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REVIEW

Emerging targets in cancer management: role of the CXCL12/CXCR4 axis

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Monica Cojoc[1]
Claudia Peitzsch[1]
Franziska Trautmann[1]
Leo Polishchuk[2]
Gennady D Telegeev[2]
Anna Dubrovska[1]

[1]OncoRay National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden, Germany; [2]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

Abstract: The chemokine CXCL12 (SDF-1) and its cell surface receptor CXCR4 were first identified as regulators of lymphocyte trafficking to the bone marrow. Soon after, the CXCL12/CXCR4 axis was proposed to regulate the trafficking of breast cancer cells to sites of metastasis. More recently, it was established that CXCR4 plays a central role in cancer cell proliferation, invasion, and dissemination in the majority of malignant diseases. The stem cell concept of cancer has revolutionized the understanding of tumorigenesis and cancer treatment. A growing body of evidence indicates that a subset of cancer cells, referred to as cancer stem cells (CSCs), plays a critical role in tumor initiation, metastatic colonization, and resistance to therapy. Although the signals generated by the metastatic niche that regulate CSCs are not yet fully understood, accumulating evidence suggests a key role of the CXCL12/CXCR4 axis. In this review we focus on physiological functions of the CXCL12/CXCR4 signaling pathway and its role in cancer and CSCs, and we discuss the potential for targeting this pathway in cancer management.

Keywords: epithelial-to-mesenchymal transition, cancer stem cells, metastasis

Introduction to the CXCL12/CXCR4 axis

Chemokines are a class of small (8–10 kDa) inflammatory or homeostatic cytokines sharing a common biological activity in stimulating the migration of different types of cells including lymphocytes, monocytes, neutrophils, endothelial cells, mesenchymal stem cells, and malignant epithelial cells.[1,2] Chemokines are classified into four conserved groups – CXC, CC, C, and CX3C – based on the number and spacing of their N-terminal cysteine residues: CXC chemokines have a single nonconserved amino acid residue (X) between the first N-terminal cysteine residues (C); CC chemokines have these two cysteine residues adjacent; C chemokines have only one N-terminal cysteine; whereas CX3C chemokines contain three nonconserved amino acid residues separating the N-terminal cysteine pair. More than 50 chemokines have been discovered so far.[1,3] The classification of the chemokine receptors is based on the type of their ligands. For example, CXC receptors bind CXC ligands, while CC receptors bind CC ligands, etc.[3]

To date, over 20 chemokine receptors have been identified.[1,3–5] Chemokine receptors belong to a family of G protein-coupled receptors (GPCRs) containing seven transmembrane-spanning α-helix domains. One of the intracellular loops of the chemokine receptors couples with heterotrimeric G proteins that mediate a cascade of intracellular signaling following ligand binding.[4] The heterotrimeric G protein is composed of the Gα, Gβ, and Gγ subunits. Both Gα and Gβ subunits have covalently

Correspondence: Anna Dubrovska
OncoRay National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden, Fetscherstr 74/PF41, 01307 Dresden, Germany
Email anna.dubrovska@oncoray.de

Gennady D Telegeev
Institute of Molecular Biology and Genetics, NAS of Ukraine, Zabolotnogo Str, 150 Kyiv 03143, Ukraine
Email g.d.telegeev@imbg.org.ua

attached lipid tails that anchor G proteins to the plasma membrane. In the inactive or basal state, the Gα subunit contains the guanine nucleotide diphosphate (GDP).

Upon activation, GPCR acts as a GEF (guanine nucleotide exchange factor) and promotes the conformational change of the Gα subunit and replacement of the bound GDP by guanine nucleotide triphosphate (GTP). This exchange triggers the further conformation changes within the Gα subunit, which allows the trimeric G protein to be released from the receptor, and to dissociate into the GTP-bound Gα subunit and Gβ/Gγ dimer. Both the activated components interact with various effector proteins and initiate unique intracellular signaling cascades, such as activation of phospholipase C (PLC), regulation of adenylate cyclase, triggering of different kinase cascades including mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), p38, and the phosphoinositide 3-kinase (PI3K) routes (Figure 1).[5–9]

The distinct routes of the GPCRs signaling depend on the coupled Gα subunits, which are classified into four families; $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$. GPCRs coupled to the $G\alpha_s$ stimulate adenylyl cyclase whereas $G\alpha_i$ bound GPCRs inhibit it. The adenylyl cyclase serves as an effector enzyme that catalyzes 5′adenosine triphosphate into cyclic adenosine monophosphate (cAMP) and thereby activates cAMP-dependent protein kinase, which regulates a host of other downstream effectors including MAPK signaling pathway.[5,10] Activated $G\alpha_i$ is also able to activate the Src family of tyrosine kinases, which play an important role in signal integration.[3,6] GPCRs coupled to $G\alpha_q$ act through PLCβ, which cleaves phosphatidylinositol 4,5-bisphosphate to form the second messenger molecules called diacylglycerol and inositol-1,4,5-trisphosphate (IP_3).

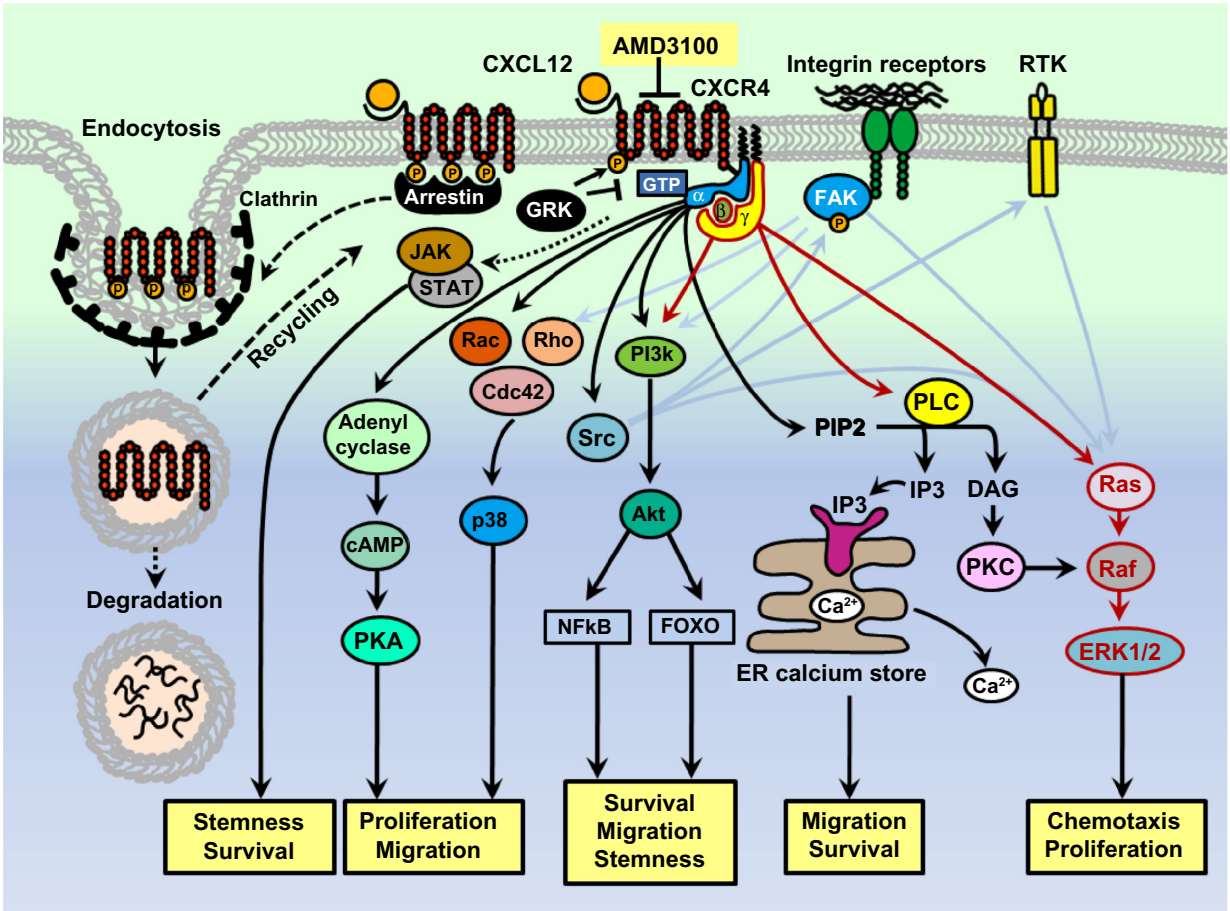

Figure 1 A schematic of the CXCL12/CXCR4 signaling pathways.

Abbreviations: ER, endoplasmic reticulum; GTP, Guanosine-5′-triphosphate; RTK, receptor tyrosine kinase; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C chemokine receptor; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; JAK, Janus kinase; STAT, signal transducer and activator of transcription; Cdc42, Cell division control protein 42 homolog; Rac, Ras-related C3 botulinum toxin substrate; Rho, Ras homolog gene family; GRK, G protein-coupled receptor kinase; FOXO, Forkhead box protein; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; PIP2, phosphatidylinositol bisphosphate; FAK, focal adhesion kinase; PLC, phospholipase C; PKC, protein kinase C; Ras, Rat sarcoma protein family; ERK, extracellular-signal-regulated kinase.

Diacylglycerol activates another enzyme called protein kinase C (PKC), whereas IP_3 diffuses to the endoplasmic reticulum where it opens calcium channels and triggers the release of calcium from intracellular stores into the cytoplasm. This intracellular calcium mobilization is frequently used for analysis of chemokine receptor activity.[11] GPCRs coupling to $G\alpha_{12}$ alternatively act via the Rho-GEF, which in turn activates the small G protein RhoA (Figure 1).[5,12]

Activation of PI3K by GPCRs is thought to be dependent on the direct binding of Gβγ subunits.[13] PI3K activation triggers a signaling cascade leading to the activation of AKT (also called protein kinase B) and its downstream targets including phosphoinositide-dependent kinase 1 (PDK1), glycogen synthase kinase 3 (GSK3), mammalian target of rapamycin (mTOR), p70 ribosomal protein S6 kinase ($p70^{S6K}$), forkhead family transcription factors (FOXO), and other signaling proteins. Notably, PI3K activation in response to the GPCR-mediated signaling results in the activation of focal adhesion kinase (FAK), which induces migratory activity in different types of cells, including tumor cells.[6,14,15]

The duration of the GPCR signaling depends on the Gα subunit lifespan in the GTP-bound state. Hydrolysis of the GTP of Gα-GTP to GDP leads to the inactivation of the Gα subunit and to its reassociation with the Gβ/Gγ dimer, which terminates all effector interactions.[16,17] In addition, chemokine receptor signaling is tightly regulated by the process of internalization and lysosomal degradation. Upon GPCR signaling activation, intracellular domains of receptors are phosphorylated by the second messenger kinases such as G protein-coupled receptor kinases (GRKs), followed by the binding of the phosphorylated receptors with regulatory proteins called arrestins. Arrestins impair communication of GPCRs with the G proteins and target them for lysosomal degradation following protein internalization and trafficking (Figure 1).[18,19]

Interestingly, it has been reported that several chemokine receptors including CCR2, CCR5, CXCR1, CXCR2, CXCR4, and CXCR7 can undergo homo- or hetero-dimerization upon ligand binding; a process that was proposed to regulate distinct intracellular signaling pathways.[7,20,21] Chemokines and their receptors display a high degree of redundancy in that most chemokines bind to multiple receptors and vice versa. The chemokine stromal cell-derived growth factor 1 (SDF-1), also known as CXCL12, binds primarily to its cognate receptor CXCR4, which is also a coreceptor for the entry of the Human immunodeficiency virus (HIV) into the target immune cells (T helper cells) besides the CD4 receptor.[22]

The assumption that CXCR4 is the only receptor for CXCL12 was recently challenged, since it was demonstrated that this chemokine also binds to the orphan receptor called CXCR7, which is a receptor for the interferon-inducible T cell chemoattractant CXCL11/I-TAC. Moreover, CXCR7 constitutively forms heterodimers with the CXCR4 receptor. Growing evidence indicates that binding of CXCL12 to CXCR7 does not result in activation of signaling pathways typical of G proteins. It has been proposed that CXCR7 serves as a ligand scavenger or acts as a "decoy" receptor.[23,24] Very recently, this receptor has been described as an activator of various signaling pathways in a CXCL12-dependent manner.[23,24] CXCR7 is broadly expressed in normal tissues including the heart, brain, spleen, kidney, lung, testis, ovary, thyroid, and human placenta.[23] A germline deletion of CXCR7 resulted in perinatal lethality, and expression of CXCR7 was associated with cardiac development.[25] Moreover, CXCR7 is upregulated in many malignant cells, including breast, lung, cervical, pancreatic, and prostate cancer cells, and found to be involved in tumor cell growth, survival, and metastasis.[21,22,24]

Physiological function of the CXCL12/CXCR4 signaling

CXCL12 is a small (8 kDa) homeostatic chemokine that was originally described as an efficacious lymphocyte chemoattractant and regulator of hematopoiesis, and was soon after also characterized as a modulator of multiple physiological processes.[26–29] CXCL12 is a pleiotropic chemokine that is widely expressed in different organs including the brain, lung, colon, heart, kidney, and liver where it acts as a chemoattractant for immature and mature hematopoietic cells; it thus plays an important role in inflammation and immune surveillance of tissues. Additionally, CXCL12 serves as an emergent salvage signal for initiating tissue regeneration and repair. Of note, various tissues respond to the chemical or physical insults such as toxic agents, hypoxia, and irradiation by increasing expression and secretion of CXCL12, which is important for recruitment of CXCR4 positive stem and progenitor cells to a site requiring tissue regeneration.[29] CXCL12 is expressed from a single gene in six splice variant isoforms known as SDF-1α, SDF-1β, SDF-1γ, SDF-1δ, SDF-1ε, and SDF-1φ.[30–32] These CXCL12 isoforms share the same first three exons but contain different fourth exons. Different splice variants are characterized by distinct properties such as stability and tissue of origin. SDF-1α is constitutively produced in many organs but tends to undergo rapid degradation in the blood. In contrast, SDF-1β displays high proteolytic stability and is expressed in highly vascularized organs such as the liver, spleen, and kidney. SDF-1γ is present in less vascularized organs such as the heart and brain.[30–32]

Dissection of the functional differences between the CXCL12 isoforms warrants further investigation.

The expression of CXCL12 cognate receptor CXCR4 has been shown to be highest in hematopoietic cells but it is also widely and constitutively expressed by numerous cells types including hematopoietic stem cells, endothelial stem cells, liver oval stem cells, neural stem cells, skeletal muscle satellite cells, primordial germ cells, retina pigment epithelium stem cells, and embryonic stem cells. All of these cells not only express functional CXCR4 on their surface but also follow a CXCL12 gradient.[26–29,33–39] A growing body of evidence suggests that the CXCL12/CXCR4 axis is essential for the migration of progenitor cells during embryonic hematopoiesis and organogenesis as well as for organ homeostasis, vascularization, and tissue regeneration.[38–51] Due to the apparent redundancy within the chemokine system, knockout of one of the chemokine/GPCR axes could be compensated to a large extend by other GPCR routes. However, mice lacking the *CXCR4* or *CXCL12* genes exhibit a significant defect in the colonization of embryonic bone marrow (BM) by hematopoietic stem cells (HSC), and show defects in the development of other organs including the heart, brain, and blood vessels. In the case of *CXCL12* and *CXCR4*, ablation of either gene is lethal and these embryos die in utero. Thus, the CXCL12/CXCR4 axis appears to have a fundamental physiological role in normal tissue development.[26,27,49–52]

Role of the CXCL12/CXCR4 axis in cancer and cancer stem cells

The chemokine CXCL12 and its cognate receptor CXCR4 were first identified as regulators of trafficking and tissue localization of B cells from patients with chronic lymphocytic leukemia.[53,54] Shortly thereafter CXCR4 was proposed to regulate the trafficking and invasion of breast cancer cells to sites of metastases.[55] More recently, it has been established that CXCR4 plays a central role in tumor cell dissemination

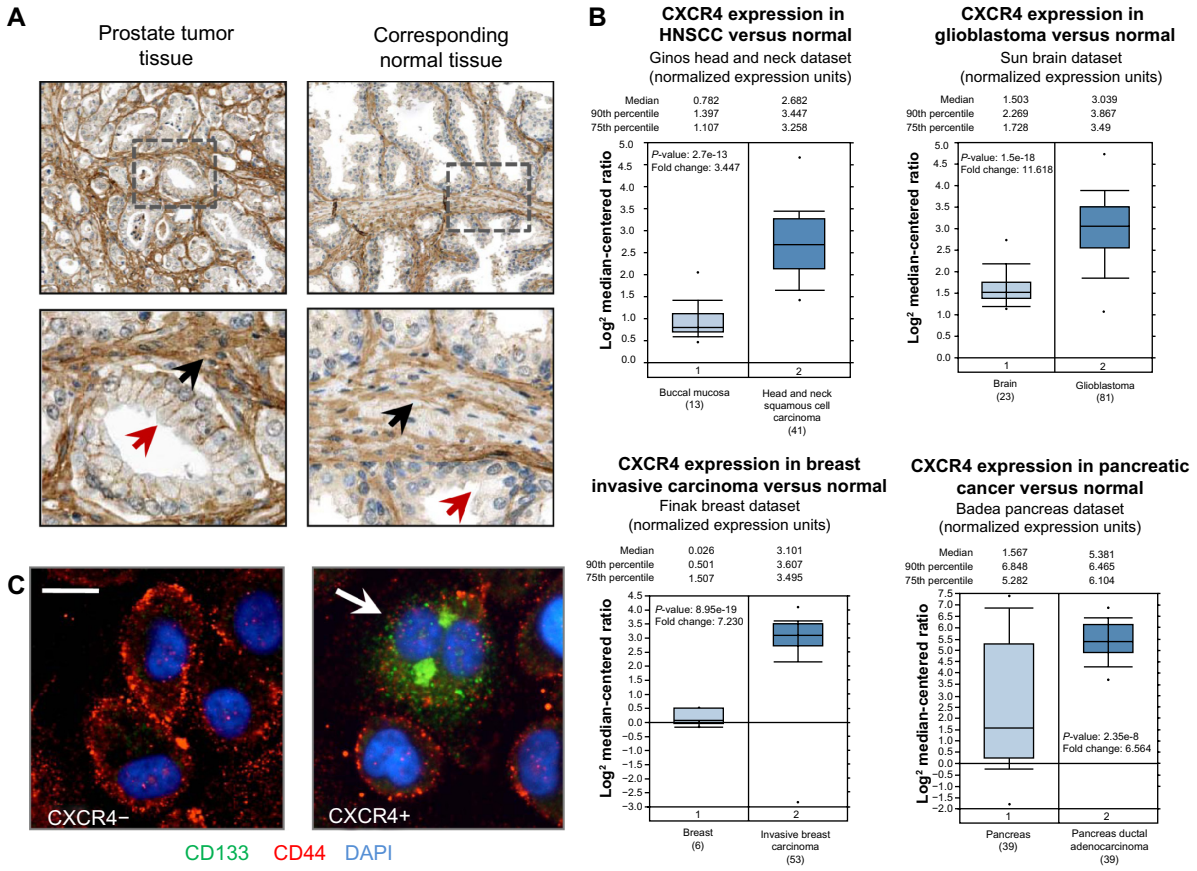

Figure 2 CXCR4 expression in cancer cells and tumor stroma.

Notes: (**A**) Prostate tumor cells have a higher expression of CXCR4 than corresponding normal epithelial tissues (shown with red arrows). Tumor and normal prostate stroma cells also express CXCR4 protein (shown with black arrows). (**B**) The Oncomine (Life Technologies, Carlsbad, CA, USA) data sets indicate a high expression of *CXCR4* mRNA in HNSCC, glioblastoma, breast, and pancreatic tumors compared to normal tissues. (**C**) Prostate cancer PC3 CXCR4+ cell population has a higher proportion of CD44+/CD133+ cells compared to PC3 CXCR4- cell population. Cells expressing high or low levels of CXCR4 were FACS-purified and plated in 384-well black clear-bottom plates in serum-free epithelial basal medium as described previously. Adapted from Dubrovska A, Elliott J, Salamone RJ, et al. CXCR4 Expression in Prostate Cancer Progenitor Cells. *PLoS ONE*. 2012;7(2):e31226.[110] The cells were fixed after 18 hours and stained with anti-CD133 and anti-CD44 antibodies. Scale bar indicates 15 μm.

Abbreviations: CXCR, CXC chemokine receptor; CD, cluster of differentiation; DAPI, 4',6-diamidino-2-phenylindole; HNSCC, head and neck squamous cell carcinoma; FACS, fluorescence-activated cell sorting.

and metastasis development in more than 75% of all cancers including breast, ovarian, lung, colon, prostate, kidney, melanoma, brain, esophageal, pancreatic, and many forms of leukemia.[56–60] Whereas CXCR4 expression is low or absent in many normal tissues, it is highly expressed in more than 23 different types of tumors including prostate, brain, breast, kidney, pancreas, ovarian cancer, and melanoma (Figure 2).[56] CXCL12 protein levels are highest in organs that are known to be the common sites of metastasis including the liver, bone marrow, and lungs, suggesting that tumor cells may use chemokine-mediated trafficking patterns, which are normally utilized during organogenesis, vascularization, and tissue regeneration.[30–32,55]

The CXCR4/CXCL12 axis also indirectly promotes tumor metastasis by mediating invasion and proliferation of tumor cells, and enhancing tumor-associated neoangiogenesis.[61–63] The stromal fibroblasts found within carcinomas and termed carcinoma-associated fibroblasts (CAFs) constitute the major cellular component of the tumor microenvironment. For a long period of time, neither the source of CAFs nor the differences between CAFs and fibroblasts from nonneoplastic tissue have been defined. Recent studies have shown that CAFs can arise from multiple origins including resident tissue fibroblasts, epithelial cells, human mesenchymal stem cells, and HSC.[64–66,67,68]

Analogous to their normal counterparts, which are present in inflammatory environments and aid in wound healing through tissue remodeling and repair, CAFs promote angiogenesis and matrix degradation through elevated secretion of CXCL12 and matrix metalloproteinase-1 (MMP-1).[66,69] During the past 25 years, the accumulation of evidence supports a critical and complex role of MMPs in tumor development. For example, MMPs can increase genomic instability in normal epithelium, which may result in tumor formation. MMPs are the key enzymes responsible for degradation of the extracellular matrix, which is the physical barrier for cancer cell invasion.[70] A recent study has identified *CXCR4* as a CAF-associated gene, implying an existence of an autocrine feedback loop (Figure 2).[71] The CXCR4/CXCL12 signaling in CAFs results in a multitude of cellular functions, including migration within the tumor microenvironment, adhesion, proliferation, and secretion of MMPs.[72–75]

A large number of studies demonstrated that CAF-derived CXCL12 not only stimulates carcinoma cell growth directly through the CXCR4 receptor displayed on tumor cells, or indirectly through MMP-mediated tissue remodeling, but also serves to recruit endothelial progenitor cells into tumors and thereby promotes neoangiogenesis.[64,65]

Moreover, CAF-mediated CXCL12 promotes an epithelial-to-mesenchymal transition (EMT) in primary tumors.[76] The acquisition of the EMT program is a critical process for the progression of cancers from local carcinomas to invasive malignancies, which is often associated with the loss of epithelial differentiation and gain of mesenchymal phenotype. Recent studies have shown a molecular link between EMT and self-renewal and demonstrated that cancer cells undergoing EMT gain the cancer stem cell (CSC) phenotype and tumorigenicity.[77–80]

A growing body of evidence indicates that a subset of cancer cells referred to as CSCs play a critical role in tumor initiation and resistance to anticancer therapy.[77] Similarly to normal stem cells, CSCs possess the ability to self-renew and to differentiate into all cell populations within the tumor mass.[81] This stem cell concept of tumorigenesis was proven the first time in 1994 by John Dick et al who demonstrated that $CD34^{+}CD38^{-}$ acute myeloid leukemia cell subset is a cell population initiating the malignant disease in immunodeficient mice.[82] Clarke et al introduced the CSC concept in solid tumors in 2003.[83] In this study they identified $CD44^{+}CD24^{-/low}$ breast cancer CSCs. As few as 100 cells with the $CD44^{+}CD24^{-/low}$ phenotype were able to form tumors in mice, whereas tens of thousands of cells with alternate phenotypes were unable to initiate tumor growth.[83] During the last few years, similar discoveries were made in other tumor types. Moreover, this population has been implicated to therapy resistance and tumor recurrence.[84–86]

Several lines of evidence suggest a link between the EMT and CSC characteristics in various types of cancer. Alterations in genes associated with developmental pathways such as Wnt, SHH and Notch are common in CSC populations, and could facilitate the EMT process. Analogous to differentiated somatic cells, which can be reset to a pluripotent stage by the process of induced dedifferentiation, non-CSC tumor cells can be reprogrammed by the activation of developmental pathways and EMT programs that change their self-renewal and differentiation potency.[79,80,85,87,88]

The positive correlation between EMT and CSC properties could lead to the concept of "migrating cancer stem cells" as the basis of metastatic colonization.[89,90] Recent findings demonstrated that a distinct subpopulation of CSCs can initiate tumor growth at secondary sites. The features of CSCs such as invasion, attachment-independent survival, and the ability to interact with micromilieu at the extravasation site support their involvement in metastatic dissemination.[84,91] Moreover, recent studies have revealed striking similarities of the signaling pathways regulating

CSC and driving metastasis formation. For example, Liu and colleagues reported a 186-gene "invasiveness" gene signature (IGS) that was identified by comparing the gene expression profiles of normal breast epithelium and breast CSCs with $CD44^{+}/CD24^{-/low}$ phenotype. Among 295 breast cancer patients, there was a significant association between the IGS signature and metastasis-free survival. The IGS was also applied to discriminate low- and high-risk patients with medulloblastoma, lung, and prostate cancers, demonstrating the prognostic value of markers that define CSCs.[92] More specifically, several reports demonstrated that CSCs are involved in metastatic dissemination in xenograft models of breast, pancreatic, and colorectal carcinoma.[83,93,94]

In support of the hypothesis that metastatic tumor cells could have both EMT and CSC phenotypes, clinical studies have shown that the majority (>80%) of the circulating tumor cells (CTCs) in patients with metastatic castration-resistant prostate cancer coexpress stem cell marker CD133 and mesenchymal proteins including vimentin, N-cadherin, and O-cadherin.[95] Study of CTCs from metastatic breast cancer patients revealed that 62% of the cells were positive for at least one of three EMT markers – Twist1, AKT2, PI3Kα – and 69% of the total

Table 1 CXCR4 as a marker for putative cancer stem cell populations in solid tumors

Tumor type	Additional CSC markers analyzed in combination with CXCR4	Biological functions of the CXCL12-CXCR4 axis	Methods used	References
Renal	N/A	Clonogenicity, tumorigenicity, drug resistance	– Analysis of the FASC sorted patient-derived cells for their spherogenicity and tumorigenicity; – Inhibition of CXCR4 expression by siRNA; – Chemical inhibition of CXCR4 functions in vitro; – Combination treatment with AMD3100 and pazopanib, sunitinib and sorafenib	161
Prostate	CD133, CD44	Spherogenicity, tumorigenicity, differentiation potential, drug resistance	– Analysis of the FASC sorted cells from the established cell lines for their differentiation potential, cell adhesion, clonal growth, spherogenicity, and tumorigenicity; – Inhibition of CXCR4 in vitro and in vivo by using chemical inhibitor and neutralizing antibody; – Combination treatment with AMD3100 and Taxotere (Sanofi SA) (docetaxel)	110
Colon	CD133	Migration and metastasis	– Analysis of the FASC sorted patient-derived cells for their migratory potential in vitro and metastatic properties in vivo	204
Pancreas	CD133	Migration, tumorigenicity, drug resistance and metastasis	– Analysis of the FASC sorted patient-derived cells for their migratory properties in vitro, tumorigenic and metastatic properties in vivo; – Analysis of the FASC sorted patient-derived cells for their resistance to the standard chemotherapeutic agent gemcitabine in vitro and in vivo	205
Glioma	CD133	Clonogenicity, spherogenicity, tumorigenicity, differentiation potential, chemo- and radioresistance	– Analysis of the FASC sorted patient-derived cells for their clonogenic properties, spherogenicity, and differentiation potential in vitro, and tumorigenicity in vivo; – Analysis of the FASC sorted patient-derived cells for their resistance to the standard chemotherapeutic agent temozolomide and radiation treatment	206
Gefitinib-resistant non-small cell lung cancer	N/A	Spherogenicity, chemo- and radioresistance, tumorigenicity	– Analysis of the FASC sorted A549/GR cells for their sphere forming and self-renewal capacity in vitro and tumorigenic properties in vivo; – Analysis of the FASC sorted A549/GR cells for their resistance to irradiation and to the standard chemotherapeutic agent gefitinib and cisplatin in vitro	176

Abbreviations: CXCR, CXC chemokine receptor; CXCL, chemokine (C-X-C motif) ligand; CSC, cancer stem cell; FACS, fluorescence-activated cell sorting; siRNA, small interfering RNA.

CTC population were positive for the CSC marker aldehyde dehydrogenase (ALDH).[96]

Although the signals generated by the metastatic niche that regulate CSCs are not fully understood, recent studies provide strong evidence for the important role of the chemokine receptor CXCR4 for CSC maintenance, dissemination, and consequent metastatic colonization. The role of CXCR4 has been examined in the CSC context in various types of cancer including pancreatic, colon, renal, brain, lung, and prostate cancer (Table 1 and Figure 2). Moreover, Fusi et al showed that CTCs from patients with metastatic carcinoma or melanoma are positive for CXCR4 expression.[97]

All together, these findings demonstrate that activation of the CXCL12/CXCR4 signaling can be indicative of the metastatic CSC population and suggest that therapeutic modulation of the CXCR4/CXCL12 axis may be essential for inhibition of metastatic and tumorigenic potential of CSCs.

Regulation and biological effect of CXCR4

The CXCR4/CXCL12 signaling in tumor cells is regulated at several levels.[49] First, expression of the CXCR4 and CXCL12 genes are regulated at the transcriptional level by hypoxia. Several reports suggest that low tumor oxygenation and other signals from the tumor microenvironment such as growth factors collaborate to promote EMT associated with high invasiveness and resistance to chemo- and radiotherapy.[98] In hypoxia, the lack of oxygen leads to the hypoxia inducible factor 1α (HIF1α)-dependent activation of the CXCR4 and CXCL12 expression.[99,100] In addition, tumor fibroblasts and macrophages within the irradiated tumor niche start to produce growth factors and cytokines including CXCL12 which may lead to the invasive behavior of CSCs.[101,102] The overlapping signaling mechanisms that govern tumor resistance to conventional treatment and invasiveness could explain why, in some cases, cancer, which relapses after treatment, can develop into more aggressive metastatic disease, which is difficult to treat, and is associated with poor clinical prognosis.[103–106]

Studies of the molecular interaction between stroma and tumor cells suggest that CSCs can acquire resistance to chemo- and radiotherapy by adhesion to extracellular matrix or accessory cells via integrin signaling and, thus, may be responsible for residual disease and relapses. At the molecular level, CXCR4 is an important mediator of the interaction of tumor cells with extracellular matrix proteins such as laminin, fibronectin, and collagen, which contributes to metastatic spread.[107–109] Our recent study demonstrated that CXCL12/CXCR4 signaling pathway regulates the adhesion of $CD133^+/CD44^+$ prostate cancer progenitors to the extracellular protein fibronectin that is important for metastatic process. Moreover, the expression of α5 and β3 integrin subunits, which form receptors for fibronectin, are strongly upregulated in $CD133^+/CD44^+$ progenitor cells compared to $CD133^-/CD44^-$ prostate cancer cells.[110] Despite the fact that CXCR4 does not directly modulate cell attachment, CXCR4 receptor engagement by CXCL12 plays an essential role in managing cell adhesion by modulation of integrin expression, FAK phosphorylation, and activation of p38 MAPK and ROCK kinases.[108,111] The disruption of the interaction of cancer cells with their microenvironmental milieu by CXCR4 inhibition leads to their sensitization to the cytotoxic therapeutic agents.[112,113] These findings are consistent with data of high CXCR4 expression by nasopharyngeal carcinoma cells in postradiotherapy patients.[114] Moreover, elevated CXCR4 expression shows prognostic value for patients with renal, colorectal, and breast carcinoma.[115–119]

In addition to HIF-1α, some other transcription factors can influence CXCR4 transcription, including v-ets erythroblastosis virus E26 oncogene homolog 1 and NF-kB nuclear factor kappa-light-chain enhancer of activated B cells, which mediate CXCR4-dependent tumor invasion upon stimulation with hepatocyte growth factor.[37,120,121] Furthermore, a novel vesnarinone-responsive molecule Krüppel-like factor 2 and histone deacetylase 3-interacting protein CREB3 were also shown to activate the transcription of the CXCR4 and, therefore, contribute to cell migration.[122,123] CXCR4 expression and function are positively regulated by the developmental signaling pathways Wnt, SHH and Notch and the oncogenic pathways PI3K/AKT, NF-kB, and JAK/STAT that are also strongly implicated as CSC regulators.[124–128] In turn, activation of the CXCL12/CXCR4 signaling may affect these pathways, suggesting a positive feedback loop between CXCR4 and the signaling routes regulating self-renewal capacity and tumorigenicity of cancer cells.[126,127,129–133] At the intracellular level, CXCL12/CXCR4 signaling triggers several phosphorylation cascades controlled by Src and AKT.

The PI3K/AKT axis serves as the central route in the CXCL12/CXCR4 signaling cascade.[134–135] Recently, we showed that activation of the CXCL12/CXCR4 and PI3K/AKT signaling pathways is important for self-renewal and tumorigenicity of prostate cancer cells with stem cell characteristics.[136,137] PI3K/AKT signaling regulates transcription through the FOXO by phosphorylating conserved serine/threonine residues. Transcriptionally active FOXOs affect a wide range of biological processes, including cell survival,

DNA repair, oxidative stress response, and longevity.[138] Among the members of the FOXO family, FOXO3A has been shown to be important for the maintenance of neural, hematopoietic, endothelial stem cells,[139–141] and cancer stem-like cell populations.[136,137] The chromatin immunoprecipitation assay demonstrated that FOXO3A binds to the CXCR4 promoter.[110] These data suggested that the CXCR4/AKT positive feedback system may play a role in the maintenance and dissemination of the prostate cancer progenitors.

In addition, CXCL12/CXCR4 signaling may promote tumor growth through transactivation of receptor tyrosine kinases such as EGFR, IGF-1R, and FGFR, which contributes to enhanced invasive signals and metastatic growth of breast, prostate, and ovarian tumors.[142–144]

CXCR4 has also been demonstrated to elicit intracellular signals through interaction with the scaffolding proteins independently of heterotrimeric G-protein coupling.[145] For example, CXCR4 signaling can be modulated by β-arrestin that induces CXCR4 internalization and attenuates CXCR4-mediated G protein activation. β-arrestin can be recruited to the CXCR4/CXCR7 heterodimeric complex resulting in potentiation of downstream β-arrestin-dependent cell signaling pathways, including ERK1/2, p38 MAPK, and SAPK/JNK, which enhances cell migration in response to CXCL12 stimulation.[21,24,25,146–148]

Finally, recent studies support a new view of CXCR7 as a signaling receptor independent of G proteins.[149] CXCL12 binding to CXCR7 activates the PI3K/AKT, PLC/MAPK, and protein kinase C pathways and promotes tumor growth, neovascularization, and dissemination.[24,25,146]

In summary, activation of CXCL12/CXCR4 axis may be critical for different aspects of tumor initiation, progression, metastasis, and therapy resistance, and targeting CXCR4 signaling might be beneficial in cancer treatment.

Critical analysis of the potential for targeting the CXCL12/CXCR4 axis in cancer management

Multiple agents are currently being developed to target CXCL12/CXCR4 signaling in cancer. Among these inhibitors is anti-CXCR4 drug AMD3100, also known as plerixafor (Mozobil; Sanofi SA, Paris, France), which is approved for stem cell mobilization in patients with non-Hodgkin's lymphoma and multiple myeloma, while the CXCL12 analog CTCE-9908 (Chemokine Therapeutics Corp, Vancouver, BC, Canada) is approved for clinical use in patients with osteosarcoma. Novel CXCR4 antagonists BKT140 (Emory University), POL6326 (Polyphor Ltd, Allschwill, Switzerland), and TG-0054 (ChemoCentryx, Inc, Mountain View, CA, USA), which were characterized as powerful human stem cell mobilizers, are currently in clinical trials for multiple myeloma, leukemia, and lymphoma. NOX-A12 (Noxxon Pharma AG, Berlin, Germany) is the only anticancer agent in active clinical development that neutralizes CXCL12 resulting in a complete block of CXCL12 signaling through its two receptors, CXCR4 and CXCR7. The anti-CXCL12 aptamer NOX-A12 is in clinical trial for the treatment of chronic lymphocytic leukemia and multiple myeloma. CXCR4 inhibitor MSX-122 (Altiris Therapeutics Inc, Tucker, GA, USA) is in Phase I trials for advanced malignant diseases that are metastatic or unresectable and that are resistant to standard therapy, while CXCR7-specific inhibitor CCX2066 (ChemoCentryx, Inc) is in preclinical studies.[134,150]

CXCR4 antagonist AMD3100 is the most studied among the agents that inhibit CXCL12/CXCR4 signaling. AMD3100 was initially studied as an anti-HIV agent and then it was discovered that this compound increases white blood cell counts in the blood and is able to mobilize stem cells from the bone marrow. This observation led to the examination of its anticancer activity.[151] AMD3100 has already been shown to decrease metastatic potential in animal models for different types of tumors, including breast, ovarian and colorectal cancer, melanoma, and oral squamous cell carcinoma.[152–158] Similarly, blocking CXCR4 receptor function by a monoclonal antibody or polypeptide inhibits cancer cell proliferation, motility, and invasion in multiple preclinical models both in vitro and in vivo.[110,113,159,160]

The fact that CXCR4 is present in normal and cancer stem-like cells in various tissues suggests that this molecule could be essential for maintenance and viability of tumor progenitor cell population. Indeed, recent data suggest that inactivation of the CXCL12/CXCR4 axis by neutralizing antibody or with the CXCR4-specific small molecule antagonist AMD3100 inhibits glioma, renal, colon, pancreas, and prostate cancer progenitors as well as tumor initiating population within gefitinib-resistant lung cancer and tamoxifen-resistant breast cancer cells in vitro and in animal models (Table 1).[107,161–163]

Preclinical and clinical data demonstrated that tumor cells can be protected from the effect of ionizing radiation by hypoxia, and determination of microenvironmental parameters such as tumor hypoxic fraction, vasculature, and perfusion may have a prognostic value for the response to radiotherapy.[164–166] Moreover, low oxygen tension is a critical microenvironmental factor in regulating tumor initiating

cells.[98,167] Hypoxia promotes expansion of glioma and colon CSCs and converts non-stem cancer cells into CSC populations with increased self-renewal capacity.[168,169] The effects of reduced oxygen tension on CSCs are mediated at least in part through the activation of the HIF signaling pathway.[167] As described above, CXCR4 expression is also induced under hypoxic stress via activation of the HIF pathway.[99,100]

Pharmacologic inhibition of the CXCL12/CXCR4 interaction by AMD3100 or neutralizing antibody prevents the recurrence of glioblastoma after irradiation in mice by inhibition of vasculogenesis.[170] Preclinical studies have shown that radiation upregulates HIF-1 expression level and activity in vivo.[171,172] This induces the *CXCL12* gene expression and promotes the mobilization of $CD11b^+$ monocytes from the BM, recruitment of these BM derived cells into the tumors, and development of functional tumor vasculature,[170,173,174] thereby supporting all remaining viable tumor cells. Similarly, concomitant treatment with local irradiation and AMD3100 induced a significant tumor growth delay and increased radiocurability in lung tumors by retention of BM derived cells.[175]

Another study demonstrated the role of CXCR4 in tumor radioresistance more definitively and showed that activation of CXCR4-mediated STAT3 signaling in non-small cell lung cancer cells (NSCLC) is functionally crucial for the maintenance of stemness and resistance to radiotherapy.[176] Another molecular route that can underlie the CXCR4-mediated radioprotection is the integrin signaling pathway. Accumulating evidence suggests that CXCR4 route enhances integrin-mediated adhesion and cooperates with integrin signaling in mediating chemoresistance.[177–179] In fact, CXCR4 engagement by CXCL12 induces expression of the integrin receptors such as α3, α5, β1, and β3 subunits, activation of FAK, and integrin-linked kinase, which is accompanied by the up-regulation of ERK1/2, JNK, and p38 phosphorylation.[108,179–182] Previous findings showed that integrins might induce tumor radioresistance via activating SAPK/JNK, MEK1/2, PI3K/AKT, and NF-kB signaling pathways.[183–185]

Collectively, these clinical and preclinical data are consistent with the CXCL12/CXCR4 pathway being a potential target to inhibit tumor growth and neovascularization, metastatic dissemination, and therapy resistance. Furthermore, the availability of pharmacologic inhibitors impinging CXCL12/CXCR4 signaling pathway opens novel opportunities for translational and clinical studies. However, important challenges remain prior to the clinical use of CXCR4 inhibitors in patients with solid tumors. First, CXCR4 is expressed by numerous types of healthy tissues.[26–29,33–39,183–188] Interference with CXCL12/CXCR4 signaling leads to deficiencies in hematopoiesis and organ homeostatic functions as well as in tissue repair after various stresses and insults, including cytotoxic drugs and radiation injury. Given the ubiquitous expression of CXCR4 and the functional importance of the CXCL12/CXCR4 signaling axis, this may impede the use of the CXCR4-targeted therapeutic tools in the clinic. Clinical studies of AMD3100 as a mobilizer of HSC in non-Hodgkin's lymphoma and multiple myeloma patients have demonstrated that AMD3100 has minimal side effects. However, in the clinic, AMD3100 is given to the patients as a daily subcutaneous injection with granulocyte colony-stimulating factor for a limited time (1–7 days),[189] whereas in preclinical studies for evaluating the antitumor efficacy of AMD3100, it is often delivered continuously via a subcutaneous osmotic infusion pump.[110,162,190,191] In clinical study for HIV treatment, AMD3100 was administered as a daily intravenous infusion or subcutaneous injection for a period of 11–14 days and, despite its efficacy for the treatments for HIV patients, the trials were discontinued due to cardiac toxicity.[192,193]

Accumulating experimental evidence suggests that combinatorial strategies based on bulk tumor reduction and CSC-specific pathway inhibition offer a promising treatment modality and are predicted to have a greater efficacy in tumor reduction and prevention of relapse than monotherapies.[88,110,137] If these conditions can be met, the use of two types of therapy in low-dose combination can provide better therapeutic effects with less side-effect toxicity. Recent prostate tumor xenograft studies in mice showed that a combination of AMD3100, which targets prostate cancer stem-like cells, and the conventional chemotherapeutic drug Taxotere (Sanofi SA), which targets the bulk tumor, is significantly more effective in eradicating tumors as compared to monotherapy.[110,136,137,194] However, efficacy of CXCR4 inhibitors against CSC function in cancer patients remains to be determined. The ongoing clinical trials for CXCR4 inhibitors as chemosensitizers in acute myeloid leukemia and other hematological malignancies will help to elucidate this question.

Conclusion

Although the collective evidence from the preclinical and clinical studies support the potential efficacy of CXCR4 inhibitors for development of innovative approaches to cancer treatment, several significant challenges remain before translation of these inhibitors into the clinic. A major

factor that can prevent a successful clinical use of the CXCR4-targeting anticancer therapy is a potential side effect on the stem cell compartment in normal tissues. This may be especially important when this treatment is combined with radiotherapy and other cytotoxic therapy associated with depletion of normal tissue progenitors. Thus, biology-driven rational design of novel combination therapies will be critical for the development of low-side-effect cancer treatment and can be based on the synergistic antitumor effect of CXCR4 inhibition and conventional therapy. There is also a need for the evaluation of the relationship between CXCR4 and tumor initiating cells in cancer patients. In fact, evaluation of the CXCR4 and CXCL12 expression level may have significant prognostic value in various types of cancer, including glioma, prostate, breast, colon, ovarian, pancreatic, and lung cancer where high expression of CXCR4 or CXCL12 predicts poor patient outcome.[195–203] However, direct proof for stemness of CXCR4$^+$ cells from primary human tumor tissues is still missing. A better understanding of the role of CXCR4 pathway for the maintenance of tumor progenitor population may be necessary for the development of screening tests to identify the patients who are likely to respond to CXCR4 inhibition. In addition, recent discovery of the cancer stem cell plasticity and heterogeneity can make the CXCR4+ tumor cell population a moving target that could be hard to track and eradicate.[85] Nevertheless, the key role of CXCR4 in tumor initiation, vascularization, dissemination, and therapy resistance underscore the importance of CXCR4 inhibition for the optimization of current anticancer treatment strategies.

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Disclosure

The authors report no conflicts of interest in this work.

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