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 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 Goβγ/GDP protein complex leads to a GTP-for-GDP exchange, allowing Gαi to dissociate from the Gβγ subunit, leading to activation of ERK1/2, IκBα, JNK, Akt, p38 MAPK, and GSK-3β. In nonmetastatic cells, CXCR4 was able to independently form a complex with Gαi or Gβ subunits, but no Goβγ heterotrimer could associate with CXCR4 and, ultimately, Gβγ-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