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Review Article

C-X-C motif chemokine ligand 12—C-X-C chemokine receptor type 4 signaling axis in cancer and the development of chemotherapeutic molecules

*Jui-Hung Yen[a†], Chun-Chun Chang[b,c†], Hao-Jen Hsu[d†], Chin-Hao Yang[e], Hemalatha Mani[e], Je-Wen Liou[c,d,e]**

[a]Department of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan, [b]Department of Laboratory Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, [c]Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan, [d]Department of Biomedical Sciences and Engineering, Tzu Chi University, Hualien, Taiwan, [e]Department of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan

†These authors contributed equally to this work.

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Abstract

Chemokines are small, secreted cytokines crucial in the regulation of a variety of cell functions. The binding of chemokine C-X-C motif chemokine ligand 12 (CXCL12) (stromal cell-derived factor 1) to a G-protein-coupled receptor C-X-C chemokine receptor type 4 (CXCR4) triggers downstream signaling pathways with effects on cell survival, proliferation, chemotaxis, migration, and gene expression. Intensive and extensive investigations have provided evidence suggesting that the CXCL12-CXCR4 axis plays a pivotal role in tumor development, survival, angiogenesis, metastasis, as well as in creating tumor microenvironment, thus implying that this axis is a potential target for the development of cancer therapies. The structures of CXCL12 and CXCR4 have been resolved with experimental methods such as X-ray crystallography, NMR, or cryo-EM. Therefore, it is possible to apply structure-based computational approaches to discover, design, and modify therapeutic molecules for cancer treatments. Here, we summarize the current understanding of the roles played by the CXCL12-CXCR4 signaling axis in cellular functions linking to cancer progression and metastasis. This review also provides an introduction to protein structures of CXCL12 and CXCR4 and the application of computer simulation and analysis in understanding CXCR4 activation and antagonist binding. Furthermore, examples of strategies and current progress in CXCL12-CXCR4 axis-targeted development of therapeutic anticancer inhibitors are discussed.

Keywords: *Cancer progression, Chemotherapeutic agents, CXCL12, CXCR4, Stromal cell-derived factor 1*

Introduction

Chemokines are a large family of small cytokines with molecular weights of between 8 and 10 kDa. These secreted proteins play crucial roles in embryogenesis, hematopoiesis, mitogenicity, and innate and adaptive immunity [1], and are involved in the regulation of immune cell migration and trafficking, directing movement of cells throughout the body in response to infection, inflammation, and other immune responses [2]. Based on the arrangement of conserved cysteine residues in their amino acid sequences, chemokines are classified into four major subfamilies: CXC, CC, CX3C, and XC [3,4]. Chemokines exert their effects by binding to specific cell surface chemokine receptors, typically G-protein-coupled receptors (GPCRs). Upon chemokines binding to their specific receptors, the interactions trigger intracellular signaling pathways, which are involved in various immune responses, including inflammation, infection, and the development of immune cells. They help to recruit immune cells to infection sites, and facilitate the removal of pathogens and damaged cells [5]. Chemokines also play roles in maintaining homeostasis by regulating the migration of immune cells under normal physiological conditions, involving in the immune surveillance and the maintenance of tissue integrity [5-7]. Dysregulation of chemokine signaling has been implicated in various diseases, including inflammatory disorders, autoimmune diseases, and certain cancers [2,4,8]. Therefore, chemokines and their receptors have been considered targets for the development of therapeutic strategies. C-X-C motif chemokine ligand 12 (CXCL12), also known as stromal cell-derived factor 1 (SDF-1), is one of the most important chemokines in the body. CXCL12 has been found to bind only to two receptors, C-X-C chemokine receptor type 4 (CXCR4) and CXCR7. It binds and activates

**Address for correspondence:* Prof. Je-Wen Liou, Department of Biochemistry, School of Medicine, Tzu Chi University, 701, Zhongyang Road, Section 3, Hualien, Taiwan.
E-mail: jwliou@mail.tcu.edu.tw

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the receptor CXCR4 triggering intracellular signaling through several pathways related to chemotaxis, cell survival and/or proliferation, elevation of intracellular calcium level, and gene transcriptions [9]. The CXCL12-CXCR4 axis is also suggested to be involved in several aspects of tumor progression including angiogenesis, metastasis, and survival [9]. This article aims to review the studies on CXCL12, CXCR4, CXCL12-CXCR4 signaling in tumor progression, as well as the developments of anticancer therapeutics targeting this signaling axis.

C-X-C motif chemokine ligand 12 and C-X-C chemokine receptor type 4

CXCL12, also known as SDF-1, is constitutively expressed in most tissues, and it is a strong factor inducing chemotaxis. Initially, two different spliced isoforms, SDF-1α and SDF-1β, were identified. These two splice forms contain identical amino acid sequences, except that SDF-1β contains 4 additional amino acids at the carboxy terminus [10]. Later, additional four splice variants, SDF-1γ, SDF-1δ, SDF-1ε, and SDF-1ϕ, containing different lengths of amino acid extensions at the carboxyl terminus have been identified [11,12]. CXCL12 exerts its functions through binding its primary receptor CXCR4, subsequently activating the downstream signaling. It has been suggested that CXCL12 can also bind to CXCR7 (also called atypical chemokine receptor 3, ACKR3) [13], which is a scavenger receptor with multiple ligands. In addition to CXCL12, ligands such as CXCL11, macrophage inhibitory factor, adrenomedullin, and opioid peptides, such as dynorphin, enkephalin, and nociception, have also been identified to bind to CXCR7 [14-16]. On the other hand, for CXCR4, CXCL12 is the only identified ligand. CXCR4, originally called fusin, received great attention when it was discovered to be an important receptor for HIV entry to host cells [17,18]. CXCR4 is constitutively expressed in T-lymphocytes and regulates T-cell migration along gradients of CXCL12 [19]. It has been indicated that the CXCL12-CXCR4 signaling plays an essential role in maintaining hematopoietic stem cell pool in bone marrow stromal cell niches [20]. CXCL12-CXCR4 axis has also been suggested to be responsible for B-cell lymphopoiesis and bone marrow myelopoiesis [21].

Activation of C-X-C motif chemokine ligand 12—C-X-C chemokine receptor type 4 signaling

When the ligand CXCL12 binds to CXCR4, the interactions stimulate a variety of downstream signaling pathways which regulate chemotaxis, cell survival, proliferation, and related gene expression. These pathways are summarized in Figure 1. The actual signal transduction can vary in different cell types and tissues. CXCR4 is a GPCR. Upon activation by CXCL12 binding, CXCR4 is coupled with a G-protein containing $G_{\alpha i}$, G_{β}, and G_{γ} subunits. After GDP/GTP exchange, the $G_{\alpha i}$ subunit and $G_{\beta\gamma}$ dimer dissociate from the receptor. cAMP and its downstream signaling have been found to inhibit cell migration [22-24]. The dissociated $G_{\alpha i}$ inhibits the production of cAMP, thus promoting chemotaxis and cell migration. The dissociated $G_{\alpha i}$ is also found to promote cell migration through activation of the Ras-Raf-MEK-ERK pathway [25,26].

The dissociated $G_{\beta\gamma}$ dimer activates phospholipase C-β, leading to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce the secondary messengers, inositol 1,4,5-trisphosphate (IP3), and diacylglycerol (DAG). IP3 subsequently facilitates the release of calcium from intracellular stores in cells [27], and the elevated cytosolic calcium level is crucial for cell migration [28]. The produced DAG can activate protein kinase C and mitogen-activated protein kinase (MAPK), also contributing to chemotaxis

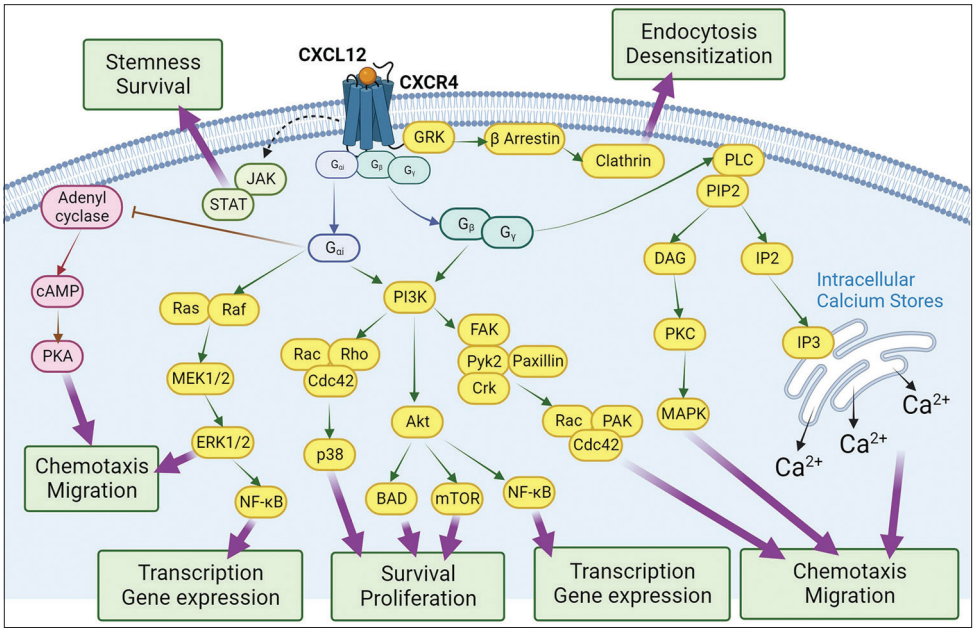

Figure 1: Intracellular transduction pathways and functions of C-X-C motif chemokine ligand 12 (CXCL12)—C-X-C chemokine receptor type 4 (CXCR4) signaling

and cell migration [29,30]. The CXCL12-CXCR4 axis was found to activate the phosphoinositide 3-kinase (PI3K)/Akt pathway [31]. One of the most studied functions of $G_{\beta\gamma}$ dimer is the activation PI3K [32,33], leading to the activations of several key signaling pathways linking to cell survival, proliferation, and migration. The roles played by $G_{\alpha i}$ in PI3K activation have also been suggested [34,35]. Upon activation, PI3K activates downstream signaling pathways, including Cdc42, Rac, and p21-activated kinase (PAK) pathway [36], focal adhesion kinase (FAK), proline-rich tyrosine kinase 2 (Pyk2), Crk, paxillin pathway [37], as well as Akt and its downstream pathways [31]. The Cdc42, Rac, and PAK pathway is believed to be involved in cell migration, and has been identified to regulate cell colony spreading [38]. Dynamics and associations of FAK, Pyk2, Crk, and paxillin are suggested to be involved in focal adhesion and thus cell migration [39,40]. PI3K can also regulate cell survival and cell proliferation through activations of Rac, Rho, and p38 MAPK pathways [41,42]. The activation of the serine-threonine kinase Akt leads to activations of the downstream transcription factor nuclear factor-κB (NF-κB), BCL2-associated agonist of cell death (BAD), and mTOR pathways. Both Akt and ERK can activate NF-κB. NF-κB is an important transcription factor inducing the expression of many pro-inflammatory genes and participating in inflammasome regulation [43]. NF-κB also plays a key role in regulating the survival, activation, and differentiation of a variety of cells [44-46]. Akt activates BAD and mTOR upon CXCR4 activation by CXCL12. Both of these two pathways are linked to cell survival and proliferation [47-49].

The CXCL12-CXCR4 binding also triggers G-protein-independent signaling pathways. Upon CXCL12 binding, JAK2 and JAK3 associate with CXCR4, and are both activated. This activation enables the recruitment and tyrosine phosphorylation of several members of the STAT family factors [50]. The activation of JAK-STAT signaling leads to cellular responses in favor of maintaining cell stemness and survival [51,52]. CXCL12 binding also leads to rapid phosphorylation at multiple serine and threonine sites of the intracellular C-terminus of CXCR4 by G-protein-coupled receptor kinases. This phosphorylation of receptor results in recruitment of β-arrestin and clathrin-mediated endocytosis, causing internalization of the receptor and thus desensitization of the signaling [53,54]. The activation of CXCL12-CXCR4 axis induces cellular functions through both G-protein-dependent and G-protein-independent pathways. The signaling map of CXCL12-CXCR4 axis is also illustrated in Figure 1.

C-X-C Motif chemokine ligand 12—C-X-C chemokine receptor type 4 signaling in cancers

As mentioned, the CXCL12-CXCR4 signaling is linked to cell survival, proliferation, and migration. Cancer cells are thought to take advantages of this signaling axis for their proliferation, chemotaxis, invasion, and angiogenesis in cancer development, progression, and metastasis. CXCR4 is found to be overexpressed in many types of cancers [55], including leukemia [56,57], breast cancer [58], pancreatic cancer [59], oral cancer [60,61], cervical cancer [62,63], gastric cancer [64], colorectal cancer [65], liver cancer [66,67], thyroid cancer [68], ovarian cancer [69], prostate cancer [70], lung cancer [71], kidney cancer [72], brain cancer [73,74], and melanoma [75]. The overexpression of CXCR4 is observed in several types of cancers and has been recognized as a biomarker for poor prognosis [55]. CXCL12-CXCR4 signaling also plays a pivotal role in creating and regulating microenvironments supporting tumor growth, metastasis, and colonization [76]. CXCL12 expressed by mesenchymal stromal cells in organs/tissues, such as the liver, lung, and bone marrow, can attract and recruit CXCR4-expressing tumor cells to mesenchymal stroma niches, causing metastasis [76]. In addition, the expressed CXCL12 in mesenchymal stroma niches also attracts CXCR4+ immune/inflammatory cells, fibroblasts, vascular cells, and stromal cells to the tumor sites, further assisting tumor development and growth by the production and secretion of growth factors, cytokines, chemokines, and promoting angiogenesis [76,77].

As CXCL12-CXCR4 axis is crucial and significant in cancer development and metastasis, the CXCL12-CXCR4 pathway presents an attractive target for therapeutic intervention intended for disrupting CXCL12-CXCR4 interactions or inhibiting downstream intracellular protein activities. CXCR7 is suggested to be a scavenger for CXCL12 [78], thus should regulate the CXCR4 signaling by scavenging its ligand, and can be considered a druggable target [14]. However, as mentioned, CXCR7 is a broad-spectrum scavenger for a range of ligands for different receptors, making the effects of CXCR7 functional alterations complicated. As a result, compared with CXCR7 signaling, CXCL12-CXCR4 signaling axis is a more popular anticancer therapeutic target. Various approaches are being explored in different cancer types and conditions, including small molecular inhibitors targeting CXCR4 [79], blocking antibodies against CXCR4 or CXCL12 [80,81], and inhibitory peptide therapeutics against CXCR4 [82].

Protein structures of C-X-C motif chemokine ligand 12 and C-X-C chemokine receptor type 4

With the advances in computer technology, computational approaches have become powerful tools in the discovery, design, and development of possible therapeutic inhibitors targeting the CXCL12-CXCR4 axis. As the three-dimensional structures of CXCL12 and CXCR4 have been resolved by experimental methods, structure-based computational studies have become popular for this purpose.

Understanding the ligand–receptor interactions at submolecular levels is essential for the determination of molecular physiological and pathological functions and for developing new therapies targeting CXCL12-CXCR4 signaling. For this purpose, high-resolution three-dimensional structures for CXCL12 and CXCR4 are required. Chemokines are characterized by their amino acid primary sequence and the positions/arrangements of crucial cysteine residues that form disulfide bonds essential for maintaining the

monomeric structure of chemokine proteins. Typical structure of chemokines comprises three central β−strands, an α-helix at the C-terminal end, and a short unstructured N-terminus pivotal for receptor activation. CXCL12 is a highly conserved protein among vertebrate species, indicating its importance. The identity and similarity between amino acid sequences of human (Uniprot: P48061) and mouse (Uniprot: P40224) CXCL12 are 92% and 95%, respectively [Figure 2]. In humans, the CXCL12 gene contains a single open reading frame of 267 nucleotides encoding the CXCL12α with 89 amino acids or the CXCL12β with 93 amino acids. The three-dimensional structures of CXCL12 have been resolved with experimental methods such as solution NMR [83,84], X-ray crystallography [85,86], and cryo-EM [87]. An example crystal structure (PDB: 3GV3) [85] of CXCL12 monomer is shown in Figure 3. The N-terminal domain of CXCL12 has a highly flexible and disordered structure [83], and is responsible for the chemokine to dock into the receptor CXCR4 [86].

The receptor CXCR4 is also a highly conserved protein. The identity and similarity between amino acid sequences of human (Uniprot: P61073) and mouse (Uniprot: P70658) CXCR4 are 90% and 95%, respectively [Figure 4]. The structure of CXCR4 has also been resolved by X-ray crystallography [88,89]. Figure 5 shows a crystal structure of CXCR4 in complex with a peptide antagonist binding to its ligand binding site (PDB: 3OE0) [89]. The CXCR4 structure represents a structure of typical GPCRs which contains 7 membrane-spanning α-helices, and a G-protein binding pocket on the intracellular side of the protein. Like other GPCRs, CXCR4 is a dynamic protein receptor adopting a two-state thermodynamic equilibrium model in which the receptor conformations are shifting between active and inactive states [90,91], contributing to a constitutive activity. The binding of the ligand to the receptor stabilizes the active state, changing the equilibrium toward active conformation thus increase the receptor activity. Molecular dynamics (MD) simulation analysis reveals that during activation, transmembrane helix 6 (TM6) of CXCR4 displaces away from the helical bundle to open up intracellular pocket for G-protein coupling [92]. This outward movement of TM6 during activation is proved experimentally using single-molecule FRET [93]. Disabling the outward movement of TM6 can abolish the downstream signaling of a GPCR [94]. In addition, MD simulations indicate that the formation of water channel within CXCR4 protein is crucial for G-protein coupling upon stimulated by CXCL12 [92]. Accumulated information from CXCL12 and CXCR4 structures and their interactions have made it possible to perform structure-based design and investigations of molecular inhibitors targeting this signaling axis.

Plant compounds for inhibiting C-X-C chemokine receptor type 4 expression

The importance of the CXCL12-CXCR4 signaling in cancer development, progression, and metastasis has made it a target for the developments of therapeutics. Although direct binding and inactivation of CXCL12 has been considered [86], targeting CXCR4 is more popular for this purpose. Natural compounds and phytochemicals have been considered sources for anticancer drug discovery and development. Many plant compounds have been found to be able to inhibit tumor invasion through suppression of CXCR4 expression, and this suppression is through downregulation of NF-κB. For example, butein [Figure 6a], a plant polyphenol, xanthohumol [Figure 6b], a prenylflavonoid derived from hops plant, apigenin [Figure 6c], a natural dietary plant flavonoid, and baohuoside I [Figure 6d], a flavonoid isolated from *Epimedium* sp., were found to be able to downregulate CXCR4 expression in a variety of cancer cells [95-98]. Butein was tested to inhibit CXCL12-induced migration and invasion of breast and pancreatic cancer cells [95]. Xanthohumol was indicated to suppress the invasion ability of breast and colon cancer cells [96]. Apigenin is able to abolish migration and invasion of transformed cells induced by CXCL12 and suppress tumor growth in a mouse model [97]. Baohuoside I was found to suppress the invasion of cervical and breast cancer cells [98]. In human chronic myeloid leukemia, CXCL12 was found to enhance the resistance of cancer cells to Adriamycin by increasing the expression of CXCR4, upregulating the downstream PI3K/Akt pathway, thus decreasing the expression of apoptosis-related proteins. Oroxylin A [Figure 6e], a natural bioactive monoflavonoid, was demonstrated to enhance the sensitivity of leukemic cells to Adriamycin and promote apoptosis by suppressing the expression of CXCR4, as indicated with *in vitro* and *in vivo* mouse model experiments [99].

Compounds directly binding to C-X-C chemokine receptor type 4

Owing to the fact that the three-dimensional structure of CXCR4 has been resolved by X-ray crystallography, computational analysis on ligands or compounds interacting with CXCR4 has become a useful means in drug discovery and design. Compounds which directly interact with CXCR4 to inhibit CXCL12 binding have become hot candidates to be developed into therapeutics. Silibinin [Figure 6f], a bioactive flavanolignan isolated from milk thistle, was identified to be

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Human   1   MNAKVVVVLVLVLTALCLSDGKPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIV  60
            M+AKVV VL LVL ALC+SDGKPVSLSYRCPCRFFESH+ARANVKHLKILNTPNCALQIV
Mouse   1   MDAKVVAVLALVLAALCISDGKPVSLSYRCPCRFFESHIARANVKHLKILNTPNCALQIV  60

Human   61  ARLKNNNRQVCIDPKLKWIQEYLEKALNKRFKM  93
            ARLKNNNRQVCIDPKLKWIQEYLEKALNKR KM
Mouse   61  ARLKNNNRQVCIDPKLKWIQEYLEKALNKRLKM  93
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Figure 2: Alignment and comparison of human and mouse C-X-C motif chemokine ligand 12 (CXCL12) amino acid sequences. The identity and similarity between human (Uniprot: P48061) and mouse (Uniprot: P40224) CXCL12 are calculated to be 92% (86/93) and 95% (89/93), respectively

a CXCR4 antagonist through molecular docking screening. Biochemical experiments showed that silibinin is able to bind directly to the CXCR4 binding cavity and block CXCL12-induced CXCR4 internalization, calcium response, and phosphorylation of Akt and Erk. It was also observed that the silibinin treatment inhibited CXCL12-induced migration of breast cancer cells [100]. Mysinger *et al*. performed a large-scale molecular docking screening from more than 3 million molecules against the CXCR4 structure and tested 47 high-scoring compounds experimentally. They identified 5 antagonists with one of them resembling known ligands and lacking specificity, and the rest four dissimilar to previously known scaffolds and were apparently specific [101]. The search for novel CXCR4 small molecule compound antagonists is still a continuous task. Several CXCR4 inhibitory compounds have been developed into anticancer lead drugs and are under clinical trials. So far, probably the most successful small molecule is the AMD3100 (also known as plerixafor or Mozobil®) [Figure 6g]. AMD3100 was first identified as an antiviral against HIV by selectively blocking CXCR4 [102]. AMD3100 is a small molecule with two cyclam rings connected by a phenylene linker. Each ring specifically interacts with the carboxyl groups on CXCR4 [103], inhibiting CXCL12 binding and downstream signaling. AMD3100 obtained approval from the US Food and Drug Administration (FDA) in December 2008 for clinical application in autologous transplantation of bone marrow cells in patients with non-Hodgkin's lymphoma or multiple myeloma [102] and is currently under clinical trials for the treatments of solid cancers including colorectal and pancreatic cancers [104]. It was also tested in mice to reduce the growth of metastatic breast cancer [105], and reduce the recurrence of glioblastoma after radiotherapy [106].

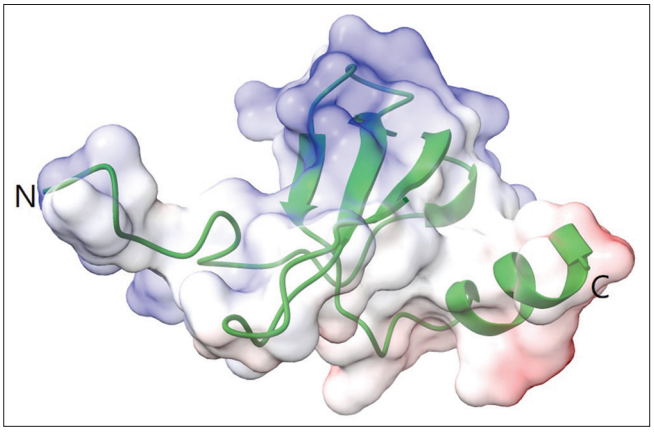

Figure 3: Crystal structure of human C-X-C motif chemokine ligand 12 (PDB: 3GV3 [85]). Protein secondary structures are presented in the backbone structure (green). The blue and red colors on the surface presentation indicate the positively charged and negatively charged regions. The N- and C-terminals of the protein are indicated with N and C, respectively

Peptide-based C-X-C chemokine receptor type 4 antagonists

Peptide-based CXCR4 antagonists for cancer therapy are also under intensive investigation and development. By exploring protein–protein interactions between CXCL12 and CXCR4, and detailed structural/residual investigations on the binding pocket of CXCR4, inhibitory peptides can be designed. Potential peptide-based antagonists with high CXCR4 affinity have been developed as lead drugs for anticancer purpose, and several have entered clinical trial stages. For example, 4F-benzoyl-TN14003 (BKT140), containing 14 amino acids, was tested to inhibit leukemia and multiple myeloma tumor growth [107]; CTCE-9908, containing 17 amino acids, was found to induce mitotic catastrophe in ovarian cancer cells [108], and inhibit primary breast tumor growth and metastasis in a mouse model [109]; LY2510924, a potent, selective cyclic peptide antagonist of CXCR4, was identified to exhibit anticancer activities in solid tumor and breast cancer in metastatic xenograft models [110]. A randomized phase II study evaluating the efficacy and safety of LY2510924 as a chemotherapeutic drug for extensive-disease small cell lung cancer has been completed [111]. BL-8040 (motixafortide), sold under the brand name Aphexda, is a peptide-based CXCR4 antagonist. The efficacy of BL-8040 has been reported by numerous preclinical studies in melanoma, neuroblastoma, and

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Human   4    ISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGNGLVILVM  63
             +SIYTSDNY+EE+GSGDYDS KEPCFR+EN +FN+IFLPTIY IIFLTGIVGNGLVILVM
Mouse   6    VSIYTSDNYSEEVGSGDYDSNKEPCFRDENVHFNRIFLPTIYFIIFLTGIVGNGLVILVM  65

Human   64   GYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSS  123
             GYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDA+A+WYFG FLCKAVH+IYTVNLYSS
Mouse   66   GYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAMADWYFGKFLCKAVHIIYTVNLYSS  125

Human   124  VLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWIPALLLTIPDFIFANVSEAD--  181
             VLILAFISLDRYLAIVHATNSQRPRKLLAEK VYVGVWIPALLLTIPDFIFA+VS+ D
Mouse   126  VLILAFISLDRYLAIVHATNSQRPRKLLAEKAVYVGVWIPALLLTIPDFIFADVSQGDIS  185

Human   182  ---DRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSCYCIIISKLSHSKGHQKRKAL  238
                DRYICDR YP+ LW+VVFQFQHIMVGLILPGIVILSCYCIIISKLSHSKGHQKRKAL
Mouse   186  QGDDRYICDRLYPDSLWMVVFQFQHIMVGLILPGIVILSCYCIIISKLSHSKGHQKRKAL  245

Human   239  KTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFENTVHKWISITEALAFFHCCLN  298
             KTTVILILAFFACWLPYY+GISIDSFILL +IKQGC+FE+ VHKWISITEALAFFHCCLN
Mouse   246  KTTVILILAFFACWLPYYVGISIDSFILLGVIKQGCDFESIVHKWISITEALAFFHCCLN  305

Human   299  PILYAFLGAKFKTSAQHALTSVSRGSSLKILSKGKRGG  336
             PILYAFLGAKFK+SAQHAL S+SRGSSLKILSKGKRGG
Mouse   306  PILYAFLGAKFKSSAQHALNSMSRGSSLKILSKGKRGG  343
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Figure 4: Alignment and comparison of human and mouse C-X-C chemokine receptor type 4 (CXCR4) amino acid sequences. The identity and similarity between human (Uniprot: P61073) and mouse (Uniprot: P70658) CXCR4 are calculated to be 90% (303/338) and 95% (322/338), respectively

breast and lung cancers [112]. BL-8040 has been approved in September 2023 by the US FDA for medical use in the treatment of multiple myeloma [113]. With continuous efforts made in this research field, it is expected that more peptide-based CXCR4 inhibitors will be available for further anticancer trials. However, the applications of peptide-based therapeutics are very often limited by *in vivo* protease/peptidase degradations. Modifications of terminals and/or key amino acids in identified peptide-based CXCR4 antagonists to enhance both the CXCR4 affinity and metabolic stability are considered also an important research direction and have also been performed [114].

Conclusions

Chemokine CXCL12 binding to its receptors CXCR4 plays a role in regulating homeostasis under normal physiological conditions. This signaling axis is linked to cell survival, proliferation, chemotaxis, and migration. However, a variety of cancer cells overexpress CXCR4 and exploit CXCL12-CXCR4 signaling for cancer growth, survival, angiogenesis, metastasis, and the development of tumor microenvironment and chemoresistance. The CXCR4 overexpression in tumors is linked to poor prognosis. Therefore, the CXCL12-CXCR4 axis is considered a potential therapeutic target for cancer treatments. Inhibitors, including small molecule based and peptide based, targeting this signaling axis are currently under intensive research and development. Several CXCR4 antagonists are currently in clinical trials at different stages, and a few have been approved by the US FDA for medical use in the treatment of cancers. The experimentally resolved structures of CXCL12 and CXCR4 have allowed the application of structure-based computational simulation and analysis to facilitate the discovery, design, and modification of therapeutic drugs. It is expected that in the near future, the CXCL12-CXCR4 axis will continue to be a research focus in the development of therapeutic strategies for cancers.

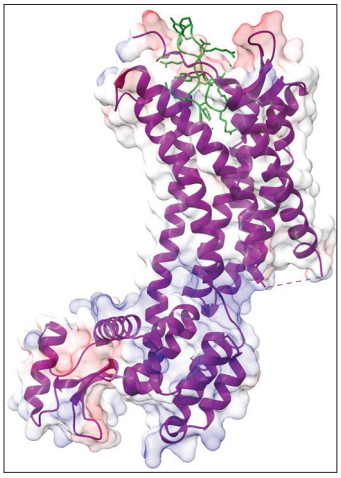

Figure 5: Crystal structure of the C-X-C chemokine receptor type 4 (CXCR4) chemokine receptor in complex with a cyclic peptide antagonist (PDB: 3OE0 [89]). The CXCR4 lysozyme chimera protein is colored in purple, and the peptide antagonist is colored in green. On the surface presentation, the blue and red colors indicate the positively charged and negatively charged regions. The bundle of helices is the transmembrane region and is highly hydrophobic

Data availability statement

Data sharing was not applicable to this article as no datasets were generated or analyzed during the current study.

Acknowledgment

The schematic diagram of signal transduction pathways [Figure 1] was prepared with software from BioRender.com. The alignments of amino acid sequences [Figures 2 and 4] were performed with the BLAST tool from the National Center for Biotechnology Information. Structures of proteins [Figures 3 and 5] were visualized with software ChimeraX from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco. The authors would like to thank Professor Ji-Hshiung Chen and Dr. Alex Yong Kwang Tan for proofreading this manuscript.

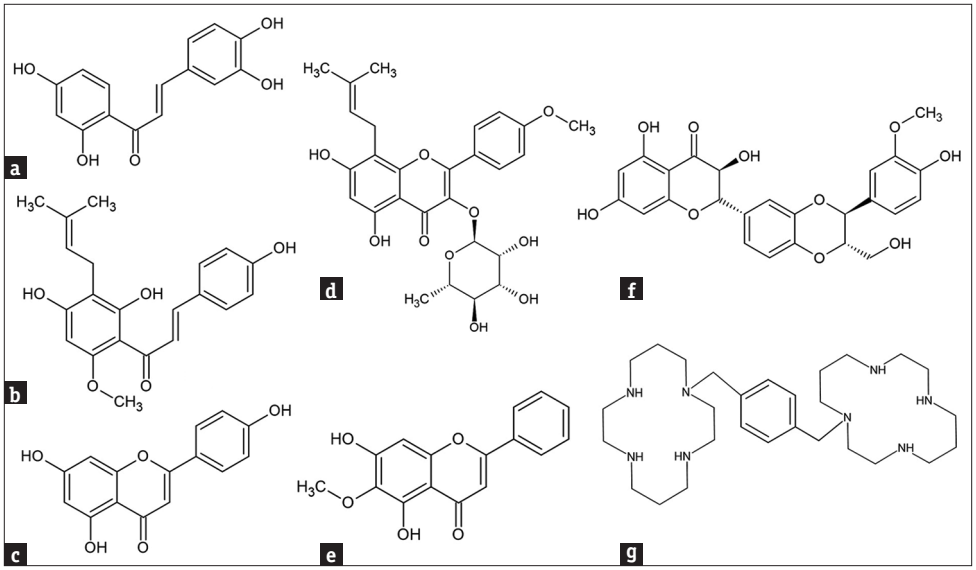

Figure 6: Chemical structures of selected compounds suppressing C-X-C chemokine receptor type 4 expression or activity. (a) butein; (b) xanthohumol; (c) apigenin; (d) baohuoside I; (e) oroxylin A; (f) silibinin; (g) AMD3100 (plerixafor)

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Conflicts of interest

There are no conflicts of interest.

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