

CXCL12 (SDF-1)/CXCR4 Pathway in Cancer

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Abstract

Chemokines, small proinflammatory chemoattractant cytokines that bind to specific G-protein-coupled seven-span transmembrane receptors, are major regulators of cell trafficking and adhesion. The chemokine CXCL12 [stromal cell-derived factor-1 (SDF-1)] binds primarily to CXC receptor 4 (CXCR4; CD184). The binding of CXCL12 to CXCR4 induces intracellular signaling through several divergent pathways initiating signals related to chemotaxis, cell survival and/or proliferation, increase in intracellular calcium, and gene transcription. CXCR4 is expressed on multiple cell types including lymphocytes, hematopoietic stem cells, endothelial and epithelial cells, and cancer cells. The CXCL12/CXCR4 axis is involved in tumor progression, angiogenesis, metastasis, and survival. This pathway is a target for therapeutics that can block the CXCL12/CXCR4 interaction or inhibit downstream intracellular signaling.

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Background

Chemokines are 8 to 12 kDa peptides that function as chemoattractant cytokines involved in cell activation, differentiation, and trafficking. Chemokines bind to specific G-protein-coupled seven-span transmembrane receptors (1–3). Most chemokines bind to multiple receptors, and the same receptor may bind to more than one chemokine. The chemokine CXCL12 (stromal cell-derived factor-1) binds to the receptors CXC receptor 4 (CXCR4, CD184) and CXC receptor 7 (RDC1, CXCR7; refs. 1, 4–7). CXCR4 or CXCL12 gene knockout in mice results in impaired hematopoiesis exhibited as a defect in trafficking of hematopoietic stem cells (HSC) from the fetal liver to the embryonic bone marrow, and defects in heart and brain development, and vascularization. Thus, CXCR4 and CXCL12 knockouts are embryonic lethal (8, 9).

CXCL12 is a homeostatic chemokine. The major function of the homeostatic chemokines is to regulate hematopoietic cell trafficking and secondary lymphoid tissue architecture. CXCL12 knockout studies show that bone marrow colonization during the third trimester of gestation is controlled by CXCL12/CXCR4 pathway function. The CXCL12/CXCR4 pathway function in adults is integral to the retention and homing of HSC in the bone marrow microenvironment and lymphocyte trafficking. CXCL12 is constitutively expressed in several organs including lung, liver, skeletal muscle, brain, kidney, heart, skin, and bone marrow. CXCL12 secretion is also associated with tissue damage such as heart infarct, limb ischemia, toxic liver

damage, excessive bleeding, total body irradiation, and after tissue damage related to chemotherapy. CXCR4 is expressed by endothelial cells and pericytes of hypoxic, injured, or pathological tissues, including injured carotid arteries and atherosclerotic plaques. Finally, endothelial precursor cells express and secrete CXCL12.

CXCR4 is widely expressed on hematopoietic cells including CD34⁺ HSC, T-lymphocytes, B-lymphocytes, monocytes and macrophages, neutrophils and eosinophils as well as by brain, lung, colon, heart, kidney, and liver, and endothelial and epithelial cells, microglia, astrocytes and neuronal cells, and progenitor cells including endothelial and smooth muscle progenitors. Functional CXCR4 is expressed on embryonic pluripotent stem cells and several types of tissue-committed stem cells, for example, neural tissue, skeletal muscles, heart, liver, endothelium, and renal tubular- and retina pigment-epithelium (8). These cells express functional CXCR4 and migrate and/or invade along CXCL12 gradients. CXCR4⁺ proangiogenic cells include immature and mature hematopoietic cells, endothelial precursor cells, and smooth muscle cell progenitors, which have direct or indirect proangiogenic properties. CXCL12 plays a role in the mobilization and recruitment of these cells to the neoangiogenic niches supporting revascularization of ischemic tissue and tumor growth (10).

The expression of CXCR4 on malignant epithelial cells and on cells from several hematopoietic malignancies implies that the CXCL12/CXCR4 pathway may influence the biology of cancer and play a pivotal role in directing the metastasis of CXCR4⁺ tumor cells to organs that express CXCL12 (e.g., lymph nodes, lungs, liver, or bones). Several CXCR4⁺ cancers metastasize to the bones and lymph nodes in a CXCL12-dependent manner in which the bone marrow in particular can provide a protective environment for tumor cells (11). CXCR4 can also promote tumor vascularization (see above) and

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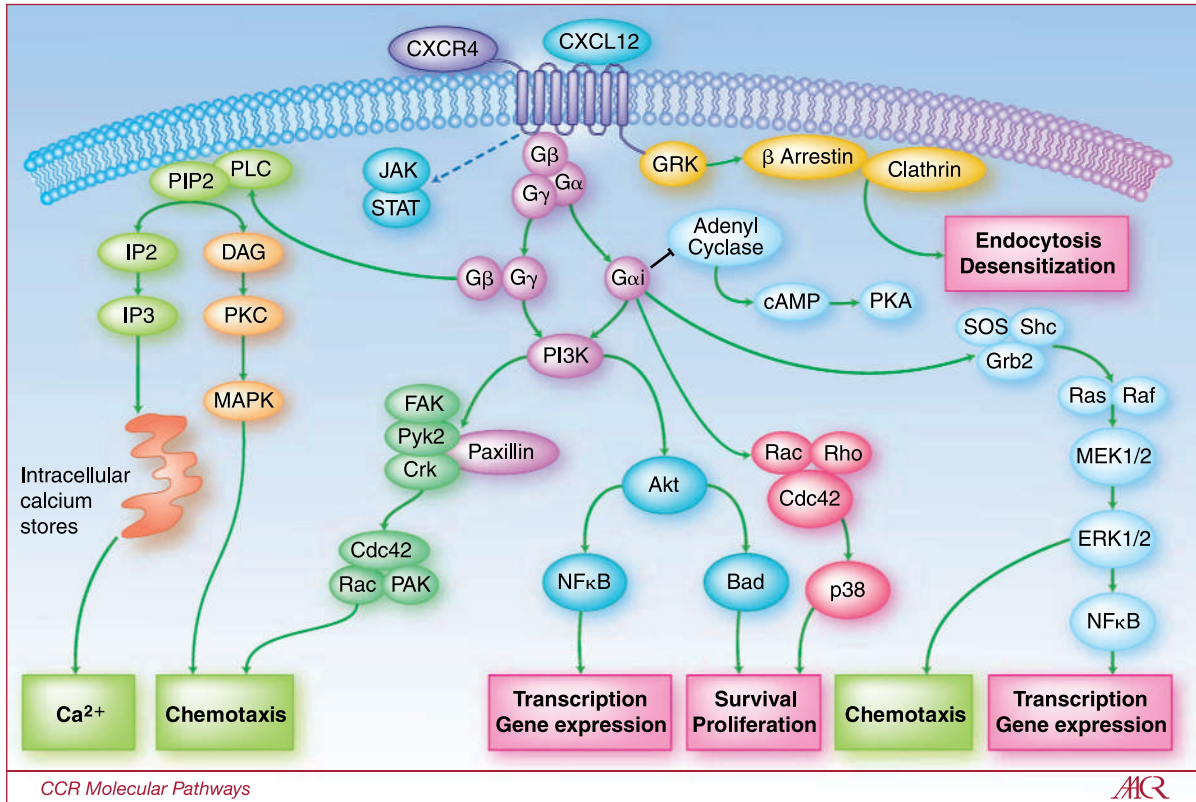


Fig. 1. A schematic of the CXCL12/CXCR4 intracellular signal transduction pathways.

act as a survival or growth factor; the implications of this are discussed further below.

CXCR4-activating mutations involving CXCR4 C-terminus truncations have been described in patients suffering from warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome (12). Myelokathexis manifests as severe chronic neutropenia, lymphopenia, and hypercellular bone marrow, attributed to a defect in the release of neutrophils with an accompanying apoptosis of mature myeloid cells.

CXCL12/CXCR4 Pathway

The binding of CXCL12 to CXCR4 initiates divergent signaling pathways downstream of ligand binding, which can result in a variety of responses such as chemotaxis, cell survival and/or proliferation, increase in intracellular calcium, and gene transcription. Figure 1 represents some of the key signaling pathways thought to be involved in CXCR4 signal transduction. The precise nature of these pathways may be tissue-dependent and thus may differ between cell types.

As a G-protein-coupled receptor (GPCR) the mechanism of CXCR4 receptor activation is mediated by coupling to an intracellular heterotrimeric G-protein associated with the inner surface of the plasma membrane.

The heterotrimer is composed of $G\alpha$, $G\beta$ and $G\gamma$ subunits, which in its basal state binds the guanine nucleotide GDP. Upon activation by ligand binding, GDP is released and replaced by GTP, which leads to subunit dissociation into a $\beta\gamma$ dimer and the α monomer to which the GTP is bound. The GTP is rapidly hydrolyzed to GDP resulting in reassociation of the receptor and the trimeric G-protein complex. On the basis of sequence similarity, the $G\alpha$ subunits have been divided into four families: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$. Each $G\alpha$ subunit relays the GPCR signal via different routes (13, 14). The $G\alpha_s$ subunit stimulates adenyl cyclase whereas $G\alpha_i$ inhibits adenyl cyclase. The $G\alpha_q$ family acts via PLC, such as PLC β , to activate phosphatidylinositol-specific phospholipases, which hydrolyze PIP2 to generate two second messengers, IP3 and DAG. IP3 and DAG increase the intracellular concentrations of free Ca^{2+} and activate a number of protein kinases, including PKC. $G\alpha_q$ activates the transcription factor NF κ B through PYK2. Both $G\alpha_i$ - and $G\alpha_q$ -coupled receptors stimulate mitogen-activated protein kinase (MAPK) activation. $G\alpha_{12}$ is associated with low molecular weight G proteins such as Rho and Ras. One of the features of chemokine receptors is that they are primarily $G\alpha_i$ -coupled receptors and as such can be inhibited by pertussis toxin. Ligand stimulation can result in an increase in intracellular calcium. This calcium flux can be readily measured and is frequently used as a measure of chemokine

activity. Interestingly, calcium flux is not associated with $G\alpha_i$ but with $G\alpha_q$, which raises the question of how chemokines elicit a calcium flux. This process seems to be achieved via the $G_{\beta\gamma}$ subunit, which can trigger PLC activation and formation of IP3 and DAG, resulting in mobilization of Ca^{2+} from intracellular stores (14). There are recent data, however, that suggest that CXCR4 signaling may not be limited to $G\alpha_i$ as first thought, and that CXCR4 can couple to other $G\alpha$ proteins such as $G\alpha_q$, $G\alpha_o$, and $G\alpha_s$ (15).

One of the major functions of chemokines is lymphocyte trafficking. CXCR4-mediated chemotaxis is mediated by PI3 kinase (PI3K; refs. 14, 16). PI3K can be activated both by $G_{\beta\gamma}$ and $G\alpha$ subunits. PI3K activation can result in the phosphorylation of several focal adhesion components such as proline-rich kinase-2 (Pyk-2), Crk-associated substrate (p130Cas), focal adhesion kinase (FAK), paxilin, Nck, Crk, and Crk-L (17, 18). Crk, which belongs to the adaptor family of proteins composed of SH2 and SH3 domains, has a putative role in signaling. JNK is activated by v-Crk. The activation of JNK by v-Crk may involve the guanine nucleotide exchange proteins SOS or C3G, both of which bind to the Crk SH3 domain. JAK2, JAK3, and Tyk-2 may also associate with CXCR4 and be activated by transphosphorylation in a $G\alpha_i$ -independent manner. Chemotaxis has been shown to be mediated via MAPK either through PKC, or through $G\alpha_i$, which can signal through Erk1/2 (14, 19). CXCR4 signaling has been shown to involve the Ras-activated signaling pathway, several src-related kinases such as Src, Lyn, Fyn, and Lck, T-cell activating molecule ZAP-70, and vav and small GTPases.

PI3K can lead to the activation of the serine-threonine kinase AKT, which has been found to play a key role in tumor cell survival, and possibly proliferation (20). However AKT may not be the only player in cell survival signaling. Both p38 and Erk1/2 have been implicated in tumor cell survival (21). CXCL12 can promote cell survival through the PI3-kinase- and MAP-kinase cascades without cell cycle progression. The proapoptotic Bcl-2 antagonistic of cell death protein BAD can be inactivated by the CXCL12-mediated activation of MAPK extracellular signal regulated kinases (MEK), extracellular signal-regulated kinase-ribosomal S6 kinases, and PI3K-pathways. Genes associated with cell survival can be up-regulated upon CXCL12 exposure. Thus, CXCL12 may promote cell survival by two mechanisms: post-translational inactivation of the cell death machinery and an increased transcription of cell survival-related genes (22).

Signaling through $G\alpha_i$ has been linked to transcription and expression through the PI3K-AKT-NF- κ B axis, and also via MEK1/2 and Erk 1/2. ERK can phosphorylate and activate other cellular proteins (like p90RSK), as well as translocate into the nucleus and phosphorylate and/or activate transcription factors, leading to changes in gene expression and cell cycle progression. Adhesion molecules are key proteins involved in tumor cell invasion, and CXCL12 can up-regulate expression of adhesion molecules such as VLA-4 (23, 24).

GPCR oligomerization has been postulated to play a role in modulating GPCR signaling. Both CXCR4 homodimers and heterodimers have been reported. Homodimerization of CXCR4 has been suggested to result in G-protein-independent signaling through the JAK/Stat signaling pathway (14). However, this is still a subject of debate and the role of JAK/Stat in CXCR4 signal transduction has been questioned (25).

CXCR4 signaling is rapidly desensitized after ligand binding by receptor internalization. The intracellular C-terminus of CXCR4 is rapidly phosphorylated at serine sites by G-protein receptor kinases (GRK) after ligand binding (15). This process is followed by recruitment of β -arrestin and clathrin-mediated endocytosis. The neutrophils from patients with the rare autoimmune disease WHIMS have enhanced chemotactic responsiveness to CXCL12 caused by impaired desensitization and internalization of CXCR4, attributed to truncations in the CXCR4 C-terminus resulting in dysregulation of the normal attenuation of CXCR4 function by GRK3 (26). However, GRK- β -arrestin interactions may play more subtle roles in CXCR4 signaling with different GRKs having differential effects on receptor internalization, calcium flux, and Erk activation (26).

CXCR4 and Cancer

The CXCL12/CXCR4 axis is involved in several aspects of tumor progression including angiogenesis, metastasis, and survival (1, 27–42). The bone marrow microenvironment facilitates the survival, differentiation, and proliferation of normal hematopoietic cells, malignant hematopoietic cells, and epithelial tumor cell bone metastasis. Bone marrow factors produced, such as CXCL12 and interleukin 6 (IL-6), mediate homing, survival, and proliferation of tumor cells, and integrin-mediated adhesion sequesters tumor cells to this niche. The CXCL12/CXCR4 pathway is responsible for retention of acute lymphoid leukemia and acute myeloid leukemia cells in the bone marrow (43, 44). Environment-mediated drug resistance is induced immediately by the microenvironment and is independent of epigenetic or genetic changes caused by drug exposure, and therefore is a form of *de novo* drug resistance (11).

Clinical-Translational Advances

CXCL12/CXCR4 antagonists

The CXCL12/CXCR4 pathway is a target for therapeutics that block CXCL12/CXCR4 interaction or inhibit downstream intracellular enzyme activities. Small molecular inhibitors of CXCR4, such as plerixafor or BKT140, and blocking antibodies toward CXCR4 or CXCL12 are being investigated in various cancer settings (45). Plerixafor is a small molecule with two cyclam rings connected by a phenylene linker. At physiological pH, two nitrogens on each ring are protonated allowing specific charge-charge interactions with the carboxylate groups on CXCR4, thus

inhibiting CXCL12 binding and downstream signaling events (46–50).

Hematopoietic stem cell transplant (HSCT) is an important treatment option for hematologic malignancies. Autologous HSCT following high dose chemotherapy or radiotherapy is used for non-Hodgkin's lymphoma and multiple myeloma, and allogeneic HSCT is used for several leukemias. Hematopoietic growth factors such as granulocyte-colony stimulating factor (G-CSF), either alone or in combination with chemotherapy, are used for mobilizing and collecting HSC for transplant. However, some patients fail to mobilize adequate HSC for transplant when treated with G-CSF.

CXCL12/CXCR4 disruption is essential for the egress of hematopoietic stem and/or progenitor cells from bone marrow into circulation. Conversely, CXCL12/CXCR4 function is essential for homing and/or engraftment of hematopoietic stem cells to the bone marrow after transplantation. Clinical trials showed that non-Hodgkin's lymphoma and multiple myeloma patients treated with plerixafor and G-CSF resulted in significantly more patients collecting the optimal number of HSC for autologous transplant compared with G-CSF alone (51–53).

The bone marrow provides protection for leukemic cells from chemotherapeutic agents conferred by interactions with stromal cells, in part mediated by CXCR4 and CXCL12. In a mouse model of acute promyelocytic leukemia, treatment with a CXCR4 antagonist made the leukemia cells more sensitive to cytarabine and prolonged the

survival of tumor bearing mice compared with both untreated mice, and mice treated with cytarabine alone, thus suggesting a role for CXCL12/CXCR4 inhibition in hematological malignancy treatment (44). Clinical trials are ongoing investigating the potential of CXCL12/CXCR4 inhibitors as chemosensitizers in acute myeloid leukemia and other hematological malignancies.

Conclusion

Inhibition of CXCR4 with plerixafor has shown utility by facilitating mobilization of hematopoietic stem cells for autologous transplant in non-Hodgkin's lymphoma and multiple myeloma. There are early indications that CXCR4 blockade may have further applications in certain hematologic cancers. CXCR4 signals via divergent signaling pathways mediating functions that include cell migration and survival. The usurping of these pathways by tumor cells for metastasis and protection from apoptosis may provide therapeutic opportunities for CXCR4 blockade in cancer. However, much remains to be done to define these signaling pathways and the role of CXCR4 in tumor progression.

Disclosure of Potential Conflicts of Interest

B. Teicher, S. Fricker, employment, ownership interest, Genzyme.

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