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CXC Chemokines in Cancer Angiogenesis and Metastases

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Abstract

The tumor microenvironment is extremely complex that depends on tumor cell interaction with the responding host cells. Angiogenesis, or new blood vessel growth from preexisting vasculature, is a preeminent feature of successful tumor growth of all solid tumors. While a number of factors produced by both the tumor cells and host responding cells have been discovered that regulate angiogenesis, increasing evidence is growing to support the important role of CXC chemokines in this process. As a family of cytokines, the CXC chemokines are pleiotropic in their ability to regulate tumor-associated angiogenesis, as well as cancer cell metastases. In this chapter, we will discuss the disparate activity that CXC chemokines play in regulating cancer-associated angiogenesis and metastases.

I. INTRODUCTION

Angiogenesis plays a critical role in the development, growth, and metastatic potential of cancer. Chemokines are a family of small heparin-binding proteins (8–10 kDa in size) that were originally described for their role in mediating leukocyte recruitment to sites of inflammation (Charo and Ransohoff, 2006). Within the chemokine family, there are four subgroups (CXC, CC, CX₃C, and C chemokines) that are defined by the positioning of the conserved cysteines near the amino-terminus. The CXC subgroup, the focus of this review, has been shown to play a pivotal role in angiogenesis in both physiologic and pathologic settings (Keeley *et al.*, 2008; Mehrad *et al.*, 2007; Vandercappellen *et al.*, 2008). The CXC chemokine family is further categorized on the basis of the presence or absence of a three amino acid sequence, glutamic acid-leucine-arginine (called the “ELR” motif) proximal to the CXC sequence. The “ELR” motif is important since the ELR containing (ELR+) CXC chemokines are potent promoters of angiogenesis, while the interferon (IFN)-inducible, non-ELR containing (ELR–) CXC chemokines are potent inhibitors of angiogenesis (Table I) (Strieter *et al.*, 1995). In this review, we will discuss the unique role that the ELR+ and ELR – CXC chemokines play in cancer angiogenesis and metastases.

II. ANGIOGENIC CXC CHEMOKINES AND RECEPTORS

The angiogenic CXC chemokine family includes CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 (Table I). In the mouse, all ELR+ CXC chemokines signal via CXCR2, whereas in humans, ELR+ CXC chemokine ligands can signal via both CXCR2 and CXCR1 (Mehrad *et al.*, 2007). CXCR2, however, is considered the major angiogenic receptor in humans since the expression of CXCR2 alone is required for endothelial cell chemotaxis despite the fact that both CXCR1 and CXCR2 are detected on endothelial cells (Addison *et al.*, 2000; Murdoch *et al.*, 1999); and immunoneutralization of CXCR2 blocks the response of human endothelial cells to CXCL8 (Heidemann *et al.*, 2003). Lastly, while

only CXCL8 and CXCL6 bind to CXCR1, all the human ELR+ CXC chemokines mediate angiogenesis (Mehrad *et al.*, 2007).

In addition to CXCR2, a unique promiscuous, nonsignaling chemokine receptor, the red blood cell Duffy antigen for chemokines (DARC) binds CXCL1, CXCL5, and CXCL8 and is thought to function as a “decoy” for excess ELR+ CXC angiogenic chemokines, thus creating a less angiogenic environment leading to inhibition of tumor growth and metastasis (Addison *et al.*, 2004). When transfected and overexpressed in a human non-small cell cancer tumor line, and implanted into animals, the DARC-expressing tumors had greater necrosis, decreased blood vessel density, and decreased potential of metastases (Addison *et al.*, 2004); similar findings have been shown using breast cancer cell lines (Wang *et al.*, 2006a). In a transgenic adenocarcinoma mouse model of prostate cancer, DARC-knockout mice developed larger, more aggressive tumors, and the tumors had increased blood vessel density and increased levels of angiogenic ELR+ CXC chemokines compared to wild-type mice (Shen *et al.*, 2006). Moreover, in a separate study, transgenic expression of DARC by mouse endothelial cells resulted in an attenuated angiogenic response to ELR+ CXC chemokines *in vivo* (Du *et al.*, 2002). From a clinical perspective, since approximately 80% of individuals of African descent lack DARC, it has been suggested that the decreased clearance of angiogenic chemokines may be the mechanism behind their increased mortality from prostate cancer (Shen *et al.*, 2006).

III. ANGIOSTATIC CXC CHEMOKINES AND RECEPTORS

The angiostatic CXC chemokine family includes the IFN-inducible CXCL4, CXCL9, CXCL10, CXCL11, and CXCL14 (Table I). The major angiostatic receptor for CXCL9, CXCL10, and CXCL11 is a unique G protein-coupled receptor called CXCR3 (Ehlert *et al.*, 2004;Loetscher *et al.*, 1998;Luster *et al.*, 1998;Rollins, 1997).More recently, however, two novel receptors derived from alternative splicing of the CXCR3 gene product have been described. In humans, therefore, the CXCR3 receptor exists in at least three distinct mRNA splice variants: CXCR3A, CXCR3B, and CXCR3-alt (Lasagni *et al.*, 2003;Mehrad *et al.*, 2007). In one study, human microvascular endothelial cell line-1, transfected with CXCR3A or CXCR3B, was found to bind to CXCL9, CXCL10, and CXCL11; CXCL4, however, showed high affinity for CXCR3B alone (Lasagni *et al.*, 2003). These investigators concluded, therefore, that CXCR3B acts as a functional receptor for CXCL4. CXCR3-alt, which is generated by posttranscriptional exon skipping, has been shown to exhibit a reduced response to CXCL9 and CXCL10 while maintaining its signaling activity to CXCL11 (Ehlert *et al.*, 2004). The roles of the receptors CXCR3-alt and CXCR7 (a novel receptor for CXCL11 and CXCL12) (Balabanian *et al.*, 2005;Burns *et al.*, 2006) in regard to angiogenesis remain undefined.

CXCR4 is the main receptor for CXCL12, a non-ELR containing CXC chemokine. The CXCL12–CXCR4 biological axis has been associated with tumor invasion and metastases (Bachelder *et al.*, 2002; Belperio *et al.*, 2004; Pan *et al.*, 2006a; Phillips *et al.*, 2003), but its role in cancer angiogenesis is less clear. Previous studies have shown that stimulation of human umbilical vein endothelial cells (HUVEC) with VEGF or bFGF resulted in upregulation of cell surface expression of CXCR4 and increased migration of the cells toward CXCL12 (Salcedo *et al.*, 1999). In addition, the same investigators injected CXCL12, VEGF, or saline (control) subcutaneously in mice and evaluated the extent of microvessel formation (Salcedo *et al.*, 1999). They found that the mice that received subcutaneous injections of CXCL12 and VEGF had significant increase in microvessel formation within 4 days compared to those received saline control (Salcedo *et al.*, 1999). Using a different murine model in a study evaluating the function of CXCR4 during embryogenesis, investigators found that CXCR4 was expressed in developing vascular

endothelial cells and that mice lacking CXCR4 or CXCL12 had defective formation of the large vessels supplying the gastrointestinal tract (Tachibana *et al.*, 1998). Moreover, they showed that mice lacking CXCR4 or CXCL12 died *in utero* and were defective in vascular development, hematopoiesis, and cardiogenesis (Tachibana *et al.*, 1998). However, in animal models of breast, renal cell, and non-small cell lung cancer, immunoneutralization of CXCL12 or CXCR4 attenuated tumor metastases, but had no effect on the extent of angiogenesis or tumor size of the primary tumor (Muller *et al.*, 2001; Phillips *et al.*, 2003), suggesting that the CXCL12–CXCR4 biological axis mediates metastases independent of angiogenesis. These discrepant findings suggest that, although CXCL12–CXCR4 axis can mediate angiogenesis in other models, within the microenvironment of the tumor, any CXCL12 produced is most likely cleared by CXCR4 expressed by tumor cells and does not induce tumor angiogenesis.

The ELR-negative CXC chemokines, CXCL4 and CXCL14, have been shown to exhibit unique angiostatic properties. CXCL4, the original chemokine shown to inhibit angiogenesis (Maione *et al.*, 1990), signals via the receptor CXCR3. Moreover, it has been shown to inhibit angiogenesis via interaction with cell surface glycosaminoglycans or with angiogenic mediators and their receptors such as bFGF or CXCL8 (Bikfalvi and Gimenez-Gallego, 2004; Dudek *et al.*, 2003; Perollet *et al.*, 1998). CXCL4 also exists as a nonallelic gene variant, CXCL4L1, which is a very potent inhibitor of angiogenesis in both *in vitro* and *in vivo* models (Struyf *et al.*, 2004).

CXCL14 is a non-ELR CXC chemokine that was first identified in head and neck carcinoma where it was found to be downregulated in the tumor specimens (Frederick *et al.*, 2000; Hromas *et al.*, 1999; Sleeman *et al.*, 2000). Several studies support the notion that the loss or decreased expression of CXCL14 is associated with tumor formation and growth. In one study, CXCL14 was found to inhibit endothelial cell chemotaxis to CXCL8, VEGF, and bFGF *in vitro*, and to be a potent inhibitor of angiogenesis *in vivo* (Shellenberger *et al.*, 2004). In an animal model of prostate cancer, CXCL14 inhibited tumor growth when transfected into prostate cancer cells (Schwarze *et al.*, 2005). The mechanisms of action of CXCL14, as well as its receptor, remain to be elucidated.

IV. IMMUNOANGIOSTASIS

CXCR3 and its ligands contribute to antitumor defenses by two distinct mechanisms, inhibition of angiogenesis, as discussed above, and in addition, promoting Th1-dependent immunity through recruitment of CXCR3-expressing T and NK cells (Balestrieri *et al.*, 2008; Loetscher *et al.*, 1996; Luster, 1998; Qin *et al.*, 1998; Rabin *et al.*, 1999). This concept has been described as “immunoangiostasis” (Pan *et al.*, 2006b; Strieter *et al.*, 2004). In animal models of non-small cell lung cancer, investigators showed Th1 cytokine-induced cell-mediated immunity and inhibition of angiogenesis resulted in suppression of tumor growth (Hillinger *et al.*, 2003; Sharma *et al.*, 2000, 2003). In one study (Sharma *et al.*, 2000), CCL19 was shown to promote recruitment of dendritic cells and T cells and to induce a reduction in tumor size via increases in the angiostatic CXC chemokines, CXCL9 and CXCL10. In another study, the intratumor injection of CCL21 resulted in complete tumor eradication in some of the treated mice that was entirely attenuated by inhibiting CXCL9 and CXCL10, suggesting the antitumor response is secondary to CXCR3 ligands inducing a local immunoangiostatic environment (Sharma *et al.*, 2003). In a murine model of renal cell carcinoma (Tannenbaum *et al.*, 1998), IL-12 treatment resulted in regression of the tumor, however, this effect was lost when the CXCR3 ligands were depleted, thus, underscoring the importance of the CXCR3/CXCR3 ligand biology in tumorigenesis. The concept of immunoangiostasis has been demonstrated by investigators using a murine model of renal cell carcinoma (Pan *et al.*, 2006b). In this study, systemic administration of IL-2 induced the

expression of CXCR3 on circulating mononuclear cells, but impaired the CXCR3 chemotactic gradient from plasma to tumor by increasing circulating CXCR3 levels (Pan *et al.*, 2006b). Systemic IL-2 administration in CXCR3^{-/-} mice, however, did not inhibit tumor growth, suggesting that the antitumor effect of IL-2 was CXCR3-dependent. Additional experiments showed that the combined administration of systemic IL-2 with intratumor CXCL9 resulted in a significant reduction in tumor growth and angiogenesis, and an increase in tumor necrosis and intratumor infiltration of CXCR3⁺ mononuclear cells compared to treatment with IL-2 or CXCL9 alone. These results support the notion of immunoangiostasis by demonstrating the following: (1) optimization of systemic immunotherapy by combining systemic activation of mononuclear cells to express CXCR3 and enhancing the CXCR3 chemotactic gradient to promote mononuclear cell extravasation within the tumor, (2) induction of type 1 cytokine-dependent cell-mediated immunity, and (3) inhibition of tumor angiogenesis. The unique capacity to be angiostatic and to demonstrate Th1 cell-mediated immunity underscores the potential therapeutic role that the CXCR3/CXCR3 ligand axis may have in cancer.

V. CHEMOKINE-INDUCED ANGIOGENESIS IN TUMOR MODELS

Angiogenesis is essential for the growth of tumors. While much of the tumor angiogenesis research thus far has focused on the contribution of the VEGF family (Kerbel, 2008), CXC chemokine-mediated angiogenesis has been shown to play a critical role in malignancies affecting a multitude of organs including skin, pancreas, ovary, colon, stomach, lung, prostate, brain, head and neck, and kidneys. Most human malignancies express one or more of the CXC chemokine receptors including CXCR4, CXCR3, and the novel receptor for CXCL11 and CXCL12, CXCR7 (Koizumi *et al.*, 2007).

A. Melanoma

The angiogenic chemokines, CXCL1, CXCL2, and CXCL3, were originally identified from culture supernatants of melanoma cell lines (Richmond and Thomas, 1988), and are highly expressed in human melanoma (Luan *et al.*, 1997; Owen *et al.*, 1997). Sustained transgenic expression into immortalized murine melanocytes transformed their phenotype from one that did not normally form tumors, to one that formed highly vascular tumors in immunocompetent mice. These same investigators also demonstrated that depletion of CXCL1, CXCL2, or CXCL3 in the host resulted in significant reduction in angiogenesis and tumor growth (Luan *et al.*, 1997; Owen *et al.*, 1997). The chemokine receptor, CXCR2, is also responsible for the angiogenic activity mediated by these chemokines (Addison *et al.*, 2000). A point mutation in CXCR2 results in constitutive signaling which promotes preneoplastic to neoplastic cellular transformation (Burger *et al.*, 1999). Lastly, a direct correlation between levels of CXCL8 and CXCR2, and the aggressiveness of the tumor, has been found in melanoma (Varney *et al.*, 2006).

B. Pancreatic Cancer

The ELR⁺ CXC chemokines and CXCR2 have been found to play important roles in human pancreatic cancer. In one study, investigators found that Capan-1, a human pancreatic cell line, expressed the angiogenic CXC chemokines CXCL1 and CXCL8 as well as CXCR2. Moreover, they found that growth of Capan-1 cells was inhibited when anti-CXCL1 or anti-CXCL8 monoclonal antibodies were added into the culture medium (Takamori *et al.*, 2000). In a separate study using a rat corneal micropocket model, investigators showed that blockade of the chemokine receptor CXCR2 inhibited pancreatic cell-induced angiogenesis (Wente *et al.*, 2006). In this study, secreted CXC chemokine levels of CXCL3, CXCL5, and CXCL8 in the supernatant of the cell lines were analyzed by ELISA; the expression of all three CXC chemokines in the supernatant of two cell lines was confirmed (Wente *et al.*,

2006). Using the corneal micropocket assay and pelleted supernatant of all three cell lines, the investigators showed that neovascularization was induced, and the angiogenesis could be significantly inhibited by the addition of anti-CXCR2 antibody (Wente *et al.*, 2006).

In a study using an orthotopic xenograft model, investigators found that constitutive NF- κ B activity directly correlated with tumor angiogenesis, tumor growth, and metastases of human pancreatic cancer cells (Xiong *et al.*, 2004). In this study, blockade of the NF- κ B activity significantly inhibited the *in vitro* and *in vivo* expression of VEGF and CXCL8, attenuated tumor growth, and suppressed metastases. CXCL8 has also been shown to promote the proliferation of pancreatic carcinoma cells (Kamohara *et al.*, 2007).

Recently, investigators have shown that the CXC chemokine/CXCR2 axis promotes pancreatic cancer tumor-associated angiogenesis both *in vivo* and *in vitro* (Matsuo *et al.*, 2009). Specifically, the investigators prospectively collected secretin-stimulated exocrine pancreatic secretions from normal individuals and from patients with pancreatic cancer. They found that the concentrations of the ELR+ CXC chemokines were significantly higher in patients with pancreatic cancer compared to normals (Matsuo *et al.*, 2009). Moreover, *in vitro*, they found that the ELR+ CXC chemokine levels in the supernatants from several different pancreatic cancer cell lines were significantly higher than those seen in the supernatant from a human pancreatic ductal epithelial cell line (Matsuo *et al.*, 2009). Lastly, using an orthotopic nude mouse model of pancreatic cancer, they showed significant reduction in tumor volume and microvessel density following administration of anti-CXCR2 antibody (Matsuo *et al.*, 2009).

C. Ovarian Cancer

The angiogenic CXC chemokine, CXCL8, has been implicated in the biology of ovarian carcinoma. The expression of CXCL8, VEGF, and bFGF was evaluated in human ovarian cancer cell lines (Yoneda *et al.*, 1998). In this study, all cancer cell lines expressed similar levels of bFGF *in vitro*, but levels of CXCL8 and VEGF were different across the lines (ranging from high to low expression). When implanted into the peritoneum of immunocompromised mice, the high-expressing CXCL8 tumors were associated with a significant increase in mortality (all animals died within 51 days), and the expression of CXCL8 was associated with increased tumor vascularity. In the same study, VEGF and bFGF expression were not correlated with tumor vascularity or mortality, although VEGF was associated with the presence of ascites (Yoneda *et al.*, 1998). Importantly, these findings have been validated in a separate study where investigators found that the angiogenic activity of ascites fluid from patients with ovarian cancer corresponded to levels of CXCL8 (Gawrychowski *et al.*, 1998).

D. Gastrointestinal Cancer

Several angiogenic and angiostatic CXC chemokines have been studied in cancers of the gastrointestinal tract including gastric and colorectal cancers. The angiogenic CXC chemokine, CXCL5, binds to CXCR2 and its expression has been shown to correlate with late stages of gastric cancer (Park *et al.*, 2007). Another angiogenic ELR+ CXC chemokine, CXCL6, has been shown to be expressed by endothelial cells within gastrointestinal tumors and has been associated with neovascularization (Gijbbers *et al.*, 2005). In one study, prostaglandin E2 was found to induce *in vivo* tumor growth by inducing expression of the angiogenic CXC chemokine, CXCL1 (Wang *et al.*, 2006b). In another study, investigators demonstrated that high expression of CXCL12 (defined as CXCL12 positivity in 50% or more of tumor cells) in colorectal cancer cells was associated with shorter survival compared to those with low expression of CXCL12 (Akishima-Fukasawa *et al.*, 2009). Lastly, in one study using colorectal cancer cell lines, CXCL10 significantly upregulated

invasion-related properties in colorectal cancer cells by promoting matrix metalloproteinase-9 expression, adhesion to laminin, and inducing colorectal carcinoma cell migration. Their findings suggest that CXCL10 may promote progression of colorectal carcinoma (Zipin-Roitman *et al.*, 2007).

E. Bronchogenic Cancer

The angiogenic CXC chemokines CXCL5 and CXCL8 have been shown to play important roles in lung cancer angiogenesis. In a murine model of non-small cell lung cancer (human non-small cell lung cancer/SCID mouse chimera), tumor-derived CXCL8 correlated with tumorigenesis (Arenberg, 1995), and the depletion of CXCL8 in this model was associated with a greater than 40% reduction in tumor size, and a reduction in spontaneous metastases. These findings were directly correlated to decreased tumor angiogenesis (Arenberg, 1995). Other investigators have shown that non-small cell lung cancer cell lines that constitutively express CXCL8 are more virulent, and have greater angiogenic activity (Smith *et al.*, 1994; Yatsunami *et al.*, 1997). In a study of human bronchogenic carcinoma, the angiogenic CXC chemokine, CXCL8, was shown to be directly associated with tumor angiogenesis (Smith *et al.*, 1994). These investigators found that CXCL8 levels were four times higher in human tissue homogenates of non-small cell bronchogenic carcinoma compared to normal lung tissue. Moreover, functional studies using tissue homogenates of tumors demonstrated the induction of *in vitro* endothelial chemotaxis and *in vivo* corneal neovascularization; and addition of anti-CXCL8 antibodies resulted in marked attenuation of both endothelial cell chemotaxis and neovascularization (Smith *et al.*, 1994), suggesting that tumor production of CXCL8 is critical for the neovascularization necessary for the initiation and maintenance of tumor growth.

CXCL5 has also been shown to be associated with non-small cell lung cancer angiogenesis (Arenberg *et al.*, 1998). These investigators found elevated levels of CXCL5 in human specimens of non-small cell lung cancer and that these levels were strongly correlated with the vascularity of the tumor. In a SCID mouse model of human non-small cell carcinoma, these investigators showed that expression of CXCL5 in the tumors correlated with tumor growth (Arenberg *et al.*, 1998). Passive immunization of non-small cell lung cancer tumor-bearing mice with neutralizing anti-CXCL5 antibodies reduced tumor growth, tumor vascularity and metastases, but neither the *in vitro* nor *in vivo* proliferation of non-small cell lung cancer cells was affected by CXCL5 (Arenberg *et al.*, 1998). While the lack of complete tumor inhibition is likely due to a functional redundancy among the angiogenic chemokines, overall, expression of the ELR+ CXC chemokines in human non-small cell lung cancer samples correlates with worse survival (Chen *et al.*, 2003; White *et al.*, 2003).

The common receptor for the ELR+ CXC chemokines, CXCR2, is important in mediating angiogenesis. In a murine model of heterotopic and orthotopic syngeneic Lewis lung carcinoma in C57Bl/6 mice, investigators found a correlation between the expression of endogenous ELR+ CXC chemokines and tumor growth and metastatic potential of the tumors (Keane *et al.*, 2004). In addition, tumors in the CXCR2^{-/-} mice were smaller and had increased tumor necrosis, reduced vascular density, and a marked reduction in spontaneous metastases (Keane *et al.*, 2004). In a separate murine model in which Kras^{LA1} mice develop spontaneous lung adenocarcinoma via somatic activation of a KRAS allele carrying an activating mutation in codon 12, elevated levels of ELR+ CXC chemokines were found in premalignant lesions of Kras^{LA1} mice, and inhibition of CXCR2 blocked the expansion of early alveolar neoplastic lesions, and induced apoptosis of vascular endothelial cells within the alveolar lesions (Wislez *et al.*, 2006).

F. Prostate Cancer

In one study, investigators determined whether the expression of CXCL8 by human prostate cancer cells correlates with induction of angiogenesis, growth, and metastatic potential (Kim *et al.*, 2001). In this study, low and high CXCL8-producing cancer clones were isolated from the heterogeneous PC-3 human prostate cancer cell line. Titration studies showed that the PC-3 cells expressing high levels of CXCL8 were highly vascularized, rapidly growing, and had a 100% incidence of lymph node metastasis (Kim *et al.*, 2001). In a human/SCID mouse chimeric model of heterotopic prostate cancer, three human prostate cancer cell lines were examined for constitutive production of the ELR+ CXC chemokines (Moore *et al.*, 1999). These cancer lines were found to use different ELR+ CXC chemokines as angiogenic mediators: depletion of CXCL1, but not CXCL8, inhibited tumor growth and angiogenesis in some lines, and the converse occurred in other lines (Moore *et al.*, 1999). These findings suggest that different prostate cancers use different CXC chemokines to mediate tumor angiogenesis, a finding that has been described by other investigators (Kim *et al.*, 2001) as well as with other types of cancers (Chen *et al.*, 1999; Cohen *et al.*, 1995; Kitadai *et al.*, 1998; Miller *et al.*, 1998; Richards *et al.*, 1997; Singh *et al.*, 1994).

G. Glioblastoma

The hallmark of glioblastoma multiforme is the presence of marked angiogenesis. The mechanism underlying the angiogenesis is incompletely defined. A candidate tumor suppressor gene, ING4, was found to be downregulated in human glioblastoma specimens (Garkavtsev *et al.*, 2004). Specimens with the lowest expression of ING4 had the greatest growth and angiogenesis when implanted into immunocompromised mice. Inhibition of CXCL8 *in vivo*, however, markedly reduced tumor-associated angiogenesis and growth of the tumor. These findings suggest a connection between ING4 and the expression of ELR+ CXC chemokines in human cancer, and may provide a unique opportunity to target ELR+ CXC chemokine-mediated angiogenesis.

H. Head and Neck Cancer

The non-ELR CXC chemokine, CXCL14, was found to be downregulated in head and neck squamous cell carcinoma specimens as compared to normal adjacent tissue (Frederick *et al.*, 2000). Subsequently, other investigators showed that CXCL14 inhibits *in vitro* endothelial cell chemotaxis in response to CXCL8, VEGF, and bFGF, and also inhibits angiogenesis in response to these mediators *in vivo* (Shellenberger *et al.*, 2004). In addition, the angiogenic CXC chemokine, CXCL5, has been associated with increased proliferation and invasion of head and neck squamous cell carcinoma thought to be, at least in part, a function of increased angiogenesis (Miyazaki *et al.*, 2006).

I. Renal Cell Cancer

In a study of patients with metastatic renal cell carcinoma, investigators evaluated tumor specimens and plasma for levels of ELR+ CXC chemokines and expression of CXCR2 (Mestas *et al.*, 2005). They found that the proangiogenic CXCR2 ligands, CXCL1, CXCL3, CXCL5, CXCL8, as well as VEGF, were elevated in the plasma and were also expressed within the tumors. Moreover, CXCR2 was expressed on endothelial cells within the tumors. These investigators also used a model of syngeneic renal cell carcinoma in BALB/c mice (Mestas *et al.*, 2005). In the CXCR2^{-/-} mice, there was a marked reduction in tumor growth which correlated with decreased angiogenesis, increased tumor necrosis, and decreased metastatic potential. These findings suggest that CXCR2 and its ligands play an important role in renal cell carcinoma-associated angiogenesis and tumorigenesis.

VI. CHEMOKINES AFFECT ON CANCER METASTASES

The word “metastasis” (from the Greek for “displacement”) refers to the migration of malignant cells to areas distant from the primary tumor. Tumor metastasis is an organized, organ-specific process that occurs in a stepwise fashion: (1) malignant cells are released from the primary tumor, (2) the released malignant cells invade blood vessels or lymphatics and are transported to the capillary bed of a distant organ, and (3) the malignant cells travel from the circulation to the organ parenchyma of the distant site and proliferate (Chambers *et al.*, 2002; Geiger and Peeper, 2009). Since the vast majority of cancer deaths occur due to metastasis of the tumor, rather than growth of the primary tumor, understanding this multistep process is critical in cancer biology and treatment (Kruizinga *et al.*, 2009; Leber and Efferth, 2009).

In addition to mediating cellular migration, chemokines and their receptors have been shown to affect many cellular functions including survival, adhesion, invasion, proliferation, and circulating chemokine levels. A growing body of evidence supports a chemokine-mediated mechanism for the metastatic spread of tumor cells: *in vitro* and *in vivo* models have shown that chemokines regulate tumor-associated angiogenesis (a prerequisite for metastasis), activate host tumor-specific immunologic responses, and direct tumor cell proliferation in an autocrine fashion (Ben-Baruch, 2009; Gerber *et al.*, 2009; Kruizinga *et al.*, 2009).

A. The CXCL12–CXCR4 Axis in Mediating Homing of Metastases

The ELR– CXC chemokine, CXCL12, plays an important role in stem cell motility (Hattori *et al.*, 2001) as well as tumor invasion (Chu *et al.*, 2007). While distinguishing the angiogenic activity of a chemokine from its metastatic effect may be difficult in some experimental systems, it is generally agreed that the CXCL12–CXCR4 axis plays a critical role in tumor metastases. Moreover, investigators have shown that, *in vivo*, CXCR4 is upregulated in tumor cells by the presence of hypoxia via hypoxia-inducible factor-1 α (HIF-1 α) (Schioppa *et al.*, 2003; Schutyser *et al.*, 2007).

The CXCL12–CXCR4 axis has been shown to be a critical factor in cancer biology in that it promotes the migration of tumor cells into metastatic sites. In fact, CXCR4 is the most common chemokine receptor that has been shown to be overexpressed in human cancer (Koizumi *et al.*, 2007). The increased expression of CXCR4 has been associated with increased metastatic potential and poor prognosis in many solid tumors, including esophageal cancer (Kaifi *et al.*, 2005; Sasaki *et al.*, 2009; Wang *et al.*, 2009), colorectal cancer (Kim *et al.*, 2005; Matsusue *et al.*, 2009; Mongan *et al.*, 2009; Speetjens *et al.*, 2009), non-small cell lung cancer (Belperio *et al.*, 2004; Oonakahara *et al.*, 2004; Phillips *et al.*, 2003; Wagner *et al.*, 2009), melanoma (Murakami *et al.*, 2004; Scala *et al.*, 2005), breast cancer (Kato *et al.*, 2003; Muller *et al.*, 2001; Smith *et al.*, 2004), ovarian cancer (Scotton *et al.*, 2002), prostate cancer (Taichman *et al.*, 2002), pancreatic cancer (Saur *et al.*, 2005), neuroblastoma (Geminder *et al.*, 2001; Russell *et al.*, 2004), osteosarcoma (Oda *et al.*, 2006), renal cell cancer (Pan *et al.*, 2006a), and gastric cancer (Yasumoto *et al.*, 2006).

CXCL4L1, a variant of CXCL4 isolated from thrombin-stimulated platelets, has been shown to be a more potent inhibitor of endothelial cell chemotaxis compared to CXCL4 *in vitro*, and more effective than CXCL4 in inhibiting bFGF-induced angiogenesis in rat corneas (Struyf *et al.*, 2004). In a separate study using different tumor models of melanoma (B16 melanoma orthotopically propagated in C57Bl/6 mice) and lung carcinoma (A549 adenocarcinoma and Lewis lung carcinoma cell lines orthotopically propagated in C57Bl/6 and SCID mice), the same investigators showed that CXCL4L1 is a more potent inhibitor of tumor growth and metastasis than CXCL4 (Struyf *et al.*, 2007). They also demonstrated that while CXCL4L1 was more potent than CXCL10 in preventing tumor metastasis in

immunocompromised mice, it had equal antitumoral activity as CXCL9 in immunocompetent mice (Struyf *et al.*, 2007). Together these data support the contention that CXCL4L1 is a highly potent antitumoral chemokine, and that it prevents the development and metastasis of tumors through its potent antiangiogenic properties.

B. CXCR7, A Novel Receptor for CXCL11 and CXCL12

While CXCL12 primarily binds to CXCR4, and CXCL11 to CXCR3, a novel receptor, CXCR7, has also been identified (Burns *et al.*, 2006). CXCR7 can regulate CXCL12-mediated migratory cues and may play a critical role in tumor cell metastases and tissue invasion (Zabel *et al.*, 2009). In a study of heterotopic transfer of human breast cancer cell line into SCID mice, and lung cancer cell line into immunocompetent mice, investigators sought to determine the role of CXCR7 in the growth of these tumors (Miao *et al.*, 2007). Using a combination of overexpression and RNA interference, they showed that CXCR7 was expressed on breast and lung cancer cell lines and promoted growth of both tumors (Miao *et al.*, 2007). In addition, they found that the expression of CXCR7 on breast cancer cells enhanced the ability of the cells to seed and proliferate in the lung, a common site of metastatic breast cancer (Miao *et al.*, 2007). Lastly, in another study using high-density tissue microarrays constructed from clinical samples from patients undergoing radical prostatectomy, CXCR7 expression was increased in tumors that were more aggressive and with increasing tumor grade (Wang *et al.*, 2008).

VII. CONCLUSION

The CXC chemokines and their receptors play a critical role in tumor angiogenesis, growth, aggressiveness, and ultimately metastasis. Understanding the mechanisms through which they work may lead to novel therapies for a wide range of human malignancies.

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Table I**CXC Chemokine Ligands and Receptors Involved in Cancer Angiogenesis and Metastasis**

Systemic name	Human ligand	Receptor	Promotes angiogenesis	Promotes metastasis
<i>ELR+ CXC chemokines</i>				
CXCL1	Gro- α	CXCR2	+	+
CXCL2	Gro- β	CXCR2	+	+
CXCL3	Gro- γ	CXCR2	+	+
CXCL5	ENA-78	CXCR2	+	+
CXCL6	GCP-2	CXCR1/CXCR2	+	+
CXCL7	NAP-2	CXCR2	+	?
CXCL8	IL-8	CXCR1/CXCR2	+	+
<i>ELR- CXC chemokines</i>				
CXCL4	PF-4	CXCR3	–	?
CXCL4L1	PF-4var	?	–	–
CXCL9	Mig	CXCR3	–	?
CXCL10	IP-10	CXCR3	–	–
CXCL11	I-TAC	CXCR3/CXCR7	–	?
CXCL12	SDF-1	CXCR4/CXCR7	?	+
CXCL14	BRAK	?	–	?