

CXCR4 in breast cancer: oncogenic role and therapeutic targeting

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Abstract: Chemokines are 8–12 kDa peptides that function as chemoattractant cytokines and are involved in cell activation, differentiation, and trafficking. Chemokines bind to specific G-protein-coupled seven-span transmembrane receptors. Chemokines play a fundamental role in the regulation of a variety of cellular, physiological, and developmental processes. Their aberrant expression can lead to a variety of human diseases including cancer. C-X-C chemokine receptor type 4 (CXCR4), also known as fusin or CD184, is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12). CXCR4 belongs to the superfamily of the seven transmembrane domain heterotrimeric G protein-coupled receptors and is functionally expressed on the cell surface of various types of cancer cells. CXCR4 also plays a role in the cell proliferation and migration of these cells. Recently, CXCR4 has been reported to play an important role in cell survival, proliferation, migration, as well as metastasis of several cancers including breast cancer. This review is mainly focused on the current knowledge of the oncogenic role and potential drugs that target CXCR4 in breast cancer. Additionally, CXCR4 proangiogenic molecular mechanisms will be reviewed. Strict biunivocal binding affinity and activation of CXCR4/CXCL12 complex make CXCR4 a unique molecular target for prevention and treatment of breast cancer.

Keywords: breast cancer, CXCR4, drug target, chemokine, angiogenesis

CXCR4 structure, functions, and signaling

Chemokines are a superfamily of small molecule chemoattractive cytokines that regulate many cellular functions.¹ The CXC chemokine stromal-derived-factor-1 (SDF-1 or CXCL12) is expressed in a variety of cells, including stromal cells (fibroblasts and endothelial cells).^{2,3} Chemokine receptors are a family of G protein-coupled cell surface receptors (GPCRs) with seven transmembrane-spanning domains. Chemokines are small, structurally related proteins that play a significant role in leukocyte trafficking and are divided into four groups (CXC, CC, C, and CX3C) based on the position of the first two conserved cysteines.^{4,5} The chemokine receptor CXCR4 is a 352-amino acid rhodopsin-like GPCR that selectively binds to the CXC chemokine SDF-1 or CXCL12. CXCR4 is the physiological receptor for SDF-1, in addition to CXCR7.⁶ The SDF-1/CXCR4 axis plays an important role in many physiological processes, including trafficking and homeostasis of immune cells such as T-lymphocytes⁷ and homing and retention of hematopoietic stem cells within the bone marrow.⁸ The interaction between SDF-1 and CXCR4 leads to the activation of various intracellular signaling transduction pathways and downstream effectors that mediate cell survival, proliferation, chemotaxis, migration, and adhesion.¹ CXCR4 is highly expressed in various types of cancers, including oral cancer,^{9,10} esophageal cancer,^{11–13} gastric cancer,¹⁴ colon cancer,¹⁵ liver cancer,¹⁶ pancreatic

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cancer,¹⁷ thyroid cancer,^{18,19} breast cancer,¹ ovarian cancer,²⁰ prostate cancer,^{21,22} lung cancer,^{23,24} kidney cancer,^{25,26} brain cancer,^{27,28} melanoma,^{29,30} and leukemia,^{31,32} and is associated with chemotaxis, invasion, angiogenesis, and cell proliferation contributing tumorigenesis and cancer progression. A potential mechanism of CXCR4's involvement in tumor dissemination and metastasis is through promoting its transendothelial migration at the primary site.

Regulation of CXCR4 expression

In a typical model, CXCR4 mediates virtually all signaling pathways via its coupled G proteins which triggers a highly conserved DRY (Asp-Arg-Tyr) motif located in the second intracellular loop. This concept has changed after the finding of activation of downstream MAPK (mitogen-activated protein kinase) pathways through β -arrestins in the absence of detectable G protein activities. The latter delivers signals through the C-terminal (CT) tail of GPCRs which is rich in serines and threonines.³³ CXCR4 is expressed constitutively by lymphatic tissues, thymus, brain, spleen, stomach, and small intestine.³⁴ With regard to breast tissues, CXCR4 protein has no or low-level expression in normal breast epithelium and is highly expressed in ductal carcinoma, progressively increasing from atypical hyperplasia to carcinoma.³⁵

Transcriptional mechanism

Hypoxia inducible factor 1 alpha (HIF-1 α) and HIF-2: CXCR4 promoter has a functional hypoxia response element, so HIF can activate CXCR4 transcription. In normoxia, Von Hippel Lindau (VHL) protein, a negative regulator of HIF, degrades CXCR4 protein through HIF downregulation. In hypoxia, due to lack of VHL protein in a variety of cancer cell lines, HIF-1 and HIF-2 are stabilized and then induce CXCR4 transcription, expression, and, subsequently, CXCR4/CXCL10 pathway activation.^{36–38}

Nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B): Similar to HIF, there is an NF- κ B binding site within in the CXCR4 gene promoter. Upon stimulation of some ligands such as hepatocyte growth factor (HGF), NF- κ B subunits p65 and p50 bind at -66 to +7 of the CXCR4 promoter, transcriptionally activate CXCR4, and regulate tumor invasion.^{39–44}

Other factors: Vesnarinone-responsive molecule Kruppel-like factor 2 and histone deacetylase 3-interacting protein CREB3 have also been reported to inactivate the transcription of CXCR4 and, therefore, regulate cell migration.^{45,46} Transcription factor YY1, a 65 kDa DNA-binding protein, which can either repress or activate transcription depending on the context, is phosphorylated by carboxyl-terminal

Src kinase homologous kinase (CHK), and activated YY1 represses expression of CXCR4 in breast cancer cells.^{47,48}

Posttranscriptional and translational mechanism

CXCR4-mediated feedback loop of multiple signaling pathways

CXCR4 expression is positively regulated by the developmental signaling pathways Wnt/ β -catenin⁴⁹ (while knock-down of CXCR4 could obviously reduce proliferation and invasion of ovarian cancer cell and inhibit Wnt target genes^{50–52}), SHH-GLI-NANOG⁵³ (while a functional CXCR4 pathway is necessary to maintain the SHH-GLI1-NANOG network and self-renewal properties⁵⁴), and Notch as well as oncogenic pathways phosphoinositide 3-kinase (PI3K)/AKT,^{55,56} NF- κ B,^{44,57} and Janus kinase (JAK)/signal transducer and activator of transcription (STAT).^{58,59} In turn, the activation of the SDF-1/CXCR4 signaling activates MAPKs signaling, which promotes chemotaxis and proliferation,^{55,60} induces phospholipase C (PLC)/protein kinase C (PKC)-Ca²⁺ signaling promoting cell migration, and affects PI3K/AKT promoting cell survival.^{55,56} This indicates a positive feedback loop between CXCR4 and the signaling pathways mediating tumorigenicity of cancer cells (Figure 1). In addition, SDF-1/CXCR4 signaling pathway may transactivate epidermal growth factor receptor (EGFR)/HER2-neu signaling to promote invasive signals and metastatic growth of breast,⁶¹ prostate,⁶² and ovarian cancers.⁶³

Interestingly, CXCR4 can induce intracellular signals independent of heterometric G-protein coupling.⁶⁴ Binding of CXCR4 to SDF-1 can activate G protein-coupled receptor kinase (GRK) and the downstream molecule β -arrestin. β -arrestin can cause internalization and desensitization of CXCR4. However, if β -arrestin is recruited and activated by the CXCR4/CXCR7 heterodimeric complex, the downstream signaling cascades such as PI3K/AKT, extracellular signal-regulated kinase (ERK)1/2, PLC/MAP, p38 MAPK, and stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK) are enhanced, and thus tumor growth, cell migration, dissemination, and neovascularization are facilitated.^{65–69}

Vascular endothelial growth factor (VEGF) level is elevated in both breast cancer cell lines and clinical breast cancer samples. VEGF potentiates the metastasis of breast cancer cells in two ways:⁵⁵ in a paracrine manner, VEGF increases vascular permeability by mediating actin rearrangement and affecting gap junction, thus enhancing angiogenesis and tumor cell extravasation;⁷⁰ in an autocrine manner, VEGF promotes breast cancer cell migration and metastasis by inducing CXCR4 expression.

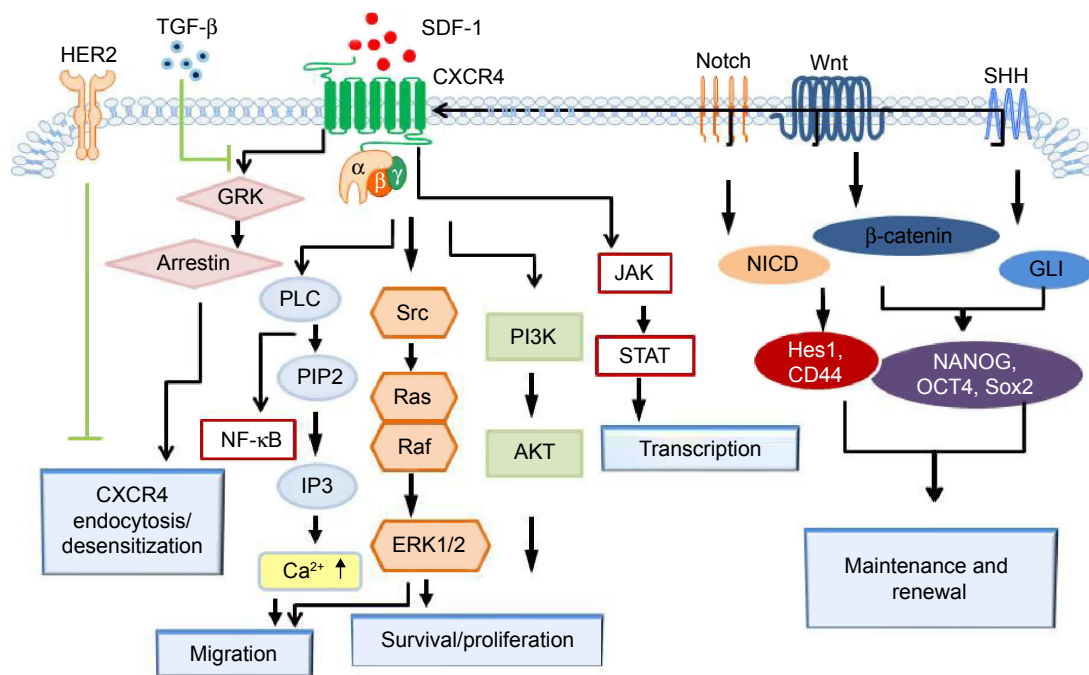


Figure 1 CXCR4-mediated feedback loop of multiple signaling pathways.

Notes: CXCR4 (C-X-C chemokine receptor type 4) expression is positively regulated by the developmental signaling pathways Wnt/ β -catenin,^{50–52} Sonic hedgehog (SHH)-GLI-NANOG⁵⁴ and Notch as well as oncogenic pathways phosphoinositide 3-kinase (PI3K)/AKT,^{55,56} NF- κ B,^{44,57} and Janus kinase (JAK)/signal transducer and activator of transcription (STAT)^{58,59} (not shown for clarity). In turn, the activation of the SDF-1/CXCR4 signaling activates mitogen-activated protein kinase (MAPKs) signaling promoting chemotaxis and proliferation,^{55,60} induces phospholipase C (PLC)/Protein kinase C (PKC)- Ca^{2+} signaling promoting cell migration, and affects PI3K/AKT promoting cell survival.^{55,56} This indicates a positive feedback loop between CXCR4 and the signaling pathways mediating tumorigenicity of cancer cells. Interestingly, CXCR4 can induce intracellular signals independent of heterometric G-protein coupling.⁶⁴ Binding of CXCR4 to SDF-1 can activate G protein-coupled receptor kinase (GRK) and downstream molecule β -arrestin causes internalization and desensitization of CXCR4. HER2 and TGF- β inhibit CXCR4 internalization and degradation.

Abbreviations: CXCR4, C-X-C chemokine receptor type 4; HER2, human epidermal growth factor receptor 2; TGF- β , transforming growth factor beta; SDF-1, stromal-derived-factor-1; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; NF- κ B, nuclear factor- κ B; ERK, extracellular signal-regulated kinase; NICD, notch intracellular domain; GLI, Glioma-associated oncogene homolog; CD44, cluster of differentiation-44; OCT, octamer-binding transcription factor.

Levels of CXCR4 protein are also regulated via post-transcriptional mechanisms. Prolonged exposure of cells to SDF-1 under physiological situations desensitizes them to SDF-1 through endocytosis of CXCR4 receptor. The receptor is ubiquitinated by the E3 ligase atrophin-1-interacting protein 4 (AIP4), which targets the CXCR4 receptor to the endosome and degrades it in lysosomes.⁷¹ However, Luker and Luker³⁵ and Basu and Broxmeyer⁷² report that the hematopoietic stem cells and myeloid progenitor cells retain responsiveness to SDF-1 even in the presence of sustained exposure to SDF-1. EGFR seems to be important for the CXCR4 internalization. The oncogene Her2 not only increases the translation of CXCR4 mRNA by 2.5-fold over controls, but it also blocks the ubiquitination and the degradation of CXCR4 after the binding of CXCL12 in breast cancer cells.^{35,73} Similar to Her2, another EGFR variant, EGFvIII affects CXCR4 expression at multiple levels. For instance, it increases CXCR4 expression transcriptionally through the upregulation of HIF-1 α . It can also enhance CXCR4 expression posttranslationally by activating p38 MAPK and inactivating AIP4 and β -arrestin1/2 to reduce

CXCR4 internalization, cellular trafficking, and lysosomal degradation.⁷⁴

CXCR4 in primary breast cancer

The role of CXCR4 in promoting breast cancer cell proliferation

Due to the similarity between the migration and invasion of cancer cells and chemotaxis of immune cells, initial studies of CXCR4 focused on its functions in metastatic breast cancer. In addition to metastasis, the SDF-1/CXCR4 signaling pathway plays a role in growth and proliferation of primary breast cancer. The first evidence for this is that Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line with low expression of CXCR4 formed much smaller tumor mass at inoculated sites when the cells were inoculated subcutaneously in the SCID (severe combined immunodeficiency) mice compared to those generated by MD Anderson-metastatic breast (MDA-MB)-231 cell line with high expression of CXCR4.¹ In addition, tumors cells with downregulated expression of CXCR4 by RNA interference and stable RNA interference grew more slowly than cells with upregulated

or normal levels of CXCR4 when the cells were implanted into syngeneic, immunocompetent mice.^{75,76}

The mechanism by which CXCR4 promotes breast cancer growth

The molecular mechanisms by which CXCR4 facilitates the growth of primary breast cancers are widely investigated, and can be summarized as follows:

1. Angiogenesis: it has been found that disruption of the interaction between SDF-1 and CXCR4 with neutralizing antibodies dampens the growth of human breast cancer xenografts in vivo, partly through reducing numbers of blood vessels.⁷⁷ Stromal cells present in primary breast cancers are the important sources of SDF-1. SDF-1 produced in the tumor microenvironment recruits circulating endothelial progenitors to sites of primary breast tumors and generates more microvessels thus increasing density of microvessels in cancer.⁷⁸
2. Typical signaling pathways related to cell proliferation: a number of downstream effectors of CXCR4 (PI3K/AKT, Src/ERK1-2, NF- κ B, and STAT3) which are well known for their growth-promoting ability have been identified to contribute to the growth of primary breast tumors (Figure 1). The cross talk between CXCR4 and other stem cell-related pathways such as Notch, Wnt, and SHH are also likely to be involved in breast cancer cell proliferation (Figure 1).

3. Recruitment of immune cells: in addition to endothelial progenitors, dendritic cells are also recruited to tumor sites through SDF-1. Dendritic cells suppress antitumor immunity by inactivation of cytotoxic T-cells, promoting tumor growth.⁷⁹ The schematic effects of CXCR4 on primary and metastatic breast cancer are shown in Figure 2.

Recently, some studies have successfully shown the relationship between hormone-dependent breast cancer cell proliferation and CXCR4 signaling. Stimulation of estrogen E2 on the two receptors of CXCL12, CXCR4, and CXCR7 promotes E2-mediated breast cancer growth. Estrogen E2 was found to stimulate CXCR4 transcription via estrogen receptor (ER)-binding sites at the CXCR4 promoter enhancing E2-mediated cellular growth.⁸⁰ In contrast, another receptor for the chemokine SDF-1 (CXCL12), CXCR7 expression was repressed by estrogen through inhibition of the recruitment of transcription factor NF- κ B at the CXCR7 promoter. CXCR7 overexpression decreases the growth effect of E2, although it can increase the basal MCF-7 cell growth rate.^{80,81} In addition, a nuclear receptor COUP-TF is overexpressed in MCF-7 breast cancer cells and inhibits the expression of the chemokine CXCL12, while enhancing the expression of its receptor CXCR4. Through the activation of epithelial growth factor (EGF), COUP-TFI increases the invasive potential of ER-positive breast cancer.⁸²

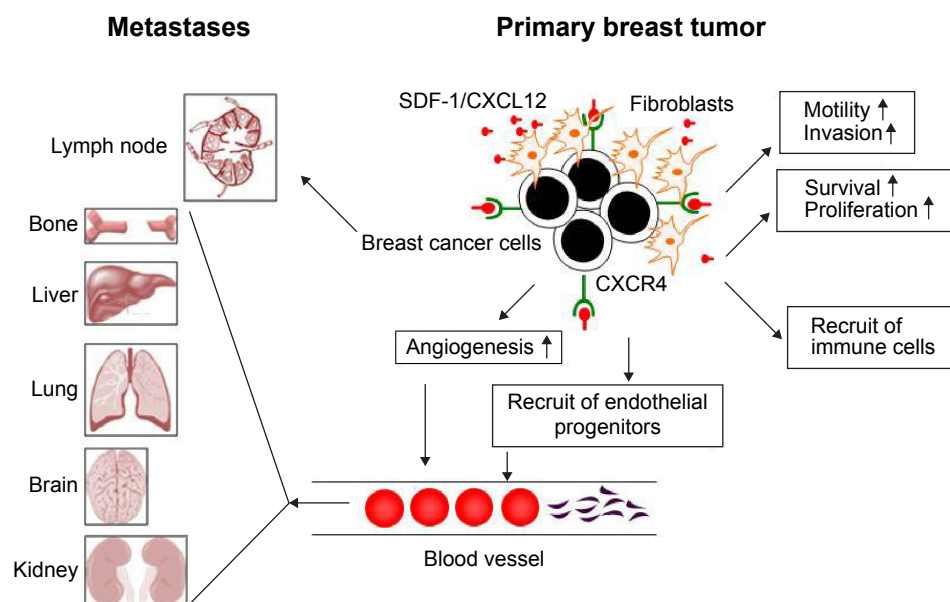


Figure 2 Potential roles for CXCR4 in breast cancer.

Notes: Breast tumor cells express CXCR4 and both breast cancer cells and fibroblasts produce SDF-1 (CXCL12). Tumor expressed CXCR4 directs metastasis to sites such as bone, liver, lung, brain, lymph node, and kidney. In addition, SDF-1/CXCR4 interacts locally in autocrine and paracrine manners to increase primary tumor growth. Tumor and tumor microenvironment secreted SDF-1 promote tumor cell survival and growth and also direct migration of immune and bone marrow derived cell into the tumor environment.

Abbreviations: CXCR4, C-X-C chemokine receptor type 4; SDF-1, stromal-derived-factor-1.

CXCR4 in metastatic breast cancer

Metastasis of cancer cells to distant locations needs several sequential steps, such as intravasation from the primary tumor sites into blood vessels, survival in the circulation, migration to secondary organs, adhesion, and proliferation of cancer cells in targeting organs and tissues.⁸³ CXCR4 promotes breast cancer metastasis to organs (bone, liver, and lung) where its ligand, SDF-1, is generated in large quantity. The cytokine SDF-1 is released in large amounts by lung, bone, and liver, which are the sites commonly affected by metastatic breast cancer. The interaction between SDF-1 and CXCR4 makes breast cancer cells move out of the circulation and traffic into organs with high amounts of chemokines, and thus forming metastatic tumors.² SCID mice inoculated with CXCR4-low-expressing MCF-7 did not demonstrate organ metastasis, while SCID mice inoculated with CXCR4-high-expressing MDA-MB-231 cells exhibited spontaneous metastasis in lung.¹ Moreover, the metastatic lung cancer cells express more CXCR4 than primary lung cancer cells.⁴⁴

Bone

Increased CXCR4 expression is present in breast cancer cells metastasized to bone.⁸⁴ Some bone cells such as osteoblasts produce SDF-1 (CXCL12), and this is the reason that breast cancer cells can easily metastasize to bone. The same mechanisms apply to liver and lungs, the other two target sites of breast cancer metastasis where large amount of SDF-1 are produced. Binding of SDF-1 (CXCL12) to CXCR4 activates the tyrosine kinase Src, and downstream effector AKT enhances the survival of breast cancer cells residing in the bone.⁸⁵

Brain

Tumor cells have the ability of breaching the blood–brain barrier, a tightly sealed area around the brain with high electrical resistance produce by tight junctions, to generate a metastatic cerebral tumor.⁵⁵ An in vitro study demonstrated that SDF-1 introduces the breast cancer cells into the brain via increased vascular permeability. Breast cancer metastasizing to the brain involves several signaling pathways, particularly those involving growth factors and chemokines. Several cofactors of CXCR4 are involved in breast cancer cell invasion. The CXCR4/SDF-1-mediated activation of PI3K/AKT and phosphorylation of focal adhesion kinase (FAK) and the forkhead transcription factor like 1 (FKHRL1) were necessary for breast cancer cell migration through the blood–brain barrier.⁷⁰ The mutual interplay between the CXCR4-VEGF,⁷⁰

CXCR4-NFκB,⁸⁴ and CXCR4-EGFR-ERK1/2^{44,86} pathways have been observed to promote breast cancer cell migration and metastasis.

Lung

As stated previously, CXCR4 signaling activated by its ligand SDF-1 causes chemotaxis and migration of breast cancer cells. Breast cancer cells that metastasized to the lungs exhibited high levels of CXCR4 expression compared to their parental cells, supporting the role of CXCR4 in organ directed-metastasis and invasion of breast cancer. Neutralizing antibodies to CXCR4 remarkably inhibited metastases of breast cancers to lymph nodes and lung.³⁵

Lymph node, liver, and kidney

Local lymph node involvement in breast cancer patients has been widely investigated; a recent meta-analysis showed that breast cancer with CXCR4 expression were associated with lymph node status (RR = 0.99, 95% CI: 1.01–1.43). Systemic studies of the role of CXCR4 on liver and kidney metastasis of breast cancer are not available at present.

CXCR4 and prognosis of breast cancer patients

A number of studies have assessed the effects of CXCR4 expression on breast cancer prognosis. Kato et al⁸⁷ systematically analyzed the expression levels of CXCR4 in 79 surgically resected invasive ductal carcinomas and the relation between the immunohistochemical staining pattern and clinicopathological outcomes. They found that the patients with high levels of CXCR4 had more extensive metastasis to lymph nodes compared to those with low levels of CXCR4.⁸⁷ Recently, more powerful meta-analyses were performed by two different groups quantitatively analyzing the impact of CXCR4 expression on breast cancer prognosis. Zhang et al⁸⁸ included 13 eligible studies consisting of 3,865 participants from USA, Canada, China, Japan, Korea, and Taiwan. Their meta-analysis showed that CXCR4 overexpression was associated with lymph node infiltration (risk ratio RR = 1.20, 95% CI [confidence interval]: 1.01–1.43, $P < 0.001$) and distant metastasis (RR = 1.52, 95% CI: 1.17–1.98). CXCR4 overexpression significantly reduced disease free survival (DFS) (RR = 0.7, 95% CI: 0.70–0.86) and overall survival (OS), with the risk ratio RR being 0.77 (95% CI: 0.70–0.86).⁸⁸

Xu et al⁶ investigated 15 published studies that included 3,104 patients which are from USA, Canada, Germany, France, China, and Japan. Their results are consistent with Zhang et al's⁸⁸ results and showed that the OS and DFS were

found to be significantly reduced in breast cancer patients with high levels of CXCR4 expression relative to those with low levels of CXCR4 expression, with the hazard ratio being 1.65 (95% CI: 1.34–2.03, $P < 0.00001$) and 1.94 (95% CI: 1.42–2.65, $P < 0.00001$), respectively. Stratified analysis indicated that high expression of CXCR4 in whole cells, cytoplasm, and nucleus predicts reduced OS, while only elevated levels of CXCR4 in whole cells and cytoplasm predicts a poor DFS.⁶ Therefore, CXCR4 is an efficient prognostic factor for breast cancer.

CXCR4 as a potential therapeutic target for breast cancer

Beyond potential applications for diagnosis of breast cancer, a variety of studies have shown that peptides or small molecule inhibitors of CXCR4 attenuate the growth of breast cancer in vivo and in vitro. Several novel CXCR4 antagonists have shown promising results in both in vitro and in vivo anticancer activity in several tumor cell types (using animal tumor models), including those derived from breast cancer. We summarize the effects of various CXCR4 inhibitors on breast cancer in Table 1.

T140 and its analogs as CXCR4 antagonists

Fourteen-mer peptides, T140 and its analogs, were originally developed as specific CXCR4 antagonists, as HIV-entry inhibitors, and as antirheumatoid arthritis agents.⁸⁹ CXCR4 antagonist T140 and its analogs actually block the CXCR4 receptor binding to its ligand SDF-1.⁸⁹ In 2003, Tamamura et al⁹⁰ first reported that T140 analogs, peptidic CXCR4

antagonists composed of 14 amino acid residues which were previously developed as anti-HIV agents, effectively inhibited SDF-1-induced migration, proliferation, and metastasis in human breast cancer MDA-MB-231. In the following year, Liang et al⁹¹ improved the compound T140 by substituting basic residue of the COOH-terminal with nonbasic polar amino acids to compromise the total positive charges of the molecule to generate a synthetic antagonist 14-mer peptide compound, TN14003. TN14003 (BKT140) is far less cytotoxic and more stable in serum relative to T140, and TN14003 not only proved to be effective in reducing metastases of breast cancer by inhibiting migration, but also is useful as a diagnostic tool for detecting CXCR4 receptor-positive tumor cells in cultured cells, living animals, and probably also patients, using positron emission tomography.^{35,91} However, lack of oral availability prompted Zhan et al⁹² to further look for alternatives that possess better pharmacologic profiles. Using TN14003 as their benchmark, a first nitrogen atom substitution of the terminal aromatic rings (ie, pyridyl instead of phenyl) was designated as WZ811,⁹² while a second nitrogen atom substitution in the terminal aromatic rings (ie, pyrimidyl) was designated as MSX-122. Compared to WZ811, MSX-122, which was prepared in a single chemical step using a reductive amination reaction, further improved the pharmacokinetic profile.⁹³ Both WZ811 and MSX-122 block lung metastasis of breast cancer. However, the advantage of MSX-122 is its ability to intervene the Gai-signaling pathway (cAMP modulation), but not the Gq-pathway (calcium flux).⁹⁴ Consequently, MSX-122 blocks chemotaxis and the homing of the CXCR4-positive cells (including cancer cells) to distant

Table 1 Effects of CXCR4 inhibitors on breast cancer

Compound	Type of studies	Biological function on breast cancer	References
T140	In vivo	Inhibition of SDF-1 induced BC migration	90
TN14003	In vivo	Limiting metastases of breast cancer, a radiotracer to detect CXCR4-positive cells	91
AMD3100	In vivo	Reduce lung metastases of BC	76
GST-NT21MP	In vivo	Decrease in SDF-1-induced cell growth, adhesion, migration	97
CTCE-9908	In vivo	Antitumor growth and antimetastatic effects	99–101
ALX40-4C	In vitro	Prevent breast cancer spread	102
Baohuoside	In vitro	Antimetastatic effect on BC through the downregulation of CXCR4 expression	103
Ginsenoside Rg3	In vitro	Reduce CXCR4 expression and migration and invasion	104
AKBA	In vitro	Abolish breast tumor cell invasion	105
Butein	In vitro	Reduce SDF-1-induced migration and invasion of BC	110
WZ811	In vitro	Inhibition of Matrigel invasion	92
MSX-122	In vitro, in vivo	Inhibition of cAMP and Matrigel invasion	93
AMD3465	In vitro, in vivo	Reduction of cancer growth and metastasis	98

Abbreviations: BC, breast cancer; cAMP, cyclic adenosine monophosphate; CXCR4, C-X-C chemokine receptor type 4; SDF-1, stromal-derived-factor-1.

organ sites when enriched with CXCL12 in the stroma without disturbing the retention of hematopoietic progenitor cells in the bone marrow. Therefore, MSX-122 could have a unique therapeutic application for cancer metastasis or inflammation.

Cyclic pentapeptides, such as FC131 [cyclo(D-Tyr-Arg-Arg-L-3-(2-naphthyl)alanine-Gly)], were also previously developed as CXCR4 antagonists based on pharmacophores of T140.^{89,95,96} Binding models for FC131 in CXCR4 have previously been suggested based on molecular docking guided by structure–activity relationship data; however, none of these have been verified by in vitro experiments.⁸⁹

Non-T140-related CXCR4 antagonists

To date, several non-T140-related CXCR4 antagonists have been reported. Inhibition of CXCR4 with ABD3100 (plerixafor [Mozobil, Sanofi, Bridgewater, NJ, USA]), a chemokine receptor antagonist and a small molecule inhibitor of CXCR4 receptor has significantly limited lung metastases from orthotopically transplanted breast cancer cells.⁷⁶ Yang et al's⁹⁷ data also suggest that GST-NT21MP could be a potential anticancer agent for the treatment of breast cancer. A highly purified recombination polypeptide (GST-NT21MP), which is a synthetic 21-mer peptide antagonist of CXCR4 (NT21MP) derived from the viral macrophage inflammatory protein II, and was found to block the CXCR4 pathway, thus decreasing SDF-1-induced cell growth, adhesion, and migration capacities in breast cancer cells. Furthermore, GST-NT21MP-mediated antitumor activity was found to be associated with reduced phosphorylated Src, AKT, FAK, and ERK1/2 as well as decreased Bcl-2. A small molecule antagonist of CXCR4, AMD3465, can inhibit breast cancer growth and metastasis and demonstrate the biologically relevant modulation of oncogenic signaling and tumor microenvironment.⁹⁸ Ling et al reported that AMD3465 triggers a reduction in breast cancer cell invasiveness both in vitro, and in vivo in murine syngeneic immunocompetent breast cancer models, and they found that AMD3465 inhibited breast tumor initiation and metastases to the lung and liver. Not only negatively regulating breast tumor cells, AMD3465 also reduces the infiltration of immune cells at the metastatic sites as well as at the spleen. CTCE-9908 (Chemokine Therapeutics Corp, Vancouver, BC, Canada) is a peptide analog of SDF-1 consisting of a dimer of the first eight amino acids of SDF-1, which competitively binds to CXCR4 and serves as a competitive inhibitor to SDF-1. To test the effects of CTCE-9908 in breast cancer, a transgenic mouse mammary tumor virus-driven Polyoma Middle

T Antigen (PyMT) model has been widely used based on the overexpression of HER2/neu and stepwise progression from hyperplasia to preinvasive, invasive, and distant metastasis in this model. Increasing doses of CTCE-9908 alone slowed the rate of tumor growth. Once combined with docetaxel or DC101 (an anti-VEGFR2 monoclonal antibody) or an anti-angiogenic agent, the effects were induced much more than that observed with docetaxel alone. The markedly increased antitumor and antimetastatic effects of CTCE-9908, which indicated that it is a potentially new effective combinatorial therapeutic strategies in the treatment of breast cancer, is involved in targeting the SDF-1/CXCR4 ligand/receptor pair.^{99,100} Another study to evaluate the efficacy of CTCE-9908 in a xenograft mouse model did not show inhibition of primary tumor growth with or without paclitaxel (a chemotherapeutic) relative to control, but it still inhibited organ-specific metastasis to leg.¹⁰¹ Another CXCR4-neutralizing peptide ALX40-4C (N-alpha-acetyl-nona-D-arginine amide; American Peptide Company, Sunnyvale, CA, USA) blocks the binding of SDF-1 to CXCR4 without influencing the binding of other chemokines to their respective GPCRs.^{17,18} To establish a role for CXCR4 in invasion, the effect of ALX40-4C was tested in MDA-MB-231.¹⁰² This peptide significantly inhibited Matrigel invasion by 75% but did not reduce cell viability, which suggest a distinct contribution of CXCR4 to the invasion but not the survival of breast carcinoma cells.

Natural products inhibiting CXCR4

A few of the new CXCR4 inhibitors from natural product have also been reported. Kim and Park¹⁰³ investigated baohuoside I, a component of *Epimedium koreanum*, as a regulator of CXCR4 expression and function in cervical cancer and breast cancer cells. They observed that baohuoside I downregulated CXCR4 expression in a dose- and time-dependent manner. The decrease in the level of CXCR4 expression caused by baohuoside I correlated with inhibition of the CXCL12-induced invasion of both cervical and breast cancer cells, indicating that it plays a role in the suppression of cancer metastasis.¹⁰³ Ginsenoside Rg3 is a component extracted from puffed ginseng (*Panax ginseng* C.A. Meyer). In Chen et al's¹⁰⁴ study, at a dosage without obvious cytotoxicity, Rg3 treatment reduces CXCR4 expression, decreases the ability of migration and invasion of breast cancer MDA-MB-231 cells induced by CXCL12 suggesting that Rg3 is a new CXCR4 inhibitor from a natural product. Acetyl-11-keto-b-boswellic acid (AKBA) is a derivative of boswellic acid, which is the main component of a gum resin from

Boswellia serrata. AKBA has been used traditionally to treat a number of inflammatory diseases, including osteoarthritis, chronic colitis, ulcerative colitis, Crohn disease, and bronchial asthma. AKBA abolished breast tumor cell invasion, and this effect correlated to the downregulation of both the CXCR4 mRNA and CXCR4 protein.¹⁰⁵ Butein (3, 4, 20, 40-tetrahydroxychalcone), a novel regulator of CXCR4 expression and function, which is derived from numerous plants, including the stem bark of cashews (*Semecarpus anacardium*) and the heartwood of *Dalbergia odorifera*, has substantial antitumor activities, as indicated by inhibition of proliferation of a wide variety of tumor cells,^{106,107} suppression of phorbol ester-induced skin tumor formation,¹⁰⁸ and inhibition of carrageenan-induced rat paw edema.¹⁰⁹ The decrease in CXCR4 expression induced by butein was not cell type-specific, and the downregulation of CXCR4 was due to transcriptional regulation. Suppression of CXCR4 expression by butein correlated to the inhibition of CXCL12-induced migration and invasion of breast cancer cells, suggesting that butein is a novel inhibitor of CXCR4 expression and thus has a potential in suppressing metastasis of cancer.¹¹⁰

Recombinant chimeric protein CXCL12/54R

In a transgenic mouse with mutant CXCL12, obtained by deleting the 55th to 67th residues of its COOH terminus (CXCL12/54R), SDF-1 was unable to bind to CXCR4. CXCR4 was quickly internalized, subsequently downstream signals mediated by CXCR4 were inactivated, resulting in the inhibition of tumor cell migration.¹¹¹ However, the inhibitory function of CXCL12/54R tends to be temporary and reversible, and TAT/54R/KDEL can produce a longer or more permanent inhibition of CXCR4 expression on the cellular surface.

TAT/54R/KDEL

A novel recombinant chimeric protein, TAT/54R/KDEL was developed, in which TAT and KDEL were linked to the NH₂-terminal and COOH-terminal of CXCL12/54R, respectively. TAT, which is from HIV-1 TAT (47–57, YGRKKRRQRRR), is able to permeate the plasma membrane of cells either alone or fused with full-length proteins or peptides^{112,113} can deliver proteins ranging from 10 to 120 kDa into the cells without any damage to cells.^{114–116} Four-peptide KDEL or DDEL is a site-specific signal which detained the soluble endoplasmic reticulum-resident proteins in ER for degradation.^{117,118} The systemic treatment of TAT/54R/KDEL could impair lung metastasis of a highly metastatic, triple-negative mammary cancer cell line, 4T1, with decrease of CXCR4 on their

membrane, suggesting that the phenotypic knockout strategy of CXCR4 using a novel recombinant protein TAT/54R/KDEL could potentially be a possible approach for inhibiting relative tumor metastasis mediated by CXCR4/CXCL12 interaction. Taken together, CXCR4 may be an effective therapeutic in preventing breast cancer spread.

In addition to breast cancer, some studies have successfully demonstrated that blockade of CXCR4 or SDF-1/CXCR4 interaction by small molecule inhibitor of CXCR4 suppresses prostate cancer (eg, CTCE-9908)¹¹⁹ and lung cancer (eg, TN14003).²³ At present, clinical trials involving CXCR4 inhibition are tested in hematological malignancies.²⁴ Administration of a CXCR4 antagonist would probably not be used alone; combinations with established chemotherapy would be likely. Clinical trials of CXCR4 antagonists in breast cancer patients are rarely available; the likely reason might be as a result of intervention failures and high attrition rates of candidate drugs that show success in animal models but fail in human clinical trials.

Conclusion

In the past 10 years, numerous investigations have been conducted on the role of SDF-1/CXCR4 signaling pathway in solid tumors, including breast cancer. The antagonists of CXCR4 could be promising agents for prevention and treatment of breast cancer metastasis. However, we must keep in mind that CXCR4 plays a critical role in embryogenesis, homeostasis, and inflammation in the fetus, especially in the embryonic development of hemopoietic, cardiovascular, and central nervous systems. Therefore, caution should be taken when inhibition of the SDF-1-CXCR4 signaling pathway is applied in human subjects.⁸⁹ Inhibition of CXCR4 signaling attenuates the immune responses, therefore moderate activation of CXCR4 pathway contributes to depression of inflammation and is beneficial for the cancer patients. However, excessive activation of CXCR4 pathway might dampen the hosts' immune responses and decrease anticancer ability. In addition, preclinical data have shown that blockade of CXCR4 may increase osteoclastic bone resorption therefore promoting tumor cell growth in bone.¹²⁰ Caution should be taken when the utility of blockade of the SDF-1/CXCR4 axis is evaluated.

Acknowledgment

This work was supported by Zhejiang Provincial Natural Science Foundation of China (Grant number LQ12H16006) and Zhejiang Provincial Natural Science Foundation of China (LQ12H16006).

Disclosure

The authors have no financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in the manuscript. The authors report no conflicts of interest in this work.

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