The CXCR4/SDF-1 Chemokine Receptor Axis

A New Target Therapeutic for Non-small Cell Lung Cancer

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Abstract: Chemokines are proinflammatory chemoattractant cytokines that regulate cell trafficking and adhesion. The CXCR4 chemokine receptor and its ligand, stromal cell derived factor (SDF-1), constitute a chemokine/receptor axis that has attracted great interest because of an increasing understanding of its role in cancer, including lung cancer. The CXCR4/SDF-1 complex activates several pathways that mediate chemotaxis, migration and secretion of angiopoietic factors. Neutralization of SDF-1 by anti-SDF-1 or anti-CXCR4 monoclonal antibody in preclinical in vivo studies results in a significant decrease of non-small cell lung cancer metastases. Since anti-SDF-1/CXCR4 strategies have already been developed for use in combating human immunodeficiency virus infections, it is likely that these approaches will be used in clinical trials in non-small cell lung cancer in the very near future.

Key Words: CXCR4, SDF-1, Non-small cell lung cancer, Chemokines, Metastasis, Migration.

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Lung cancer has been the leading cause of cancer death worldwide for many decades. Despite this sobering statistic and years of research, treatments that meaningfully change the natural history of lung cancer remain elusive. Because metastatic spread constitutes the primary source of morbidity and mortality, a thorough understanding of the metastatic process is likely to be crucial to developing effective new therapies for lung cancer. Recently, a growing appreciation of the role of chemokines in cancer has unlocked the secrets of some of the molecular pathways underpinning this process, creating an understanding that reflects Paget's "seed and soil hypothesis" and which may lead to new approaches to lung cancer management. This brief review focuses on one such chemokine receptor axis, namely the CXCR4 and SDF-1 axis, and its emerging role in non-small cell lung cancer (NSCLC).

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Chemokines, a class of small (8–14 kDa) proinflammatory chemoattractant cytokines,[1] are the major regulators of cell trafficking and adhesion in the body.[2] Chemokines bind and activate a subset of seven transmembrane spanning G protein coupled receptors present on the surface of target cells.[3] They play a crucial role in the host immune defense system by recruiting specific leukocyte subpopulations to sites of tissue damage and infection, and are highly up-regulated by inflammatory stimuli such as antigens, polyclonal stimulants, cell irritants, and cytokines.[4,5] Chemokines also have a role in dendritic cell maturation, B and T cell development, T cell responses, infection response and angiogenesis. Most intriguing is the accumulating evidence pointing to their role in tumor growth and metastasis.[1]

Basic Biology of the CXCR4/SDF-1 Axis

The CXCR4 chemokine receptor and its ligand, stromal cell derived factor (SDF-1), constitute a chemokine/receptor axis that has attracted great interest partly because CXCR4 is a target for Human Immunodeficiency Virus (HIV) binding and entry into cells,[6,7] but also because of an increasing understanding of its role in cancer. CXCR4, although initially cloned from leukocytes,[8] is now known to be expressed by many cell types and has a role in multiple facets of cellular movement.[9] Its ligand, SDF-1 (also called CXCL12), a member of the alpha family of chemokines,[1] was initially isolated from a bone marrow stromal cell line[10] and is a potent lymphocyte chemoattractant.[11] However, SDF-1 is also constitutively secreted by fibroblasts in several different organs/tissues including bone marrow, lymph nodes, lung, liver, and muscle.[12,13] During embryogenesis the CXCR4/SDF-1 axis is involved in both hematopoiesis and organogenesis as demonstrated by knock-out studies.[12,14–16] Later, the CXCR4/SDF-1 axis plays a critical role in the homing and retention of hematopoietic/lymphopoietic stem cells, pre T and B lymphocytes in the bone marrow,[17,18] and the trafficking of these cells to sites of tissue inflammation and damage.[11,19] Both CXCR4 and SDF-1 can be up-regulated under certain conditions, especially in response to hypoxic stimuli. CXCR4[20] and SDF-1[21] gene expression are up-regulated in response to hypoxia inducible factor-1α (HIF-1α) in endothelial cells and in tumor cells in response to both HIF-1α[20,22] and NF-κB.[23] Significantly, it has been shown that activated epidermal growth factor receptor also increases the expression of CXCR4 in lung cancer tumor cells.[22] Although it had been thought that SDF-1 bound exclusively to CXCR4, and

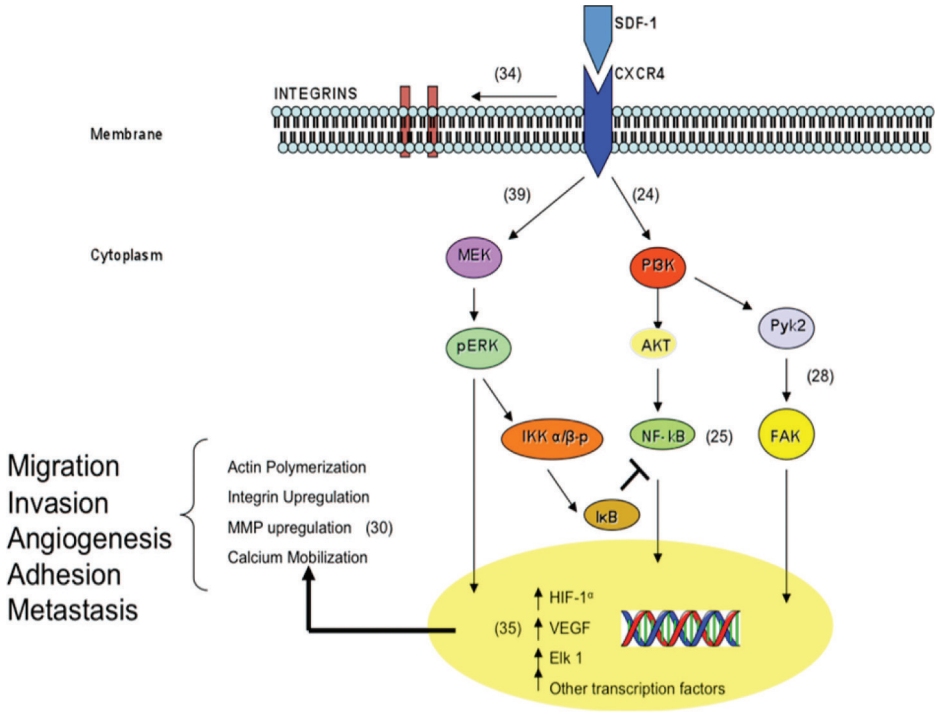

FIGURE 1. Schematic illustrating molecular pathways by which CXCR4 acts. Stromal cell derived factor (SDF-1) bound-CXCR4, possibly after incorporation into lipid rafts, acts via Gαi, to activate the phosphoinositol-3-kinase (PI3K) and mitogen activated protein kinase (MAPK) signaling pathways. Activated CXCR4 increases intracellular calcium mobilization and induces phosphorylation of focal adhesion components such as FAK and Pyk2. The activated signal transduction pathways contribute to chemotaxis, cell migration, and secretion of various matrix metalloproteinases (MMP's) including MMP-2 and MMP-9. Supporting references in brackets.

CXCR4 had SDF-1 as its only ligand,[24] there is now evidence that SDF-1 may signal through another chemokine receptor, CXCR7/RDC1,[25,26] but its role in cell trafficking and migration remains undefined.[27,28]

Molecular Pathways Associated with CXCR4/SDF-1 Axis

Investigations into the mechanisms of CXCR4 activation have demonstrated that SDF-1 bound-CXCR4 acts via Gαi, to activate the phosphoinositol-3-kinase (PI3K)[29] and mitogen activated protein kinase (MAPK)[30] signaling pathways (Figure 1). It has been suggested that the CXCR4/SDF-1 complex is incorporated into lipid rafts, and may associate with members of the Src family of kinases.[31] Activated CXCR4 increases intracellular calcium mobilization and induces phosphorylation of focal adhesion components such as FAK and Pyk2.[22,32–34] The PI3K/AKT and MAPK signal transduction pathways contribute to chemotaxis, cell migration,[30,35] and secretion of various matrix metalloproteinases (MMP's) including MMP-2 and MMP-9,[34,36] which are involved in the invasion of cells through the basement membrane.[37] They also induce expression of several cell surface integrins such as VLA-4 and VLA-5[38–40] and secretion of the angiopoietic factor, VEGF.[41] Furthermore, blocking either the PI3K or MAPK pathway inhibits CXCR4 activated cell migration in a pre B cell line.[30] Ultimately, through these pathways, SDF1-bound CXCR4 can induce cytoskeletal rearrangement, adhesion to endothelial cells, polarized migration of cells to specific organs and the secretion of angiopoietic factors, all important components of the metastatic process.[42–45]

CXCR4 and SDF-1 Role in Cancer

Involvement of the CXCR4/SDF-1 axis in cancer is attractive because it helps explain some of the well described phenomena associated with metastasis. The two key components of metastasis–acquisition of the capability to break away from the primary tumor to become blood or lymph borne and the subsequent ability to home in on its metastatic destination-can both be influenced by the CXCR4/SDF-1 axis. The molecular changes described above confer on the malignant cell a greater ability to migrate and invade,[46,47] while the constitutive release by stromal cells from common sites of metastasis such as bone marrow, lung, and liver, can guide the circulating cell to home in on its metastatic destination.[48] The role played by the CXCR4/SDF-1 axis in leukocyte trafficking and homing of stem cells[49,50] is likely analogous to organ selective metastasis of cancer stem cells.[51] In this model, a cell's metastatic potential will be determined by surface CXCR4 expression while its destiny will be influenced by local SDF-1 secretion at distal sites. As such, the CXCR4/SDF-1 axis can help explain the "nature" underlying metastatic tendency-CXCR4 expression-as well as the "nurture" of that tendency–SDF-1 secretion at metastatic sites.

The first evidence of a direct role for CXCR4/SDF-1 in tumor metastasis came from a pivotal study by Muller et al.,[52] which demonstrated that the most abundant of the chemokine receptors in breast cancer was CXCR4. Specifically, CXCR4 expression was markedly up-regulated in breast cancer cells compared with normal mammary epithelial cells in which CXCR4 was undetectable. Furthermore, in vivo administration of an anti-CXCR4 monoclonal antibody markedly re-

duced lung metastasis in a mouse model of breast cancer, providing the first evidence that CXCR4 inhibition may be a useful antimetastatic strategy. Subsequent studies have suggested that the CXCR4/SDF-1 axis plays a role in the metastasis of many types of tumor cells. Retrospective studies in breast,[47] especially triple negative breast cancer[53] and other cancers including nasopharyngeal[54] and ovarian carcinomas[55] have provided evidence that high expression of the CXCR4 receptor on primary tumor specimens correlates with a poorer clinical outcome. In vitro studies[54,56,57] in various tumor cell lines have shown that inhibition of the CXCR4/SDF-1 chemokine axis results in decreased migration and invasion of these cell lines. Lastly, in vivo studies have revealed that inhibition of the CXCR4/SDF-1 axis significantly decreased the amount of metastasis seen in mouse models of breast,[52,58] renal,[59] and head and neck cancers.[60]

Lung Cancer and the CXCR4/SDF-1 Axis

Increasing evidence suggests that the CXCR4/SDF-1 chemokine axis also plays a pivotal role in the metastasis of lung cancer, particularly in NSCLC. Firstly, in vitro studies have shown that NSCLC cell lines express high levels of CXCR4 but not SDF-1 and that SDF-1-activated CXCR4 promotes migration and invasion of these cell lines in vitro.[61] Interruption of this axis by CXCR4 down-regulation via transfection with a CXCR4 antisense nucleotide fragment, or by incubating cells with a CXCR4 neutralizing antibody, significantly decreases migration, invasion, and adhesion of NSCLC cell line cells in vitro.[62] Secondly, in vivo studies using a SCID mouse model of heterotopic or orthotopic xenoengraftment of human NSCLC cells show that preferential sites of lung cancer metastases have significantly higher levels of SDF-1 protein expression than the primary tumor or plasma levels[61] suggesting that a chemotactic gradient may be established between the site of the primary tumor and metastatic sites. In vivo neutralization of SDF-1 by an anti-SDF-1 or anti-CXCR4 monoclonal antibody resulted in a significant decrease of NSCLC metastases to several organs including the adrenal glands, liver, lung, brain, and bone marrow.[22,61] Importantly, this is supported by retrospective clinical studies that show a correlation between primary tumor CXCR4 expression and clinical outcome in NSCLC patients. Patients with high CXCR4 expressing tumors seem more prone to metastasis than patients with low expressing tumors.[62] When CXCR4 expression is limited to the nucleus only, this correlation is lost and a better prognosis is seen clearly suggesting that the metastatic potential is conferred by presumed membrane localization of the receptor.[63] Taken together, these studies provide a sound rationale for pursuing anti-CXCR4/SDF-1 approaches in clinical trials.

Anti-CXCR4 Strategies in Clinical Trials

Given the accumulating evidence supporting a role for the CXCR4/SDF-1 axis in NSCLC it is not surprising that attention is now focusing on it as a therapeutic target. Although disruption of the CXCR4/SDF-1 axis has been explored in HIV treatment, there is a dearth of experience using this approach in the oncology world. Several CXCR4 inhibitors have been tested in preclinical studies to assess their efficacy in preventing the growth and spread of various tumor cell types, but only recently have these agents entered into clinical trials. Two of these deserve a mention. AMD 3100 is a small molecule specific antagonist of the CXCR4 receptor which competitively binds and prevents the interaction of the receptor with SDF-1. A recent clinical trial showed that the administration of AMD 3100 in combination with granulocyte colony-stimulating factor to patients with non-Hodgkin lymphoma or multiple myeloma significantly increased the mobilization of hematopoietic progenitor cells to be used for autologous transplantation.[64] That this approach can influence the distribution of progenitor cells in vivo is a very significant proof of principle. Another agent, CTCE-9908, a small peptide CXCR4 antagonist, is well tolerated and preliminary evidence from phase I/II clinical trials with 26 patients, including 3 lung cancer patients, indicate that this agent is efficacious in late stage solid tumors.[65] To date there are no other published trials of this approach in early or metastatic disease. There have been no trials of anti-CXCR4 or anti-SDF-1 monoclonal antibodies and no trials as yet of CXCR4 antisense approaches.

CONCLUSION

Paget's concept of seed and soil implies that a number of factors involving both the "nature" of the malignant cell and the "nurture" provided by the distal environment must collaborate to allow metastasis to occur. Clearly, chemokines and their receptors provide a molecular explanation for this "seed and soil" phenomenon. The increasing evidence supporting a role for the CXCR4/SDF-1 axis in the metastatic evolution of several cancers, and especially NSCLC, provides the rationale for targeted disruption of this axis in future clinical trials. Because of its well-defined role as a portal of entry for the HIV, a number of inhibitors of this axis have already been developed and may be promptly tested in preclinical models and clinical trials. It is very likely that the detailed understanding of the role of the CXCR4/SDF-1 axis in NSCLC will lead to new treatment strategies that may allow us to more meaningfully change the clinical course of this disease in the very near future.

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