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The Intricate Role of CXCR4 in Cancer

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Abstract

Chemokines mediate numerous physiological and pathological processes related primarily to cell homing and migration. The chemokine CXCL12, also known as stromal cell-derived factor-1, binds the G-protein-coupled receptor CXCR4, which, through multiple divergent pathways, leads to chemotaxis, enhanced intracellular calcium, cell adhesion, survival, proliferation, and gene transcription. CXCR4, initially discovered for its involvement in HIV entry and leukocytes trafficking, is overexpressed in more than 23 human cancers. Cancer cell CXCR4 overexpression contributes to tumor growth, invasion, angiogenesis, metastasis, relapse, and therapeutic resistance. CXCR4 antagonism has been shown to disrupt tumor–stromal interactions, sensitize cancer cells to cytotoxic drugs, and reduce tumor growth and metastatic burden. As such, CXCR4 is a target not only for therapeutic intervention but also for noninvasive monitoring of disease progression and therapeutic guidance. This review provides a comprehensive overview of the biological involvement of CXCR4 in human cancers, the current status of CXCR4-based therapeutic approaches, as well as recent advances in noninvasive imaging of CXCR4 expression.

1. INTRODUCTION

Chemokines are a family of cytokines defined by their ability to induce gradient-dependent directional chemotaxis and are secreted by a variety of stromal and epithelial cells (Howard, Ben-Baruch, & Oppenheim, 1996; Smith, Whittall, Weksler, & Middleton, 2012). These small proteins (8–10 kDa) possess a common structural feature of conserved cysteine residues at the N-terminus (Baggiolini, 1998). Based on the number and relative spacing of the N-terminal cysteine residues, chemokines are divided into CXC, CX3C, CC, and C subfamilies with CXC chemokines characterized by one amino acid (X) between the two N-terminal cysteine residues (C) and CX3C chemokines with two N-terminal cysteine residues separated by three amino acids, etc. (Le, Zhou, Iribarren, & Wang, 2004). To date, nearly 50 chemokines have been discovered (Balkwill, 2004a; Viola & Luster, 2008). Chemokines exert their biological function through interaction with chemokine receptors, seven transmembrane G-protein-coupled receptors (GPCRs; Gilman, 1987), present on the target cells (Baggiolini, 1998). Chemokine receptors are grouped into four different families as CXC, CX3C, CC, and XC based on the chemokines they primarily interact with for signaling. Thus far, nearly 20 chemokine receptors have been identified (Balkwill, 2004a;

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Gilman, 1987; Pierce, Premont, & Lefkowitz, 2002; Viola & Luster, 2008). The large number of chemokines, compared to chemokine receptors, implies considerable redundancy in chemokine receptor interactions with multiple ligands binding to the same receptor and vice versa. The chemokine receptor 4 (CXCR4) is unique in that it exclusively interacts with the endogenous ligand CXCL12 (Oberlin et al., 1996).

CXCR4, also known as "fusin," is one of the most well-studied chemokine receptors due to its earlier found role as a coreceptor for HIV entry (Feng, Broder, Kennedy, & Berger, 1996). The chemokine stromal cell-derived factor-1, now renamed as CXCL12, was established as the specific ligand for CXCR4 (Bleul, Fuhlbrigge, Casasnovas, Aiuti, & Springer, 1996; Oberlin et al., 1996). Although CXCL12 is the only known chemokine that binds CXCR4, recent studies suggest that extracellular ubiquitin also acts as an immune modulator through CXCR4-mediated signaling (Saini, Marchese, & Majetschak, 2010; Tripathi et al., 2013). Although CXCR4 is known to bind only CXCL12, in 2005 another chemokine receptor CXC receptor 7 (CXCR7, ACKR3, RDC1, CMKOR1, or GPR159) was established as a receptor for CXCL12 (Balabanian et al., 2005; Burns et al., 2006). CXCR7 functions to control the CXCL12 gradients through high-affinity binding and rapid degradation (Hoffmann et al., 2012). Thus, the role of the CXCR4–CXCR7–CXCL12 axes has become more intricate in the regulation of numerous biological processes involving cell survival and migration. Comprehensive studies will be required to delineate the exact role of CXCR4–CXCR7–CXCL12 axes in cell migration. Roles of CXCR7 and CXCL12 in biology and disease have been reviewed in detail by others (Hattermann & Mentlein, 2013; Liao et al., 2013; Sun et al., 2010).

2. CXCR4/CXCL12 SIGNALING

CXCL12 binding to CXCR4 initiates various downstream signaling pathways that result in a plethora of responses (Fig. 2.1) such as increase in intracellular calcium, gene transcription, chemotaxis, cell survival, and proliferation (Ganju et al., 1998), which will be briefly discussed here. Chemokine receptors are pertussis toxin-sensitive GTP-binding proteins of Gi type. After chemokine binding, the heterotrimeric G protein is activated by the exchange of GDP for GTP and dissociates into the GTP-bound α and the βγ subunits (Goldsmith & Dhanasekaran, 2007; Mellado, Rodriguez-Frade, Manes, & Martinez, 2001). The dissociated βγ subunit activates two major signal transduction enzymes, a phospholipase C-β (PLC-β), which is specific for phosphatidylinositol, and a phosphatidylinositol-3-OH kinase (PI3K). The PLC-β cleaves phosphatidylinositol (4,5)-bisphosphate into two secondary messengers, inositol (1,4,5)-trisphosphate (IP3) and diacylglycerol (DAG). Through binding to its specific receptor in the endoplasmic reticulum, IP3 induces the release of Ca^{2+} from intracellular stores. Acting in conjunction with Ca^{2+}, DAG activates protein kinase C and mitogen-activated protein kinase (MAPK), contributing to cell migration (Bendall, Baraz, Juarez, Shen, & Bradstock, 2005; Mellado et al., 2001).

Gβγ or Gα subunits activate PI3K leading to phosphorylation of many focal adhesion components including focal adhesion kinase, proline-rich kinase-2, Crk-associated substrate (p130Cas), cytoskeletal protein paxillin, Crk, Nck, and CrkL (Wang, Park, & Groopman, 2000; Zhang, Wang, Matczak, Proper, & Groopman, 2001) and contribute to reorganization

of the actin cytoskeleton and changes necessary for cell migration. The activated PI3K rapidly generates phosphatidylinositol (3,4,5)-trisphosphate and initiates the activation of the AKT pathway (Mellado et al., 2001; Ward, 2006). Activated AKT plays a key role in tumor cell survival through inactivation of BCL-2 antagonist proapoptotic BAD resulting in cell survival. CXCR4 signaling via AKT also leads to inactivation of GSK3β and stabilization of β-catenin. Stabilized β-catenin moves to the nucleus and activates gene transcription and promotes proliferation (Mo et al., 2013).

Signaling through $G\alpha_i$ has been linked to transcription and gene expression through the PI3K-AKT-NF-κB, MEK1/2, and ERK1/2 axes. In addition, Gα subunit also activates the Ras and Rac/Rho pathways, leading to the phosphorylation of ERK and P38 proteins, respectively (Vlahakis et al., 2002). Activated ERK can phosphorylate and regulate other cellular proteins, as well as translocate into the nucleus and phosphorylate and regulate transcription factors, leading to changes in gene expression and cell cycle progression.

Homodimerization of CXCR4 has been suggested to result in G-protein-independent signaling through the JAK/STAT signaling pathway (Mellado et al., 2001; Vila-Coro et al., 1999). JAK/STAT pathway, possibly in conjunction with other signaling pathways, promotes changes in cell morphology, collectively known as polarization, leading to chemotactic responses (Mellado et al., 2001).

In addition to these general signaling cascades, other chemokine-specific signaling mechanisms also exist, making the outcomes of specific targeting of these pathways unpredictable. Even though several chemokine-targeted agents are in use, redundancy in chemokine signaling suggests that receptor-targeted strategies that eliminate redundant functions of chemokine signalingmay have greater effect than agents that solely target the effects of chemokines.

3. EXPRESSION AND PHYSIOLOGICAL FUNCTIONS OF THE CXCR4/CXCL12 AXIS

CXCR4 is commonly expressed on most hematopoietic cell types including macrophages, monocytes, T and B lymphocytes, neutrophils, hematopoietic, endothelial progenitor, and stem cells in the blood or bone marrow, dendritic cells, Langerhans cells (Bleul et al., 1996; Zabel et al., 1999), vascular endothelial cells (Gupta, Lysko, Pillarisetti, Ohlstein, & Stadel, 1998), neurons and neuronal stem cells (Hesselgesser et al., 1997), microglia and astrocytes (He et al., 1997), as well as embryonic stem cells (Kucia et al., 2005; Ratajczak et al., 2003). CXCR4-expressing cells respond to and migrate along CXCL12 gradients and contribute to several physiological functions and organ development (Kucia et al., 2004; Zou, Kottmann, Kuroda, Taniuchi, & Littman, 1998).

The chemokine CXCL12, an effective lymphocyte chemoattractant and hematopoiesis regulator (Aiuti, Webb, Bleul, Springer, & Gutierrez-Ramos, 1997; Bleul, Fuhlbrigge, Casasnovas, Aiuti, & Springer, 1996; Ma et al., 1998; Zou et al., 1998), is a modulator of several biological processes through its interaction with CXCR4. CXCL12 is expressed and secreted in different organs such as the liver, lung, kidney, brain, and bone marrow where it

retains or chemoattracts CXCR4-expressing cells (Janowski, 2009; Kucia et al., 2004; Yu et al., 2006). The CXCR4/CXCL12 axis is indispensable for cell migration during embryonic hematopoiesis, organogenesis, vascularization, and organ homeostasis (Petit, Jin, & Rafii, 2007; Ratajczak et al., 2006; Schober & Zernecke, 2007). CXCR4 or CXCL12 knockout mice show significant defects in hematopoiesis, heart, blood vessels, and brain. Ablation of either CXCR4 or CXCL12 gene is lethal at embryonic stage (Lazarini, Tham, Casanova, Arenzana-Seisdedos, & Dubois-Dalcq, 2003; Ma et al., 1998; Nagasawa et al., 1996; Ratajczak et al., 2006; Vagima et al., 2011) underscoring the importance of this axis in organ development and physiological function. The CXCR4/CXCL12 axis also plays an important role in inflammation and immune surveillance of tissues (Viola & Luster, 2008). Different tissue damaging conditions, such as hypoxia, toxins, or irradiation, increase the expression of CXCL12, thereby recruiting CXCR4-positive stem cells to the site that requires tissue repair or regeneration (Gambaryan et al., 2011; Kucia et al., 2004; Ratajczak et al., 2006; Yu & Hales, 2011). Vital roles of the CXCR4/CXCL12 axis in normal biological processes suggest a cautious approach for therapeutic targeting.

CXCR4 and CXCL12 are highly conserved between species such that the amino acid sequence homology between human and murine CXCR4 and CXCL12 is 91% and 99%, respectively (Heesen, Berman, Benson, Gerard, & Dorf, 1996; Schabath et al., 1999). Very few mutations in CXCR4 have been reported. A specific dominant germline mutation in CXCR4 gene causes WHIM syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) (Bachelerie, 2010; Hernandez et al., 2003), a immunodeficiency disorder characterized by neutropenia. Following CXCL12 stimulation, the C-terminal mutations (both nonsense and frameshift have been observed) block receptor internalization due to distal truncations of the receptor's carboxy terminal tail that removes potential phosphorylation sites involved in the receptor attenuation or desensitization processes. This results in persistent CXCR4 activation and failure of mature neutrophils to reach the bloodstream from bone marrow, leading to neutropenia.

First ever somatic mutations in CXCR4 in cancer were recently identified by Hunter et al. (2012) in 27% of Waldenström macroglobulinemia (WM), an indolent B-cell malignancy, patients. While several nonsense and frameshift mutations were identified in the C-terminus, most frequent mutations were observed at S338 of CXCR4. The recent success in treating WHIM patients with very low doses of the CXCR4 inhibitor (AMD3100/plerixafor at 001–0.02 mg/kg) (McDermott et al., 2014) suggests that similar targeted therapy approaches could be used for WM patients.

In addition to cancer, the CXCR4/CXCL12 axis is implicated in the pathology of several diseases due to its role in mediating the immune cell movement. Role of CXCR4 in autoimmune and inflammatory diseases can be found elsewhere (Buckley et al., 2000; Choi, Duggineni, Xu, Huang, & An, 2012; Chong & Mohan, 2009; Chung et al., 2010; Debnath, Xu, Grande, Garofalo, & Neamati, 2013; De Klerck et al., 2005; Gulino, 2003; Hsu, Rosenquist, Ansari, & Gershwin, 2005).

4. ROLE OF CXCR4 IN CANCER

Although initial studies were centered on the participation of CXCR4 in HIV infection of T-cells, its connection to cancer became an intense research topic with the discovery of its involvement in B-cell trafficking and tissue localization in chronic leukemia patients (Burger, Burger, & Kipps, 1999; Mohle, Failenschmid, Bautz, & Kanz, 1999) as well as regulation of organ-specific metastasis in breast cancer models (Muller et al., 2001). CXCR4 is overexpressed in more than 23 different types of human cancers including kidney, lung, brain, prostate, breast, pancreas, ovarian, and melanomas and contributes to the tumor growth, angiogenesis, metastasis, and therapeutic resistance (Balkwill, 2004b; Darash-Yahana et al., 2004; Furusato, Mohamed, Uhlen,& Rhim, 2010; Muller et al., 2001; Vandercappellen, Van Damme, & Struyf, 2008; Zlotnik, 2008).

Cancer cells are thought to hijack the CXCR4/CXCL12 axis to establish distant organ metastasis. Supporting this hypothesis, CXCL12 expression levels are highest in common sites of metastasis such as brain, bone marrow, lungs, and liver (Ho, Shiwen, Abraham, Tsui, & Baker, 2012; Janowski, 2009; Muller et al., 2001; Yu et al., 2006). Further supporting this hypothesis, abrogation of the CXCR4/CXCL12 axis results in reduced metastatic burden in a variety of mouse models of cancer.

Elevated CXCR4 expression observed in several cancers has been identified as a poor prognostic biomarker and will be discussed in detail in the later sections. Several factors contribute to the upregulation of CXCR4 in malignant cells. Notably, the hypoxia-inducible factor (HIF)-1α (Ishikawa et al., 2009; Zagzag et al., 2006), growth factors such as basic fibroblast growth factor, vascular endothelial growth factor (VEGF) (Salcedo et al., 1999), and epidermal growth factor (EGF) (Phillips et al., 2005), and transcription factors like nuclear respiratory factor-1 (Wegner et al., 1998) positively upregulate CXCR4 expression.

The CXCR4/CXCL12 axis plays a critical role in therapeutic resistance by (i) directly promoting cancer cell survival, invasion, and cancer stem (or tumor-initiating) cell phenotype; (ii) recruiting myeloid bone marrow-derived cells to indirectly facilitate tumor recurrence and metastasis; and (iii) promoting angiogenesis directly or in a paracrine manner (Duda et al., 2011; Teicher, 2011). Several studies have also identified increased expression of CXCR4 in cancer-associated fibroblasts (CAFs), which play an important role in tumorigenesis and have been implicated in neoplastic progression, tumor growth, angiogenesis, and metastasis (Eck, Cote, Winkelman, & Brinckerhoff, 2009; Kojima et al., 2010; Orimo et al., 2005). Data from these studies suggest that soluble breast cancer factors initiate the transdifferentiation of normal human mammary fibroblasts to tumor-promoting CAFs through the induction of matrix metalloproteinase-1 (MMP-1) and CXCR4 expression (Kwong, Kulbe, Wong, Chakravarty, & Balkwill, 2009). Similarly, the CXCR4/CXCL12 axis is critical for mesenchymal stem cell recruitment to the tumors (Domanska et al., 2012). In mouse models of human breast cancer (Orimo et al., 2005) and prostate cancer (PCa) (Olumi et al., 1999), high intratumoral CXCL12 levels have been shown to attract CXCR4-positive inflammatory, vascular, and stromal cells into the tumors, where they eventually support tumor growth by secreting growth factors, cytokines, chemokines, and proangiogenic factors.

In addition to contributing to the tumor–stromal interactions, CXCR4 is also expressed on cancer stem-like cells and contributes to cancer recurrence. Recent studies have shown the presence of a small subset of cancer cells, with very similar characteristics to stem cells, known as cancer stem cells (CSCs), which mediate tumor growth, metastasis, recurrence, as well as therapeutic resistance (Baccelli & Trumpp, 2012; Baumann, Krause, & Hill, 2008; Peitzsch, Kurth, Kunz-Schughart, Baumann, & Dubrovska, 2013). CXCR4 expression in CSCs confers increased invasiveness and metastatic potential as well as improved self-renewal and survival capacity (Gatti et al., 2013; Hermann et al., 2007). Hermann et al. (2007) reported that in the invasive front of pancreatic tumor, CD133+ pancreatic CSCs contained a subpopulation, characterized by CXCR4 coexpression, capable of evading the primary tumor and establishing distant metastases. The role of CXCR4 signaling in CSC survival and self-renewal was further demonstrated by Gatti et al. (2013) in human glioblastoma stem-like cells using the CXCR4 antagonist AMD3100, which reduced self-renewal and survival with greater efficacy in the cultures releasing higher levels of CXCL12.

Expression, specific involvement, and therapeutic targeting of CXCR4 in different types of cancers will be discussed in detail in the following sections.

4.1. Leukemia

Leukemia causes approximately 281,500 deaths annually and represents roughly 3% of all cancer deaths in the world (Lozano et al., 2012). Spoo, Lubbert, Wierda, and Burger (2007) reported that CXCR4 expression in acute myeloid leukemia cells in patients varied from low (n = 32), medium (n = 26) to high (n = 32) and that CXCR4 expression is an independent prognostic predictor of disease relapse. Similar observations were made in other hematological cancers such as chronic myeloid leukemia (CML), acute myelogenous leukemia (AML), and multiple myeloma (MM) (Barretina et al., 2003; Ko et al., 2014; Konoplev et al., 2011; Peled & Tavor, 2013), where CXCR4 expression on cancer cells contributed to therapeutic resistance (Chen et al., 2013; Sison, McIntyre, Magoon, & Brown, 2013). In chronic lymphocytic leukemia (CLL), CXCR4-expressing cancerous B-cells are attracted toward bone marrow stromal cells, which secrete high levels of CXCL12 (Burger et al., 1999), resembling the homing of normal hematopoietic stem cells to bone marrow (Burger & Peled, 2009; Konopleva & Jordan, 2011). The CXCR4/CXCL12 interaction within the bone marrow microenvironment protects the cancer cells from chemotherapy-induced apoptosis (Burger, 2010; Nervi et al., 2009). Stromal cells exert the protective effects, at least partially, through phosphorylation of PI3K/AKT, MAPK, and ERK (Zeng et al., 2009). In addition, the CXCR4/CXCL12 axis promotes tumor cell invasion in the stromal layer, thereby facilitating attachment to the stromal cells (Burger & Peled, 2009; Meads, Hazlehurst, & Dalton, 2008). Active adhesion molecules, such as integrins, convey prosurvival signals to the malignant cells, leading to adhesion-mediated drug resistance. Because CXCR4/CXCL12 interactions are crucial for homing of tumor cells to the bone marrow microenvironment and drug resistance, CXCR4 antagonists have been explored as chemosensitizers in leukemia treatment (Dillmann et al., 2009; Nervi et al., 2009). The CXCR4 inhibitor plerixafor is used in combination with other chemotherapy drugs, to disrupt cancer cell adhesion to the stromal cells (Dillmann et al., 2009), leading to

mobilization of cancer cells into systemic circulation and subsequent exposure to the cytotoxic chemotherapeutic agents (Nervi et al., 2009). AMD3100 is also the first FDA-approved CXCR4 antagonist used to mobilize hematopoietic stem cells from bone marrow for collection and subsequent autologous transplantation in non-Hodgkin's lymphoma and MM patients (DiPersio et al., 2009; DiPersio, Uy, Yasothan, & Kirkpatrick, 2009).

4.2. Multiple myeloma

Although MM is relatively rare, it is the second most prevalent hematological cancer after non-Hodgkin's lymphoma (Collins, 2005), accounting for 114,251 new cases and 80,015 deaths each year (WHO, 2012). CXCR4 overexpression was detected in 43.2% of MM patients (Bao et al., 2013). CXCL12 and CXCR4 have been shown to be involved in cancer cell homing and migration in MM by attracting and activating plasma cells in bone marrow (Alsayed et al., 2007). The CXCR4/CXCL12 axis plays an important role on the biological behavior of MM cells by mediating the effect of adhesion molecules such as ICAM-1, integrin $\alpha_4\beta_1$ (Li et al., 2003; Parmo-Cabanas et al., 2004). MM patients overexpressing CXCR4 were shown to be more sensitive to therapy with significantly longer median survival time than CXCR4-negative patients (48 months vs. 42 months, respectively, $P< 0.05$) (Bao et al., 2013; Pei et al., 2011; Stessman et al., 2013). The CXCR4 antagonist 4F-benzoyl-TN14003 (BKT140) was shown to inhibit MM tumor growth (Beider et al., 2011). Taking advantage of the CXCR4 expression in MM cells, Kuhne et al. (2012) demonstrated that MDX-1338, a fully humanized anti-CXCR4 antibody, significantly inhibited proliferation of MM cell lines *in vitro* and reduced tumor growth of MM xenografts in mouse models. CXCR4 antagonists, AMD3100 and BKT140, are used either alone or in combination with G-CSF to mobilize hematopoietic stem cells for autologous transplantation following high-dose chemotherapy (Lanza et al., 2014; Peled et al., 2014; Steinberg & Silva, 2010).

4.3. Breast cancer

Breast cancer is the most prevalent invasive cancer in women worldwide. It comprises 16% of all female cancers causing approximately half a million deaths every year (WHO, 2014a). The concept of cancer cells hijacking chemokine receptor pathways was first demonstrated in breast cancer models (Muller et al., 2001). It is now well established that the CXCR4/CXCL12 axis plays an important role in regulation of breast tumor growth, invasion, and metastasis (Liang et al., 2005, p. 89; Muller et al., 2001). Most breast tumors express higher levels of CXCR4 with very low expression levels reported in normal breast tissues. While all breast tumor tissues have some level of CXCR4 expression, more than 40% of the tumors have elevated CXCR4 levels (Salvucci et al., 2006). This expression also increases with tumor grade from 20% in normal breast tissue to 43% in ductal carcinoma *in situ* (DCIS) to 67% in invasive cancer (Salvucci et al., 2006). More importantly, nearly 75% of triple-negative (TN) breast cancer patients express high levels of activated CXCR4 (Chu et al., 2010; Hassan et al., 2009). A meta-analysis by Xu, Shen, Liu, and Shu (2013) showed that overall survival (OS) and disease-free survival (DFS) in breast cancer patients were negatively correlated to CXCR4 expression, with the hazard ratios (HRs) 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. CXCR4 is one of the few genes that is highly enriched in metastatic breast cancer

subpopulation and significantly overexpressed in bone metastases (Kang et al., 2003; Liang et al., 2005). Overexpression of CXCR4 in primary tumors is directly correlated to the degree of lymph node metastasis and poor survival rates in breast cancer patients (Kang, Watkins, Douglas-Jones, Mansel, & Jiang, 2005; Kato, Kitayama, Kazama, & Nagawa, 2003). The CXCR4/CXCL12 axis transactivation of HER-2 receptor and upregulation of CXCR4 are essential for HER-2-mediated metastasis (Balkwill, 2004a; Cabioglu et al., 2005). A study by Holm et al. demonstrated that CXCR4 overexpression occurs in most breast cancer patients. HER-2-negative breast tumors with higher levels of CXCR4 expression exhibit more aggressive behavior and are more likely to recur compared to tumors with lower CXCR4 expression (Holm et al., 2007). Similarly, CXCR4 has been shown to mediate estrogen-independent tumorigenesis, metastasis, and resistance to endocrine therapy. CXCR4 overexpression is correlated with worse prognosis and decreased patient survival rates, irrespective of the ER status (Rhodes et al., 2011). Involvement of CXCR4 in breast cancer invasion and metastasis was further supported by the findings that blocking the CXCR4/CXCL12 axis by low molecular weight (LMW) agents, peptides, or antibodies inhibited tumor growth and metastasis in breast cancer models (Huang et al., 2009; Muller et al., 2001; Smith et al., 2004). Similar results were also observed with siRNA-based downregulation of CXCR4 in breast cancer cell lines and mouse xenograft models (Liang et al., 2005).

4.4. Prostate cancer

PCa has the highest incidence among men in the United States (CDC, 2013). Although early detection improves prognosis, majority of PCa patients eventually develop bone metastasis. CXCR4 is one of the crucial factors involved in the bone metastasis of PCa (Gladson & Welch, 2008; Wang et al., 2005). Nearly 90% of all PCas exhibit high levels of CXCR4 resulting in poor clinical outcome (Akashi et al., 2008). Increased CXCR4 expression in PCas is associated with an aggressive phenotype and poor patient survival rates (Akashi et al., 2008). High-density prostate tumor tissue microarray analyses have revealed that (a) levels of CXCR4 protein expression in the malignant epithelia is greater than that of benign epithelia; (b) both prostatic intraepithelial neoplasia and some atrophic lesions, thought to be potentially precancerous, demonstrate positive staining for CXCR4; (c) CXCR4 expression increases with increasing tumor aggressiveness; and (d) metastases have elevated CXCR4 expression compared to primary tumors (Sun et al., 2003). In addition, the CXCR4/CXCL12 axis regulates the angiogenic phenotype in PCa (Darash-Yahana et al., 2004). As a result, CXCR4 expression has been proposed as a prognostic factor and considered a therapeutic target in PCa (Darash-Yahana et al., 2004; Wang et al., 2005). The CXCR4/CXCL12 interaction and subsequent downstream signaling pathways elicit multiple responses in PCa cells. CXCL12 treatment of human PCa cell line PC3 results in MEK/ERK signaling and activation of NF-κB, which are important for tumor cell survival (Fernandis, Cherla, Chernock, & Ganju, 2002). CXCR4/CXCL12 interaction, through upregulation of integrins, increases the attachment of PCa cells to the endothelial cell layer or to the stromal collagen, fibronectin, and laminins (Engl et al., 2006; Kukreja, Abdel-Mageed, Mondal, Liu, & Agrawal, 2005). *In vivo* studies in animal model of PCa metastasis demonstrated that CXCR4 inhibitory antibody reduces the extent of metastases to bones and CXCR4-blocking peptide limits intraosseous growth of PCa (Sun et al., 2005). CXCL12 is highly expressed in

prostate tumor-associated blood vessels and basal cell hyperplasia (Darash-Yahana et al., 2004). Overexpression of CXCR4 in subcutaneous PC3 mouse xenografts induced greater vasculature and invasiveness of tumor cells into the adjacent tissues. On the other hand, CXCR4 inhibitory antibody blocked CXCR4-dependent vascularization and tumor growth (Darash-Yahana et al., 2004). In addition, inhibition of CXCR4/CXCL12 signaling by antibodies, peptide analogues, or small molecules has been found to reduce metastatic burden in various orthotopic and metastatic mouse xenograft models of PCa (Porvasnik et al., 2009; Sun et al., 2005; Taichman et al., 2002). The above findings illustrate the importance of CXCR4/CXCL12 interaction in tumor growth and metastasis of PCa.

4.5. Ovarian cancer

Ovarian cancer is the fifth leading cause of cancer deaths in women with <25% 5-year survival rates in the United States alone (ACS, 2014). Ovarian cancers often metastasize to lymph nodes, uterus, fallopian tube, and lungs. Advanced or metastatic ovarian cancers express high levels of CXCR4 and CXCR4/CXCL12 interactions are shown to regulate metastasis of epithelial ovarian cancer (EOC) (Barbolina et al., 2010). Nearly 59% of ovarian tumors are positive for CXCR4 (Jiang, Wu, Shi, Wu, & Yin, 2006). A recent meta-analysis showed that higher CXCR4 expression in ovarian cancer is associated with a poor progression-free survival (HR, 8.48; 95% CI: 2.13–33.70; $P = 0.002$) and a lower OS (HR, 2.81; 95% CI: 1.16–6.80; $P = 0.022$) (Liu et al., 2014). The CXCL12/CXCR4 axis together with multiple other factors regulates tumor growth and metastasis in ovarian cancer. Increased CD164 expression is reported to be involved in ovarian cancer progression via the CXCL12/CXCR4 axis (Huang et al., 2009). Concomitant expression of CD40 and CXCR4 in ovarian carcinoma tissues strongly correlates with pelvic metastasis (Qu et al., 2013). Higher expression of CXCL12, seen in 20% of the ovarian tumors, has also been demonstrated to enhance ovarian cancer cell invasion through $\alpha_v\beta_6$-mediated urokinase-type plasminogen activator expression via the p38 MAPK and PI3K/AKT pathways (Xue et al., 2013). In ascites isolated from ovarian cancer patients, both CXCR4 and CXCL12 were shown to be regulated by the tumor-associated inflammatory mediator prostaglandin E2 (PGE2), which induces chemotaxis of myeloid-derived suppressor cells into the ascites' microenvironment (Obermajer, Muthuswamy, Odunsi, Edwards, & Kalinski, 2011), contributing to therapeutic resistance.

In vitro knock down of CXCR4 by small interfering RNA reduced cell proliferation while increasing apoptosis and chemosensitivity in EOC cell lines (Chen et al., 2012). Inhibition of CXCR4 by AMD3100 has been shown to delay CXCR4-mediated metastasis and invasion of EOC (Kajiyama et al., 2008). Similarly, the CXCR4 antagonist peptide CTCE-9908 increased the cytotoxicity of paclitaxel by inducing mitotic catastrophe in ovarian cancer cell lines (Kwong et al., 2009).

4.6. Lung cancer

Lung cancer is the most common cancer in the world and the leading cause of cancer-related death in the United States (ACS, 2014). Nonsmall-cell lung cancer (NSCLC) accounts for 85–90% of lung cancer cases with a 5-year survival rate of 15% (Siegel, Naishadham, & Jemal, 2012). Metastatic spread accounts for >70% of NSCLC deaths with the major sites of

NSCLC metastasis being brain, bone, adrenal gland, and liver (Hubbard, Fu, Margevicius, Dowlati, & Linden, 2012; Murthy et al., 2010; Saintigny & Burger, 2012; Triano, Deshpande, & Gettinger, 2010). Numerous studies have established a strong correlation between CXCR4 expression and poor prognosis in NSCLC (Burger, Stewart, Wald, & Peled, 2011; Saintigny & Burger, 2012; Spano et al., 2004; Su et al., 2005; Wagner et al., 2009). CXCR4 expression levels are elevated in primary and metastatic tumor tissue compared to normal lung. In NSCLC, disease prognosis correlates with localization of CXCR4 to the nuclear and/or the cytomembranous compartment (Spano et al., 2004; Su et al., 2005; Wagner et al., 2009). Higher CXCR4 expression in cytomembranous compartment is correlated with a higher tendency to locally invade neighboring tissues and with increased propensity of tumor cells to form distant metastases. CXCR4 overexpression in cytomembranous compartment is also linked to greater density of microvasculature in tumors and associated with increased microvessel invasion by tumor cells (Franco et al., 2011; Su et al., 2005). Cytomembranous overexpression of CXCR4 is also associated with poor survival in stage IV NSCLC patients (Otsuka et al., 2011). On the contrary, stronger nuclear CXCR4 expression was linked with better outcomes in early-stage patients (Spano et al., 2004). CXCL12 is also overexpressed in NSCLC in more than 80% of cases (Wald et al., 2006) and elevated CXCL12 expression was correlated with higher recurrence rate and lymph node metastasis (Wagner et al., 2009). Overall, these findings demonstrate the crucial role of the CXCR4/CXCL12 axis in tumor growth and metastasis of NSCLC.

CXCR4 expression in lung cancer is modulated by other tumor microenvironmental factors such as hypoxia or the EGF (Liu et al., 2009). In NSCLC cells, increase in CXCR4 expression due to activation of EGF receptor (EGFR) is remarkably enhanced under hypoxic conditions. Augmented expression of CXCR4 is regulated by the PI3K/PTEN/AKT/mammalian target of rapamycin (mTOR) signal transduction pathway which upregulates HIF-1α and increases CXCR4 gene transcription (Phillips et al., 2005).

The *in vitro* cisplatin treatment of NSCLC cells results in enrichment of the cell population positive for CD133, a marker for CSCs. Supporting these observations, *in vivo* cisplatin treatment of NSCLC xenografts derived from primary tumors also demonstrated an enrichment of a subpopulation of CD133+/CXCR4+ cells (Bertolini et al., 2009) suggesting involvement of CXCR4 in chemotherapy resistance and recurrence of lung cancer.

Small-cell lung cancer (SCLC) accounts for nearly 13% of lung cancers. SCLC often presents with extensive spread of the disease. Although investigated in only a few patients, CXCR4 is highly expressed in all the tested cases. CXCR4 is also overexpressed in almost all SCLC cell lines. Activation of the CXCR4/CXCL12 axis induces actin polymerization and activation of MAPK signaling, leading to firm adhesion of SCLC cells to the surrounding extracellular matrix (ECM) of stromal cells (Burger et al., 2003; Sethi et al., 1999). This increased adhesive interaction between SCLC and ECM of stromal cells increases the expression of β1, α3, α6, and αv integrins, as well as increased tyrosine-kinase activity that prevents caspase activation, resulting in decreased chemotherapy-induced cell death (Sethi et al., 1999). Furthermore, integrin activation increases MMP-9 expression and enhances the invasiveness of SCLC cells (Han, Ritzenthaler, Sitaraman, & Roman, 2006). Previously, CXCR4 was known to be involved in the activation of the JAK2/STAT3

pathway (Ahr, Denizot, Robert-Hebmann, Brelot, & Biard-Piechaczyk, 2005). It was found that due to CXCR4/CXCL12 activation, STAT3 always remains phosphorylated in SCLC cells. Blocking JAK2 or CXCR4 reduces cell growth in soft agar, demonstrating the role of CXCR4 and JAK2/STAT3 signaling in adhesion-independent SCLC cell growth (Ahr et al., 2005; Pfeiffer et al., 2009).

CXCR4 antagonists reduce the tumor growth and metastatic burden in lung cancer models (Fahham et al., 2012; Jung et al., 2013). Fahham et al. (2012) reported that targeting the CXCL12/CXCR4 axis with the antagonist BKT140 attenuated NSCLC cell tumor growth and augmented the effects of chemotherapy and radiotherapy. Taken together, CXCR4/CXCL12 plays an important role in tumor growth, invasion, metastasis, chemoresistance, and relapse of lung cancer, and CXCR4 antagonists combined with cytotoxic chemotherapy may improve the therapeutic response.

4.7. Gastrointestinal cancers

CXCR4 is involved in tumor growth and metastasis in various gastrointestinal cancers, in particular colorectal, pancreatic, hepatocellular, gastric, and esophageal cancers.

Each year, more than one million people are diagnosed worldwide with colorectal cancer (CRC), which is the fourth most common cause of cancer-related death (WHO, 2012). In colorectal cancer patients, CXCR4 expression in primary tumor cells correlates with survival, metastasis, and recurrence (Kim et al., 2006). All the CRC samples stained for CXCR4 by IHC were positive with nearly 58% demonstrating strong expression (Schimanski et al., 2005). Similarly, analysis of cell lines, 100 CRC tumors and 39 liver metastases by qRT-PCR demonstrated higher CXCR4 expression in the cell lines and tumors (Kim et al., 2005). Patients with high CXCR4-expressing tumors had increased risk of local recurrence and distant metastases, lymph node involvement, as well as significantly decreased OS (median, 9 months vs 23 months; log-rank $P = 0.03$) (Kim et al., 2005). Also, CXCR4 expression was higher in the liver metastases compared to primary tumors. In 12 of 14 paired tumors and metastases, CXCR4 expression was higher in the metastases than the primary tumor (Kim et al., 2005; Schimanski et al., 2005). These observations reiterate the role of CXCR4 expression in CRC growth, recurrence, and metastasis. Studies in an animal model using CT-26, a mouse colorectal cancer cell line, revealed that CXCR4 is important for metastasis of colon cancer to liver but not involved in tissue invasion (Zeelenberg, Ruuls-Van Stalle, & Roos, 2003). Interestingly, CXCR4 surface expression levels were found to be low or absent in colon cancer cell lines *in vitro* while high expression levels were observed *in vivo* in animal models of liver metastasis (Zeelenberg et al., 2003). These findings suggested that CXCR4 expression on colon cancer cells is regulated by tumor microenvironment and isolated metastatic cells utilize CXCR4 signaling for proliferation.

Pancreatic cancer has a very poor prognosis and limited early detection options with a 5-year survival of less than 5% (ACS, 2014). In pancreatic cancer, the CXCR4/CXCL12 axis plays an important role in tumor cell proliferation, migration, and angiogenesis. Nearly 85% of pancreatic tumor samples tested were positive for CXCR4 expression. Patients with high CXCR4-expressing tumors had a worse outcome than those with low CXCR4 expression with OS: 9.7 months (95% CI: 6.0–13.4) versus 43.2 months (95% CI: 16.3–78.1), $P =$

0.0006 (Marechal et al., 2009). In another study, high CXCR4 expression in pancreatic adenocarcinoma was observed to be an independent negative prognostic biomarker (HR = 1.74; $P<0.0001$) and associated with distant relapse (HR = 2.19; $P<0.0001$) (Bachet et al., 2012, 2012). Also, a subpopulation of CSCs expressing CD133 and CXCR4 in invasive pancreatic tumors was found to be the determinant of metastasis (Hermann et al., 2007). While HIF-1α is known to be a major factor contributing to CXCR4 expression in pancreatic and other cancers, recent studies in pancreatic cancer cells and tumors demonstrated that transcription factors such as SOX9 upregulate CXCR4 expression independently of HIF-1α, which may have consequences not only for pancreatic cancer but also for other cancers such as SCLC where SOX transcription factors are known to be overexpressed (Camaj et al., 2014). CXCR4 antagonist AMD3100 significantly inhibited the proliferation, migration, and invasion of pancreatic cancer cells (Gao, Wang, Wu, Zhao, & Hu, 2010). Mori et al. (2004) reported that CXCR4 antagonist TN14003 inhibited the migration and invasion of pancreatic cancer cells. Singh, Srivastava, Bhardwaj, Owen, and Singh (2010) demonstrated that inhibition of the CXCR4/CXCL12 axis by AMD3100 arrested the pancreatic cancer cell growth and abrogated gemcitabine resistance. Ma, Hwang, Logsdon, and Ullrich, 2013 showed that AMD3100 treatment reduced tumor growth in animal models of pancreatic ductal adenocarcinoma by blocking CXCR4-dependent mast cell migration.

Hepatocellular carcinoma (HCC) is one of the most common cancers and causes 745,000 deaths each year (WHO, 2014b). Roughly, 50% of HCC tumor specimens were identified as CXCR4 positive (Xiang et al., 2009). In HCC, the expression of CXCR4 was found to be correlated with tumor progression, lymphatic metastasis, distant dissemination, and a reduced 3-year survival rate (Schimanski et al., 2006). The CXCR4/CXCL12 axis was reported to regulate angiogenesis, essential for growth and progression of HCC (Li, Gomez, & Zhang, 2007). Li et al. (2007) found a higher expression of the CXCL12 and CXCR4 in sinusoidal endothelial cells in HCC specimens than in normal liver tissues. Findings by Mavier et al. (2004) suggested that CXCR4/CXCL12 axis promotes the proliferation of oval cells and abnormal differentiation of these cells may be associated with HCC. A study by Chu et al. (2007) indicated that the CXCR4/CXCL12 axis mediates active MMP-9 and MMP-2 secretion, thereby facilitating metastasis. CXCR4 inhibition by AMD3100 in combination with sorafenib treatment was reported to inhibit HCC growth (Chen et al., 2014).

In esophageal cancer, CXCR4 expression was found to be correlated with increased lymph node and bone marrow metastases (Sasaki et al., 2008). Approximately 85% of esophageal cancer tumors are CXCR4 positive (Sasaki et al., 2009). A study by Gockel et al. (2006) showed that patients with CXCR4-expressing tumors have a lower median OS of 20 months compared to a median OS of 76 months for patients with CXCR4-negative tumors. Supporting these observations, CXCR4 gene silencing by lentivirus shRNA inhibited proliferation of the EC9706 human esophageal carcinoma cell line and reduced the growth of tumor xenografts in mouse models (Wang, Lou, Qiu, Lin, & Liang, 2013).

Gastric and stomach cancers cause 723,000 deaths every year (WHO, 2014b) and have a poor prognosis with less than 10% 5-year survival rate (Orditura et al., 2014). Positive

staining for CXCR4 was identified in 80% of the primary gastric tumor tissues (Han et al., 2014). CXCR4 expression in primary gastric carcinomas is associated with the development of peritoneal carcinomatosis and malignant ascites which contained high levels of CXCL12 (Yasumoto et al., 2006). CXCR4 expression in primary gastric tumors was positively correlated with lymph node metastasis (Ying, Xu, Zhang, Liu, & Zhu, 2012). A meta-analysis by Han et al showed that CXCR4 expression is associated with poor prognosis in gastric cancer patients. In this study, OS was found to significantly correlate with CXCR4 expression, with the HR of 2.63 (95% CI: 1.69–4.09; $P<0.0001$), and a significant association was also detected between CXCR4 expression and tumor stage (odd ratio (OR): 0.52, 95% CI: 0.32–0.83; $P = 0.007$), depth of invasion (OR: 0.44, 95% CI: 0.27–0.73; $P = 0.001$), lymph node metastasis (OR: 2.30, 95% CI: 1.57–3.36; $P<0.0001$), and vascular invasion (OR: 0.72, 95% CI: 0.53–0.98; $P = 0.04$) (Han et al., 2014). Fakhari et al. (2014) reported that *Helicobacter pylori* infection increased CXCL12 secretion by gastric epithelial cell line, upregulated CXCR4 expression in bone marrow-derived-mesenchymal stem cells, and enhanced their migration toward CXCL12 gradient. Findings by Oh et al. (2012) indicate that hypoxia upregulates CXCR4 in gastric cancer cells in a HIF-1α-dependent manner and that upregulation of CXCR4 is involved in gastric cancer cell migration and invasion. Iwanaga et al. showed that CXCR4 blockers AMD3100 and KRH3955 inhibited the growth of gastric cancer xenografts in a mouse model (Iwanaga, Iwasaki, Ohashi, Nunobe, & Iwagami, 2007; Iwanaga et al., 2012).

4.8. Renal cell carcinoma

Nearly 337,860 new cases of renal cancer are diagnosed and an estimated 143,369 related deaths are reported every year in the world (WHO, 2012). The majority of reported renal cell cancer (RCC) cases are clear cell renal cell carcinoma (ccRCC). Loss of function of the von Hippel–Lindau (VHL) tumor suppressor protein is the genetic hallmark of most ccRCCs (Zagzag et al., 2005). Native VHL targets HIF-1α for degradation under normoxic conditions. In ccRCCs with known VHL mutation, this process is suppressed due to loss of function of VHL or hypoxic conditions, leading to stabilization of HIF-1α and enhanced CXCR4 expression through interaction of HIF-1α with the promoter region of the CXCR4 gene (Staller et al., 2003; Zagzag et al., 2005). As such, CXCR4 is highly overexpressed in ccRCC. In a recent study, CXCR4 expression was found in 65.4% of ccRCC patients with 36.8% demonstrating strong immunoreactivity (Li et al., 2013). High CXCR4 expression was associated with a poor prognosis in ccRCC patients (Li et al., 2013). A significant aspect of VHL mutation is that it does not automatically drive ccRCC into metastasis or correlate with poor clinical outcome, but rather it is the CXCR4 expression that contributes to poor prognosis (Vanharanta et al., 2013). Recent studies demonstrate that genes beneficial for tumor cell survival and metastasis, such as CXCR4 and CYTIP, are selected for and induced through selective epigenetic changes in polycomb repression complex 2 (PRC2), eventually driving the metastatic phenotype of a subpopulation of VHL-mutated ccRCCs (Vanharanta et al., 2013). In essence, CXCR4 expression is an important prognostic factor in ccRCCs (Li et al., 2013). Also, in patients with known metastatic RCC, CXCR4 was shown to be expressed on circulating pan-cytokeratin-positive RCC cells (Reckamp et al., 2009). Several studies in preclinical models also confirmed that the CXCR4/CXCL12 axis regulates invasiveness, angiogenesis, and organ-specific metastasis of RCC (D'Alterio et al.,

2010; Gahan et al., 2012; Wang et al., 2012, 2009). CXCR4 expression in RCC is correlated with metastatic potential in orthotopic mouse models of human RCC xenografts (Pan et al., 2006). Abrogation of the CXCR4/CXCL12 axis through CXCR4 inhibition resulted in reduced metastasis to distant organs (Portella et al., 2013).

4.9. Melanoma

Melanoma is the malignant tumor of melanocytes, which mostly occurs on skin (Jerant, Johnson, Sheridan, & Caffrey, 2000). A study by Longo-Imedio, Longo, Trevino, Lazaro, and Sanchez-Mateos (2005) showed that CXCR4 expression by melanoma cells in primary lesions was correlated with increased tumor thickness, ulceration, higher risk of regional and distant metastases, and higher mortality rates. Scala et al. reported CXCR4 expression in 43.6% primary cutaneous melanomas of which the CXCR4 expression levels were low in 21%, moderate in 14%, and high in 8% of cases. Tumor cell CXCR4 expression correlated with an unfavorable prognosis with a median DFS of 22 months and OS of 35 months. CXCR4 expression was able to predict the prognosis for both DFS ($P = 0.0154$) and OS ($P = 0.0009$). In patients with CXCR4-positive tumors, the HRs of relapse and death, compared with patients with CXCR4-negative tumors, were 2.5 (95% CI) and 3.1 (95% CI), respectively (Scala et al., 2005).

The role of CXCR4 in lung-specific metastasis of melanoma cells was demonstrated by Murakami et al. The authors reported that murine B16 melanoma cells transduced with CXCR4 showed more than 10-fold increase in lung-specific metastasis, which was completely inhibited by T22, a small peptide CXCR4 antagonist (Murakami, Cardones, & Hwang, 2004; Murakami et al., 2002). CXCR4 and membrane-type 1 matrix metalloproteinase (MT1-MMP) were found to coordinate their activities at different stages during metastasis of melanoma cells to the lungs. CXCR4 was essential at initial phases of melanoma cell migration and homing to lungs. On the contrary, MT1-MMP was not required at the initial stage but helped subsequent dissemination and invasion of CXCR4-positive tumor cells (Bartolome et al., 2009). Similarly, metastasis of chemoresistant CXCR4+/CD133+ stem-like melanoma cells to the target organs is stimulated by secretion of CXCL12 from lymphatic vessels (Kim et al., 2010). Blockade of CXCR4 by AMD3100 coupled with cytotoxic drug dacarbazine significantly inhibited tumor growth and metastasis of melanoma compared to dacarbazine alone (Kim et al., 2010). Recently, another CXCR4 inhibitor AMD11070 was shown to abrogate melanoma cell migration significantly and more effectively than AMD3100 (O'Boyle et al., 2013).

4.10. Brain tumors

Several studies demonstrated that the CXCR4/CXCL12 axis is involved in tumor cell proliferation, angiogenesis, invasion, and metastasis in malignant gliomas as well as in other brain tumors. Brain tumor cell lines, primary tumors, and metastases have high concentrations of CXCR4 receptors compared to normal brain parenchyma (Rempel, Dudas, Ge, & Gutierrez, 2000; Rubin et al., 2003; Sehgal, Keener, Boynton, Warrick, & Murphy, 1998; Woerner, Warrington, Kung, Perry, & Rubin, 2005). Expression analysis using cDNA expression arrays revealed that CXCR4 is overexpressed in 57% of primary glioblastoma multiforme tumor tissues and in 88% of glioblastoma cell lines analyzed (Sehgal et al.,

1998). CXCR4 and CXCL12 expression varies in glioma cells with low-grade tumors expressing intermediate level of CXCR4 and CXCL12 and advanced gliomas expressing higher levels of CXCR4 (Gagliardi et al., 2014; Rempel et al., 2000). The invading regions of glioblastomas and satellite tumors, which are the primary reason for recurrence, are also known to express high levels of CXCR4 (Zagzag et al., 2008). As such, CXCR4 expression is considered a prognostic marker in gliomas. In addition, patients with CXCR4-positive glioblastoma multiforme exhibited poorer postoperative life expectancy when compared to patients with CXCR4-negative tumors.

Schuller et al. (2005) showed that 17/18 and 6/7 of desmoplastic and nodular medulloblastomas have elevated CXCR4 expression. In another study, Smith et al. (2007) demonstrated that 80% of malignant B-cells from patients with primary central nervous system lymphoma stained positive for CXCL12 and CXCR4. More than 50% of astrocytomas, regardless of grade, were shown to be positive for CXCR4 expression. More importantly, 100% of expressed CXCR4 was present in the phosphorylated state (pCXCR4/CXCR4 ratio) in grades 2–4 astrocytomas compared with only 76% in grade 1 astrocytomas suggesting that staining for CXCR4 alone may not be sufficient but staining for activated CXCR4 may provide more information regarding the contribution of the CXCR4/CXCL12 axis to the tumor biology (Woerner et al., 2005).

CXCR4 expression in brain tumors is upregulated by factors such as HIF-1α and hepatocyte growth factor (Esencay, Newcomb, & Zagzag, 2010; Zagzag et al., 2008). CD133+/CXCR4+ glioma stem-like cells mediate VEGF production and promote tumor angiogenesis (Ping et al., 2011). Taking advantage of the CXCR4 expression observed in brain tumors, Rubin et al. (2003) demonstrated that systemic administration of the CXCR4 antagonist AMD3100 inhibits intracranial growth of glioblastoma xenografts by inducing apoptosis and reducing tumor cell proliferation. Furthermore, combination treatments, incorporating CXCR4 inhibition, have resulted in a synergistic effect with cytotoxic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-based chemotherapy in glioblastoma models (Redjal, Chan, Segal, & Kung, 2006).

4.11. Soft tissue sarcomas

Soft tissue sarcomas are relatively rare cancers accounting for approximately 12,000 cases (0.7% of all cancer cases) and 4700 deaths (0.8% all cancer-related deaths) each year in the United States (ACS, 2014). Soft tissue sarcoma is one of the most therapy resistant forms of cancer. The neurofibromatosis type 1 (NF1) disorder, a genetic disorder of the nervous system, results in the development of neurofibrosarcomas called the malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs have limited therapeutic options and a cause of significant mortality in NF1 patients. Tissue microarray analysis of NF1 tumors has indicated that 94% of neurofibromas, 98% of NF1-deficient MPNSTs, and 66% of sporadic MPNST tissues showed CXCR4 immunoreactivity (Mo et al., 2013). CXCR4 expression, in association with CXCL12, forms an autocrine loop and activates the AKT/GSK-3b/β-catenin pathway, resulting in malignant transformation of cells. In addition, CXCR4 downregulation or inhibition results in cyclin D1-mediated cytostatic effects on tumor

growth (Mo et al., 2013) suggesting that inhibition of CXCR4 in these patient populations may have therapeutic benefit.

5. CXCR4 ANTAGONISTS AS THERAPEUTIC AND IMAGING AGENTS

Considering the critical role of the CXCR4/CXCL12 axis in various disease states, there is currently significant interest in the discovery and development of antagonists and imaging probes for therapeutic targeting and noninvasive monitoring of CXCR4 expression. Reports on CXCR4 and CXCL12 NMR and homology models have contributed significantly to our understanding of CXCR4–ligand interactions, thereby facilitating the development of highly specific CXCR4 inhibitors. A recent study by Wu et al. (2010) described the crystal structure of ligand-activated human CXCR4, thereby further illuminating probable receptor-ligand binding modes. This study utilized three stabilized constructs of CXCR4, expressed in baculovirus-infected *Spodoptera frugiperda* (Sf9) insect cells, for structural evaluation based on thermal stability, monodispersity, and lipid matrix diffusion. The small-molecule isothiourea derivative (IT1t, Fig. 2.2-**1**) and the 16-amino acid cyclic peptide analogue CVX15 (Fig. 2.2-**2**), both potent CXCR4 ligands, were selected based on solubility, affinity, and thermostability. IT1t was shown to interact with side chains from helices I, II, III, and VII while making no contact with helices IV, V, and VI. Nitrogen 4, or potentially nitrogen 3 (in the flipped thiourea conformation), of the isothiourea group formed a salt bridge with the Asp97 residue, thus indicating the crucial role of both N4 and N3 as also previously illustrated by the drastic reduction in binding affinity (roughly 100-fold) following methylation of only one nitrogen atom (Thoma et al., 2008). In case of CVX15, the cyclic peptide fills most of the binding cavity volume and forms a disulfide-stabilized (Cys4–Cys13) β-hairpin structure with D-Pro8-Pro9 at the extracellular turn. Hydrogen bonding is observed between the N-terminus of the peptide and residues Asp187 to Tyr190 of the CXCR4 backbone. The NaI3 group is anchored into the hydrophobic region neighboring helix V. Arg14 in particular forms hydrogen bonds with Asp262 and Tyr5. The C-terminus D-proline establishes a water-mediated interaction with Asp288 of CXCR4. Although binding modes of the small-molecule inhibitor and peptidic ligand overlap to some extent, conformational variations in CXCR4 induced by latter are more pronounced. For instance, CVX15 binding induces structural reconfiguration in the base of the receptor N-terminus as well as the extracellular regions of the helices. The binding interaction of CXCL12 to CXCR4 takes place with the RFFESH loop of CXCL12α interacting with the N-terminus of CXCR4, while the N-terminus of CXCL12 simultaneously interacts with the binding pocket comprising the transmembrane helices and the extracellular loops (Zhong et al., 2013). While this study has shed more light on CXCR4–ligand interactions, the observed variability in binding modes has further complicated the rationale design and predictability of essential structural backbones necessary for production of high-affinity CXCR4 binding ligands. Currently, the majority of CXCR4-targeted scaffolds have been derived from optimization of preexisting compounds or library hits known to have sufficient affinity for CXCR4. Numerous agents, including peptide, small-molecule and antibody-based therapeutics, and imaging probes have been developed for the monitoring of CXCR4 expression, each of which will be discussed categorically in more detail in the following sections. Most of these agents have cationic parts that can bind chiefly anionic extracellular domains of CXCR4.

Considering the large number of therapeutics and imaging agents reported, only those routinely used as therapeutics or for direct *in vivo* imaging of CXCR4 expression will be discussed. Existing CXCR4 antagonists can be broadly categorized into (1) peptide-based CXCR4 antagonists, (2) antibodies against CXCR4, and (3) LMW agents.

6. PEPTIDES AND PEPTIDOMIMETICS

6.1. CXCL12-based peptides

CXCL12 binds both CXCR4 and CXCR7 receptors and is commonly utilized in *in vitro* competition binding assays and a natural choice to derive peptides that bind to CXCR4 (Kryczek, Wei, Keller, Liu, & Zou, 2007; Sun et al., 2011). As a result, several CXCL12-derived peptides were developed as therapeutics based on known CXCR4/CXCL12 interactions. CTCE-9908, a 17-amino acid peptide analogue of CXCL12, has been shown to reduce the growth and adhesion of tumor cells as well as metastatic dissemination of cancer cells in a variety of tumor models (Kim et al., 2008; Wong & Korz, 2008). Currently, CTCE-9908 is in phase I/II clinical trial in patients with advanced solid tumors (Wong & Korz, 2008). Spiegelmers are RNA oligonucleotides in L-configuration that can be selectively designed for a protein of choice. NOX-A12 is a PEGylated spiegelmer that binds and neutralizes CXCL12 and inhibits CXCR4/CXCL12 interactions. NOX-A12 was shown to mobilize stem cells in mice and humans and chemosensitize CLL cells through disruption of CXCR4/CXCL12 interactions (Vater et al., 2013). Furthermore, combination of NOX-A12 with bendamustine and rituximab is in phase II clinical trial for the treatment of relapsed CLL (NCI, 2014).

Similarly, CXCL12-based imaging agents are beneficial to both *in vitro* evaluation as well as *in vivo* imaging applications of chemokine receptor expression. Meincke, Tiwari, Hattermann, Kalthoff, and Mentlein (2011) reported the development and evaluation of an IRDye 800CW-CXCL12 conjugate, via optical imaging, in nude mice with MCF-7 breast (high expression of CXCR4 and CXCR7) and A764 glioma (high expression of only CXCR7) tumor xenografts. Both tumors were visible from 24 to 96 h post probe injection; however, significant nonspecific uptake was also observed in the liver, brain, and bone marrow. Images obtained 96 h p.i. indicated almost complete clearance of the tracer from nonspecific organs and A764 tumor, while maintaining retention in MCF-7 tumors.

Misra et al. (2008) reported the development and biological analysis of ^{99m}Tc-labeled CXCL12 for monitoring changes in CXCR4 expression before and after myocardial infarction (MI). The pharmacokinetics of [^{99m}Tc-MAS_3]-CXCL12 were evaluated and quantified in Sprague-Dawley rats before and after experimentally induced MI. The tracer exhibited high CXCR4 specificity and affinity (2.7±0.9 nM) on the surface of living rat neonatal cardiomyocytes as well as rapid clearance within 2 h p.i. with less than 26.2±6.1% of injected dose remaining with low nonspecific uptake (<0.1%) in the liver, spleen, lung, and heart. Following MI induction, a 5-fold increase in tracer uptake was observed and correlated with increased CXCR4 expression, as indicated by confocal immunofluorescence, confirming the capability of this tracer to monitor CXCR4 expression prior to and following onset of MI. [^{99m}Tc-MAS_3]-CXCL12 was not tested in cancer models.

6.2. Synthetic peptide CXCR4 antagonists

The development of current CXCR4-targeted peptide analogues has taken place over the past 23 years, starting with the discovery of the antiviral activity of tachyplesin I and II as well as polyphemusin I and II (isolated from *Tachypleus tridentatus* and *Limulus polyphemus*, respectively) against HIV-1 (Masuda et al., 1992; Miyata et al., 1989; Morimoto et al., 1991; Nakamura et al., 1988). Numerous lower molecular weight peptide analogues (e.g., T22, TW70, and T134) have since been developed exhibiting reduced cytotoxicity levels (Nakashima et al., 1992; Tamamura et al., 1998; Waki et al., 1996). Following the discovery of TC134, a high-affinity (IC_{50} = 4 nM for CXCR4) 14 amino acid cyclic structural analogue, T140, was developed, which has since been the cornerstone for the development of various peptide analogues such as CVX15 (IC_{50} = 0.6 nM) and Ac-TZ14011 (IC_{50} = 5.2 nM) (Tamamura et al., 2003, 1998). The latter has also been heavily utilized for molecular imaging applications of CXCR4 expression utilizing optical and nuclear imaging modalities.

Numerous *in vitro* and *in vivo* preclinical studies have established the efficacy of T140 and its analogues for blocking CXCR4 thus reducing tumor growth and metastasis in breast cancer, melanoma, chronic leukemia, MM, and pancreatic cancer mouse models (Burger, 2009).

The 14-residue polypeptide 4F-benzoyl-TN14003 (BKT140), derived from a naturally occurring horseshoe crab protein, exemplifies this category of peptides. BKT140 binds CXCR4 with high specificity and affinity (Tamamura et al., 2003). Beider et al. (2011) demonstrated that BKT140 treatment can significantly reduce acute myeloid leukemia and MM xenografts in animal models, while Peled et al. (2014) have shown that BKT140 treatment induces a robust mobilization of CD34+ cells in patients with MM. BKT140 in combination with imatinib, a tyrosine-kinase inhibitor, was shown to overcome the protective effects of stroma in CML models (Fahham et al., 2012). POL6326 is another selective CXCR4 peptidomimetic antagonist developed based on protein epitope mimetics technology (DeMarco et al., 2006; Lederer et al., 2007). POL6326 is in phase II trial in MM patients (Schmitt et al., 2010).

Peptides have been used as imaging agents owing to their relatively faster clearance compared to antibodies and simplified bifunctional chelation chemistry allowing for use of various radiometals. Based on the discussed peptide scaffolds, several peptide-based CXCR4 imaging agents were investigated. The group of van Lueewen has developed both mono and multimodality imaging agents encompassing Ac-TZ14011 analogues for CXCR4 imaging. In one report by Kuil, Buckle, Yuan, et al. (2011), Ac-TZ14011 was conjugated to DTPA for In-111 labeling and SPECT imaging, as well as a Cy5.5-like dye for optical imaging (Fig. 2.2-**3**). Flow cytometry and confocal microscopy demonstrated the receptor specificity of this dual modality peptide while SPECT–CT imaging in a mouse model bearing CXCR4-positive (MIN-O) and CXCR4-negative (4T1) tumors indicated CXCR4-specific tumor uptake. Although tumor uptake (%ID/g) values were not provided, tumor-to-muscle ratios of 4.55±0.68 and 1.20±0.12 for MIN-O and 4T1 tumors, respectively, were observed indicating enhanced uptake with targeting. Bunschoten et al. (2012) reported

a ^{99m}Tc-labeled human serum albumin (HSA) nanoparticle coated with Ac-TZ14011 and the NIR dye IR783 for use in CXCR4-specific sentinel lymph node procedures. Tracer uptake in lymph nodes was confirmed by *ex vivo* detection of ^{99m}Tc-HSA and *ex vivo* fluorescence imaging of excised mouse lymph nodes, thereby illustrating the application of this probe for use in studying lymphatic drainage.

Kuil, Buckle, Oldenburg, et al. (2011) also reported the *in vivo* evaluation of dimeric and tetrameric Ac-TZ14011 analogues (Figs. 2.2-**4** and 2.2-**5**) using a multifunctional single attachment point (MSAP) label which contained DTPA for In-111 conjugation and a Cy5.5-like fluorophore for optical imaging. Although peptide multimerization was shown to reduce nonspecific muscle uptake from 0.31±0.01 to 0.08±0.02, the tumor uptake (%ID/g) in MIN-O tumor-bearing mice was fairly consistent at 1.10±0.60. Of note was the higher toxicity and lower tumor-to-muscle ratios of the tetrameric compound (7.41±1.87 for the dimer vs. 5.47±0.50 for the tetramer) compared to the dimeric analogue. Buckle et al. (2012) reported the use of the ^{111}In-labeled monomeric analogue to study the extent of CXCR4 expression in MIN-O mouse tumor models, which resemble human DCIS. Utilizing ^{111}In-cDTPA-[RGDfK], the authors aimed to study and correlate CXCR4 expression with tumor angiogenesis. While CXCR4-expression levels were distinguished using ^{111}In-Ac-TZ-14011, angiogenesis in MIN-O lesions and low CXCR4-expressing control tumors was too similar to differentiate.

A fluorine-18-labeled T140 analogue (Fig. 2.2-**6**) was reported by Jacobson, Weiss, Kiesewetter, Farber, and Chen (2010) and studied in mouse models bearing tumors of CHO cells stably transfected with CXCR4. While this tracer did exhibit some receptor-specific accumulation in the CXCR4-positive tumor, it also significantly bound to red blood cells, requiring the coinjection of cold peptide for direct *in vivo* tumor visualization. The highest tumor %ID/g obtained following this methodology was 3.03±0.31. Jacobson et al. (2011) later reported the development of a Cu-64-labeled T140 analogue which was substituted with two DOTA molecules at both lysine residues in the peptide backbone (Fig. 2.2-**7**). This tracer also suffered from the same limitation as the F-18T140 analogue and was administered with a low specific activity to circumvent red blood cell uptake. While the Cu-64-labeled peptide showed similar tumor uptake compared to the F-18 analogue, it also demonstrated higher accumulation in metabolic organs such as liver, kidneys, and intestines most likely owing to the transchelation of Cu-64 *in vivo*. Following this study, Zhang et al. (2013) reported the synthesis and biological evaluation of two F-18-labeled Ac-TC14012 (a T140 analogue) peptides in mice bearing CHO-CXCR4 and control CHO tumors. The differentiating factor between the two reported analogues was the prosthetic group used for F-18 labeling, namely fluoropropionate (Fig. 2.2-**8**) and fluorobenzoate (Fig. 2.2-**9**) groups. Analogue **6** showed superior pharmacokinetics with a tumor %ID/g of 4.30±0.86% and 0.93±0.06% in the CXCR4-positive and negative tumors, respectively. This peptide exhibited lower binding to red blood cells (0.7%), when compared to **4**, with hepatic and renal retention of 25% and 15%, respectively. Jacobson et al. also reported two ^{64}Cu-labeled T140 analogues denoted as DOTA-NFB (Fig. 2.2-**10**) and NOTA-NFB (Fig. 2.2-**11**) with very similar biodistribution as that of compound **6** (Jacobson et al., 2012).

George et al. (2014) recently reported the synthesis and biological evaluation of a ^{68}Ga-labeled TN14003 (a T140 analogue) peptide (Fig. 2.2-**12**) in CXCR4-positive and negative U87-CD4 tumor mouse models). Tracer uptake in CXCR4-positive and negative tumors was 5.5% and 1.5%, respectively. High nonspecific retention was observed in liver (35%), kidneys (35%), and gallbladder (55%).

6.3. Small cyclic peptide analogues

LMW cyclic peptides have garnered considerable attention as imaging agents owing to their faster overall clearance rates, *in vivo* stability, and low nonspecific retention. Fujii et al. (2003) were the first to report small cyclic peptide analogues of T140. Their previous study using T140 analogues indicated the four amino acids Arg2, NaI3, Tyr5, and Arg14 of T140 to be essential for intrinsic bioactivity (Tamamura et al., 2000). Based on those observations, two orthogonal peptide libraries were designed based on sequence and conformation, leading to the discovery of the cyclic pentapeptide FC131 (also known as CPCR4), which exhibited a similar bioactivity to T140. Demmer et al. were first to report the synthesis, structural optimization, and *in vivo* evaluation of CPCR4 for PET imaging (Fig. 2.2-**13**; Demmer, Gourni, Schumacher, Kessler, & Wester, 2011). In that report, CPCR4 was optimized for Ga-68 labeling and evaluated *in vivo* in nude mice harboring OH-1 human small-cell lung tumors. The tracer exhibited a 6.16±1.16% uptake 60 min post-tracer injection in the CXCR4-expressing tumor with a 16.55±3.84 tumor-to-muscle ratio. That uptake was successfully blocked (CXCR4-tumor %ID/g 1.88±0.30) with 50 μg of the cold peptide. After tumor, the highest uptake was observed in kidneys (3.06±0.63), indicating rapid clearance of the tracer from the blood pool and nonspecific organs. Surprisingly, very little uptake was observed in tissues, such as liver and spleen, known to express CXCR4. A plausible explanation for the lack of uptake in other tissues is that CPCR4 may not bind to mouse CXCR4 and may only bind human CXCR4 receptors exclusively expressed on xenografted tumor cells. Although this tracer has thus far produced the “cleanest” PET images of CXCR4 expression, it may be of limited use in evaluating the comprehensive effects of CXCR4 inhibition or expression levels in preclinical models. An additional key finding of this report was that chelation of metals to DOTA significantly enhanced the binding affinity of CPCR4 toward CXCR4 receptors with indium and gallium enhancing the binding affinity by 3.4 and 30-fold, respectively. The gallium complex had a virtually identical binding affinity to the unmodified native CPCR4 peptide.

Demmer et al. (2011) also reported the synthesis and biological evaluation of a ^{68}Ga-labeled CPCR4 dimer (Fig. 2.2-**14**) in the OH1 tumor model. This tracer exhibited lower tumor uptake (2.08±0.48%) and higher nonspecific hepatic uptake (44.31±5.56%) relative to the monomeric compound reported by Wester et al. Authors attributed the variation in the observed pharmacokinetics to the high lipophilicity of dimeric peptide species.

6.4. Antibodies against CXCR4

Monoclonal antibodies (mAbs) are gaining attention as therapeutics due to their long half-life and antigen specificity and affinity. In animal models, anti-CXCR4 antibodies markedly reduced metastasis and disease progression of breast cancer, lung cancer, lymphoma, and prostate tumors (Bertolini et al., 2002; Engl et al., 2006; Phillips et al., 2003). 12G5 is the

most commonly used anti-CXCR4 antibody. Inhibition of CXCR4/CXCL12 interaction by 12G5 showed significant impairment of tumor cell migration *in vitro* (Engl et al., 2006). Kuhne et al. (2012) developed a fully humanized antibody, MDX1338, against CXCR4 and applied it for therapy in AML, non-Hodgkin lymphoma (NHL), CLL, and MM. These authors showed that MDX1338 antibody alone effectively inhibited cancer cell proliferation and retarded tumor growth in xenograft models by antibody-induced apoptosis. Therapeutic mAb development against CXCR4 may be complicated due to conformational heterogeneity of CXCR4 (Baribaud et al., 2001).CXCR4 exhibits significant conformational heterogeneity on primary and transformed B, T, and other hematopoietic cell types. Although the exact reason behind this conformational heterogeneity is not fully understood, altered glycosylation and glycation patterns in cancer cells may partially contribute to this heterogeneity (Farzan et al., 2002). These factors may create differences or change antibody specificity to CXCR4 in cancer cells.

Optically tagged CXCR4 antibodies are currently commercially available and thus far only utilized in flow cytometry or fluorescence microscopy experiments. The use of ^{125}I-12G5 was reported by Nimmagadda, Pullambhatla, and Pomper (2009) for SPECT–CT imaging of mouse models bearing U87 tumors. Tracer uptake in the tumor was of 7% 48 h p.i. relative to the 3% uptake observed for the isotype antibody, suggesting the application of this tracer for *in vivo* SPECT imaging of CXCR4 expression.

6.5. LMW CXCR4 antagonists

In the early 1990s, a series of bicyclam analogues were developed to screen for anti-HIV activity. Among those compounds, AMD3100 was found to be a specific antagonist of CXCR4, binding to CXCR4 and inhibiting CXCR4/CXCL12 interactions with no cross-reactivity with other chemokine receptors (De Clercq, 2003; Fricker et al., 2006;Hatse, Princen, Bridger, De Clercq, & Schols, 2002). Although the initial focus of this drug development was on anti-HIV activity, soon it was found in clinical trials that AMD3100 induced leukocytosis caused by the mobilization of hematopoietic cells to the peripheral blood (Hendrix et al., 2004, 2000). Further preclinical studies and clinical trials demonstrated that AMD3100 alone and in combination with granulocyte colony-stimulating factor (G-CSF) can effectively mobilize hematopoietic cells from bone marrow (De Clercq, 2010; Devine et al., 2008; Liles et al., 2003). Therapeutic use of AMD3100 to inhibit CXCR4/CXCL12 axis in different cancer types has been mentioned in previous sections. An extensive array of CXCR4 binding LMW agents has been developed and discussed in detail by others (Mosley, Wilson, Wiseman, Skudlarek, & Liotta, 2009), and a few have been extensively validated. Ling et al. (2013) reported that the CXCR4 antagonist AMD3465 inhibited breast cancer growth and metastases by acting on tumor cells as well as immune cells that constitute the tumor microenvironment. AMD11070, an orally bioavailable CXCR4 inhibitor, was demonstrated to be more effective than AMD3100 in inhibiting melanoma tumor cell migration (O'Boyle et al., 2013). Liang et al. (2012) showed that MSX-122, a partial CXCR4 antagonist without mobilizing stem cells, blocked breast cancer and melanoma metastasis in animal models. KRH3955, a nonpeptide CXCR4 inhibitor, was shown to reduce the growth of gastric tumor xenograft in animal models (Iwanaga et al.,

2012). TG-0054, a small-molecule CXCR4 antagonist, is in phase II trial for MM, non-Hodgkin lymphoma, and Hodgkin disease (Chung et al., 2009).

LMW compounds are a primary choice as imaging agents due to their high affinity and superior pharmacokinetics. LMW imaging probes for CXCR4 expression have mainly focused on the bicyclam compound AMD3100. The first report of radiolabeling (Fig. 2.2-**15**) and *in vivo* PET imaging of AMD3100 was carried out by Jacobson, Weiss, Szajek, Farber, and Kiesewetter (2009) in C57Bl/6 mice. High accumulation of the tracer was noted in the liver (41%), kidneys (10%), as well as in the immune-related organs such as spleen (13%), bone marrow (14%), and lymph nodes (10%), 1 h post tracer injection. Blocking with unlabeled AMD3100 resulted in significant reduction of signal in the spleen, liver, lymph nodes, and bone marrow implying CXCR4-specific uptake. The remaining uptake in liver tracer retention post blocking (12%) was attributed to nonspecific tracer uptake. The exact mechanism of action for this uptake still remains to be elucidated.

Nimmagadda et al. (2010) have established the utility of ^{64}Cu-AMD3100 in imaging graded levels of CXCR4 expression in human tumor xenografts preselected for CXCR4 expression. Proof-of-principle imaging and biodistribution studies were carried out in NOD/SCID mice bearing (1) U87-stb-CXCR4 and U87 control tumors, (2) orthotopic breast MDA-MB-231 (low CXCR4) and DU4475 (high CXCR4) tumors, or (3) MDA-MB-231-derived lung metastases. In the U87 mouse model, tumor uptake was shown to be CXCR4 specific reaching 35% at 60 min p.i. in the U87-stb-CXCR4 tumors (Fig. 2.3A). Specificity for CXCR4 was confirmed using *in vivo* blocking studies with unlabeled AMD3100. Similar results were obtained in breast cancer models, indicating a tumor uptake of 6% in the DU4475 tumors compared to 2% in MDA-MB-231 tumors. In mice bearing lung metastases, ^{64}Cu-AMD3100 was able to identify metastatic nodules in the lung (Fig. 2.3B), which was further validated by flow cytometry and immunohistochemistry. This report clearly illustrated the utility of ^{64}Cu-AMD3100 for imaging of graded levels of CXCR4 expression.

A ^{99m}Tc-labeled AMD3100 analogue was reported and evaluated in mouse models bearing the human hepatocyte carcinoma cell line, Hep-G2 (Zhang, Tian, Li, Guo, & Shen, 2010). While biodistribution studies were not carried out, SPECT images indicated tumor uptake. Significant uptake was also observed in the liver, spleen, and kidneys at 60 min postinjection. More recently, Hartimath, Domanska, Walenkamp, Dierckx, and de Vries (2013) reported the evaluation of [^{99m}Tc]O_2-AMD3100 in mice bearing PC3 tumors. Tumor uptake was reported at 1.7% at 5 min post injection decreasing to 0.7% over 60 min post-tracer injection. This uptake, though low, was blocked with excess unlabeled AMD3100 confirming CXCR4-mediated uptake.

De Silva et al. (2011) reported the *in vivo* evaluation of CXCR4 expression using the AMD3100 analogue AMD3465 (Fig. 2.2-**16**) in NOD/SCID mice harboring U87-stb-CXCR4 and U87 control tumors (Fig. 2.3C). TheU87-stb-CXCR4 tumor uptake was 96.29±13.98%at 90 min p.i. compared to 4.15±0.94% in U87 control tumors at the same time point. In addition, tumor-to-muscle and tumor-to-blood ratios were 362.56±153.51 and 138.78±30.87, respectively. These values are much higher than those previously reported

for ^{64}Cu-AMD3100 (tumor to muscle: 47.36±6.93; tumor to blood: 16.93±3.40). This tracer was further validated in mice with CXCR4 expressing colorectal adenocarcinoma HT-29 tumors. The HT-29 tumor uptake was 5.62±0.90% 90 min p.i. correlating with the lower CXCR4 expression (roughly 10-fold) when compared to that in U87-stb-CXCR4. This study validated the use of ^{64}Cu-AMD3465 for *in vivo* imaging of graded levels of CXCR4 expression while showing higher tumor accumulation and tumor-to-muscle and tumor-to-blood ratios when compared to ^{64}Cu-AMD3100 in the same mouse xenografts.

7. CONCLUSION

Diverse roles of CXCR4 in different types of cancers as well as in HIV infection and other pathological states have established CXCR4 as an important target for therapeutic intervention. Interaction of cancer cells with the tumor microenvironment, which protects the malignant cells from cytotoxic chemotherapy, is becoming an attractive target for improved anticancer treatment. CXCR4 antagonists through disruption of tumor–stromal cell interactions could play a significant role in sensitizing tumor cells to chemotherapy. Existing CXCR4-targeted imaging agents can detect graded levels of CXCR4 expression and have potential for therapeutic guidance and monitoring of cancer treatment. In spite of our improved understanding of CXCR4 biology, multiple challenges still remain such as delineating the role of CXCR7–CXCL12 axis contribution to these observations, identifying chemotherapeutics that modulate CXCR4 expression and developing suitable combination treatments with existing therapies.

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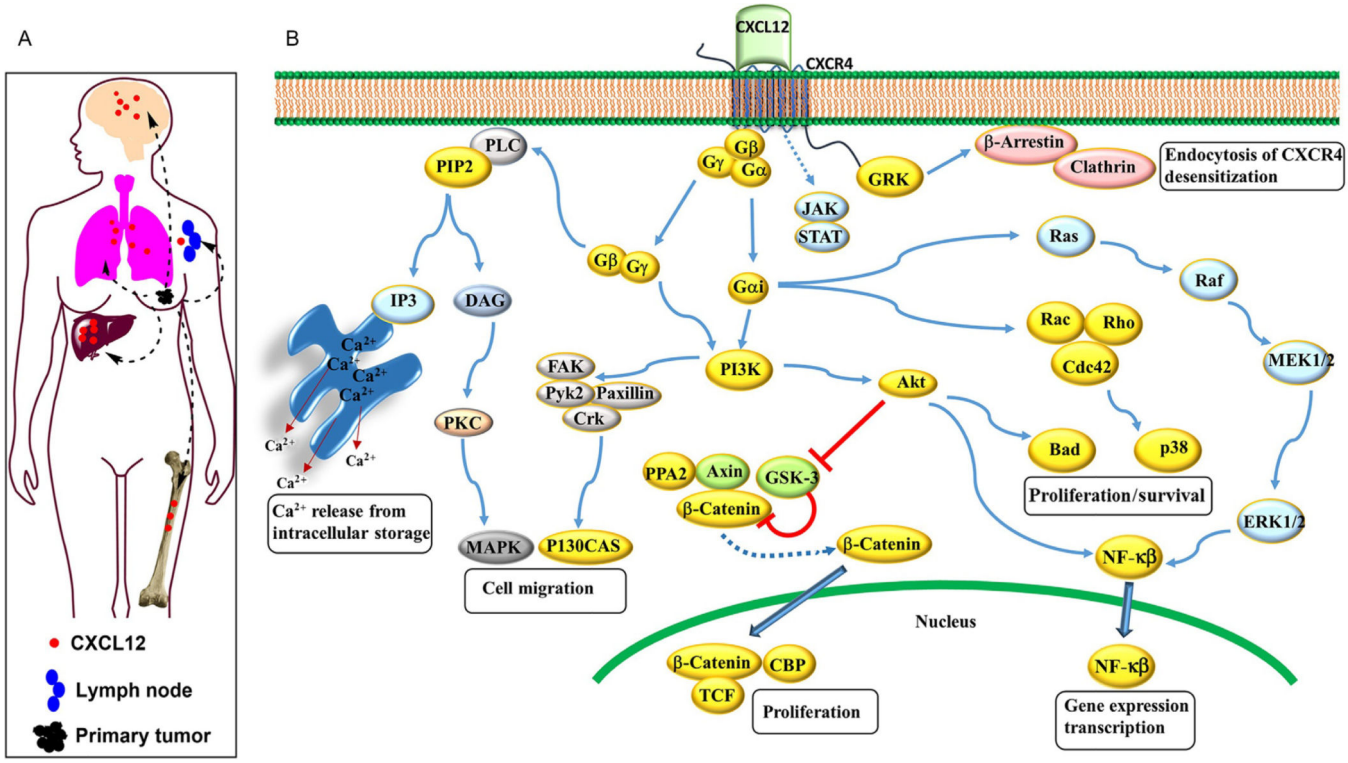

Figure 2.1.

(A) CXCL12 is highly expressed in tissues like lungs, liver, and bone marrow and is also secreted by tumor and stromal cells. CXCR4/CXCL12 interaction results in increased proliferative, migratory, and invasive properties of tumor cells that enable them to escape from primary tumors. CXCR4-expressing tumor cells migrate toward CXCL12 gradient and home to organs that release CXCL12. (B) Schematic diagram of CXCR4/CXCL12 signaling pathway.

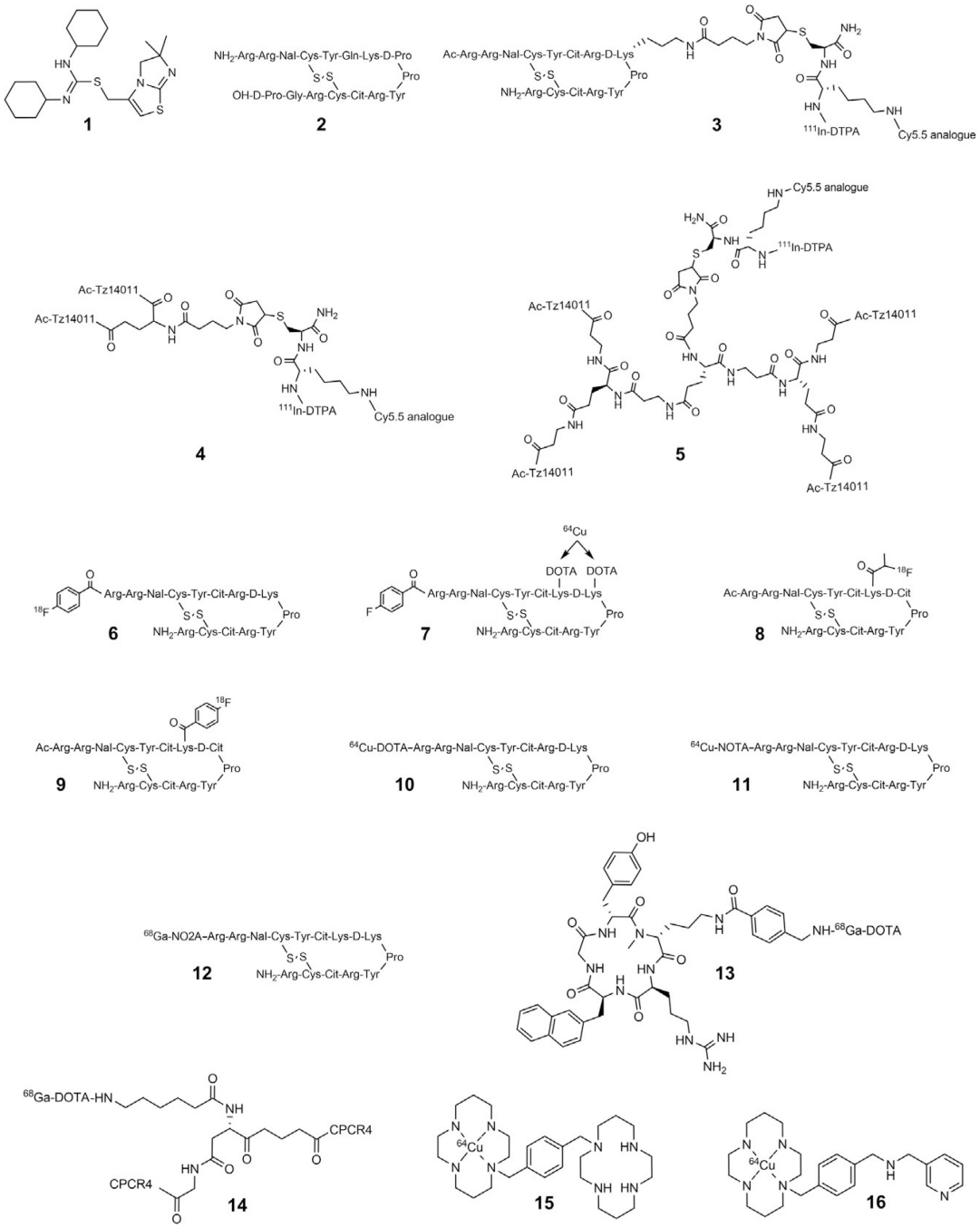

Figure 2.2.

Representative structures of CXCR4-targeted tracers employed in *in vivo* imaging applications.

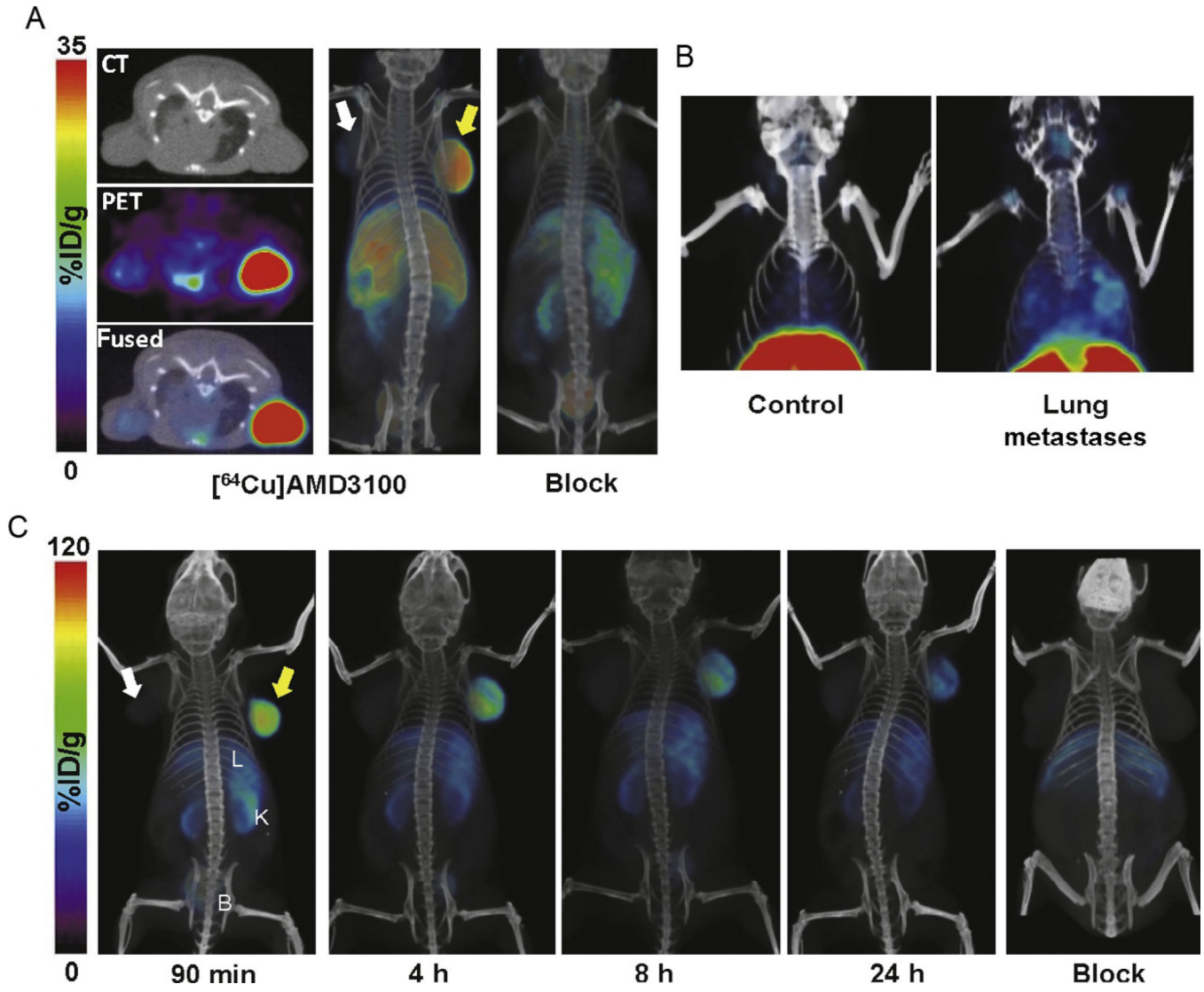

Figure 2.3.

Volume rendered whole body PET/CT images of CXCR4 expression in subcutaneous NOD–SCID mice bearing U87-stb-CXCR4 (yellow arrow) and U87 control (white arrow) tumors (A) and MDA-MB-231-derived lung metastases (B) following injection of 300 μCi of ^{64}Cu-AMD3100; U87-stb-CXCR4 (yellow arrow) and U87 control (white arrow) tumors following administration of 250 μCi of ^{64}Cu-AMD3465 or a 25-mg/kg AMD3465 blocking dose followed by 250 μCi of ^{64}Cu-AMD3465 (C). *Images (A,B) were recreated from* Nimmagadda et al. (2010) *and (C) from* De Silva et al. (2011).