Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis

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Kryczek I, Wei S, Keller E, Liu R, Zou W. Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am J Physiol Cell Physiol 292: C987–C995, 2007. First published August 30, 2006; doi:10.1152/ajpcell.00406.2006.—The chemokine stroma-derived factor (SDF-1/CXCL12) plays multiple roles in tumor pathogenesis. It has been demonstrated that CXCL12 promotes tumor growth and malignancy, enhances tumor angiogenesis, participates in tumor metastasis, and contributes to immunosuppressive networks within the tumor microenvironment. Therefore, it stands to reason that the CXCL12/CXCR4 pathway is an important target for the development of novel anti-cancer therapies. In this review, we consider the pathological nature and characteristics of the CXCL12/CXCR4 pathway in the tumor microenvironment. Strategies for therapeutically targeting the CXCL12/CXCR4 axis also are discussed.

migration; immune suppression; tumor angiogenesis; tumor metastasis; stem cells

STROMA-DERIVED FACTOR 1 (SDF-1, or CXCL12) was initially cloned by Tashiro et al. (135) and later identified as a growth factor for B cell progenitor cells, a chemotactic factor for T cells and monocytes, and in B cell lymphopoiesis and bone marrow myelopoiesis. CXCL12 is a 68-amino acid small (8 kDa) cytokine that belongs to the CXC chemokine family. CXCL12 is expressed in two isoforms, SDF-1α and SDF-1β, from a single gene that encodes two splice variants. The two encoded proteins are almost identical, except for the last four amino acids of SDF-1β, which are absent in SDF-1α. Biological and functional differences between the CXCL12 isoforms have not been described. The CXCL12 gene is mapped in chromosome 10, whereas most of the other genes encoding CXC chemokines reside on chromosome 4 (126).

It was long thought that CXCL12 bound exclusively to CXCR4 and that CXCR4 was its sole receptor. However, CXCR7 was identified as another receptor for CXCL12 at the end of 2005 (4). The immunological activities of CXCL12/CXCR4 have been largely studied in the context of immune cell trafficking. Interestingly, both CXCL12 and CXCR4 knockout mice are embryonic lethal, with any surviving pups dying within an hour of birth, suggesting that the CXCL12/CXCR4 pathway mediates multiple biological activities (93, 132). The lethal effect of CXCL12 and CXCR4 knockout is related to the pleiotropic activity of CXCL12 and CXCR4, which are critical for hematopoietic, neural, vascular, and craniofacial organogenesis (80, 93, 132).

In this review, we focus on the pathological nature and characteristics of CXCL12 in the tumor microenvironment. We discuss therapeutic strategies of targeting the CXCL12/CXCR4 pathway in the treatment of human cancer.

EXPRESSION OF CXCL12 IN HUMAN TUMOR ENVIRONMENT

CXCL12 was initially cloned from bone marrow stromal cells (135). Strikingly, CXCL12 is widely expressed in various organs including heart, liver, brain, kidney, skeletal muscle, and lymphoid organs. Vascular endothelial cells, stromal fibroblasts, and osteoblasts are the major cellular source for CXCL12 in these organs (36, 59, 99, 102, 157). Interestingly, high levels of functional CXCL12 were first reported in human ovarian cancer in 2001 (65, 119, 156). Subsequent studies documented a strong correlation between CXCL12 expression and bone marrow and lymph node metastasis of breast (90) and prostate cancer (133). Interest in the role of CXCL12/CXCR4 in tumor pathology was provoked by these studies. In addition to ovarian cancer, CXCL12 expression is reported in breast cancer (3, 57), glioblastoma (6, 106), pancreatic cancer (64, 81), prostate cancer (23, 131), thyroid cancer (51), and many other human tumors (Table 1). This list continues to grow, following the current interest of studying chemokines and chemokine receptors in human tumor pathogenesis.

Tumor stroma is an active element of tumor microenvironment. Recently, it was shown that in breast cancer, activated stroma fibroblasts produce CXCL12 (1, 96) and contribute to tumor vascularization by endothelial stem cell attraction (96). It also has been suggested that CXCL12 is involved in prostate epithelial cell transformation induced by aging fibroblasts (8). Although CXCL12 does not directly induce transformation, CXCL12 may provide conditions supportive of a transforming event. Therefore, stroma and cancer cells, two main components of tumor microenvironment, can produce CXCL12.

REGULATION OF CXCL12 EXPRESSION IN HUMAN TUMOR

Strikingly, regulation of CXCL12 expression in the tumor microenvironment has been poorly studied. It has been reported that estradiol activates estrogen receptor and induces the production of CXCL12 by tumor cells (39). We have observed that hypoxia triggers CXCL12 expression by primary human ovarian tumor cells (66) and prostate tumor cell lines (unpublished data). Hypoxia-inducible factor (HIF)-1 is the central mediator of the cellular response to hypoxia (123). In the promoter region of CXCL12 gene, there are two potential HIF-1-binding sites, termed HBS1 and HBS2. It is thought that the HBS1 region is responsible for HIF-1-dependent induction.
of CXCL12 synthesis in endothelial cells (16). Hypoxia also induces CXCL12 expression in synovial fibroblasts (48) and hematopoietic stem cells (HSC) (16). These data suggest that hypoxia may be a common condition to induce CXCL12 expression. Altogether, CXCL12 is widely expressed in various human tumors. CXCL12 expression would be regulated by hypoxia-and hormone-triggered signal pathway.

**CXCL12, TUMOR PROLIFERATION, AND SURVIVAL**

There is evidence to demonstrate that CXCL12 can modulate tumor cell proliferation and survival. Sehgal et al. (121) provided the first evidence for mitotic CXCL12 activity in human tumors, where transfection of an antisense RNA that blocks CXCR4 translation inhibited glioma cell proliferation. Later, Barbero et al. (6) confirmed that glioma cell proliferation can be induced by exogenous CXCL12. CXCL12-dependent proliferation correlated with the activation of ERK1/2 and AKT pathways. Both these pathways are known to be involved with the transduction of proliferative signals in normal and tumor glial cells (128).

In addition to glioma cells, CXCL12 can induce proliferation of several tumor cell lines, including ovarian carcinoma (120), small cell lung cancer (100), prostate cancer (23), neck squamous cell carcinoma (58), and pancreatic cancer (81). Mechanistically, CXCL12-dependent cell proliferation is linked to ERK activation (23, 60, 81, 100, 120).

CXCL12/CXCR4-mediated tumor cell proliferation may be regulated through estrogen signaling (39). About 60% of human ovarian and breast cancers are hormone dependent and overexpress the progesterone and/or estrogen receptors (55, 77). Hall et al. (39) demonstrated that CXCL12 was required for estrogen-induced proliferation of both breast and ovarian cancers.

CXCL12 also can regulate tumor cell apoptosis. CXCL12 activates NF-κB (45), which in turn inhibits radiation-induced tumor necrosis factor-α (TNF-α) production and tumor apoptosis (140). Moreover, activation of NF-κB can sensitize cancer cells to CXCL12 stimulation through upregulation of CXCR4 expression (45, 70).

Many chemotherapeutic drugs exert their effects by inducing apoptosis in the targeted cell population. CXCL12 can protect tumor cells from drug-induced apoptosis directly through the activation of antiapoptotic pathways but also indirectly by modulating the adherence of cancer cells. For example, CXCL12 mediates adhesion of small-cell lung cancer cells (SCLC) to marrow stroma cells and protects SCLC against etoposide-induced apoptosis. The protective effect could be antagonized by CXCR4-specific inhibitors as well as by blocking integrin α4 (41, 124). Similar observations are found in myeloma (44), glioma cells (138), and head and neck cancer (91). In support of this observation, CXCL12 activates integrin α4 on vascular endothelial cells and protects plasmacytoid dendritic cell from apoptosis in patients with ovarian cancer (156). Thus CXCL12 signals may be implicated in tumor cell proliferation and survival.

The role of CXCL12 in controlling tumor growth and survival has been demonstrated in vitro models. However, in some cases, the in vitro observations are not fully supported by in vivo experimental data. For example, glioma cells proliferate in vitro in response to CXCL12 (6); however, they proliferate in vivo independently of CXCL12 (106). Furthermore, tumor cells exhibit low proliferation in glioblastoma tissues, where high levels of CXCL12 expression are observed. Analysis of CXCR4/CXCL12 localization revealed an association of both CXCL12 and CXCR4 with regions of necrosis and angiogenesis (106), suggesting a role of CXCL12 in angiogenesis in vivo.

**CXCL12 AND TUMOR VASCULARIZATION**

The CXC chemokine family can be divided into two subfamilies, depending on the presence or absence of the highly conserved three-amino acid motif Glu-Leu-Arg (ELR) situated at the NH2 terminus. Members of the CXC chemokine family containing the ELR motif are potent inducers of angiogenic...
activity, whereas chemokines that lack the ELR motif are rather angiostatic.

CXCL12 is an ELR− CXC chemokine; however, it exhibits angiogenic activity. Initially, the angiogenic role of CXCL12 was observed in mice lacking CXCL12 or CXCR4 (80, 132). These mice had defective formation of large vessels supplying the gastrointestinal tract. Subsequent in vitro studies suggested a potential effect of CXCL12 on blood vessel formation. For example, CXCL12 stimulates the formation of capillary-like structures with human vascular endothelial cells (83, 86, 109). Interestingly, although high concentrations of CXCL12 are able to induce angiogenesis in vivo (83), our studies have shown that pathological levels of CXCL12 alone failed to induce meaningful vascularization in vivo. However, pathological concentrations of CXCL12 induced potent neoangiogenesis in vivo in the presence of low concentrations of vascular endothelial growth factor (VEGF) (66), revealing profound synergistic effects between CXCL12 and VEGF. Furthermore, CXCL12 attracts plasmacytoid dendritic cells (DCs) into the tumor environment, and in turn tumor plasma cytoid DCs induce neoangiogenesis through production of IL-8 and TNF-α (21). Therefore, a multifactor model is proposed to explain the mechanism whereby CXCL12 induces vascularization (109, 147). In this model, CXCL12 synergizes with soluble factors, including fibroblast growth factor (FGF) family members and VEGF (66, 92), and coordinates with immune cells, including plasmacytoid DCs (21), to induce potent vascularization in vivo.

Migration, expansion, and survival of vascular endothelial cells form the essential functional network of angiogenesis. Vascular endothelial cell migration is strongly dependent on CXCL12 (37, 109). In support of this, neutralizing antibodies against CXCL12 inhibit endothelial cell invasion into subcutaneously injected Matrigel (111). Hypoxia simultaneously stimulates CXCR4 expression (16, 129) and CXCL12 (66) production. Therefore, it is reasoned that hypoxia would promote vascular endothelial cell migration toward CXCL12 and induce tumor vascularization in a CXCL12-dependent manner.

**CXCL12 AND TUMOR METASTASIS**

Tumor metastasis was once viewed as a passive consequence of a single tumor cell simply “escaping” from a primary tumor and traveling great distances through draining lymph nodes and blood, lodging in small blood vessels and thereby forming micrometastases (152). Recent data, however, have demonstrated that tumor metastasis is an active process employing multiple molecular and cellular mechanisms (17, 19). The interaction between tumor cells and stroma is crucial for tumor metastasis (19).

**CXCL12 and Tumor Cell Adhesion**

Cancer dissemination can be viewed as a tissue remodeling process that involves proteolytic degradation of extracellular matrix. Metalloproteases (MMPs) are a family of enzymes involved in the degradation of extracellular matrix in the surrounding normal tissue and known to mediate cancer invasion and metastases (28). Activation of MMPs breaks down the physical barriers of metastasis, thus promoting invasion by cancer cells (49). Several studies have documented that CXCL12 induces MMP synthesis in different cell types (62, 75, 81, 127, 148) and facilitates tumor cell adhesion and colonization.

CXCL12 also modulates the expression and function of cell surface integrin molecules and, in turn, promotes tumor cell adhesion. Integrins are a large family of heterodimeric transmembrane glycoproteins that attach cells to extracellular matrix proteins of the basement membrane or to ligands on other cells. CXCL12 induces adhesion of SCLCs to VCAM-1, fibronectin, and collagen (13, 41).

**CXCL12 and Tumor Cell Migration**

The CXCL12/CXCR4 pathway is involved in the “homing” of lymphocytes. It was hypothesized that chemokines and chemokine receptors including CXCL12/CXCR4 might mediate cancer cells to “home” to specific secondary sites, thereby promoting organ-specific metastasis. In 2001, Muller et al. (90) provided the first evidence that the CXCL12/CXCR4 pathway mediates human breast cancer metastasis. In vivo blocking of CXCR4 with the use of a specific antibody (90) or selective synthetic polypeptide (73) or siRNA (74) resulted in significant inhibition of breast cancer metastasis to regional lymph nodes as well as in the lung. In the presence of neutralizing CXCL12 antibodies, NSCLC tumor metastases also were significantly reduced (100). Blocking of CXCR4 expression on the cell surface greatly reduced the ability of colon cancer cells to metastasize to the liver and lungs (150). Furthermore, antibody-mediated neutralization of CXCR4 was found to limit skeletal metastasis in prostate cancer (130). Induction of CXCR4 expression resulted in a dramatic increase in pulmonary metastases of melanoma cells, a situation that could be blocked using potent CXCR4 inhibitors. Subcutaneously injected prostate cancer cells transfected with CXCR4 grew larger tumors with increased muscle invasion compared with parental cells (23). In further support of the role of CXCL12 in tumor metastasis, high levels of CXCL12 are often found in lymph nodes, lung, liver, and bone marrow, where tumors frequently metastasize (90, 100, 101, 130). Nonetheless, although the molecular mechanism of action has yet to be established, these studies demonstrate the pivotal role of CXCL12/CXCR4 in tumor metastasis.

Hypoxia induces CXCR4 expression on tumor cells (54, 117), which would sensitize tumor cells to CXCL12 signals and promote tumor metastasis. However, hypoxia simultaneously stimulates both CXCR4 and CXCL12 expression (66). Human cancer cells including neuroblastoma (32), glioblastoma (6, 106), ovarian (66, 120, 156), breast (3, 57), colon (54), pancreas (64), and prostate (131) express CXCL12 (Table 1). It is reasoned that endogenous CXCL12, together with CXCR4 on tumor cells, should keep cancer cells within the primary tumor environment, rather than facilitate metastasis over a long distance. Nonetheless, the effects of CXCL12/CXCR4 on tumor metastasis may be explained by multiple factors in the tumor environment.

**Heterogeneous CXCL12 expression in different tumors.** Ovarian cancers constitutively express high levels of CXCL12 (65, 66, 156). CXCL12 expression may be restricted to either the metastatic lesion or limited fragments of primary tumor. **CXCL12 sensitivity.** The uncoupling of CXCR4 following receptor internalization by endocytosis may persist even after the receptor is recycled to the cell surface (125). Tumor cells
CXCL12 plays a pivotal role in the regulation of trafficking of normal HSCs and their homing in bone marrow (2, 63, 98). Moreover, CXCR4 is also expressed on nonhematopoietic stem cells (12, 35, 52). Stem cells may be the origin of vascular endothelial cells for tumor neovascularization (42, 79, 134). In vivo, CXCL12 secretion (96). It is postulated that CXCL12 in the tumor microenvironment may be critical for recruiting endogenous HSCs and their homing in bone marrow (2, 63, 98). Mizokami et al. (85) demonstrated that expression of CD26/DPPIV in endometrial carcinoma can directly modulate CXCL12 functions. CD26 is expressed on the surface of normal epithelial cells, but it is often lost in various cancers (143, 144). Hypoxia can upregulate CD26 expression on tumor cells (114) and, in turn, could decrease the sensitivity of tumor cells to local CXCL12.

Tumor cells moving away from high local levels of CXCL12. Although the mechanism remains to be defined, antigen-induced T-cell recruitment into the peritoneal cavity can be reversed by high but not low concentrations of CXCL12 (104). Analogously, one may speculate that high levels of CXCL12 in the tumor environment would trigger tumor cells migrating away from primary tumor and foster tumor metastasis.

CXCL12 is not the exclusive chemokine that regulates tumor cell trafficking. In summary, although other factors need to be considered, it is evident that the CXCL12/CXCR4 pathway is implicated in the mechanistic process of tumor metastasis, including tumor cell adhesion and migration.

CXCL12 AND CANCER STEM CELLS

CXCL12 plays a pivotal role in the regulation of trafficking of normal HSCs and their homing in bone marrow (2, 63, 98). Moreover, CXCR4 is also expressed on nonhematopoietic stem cells (12, 35, 52). Stem cells may be the origin of vascular endothelial cells for tumor neovascularization (42, 79, 134). In support of this, stromal fibroblasts in invasive human breast carcinomas promote tumor growth and angiogenesis through CXCL12 secretion (96). It is postulated that CXCL12 in the tumor microenvironment may be critical for recruiting endothelial stem cells to initiate tumor vascularization (96). On the other hand, cancer stem cells also express CXCR4 (69). Therefore, CXCL12 may mediate cancer stem cell trafficking and metastasis to organs that highly express CXCL12, such as bone marrow, lymph nodes, liver, and lung.

CXCL12 AND TUMOR IMMUNOSUPPRESSION

Appropriate trafficking and retention of immune cells is indispensable to mediate efficient immune responses in vivo (78). Multiple immune suppressive modes of action are involved in tumor immune evasion (22, 67, 68, 156). These mechanisms are extensively reviewed in the literature (154, 155). CXCL12 contributes to tumor immunosuppression through recruiting of specific immune cell populations. We focus our discussion on CXCL12 in the tumor environment and its role in tumor immunosuppression.

CXCL12 and CD44/CD25+ regulatory T cells. Bone marrow is a common site for human tumor metastasis, suggesting that bone marrow may provide an immunosuppressive environment for tumor retention and growth. Interestingly, a number of reports have demonstrated that functional memory T cells exist in bone marrow (7, 82). Bone marrow can serve as a site for naïve tumor-associated antigen (TAA)-specific T cell priming (7, 31, 82, 136). Indeed, TAA-specific T cells isolated from the bone marrow of tumor-bearing mice and cancer patients are functional in vitro and are able to prevent tumor growth when transferred to another host. These data suggest that these TAA-specific T cells are functionally suppressed in the bone marrow (30, 31, 82, 136). This notion was supported by our recent observation that large numbers of functional CD4+ regulatory T (Treg) cells accumulate in the bone marrow of healthy volunteers and mice (153). This observation was confirmed in a FOXP3 bicistronic reporter knock-in mouse model (139). In this model, a bicistronic reporter expressing a red fluorescent protein was knocked into the endogenous FOXP3 locus. High levels of FOXP3-expressing T cells (with red fluorescence) were found in the bone marrow (139).

Strikingly, bone marrow CD4+ Treg cells express functional CXCR4, and CD4+ Treg cell release from bone marrow is achieved through granulocyte-colony-stimulating factor reducing marrow expression of CXCL12 (153). Activation of Treg cells upregulates CXCR4 expression and enables them to migrate to the bone marrow in a CXCL12-dependent manner (153), suggesting that bone marrow could serve as a functional reservoir for activated Treg cells. Thus CXCR4/CXCL12 sig-

Fig. 1. Multiple modes of actions are implicated in CXCL12-mediated tumor biology. 1) CXCL12 promotes tumor growth and malignancy. 2) CXCL12 enhances tumor neovascularization. 3) CXCL12 fosters an immunosuppressive environment. 4) CXCL12 mediates tumor cell migration, adhesion, and invasion. mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic cells.
nals are crucial for bone marrow trafficking of activated CD4+ Treg cells. High levels of Treg cells in the bone marrow may provide an immune shield to facilitate bone marrow metastasis. Therefore, CXCL12 may contribute to tumor bone marrow metastasis by recruiting Treg cells.

**CXCL12 and plasmacytoid DCs.** Functional plasmacytoid DCs are found in the tumor environment of patients with ovarian cancer (156), melanoma (110), and head and neck squamous cell carcinoma (HNSCC) (40). Tumor cells produce CXCL12 and plasmacytoid DCs express VLA-5 and CXCR4, the key molecules that mediate plasmacytoid DC tumor trafficking (156). CXCL12 further protects tumor plasmacytoid DCs from apoptosis (156). Strikingly, tumor-associated plasmacytoid DCs induce significant IL-10 production by T cells that suppresses myeloid DC-induced TAA-specific T cell effector functions (156). Tumor plasmacytoid DCs induced IL-10+CCR7+CD8+ T cells to home to the draining lymph nodes and suppress TAA-specific central priming (142). The fact that allogeneic plasmacytoid DCs are able to induce CD4+ (89) and CD8+ (34) suppressive regulatory T cells supports these data. A large amount of plasmacytoid DCs, but not functional mature myeloid DCs, accumulate in the tumor environment (156).

Notably, it has been shown in mouse models that locally accumulated CXCL12 can lead to the inhibition of tumor growth by facilitating the chemotraction of leucocytes into the tumor area. In this respect, mouse leukemia and melanoma cells transfected with CXCL12 were rejected following injection into syngeneic mice (26). CXCL12 produced by melanoma cells attracts cytotoxic T cells through their binding to CXCR4. Blockade of CXCL12/CXCR4 signal inhibits CTL migration toward tumor cells (151). Likewise, depending on circumstances, it is possible that CXCL12 stimulates T cell immunity against a tumor. However, CXCL12 elicits potent effects on the tumor growth, angiogenesis, and immunity. The net effects of CXCL12 in human tumor appear not to be beneficial.

**CXCL12 AND THERAPEUTIC APPLICATIONS**

Compelling evidence demonstrates that CXCL12/CXCR4 signal is implicated in tumor proliferation, survival, vascularization, metastasis, and immunosuppression (Fig. 1). The in vivo blockade of this pathway reduces tumor growth and metastasis in mouse models (10, 90, 108, 150). Statistical studies suggest a possible negative association between high levels of CXCR4 expression and patient outcome in certain human tumors (57, 61, 64, 115). Targeting CXCL12/CXCR4 pathway is a logic strategy in treating cancer patients.

CXCR4 is one of the coreceptors for human immunodeficiency virus (HIV). AMD3100 is a CXCR4 antagonist and has been used in human clinical trials for treatment of HIV infection (24, 46). Phase I pharmacokinetic studies demonstrated the feasibility of intravenous dosing and showed that AMD3100 was well tolerated by the healthy volunteers (47). AMD3100 also mobilized CD34+ cells from the bone marrow into the peripheral blood of healthy volunteers as well as cancer patients (25, 76). Although these studies did not test AMD3100 as an anti-cancer intervention, the observations suggest that CXCL12/CXCR4 inhibitors would be potentially useful in clinical trials in treating cancer patients.

On the other hand, thousands of patients worldwide have received treatment with angiogenesis inhibitors or antagonists. Bevacizumab, a monoclonal antibody against VEGF, is one of them (53). Although administration of bevacizumab results in increased patient survival with certain cancers (20, 50, 56, 145), the clinical efficacy needs significant improvement. CXCL12 and VEGF synergistically induce tumor vascularization (66). It is thus expected that combination of anti-VEGF and anti-CXCL12 may be more effective.

Notably, CXCR4 and CXCL12 are expressed on multiple immune cells, vascular endothelial cells as well as stem cells. It is possible that targeting CXCR4/CXCL12 pathway may yield unexpected clinical effects. Nonetheless, it is evident that CXCR4/CXCL12 pathway is actively implicated in tumor pathogenesis and plays a significant role in tumor immunopathogenesis. Therefore, manipulation of this pathway represents new strategy for cancer treatment (154). Of course, we need to bear in mind that although targeting CXCR4/CXCL12 is an attractive option in treating human tumors, it is highly likely that to attain effective, reliable, and consistent clinical efficacy, a complicated combinatorial therapeutic regimen may be warranted.

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