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Chemokines and chemokine receptors in stem cell circulation

Katia Beider[1], Michal Abraham[1], Amnon Peled[1]

[1]Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Hospital, Jerusalem, 91120 P.O.B 12000, Israel

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1. ABSTRACT

Stem cells are rare, pluripotent, self-renewing cells that give rise to all mature cells during development and adult life. Due to their proliferative capabilities and their ability to home and contribute to the regeneration of damage tissue, stem cells can be transformed into established tumors. Stem cells can function as a double-edged sword -- they have the ability to circulate and migrate throughout the developing and mature adult organism, which is essential for their normal function; however, transformed stem cells are also endowed with the machinery to metastasize into various organs. Chemokine and chemokine receptors play a critical role in directing the trafficking of these cells. It is therefore evident that understanding the role of chemokines and their receptors in stem cell circulation is critical for the successful use of these cells in therapy for a wide variety of pathological conditions.

2. INTRODUCTION

Chemokines belong to a large family of small, chemotactic cytokines characterized by a distinctive pattern of four conserved cysteine residues. They are divided into two major (CXC and CC) and two minor (C and CX3C) groups, dependent on the number and spacing of the first two conserved cysteine residues. To date, the chemokine superfamily is composed of over 42 different human ligands and 19 overlapping chemokine receptors. Although originally identified on the basis of their ability to regulate the trafficking of immune cells, the biological role of chemokines have been shown to be involved in a number of biological processes, including growth regulation, hematopoiesis, embryologic development, angiogenesis, stem cell trafficking and tissue localization. Chemokine receptor-like sequences have been identified in mammals, birds, and fish, but not in invertebrates, plants, yeast or bacteria, suggesting a role for these proteins in the

Table 1. Chemokine receptor expression and function in stem cells

Stem Cell Type	Chemokine Receptor	Relevant Chemokine	Biological Activity	References
Hematopoietic Progenitor Cells (HPCs) and Stem Cells (HSCs)	CCR1 CCR5	CCL3	•Inhibits HSC proliferation •Induces mobilization of HSCs • Reduces hematopoietic damage	10 11 12
	CXCR1 CXCR2	CXCL8	• Induces mobilization of HPCs and HSCs	18,19,20,22, 23
	CXCR2	CXCL2	• Induces mobilization of HPCs and HSCs	26,27,28,29
	CXCR4	CXCL12	• Regulates migration and retention of HSCs and HPCs during embryo development • Regulates mobilization of HSCs to periphery, • Controls homing and engraftment of transplanted HSCs • Regulates mobilization of HSCs to non hematopoietic damaged organs (liver, kidney) and promotes regenerative processes	43,44 54,57-62 48,50 8,95
Primordial Germ Cells (PGCs)	CXCR4	CXCL12	•Regulates directional migration and survival	84-86
Murine embryonic stem cells (ESCs)	CXCR4	CXCL12	•Promotes chemotaxis, survival and differentiation of murine ESCs	88
Endothelial Progenitor Cells (EPCs)	CXCR4 CCR1, CCR3, CCR5 CCR2, CCR5	 CXCL12 CCL5 CCL2, CCL3-CCL5	•Regulates recruitment of EPCs and supports re-vascularization of ischemic tissue and tumor growth • Regulates homing and participation of EPCs in glomerular repair • Direct migration of EPCs into tumors	76 96 97
Neural Stem Cells (NSCs)	CXCR4	CXCL12	•Enhances proliferation of NSCs •Promotes migration of NSCs toward CNS damaged area	98-100
Progenitor Epithelial Cells (PECs)	CXCR4	CXCL12	• Promotes migration of PECs and re-epitheliazation of airways	101,102
Mesenchymal Stem Cells (MSCs)	CXCR4	CXCL12	• Regulates migration of MSCs to the site of myocardial infarction • Promotes development, survival and growth of MSCs • Regulates migration of MSCs under hypoxic conditions	108,109 110 113
	CCR2	CCL2	• Regulates migration of MSCs to the damaged brain	111,112
		CCL3, CXCL8	• Regulates migration of MSCs to the damaged brain	112
	CX3CR1	CX3CL1	• Regulates migration of MSCs under hypoxic conditions	113
Liver Oval Stem Cells	CXCR4	CXCL12	• Regulates migration of oval cells	77
Pancreatic Cancer Stem Cells (CSCs)	CXCR4	CXCL12	• Promotes tumor stem cell migration	135
Prostate CSCs	CXCR4	CXCL12	• Promotes tumor stem cell migration	136
Stem-Like Glioblastoma Cells	CXCR4	CXCL12	• Regulates survival and proliferation of CSCs	138
AML Stem Cells	CXCR4	CXCL12	• Regulates survival and proliferation of CSCs	139
Breast CSCs	CXCR4	CXCL12	•Metastatic spread of breast CSCs	130

A summary of Chemokine receptor expression and function in various stem cells is summarized and the relevant references are indicated

0rganization and surveillance of complex multi-cellular organisms. This review summarized the current knowledge on chemokine receptor expression and function in stem cells (Table 1).

3. THE ROLE OF CHEMOKINES IN HEMATOPIETIC STEM CELL CIRCULATION

Hematopoietic stem cells (HSCs) are rare, pluripotent, self-renewing cells that give rise to all mature blood cells through intermediates that are termed hematopoietic progenitor cells (HPCs) (1,2). The trafficking of HSCs is an essential physiological process which occurs during development. During fetal development, HSCs migrate from the aorta-gonad-mesonephros (AGM), into the embryonic liver and thereafter into the bone marrow (3,4). In the normal adult, the main site for HSCs retention and hematopoiesis is the bone marrow. HSCs continuously migrate between bone marrow and blood in such a way that, although circulating in the peripheral blood at very low frequency, HSCs are always available to correct potential deficiencies (5,6). HSCs have also been found to migrate into injury sites and this migration may serve as a potential source of pluripotent cells for the repair of damaged organs (7,8). Migration of HSCs also plays an essential role under some clinical and pathological conditions. Stem cell frequencies in the peripheral blood can be considerably increased in response to various growth factors. Recently, the use of the peripheral blood as a source of stem cells for patients undergoing autologous and allogeneic transplantation has replaced bone marrow as the preferred source of hematopoietic rescue. Therefore, an increased number of HSCs in blood following mobilization should improve transplantation efficiency. Mobilization of HSCs and HPCs is a complicated multifactorial multistep process involving multiple cell types, the microenvironment, proteases, growth factors, cytokines, adhesion molecules and chemokines. Experimentally, HSCs and HPCs are relatively easy to study; thus, accumulating evidence on

stem cell trafficking in general originates from studies performed using these cells. A number of chemokines have been reported to be involved in the homing, retention and mobilization as well as in the proliferation and survival of hematopoietic stem and progenitor cells.

3.1. Macrophage inflammatory protein- lα (MIP-lα)/CCL3

MIP-1α, a C-C chemokine known to be a monocyte chemoattractant *in vitro,* was reported to have chemoattractant and adhesive effects on lymphocytes. The functional receptors for MIP-1α have been identified as CCR1 and CCR5 (9). MIP-1α has been recognized as an inhibitor of hematopoietic stem cell proliferation that enhances stem cell recovery following cytotoxic treatment (10). MIP-1α, or a genetically engineered variant of human MIP-1α, BB10010, was found to induce mobilization of stem cells into the peripheral blood (11). BB-10010 was found to reduce the degree of accumulated hematopoietic damage after repeated sub-lethal irradiations (12) and, in a further model, enhanced leukocyte recovery and progenitor cell mobilization after cyclophosphamide (13). BB-10010 was also found to induce a rapid increase in the number of circulating hematopoietic progenitors and to further enhance the numbers by pretreatment with G-CSF. This effect was evaluated by bone marrow repopulating activity and CFUs enumeration. (11). Clinical use of human MIP-1α or BB-10010 during chemotherapy has demonstrated a significant, but modest, myeloid progenitor cell mobilizing capacity in patients with breast cancer (14), however it did not improve the ability of patients with high grade non-Hodgkin lymphoma to tolerate intensive chemotherapy (15).

3.2. Interleukin 8 (IL-8) /CXCL8

IL-8, a member of the C-X-C chemokine family, was previously known as neutrophil-activating protein (NAP-1) (16). IL-8 binds to both the CXCR1 and the CXCR2 receptors, and is responsible for a variety of biological effects including neutrophil activation and chemotaxis (17). In mice, injection of IL-8 alone results in the rapid mobilization of progenitor cells and pluripotent stem cells that are able to rescue lethally irradiated mice and to completely and permanently repopulate host hematopoietic tissues (18). In monkeys, a single intravenous injection of interleukin-8 induces a rapid mobilization of hematopoietic progenitor and stem cells (19). Supplementing mice pretreated with either G-CSF or SCF with IL-8 resulted in improved hematopoietic progenitor and stem cell mobilization compared with any of the agents used alone (20).

Mobilization induced by IL-8 was also found to involve activation of MMP-9 and the β2 integrin LFA-1, since anti-MMP-9 or anti-LFA-1 antibodies significantly reduced mobilization (17). MMP-9 was not found to be indispensable for mobilization induced by IL-8, since it was demonstrated that IL-8-induced mobilization was not affected in MMP-9-deficient mice. However, neutrophils were found to be indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice since IL-8-induced HSC/HPC mobilization was found to be abolished in neutropenic mice following administration of a depleting anti-GR-1 Ab (21). Taken together, these data suggest that the mechanisms by which IL-8 induced HSCs/HPCs mobilization appear to be dependent on sufficient numbers and normal function of circulating neutrophils, which are associated with increased MMP-9 activity, presumably released by mature neutrophils (22,23).

3.3. GROβ/CXCL2

GROβ is a member of the C-X-C family of chemokines which was found to promote neutrophil and basophil chemotaxis and degranulation. Like IL-8, GROβ protein is the ligand for the CXCR2 receptor, but unlike IL-8, it does not bind to the CXCR1 receptor (24). GROβ/CXCL2 and its N-terminal peptidase-processed variant (25) rapidly and transiently mobilized hematopoietic stem cells and neutrophils into the peripheral blood after a single injection, both in mice and in rhesus monkeys. Combination of GROβ with G-CSF resulted in augmented stem and progenitor cell mobilization compared with the use of G-CSF or GROβ alone (26,27). Peripheral blood stem cells mobilized by GROβ were found to reconstitute long-term hematopoiesis resulting from differential mobilization of early stem cells with enhanced homing and long-term repopulating capacity (28). The mechanisms by which GROβ induced mobilization was found to resemble those of IL-8 induced mobilization. Release and activation of MMP-9 has been implicated as a key mechanism involved in GROβ-induced stem cell mobilization. Analysis of MMP-9 activity by zymography in plasma of mice treated with GROβ variant demonstrates that plasma elevation of MMP-9 activity temporally precedes the detection of HPCs in blood (29). Similar data have been reported for the other CXCR2 ligand, IL-8-induced HPCs mobilization in primates (30). The ability of GROβ variant or IL-8 to mobilize hematopoietic stem and progenitor cells was found to be dependent on the CXCR2 receptor. In mice in which the CXCR2 receptor has been functionally deleted, neither IL-8 nor GROβ were able to mobilize HPCs into the peripheral blood (31,32). Furthermore, blocking CXCR1 had no effect on IL-8-mediated MMP-9 release, whereas blocking CXCR2 significantly reduced MMP-9 release. In addition, stimulating CXCR2 alone was sufficient to induce MMP-9 release (22).

3.4. Stromal cell-derived factor-1 (CXCL12/SDF-1)/CXCL12

CXCL12/SDF-1 is a member of the C-X-C chemokine family. It is generally believed that CXCL12/SDF-1 mediates its activity via a single high-affinity receptor, known as chemokine receptor 4 (CXCR4). CXCR4 is widely expressed by a variety of cell types including hematopoietic, endothelial, stromal and neuronal cells (33). Recently, an alternate receptor, known as CXCR7, was characterized to bind with high affinity to CXCL12/SDF-1 and to a second chemokine, interferon-inducible T cell alpha chemoattractant (I-TAC; also known as CXCL11) (34,35). Although most of the studies concerning the role of CXCL12/SDF-1 in mobilization were related to the CXCR4 receptor, we should take into account the existence of another receptor, CXCR7.

CXCL12/SDF-1 is constitutively produced in the bone marrow by stromal cells (36), by epithelial cells in many organs and by human and murine bone marrow endothelium (37). CXCL12/SDF-1 was found to be implicated in migration, proliferation, differentiation and survival of many cell types including human and murine hematopoietic stem and progenitor cells (38-42). The roles of the CXCL12/SDF-1/CXCR4 axis in the trafficking of hematopoietic cells have been extensively studied. Accumulating evidence suggests a pivotal role of the CXCL12/SDF-1/CXCR4 interaction in the mobilization and retention of HSCs and HPCs. Murine embryos lacking the chemokine CXCL12/SDF-1 or its receptor CXCR4 have multiple lethal defects. These studies revealed that these genes are necessary for the normal migration of HSCs from the fetal liver to the bone marrow and in the efficient retention of HSCs/HPCs in the adult bone marrow (43,44). Moreover, mobilized human CD34 progenitors were found to express reduced levels of the CXCL12/SDF-1 receptor CXCR4, which correlates with improved mobilization (45,46). Plasma elevation of CXCL12/SDF-1, delivered via an adenoviral vector, induced mobilization of mature and immature hematopoietic progenitor and stem cells with long-term repopulating ability (47). On the other hand, increasing CXCL12/SDF-1 levels within the recipient bone marrow led to higher levels of homing and repopulation (48). These data suggest that the mobilization of HSCs is controlled by the CXCL12/SDF-1 concentration gradient between peripheral blood and bone marrow (49). Preventing CXCL12/SDF-1CXCR4 interactions by neutralizing Ab directed against CXCR4 or CXCL12/SDF-1, or desensitizing CXCR4 expression on CD34+ cells prevented engraftment of repopulating stem cells, suggesting that homing and repopulation in transplanted mice with human CD34 stem cells is dependent on CXCR4 signaling (48,50). Furthermore, treatment with specific antagonists of CXCR4 induced rapid and robust HSC mobilization in both humans and mice (14,51,52). Granulocyte colony-stimulating factor (G-CSF) is routinely used in the clinic as a mobilizer of HSCs for transplantation. There is accumulating evidence that disruption of CXCL12/SDF-1/CXCR4 interaction is also a key step in HSC mobilization by G-CSF. During G-CSF-induced mobilization, the protein levels of CXCL12/SDF-1 in the bone marrow were found to drop sharply (53-55). This decrease was found to correlate with the magnitude of HSC mobilization (56). Moreover, a small-molecule antagonist of CXCR4 that inhibits CXCL12/SDF-1 signaling inhibited G-CSF–induced mobilization (54). The mechanism by which CXCL12/SDF-1/CXCR4 interaction mediates mobilization of HSCs is well characterized (57-60). CXCL12/SDF-1 was found to mediate the secretion of MMP-2 and MMP-9 from human CD34 cells (61), and these proteolytic enzymes inactivate CXCL12/SDF-1 by cleaving a few amino acids in the N terminus (62). G-CSF was found to reduce CXCL12/SDF-1 while increasing CXCR4 in the bone marrow. This gradual decrease of CXCL12/SDF-1 bone marrow concentration was found to be mostly due to its degradation by neutrophil elastase, correlated with stem cell mobilization (54). Furthermore, in addition to inactivation of bone marrow CXCL12/SDF-1 by proteolytic enzymes, these enzymes can also cleave part of the CXCR4 receptor in the bone marrow (53). As suggested by Cottler-Fox at al., the bone marrow serves as a reservoir for hematopoietic cells that can be released into the circulation upon stress signals, migrate to injured sites, and contribute to host defense and tissue repair. Disruption of the steady-state balance in the bone marrow leads to the transient increased production of CXCL12/SDF-1, and proliferation and activation of neutrophils and osteoclasts. The release of proteolytic enzymes is followed by shedding of membrane-bound SCF, proliferation of hematopoietic progenitors, increased surface CXCR4 expression and inactivation of CXCL12/SDF-1, G-CSF, the bone marrow adhesion machinery, and extracellular matrix (ECM). These events are intensified in each cycle of stimulation by G-CSF, eventually leading to the release of progenitors into the circulation (60). In summary, the bone marrow is a complex and dynamic tissue that contains a variety of cells organized among different microenvironments. The stem cell niche is a specialized microenvironment where the stem cell pool, cells with low proliferation and motility rate, are self renewing. The progenitor's microenvironments are made of highly proliferating cells with increased migratory potential. The mature cell microenvironments are areas where proliferation is reduced and cells are hyper-motile and looking for their release. The ability of cells to mobilize from their microenvironments into the circulation is dependent on the retention signals provided by CXCL12/SDF-1 and the adhesion molecules such as VLA-4 and LFA-1 and their intrinsic motility. These important factors can be regulated by the production of proteases from neutrophils that will “distract” the CXCR4/CXCL12/SDF-1 axis. Under normal conditions, these processes will be sufficient for the release of neutrophils, although stem and progenitor cells will be released into the blood only at low levels. Under stress, or after injection of G-CSF, GM-CSF, CXCR1/2 and other neutrophil-activating factors, the CXCR4/CXCL12/SDF-1 axis are blocked leading to the release of stem and progenitor cells from their microenvironments. Their release into the circulation is also a result of increase in their intrinsic motility. Indeed, distracting the CXCR4/CXCL12/SDF-1 axis with the CXCR4 antagonist T140 induces the release of all types of stem, progenitor, and mature cells such as T, pre B cells, neutrophils from the bone marrow (51).

4. THE ROLE OF CHEMOKINES IN EMBRYONIC AND TISSUE-SPECIFIC STEM CELL CIRCULATION

The chemokine receptor CXCR4 is unusually widely expressed in most mature and hematopoietic stem and progenitor cell types, including neutrophils, monocytes, T lymphocytes, B cells, B cell precursors, CD34+ progenitor cells from blood and bone marrow, blood-derived dendritic cells, Langerhans cells, T cells and macrophages, and both mature and immature T cells in thymus (63-66). It is also expressed at high levels on vascular endothelial cells (67), neurons from both the central and peripheral nervous systems (68), and microglia and astrocytes (69). Thus, the role of the CXCL12/SDF-1–CXCR4 axis was initially investigated with respect to

hematopoietic cells. However, recent evidence indicates that other than HSCs, functional CXCR4 is also expressed on the surface of various kinds of embryonic and tissue-specific stem cells such as neural stem cells (70,71), skeletal muscle satellite (72), myocardial (73), endothelial progenitors (74-76), liver oval cells (77) and retinal pigment epithelium (78,79). However, in contrast to mature cells which express a variety of chemokine receptors, stem cells express a limited number of chemokine receptors and predominantly use the CXCR4 receptor for trafficking and survival.

It is therefore not unexpected when it was reported that genetic disruption of the chemokine receptor/chemokine CXCR4/CXCL12/SDF-1 axis in mice resulted in a lethal phenotype (80-83). The animals died in the perinatal period from a variety of ailments including ventricular septal defects, defective gastric vasculogenesis and cerebellar development, abnormal bone marrow myelopoiesis, and defective B cell lymphopoiesis. Moreover, recent studies have shown that knocking down CXCL12 or its receptor CXCR4 resulted in a severe primordial germ cell (PGCs) migration defect in zebrafish. Specifically, PGCs not receiving the CXCL12 signal exhibit lack of directional movement toward their target and arrive at ectopic positions within the embryo (84,85). In mice, germ cell migration and survival require the CXCL12/SDF-1/CXCR4 pair, and it is specifically required for the colonization of the gonads by primordial germ cells. However, this involvement is not necessary in the earlier stages of germ cell migration, demonstrating a remarkable degree of evolutionary conservation (86). Murine embryonic stem cells (ESCs) secreted low levels of CXCL12/SDF-1 and expressed low levels of CXCR4; however, both increased with differentiation of ESCs. Endogenously produced or exogenously added CXCL12/SDF-1 enhanced survival of ESCs in the presence of the leukemia inhibitory factor. Furthermore, the CXCL12/SDF-1/CXCR4 axis induced chemotaxis of ESCs. The endogenous and exogenous CXCL12/SDF-1/CXCR4 axis enhanced embryoid body production of primitive and definitive erythroid cells, granulocyte-macrophage, and multipotential progenitors. These results suggest a role for the CXCL12/SDF-1/CXCR4 axis in the survival, chemotaxis, and differentiation of murine ESCs (87). Indeed, recent studies have demonstrated that activin A–induced endoderm in ES cell differentiation cultures can be enriched using the CXCR4 surface receptors (88). Thus, the CXCR4/CXCL12/SDF-1 axis plays an important role in the organization of tissues during development by regulating stem cell trafficking survival and localization.

In addition to their critical role in the development of various tissues, stem cells play a critical role in the regeneration of tissues following damage. A fine interaction between the supportive stroma, the functional cells, neovascularization, and the immune system will govern successful repair and regeneration of damaged tissue. During this process, in epithelial, mesenchimal, neuronal, endothelial, and hematopoietic tissues, specific stem cells play an important therapeutic role in the reconstruction of the functional tissue. These stem cells can either enter from the circulation or migrate from within the tissue into the damaged area. The ability of such stem cells to home to injured tissues and migrate within the damage tissues into their final localization for tissue repair is critical for their function. Both CXCR4 and its ligand CXCL12/SDF-1 are upregulated in response to hypoxia (89,90). CXCL12/SDF-1 is also upregulated following chemotherapy, irradiation and burns (42,91). CXCL12/SDF-1 gene expression is regulated by the transcription factor hypoxia-inducible factor-1 (HIF-1) in endothelial cells, resulting in the selective in vivo expression of CXCL12/SDF-1 in ischemic tissue in direct proportion to reduced oxygen tension (92,93). Indeed, overexpression of CXCL12/SDF-1 in the heart following myocardial infarction was shown to induce the recruitment of endothelial progenitor cells (EPCs) that express CXCR4 and to accelerate the recovery of the wounded muscle (90,94).

Bone marrow (BM) hematopoietic stem cells (HSCs) have been shown to facilitate regeneration in multiple nonhematopoietic tissues by either generating epithelial cells or altering the inflammatory response. Depending on injury type, the predominant mechanism of epithelial lineage regeneration occurs by spontaneous cell fusion or transdifferentiation. Irrespective of the mechanism, mobilization from the BM is a prerequisite. Mechanisms by which HSCs mobilize into damaged organs are currently unclear. Murine and human studies have shown that the chemokine CXCL12/SDF-1 and its receptor CXCR4 participate in the mobilization of HSCs from the BM and in the migration of HSCs to injured liver (8). CXCL12/SDF-1 is a potent HSC chemoattractant and is produced by the liver. This production is increased during liver injury leading to increased HSC migration to the liver, a finding diminished by neutralizing anti-CXCR4 antibodies. Additional factors have been implicated in the control of hepatic migration of HSCs such as the chemokine IL-8, hepatocyte growth factor, and MMP-9 (8). Hepatic oval “stem” cells are recognized as playing an important role in the etiology of liver growth and development, as well as in hepatic carcinogenesis. In massive liver injury models where oval cell repair is involved, hepatocytes up-regulate the expression of CXCL12/SDF-1, potent chemoattractants for hematopoietic and endothelial stem cells. Furthermore, oval cells express CXCR4 and migrate in response to CXCL12/SDF-1. The CXCR4/CXCL12/SDF-1 axis may therefore play a role in both an autocrine and a paracrine manner to support oval-dependent liver regeneration (77).

The capacity of the kidney to regenerate functional tubules following episodes of acute injury is an important determinant of patient morbidity and mortality in the hospital setting. Recent studies have indicated that adult stem cells, either in the kidney itself or derived from the bone marrow, could participate in this repair process and might therefore be utilized clinically to treat acute renal failure (ARF). The injured murine kidney has been shown to recruit different leukocyte populations, including bone-marrow-derived and peripheral-blood stem cells. CXCL12/SDF-1 is expressed in the normal mouse kidney

and tubular cells express CXCR4. CXCL12/SDF-1 expression in the kidney increases after ischemia/reperfusion (I/R)-induced ARF and decreases in the bone marrow, thereby reversing the normal gradient between BM and the periphery. This process may cause mobilization of CD34-positive cells into the circulation and their subsequent homing to the kidney with ARF. In vitro and in vivo chemotaxis of bone marrow cells toward damaged kidney epithelium was reversibly inhibited by anti-CXCR4 antibodies. These results suggest that CXCL12/SDF-1 may have a role in kidney repair (95). The chemokine RANTES (regulated upon activation, normal T-cell expressed and secreted, CCL5) has been proposed as a promising target for therapeutic intervention in renal disease. RANTES is a member of the CC-chemokine family and a ligand for a number of chemokine receptors, including CCR1, CCR3, and CCR5. Inhibition of the RANTES receptor, CCR5, was shown to be associated with decreased inflammatory infiltrates and reduced inflammatory reaction in several glomerulonephritis models. The participation of BM-derived endothelial stem cells in glomerular repair, glomerular monocyte infiltration, and proteinuria was evaluated using the RANTES antagonist Met-RANTES. RANTES receptor inhibition specifically reduced the participation of BM-derived cells in glomerular vascular repair by more than 40% without impairing monocyte influx. Blockade of RANTES receptors on CD34+ cells in vitro partially inhibited platelet-enhanced, shear-resistant firm adhesion of the CD34+ cells to activated endothelium. These data suggest that RANTES receptors may be involved in the homing and participation of BM-derived endothelial cells in glomerular repair (96). Overall, the significance of recruited BM-derived stem cells to the regenerating liver and kidney and the role of chemokines and their receptors are still fully unclear.

Proangiogenic bone marrow-derived EPCs are mobilized from the bone marrow into the circulation and then enter into damaged tissues; under permissive conditions, EPCs have the ability to differentiate into functional vascular cells. Recent evidence demonstrates that the CXCR4/ CXCL12/SDF-1 axis plays a major role in the mobilization, recruitment and retention of CXCR4 + EPCs to the neo-angiogenic niches supporting revascularization of ischemic tissue and tumor growth (76). In addition to the CXCR4/CXCL12/SDF-1 axis, it was recently reported that EPCs express functional CCR2 and CCR5 and that their trafficking into tumors may be also directed by tumor cells expressing the chemokines CCL2 and CCL3-5 (97).

Intra-tissue migration toward pathology is a critical step in stem cell engagement during regeneration. Neural stem cells (NSCs) migrate through the parenchyma along non stereotypical routes in a precisely directed manner across great distances to injury sites in the CNS. NSCs home similarly to pathologic sites derived from disparate etiologies in the brain suggesting common molecular mechanisms. Indeed, it was demonstrated that human NSCs migrate in vivo toward an infracted area (a representative CNS injury), where local astrocytes and endothelium up-regulate CXCL12/SDF-1. NSCs express CXCR4 and the exposure of CXCL12/SDF-1 to quiescent NSCs enhances proliferation, promotes chain migration and transmigration. CXCR4 blockade abrogates pathology-directed chain migration of NSCs (98-100). These results implicate the CXCR4/CXCL12/SDF-1 axis as a key step in the intra-tissue trafficking of NSCs towards damaged areas. Recipient airway epithelial cells are found in human sex-mismatched lung transplants, suggesting that circulating progenitor epithelial cells contribute to the repair of the airway epithelium. In mice, it was recently demonstrated that a population of PECs exists in the bone marrow and the circulation. These cells are positive for the early epithelial marker cytokeratin 5 (CK5) and the chemokine receptor CXCR4. Using a mouse model of sex-mismatched tracheal transplantation, it was found that CK5 circulating progenitor epithelial cells contributed to re-epithelialization of the airway and that the re-establishment of the pseudostratified epithelium was dependent on CXCR4/CXCL12/SDF-1 usage. The presence of CXCL12/SDF-1 in tracheal transplants provided a mechanism for CXCR4-dependent trafficking to this site (101,102).

Human MSC populations are located in the bone marrow and are distinguishable in their behavior from HSCs by being negative for the cell surface markers CD14/CD34 and CD45. As previously alluded, MSCs differentiate into osteogenic cells; their differentiation into bone, cartilage and fat has been extensively characterized and is induced in vitro using specific growth factors and chemical agents (103-105) . Moreover, under specific conditions, MSCs can differentiate into HSCs, neural cardiac cells, skeletal muscle and smooth muscle (105). MSCs have been shown to differentiate into lineage-specific cells within the microenvironment into which they are transplanted. Recently, the use of MSCs has provided clinical benefit to patients with osteogenesis imperfecta, graft-versus-host disease and myocardial infarction. MSCs can be easily isolated from the bone marrow (106,107). However, due to their low numbers, attempts to isolate MSCs from peripheral blood have been characterized by a low yield. In contrast to HSCs that express mainly the CXCR4 receptor, MSCs have been shown to express a variety of chemokine receptors, and to date CCR1, CCR2, CCR3, CCR4, CCR6, CCR7, CCR9, CCR10, CXCR4, CXCR5,CXCR6,CX3CR1 have been detected in human MSCs (105). However, an in vivo functional role of MSC trafficking was only documented for CXCL12/CXCR4. Using the CXCR4 antagonist AMD3100, it was found that the interaction of CXCL12 with CXCR4 was critical for the migration of MSCs to the damaged heart; moreover, CXCL12/SDF-1 expression by mesenchymal stem cells resulted in trophic support of cardiac myocytes after myocardial infarction (108,109). Furthermore, the CXCR4 ligand, CXCL12/SDF-1, was shown to promote the growth, survival and development of MSCs; in addition, MSCs are known to be able to synthesize CXCL12/SDF-1, thus having the potential to act in an autocrine manner via CXCR4 (110). A role for the chemokine CCL2 in mediating MSC migration to the brain was suggested. After ischemic brain injury, the level of CCL2 was

significantly higher in ischemic brain tissue extract. The brain tissue extract was chemotactic for MSCs in vitro and this migration was significantly diminished in the presence of a neutralizing CCL2 antibody (111,112). CCL3 and CXCL8 were also suggested to be involved in MSC migration to damaged cerebral tissue (112). Recently, it was shown by Hung SC et al., that when MSCs were exposed to hypoxic conditions, they can upregulate the chemokine receptors CX3CR1 and CXCR4 and migrate better in response to their ligands fractalkine and CXCL12/SDF-1. Blocking antibodies for the chemokine receptors significantly decreased the migration. Xenotypic grafting into early chick embryos demonstrated cells from hypoxic cultures engrafted more efficiently than cells from normoxic cultures and generated a variety of cell types in host tissues. The results suggest that culture of MSCs under hypoxic conditions may provide a general method of enhancing their engraftment in vivo into a variety of tissues (113).

Stem cells from various sources are being explored for the development of novel therapeutics for a variety of genetic disorders and pathological conditions such as Parkinson, multiple sclerosis, cancer, etc. If stem cell therapies are to succeed, it is of paramount importance to understand and control the homing, intra-tissue localization, and retention of stem cells to and within their target organ. Understanding the role of chemokine and chemokine receptor expression and function in stem cells and in the target organs is therefore a prerequisite for the future treatment and management of life-threatening diseases.

5. THE ROLE OF CHEMOKINES IN CANCER STEM CELL CIRCULATION

Recent evidence suggests that stem cells may play an important role not only in the maintenance of normal tissues, but also in the development and progression of malignant tumors (114,115). Accumulated data support the hypothesis that tissue-committed stem cell distribution to different organs may be the origin of cancer development. Long-lived stem cells may become preferential targets of accumulating oncogenic mutations that may promote initiation and progression of cancer (116). Similar to normal stem cells, cancer stem cells (CSCs) have an ability to self-renew and generate heterogeneous phenotypes of tumors (117,118). The first experimental evidence for defining CSCs was provided by the John Dick group on human leukemia (58,119). Later, human CSCs were identified in several solid tumors including breast (120), brain (121), lung (122), colon (123), prostate and pancreas (118). There are two possible origins of CSCs and experimental evidence supporting both theories exists. CSCs can derive from normal stem cells and due to their accumulated oncogenic mutation, acquire a cancerous phenotype (124). Alternatively, oncogenic transformation can occur in committed progenitor cells that will subsequently regain the ability to self-renew and become a source of CSCs (125-127). Similar to normal stem cells, CSCs exist in a prolonged state of quiescence; this makes CSCs relatively resistant to chemotherapy agents that target only dividing cells, and can thus explain the recurrence of tumors after therapy. Additionally, CSCs were proposed to be responsible for tissue-specific metastasis (118). Surface markers have been used to describe and identify different CSCs. For example, hematopoietic CSCs were identified as CD34+CD38- cells (119), and breast CSCs - as CD44+CD24-/low ESA+ cells (117). Another common surface marker of CSCs is a neural stem cell marker CD133, which is expressed by brain (121), prostate (128) and pancreatic CSCs (118).

Therefore, CSCs are a small population of tumor cells capable of initiating tumor formation, producing heterogeneous cell hierarchy of tumor, contributing to tumor maintenance and, most importantly, can promote metastasis and tumor growth at distinct sites. It is important to understand the molecular mechanisms regulating CSCs properties so that novel therapeutic strategies for specific CSC eradication can be developed. Recent evidence indicates that chemokines and chemokine receptors may play a crucial role in the several steps leading to tumorigenesis and metastasis. Chemokines regulate tumor cell survival and growth, tumor angiogenesis, and metastatic spread (129). However, the most commonly found chemokine receptor in cancer cells is CXCR4. The role of the CXCR4/ CXCL12/SDF-1 axis was extensively investigated in cancers of different origins. Current reports suggest that CXCR4 may be a key regulator of tumor invasiveness leading to local progression and tumor metastasis (98,130,131). Originally, the CXCR4/ CXCL12/SDF-1 pair was identified as responsible for the trafficking and homing of hematopoietic stem cells, pre-B lymphocytes and T lymphocytes (39,132,133). Later on, functional CXCR4 was detected on the surface of various tissue-committed stem cells. CXCR4 is involved in stem cell trafficking during development, tissue injury and regeneration. Since CXCR4 is involved in both normal stem cell function and carcinogenesis, it is reasonable to assume that CXCR4 plays an important role in cancer stem cell biology. Since CXCR4 is expressed in normal tissue-committed stem cells, it may help explain the common presence of CXCR4 on the surface of tumor cells derived from these tissues. It has been postulated that the metastatic spread of cancer cells on the one hand, and trafficking of normal stem cells on the other, share similar mechanisms (134). Due to their tumor-initiating capacity, it is now generally assumed that circulating CSCs are responsible for organ-specific metastasis.

Cancer metastasis is a complex, multi-step process in which cancer cells detach from the primary tumor mass, enter the blood circulation, sense a chemoattracting gradient, attach to the endothelium, invade the tissue and finally survive and proliferate in a foreign microenvironment. The possible role of the CXCR4/ CXCL12/SDF-1 pair in the characteristic successive steps of the CSCs metastatic process will be discussed below. It has not yet been investigated whether the CXCR4/ CXCL12/SDF-1 axis participates in the detachment of CSCs from the primary tumor, similarly to its role in the mobilization of hematopoietic stem cells from the bone marrow into the blood. However, numerous experimental data support the possible role of CXCR4 in motility and directed chemotaxis of CSCs. The study of Hermann P and

colleagues defined a subpopulation of pancreatic CSCs that co-expressed CD133 and CXCR4 and determined the metastatic phenotype of the tumor. Migration of invasive pancreatic CSCs was primarily mediated through activation of the CXCR4 receptor. Orthotopic injection of tumor-initiating CXCR4+ cells produced local tumor and metastasis. However, the depletion of CXCR4+ cells from the CSCs pool abrogated the metastatic phenotype of pancreatic tumor without affecting their tumorigenic potential (135). In addition, the role of CXCR4 in mediating the motility and migration of CSCs was shown in prostate CSCs. Miki and colleagues used the model of hTERT-immortalized malignant RC-92a tumor-derived prostate epithelial cells that retain stem cell properties with a CD133+CD44+α2β1 phenotype. In their report, it was demonstrated that RC-92a/hTERT stem-like cells responded to CXCL12/SDF-1 stimulation with an increased migration, which was inhibited using anti-CXCR4 antibodies. Co-expression of CD133 and CXCR4 was also detected by immunostaining in clinical human prostate cancer specimens, further supporting the involvement of CXCR4 in prostate CSC pathophysiology (136).

CXCR4 may regulate the survival and proliferation of CSCs. Mitotic activity of CXCR4 was demonstrated in various tumor cells, including glioma (137). In a study by Andrea Salmaggi and team, CXCR4 expression was determined in cancer stem-like cells obtained from human glioblastoma (tumospheres). These CXCR4-positive tumorospheres demonstrated a proliverative response to CXCL12 in vitro, and following transplantation into the brain of NOD/SCID mice, tumors that clearly expressed both CD133 marker and CXCR4 were produced (138). It was also shown that CXCR4 regulates the motility and survival of human AML stem cells (139). CXCR4 may also be involved in the metastatic spread of breast CSCs. As mentioned above, breast CSCs have been identified as CD44+CD24-/low ESA+ cells (117). It was previously shown that the expression levels of CD24 and CXCR4 were inversely correlated with each other (140) and that the CXCR4/ CXCL12/SDF-1 pathway was involved in the metastasis of breast cancer cells (130). Thus, it can be postulated that CXCR4-expressing breast CSCs spread into the distinct organs in a CXCL12/SDF-1-dependent manner. However, this has not yet been proven.

Taken together, summarized data indicates a critical role of the CXCR4/CXCL12 pathway in regulating the retention, mobilization, invasion and outgrowth of human CSCs from different origins. Nevertheless, many questions regarding the interplay of chemokines and other multiple molecular pathways in human CSC biology presently remain unanswered. Further investigations may enable to determine and target the specific chemokine/chemokine receptor pathways critical to CSCs and possibly help eradicate resistant tumor-forming CSCs.

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Abbreviations: CCL: CC chemokine ligand; CXCL: CXC chemokine ligand; CX3CL: CX3C chemokine ligand; XCL: C chemokine ligand; CCR: CC chemokine receptor; CXCR: CXC chemokine receptor; MSC: mesenchimal stem cells; HCS: Hematopoietic stem cells; HPCs: hematopoietic progenitor cells; PGC: primordial germ cell; EPCs: endothelial progenitor cells; NSCs: neural stem cells; CSCs: cancer stem cells.

Key Words: Chemokine, Chemokine receptor, Stem cells, Review

Send correspondence to: Amnon Peled, Hadassah Hebrew University Hospital, Gene Therapy Institute, Jerusalem, 91120 Israel, Tel: 972-2-677-8780, Fax: 972-2-6430982, E-mail: peled@hadassah.org.il

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