The Chemokine Receptors CXCR4 and CXCR3 in Cancer

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Chemokines comprise a superfamily of at least 46 cytokines that were initially described based on their ability to bind to 18 to 22 G protein–coupled receptors to induce the directed migration of leukocytes to sites of inflammation or injury. In addition to mediating cellular migration, chemokine/chemokine receptor pairs have been shown to affect many cellular functions, including survival, adhesion, invasion, and proliferation, and to regulate circulating chemokine levels. Most malignancies also express one or more chemokine receptors. Early studies established a role for CXCR4 and CXCR7 in mediating breast cancer metastasis, but other chemokine receptors, including CXCR3, now are implicated in several malignancies as biomarkers of tumor behavior as well as potential therapeutic targets. This review summarizes our current understanding regarding the contribution of CXCR4 and CXCR3 to tumor behavior and how receptor expression is regulated, transduces intracellular signals, and contributes at the molecular level to tumor behavior. It also describes recent therapeutic approaches that target these receptors or their ligands.

Introduction

Chemokines and chemokine receptors are now being examined to determine their contributions to tumor behavior and as potential therapeutic targets. The family of chemotactic cytokines, comprising more than 46 members, is subdivided into four groups based on the position of the first two cysteine residues in their amino acid sequence (CC, CXC, C, and CX3C). Chemokines also can be divided functionally into inflammatory chemokines mediating resistance to infections and homeostatic chemokines that contribute to development and other physiologic processes. Chemokines bind an 18-member family of G protein–coupled receptors to mediate intracellular signaling. The physiologic function of these receptor/ligand pairs is to direct the migration of leukocytes, expressing a subset of receptors, to sites of tissue injury or inflammation; some ligand/receptor pairs also mediate tissue homeostasis. One of the first reports to investigate the relationship of chemokine receptors to tumor metastasis was published in 2001, but the field has grown rapidly since then. More than half the known chemokine receptors have been implicated as determinants of malignant behavior. This review focuses on two of these receptors, CXCR4 and CXCR3, and highlights recent advances in our understanding of the role these receptors play in cancer.

CXCR4 Properties

CXCR4 is an evolutionarily highly conserved seven-transmembrane G protein–coupled receptor that binds the ligand SDF-1α (stromal-derived factor 1α, CXCL12) [1]. Disruption of CXCR4 is embryonically lethal, resulting from failure of hematopoiesis, organ vascularization, and neuronal migration. Upon ligand binding, the CXCR4 receptor forms a complex with the Gαi subunit G protein, resulting in inhibition of adenylyl cyclase–mediated cyclic adenosine monophosphate (cAMP) production and mobilization of intracellular calcium. Dissociation of the Gαi subunit from Gβγ leads to activation of multiple effectors downstream, including ERK1/2, MAPK, JNK, and AKT. Ligand-stimulated chemotaxis is accompanied by cytoskeletal rearrangements, actin polymerization, polarization, pseudopodia formation, and integrin-dependent adhesion to endothelial cells and other biologic substrates.

CXCR4 in Cancer

Early evidence that chemokine receptors might play a role in cancer metastasis was provided in the study by Muller et al. [2], in which breast cancer and melanoma cell lines were probed by quantitative reverse transcription

polymerase chain reaction for expression of the known chemokine receptors. That study identified CXCR4 and CCR7 as the two receptors commonly elevated in malignant versus normal mammary epithelial cell lines. Breast cancer cells migrated in vitro in response to CXCL12 and CCL21, the ligands of CXCR4 and CCR7, respectively. Blocking antibody to CXCR4 inhibited metastasis in a mouse xenograft model using MDA-MB-231 human breast cancer cells. These data formed the basis of the hypothesis that malignant cells, like leukocytes, employ chemokine receptors to migrate toward chemokine ligands expressed at common metastatic sites, such as the lungs, bone marrow, and lymph nodes. In addition to that landmark study, more than 1000 reports have documented that CXCR4 is the most widely expressed chemokine receptor in malignancy. In addition to breast cancer and melanoma, CXCR4 is detected in malignancies of the ovary, prostate, colon, head and neck, brain, and bladder. Abundant evidence exists to support a role for CXCR4 in breast cancer metastasis [3]. In ovarian cancer, CXCR4 is the dominant chemokine receptor [4], and an association between CXCR4 expression and aggressive behavior has been identified in other disease sites.

The functional role of CXCR4 in tumor metastasis was demonstrated in multiple studies using small interfering RNA, small molecular weight inhibitory peptides, or neutralizing antibody directed to CXCR4, which showed that inhibiting CXCR4 activity reduced tumor cell migration in vitro and inhibited metastasis in vivo [2–7]. Likewise, CXCR4 overexpression in a murine model of melanoma enhanced metastatic dissemination to the lungs but, interestingly, did not affect spread to the lymph nodes [8]. In contrast, on breast cancer cells, CXCR4 contributes to tumor cell dissemination to the lymph nodes. These early studies indicated that although CXCR4 clearly contributes to tumor metastatic capacity, the same receptor expressed on different malignant cells directs tumor cells to different secondary sites. The mechanisms underlying this tissue tropism have yet to be identified but may reflect differences in tumor cell survival at the secondary site rather than differences in initial deposition [9•]. As is described later, CXCR3 also selectively mediates tumor cell metastasis to different sites, depending on the malignant cell of origin.

In addition to supporting metastasis, CXCL12 has a direct effect on the proliferation of some tumor cells. Ovarian carcinoma cells and non-Hodgkin's lymphoma cells, among others, are stimulated to grow in vitro in the presence of CXCL12; this pro-proliferative effect is blocked with neutralizing antibody to CXCR4 or with AMD3100, a specific inhibitor of CXCR4 [5,7].

CXCR4 is upregulated in malignant cells by several mechanisms. Vascular endothelial growth factor (VEGF) is a known inducer of CXCR4 expression, and it has been shown that hypoxia-inducible factor (HIF)-1 acts upstream to induce VEGF [10]. HIF-1 is a heterodimeric transcription factor responsive to oxygen concentrations in tissues and has been shown to upregulate CXCR4 expression. Thus, in hypoxic regions of expanding tumors, chemokine receptor levels might be increased to facilitate survival and escape from the primary tumor mass. In addition to facilitating distant metastasis, HIF-1 has been shown to induce CXCR4 in gliomas, leading to enhanced proliferation, resistance to apoptosis, and local invasion.

Like hypoxia, steroid hormones also can regulate CXCR4; the androgen receptor negatively regulates CXCR4 [11]. During prostate cancer progression, androgen receptor signaling is lost, which could theoretically result in increased CXCR4 expression and a more invasive and metastatic phenotype. Treatment of a Her2-expressing breast cancer cell line with estradiol resulted in upregulation of CXCR4 protein expression [12]. CXCR4 mRNA levels were not altered, indicating a posttranscriptional mechanism of regulation. The induction of CXCR4 was shown to be mediated through the estrogen receptor and involved activation of the PI3K/AKT, MAPK, and mTOR pathways.

Although CXCR4 expression has been documented in many tumor types, and we are beginning to understand how CXCR4 expression is regulated, our understanding of how CXCR4 or any chemokine receptor directly contributes to metastasis remains to be elucidated. We also now understand that expression alone does not indicate a direct contribution to metastatic behavior. For example, CXCR4 is detected by immunohistochemistry in very early breast lesions, including atypical ductal hyperplasia and ductal carcinoma in situ. In a panel of cell lines, CXCR4 was detected in highly metastatic, nonmetastatic, and immortalized normal mammary epithelial cells [13•]. Ligand-binding studies indicated that the number and affinity of CXCR4 receptors were similar in nonmetastatic versus highly metastatic cells. Differences in cellular responses to ligand binding were observed; however, these occurred at the level of G protein activation. In metastatic cells, CXCL12 binding to the $G\alpha\beta\gamma$/GDP protein complex leads to a GTP-for-GDP exchange, allowing $G\alpha i$ to dissociate from the $G\beta\gamma$ subunit, leading to activation of ERK1/2, $I\kappa B\alpha$, JNK, Akt, p38 MAPK, and GSK-$3\alpha\beta$. In nonmetastatic cells, CXCR4 was able to independently form a complex with $G\alpha i$ or $G\beta$ subunits, but no $G\alpha\beta\gamma$ heterotrimer could associate with CXCR4 and, ultimately, $G\beta\gamma$-dependent downstream signaling did not occur. The molecular basis for the difference in G protein signaling in metastatic versus nonmetastatic cells remains to be elucidated. These studies have obvious implications for clinical studies that are examining CXCR4 protein expression but not receptor function. As observed in breast cancer cell lines, detection of CXCR4 protein does not necessarily indicate CXCR4-mediated signaling.

Although some of the downstream signaling pathways have now been identified, we still know little about how

those downstream effects determine metastatic success. It is unlikely that facilitating directed migration alone explains CXCR4's contribution to metastasis. The identification of downstream effectors is now contributing to our understanding in this regard. For example, a recent study shows that normal mammary epithelial cells secrete CXCL12, which acts on CXCR4-positive tumor cells to induce the expression of urokinase-type plasminogen activator receptor (uPAR) through a JNK-dependent pathway [14]. uPAR is an established contributor to the invasive potential of tumor cells.

In addition to specific CXCL12/CXCR4 receptor signaling, there is evidence that CXCR4 interacts with several growth factor receptor tyrosine kinases. Activation of the insulin-like growth factor (IGF) tyrosine kinase receptor by IGF-1, like CXCR4 activation, leads to enhanced tumor cell migration and invasion. In addition to activating IGF-1R, IGF-1 was shown to transactivate CXCR4 signal transduction in metastatic MDA-MB-231 cells but not to activate nonmetastatic MCF-7 cells, even though both cell lines are positive for IGF-1R and CXCR4 [15]. G protein–coupled receptors, tyrosine kinase receptors, and other cell surface receptors function in plasma membrane regions termed *lipid rafts* that are enriched in cholesterol and glycosphingolipids and contain concentrations of membrane signaling proteins. CXCR4 and the Her2 receptor are colocalized in lipid rafts of prostate cancer cells, and CXCL12 can transactivate Her2 in these cells in a Src kinase–dependent mechanism [16]. This receptor cross-talk resulted in upregulation of matrix metalloprotease 9 (MMP9) and enhanced invasion and metastatic growth in the bone. The molecular basis for receptor transactivation remains to be elucidated.

Therapeutic Potential

An extensive literature documents the association of CXCR4 with poor outcomes in several malignancies. In addition to being a useful prognostic marker, gene targeting or antibody blocking of CXCR4 inhibited growth and metastasis in several preclinical models, establishing the proof of principle that CXCR4 is a potential therapeutic target. Several CXCR4-blocking agents have shown antimetastatic activity. A peptide antagonist, TN14003, has tumor-inhibitory activity in murine models of breast and head and neck cancers [17,18]. The bicyclam antagonist AMD3100 was developed to inhibit HIV viral entry through CXCR4 acting as a co-receptor, and this and the related compound AMD4365 have demonstrable activity in models of glioma, medulloblastoma, and thyroid and other cancers [19,20•]. An analogue of CXCL12, CTCE-9908, inhibited lung colonization in melanoma and osteosarcoma models [21]. Both CTCE-9908 and AMD3100 are being evaluated in clinical trials, the results of which are eagerly awaited.

CXCR3 Properties

CXCR3 binds three ligands with high affinity: CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC). Like CXCR4, CXCR3 is a classic seven-transmembrane G protein–coupled receptor linked to several pathways, including MAPK, Src, and PI3K signaling pathways. In addition to binding multiple ligands, CXCR3 is expressed in several forms. CXCR3-A is expressed on Th1 T cells, cytotoxic $CD8^+$ T cells, and activated B and natural killer (NK) cells and mediates the directed migration of these cells to inflamed lymph nodes and other reactive sites. An alternatively spliced variant, CXCR3-B, contains a longer NH2-terminal extracellular domain. Transfection of CXCR3-A or CXCR3-B into human microvascular endothelial cells revealed that CXCR3-B inhibits DNA synthesis and induces apoptosis whereas CXCR3-A supports cell survival and chemotaxis [22]. Thus, CXCR3-B mediates the well-documented angiostatic effects of CXCL9, CXCL10, and CXCL11 on tumor-associated blood vessels. A third variant containing a truncated C-terminus (CXCR3-alt) lacks an intact third and second extracellular loop, but cells expressing this variant are capable of migrating in vitro in response to stimulation with CXCL11 [23]. In spite of these apparent similarities between CXCR3-alt and CXCR3-B, a role for CXCR3-alt in angiostasis has not been established.

CXCR3 in Malignancy

CXCR3 has been detected on many malignant cell lines and has been linked to patient outcomes in melanoma and colon and breast cancer [24•,25•,26]. For these disease sites, high CXCR3 expression is linked to more aggressive disease. In contrast, in renal cell carcinoma and chronic lymphocytic leukemia (CLL), low CXCR3 levels predict shorter survival times [27,28]. The following sections summarize CXCR3's role in melanoma, breast cancer, and colon cancer, highlighting the similarities and differences in CXCR3 function in these three malignancies.

CXCR3 in melanoma

CXCR3 is widely expressed on melanoma cell lines, and migration of cultured tumor cells is stimulated in response to CXCL9. Highly metastatic B16F10 and poorly metastatic B16F1 cell lines express comparable levels of CXCR3 [29]. Gene silencing of CXCR3 in B16F10 cells inhibited the development of lymph node metastases without affecting the number of pulmonary lesions. The locally growing tumor implant also was not affected by modulating the degree of CXCR3 expression. Thus, CXCR3 appears to play a role related to melanoma trafficking to lymph nodes but not migration to other secondary organs or to expansion at the primary site. Inducing CXCL9 and CXCL10 by injecting adjuvant into the lymph nodes facilitated homing of tumor cells to the inflamed node. CXCR3

expression was examined in two studies using primary human melanoma specimens [24•,29]. Approximately one third of the tumor samples had detectable CXCR3 by immunohistochemistry. CXCR3 expression was positively correlated with tumor thickness and the presence of distant metastases [24•]. These studies support the hypothesis that CXCR3 mediates melanoma metastasis to draining lymph nodes.

CXCR3 in breast cancer

The earliest report linking chemokine receptors to metastasis did not detect CXCR3 in breast cancer cell lines [2]; however, many studies since then have confirmed that CXCR3 is expressed and mediates cellular functions in breast and other malignant cells [30–32]. Human breast cancer cell lines express CXCR3-A and CXCR3-B, and, as observed on endothelial cells, these two receptor variants play different roles [32]. Gene silencing of CXCR3-B enhanced the proliferation of tumor cells in response to CXCL10, consistent with the previously described inhibitory activity of CXCR3-B on endothelial cell proliferation. Evidence also was provided that, unlike CXCR3-A, CXCR3-B does not appear to be linked to Gαi, as the effects on proliferation are not sensitive to pertussis toxin. Immunohistochemical examination of primary human breast tumors detected CXCR3 as well as the ligand, CXCL10. The antibody could not distinguish CXCR3-A from CXCR3-B. Other laboratories have shown that expression of both the ligand and receptor in breast cancer cell lines provides an autocrine loop in which CXCL10 stimulates proliferation of CXCR3-positive tumor cells [30].

Like human breast cancer cells, murine mammary tumor cell lines express CXCR3 [33•]. Receptor activation by CXCL9, CXCL10, or CXCL11 leads to calcium mobilization and a chemotactic response; however, the latter two ligands are more potent activators of these responses. Lung-colonizing ability or spontaneous metastasis to the lungs from a tumor implanted in the mammary gland was inhibited by systemic or local treatment with the small molecular weight CXCR3 antagonist AMG487. Likewise, gene silencing of CXCR3 compromised the lung metastatic potential of these cells [26]. Although CXCR3 likely mediates directed migration of tumor cells to distant sites, our studies also showed that neither receptor antagonism nor CXCR3 gene silencing was effective at controlling metastasis in hosts depleted of functioning NK cells. We hypothesize that compromising the ability of tumor cells to home to secondary sites is not sufficient to reduce metastasis; immune-mediated destruction of circulating tumor cells also may be required.

We examined CXCR3 protein expression in a series of 75 primary breast tumors from women with stage I or II disease at diagnosis. We detected CXCR3 in the cytoplasm and plasma membrane of malignant cells from every patient. The degree of expression was variable, however, and higher CXCR3 was an independent predictor of poor prognosis and worse long-term survival.

CXCR3 in colon cancer

Colon cancer cell lines express all known variants of CXCR3 [25•,34]. Stimulation with CXCL10 leads to cell migration, calcium mobilization, and ERK1/2 and AKT activation, which are accompanied by the induction of MMP2 and MMP9. Forced expression of CXCR3 in human colon cancer cells that do not constitutively express CXCR3 led to enhanced numbers of metastatic colonies in the para-aortic lymph nodes of animals with tumor cells implanted in the rectum. Metastatic rate to lungs or liver was not altered by changes in CXCR3 expression level. As observed in melanoma and breast cancer, altering the CXCR3 levels had no impact on the size of the primary tumor.

An examination of CXCR3 in primary human colon cancer specimens detected CXCR3 in approximately one third of tumors, whereas half the tumors were positive for CXCR4. CCR7 expression was detected in only 14% of tumors. Patients with CXCR3-positive tumors were significantly more likely to have lymph node metastases. CXCR3 was an independent risk factor indicating a poor prognosis.

Other cancers

Although CXCR3 expression appears to contribute to the aggressive behavior of melanoma, breast cancer, and colon carcinoma, and CXCR3 is expressed in other malignancies, the relationship between high CXCR3 expression and poor outcome is not true for all malignancies. In patients with renal cell carcinoma, 5-year disease-free survival was significantly better for patients with low–CXCR3-expressing tumors [28]. Poor prognosis in CLL also was correlated with lower CXCR3 [27].

CXCR3 Ligands

Dissecting CXCR3's role in tumor behavior is complicated by the fact that many cells in the tumor microenvironment potentially express CXCR3. Although tumor cell receptor expression clearly contributes to malignant behavior, many host cells—including cytotoxic T cells, Th1 cells, and NK cells, as well as endothelial cells in the tumor vasculature—may express CXCR3. There are several studies showing that forced overexpression of CXCL9, CXCL10, or CXCL11 in the local tumor inhibits tumor growth and metastasis [35–38]. In some models, the protective effect is associated with diminished angiogenesis; in other models, the protective effect of ligand expression depends on functioning T lymphocytes or NK cells. The angiostatic effect is likely mediated through CXCR3-B expressed on endothelial cells, whereas the T- and NK-dependent therapeutic effects are more likely to be mediated through

CXCR3-A expression on activated T and NK cells. In addition to attracting immune effector cells to the tumor microenvironment, we have hypothesized that by reversing the chemokine gradient, the tumor cell–expressed CXCR3 no longer supports migration to distant sites of lower ligand expression.

The importance of T-cell–expressed CXCR3 is illustrated by the observation that patients with cutaneous T-cell lymphomas had decreased surface expression of CXCR3 on circulating $CD8^+$ T cells [39]. Although CXCR3 transcript levels were normal, surface receptor expression was diminished, and, not surprisingly, these cells migrated poorly to CXCR3 ligands. Circulating CXCR3 ligands also were detected in patient plasma, leading to the hypothesis that high circulating ligand concentrations lead to downregulation of CXCR3 on $CD8^+$ cytotoxic T cells, compromising the ability of these effector cells to migrate into malignant lesions.

Therapeutic Potential

Antisense RNA targeting of CXCR3 inhibits metastasis of B16F10 melanoma to lymph nodes but does not affect the number of pulmonary metastases [29]. Administration of neutralizing antibodies against the ligands CXCL9 and CXCL10 produced a similar protective effect. Using a small molecular weight antagonist of CXCR3, AMG487, we have shown that systemic administration inhibits spontaneous metastasis of syngeneic breast tumors to the lungs [33•]. AMG487 was developed as a potential therapy for inflammatory diseases, and although efficacy was not demonstrated in clinical trials to treat psoriasis, the drug was well tolerated and might be a promising candidate to evaluate in clinical cancer trials. CXCR3 short hairpin RNA expressed in mammary tumor cells also was effective at controlling breast tumor metastasis. Regardless of whether a genetic or pharmacologic approach was used to inhibit CXCR3, the ability to affect breast cancer metastasis depended on the presence of functioning NK cells. These studies further support the idea that chemokine receptors do not function only to direct tumor cell trafficking. Although the results of these studies are encouraging, long-term systemic antagonism of CXCR3 might be anticipated to compromise the ability of CXCR3-positive T cells and NK cells to exert endogenous tumor control. Furthermore, antiangiogenic properties of CXCR3 ligands might be negated by CXCR3 antagonism. It is reassuring that, to date, studies by other laboratories as well as our own have not observed any growth-stimulating effects on the local tumor by CXCR3 downregulation or receptor antagonism. More extensive evaluation of the effect of receptor targeting on potential antitumor immune effector cell functions is needed to further address this important question.

Conclusions

In the 8 years since chemokine receptors were implicated in mechanisms of tumor metastasis, an extensive literature has developed documenting the expression of CXCR4, CXCR3, and other chemokine receptors on malignant cells. Clinical and preclinical studies have established that CXCR4 and probably CXCR3 are associated with more aggressive disease and enhanced metastatic potential in many disease sites. Preclinical studies indicate that reducing chemokine receptor function can markedly reduce metastatic potential, but the mechanisms underlying these potential benefits are largely unknown. Whether these findings can be translated into the development of effective therapies to prevent metastasis in the clinical setting will be determined in ongoing and future clinical trials.

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No potential conflict of interest relevant to this article was reported.

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