·REVIEW·

Correlations of Chemokine CXCL12 and Its Receptor to Tumor Metastasis

JIANG Yu-Ping[1], WU Xiao-Hua[2]

[ABSTRACT] Previous studies suggest that chemokine CXCL12 and its receptor CXCR4 play important roles in embryonic development, stem cell trafficking, and inflammatory reactions. It is a central factor in directing cell movement of many physiologic and pathologic processes. The mechanisms of cancer cell metastasis and white blood cell trafficking are similar. CXCL12-CXCR4 axis exerts important effects in regulating growth, invasion, metastasis, and secretion of malignant cells. Data from animal experiments suggest that CXCR4 may be an important therapeutic target in inhibiting malignant growth and metastatic behavior of tumor cells. This review focused on the role of CXCL12-CXCR4 axis in regulating tumor metastasis and progression, and the molecular mechanisms that are essential to this process.

KEYWORDS: CXCL12; CXCR4; Neoplasm; Metastasis; Molecular regulator

1. Department of Gynecology and Obstetrics, The Second Hospital, Hebei Medical University, Shijiazhuang, Hebei, 050000, P. R. China

2. Department of Gynecology and Obstetrics, The Fourth Hospital, Hebei Medical University, Shijiazhuang, Hebei, 050011, P. R. China

Correspondence to: WU Xiao-Hua
Tel: 86-311-86033941-425
Fax: 86-311-86965898
E-mail: xiaohuawu65@yahoo.com

Received: 2006-05-16
Revised: 2006-08-08

Chemokines are a large subfamily of chemoattractant cytokines which are classified into 4 highly conserved groups: CXC, CC, C and CX_3C, based on the position of the first two tyrosines adjacent to the amino terminus. Chemokines exert their effects by binding to transmembrane domain G protein-coupled receptors. Currently more than 50 different chemokines and 20 different chemokine receptors have been cloned [1]. One chemokine binds to multiple receptors, and the same receptor usually binds more than one chemokines. However, there is one exception to this rule, which is the chemokine CXCL12 (namely stromal-derived factor-1, SDF-1). This chemokine binds only to CXCR4, and CXCR4 is its only receptor.

Expression of chemokine CXCL12 and its receptor CXCR4 in tissues and signaling pathways

It must be noted that CXCR4 is not a tumor specific marker. Furthermore, both receptor CXCR4 and ligand CXCL12 are widely expressed in a range of normal tissues. Functional CXCR4 protein is found on peripheral blood lymphocytes and unprimed T cells, monocytes, plasma cells, dendritic cells, vascular smooth muscle cells, endothelial cells, retinal pigment epithelial cells, intestinal and alveolar epithelial cells, microglia,

neurons, astrocytes and liver stem cells [2]. CXCL12 is constitutively secreted by stromal cells in many tissues and the most important sources of CXCL12 are bone marrow, lymph node, muscle and lung-derived fibroblasts.

The association of CXCL12 with CXCR4 activates multiple signaling pathways (Figure 1). After binding to CXCR4, CXCL12 first triggers dimerization of this receptor [3]. In addition, in order to signal most efficiently, CXCR4 has to be included in membrane lipid rafts. After being activated, CXCR4 becomes physically associated with Gαi protein (inhibitory G protein) and tyrosine residues within the C-terminus become phosphorylated, probably through the activation and association with the receptor of JAK2 (Janus kinase 2) and JAK3 kinases [3]. Subsequently, the activated CXCL12CXCR4 complex is rapidly internalized from the cell surface and followed by the binding of β-arrestin [4]. CXCR4 endocytosis may be necessary to activate several pathways, such as the activation of MAPK (mitogen-activated protein kinase) p42/44 cascade [5]. However, it was found that CXCL12-mediated phosphorylation of MAPK p42/44 also occurs in cells transfected with a mutant CXCR4 receptor that does not undergo internalization [4]. Internalized CXCR4 may again be re-expressed on the cell surface. It is recently noticed that the internalization of CXCR4 from the cell surface may be inhibited by heparin, L-selectin specific antibodies and L-selectin binding ligands such as fucoidan or sulfatide[6].

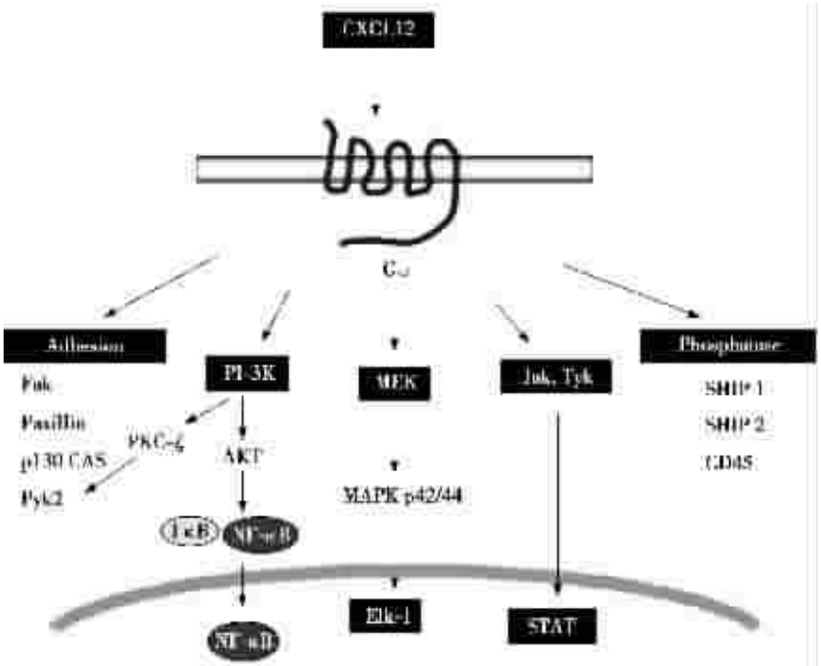

Figure 1 Signal transduction pathways activated by CXCL12-CXCR4 axis

Interaction of CXCL12 and CXCR4 activates several signal transduction pathways in the cells. Activation of these pathways differs between cell types, and regulates locomotion, chemotaxis, adhesion, and secretion of CXCR4-positive cells. PI-3k, phosphatidylinositol 3-kinase; SHIP, SH_2-containing inosital 5′-phosphatase.

The most important pathways involved in signaling from activated CXCR4 include activation of calcium flux and focal adhesion components, such as Pyk-2 (proline-rich kinase-2), p130Cas, FAK(focal adhesion kinase), paxilin, Crk and Crk-L, protein kinase C, PKC-γ (phospholipase C-γ) as well as MAPK p42/44-ELK-1 and PI-3K (phosphatidylinositol 3-kinase) -AKT-NF-κB (nuclear factor kappa B) axes [7]. CXCR4 signaling also involves several src-related kinases and T-cell activating molecule ZAP-70 [2]. JAK2 and JAK3 and Tyk-2 (Tyrosine kinase-2) may also be associated in some cell types with CXCR4 and are activated, probably by transphosphorylation, in a Gαi-independent manner [3]. As a consequence of these several members of the STAT (signal transducer and

activator of transcription) family, transcription factors may become recruited and phosphorylated. However, the involvement of STAT proteins in the signaling of activated CXCR4 may depend on the cell type. Membrane expressed haematopoietic phosphatase CD45 as well as proteintyrosine phosphatases (SHIP1 and SHIP2) are also involved in the modulation of CXCR4 signaling. Accordingly, haematopoietic cells derived from mice lacking SHIP1 showed altered patterns of chemotactic response to CXCL12. SHIP2 was also shown to regulate CXCL12 induced migration of T- and pre-B-cells [7]. Moreover, CD45 was found after stimulation by CXCL12 to associate with CXCR4 within lipid rafts [8]. This interaction could be inhibited after pretreatment of cells with β-cyclodextrin, a lipid raft formation inhibitor.

Modulators of chemokine CXCL12 and its receptor CXCR4

The N-terminus of CXCR4 and its first extracellular loop are crucial for CXCL12 binding. Hyposulfation of N-terminal tyrosine residues or enzymatic cleavage of the CXCR4 N-terminus by leukocyte-derived proteases inhibits CXCR4 signaling. In addition, CXCL12 may also be N-terminally truncated by cell-surfaceexpressed CD26. As a result, truncated CXCL12, in contrast to the full-length CXCL12, does not possess chemotactic activity and may even act as an antagonist of CXCR4[9].

Several factors have been recently identified to positively affect the sensitivity of CXCR4+ cells to a CXCL12 gradient [10] (Figure 2): ① anaphylatoxin C3a (C3 complement protein cleavage fragment), ② des-Arg C3a (product of C3a degradation by carboxypeptidase), ③ platelet derived membrane microvesicles, ④ hyaluronic acid, and ⑤ sphingosine-1 phosphate, which are found to significantly increase the chemotaxis of CXCR4+ cells to low dosages of CXCL12. Similarly, it is found that several other molecules such as fibronectin, fibrinogen, thrombin, soluble uPAR (urokinase-type plasminogen activator receptor), and VCAM-1 (vascular adhesion molecule-1) increase the chemotactic response of cells to low dosages of CXCL12 as well. Modulation of CXCR4 function by all these molecules on solid tumour cells could increase metastatic properties of CXCR4 positive

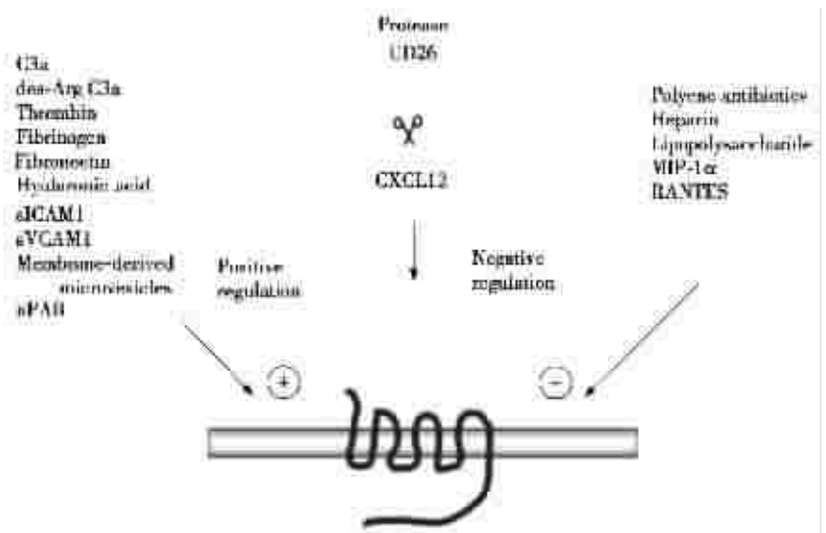

Figure 2 Modulation of CXCL12-CXCR4 axis by external factors

On one hand, leukocyte-derived proteases or cell surface-expressed CD26 may cleave both SDF-1 and the N-terminus of CXCR4; on the other hand, CXCL12-CXCR4 axis is regulated both positively (for example, by C3a, des-Arg C3a, thrombin, urokinase-type plasminogen activator receptor, fibrinogen, fibronectin, hyaluronic acid, sICAM1, sVCAM1, and cell membrane-derived microvesicles) and negatively (for example, by polyene antibiotics, heparin, MIP-1α, and RANTES).

tumour cells. Because inflammation plays an important role in tumor progression, molecules generated during inflammation could have an enhancing effect on the metastatic behavior of CXCR4+ tumor cells. Conversely, it has been reported that CXCR4 signaling may be desensitized in B lymphocytes and T lymphocytes by MIP-1α (macrophage inflammatory protein-1α ,CCL3) or RANTES (regulated on activation normal T-cell expressed and secreted,CCL5), which activate CCR5[11]. It may also be negatively modulated by heparin and lipopolysaccharide (LPS). It is likely that, whereas heparin restricts CXCL12 availability in the intercellular environment, LPS probably interferes directly with the binding of CXCL12 to CXCR4.

It was showed that this sensitization of HSC (hematopoietic stem cells) chemotaxis to a CXCL12 gradient is dependent on the cholesterol content of the cell membrane and incorporation of CXCR4 and small GTP-ase (guanosine triphosphatase) Rac-1 into membrane lipid rafts [12]. It was found that this incorporation of CXCR4 and Rac-1 in lipid rafts facilitate GTP binding of Rac-1. Recent data showed that depletion of membrane cholesterol severely affects responsiveness of CXCR4+ cancer cells to a CXCL12 gradient. Thus, drugs that perturb lipid raft formation by depleting cholesterol from cells such as polyene antibiotics could potentially negatively affect the metastatic properties of cancer cells.

The expression of CXCR4 is regulated by several transcription factors related to organ development as well as those related to stress and tissue damage. With respect to the former, it showed that expression of CXCR4 is regulated in various tissues by PAX genes (paired-box transcription factors). Sequence analysis of the CXCR4 promoter and chromatin immunoprecipitation analysis confirmed that PAX genes bind to the CXCR4 promoter. Because the pattern of expression of PAX genes is organ-specific, it is proposed that PAX proteins may regulate CXCR4 expression in a tissue-specific manner. Besides PAX genes, CXCR4 expression may also be positively regulated by transcription factors related to stress/hypoxia and tissue damage such as NF-κ B, HIF-1 (hypoxia-inducible factor), glucocorticoids, lysophosphatidylcholine, TGF-β (transforming growth factor-β), VEGF (vascular endothelial growth factor), IFN-α (interferon-α), and several interleukins (IL-2, IL-4, and IL-7)[13].

In humans there are two identified splice variant forms of CXCL12，namely α and β . The former is more abundant than the latter. Studies showed that CXCL12 expression is upregulated in endothelial cells by HIF-1, which leads to chemoattraction of CXCR4+ TCSCs (tissue-committed stem cells) that participate in tissue regeneration [14]. Expression of CXCL12 has also been found to be positively regulated by NF-κ B. Because HIF-1 and NF-κ B upregulated both the CXCR4 expression and the CXCL12 expression, this confers a pivotal role of the CXCL12CXCR4 axis in maintaining tissue repair. Conversely, the expression of CXCL12 was found to be downregulated by steroids and TGF-β 1 [2]. The downregulation of CXCL12 expression by steroids inhibits the accumulation of CXCR4+ inflammatory cells and contributes to the anti-inflammatory effect.

Expression and effect of chemokine CXCL12 and its receptor CXCR4 in tumor

The chemokine receptor most commonly found on tumour cells is CXCR4. CXCR4

expression has been reported in at least 23 different types of cancer, including cancers of epithelial, mesenchymal and haematopoeitic origin. For example tumor cells from breast, prostate, pancreatic, colon, lung and ovarian carcinomas, neuroblastoma and glioblastoma, all express CXCR4 [2]. However, there is less information on CXCL12 in malignant tissues and cell lines. The ligand is found in primary tumour sites in lymphoma, glioma, ovarian cancer and pancreatic cancer [2] and at sites of metastasis in breast and thyroid cancer, neuroblastoma and haematologic malignancies. CXCL12 is not produced by cell lines derived from breast and ovarian cancer, but has been detected in cell lines derived from prostate and pancreatic cancer as well as glioma.

Tumour metastasis is a complex and non-random multi-step process, involving in regulation of cell motility and migration, adhesion, invasion, proliferation, angiogenesis, homing to specific metastatic organs and evasion of the immune system. In this review, we will focus on the role of the CXCL12CXCR4 axis in regulating all these crucial steps for tumour metastasis.

Chemokine CXCL12 and its receptor CXCR4 and cancer cell motility and migration

It is noticed that CXCL12 increases the motility of several human tumour cells (e.g., breast cancer, rhabdomyosarcoma and small lung cancer cell lines). Activation of CXCR4 stimulates directed migration of cancer cells, invasion through matrigel, endothelial cell, bone marrow stromal, or fibroblast monolayers, towards a CXCL12 gradient [2]. CXCL12 displays significant increase in the number and thickness of F-action bundles, activation of PI-3K, augment of calcium flux and pseudopodia formation. The concentrations of CXCL12 required to stimulate migration, invasion, calcium flux and proliferation in malignant cells are comparable to those used to stimulate normal cells. Most commonly 1001000 ng/ml CXCL12 gives an optimal response in HPC, monocytes and endothelial cells and similar doses are effective on a range of cancer cell lines and primary cancer cells. The stimulation of cell movement by CXCL12 (motility and directional migration) was inhibited in normal haematopoietic cells as well as in several established cell lines after blocking the PI-3K-AKT axis by employing PI-3K antagonist LY290042 or inhibition of Gαi signaling by pertussis toxin[2].

Intra-arterial injection of MDA-MB-231 human breast cancer cells in immunodeficient mice resulted in osteolytic bone metastases. Subpopulations of cells with enhanced metastatic abilities were isolated by in vivo selection. A gene expression profile was linked with the bone metastatic clones. It is found that one of four highly over expressed genes was CXCR4, along with IL-11, osteopontin and CTGF (connective tissue-derived growth factor) [15]. In the syngeneic B16 melanoma model, CXCR4-transfected cells showed a 10-fold increase in lung metastases after i.v. injection and there was increased adhesion of these cells to dermal and pulmonary microvascular endothelial cells.

Chemokine CXCL12 and its receptor CXCR4 and cancer cell adhesion

It is known that CXCL12 modulates adhesion of cells to fibrinogen, fibronectin, stroma and endothelial cells by activation of the various adhesion molecules (e.g., integrins) [5.16]. CXCL12 was reported to activate integrins LFA-1 (lymphocyte function associated

antigen-1), VLA-4 (very late activation antigen-4) and VLA-5 on immature human haematopoietic cells [16]. It was found that CXCL12 also activates αIIb/β3 integrin (CD41) on the surface of megakaryocytic cells. Furthermore, CXCL12 induced firm adhesion and transendothelial migration in human CD34+ haematopoietic cells which was dependent on LFA-1/ICAM-1 (intracellular adhesion molecule-1) and VLA-4/VCAM-1 (VLA-4/vascular adhesion molecule-1) interactions. These interactions were inhibited by pertussis toxin and cytochalasin D, indicating the involvement of Gαi protein downstream signaling and the requirement of an intact cytoskeleton [16]. CXCL12 augments α 4β 7 mediated adhesion via activation of the GTPase RhoA.

A study has demonstrated that CXCL12 stimulation of different ovarian cancer cell lines upregulates the expression of β 1 integrin and this integrin modulation correlates with increased tumor cell adhesion to fibronectin[17]. In small cell lung cancer cells CXCL12 stimulation induced firm adhesion to marrow stromal cells via activation of α 4β 1 integrin. Overexpression of the signaling molecule Akt2 in both breast and ovarian cancer cell lines resulted in cell lines which exhibited increased adhesion to collagen IV due to upregulation of β 1 integrin. Adhesion molecules can also impact on chemokine receptor expression. In CXCR4+ lymphocytes, for example, activation of L-selectin increased cell surface CXCR4 expression. It indicates that signaling by CXCR4 modulates function of adhesion molecules on cell surface and in reverse adhesion molecules also increases CXCR4 expression [6].

Chemokine CXCL12 and its receptor CXCR4 and cancer cell secretion

Interaction of CXCR4 with CXCL12 led to the activation of NF-κB which plays an important role in cell secretion. In addition, it was found that cells after stimulation by CXCL12 secrete more MMPs (matrix metalloproteinase) (e.g., MMP-2 and -9), nitric oxide and some angiopoietic factors such as VEGF [5]. These factors play an important role in tumour migration through vascular basement membranes, in tumour vasculogenesis and in a crosstalk between CXCR4 positive haematopoietic cells and endothelium. The secretion of MMPs and VEGF by normal human megakaryocytes was dependent on PI-3K-AKT-NF-κB axis and was inhibited after exposure of cells to LY290042[18].

Chemokine CXCL12 and its receptor CXCR4 and cancer cell growth and survival

The effect of CXCL12 on cell proliferation and survival remains controversial. It has been suggested that the ratio between activated p38 stress kinase and the PI-3KAKT axis determines whether a particular chemokine will support or inhibit cell survival [2]. It was found that CXCL12 did not affect cell survival or proliferation in human CD34+ progenitor cells, 26 different established human T and B lymphoid and myeloid cell lines [5.18]. However, in normal human CD34+ cells CXCL12 induced phosphorylation of MAPK p42/44 and serinethreonine kinase AKT, which are associated with cell proliferation, yet did not affect cell proliferation and survival. In contrast, CXCL12 was found to stimulate the proliferation and survival of many solid tumors such as prostate cancer, glioma, astrocytes and epithelial ovarian cancer [19-21]. Some tumour cells were found to secrete CXCL12 and an autocrine CXCL12CXCR4 interaction had

been suggested to regulate the proliferation/survival of these cells [20]. Moreover, CXCL12 was found to be a survival factor for glioma and glioblastoma cells. This effect was correlated with a prolonged activation of the kinases AKT and MAPK p42/44 [21]. The CXCL12-CXCR4 interaction stimulated cell growth partially by phosphorylation of AkT [20.21]. These data support the notion that the activation of MAPK p42/44 and PI-3K-AKT pathways in target cells is not sufficient to stimulate cell growth /survival and also other complementary pathways have to be activated simultaneously in order to increase their proliferation/survival [18].

Chemokine CXCL12 and its receptor CXCR4 and tumor progression and prognosis

CXCR4 expression may be linked to other factors that are involved in the processes of malignancy. For example, CXCL12 stimulation of ovarian cancer cell lines and primary cells isolated from ascitic disease caused production of the cytokine TNF-α (tumor necrosis factor α)[19]. TNF-α has been implicated in tumour communication and is also an endogenous tumour promoter . There are also links between CXCR4 and VEGF. In breast cancer cell lines and glioma cells, VEGF was demonstrated to have an autocrine action and induce expression of CXCR4 which promoted migration towards CXCL12 [14]. Hormones have also been linked with CXCL12 in gynaecological cancers. It was reported that CXCL12 is a target of estrogen action in ER (estrogen receptor) positive human ovarian and breast cancer cell lines. Estradiol treatment induced expression of CXCL12 in ER+ cell lines and promoted cell proliferation which can be blocked with either addition of anti-CXCL12 antibody or ER antagonists.

Recently, clinical studies have showed that CXCR4 expression is correlated with tumor malignancy and poor prognosis. In refractory metastatic prostate cancer, CXCR4 expression was linked to the presence of metastatic disease and induced a more aggressive phenotype [22]. CXCR4 expression was higher in pancreatic cancer cell lines derived from metastatic lesions compared with those derived from primary tumors. CXCR4 expression was upregulated in invasive and advanced bladder cancer tissue. In contrast, superficial bladder tumors displayed low CXCR4 expression. In 136 esophageal tumors examined, patients with CXCR4 positive expression had significantly reduced median overall and disease-specific survival of 20 and 25 months respectively, compared with patients with CXCR4 negative expression of 76 and 97 months. CXCR4 expression was statistically associated with increased lymph node microinvolvement and bone marrow micrometastatasis and poor clinical outcome [23]. A total of 1808 invasive breast carcinomas and 214 pre-invasive breast samples could be analyzed and showed that CXCR4 cytoplasmic expression is linked to tumor aggressivity and has prognostic value with respect to disease-specific survival [24]. In addition, in patients with colorectal cancer, osteosarcoma, neuroblastoma, oral squamous cell carcinoma and malignant melanoma, CXCR4 expression increased the risk for recurrence, lymph node metastasis and poor survival [25]. However, in gastric cancer patients, the prognosis of CCR7 positive tumors was better than that with CCR7 negative tumors and no such correlation was observed for CXCR4 expression.

Most studies found that CXCL12 expression is not associated with tumor prognosis. However, the recent study using RT-PCR has showed that CXCL12 can increase the invasiveness and migration of breast cancer cells. Its level was correlated with lymph node involvement and poor survival [26].

Future directions

Functional CXCR4 is widely expressed by malignant cells. There is preliminary evidence that CXCR4 expression can confer an invasive phenotype. Moreover, the animal experiments show that CXCR4 antibodies or antagonists may inhibit cancer growth and spread. CXCL12CXCR4 axis emerges as an important regulator in tumor metastasis. All these blocking strategies could be potentially employed as new antimetastatic drugs to inhibit metastatic behaviour of CXCR4 positive cancer cells. However, CXCR4 and its ligand CXCL12 are also widely expressed in a number of homeostatic and inflammatory situations, and CXCR4 antagonists can mobilise stem cells from bone marrow and affect trafficking and development of haematopoietic stem cells, maturation of lymphocytes and platelet formation. However, the first clinical trials in HIV-infected patients where CXCR4 antagonist AMD3100 were employed to prevent binding of HIV to CXCR4 have proven safe for such therapeutic strategies. Besides the small molecular inhibitors of CXCR4, double stranded RNAi (RNA interference) could also be potentially employed to downregulate the expression of CXCR4. Downregulation of HIF-1 may lead to downregulation of CXCL12 in various tissues and inhibit spreading of CXCR4+ tumor cells. Recently, a small molecular inhibitor of transcriptional coactivation of HIF-1, called chetomin, has been identified. Systemic administration of chetomin inhibited hypoxia-inducible transcription within tumors and inhibited tumor growth in mice [27].

In this review, we have focused on a role of the CXCL12CXCR4axis in tumor metastasis. This process, however, is regulated by several other regulatory axes such as hepatocyte growth factor, VEGF and kit ligand. Relatively little is known about correlation between CXCR4 signaling and the oncogenes and growth factors in malignant disease. It would seem that further understanding of the action of CXCR4 in normal and malignant disease could lead to exciting new therapeutic options in a range of malignancies.

[REFERENCE]

[1] Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity [J]. Immunity, 2000,12(2):121-127.

[2] Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4 [J]. Semin Cancer Biol, 2004, 14(3):171-179.

[3] Vila-Coro A J, Rodriguez-Frade J M, De Ana A M, et al. The chemokine SDF-1α triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway [J]. FASEB J,1999, 13(13):1699-1710.

[4] Sun Y, Cheng Z, Ma L, et al. β-arrestin 2 is critically involved in CXCR4-mediated chemotaxis and this is mediated by its enhancement of p38 MAPK activation [J]. J Biol Chem, 2002,277 (51):49212-49219. Kijowski J, Baj-

[5] Krzyworzeka M, Majka M, et al. The SDF-1-CXCR4 axis stimulates VEGF secretion and activates integrins but does not affect proliferation and survival in lymphohematopoietic cells [J]. Stem Cells, 2001,19(5):453-466.

[6] Ding Z, Issekutz T, Downey G P, et al. L-selectin stimulation enhances functional expression of surface CXCR4 in lymphocytes: implications for cellular activation during adhesion and migration [J]. Blood, 2003,101(11):4245-4252.

[7] Chernock R D, Cherla R P, Ganju R K. SHP2 and cbl participate in α-chemokine receptor CXCR4-mediated signaling pathways [J]. Blood, 2001,97(3):608-615.

[8] Fernandis A Z, Cherla R P, Ganju R K. Differential regulation of CXCR4-mediated T-cell chemotaxis and mitogen-activated protein kinase activation by the membrane tyrosine

phosphatase, CD45 [J]. J Biol Chem, 2003,278(11): 9536-9543.

[9] Christopherson K W, Cooper S, Broxmeyer H E. Cell surface eptidase CD26/DPPIV mediates G-CSF mobilization of mouse progenitor cells [J]. Blood, 2003,101(12):4680-4686.

[10] Sbaa-Ketata E, Courel M N, Delpech B, et al. Hyaluronan-derived oligosaccharides enhance SDF-1-dependent chemotactic effect on peripheral blood hematopoietic CD34+ cells [J]. Stem Cells, 2002,20(6): 585-587.

[11] Hecht I, Cahalon L, Hershkoviz R, et al. Heterologous desensitization of T cell functions by CCR5 and CXCR4 ligands: inhibition of cellular signaling, adhesion and chemotaxis [J]. Int Immunol, 2003,15(1):29-38.

[12] Wysoczynski M, Reca R, Ratajczak J, et al. Incorporation of CXCR4 into membrane lipid rafts primes homing-related responses of hematopoietic stem/progenitor cells to an SDF-1 gradient [J]. Blood, 2005,105(1):40-48.

[13] Bachelder R E, Wendt M A, Mercurio A M. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4 [J]. Cancer Res, 2002,62(24):7203-7206.

[14] Ceradini D J, Kulkarni A R, Callaghan M J, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1 [J]. Nat Med, 2004,10 (8):858-864.

[15] Kang Y, Siegel P M, Shu W, et al. A multigenic program mediating breast cancer metastasis to bone [J]. Cancer Cell, 2003,3(6):537-549.

[16] Peled A, Kollet O, Ponomaryov T, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34+ cells: role in transendothelial/stromal migration and engraftment of NOD. SCID mice [J]. Blood, 2000,95(11):3289-3296.

[17] Scotton C J, Wilson J L, Milliken D, et al. Epithelial cancer cell migration: a role for chemokine receptors? [J]. Cancer Res, 2001,61(13):4961-4965.

[18] Majka M, Ratajczak J, Villaire G, et al. Thrombopoietin but not cytokines binding to gp130 protein-coupled receptors activates MAPKp42/44,AKT and STAT proteins in normal human CD34+ cells, megakaryocytes and platelets [J]. Exp Hematol,2002,30(7):751-760.

[19] Scotton C J, Wilson J L, Scott K, et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer [J]. Cancer Res, 2002,62(20):5930-5938.

[20] Sun Y X, Wang J, Shelburne C E, et al. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) *in vivo* [J]. J Cell Biochem, 2003,89(3):462-473.

[21] Barbero S, Bonavia R, Bajetto A, et al. Stromal cell-derived factor 1alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt [J]. Cancer Res, 2003,63(8):1969-1974.

[22] Darash-Yahana M, Pikarsky E, Abramovitch R, et al. Role of high expression levels of CXCR4 in tumor growth, vascularization, and metastasis [J]. FASEB J, 2004,8(11): 1240-1242.

[23] Kaifi J T, Yekebas E F, Schurr P, et al. Tumor-cell homing to lymph nodes and bone marrow and CXCR4 expression in esophageal cancer [J]. J Natl Cancer Inst, 2005,97(24): 1840-1847.

[24] Salvucci O, Bouchard A, Baccarelli A, et al. The role of CXCR4 receptor expression in breast cancer: a large tissue microarray study [J]. Breast Cancer Res Treat, 2006,97(3): 275-283

[25] Kim J, Takeuchi H, Lam S T, et al. Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival [J]. J Clin Oncol, 2005,23(12):2744-2753.

[26] Kang H, Watkins G, Parr C, et al. Stromal cell derived factor-1: its influence on invasiveness and migration of breast cancer cells *in vitro*, and its association with

[27] Kung A L, Zabludoff S D, France D S, et al. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway [J]. Cancer Cell, 2004,6 (1):33-43.