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CXCL12 (SDF1 α) – CXCR4/CXCR7 Pathway Inhibition: An Emerging Sensitizer for Anti-Cancer Therapies?

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Abstract

Addition of multiple molecularly targeted agents to the existing armamentarium of chemotherapeutics and radiotherapies represents a significant advance in the management of several advanced cancers. In certain tumor types with no efficacious therapy options, these agents have become the first line of therapy, e.g., sorafenib in advanced hepatocellular carcinoma or bevacizumab in recurrent glioblastoma. Unfortunately, in many cases, the survival benefits are modest, lasting only weeks to a few months. Moreover, they may not show benefit in patients with localized disease (i.e., in adjuvant setting). Recent studies have provided increasing evidence that activation of the chemokine CXCL12 (SDF1 α) pathway is a potential mechanism of tumor resistance to both conventional therapies and biological agents via multiple complementary actions: (i) by directly promoting cancer cell survival, invasion and the cancer stem/tumor-initiating cell phenotype; (ii) by recruiting “distal stroma” (i.e., myeloid bone marrow-derived cells) to indirectly facilitate tumor recurrence and metastasis; and (iii) by promoting angiogenesis directly or in a paracrine manner. Here, we discuss recent preclinical and clinical data that support the potential use of anti-CXCL12 agents (e.g., AMD3100, NOX-A12 or CCX2066) as sensitizers to currently available therapies by targeting the CXCL12/CXCR4 and CXCL12/CXCR7 pathways.

Background

The addition of anti-vascular endothelial growth factor (VEGF)-based therapies to existing cytotoxic treatments has changed clinical practice as well as the research directions in advanced glioblastoma (GBM), colorectal cancer, lung cancer and hepatocellular carcinoma (HCC) (1–4). Unfortunately, the overall survival benefits in patients with these malignancies remain modest despite exceedingly high financial costs (5–12). Clearly, development of novel approaches to maximize the efficacy of available treatments remains a major priority in oncology.

Chemokines, a family of small cytokines, play an important role in leukocyte migration (13). There are over 50 chemokines, all with four conserved cysteins that form two essential disulphide bonds. Chemokines interact with a group of over 20 C-C or C-X-C seven transmembrane domain G-protein coupled receptors (GPCRs, reviewed in (14)). Although

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Disclosure of Potential Conflicts of Interest

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initially of clinical interest for immunology and diseases such as HIV/AIDS, chemokine pathways have become an important area of investigation for cancer therapy (14,15).

An emerging chemokine target for cancer therapy is CXCL12, also known as the stromal-derived factor 1 alpha (SDF1 α), which binds and initiates signaling through its cognate receptors CXCR4 and CXCR7 (14,16,17). CXCL12 is a specific ligand for CXCR4 whereas CXCL11 binds CXCR7 as well. Knockout studies of CXCL12, CXCR4, or CXCR7 have shown lethality in mice either during embryonic development or perinatally (18–20). This emphasizes that the pleiotropic activity of CXCL12 pathway is critical for hematopoietic, neural, vascular, craniofacial organogenesis and cardiac development. Activation of CXCR4 or CXCR7 signaling may affect several major signaling pathways related to cell survival, proliferation and migration (see Figure 1). For example, CXCL12 activates PI3K/Akt, IP3, and MAPK pathways via CXCR4, thus regulating cell survival, proliferation and chemotaxis. CXCR4 signaling can be modulated by β -arrestin-mediated internalization of the receptor. Much less is known about CXCL12 signaling via CXCR7, which was initially thought to serve primarily as a sink for CXCL12 (21–23). Indeed, when CXCR7 is activated by CXCL12, the classical GPCR mobilization of Ca²⁺ is not observed (24). Rather, the β -arrestin pathway is activated and scavenges CXCL12 (22,25). CXCR4 and CXCR7 can also form heterodimers, whereby CXCR7 changes the conformation of the CXCR4/G-protein complexes and abrogates its signaling (26). Finally, CXCR7 can signal through the PLC/MAPK pathway and increase cell survival in gliomas (27).

Because CXCR4 is a marker of hematopoietic cells in adults, much research has been dedicated to its role in hematopoiesis (and in particular to stem cell trafficking) as well as its involvement in leukemias (recently reviewed by Teicher and Fricker in (28)). In solid tumors, CXCR4 can be markedly overexpressed compared with normal tissues, and is present primarily on cancer cells (14,29–35). Similarly, CXCR7 is highly expressed on certain cancer cells (14,27). Some studies have demonstrated a direct correlation between receptor upregulation in cancer cells and tumor growth/progression, neovascularization, invasion and metastasis (36–39). CXCL12 expression is also detected in various cancer cells, which is consistent with autocrine signaling (29,35). However, unlike CXCR4, this chemokine is also abundant in many normal tissues (14). In addition, in solid tumors different stromal cells express CXCL12 and/or its receptors can be involved in paracrine interactions to promote tumor progression. Stromal (myo)fibroblasts in some cases may be a major source of secreted CXCL12 in tumor tissue (17,40,41). Blood vascular endothelial cells express both CXCL12 and its receptors (27,37). Moreover, CXCL12 is significantly involved in recruitment of various bone marrow-derived cells (BMDCs) expressing CXCR4, including CD11b⁺ myelomonocytes (that differentiate into macrophages with pro-vascular activity) (42,43), and endothelial precursor cells and “hemangiocytes” that may directly incorporate in tumor vessels (40,44,45).

In gliomas, CXCL12 and both its receptors are expressed in the cancer cells themselves. The CXCR4/CXCL12 axis is particularly prevalent in pseudopalisading zones surrounding the necrotic foci and in invading glioma cells (35,46,47). A recent study revealed more complexity in the axis when CXCR4 was found on glioma stem-like cells while more abundant expression of CXCR7 was found on “differentiated” glioma cells, which mediated their resistance to apoptosis (27). Interestingly, a recent study has suggested a role for CXCR4 in modulation of neural stem cell migration and engraftment (48). Both receptors were also found on tumor-associated endothelial cells (27,46), and CXCR7 on microglia (27). On the other hand, hypoxia inducible factor 1 alpha (HIF-1 α) activation, which can induce local expression of VEGF, placenta growth factor (PlGF), VEGF receptor 1 (VEGFR1) and CXCL12, was shown to enhance the recruitment of multiple bone marrow-

derived cell (BMDC) populations via CXCR4 pathway (42). Finally, growth factors such as PDGF-D could upregulate CXCR4 and increase lymphatic metastasis in breast cancer (39).

In addition, CXCL12 signaling may indirectly promote tumor growth. For example, it can transactivate Her2/neu in breast cancer cells or trigger an “angiogenic switch” through upregulation of VEGF and IL-8 in prostate cancer (36,49,50). CXCR4 has also been identified as marker of sprouting endothelial cells (referred to as “tip cells”) (51) and can mediate VEGF expression through the transcription factor Yin Yang 1 (51,52). However, of note, CXCL12/CXCR4 pathway may also affect tumor angiogenesis independently of the VEGF pathway – the main target of currently approved antiangiogenic drugs (53,54)(see below).

In summary, activation of CXCL12 pathway may be critical for tumor progression via multiple complementary mechanisms: (i) by directly promoting cancer cell survival, invasion and stem/tumor-initiating cell phenotype; (ii) by recruiting “distal stroma” (i.e., myeloid BMDC) to indirectly facilitate tumor growth and metastasis; and (iii) by promoting angiogenesis either directly or in a paracrine manner, to support tumor growth. Future investigation should unravel the intricate roles of CXCR7 versus CXCR4 in different cancers and, in particular, confirm whether CXCL12 pathway also mediates cancer stem-like cell survival and migration, similar to normal adult stem cells. All this raises the exciting possibility that blocking CXCL12 pathway may be a valid strategy to target various components in solid tumors.

Clinical-Translational Advances

Based on these hypotheses, multiple agents are currently being developed to target the CXCL12 pathway in cancer. These agents include the anti-CXCR4 drug AMD3100, also known as plerixafor (Mozobil[®]); the CXCL12 analogue CTCE-9908 (Chemokine Therapeutics, Canada); the anti-CXCL12 aptamer Nox-A12 (Noxxon, Germany), the CXCR7-specific inhibitor CCX2066 (ChemoCentryx, Mountain View, CA) (55–57). In addition, other strategies to inhibit CXCL12 pathway [e.g., the agent chalcone 4 (C7870, Sigma) or RNA interference]—that provided important insights into regulation of the pathway—could also be potentially tested for the therapy of solid tumors (37,50,58,59). Of these, AMD3100 and CTCE-9908 are approved for clinical use in patients with leukemias (for stem cell mobilization) and osteosarcoma, respectively (14,56).

Will blockade of CXCL12 pathway alone be efficacious in solid tumors?

Multiple preclinical studies converged to the finding that anti-CXCL12 agents can significantly delay primary tumor growth and metastasis when treatment is started at or close to the time of tumor implantation (i.e., in a “preventive” setting) (14,30–34). However, blockade of CXCL12 pathway had minor antitumor effects on established tumors: Although CXCR4 antagonists did inhibit tumor growth in some cases (60,61), they were ineffective in others (62–65). Thus, these preclinical studies suggest that blocking CXCL12 pathway alone may be not be sufficient except for certain solid tumors.

One potential setting in which blockade of CXCL12 pathway may be more widely efficacious is in preventing or delaying metastasis. Indeed, previous studies have shown that CXCL12 pathway is a key mediator of metastasis in prostate, colorectal and breast cancer (33,54,58). Since VEGF blockade has failed so far to prevent metastasis in mice and in patients (66–69), evaluation of CXCL12 blockade has emerged as a potential additional or alternative target for neoadjuvant and adjuvant treatment (70). However, clinical translation of anti-CXCL12 therapy in this setting will require combinations with standard neoadjuvant and adjuvant treatments (see below).

Blocking CXCL12 pathway in combination with other therapies may prevent tumor recurrence: What is the preclinical evidence?

In contrast to monotherapy, the use of anti-CXCL12 therapy in combination with other anti-cancer treatments showed promising efficacy in most studies (see Table 1). One reason could be that CXCL12 pathway is activated in response to various therapies and may be an important mechanism of acquired resistance to them. This hypothesis is consistent with results from recent preclinical and clinical studies of antiangiogenic therapy, chemotherapy or radiation therapy. Our studies showed that treatment with the pan-VEGFR tyrosine kinase inhibitor cediranib leads to an increase in circulating CXCL12 concentrations and CXCR4⁺ cells as well as in myeloid BMDC infiltration in brain tumors as seen in clinical studies (71–74). In addition, using genetic models, we found that the CXCL12/CXCR4 pathway activation can compensate for specific inhibition of VEGFR1 activity in BMDCs and promote angiogenesis, tumor growth and metastasis by recruiting Gr1⁺ myeloid BMDCs to the tumor (54). Others have shown that certain chemotherapeutics (e.g. paclitaxel) or vascular-disrupting agents rapidly increased both circulating CXCL12 levels and the mobilization of BMDCs (75,76). Finally, local irradiation directly increased CXCL12 expression in tumors both directly and indirectly (secondary to treatment-induced hypoxia and HIF1 α activation), and increased the recruitment of myeloid BMDCs (62,63,77).

To date, several studies have evaluated the role of therapy-induced activation of CXCL12 pathway as a mechanism of resistance to therapy with cytotoxics. For example, orthotopic U87 gliomas showed a substantial growth inhibition after chemotherapy (BCNU) with AMD3100, although the two treatments had no effect when given as monotherapy (64). Similarly, combination of AMD3100 with irradiation induced a significant tumor growth delay and increased curability in brain, lung and breast tumors (62,63). However, treatment with AMD3100 commenced 5 days after irradiation had no significant effect in lung and breast tumors (62). These studies emphasize that it is critical to understand the temporal role of the CXCL12/CXCR4 pathway activation in a tumor-dependent manner in pre-clinical models to identify optimal schedules for combining inhibitors against this pathway with other therapeutic agents in cancer patients.

Blocking CXCL12 pathway for tumor sensitization to other therapies: What is the clinical evidence?

The preclinical data discussed above is strongly supported by clinical studies, particularly in brain tumors. For example, clinical correlative studies have shown that elevated expression of CXCL12, CXCR4 and CXCR7 is associated with higher tumor grade and invasion and decreased apoptosis in GBM (27,47,78). In our clinical studies, we found that circulating plasma CXCL12 levels significantly correlated with progression in recurrent GBM after treatment with the pan-anti-VEGFR agent cediranib (71,72) (Table 1). Of note, one of the most striking features in the recurrent GBMs after cediranib therapy was the emergence of an infiltrative tumor phenotype with a diffuse tumor with relatively normal blood vessels and reduced necrosis (74).

Treatment-induced increase in CXCL12 and CXCR4 expression in cancer cells has also been documented in clinical studies of neoadjuvant anti-VEGF therapy in rectal cancer (79). Importantly, increased circulating plasma CXCL12 after neoadjuvant anti-VEGF therapy with chemoradiation was associated with development of metastatic disease at three years (79). In addition, in our clinical trials we found circulating plasma CXCL12 levels significantly correlated with progression in advanced HCC and in soft tissue sarcoma after treatment with anti-VEGF agents (80,81) (Table 1). Collectively, these clinical data are consistent with CXCL12 pathway being a potential target to prevent progression in GBM

and improve neoadjuvant and adjuvant therapy for other solid tumors. However, important challenges remain prior to the translation of these promising findings into the clinic.

Clinical implications, challenges and future direction

The convergence to the CXCL12 pathway in studies of multiple tumor types strongly suggests that: (i) tumors in different organs exploit this common pathway to spread and escape from therapy; and (ii) the systemic effects of CXCL12 pathway activation on BMDCs and local stroma are critical. Blockade of CXCL12 in adjuvant setting has been proposed several years ago to prevent breast cancer cell chemotaxis (70). However, no clinical trial has tested to date this concept in solid tumors. Moreover, future research should consider the role of CXCR7, which is clearly emerging as a potentially important pathway in solid tumors. Gaining a more in depth understanding of the CXCL12/CXCR7 pathway in preclinical studies is urgently needed to inform the future translation of CXCL12, CXCR4 or CXCR7 inhibitors into the clinic.

To bridge these gaps in our knowledge, preclinical models should be developed to closely recapitulate the clinical features and the response to targeted or cytotoxic therapies as seen in patients. This will be critical in determining the causal role of CXCL12 pathway in tumor progression, and then rapidly translating the pre-clinical findings to the clinic to prolong survival in patients with advanced cancers beyond what is possible with currently approved therapies. This should be facilitated by the fact that the CXCR4 blocker AMD3100 is an FDA-approved drug with a relatively mild toxicity profile (55,82). However, the optimal dosing of AMD3100 with anti-VEGF agents, chemotherapy or radiation will be a challenge in patients with solid tumors. In preclinical studies, AMD3100 is often given continuously with an infusion pump for tumor therapy, whereas in the clinic it is given as a daily s.c. injection with granulocyte colony-stimulating factor for a limited time (1–7 days) to mobilize hematopoietic stem cells into the circulation prior to transplantation (82). Only a mechanistic understanding of action of CXCL12 pathway in solid tumors could provide novel insight into how to use this drug safely for improved cancer treatment. Moreover, CXCR4 blockade with AMD3100 may not be sufficient to block the effects of CXCL12, which may also bind to CXCR7 on cancer or stromal cells. This could be studied using Nox-A12, an aptamer against CXCL12, or with CXCR7-specific inhibitors such as CCX2066. Finally, as we move forward with the development of inhibitors of the CXCL12 pathway for solid tumors, a critical issue will become the development of biomarkers. Currently, there are no approved biomarkers for anti-VEGF agents or AMD3100 (6). Thus, mechanistic studies and biology-driven, rational design of novel combination therapies will be critical for successfully pursuing this pathway as a novel target for sensitization to existing therapies (83). Overcoming these challenges would increase the chances of realizing the promise of CXCL12 pathway inhibition as a potentially effective strategy to decrease the rates of local and distant failure after currently available therapies for solid tumor.

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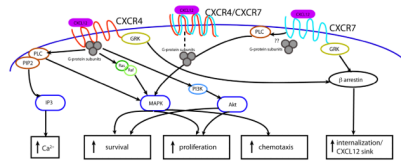


Figure 1. CXCL12 pathway

CXCL12 binds to CXCR4 and CXCR7, which are G protein-coupled receptors (GPCR) that can form homodimers or heterodimers. In the latter case, CXCR7 changes the conformation of the CXCR4/G-protein complexes and abrogates signaling. Activation of CXCR4 by CXCL12 leads to G-protein coupled signaling through PI3K/Akt, IP3, and MAPK pathways, which promote cell survival, proliferation and chemotaxis. In addition, the β -arrestin pathway can be activated through GRK to internalize CXCR4. When CXCR7 binds CXCL12, the classical GPCR mobilization of Ca^{2+} does not occur, and activation of the β -arrestin pathway may lead to scavenging of CXCL12. In certain cancer cells (e.g., glioma) CXCR7 can also signal through PLC/MAPK to increase cell survival.

Table 1

Evidence suggesting the potential use of CXCL12 inhibition for sensitization of solid tumors to other treatments

Treatment type	Preclinical data	Clinical data
Radiation therapy	Increase in CXCL12 after radiation treatment of glioblastomas, lung and breast cancers; CXCR4 blockade synergizes with radiotherapy (62,63,84)	Increase in circulating CXCL12 after anti-VEGF with chemoradiation treatment associated with metastasis in localized rectal cancer (79)
Chemotherapy	CXCR4 blockade synergizes with BCNU of glioblastomas (64) Increase in circulating CXCL12 after paclitaxel treatment of breast cancers (75) CXCR4 blockade synergizes with cyclophosphamide of lung cancers (85)	Not available
Antiangiogenic therapy	Sunitinib increases circulating CXCL12 (in non-tumor or breast tumor-bearing mice)(86)	Increase in CXCL12 and CXCR4 in cancer cells after anti-VEGF treatment in localized rectal cancer (79) Increase in circulating CXCL12 after sorafenib treatment associated with worse response in soft tissue sarcomas (81) Increase in circulating CXCL12 after sunitinib treatment associated with poor survival in hepatocellular carcinomas (80) Increase in circulating CXCL12 after cediranib or vatalanib treatment associated with poor outcomes in glioblastomas (71,72,87)
Other	Vascular disrupting agents in lung cancer increase circulating CXCL12 of lung carcinomas (e.g., OXi-4503) (76) HIF-1 α KO phenotype increases CXCL12 in glioblastomas (42) CXCR4 inhibition synergizes with cyclophosphamide or immunotherapy against lung metastases from melanoma (88) CXCL12 expression increased in glioblastomas by VEGF and PlGF (89) CXCR4 in BMDCs compensates for VEGFR1 inhibition in BMDCs and promotes lung metastasis from breast and prostate cancers (54)	CXCL12 is increased in the circulation of prostate cancer patients after hormone therapy compared to control (surgery or no treatment; Smith et al., unpublished)