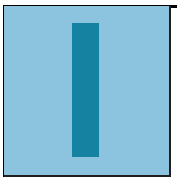

PART I
Stem cell biology

CHAPTER 1

Homing of stem cells and tissue injury

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Introduction

Hematopoietic stem cells continuously replenish the blood with immature and maturing leukocytes as part of homeostasis. Organ injury dramatically amplifies this process by the secretion of stress signals, which induce recruitment of progenitor and maturing cells from the bone marrow reservoir of leukocytes to the damaged tissue, as part of host defense and repair mechanisms. This chapter will review the reciprocal cross talk between injured tissues and the bone marrow reservoir and will point out key players in stem cell homing. The central roles of the chemokine SDF-1 (CXCL12) and its receptor CXCR4 in stem cell recruitment to inflamed/damaged tissues will be discussed.

The homing process: the roles of SDF-1 and CXCR4

Organ injury and/or inflammation because of viral or bacterial infections are accompanied by an increase in the levels of inflammatory cytokines and chemokines in the damaged organs and consequently in the peripheral blood [1]. These stress signals have been shown to recruit immature hematopoietic stem and progenitor cells as well as maturing leukocytes from the bone marrow reservoir to the circulation, which then home to the damaged liver, brain, heart and other nonhematopoietic organs [2–4], as part of host defense and repair mechanisms. The bone marrow contains a variety of cell types, including hematopoietic stem cells with self-renewal and multilineage differentiation capacity [5, 6] and nonhematopoietic stemcells, mesenchymal stem cells [7] and endothelial progenitor cells [8]. These bone marrow cells have been shown to contribute to tissue regeneration and to the recovery of damaged organs [3, 9] as well as to tissue neovascularization [8, 10].

Several physiological (e.g., physical activity [11]) and pathological (e.g., myocardial infarction, ischemia [12]) stimuli as well as clinical treatments (e.g., granulocyte-colony-stimulating factor [13], statins [14], estrogens [15]) increase the numbers of various bone marrow progenitor cell types in the circulation, with the potential of their migration to injured tissues. Major players in the regulation of this multistep process of cell mobilization and homing are the chemokine SDF-1 and its receptor CXCR4. SDF-1 (also termed CXCL12) is the only known powerful chemoattractant of murine [5] and human [16] hematopoietic stem cells. In early developmental stages, experimental deficiency of SDF-1 results in lethal cardiac defects, similar to those of CXCR4-deficient mice. CXCR4 and its ligand SDF-1 are constitutively expressed by murine and human bone marrow endothelial and endosteal bone lining stromal cells [6, 17–19], which both define the hematopoietic stem cell niches [20, 21]. Homeostatic expression of SDF-1 is also found in nonhematopoietic tissues, including skin [22], epithelial cells in the bile ducts of brain endothelium [2], liver [3], and heart [23, 24].

Many studies document the central roles of CXCR4 in navigating the homing of circulating human $CD34^+$ hematopoietic stem and progenitor cells through the blood-marrow barrier into their specialized niches in the bone marrow. This specific process is induced in response to presentation

of murine SDF-1 (which is cross-reactive with the human chemokine) on endothelial and other bone lining stromal cells in transplanted immune-deficient NOD/SCID mice (reviewed in [25]).

Certain stress-induced physiological and pathological conditions are characterized by SDF-1 elevation in the peripheral blood and within damaged organs, which contribute to the recruitment of $CXCR4^+$ homing cells. For example, after myocardial infarction, SDF-1 mRNA levels are markedly up-regulated in the murine heart and are involved in the chemoattraction of bone-marrow-derived cells [24]. In another model of ischemic cardiomyopathy in the rat heart, it was shown that forced expression of SDF-1 improves ventricular function post damage [26]. In addition, during focal cerebral ischemia, SDF-1 expression is increased in endothelial cells located within the lesioned brain areas and is assumed to induce CXCR4-dependent infiltration of circulating leukocytes [2]. A hint for the contribution of bone-marrow-derived $CD34^+$ progenitor cells to organ recovery was demonstrated using a brain stroke model in mice. The administration of human cord-blood-derived $CD34^+$ cells to mice that were previously subjected to stroke induced neovascularization by vascular endothelial growth factor (VEGF) secretion in the ischemic zone, provided a favorable environment for neuronal progenitor migration and regeneration [4]. In agreement with these observations, it was shown that during hypoxic conditions, the elevation of the transcription factor hypoxia-inducible-factor-1 in endothelial cells selectively up-regulates SDF-1 expression within ischemic regions *in vivo*, which in turn increases the recruitment of $CXCR4^+$ circulating progenitors into areas of reduced oxygen tension in murine skin, muscle, and bone marrow [10]. Furthermore, stress-induced signals such as inflammation, irradiation and hepatitis C virus infection of the murine or human liver result in elevation of SDF-1 amounts, accompanied by HGF (hepatocyte growth factor) expression, which then target human $CD34^+$ progenitors to the damaged liver [3]. Altogether, these results suggest a mechanism for tissue defense. There is also evidence for repair and regeneration by SDF-1-mediated recruitment of $CXCR4^+$ hematopoietic and endothelial precursors upon stress-induced conditions. Interestingly, selective expression of different SDF-1 isoforms has been reported. For example, in the brain, neurons express SDF-1-α, while endothelial cells selectively express SDF-1-β. During cerebral ischemia, transient and selective modulations in SDF-1 expression are believed to regulate distinct pathways for neuronal phenotype or cerebral infiltration [2]. In addition, although α and γ isoforms (but not SDF-1-β) of SDF-1 are abundantly expressed in heart tissue SDF-1, the amounts of SDF-1-α are selectively up-regulated after myocardial infarction [27]. Such modulations in SDF-1 isoform expression imply a specific functional role for different SDF-1 alternative splicing products, which has to be elucidated more broadly.

Inflammation and ischemic and hypoxic conditions are known to stimulate elevation in VEGF and SDF-1 levels [12, 28]. Both SDF-1 and VEGF are reported to be involved in sprouting and remodeling of preexisting blood vessels in the course of angiogenesis and mediate neovascularization [29, 30], for example, by recruiting endothelial cell precursors from the bone marrow [31], for wound healing or in pathological processes such as chronic inflammation or tumor growth [32].

Clinical protocols of DNA-damaging agents such as total body irradiation or chemotherapy have been shown to cause significant increase in SDF-1 levels in the bone marrow and spleen within 24–48 hours, leading to improved CXCR4-dependent homing of human $CD34^+$ stem and progenitor cells in transplanted NOD/SCID mice [17]. In contrast, repetitive administration of G-CSF (granuloycyte colony-stimulating factor), which is widely used in clinical protocols aimed at hematopoietic stem cell mobilization, markedly decreases SDF-1 expression in human and murine bone marrow [13]. Moreover, administration of the sulfated polysaccharide fucoidan, which competes with SDF-1 binding to heparan sulfate, resulted in a rapid and massive release of SDF-1 into the circulation, reduction in its levels in the bone marrow, and a significant increase in the levels of circulating hematopoietic stem and progenitor cells [33]. Supporting this notion, enforced increased levels of SDF-1 by adenoviral vectors [31], MetSDF-1 [34], or SDF-1 injections [35] lead to progenitor and stem cell mobilization. In another model of parabiotic mice with joint circulation, the dramatic elevation in the levels of G-CSF-mobilized stem cells correlated with increased repopulation

in the partner bone marrow, revealing that mobilized stem cells can efficiently home back to G-CSF-simulated bone marrow (mimicking stress injury and inflammation), in contrast to the low homing levels observed with steady state circulating stem cells to nonstimulated bone marrow [36]. This report demonstrates that during stress-induced mobilization, homing rates to the stressed organs are also augmented, confirming that mobilization and homing are sequential, physiological processes. Collectively, these results suggest that the up-regulation of SDF-1 in the injured organ and consequently in the circulation is a prerequisite first step, interfering with the steady state balance in the bone marrow reservoir, initiating mobilization and recruitment, which requires microenvironmental changes of the stem cell niche and its residents—the stem cells.

How do the distant injured organs transfer SDF-1 "stress signals" through the circulation into the bone marrow? The endothelium harbors a highly selective transport system for delivery of chemokines and other molecules across this mechanical barrier—a mechanism termed transcytosis—in which active transfer of proteins and molecules is mediated by transport vesicles (e.g., clathrin-coated pits, caveolae [37]). As such, endothelial cells in the blood-marrow barrier use their CXCR4 receptors to actively regulate SDF-1 levels by CXCR4-mediated functional transcytosis of this chemokine from the circulation into the bone marrow. This unique and potent capacity also characterizes other stromal cells, including cells of the endosteum region, which comprise the hematopoietic stem cell niche, but not bone marrow residing or circulating hematopoietic cells [19]. It is noteworthy that CXCR4-mediated translocation of functional circulating SDF-1 into the bone marrow can actively increase hematopoietic progenitor cell homing to this tissue, which is followed by stem cell mobilization and recruitment to injured organs. Importantly, this process uniquely defines the dual role of CXCR4-expressing tissue-anchored stromal cells in the bone marrow, spleen, and other organs. CXCR4 is capable of activating signaling pathways upon SDF-1 stimulation (e.g., migration, proliferation, proteolytic enzyme secretion, angiogenesis, and neovascularization) and can also regulate internalization and trafficking of its ligand potentially aimed at communication between organs and the bone marrow reservoir [19, 38].

Homing and the injured myocardium

SDF-1 appears to be a key factor that regulates trafficking of additional types of bone-marrow-derived stem and progenitor cells, such as endothelial progenitor cells [10, 39] and mesenchymal stem cells [7, 40], to ischemic/inflamed tissue. In accordance, local delivery of SDF-1 can enhance endothelial progenitor cell recruitment and neovascularization [41]. Several examples illustrate the contribution of bone-marrow-derived endothelial progenitor cells to improve cardiac function [10, 26, 41] and to enhance angiogenesis and neovascularization in several ischemic tissue models [42]. Of importance, inflammatory pathways in the injured organs also activate the recruitment of mature bone-marrow-derived cell types, which participate in mechanisms of tissue defense and repair as well. In addition, more differentiated bone-marrow-derived mature cells have been shown to establish perivascular niches prior to endothelial cell positioning and retention. Bone marrow recruitment of myeloid cells into injured heart and their retention in close proximity to angiogenic vessels is mediated by VEGF-induced expression of SDF-1 in activated perivascular myofibroblasts [43]. Moreover, it was shown that the homing of bone-marrow-derived hematopoietic c-kit$^+$ progenitor cells was accompanied by the recruitment of bone-marrow-derived mature natural killer cells in response to inflammation-secreted stress signals, which contributed to cardiac survival and repair after myocardial infarction [44]. However, in patients suffering from chronic ischemic coronary heart disease (ICMP), the ability of circulating endothelial progenitor cells to contribute to the neovascularization of the continuously inflamed heart is impaired with respect to number and functional activity. Note also that bone-marrow-derived mononuclear cells, isolated from patients with ICMP, have a significantly reduced migratory potential to a gradient of SDF-1 or VEGF, with reduced progenitor colony-forming activity *in vitro* and reduced neovascularization capacity *in vivo*, despite a similar content of hematopoietic progenitor cells, which would limit their therapeutic potential

for clinical cell therapy [45]. These results suggest that factors such as SDF-1, continuously secreted by the ischemic hearts of ICMP patients, can effectively reach the bone marrow, leading to desensitization and inhibition of endothelial and hematopoietic progenitors' migration and proliferation/differentiation.

Increased levels of SDF-1 in injured tissues are accompanied by modulations in the levels of additional factors, which actively participate in regulation of SDF-1 availability and function as well as in homing cell navigation and retention. Deficiency in nitric oxide synthase results in elevated SDF-1 levels in ischemic artery in a murine model, which is accompanied by increased numbers of circulating $Sca1^{+}c\text{-}Kit^{+}Lin^{-}$ stem cells [46]. Since this ligand also induces secretion of matrix metalloproteinases (MMPs), such as MMP2/9, SDF-1 elevation in damaged organs is also accompanied by an increase in and activation of various MMPs, which are involved in matrix degradation in the context of motility and *in vivo* migration of normal and malignant progenitor cells [3, 47].

Conclusion

In summary (schematically illustrated in Figure 1.1), dynamic SDF-1 and CXCR4 interactions regulate immature and mature bone-marrow-derived cell egress/mobilization in response to stress signals as well as their homing into injured organs aimed at tissue defense and repair mechanisms.

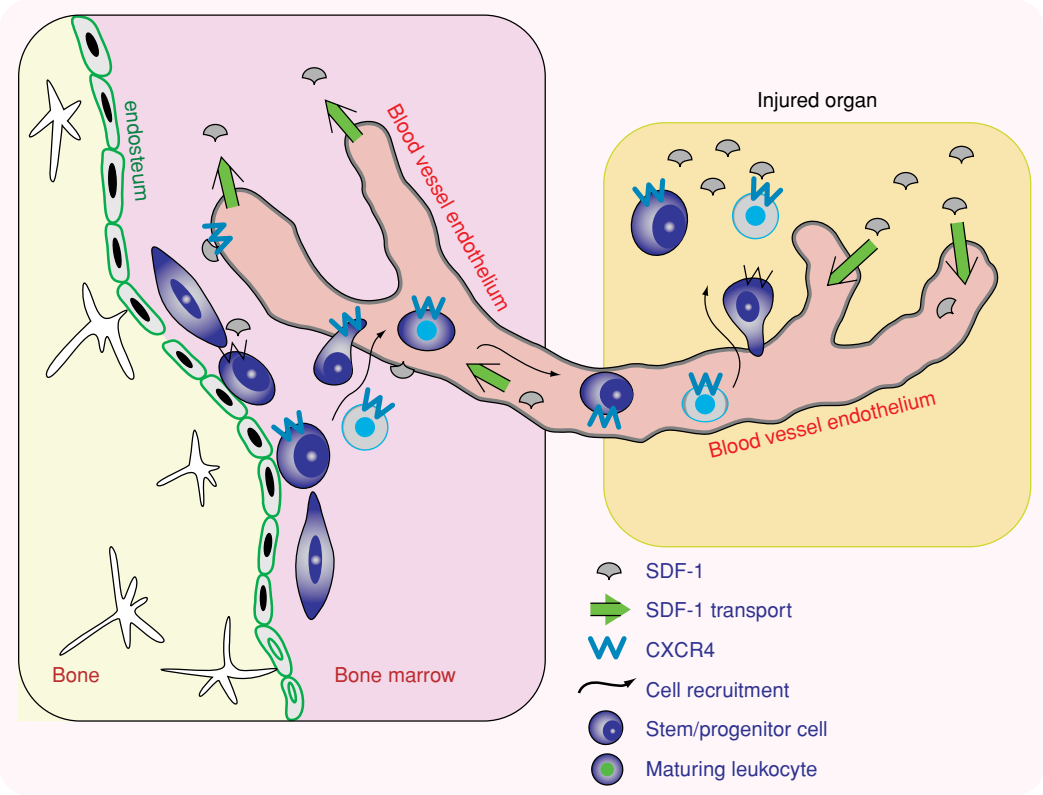

Figure 1.1 Immature and maturing cell recruitment mediated by SDF-1 transport: a model for communication between the injured organ and the bone marrow. Organ injury induces increased local production of SDF-1. Endothelial cells of the blood vessels translocate SDF-1 from the damaged tissue via the circulation into the bone marrow in a CXCR4-dependent manner. Presentation of the translocated SDF-1 by bone marrow endothelial and other stromal cells recruits CXCR4-expressing immature progenitors and stem cells as well as maturing leukocytes to the injured organ as part of host defense and organ repair.

Recent findings illustrate the participation of CXCR4 expressed by endothelial and other stromal cells in the bone marrow, spleen, and other organs, in regulating homing and retention of hematopoietic progenitors upon uptake and presentation of circulating functional SDF-1, leading to mobilization and recruitment of immature hematopoietic and endothelial progenitors to injured organs. These results suggest that CXCR4 expressed by stromal and endothelial cells actively participates in regulation of this mutual organ crosstalk during homeostasis and organ injury/damage. In cooperation with the bone marrow reservoir of hematopoietic and endothelial cells, $CXCR4^{+}$ progenitors and maturing cells with migration, proliferation, neovascularization, and defense potential participate in organ–bone marrow communication as part of host defense and repair mechanism. Taken together, these findings deepen our understanding of the significance of SDF-1 modulations in the circulation, bone marrow, and damaged organs, which accompany many pathological conditions and may contribute to the creation of improved clinical protocols.

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