The role of CXCL12 in tumor microenvironment

Wenfang Meng\textsuperscript{a,b}, Shihang Xue\textsuperscript{c}, Ye Chen\textsuperscript{a,b,*}

\textsuperscript{a} Division of Medical Genetics and Genomics, The Children’s Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
\textsuperscript{b} Institute of Genetics, Zhejiang University, Hangzhou, Zhejiang, China
\textsuperscript{c} Ningbo No.4 People’s Hospital, Ningbo, Zhejiang, China

**A B S T R A C T**

The chemokine ligand C-X-C motif chemokine ligand 12 (CXCL12) is a kind of small molecules of cytokines that widely expressed in diversified tissues. Recent evidence suggests that CXCL12 plays an important role in the communication of tumor cells with their surrounding microenvironment. The interaction of CXCL12 and its receptors subsequently excite the downstream signaling pathways to affect tumor angiogenesis, tumor cell proliferation and chemoresistance, and thus represents a potential target for cancer therapy. Outpouring molecules targeting CXCL12/CXCR4 axis in tumor microenvironment combined with traditional chemotherapy have drawn more and more attentions, which will be a promising method in anti-cancer therapies. Our review focuses on these roles of CXCL12 and summarizes strategies for treating cancer by disrupting this interaction with special emphasis on the CXCR4/CXCL12 axis.

1. Introduction

Tumor microenvironment (TME) is the local environment of tumorigenesis and tumor growth, composed of tumor cells and surroundings such as blood vessels, the extracellular matrix (ECM), other non-malignant cells such as bone marrow-derived dendritic cells, mesenchymal stem/stromal cells, fibroblasts, pericytes, immune cells, and the expression of their products, metabolic material, and also signaling molecules (Fig. 1) (Junttila and de Sauvage, 2013; Quail and Joyce, 2013). TME affect whole process of tumors from happening to transfer, and tumor cells in turn influence the physical and chemical properties of the TME to make TME beneficial even stimulative to the growth of tumor (Mahadevan and Von Hoff, 2007).

Chemokines are a very important part of the cross talk between tumor cells and the tumor microenvironment (Mantovani et al., 2008). Chemokine receptors and their specific chemokines control and regulate the tumor development, including leukocyte infiltration, tumor related angiogenesis, activation of the specific immune response to the tumor of host, stimulating tumor cell proliferation by autocrine or paracrine way, as well as control of tumor cell movements (Mbeunkui and Johann, 2009). The chemokine ligand C-X-C motif chemokine ligand 12 (CXCL12) and its receptor chemokine receptor 4 (CXCR4) are two key factors in the cross-talking, what makes them promising targets for cancer therapy. In this review, we summarize the role of CXCL12 in tumor growth, angiogenesis, metastasis, and TME-mediated chemoresistance.

2. The CXCL12/CXCR4 axis

Chemokines are small proteins possessing a common structural feature of conserved cysteine residues at the N-terminus with a molecular mass of between 8 and 10 kDa (Bagnoliini, 1998). Chemokines have a common structural composition. According to the number of and relative spacing of the N-terminal cysteine residues, chemokines are divided into four families (CXC, CX3C, CC, and C) (Le et al., 2004; Vindrieux et al., 2009). CXCL12, also known as stromal-derived factor 1 (SDF-1), is widely secreted in different tissues by stromal cells, fibroblasts and epithelial cells in different isoforms, encoded on chromosome 19q11. CXCR4 is an evolutionarily highly conserved G protein coupled receptor (GPCR) expressed on a great diversity of cell types, including lymphocytes, hematopoietic stem cells, endothelial cells, epithelial cells, stromal fibroblasts and cancer cells. This allows them to migrate along CXCL12 gradients. It has been observed that the level of CXCL12/CXCR4 is increased in many types of cancer, such as breast cancer, pancreatic cancer, ovarian carcinoma, cervical carcinoma, leukemia diseases and the rest (Mahadevan and Von Hoff, 2007; Shen...
et al., 2009; Zeng et al., 2009; Ling et al., 2013; Kim and Park, 2014; Li et al., 2016). Furthermore, CXCR4 expression has been defined as a prognostic factor in several human tumor types. As shown in the Fig. 1, in the tumor microenvironment, some conditions like hypoxia or toxins increase the levels of CXCL12 and thus generate the migration of tumor cells expressing CXCR4, leading them to safer residence constantly. And the heterotrimeric G protein dissociates into subunits (the GTP-bound α, β, and γ) when CXCR4 binding to CXCL12 (Chatterjee et al., 2014), thus contributing to the tumor angiogenesis, tumor cell survival, proliferation and chemoresistance through the excitation of a variety of downstream signaling pathways (Burger and Kipps, 2006; Wojcechowskyj et al., 2011).

3. CXCL12 enhances adhesion ability of tumor cells

CXCL12 regulates adhesion of tumor cell with laminin, fibronogen, stromal cells and endothelial cell by activating various cell surface adhesion molecules (e.g. integrins). It has been shown that CXCL12 could increase the adhesion rate of pancreatic cancer cells to laminin (the main component of basement membrane) and enhance the capacity of cells to penetrate the matrigel (artificial reconstruction basement membrane) (Mori et al., 2004). Compared with normal cells, the prostate cancer cells pretreated with CXCL12 significantly represented higher adhesion of osteosarcomas and endothelial cell lines in vitro in a dose-dependent manner (Taichman et al., 2002). In another similar assay, CXCL12 was found to increase adhesion of PC-3 cell to the human umbilical vein endothelial cell monolayer (Kukreja et al., 2005). Moreover, CXCL12 increased the level of integrin α5β3 expression in the tumor cell membrane, and thus enhanced the adhesion of prostate tumor cells to human endotholium or extracellular matrix proteins laminin, collagen, and fibronectin (Engl et al., 2006). The effects of CXCL12 on the expression and activity of integrins on cell surfaces may be crucial in adhesion to fibronectin and collagen I in prostate cancer cell (Dehghani et al., 2014). The adhesion of HeLa cells to fibronectin and laminin was increased when CXCL12 was added in the medium. In ovarian cancer cell lines, CXCL12 induced integrin β1 expression which leads to the increased adhesion of tumor cells to laminin (Shen et al., 2009). In small cell lung cancer cell lines, CXCL12/CXCR4 interaction could induce the expression of integrin and promote adhesion of cancer cells to vascular cell adhesion molecule-1 (VCAM-1), fibronectin and collagen and also vascular endothelial cells (Hartmann et al., 2005).

4. CXCL12 promotes tumor angiogenesis and metastasis

CXCL12/CXCR4 axis is closely related to angiogenesis which supplying nutrient to tumor cells and giving rise to excretion of tumor cells metabolites efficiently. CXCL12 can stimulate angiogenesis directly or indirectly. Vascular endothelial growth factor (VEGF) is an important cytokine that induces tumor angiogenesis and promotes tumor metastasis. VEGF can induce endothelial cells to express MMP-2, MMP-9, to stimulate chemotaxis of endothelial cell and the formation of capillary channels, and thus indirectly regulate angiogenesis. Salvucci and other studies have shown that CXCL12 could induce endothelial cells to express VEGF, and VEGF in turn could promote the expression of CXCL12 in vascular endothelial cell (Salvucci et al., 2002). Moreover, VEGF also induces expression of CXCR4 in cancer cells in autocrine way, thereby promotes cancer cells’ migration along CXCL12 gradients. Metastasis is an important biological characteristic of malignant tumors. CXCL12 can not only induce expression of matrix metalloproteinases (MMP) in cancer cells, but also up-regulate the activity of MMP in tumor microenvironment, which lead to the promoted tumor inva- 
sion and metastasis. CXCL12 could increase MMP-9 and MT1-MMP expression and activities, which regulated myeloma cells’ movement in the bone marrow (Parmo-Cabanias et al., 2006). By stimulating different types of cells to secrete MMP-2 CXCL12/CXCR4 axis could improve the migration of nerve cells along the corpus callosum (Mao et al., 2016).
<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Indication/condition</th>
<th>Function</th>
<th>Trial number/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-0651</td>
<td>Anti-CXCR4 antibody</td>
<td>Healthy volunteers</td>
<td>Increasing leukocyte mobilization and decreasing extracellular matrix deposition.</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
</tr>
<tr>
<td>AMD070</td>
<td>Small molecule CXCR4 antagonist</td>
<td>BLM-induced pulmonary and CC34-induced hepatic fibrosis in mice</td>
<td>NCT00010466</td>
<td></td>
</tr>
<tr>
<td>AMD3100 (Mozobil, plerixafor)</td>
<td>Small molecule CXCR4 antagonist</td>
<td>Glioma, Anaplastic Astrocytoma, Anaplastic Oligodendroglioma, Mixed Anaplastic Oligoastrocytoma</td>
<td>NCT00063804</td>
<td></td>
</tr>
<tr>
<td>AMD3465</td>
<td>CXCR4 antagonist</td>
<td>Primary Chronic Lymphocytic Leukemia, Acute Myelogenous Leukemia</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
<td></td>
</tr>
<tr>
<td>BA-2341</td>
<td>Allosteric agonist</td>
<td>Cervical cancer and breast cancer</td>
<td>Suppression of cancer metastasis</td>
<td>NCT01205397</td>
</tr>
<tr>
<td>BL-8040 (BKT-140)</td>
<td>Peptide (T140 analogue)</td>
<td>Acute Myeloid Leukemia</td>
<td>Mobilization of leukemic blasts</td>
<td>NCT02502968</td>
</tr>
<tr>
<td>BL-8040 (BKT-140)</td>
<td>Peptide (T140 analogue)</td>
<td>Multiple Myeloma</td>
<td>Mobilization of Hematopoietic Stem Cells</td>
<td>NCT00703190</td>
</tr>
<tr>
<td>BMS-936564 (MDX-1338)</td>
<td>Anti-CXCR4 antibody</td>
<td>Acute Myeloid Leukemia, Diffuse large B-cell leukemia</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
<td></td>
</tr>
<tr>
<td>CTCE-0021</td>
<td>CXCR4 agonist peptide</td>
<td>Jurkat human lymphoblastoid cell line</td>
<td>Mobilizer of polymorphonuclear neutrophils and hematopoietic stem and progenitor cells</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
</tr>
<tr>
<td>CTCE-9908</td>
<td>CXCL12 mimetic</td>
<td>Prostate cancer</td>
<td>Decreasing the invasion of tumor</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
</tr>
<tr>
<td>CTCE-0214</td>
<td>CXCL12 mimetic</td>
<td>Breast cancer</td>
<td>Mobilization bone marrow recovery</td>
<td>NCT01747503 (Chow et al., 2016) (PMID: 26998906)</td>
</tr>
</tbody>
</table>
CXCR4 was highly expressed in tumor cell of patients with liver metastases of colorectal cancer, while high expression of its ligand CXCL12 was found in the most common metastatic sites of colorectal cancer, such as lymph nodes, liver, lung (Kim et al., 2006). Sun et al. also confirmed that down-regulation of CXCR4 expression could also inhibit distant metastasis of prostate cancer (Sun et al., 2005). In addition, the use of neutralizing antibodies, specific peptides, and siRNAs to block CXCR4 expression significantly inhibited the metastasis of breast cancer to lymph nodes and lungs (Skobe et al., 2001). Though the molecular mechanism of tumor metastasis has not yet been fully elucidated, various studies have indicated that the role of CXCL12/CXCR4 axis is significant during the progress.

5. CXCL12 contributes to tumor cell growth and survival

Studies have shown that CXCL12/CXCR4 axis can regulate a variety of tumor cell proliferation (Kryczek et al., 2007; Wu et al., 2008). The binding of CXCL12 to its receptor CXCR4 can induce proliferation of various type of tumor cells by activating the extracellular signal-regulated kinase (ERK) and AKT signaling pathway. Activated ERK phosphorylates and regulates other cellular proteins and transcription factors, bringing about changes in gene expression and cell cycle process. Activated AKT plays a key role in tumor cell survival through inactivation of BCL-2 antagonist resulting in cell survival. CXCL12/CXCR4 signaling via AKT also resulted in inactivation of GSK3β and stabilization of β-catenin. Stabilized β-catenin moved to the nucleus activating gene transcription and promoting proliferation (Barbero et al., 2003). CXCL12-dependent tumor proliferation contributed to tumor growth by a sustained inhibition of cyclic AMP (cAMP) production (Yang et al., 2007). CXCL12 also protected CXCR4-positive cancer cells from apoptosis induced by both serum-free culture and chemo-administration. In addition, tumor cells could express CXCL12 in paracrine form, stimulating tumor stromal cells to produce TNF, which in turn promoted tumor cell growth. These studies suggest that the CXCL12/CXCR4 biological axis not only directly promotes cell proliferation but also indirectly through anti-apoptotic effects (Marchesi et al., 2004).

6. Targeting the CXCR4/CXCL12 axis in tumor microenvironment

Up to now, several molecules have been developed to target CXCL12 or CXCR4 for the purpose of interfering with the tumor growth, and have stronger lethal effect with combination of other pharmacuticals (Table 1). CTCE-9908 is a CXCL12 analogue with inhibited and agonist activity. In two murine models of osteosarcoma, CTCE-9908 reduced osteosarcoma cells’ growth and adhesion, and the metastatic dissemination of cancer cells (Kim et al., 2008). Compared with controls receiving scrambled protein, the treatment with CTCE-9908 experimental group showed lower primary tumor growth rate in transgenic mice model of breast cancer. In addition, CTCE-9908 gave rise to reduced protein expression of vascular endothelial growth factor (VEGF) and p-AKT/AKT (Hassan et al., 2011). Olaptesed pegol (ola-PEG), a high-affinity anti-CXCL12 spiegelmer represented as a successful agent targeting the interaction between bone marrow niches and tumor cells, thereby blocking or disrupting bone marrow colonization by multiple myeloma cells in a cancer xenograft model. It also diminished tumor mobilization, homing, and growth within the bone marrow niches. Combinations of ola-PEG with other chemotherapeutic agents were also likely to lead to synergistic effects (Roccaro et al., 2014). Plerixafor (AMD3100), a CXCR4 inhibitor and a hematopoietic stem cell mobilizer, competitively inhibited CXCL12 binding to CXCR4, which resulted in inhibition of initial establishment of prostate tumors in the bone (Conley-LaComb et al., 2016). AMD3100 blocked ligand-receptor binding of CXCL12/CXCR4 and reduced growth of ovarian cancer cells, in addition, modestly promoted overall survival of mice with metastatic ovarian cancer (Ray et al., 2011). AMD3100 undermined the
interaction between tumor and stroma, inhibited the adhesion of leukemic cells and weakened the cell migration ability to bone marrow and niche. It also brought about mobilization of the leukemia cells to enhance cytotoxicity of cytarabine(Ara-C) on account of the absence of the protection of the tumor microenvironment. Treatment of AMD3100 combined with G-CSF had a stronger impact on therapy induced by Ara-C (Shen et al., 2016). In mouse model of breast cancer, AMD3465 decreased the cancer growth and metastases of breast cancer cells, by acting on the interaction of tumor cells and the tumor microenvironment (Ling et al., 2013). Compared with the conventional method of drug delivery, the targeted delivery of CXCR4-A-mFc antagonists by oncolytic Vaccinia viruses (OVV) showed an enhanced antitumor effect in destroying tumor vasculature and inhibiting breast cancer metastasis (Gil et al., 2013). In ovarian cancer, by inhibiting the expression of CXCL12, mir-448 inhibited cell proliferation, migration and invasion, serving as tumor suppressor (Lv et al., 2015). As a component of Epithelial koreanum, Baohouside I could suppress cervical and breast cancer metastasis by downregulating CXCR4 expression (Kim and Park, 2014).

7. CXCR7 impact on CXCL12 biology in tumor study

For many years it was believed that CXCR4 was the only receptor for CXCL12. When chemotactic response to T lymphocytes of CXCL12 were studied, Balabanian et al., found that CXCL12 bound to RDC1 (previously used as an orphan receptor) and anti-RDC1 monoclonal antibodies probably inhibited the chemotactic response to T lymphocytes of CXCL12 (Balabanian et al., 2005). Burns et al. discovered that on the 13th day of the CXCR4 knockout mouse embryonic development, the liver cells can still bind CXCL12 in the study by chance. RDC1 had similarity with CXCR4 in amino acid conserved sequences, so renamed CXCR7 (Burns et al., 2006).

Membrane-associated CXCR7 is expressed on many tumor cell lines, on activated endothelial cells, on fetal liver cells, and on T lymphocytes (Balabanian et al., 2005; Burns et al., 2006). The expression of CXCR7 was found to be strongly positive in 97% (106/109) human breast cancer vascular endothelial cells detected by immunohistochemistry, but almost undetectable in normal mammary vascular endothelial cells (Miao et al., 2007). Studies have shown that CXCR7 could reduce tumor cell apoptosis and promote its proliferation. Compared with wild-type cells, after 5 days’ treatment, the number of viable cells in CXCR7-transfected MDA MB 435s cells was significantly higher, although no difference was found in the total cells (Burns et al., 2006). CXCR7 also regulated expression of pro-angiogenic factors interleukin-8 and vascular endothelial growth factor, which may be involved in the regulation of angiogenesis (Wang et al., 2008). CXCL12/CXCR7 axis is also involved in the tumor invasion and metastasis process. Compared with wild-type cells, the number of cells adhered to human dermal microvascular endothelial cells (HDMEC) was significantly higher, and the invasive ability of CXCR7-over-expressed prostate cancer or C4-2B cells was higher in the presence of CXCL12. CXCR7 enhanced the cancer growth and metastasis of tumor cells by regulating the levels of cell adhesion molecules (FN1, CDH11 and CD44) and matrix metalloproteinase (MMP), such as MMP3, MMP10, MMP11 and HPSE (Wang et al., 2008). In vitro experiments it was also confirmed that CXCR7-transfected MDA MB 435s cells were more human to umbilical vein endothelial cells (HUEVs) than untransfected wild-type cells (Burns et al., 2006). Similarly, in in vivo experiments inducing lung metastasis model in mice injected with tumor cells in the tail vein, tumors caused by injecting cells with 4T1-CXCR7-RNAi into the lungs were smaller than injecting the wild-type 4T1 WT cells, while the tumors caused by CXCR7-over-expressed cells transferred to the lungs were larger than the wild-type 4T1 WT cells’ group, indicating that CXCR7 expression in breast cancer cells enhanced the ability to metastasize to the lungs and proliferate in the lungs (Miao et al., 2007).

8. Conclusion

It is well recognized that the interaction between tumor cells and TME is a rising target to improve anti-cancer treatment caused by protection of TME (Fig. 1). Extensive bench studies indicate that targeting the CXCL12/CXCR4 axis may have beneficial actions for sensitizing tumor cells to chemical therapy; however, clinical researches to date are still lagged behind due to multiple reasons such as the limit of pharmacokinetic properties. Additional studies with new agents are required to prove whether interference of the CXCR4/CXCL12/CXCR7 axis in TME may increase the effectiveness of cancer therapy, and the process of drug approval needs to be accelerated.

Acknowledgements

The work was supported by the project from the Natural Science Foundation of Zhejiang Province (R15H080001), and the “Double First-rate” project initiatives of Zhejiang University.

Conflict of interest

The authors declare no competing financial interests.

References


sion in patients with melanoma and colorectal cancer liver metastases and the as-


Miao, W.F., Yi, X., Qin, J.B., Tian, M.L., Jin, G.H., 2016. CXCL12/CXCR4 Axis improves