and subsequent lodging of those cells in specific niches in the bone marrow. Migration of hematopoietic stem cells to the bone marrow is a highly regulated process that requires correct regulation of the expression and activity of various molecules including chemoattractants, selectins and integrins. This review will discuss recent studies that have extended our understanding of the molecular mechanisms underlying adhesion, migration and bone marrow homing of hematopoietic stem cells.

Introduction

Bone marrow homing is a rapid, coordinated process in which circulating hematopoietic stem and progenitor cells actively enter the bone marrow after transplantation. Rolling and firm adhesion of those cells on endothelial cells in small marrow sinusoids is followed by trans-endothelial migration across the endothelium/extracellular matrix barrier (**Fig. 1**). Selectins are implicated in playing an important role in bone marrow homing of hematopoietic stem cells (HSCs) by regulating initial tethering and rolling of those cells along the endothelial wall of blood vessels. In addition, inhibition of VE-cadherin is thought to be important for transendothelial migration by disturbing the integrity of bone marrow derived endothelial cell monolayers. SDF-1 mediated integrin activation subsequently induces firm adhesion of the HSCs to the endothelial wall upon which firmly attached HSCs can transmigrate through the endothelial layer and basal lamina consisting of the integrin substrates fibronectin, collagen and laminin. Finally, HSCs anchor to their specialized niches within the bone marrow compartment near osteoblasts and initiate long-term repopulation. The importance of selectins, integrins, cadherins and chemoattractants in regulation of the different stages of bone marrow homing will be described below in more detail.

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Adhesion Molecules and Bone Marrow Homing

P- and E-selectin play an important role in rolling of hematopoietic stem cells. Selectins are implicated in playing an important role in bone marrow homing of hematopoietic stem and progenitor cells by regulating initial tethering and rolling of cells along the endothelial wall of blood vessels. It has, for example, been demonstrated that coating of a surface with immobilized P- or E-selectin is sufficient to induce rolling of human $CD34^+$ hematopoietic progenitor cells (HPCs) under flow conditions.[1] In addition, intravital microscopy in bone marrow sinoids and venules of mice deficient for individual selectins revealed that rolling of HPCs involves both P and E-selectin, but not L-selectin.[2] To allow trans-endothelial migration to occur, firm adhesion of hematopoietic stem and progenitor cells to endothelial cells is required. In contrast to fluid-phase P- and E-selectin,[1] adhesion of $CD34^+$ HPCs to bone marrow derived endothelial cells under static conditions has been shown not to depend on E-selectin.[3] Experiments performed to study the importance of E-selectin in trans-endothelial migration of human HPCs yielded contradictory results. Transwell experiments performed by Voermans et al., for example, suggested that E-selectin is not important for trans-endothelial migration.[4] In contrast, Naiyer et al. have demonstrated in similar experiments that blocking of E-selectin with antibodies is sufficient to reduce trans-endothelial migration.[3] Similarly, transplantation studies with mice deficient for both P-and E-selectin revealed that the recruitment of HPCs to the bone marrow does depend on selectins.[5] Selectin ligands must be α1–3 fucosylated to form glycan determinants such as sialyl Lewis x [sLe(x)]. It has, for example, been demonstrated that inadequate α1–3 fucosylation of umbilical cord blood derived $CD34^+CD38^{-/low}$ cells results in reduced binding to P- and E-selectin. In addition, treatment of human $CD34^+$ cells with guanosine diphosphate GDP fucose and exogenous α1–3 fucosyltransferase VI improves rolling of those cells on P- and E-selectin and appears to be sufficient to enhance engraftment levels after transplantation in irradiated NOD/SCID mice.[1]

Since human $CD34^+$ HPCs exhibit a stronger E-selectin binding capacity compared with mouse $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ (LSK) cells,[6] it could be suggested that homing of human and mouse hematopoietic stem and progenitor cells are differentially regulated. Indeed, it has been shown that although the PSGL-1 glycoform CLA, CD43 and the CD44 glycoform HCELL, which

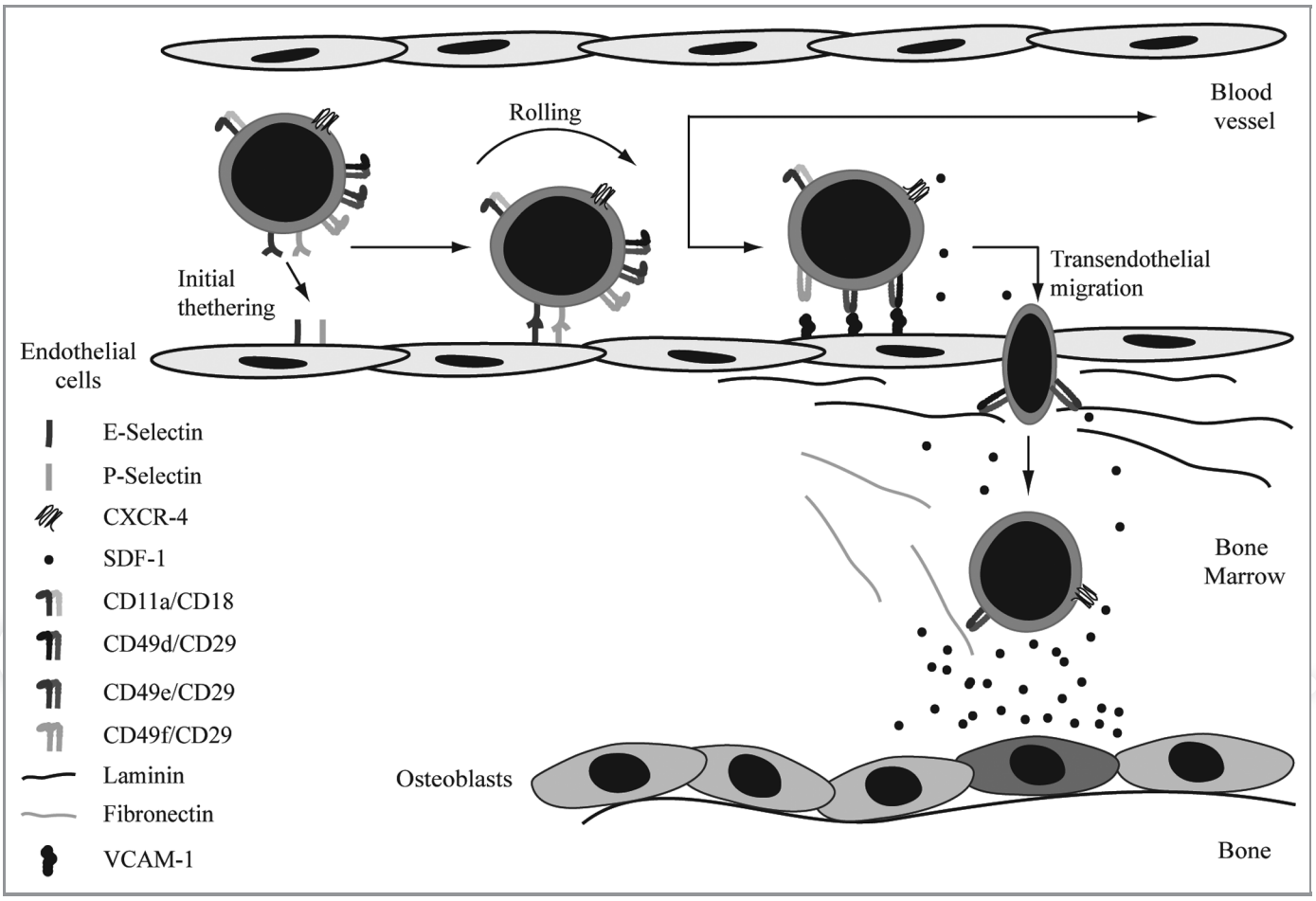

Figure 1. Homing of HSCs to the bone marrow. Initial tethering and rolling are the first steps in bone marrow homing. These processes are mediated by both E- and P-selectin. SDF-1 mediated integrin activation induces firm adhesion of the HSCs to the enodethelial wall. Firmly attached HSCs can subsequently transmigrate through the endothelial layer and basal lamina, consisting of fibronectin, collagen and laminin. Integrins involved in these steps are CD49d/CD29, CD49e/CD29 and CD49f/CD29. Finally, HSCs migrate toward the SDF-1 gradient to the osteoblasts. Chemoattractants involved in migration of HSCs.

are ligands for E-selectin, are all expressed on both mouse and human hematopoietic stem and progenitor cells,[6,7] the interaction between E-selectin and CD44 only occurs in humans but not mice.[6] A role for CD44 itself in regulation of bone marrow homing has also been determined. While shRNA mediated silencing of CD44 in human cells was shown to be sufficient to decrease E-selectin binding under physiologic shear conditions, enforced CD44 expression in mouse LSK cells conversely increased E-selectin adherence, resulting in improved bone marrow homing in vivo.[6] In addition to a role in bone marrow homing, a role for CD44 in retention of HSCs in the bone marrow has also been suggested. Treatment of mice with blocking antibodies against CD44 has been shown to increase the number of committed progenitors in the peripheral blood.[8]

Integrins play an important role in HSC adhesion and transendothelial migration. Integrins are also implicated in playing an important role in regulation of bone marrow homing. In vitro studies with blocking antibodies have, for example, shown that both CD49d/CD29 (α4β1 or VLA-4) and CD11a/CD18 (αLβ2 or LFA-1), but not CD49e/CD29 (α5β1 or VLA-5), play an important role in adhesion of hematopoietic stem and progenitor cells to endothelial cells and subsequent trans-endothelial migration.[4,9,10] Although inhibition of CD49e/CD29 alone was not sufficient to inhibit trans-endothelial migration, an additive effect was observed by a combination of antibodies directed against CD11a/CD18, CD49d/CD29 and CD49e/CD29.[10] The importance of both CD49d/CD29 and CD49e/CD29 in directional migration through the basal lamina, which is composed of the extracellular matrix proteins laminin, collagen and fibronectin, has been examined utilizing a three dimensional extracellular matrix-like gel. These experiments showed that SDF-1-induced directional migration of $CD34^+$ cells is dependent on both CD49d/CD29 and CD49e/CD29.[10] In contrast, both adhesion of $CD34^+$ HPCs to fibronectin[10] and chemotaxis of peripheral blood $CD34^+$ HPCs on recombinant fibronectin[11] were found to be primarily dependent on CD49e/CD29. In vivo transplantation experiments were performed to study the role of CD49e/CD29 and CD49d/CD29 in bone marrow homing. Pre-treatment of HPCs with an antibody directed against CD49e/CD29, prior to transplantation, was

sufficient to partially reduce homing of those cells to the bone marrow.[11,12] Similarly, the migratory capacity of cells either deficient for CD49d[13] or pretreated with CD49d antibodies[8,11,14,15] has been shown to be impaired, resulting in delayed short-term engraftment.[13] To investigate whether, in addition to CD49d/CD29, CD49d/ITGB7 (α4β7) could also be involved in bone marrow homing, either CD49d/ITGB7 (α4β7) or its substrate MadCam-1 were inhibited with blocking antibodies. These experiments resulted in a significant, but not complete, inhibition of bone marrow homing.[16] Another integrin implicated in regulation of bone marrow homing is CD49f (α6). In contrast to CD49d that appears to be predominantly involved in bone marrow homing of bone marrow derived short-term repopulating HSCs, CD49f is thought to be important for homing of both short-term and long-term HSCs.[15] However, similar experiments with fetal liver cells revealed that, in contrast to CD49d which appears to be important for homing of both hematopoietic stem and progenitor cells, CD49f is only important for homing of HPCs.[17] In contrast, bone marrow homing was not affected in a more recent study in which mice were transplanted with mouse bone marrow derived hematopoietic stem and progenitor cells pretreated with blocking antibodies directed against CD49f.[18] In addition, blocking the activity of CD49f on hematopoietic stem and progenitor cells obtained from human and primate bone marrow, but not from mobilized peripheral blood or cord blood, resulted in enhanced bone marrow homing.[18] Although additional research is required to fully understand the role of CD49f, these studies suggest that CD49f regulates bone marrow homing of specific subsets of HPCs depending on the source of those cells.

Transplantation experiments were also performed to determine the role of CD11a and CD18 in migration of HSCs to the bone marrow. These experiments revealed that, in contrast to CD49d and CD49e, both CD11a[8] and CD18[14] are not involved in bone marrow homing. However, inhibition of CD49d/CD29 in CD18 deficient HSCs resulted in more dramatic reduction in bone marrow homing in comparison to inhibition of CD49d/CD29 in wild-type mice. This suggests that CD18 can contribute to bone marrow homing when the function of CD49d/CD29 is compromised.[14] As described above, deletion of both P- and E-selectin in recipient mice significantly reduced bone marrow homing after transplantation of wild-type progenitors. Treatment of these mice with a blocking antibody against VCAM-1, thereby prohibiting interaction with CD49d/CD29, further reduced bone marrow homing after transplantation,[5] suggesting that both selectins and integrins are required for optimal bone marrow homing. With blocking antibodies, a role for integrins in retention of HSCs in the bone marrow has also been investigated. Treatment of mice with blocking antibodies against either CD49d[8] or CD49f[15] revealed that only inhibition of CD49d is sufficient to induce mobilization of hematopoietic stem and progenitor cells to the peripheral blood, indicating that CD49d, but not CD49f, plays an important role in lodging of hematopoietic progenitors in the bone marrow.

VE-cadherin plays a role in regulation of the integrity of endothelial cell monolayers. A third group of proteins implicated in regulation of bone marrow homing are cadherins. Inhibition of VE-cadherin has, for example, been shown to enhance transendothelial migration of UCB derived $CD34^+$ HPCs by reducing the integrity and enhancing the permeability of bone marrow derived endothelial cell monolayers. In addition, at the site of transmigration, $CD34^+$ cells appear to induce a loss of VE-cadherin localization.[19] The final step in bone marrow homing is anchoring of HSCs to their specialized niches within the bone marrow compartment. Based on studies in which high N-cadherin expression was observed at the junction between osteoblasts and HSCs,[20] it has been suggested that N-cadherin could play a role in the interaction of both cell types in the bone marrow. However, the mRNA and protein expression of N-cadherin appears to be very low[22-24] or absent in HSCs.[21,25] To date, the role of N-cadherin in regulation of HSCs is therefore still controversial[21] and requires further investigation.

Chemoattractants and Migration of HSCs

Chemoattractants play an important role in directing migration of hematopoietic stem and progenitor cells to the bone marrow. Several studies have demonstrated that Stromal Cell Derived Factor 1 (SDF-1), also known as CXC chemokine ligand 12 (CXCL12)[26] acts as a chemoattractant for hematopoietic stem and progenitor cells and is important for their trans-endothelial migration.[3,27,28] Analysis of a large panel of CC and CXC chemokines indicated that, within that group, the only chemokine capable of inducing migration of murine hematopoietic stem and progenitor cells is SDF-1.[29,30] Similarly, examination of a panel of chemokines and cytokines in trans-endothelial migration assays revealed that SDF-1 is also important for migration of human HPCs through a confluent layer of endothelial cells.[30] However, to a lesser extent, also other chemokines and cytokines, including CXCL10 (IP-10), CCL2 (MCP-1), CCL5 (RANTES), SCF and IL-8 could also induce trans-endothelial migration.[30] In addition, LTD4, a ligand for the G protein-coupled receptor CysLT(1), a mediator of the cysteinyl leukotriene family that is highly expressed in HPCs, has been demonstrated to upregulate integrin-dependent adhesion of HPCs[31] and to induce chemotaxis and trans-endothelial migration in vitro.[32] It has recently been demonstrated that, in addition to G protein coupled receptor recognizing chemokines, the proteolysis-resistant bioactive lipids sphingosine-1-phosphate and ceramide-1-phosphate act as chemoattractants for hematopoietic stem and progenitor cells during bone marrow homing.[33] The role of SDF-1 in migration of hematopoietic stem and progenitor cells will be discussed below in more detail.

SDF-1 plays a critical role in bone marrow homing. SDF-1 is considered to be essential for migration of HSCs to the bone marrow.[9,29,34] In the adult human bone marrow, SDF-1 was found to be expressed by endothelial cells and along the endosteum region in the bone marrow.[10,35,36] To date, six different SDF-1 splicing variants have been described: α, β, γ, δ, ε and φ.[37] From these six isoforms, SDF-1α appears to be the most abundantly expressed isoform. Whereas the expression of SDF-1β seems to be related to the vascular system, in humans and mice, SDF-1γ is primarily expressed in the heart.[38,39] While SDF-1α

consists of three exons, the other isoforms consist of a different fourth C-terminal exon. The N-terminal domain (aa 1–8) of SDF-1, which is present in all SDF-1 isoforms, is responsible for receptor binding and receptor activation.[40] The C-terminal domain, which is different in all SDF-1 isoforms, is important for stabilization of the interaction with the receptor. To date, two seven-transmembrane domain, G-protein coupled, receptors for SDF-1 have been identified, CXCR4 (LESTR/fusin) and CXCR7, of which CXCR4 appears to be the most prominent.[41,42] Mouse transplantation studies have been performed to investigate the importance of SDF-1 in migration of HSCs to the bone marrow. Upregulation of CXCR4 expression by incubation with hematopoietic cytokines (SCF and IL-6)[43] or overexpression of CXCR4 by viral transduction[44,45] was shown to be sufficient to enhance bone marrow homing of human $CD34^+$ and $CD34^+CD38^-$ cells in immune deficient mice.[43,46,47] In addition, pre-treatment of human $CD34^+CD38^{-/low}$ cells with a blocking antibody directed against CXCR4 impaired their capacity to home to the bone marrow of immune deficient recipient mice.[43,46-48] Similarly, fetal liver hematopoietic stem and progenitor cells deficient for CXCR4 displayed a reduced bone marrow homing capacity compared with wild-type cells.[49] In addition to migration to the bone marrow, SDF-1 also appears to play a critical role in retention of HSCs in the bone marrow. Enhancing the level of SDF-1 in plasma, but not bone marrow, utilizing adenoviral vectors[50] or sulfated glycans[51,52] was shown to induce mobilization of CXCR4 expressing hematopoietic stem and progenitor cells to the peripheral blood.[50,52] In addition, treatment of mice or healthy human volunteers with AMD3100, a selective CXCR4 antagonist, also resulted in enhanced numbers of HSCs in peripheral blood, again suggesting a role for CXCR4 and SDF-1 in HSC retention in the bone marrow.[53]

In contrast to CXCR4 that is expressed by hematopoietic stem and progenitor cells,[54] CXCR7 is only expressed at low levels in normal human hematopoietic stem and progenitor cells and does not appear to be important for migration of those cells.[55] However, CXCR7 was found to be highly expressed in several human myeloid leukemic cell lines and is thought to play a role in adhesion and, to a lesser extent, also in migration of those cells.[55]

Negative regulation of SDF-1 activity. Proteolytic enzymes have been implicated in negatively regulating migration of HSCs by cleaving and inactivating SDF-1.[52,56,57] Matrix metalloproteinases (MMP) 2/9 mediated cleavage of SDF-1 at the Ser^4-Leu^5 bond, has for example, been demonstrated to result in a reduced binding capacity of SDF-1 for CXCR-4 and a diminished chemoattractant activity for hematopoietic stem and progenitor cells.[58,59] A second class of SDF-1 proteases include the carboxypeptidases M and N.[60,61] Carboxypeptidase M, which is expressed by both stromal cells and $CD34^+$ HPCs,[60,62] is a membrane bound zinc-dependent peptidase that cleaves C-terminal basic residues. Carboxypeptidase M mediated cleavage of SDF-1 results in reduced chemotactic activity of hematopoietic stem and progenitor cells, which can be rescued by addition of the carboxypeptidase inhibitor DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid.[60] Carboxypeptidase N, which is present in human serum and plasma,[61] specifically cleaves SDF-1 at Lysine 68 resulting in reduced SDF-1 activity and inhibition of SDF-1 mediated induction of migration of HPCs.[61] Another membrane-bound protein involved in negatively regulating the activity of SDF-1 is CD26 (DPPIV).[63] CD26 is expressed by a small number of umbilical cord blood derived $CD34^+CXCR4^+$ cells. SDF-1α and SDF-1β appear to be differentially processed by CD26.[64] Whereas SDF-1α (1–68) undergoes processing at both the C- and N-terminal regions to produce SDF-1α (3–67), SDF-1β (1–72) is processed only at the N-terminus resulting in SDF-1β (3–72).[64] This differential processing suggests that these SDF-1 isoforms may not only have overlapping, but also unique roles. Migration experiments revealed that inhibition of endogenous CD26 activity is sufficient to enhance the migration of $CD34^+$ cells toward SDF-1, suggesting that CD26 abrogates SDF-1 induced migration of HPCs.[63,65,66] In addition, inhibition of CD26 with peptides such as diprotin A (Ile-Pro-Ile) or Val-Pyr enhanced homing and engraftment of both limited numbers of mouse bone marrow HSCs in lethally irradiated congenic mice[67] and human $CD34^+$ cells in immune deficient mice.[68-70] Furthermore, pretreatment of lethally irradiated congenic recipient mice with diprotin A enhanced the engraftment of non-treated mouse bone marrow cells.[71]

Other proteolytic enzymes implicated in negatively regulating SDF-1 activity include neutrophil elastase,[72,73] cathepsin G[72,73] and cathepsin K.[57] The importance of proteolytic enzymes for retention of HSCs in the bone marrow has also been investigated. An accumulation of various proteolytic enzymes including MMP-9, neutrophil elastase and cathepsin G or K[72,73] has, for example, been observed in mouse bone marrow upon G-CSF administration, which correlated with a gradually decrease in SDF-1 in the bone marrow.[72] Similarly, an enhanced SDF-1 plasma level was shown to result in upregulation of MMP-9 in the bone marrow and mobilization of hematopoietic stem and progenitor cells.[56] In contrast, a high level of SDF-1 in plasma was not sufficient to induce mobilization of HPCs in MMP-9 deficient mice.[56]

Molecular mechanisms underlying SDF-1 mediated regulation of migration. To understand the molecular mechanisms underlying migration of hematopoietic stem and progenitor cells, research has focused on identifying the downstream effectors of CXCR4 (**Fig. 2**). SDF-1 has, for example, been demonstrated to induce the activity of the integrins CD11a/CD18[10] and CD49/CD29[10,74] on $CD34^+$ cells which allow interactions with their substrates ICAM-1 and VCAM-1, respectively.

Small guanosine triphosphatases (GTPases) that belong to the Ras superfamily of GTPases, including Rho, Rac and Cdc42 can be activated by SDF-1.[75-78] An important mediator of SDF-1 induced HPC migration is Rho.[79-81] It has, for example, been demonstrated that SDF-1 mediated release of intracellular Ca^{2+} stores requires activation of Rho, but not Rac or Cdc42.[82] In addition, overexpresssion of dominant negative RhoA by retroviral transduction in mouse cells resulted in decreased migration of HPCs toward SDF-1 and reduced integrin-mediated adhesion.[82] Furthermore, overexpression of RhoH, a GTPase deficient type of Rho,[83] in hematopoietic stem and progenitor cells resulted in impaired activation of Rac GTPases, defective

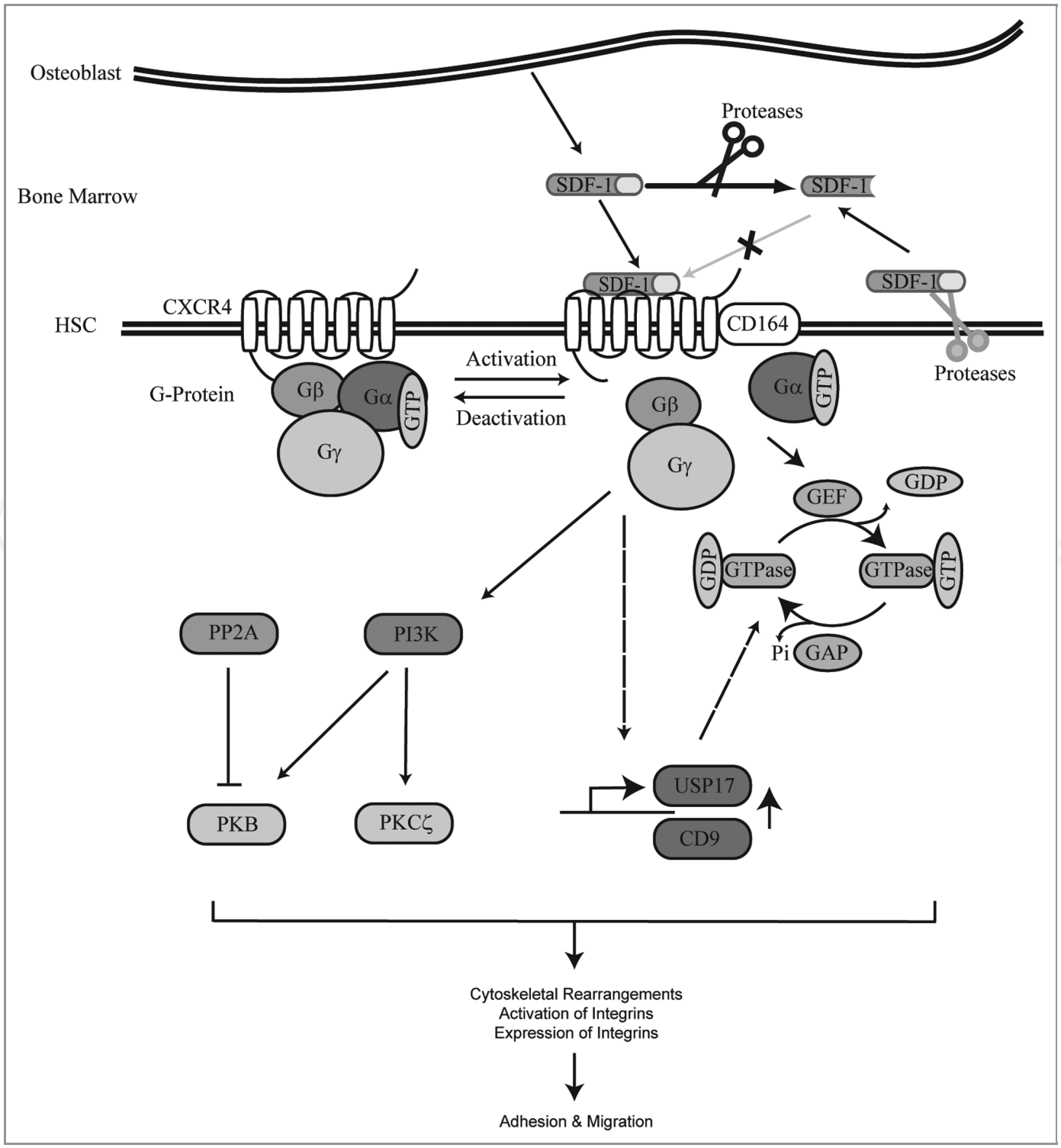

Figure 2. SDF-1/CXCR4 signaling cascade. SDF-1 is produced by bone lining osteoblasts in the bone marrow. Upon binding of SDF-1 to the G protein coupled receptor CXCR4, CD164 is recruited to the receptor and a downstream signaling cascade is activated. SDF-1 stimulation results in the activation of multiple signal transduction molecules. Proteins activated by SDF-1 that have been demonstrated to play an important role in HSC migration include PI3K and the GTPases Rac, Rho and Cdc42. In addition, activation of CXCR4 also results in upregulation of USP17 and CD9 at the transcriptional level. USP17 is involved in the translocation of Rac, Rho and Cdc42 to G-Proteins resulting in activation of these GTPases. Activation of CXCR4 eventually leads to cytoskeletal rearrangements, activation of integrins and migration of HSCs. SDF-1 can be cleaved by both extracellular and membrane-bound proteases thereby prohibiting activation of CXCR4.

actin polymerization and impaired chemotaxis. In contrast, inhibition of RhoH expression in these cells conversely stimulated SDF-1-induced migration in vitro.[84]

It has been demonstrated that SDF-1 induced chemo-attraction in vitro is, at least in part, also mediated by Rac.[76,77,85] Deletion of Rac2 was shown to be sufficient to enhance SDF-1 induced migration of hematopoietic stem and progenitor cells.[86] However, in Rac2 deficient cells, the activity of Cdc42 and Rac1 was enhanced, suggesting a compensatory role of Cdc42 and Rac1 in migration.[86] In addition, analysis of Rac2 deficient mice revealed that Rac2 is essential for lodging of HPCs in the bone marrow.[86] The hematopoietic-specific guanine nucleotide exchange factor Vav1 is an upstream regulator of Rac activity. Deletion of Vav1 in HSCs has been demonstrated to result in impaired responses to SDF1, deregulated Rac/Cdc42 activation, a reduction in in vitro migration and impaired localization in the bone marrow after transplantation.[87] Recently, R-Ras, a negative upstream regulator of Rac activity has been identified that is inhibited by SDF-1.[87] Deletion of R-Ras in hematopoietic stem and progenitor cells resulted in increased directional migration which could be reversed by inhibition of Rac.[87] Furthermore, R-Ras deficient mice showed enhanced responsiveness to G-CSF for progenitor cell mobilization and exhibited decreased bone marrow homing.[87] Finally, a role for Rap1, another GTPase, in regulation of SDF-1 induced migration has also been suggested. It has, for example, been demonstrated that Epac1, a nucleotide exchange protein for the GTPase Rap1, which is directly activated by cAMP, improves the adhesive and migratory capacity CD34$^+$ HPCs.[88]

CD164 (Endolyn), a type I integral transmembrane silomucin,[89,90] which is recruited to CXCR4 upon SDF-1 stimulation[91] was shown to play an important role in SDF-1 mediated migration of human CD133$^+$ hematopoietic stem and progenitor cells.[91] Both siRNA mediated knockdown of CD164 or inhibition of CD164 with the 103B2 mAb resulted in a specific reduction in migration of CD133$^+$ cells toward SDF-1.[91] Knock-down of CD164 resulted in a significant reduction in SDF-1 mediated activation of PI3K and PKCζ.[91] Both PI3K and PKCζ[92] have been implicated in playing an important role in SDF-1 mediated migration of CD34$^+$ cells. The role of PI3K in regulation of bone marrow homing will be discussed below.

SDF-1 not only regulates the activity of downstream effectors, but can also regulate the expression of specific target genes. A rapid increase in expression of the ubiquitin-specific protease 17 (USP17) has, for example, been observed in peripheral blood mononuclear, Jurkat and HeLa cells stimulated with SDF-1.[93] While inhibition of USP17 in HPCs was shown to be sufficient to decrease migration of these cells toward SDF-1, overexpression of USP17 conversely resulted in enhanced migration.[93] shRNA mediated inhibition of USP17 expression prohibited the transport of RAC1, Cdc42 and RhoA to the plasma membrane upon SDF-1 stimulation and resulted in decreased polymerization of actin and tubulin and reduced membrane ruffling.[93] Since CXCR4 levels were not affected by inhibition or overexpression of USP17, it is likely that USP17 modulates CXCR4 signaling. Another SDF-1 responsive gene is CD9, a member of the tetraspanin superfamily.[94,95] Inhibition of CD9, utilizing a neutralizing antibody, resulted in enhanced adhesion of progenitors to fibronectin and human umbilical vein endothelial cells and reduced transendothelial migration toward a SDF-1 gradient.[95] Transplantation experiments revealed that pre-treatment of human CD34$^+$ cells with a neutralizing CD9 antibody prior to transplantation results in reduced bone marrow homing.[95]

The PI3K/PKB Signaling Module and Bone Marrow Homing

It has been demonstrated that SDF-1 induces the activity of Phosphatidylinositol-3-Kinase (PI3K) and its downstream effector Protein Kinase B (PKB/c-Akt)[96] in leukemic cell lines.[97] A role for this signaling module in mediating SDF-1 induced migration of HSCs was therefore suggested. However, Protein Phosphatase 2A was shown to positively regulate SDF-1 induced migration of human HPCs by inhibition of PKB activity.[98] Similarly, inhibition of PKB activity in human CD34$^+$ cells for over 24 h resulted in a reduced capacity to adhere to bone marrow derived stromal cells and an induction of their basal migratory capacity.[99] Transwell migration experiments through a confluent layer of human umbilical vein endothelial cells revealed that the observed reduction in firm adhesion does not ameliorate the induced migratory capacity of CD34$^+$ cells pre-treated with a PKB inhibitor.[99] Furthermore, ectopic expression of constitutively active PKB in CD34$^+$ cells conversely induced firm adhesion and reduced the basal level of migration.[99] Although it cannot be excluded that transient activation of PI3K/PKB by SDF-1 is important for induction of migration, these studies indicate that prolonged activation of PKB activity is detrimental for migration of hematopoietic stem and progenitor cells. In agreement with this, it has been demonstrated that deletion of SHIP (SH2-containing inositol-5'-phosphatase), a negative regulator of PI3K,[100] in HSCs is sufficient to impair their ability to home to the bone marrow and spleen.[101] In addition, deletion of Phosphate and tensin homolog (PTEN), another critical negative regulator of PI3K signaling[102] in HSCs diminished their bone marrow homing capacity when these cells were transplanted into non-irradiated recipients where vacant niches are limited.[103] Constitutive activation of PKB in human HPCs cells was, similar to deletion of SHIP, shown to be sufficient to significantly inhibit migration of these cells to the bone marrow and spleen of recipient mice.[99] In addition, transient inhibition of PKB activity in human HPCs prior to transplantation was shown to conversely improve bone marrow homing.[99] However, ectopic expression of constitutively active PKB in mouse HPCs only modestly impaired bone marrow homing, 18 h after transplantation.[104] Together, these studies demonstrated that correct regulation of PI3K/PKB is essential for migration of hematopoietic stem and progenitor cells to the bone marrow after transplantation, which is a prerequisite for optimal engraftment and hematopoietic recovery.[99,101,104]

The molecular mechanisms underlying PKB mediated regulation of migration and bone marrow homing are, thus far, incompletely understood. As described above, the capacity of HSCs to migrate to the bone marrow after transplantation

depends on chemokines, integrins and selectins. A reduced CXCR4 expression was observed in SHIP deficient HSCs, suggesting that activation of PI3K impairs their response to SDF-1.[103] In addition, in NIH 3T3 fibroblasts, an important role for PKB and its downstream effector GSK-3 in recycling of the CD49e/CD29 and CD51/CD61 ($\alpha v\beta 3$) integrins to the membrane has been demonstrated.[105] Furthermore, ectopic expression of PKB in human hematopoietic stem and progenitor cells has been demonstrated to enhance the level of CD49d, while inhibition of PKB activity conversely reduces the expression of both CD49d and CD18.[99] Although it is evident that integrins play an important role in adhesion and migration of cells, the importance of these molecules in PKB mediated inhibition of migration remains to be investigated.

Conclusion and Future Perspectives

Bone marrow homing is a coordinated multistep process. While initial tethering and rolling of hematopoietic stem and progenitor cells along the endothelial wall of blood vessels are primarily regulated by specific selectins, various integrins have been shown to be involved in regulation of the next stages in this process; firm adhesion to the endothelial wall and trans-endothelial migration. Directional migration toward the hematopoietic stem cell niche in the bone marrow requires a chemokine gradient. Although SDF-1 appears to be the most prominent chemokine involved in this process, it has recently been demonstrated that proteolysis-resistant bioactive lipids can also act as chemoattractants for HSCs. The balance between migration of HSCs to and from the bone marrow is not only regulated by the level of SDF-1 in the bone marrow, but also by a variety of proteolytic enzymes that negatively regulate the activity of SDF-1. The molecular mechanism underlying SDF-1 mediated regulation of HSC migration has been investigated extensively and revealed that multiple signaling molecules, including several GTPases and PI3K, are activated upon SDF-1 stimulation. Although PI3K and its downstream effector PKB are activated upon SDF-1 stimulation, recent studies, however, have demonstrated that this signaling module plays a critical role in negatively regulating migration of HSCs and bone marrow homing.

Correct regulation of bone marrow homing process is a prerequisite for optimal hematopoietic recovery after HSC transplantation. Research has therefore focused on improving current stem cell transplantation regimes both either circumventing or enhancing bone marrow homing. Phase I/II clinical trials have, for example, been initiated to circumvent bone marrow homing by intra bone injection of UCB cells (NCT00696046; NCT01332006; NCT00295880) in patients. Thus far, only the latter trial has been terminated. However, the results of this trial did not reveal any improvement in the rate of engraftment as compared with historical results.[106] Alternatively, optimization of bone marrow homing may be achieved by improving initial tethering and rolling of HSCs to endothelial cells or enhancing chemoattractant induced migration. Preclinical studies in mouse models have revealed that both enhanced fucosylation of $CD34^+$ cells[1] and inhibition of CD26,[67-71] resulting in enhanced SDF-1 activity, is sufficient to enhance bone marrow homing and to induce engraftment levels. Phase I/II clinical trials have therefore recently been initiated to investigate whether enhanced fucosylation (NCT01471067) or CD26 peptidase inhibition using Sitagliptin (NCT00862719) would be sufficient to improve stem cell transplantation regimes in patients with hematological malignancies.

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