Published in final edited form as:
Exp Cell Res. 2011 March 10; 317(5): 685–690. doi:10.1016/j.yexcr.2010.10.020.

Chemokines as mediators of tumor angiogenesis and neovascularization

Ellen C. Keeley, M.D.[a], **Borna Mehrad, M.D.**[b], and **Robert M. Strieter, M.D.**[b]

[a]Department of Medicine, Division of Cardiology, University of Virginia, Charlottesville, Virginia

[b]Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of Virginia, Charlottesville, Virginia

Abstract

Chemokines are a superfamily of structurally homologous heparin-binding proteins that influence tumor growth and metastasis. Several members of the CXC and CC chemokine families are potent inducers of neovascularization, whereas a subset of the CXC chemokines are potent inhibitors. In this paper, we review the current literature regarding the role of chemokines as mediators of tumor angiogenesis and neovascularization.

Introduction

Neovascularization is essential to tumor growth and progression, and is a general term that incorporates three forms of new blood vessel growth: vasculogenesis (defined as de novo formation of a capillary plexus by endothelial progenitor cells), angiogenesis (defined as formation of a new capillary network from pre-existing capillaries), and arteriogenesis (defined as formation of arteries) (1). Chemokines, a superfamily of structurally homologous 8–10 kDa heparin-binding cytokine molecules, are critical mediators of neovascularization in many physiologic and pathologic states (2,3). In this review, we discuss the current literature regarding the role of chemokines as mediators of tumor angiogenesis and neovascularization.

Chemokine nomenclature

Structurally, chemokines are grouped into 4 families (designated CC, CXC, C, and CX_3C) based on the location of conserved cysteine residues near their amino-terminus (4). Most of the chemokines belong to the CC and CXC subgroups. In the CC subgroup, the first two cysteine residues are adjacent, whereas in the CXC subgroup the first 2 cysteine residues are separated by a non-conserved amino acid, constituting the Cys-X-Cys or ‘CXC’ motif. The CXC chemokine ligands are further classified on the basis of the presence or absence of another 3 amino acid sequence, glutamic acid-leucine-arginine (the ‘ELR’ motif), immediately proximal to the CXC sequence. The ELR-positive CXC chemokines and several of the CC chemokines are potent *promoters* of neovascularization, whereas the

Correspondence to: Robert M. Strieter, MD, University of Virginia, Department of Medicine, Division of Pulmonary Critical Care, Box 800466, Charlottesville, Virginia 22908-0466, Tel. 434-982-6999, Fax. 434-243-0399, Strieter@Virginia.edu.

interferon-inducible subset of the ELR-negative CXC chemokines are potent *inhibitors* of neovascularization (2) (Table).

Chemokine ligands that promote neovascularization

ELR-positive CXC chemokines

The ELR-positive CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 (Table) promote the migration and proliferation of endothelial cells and are potent promoters of neovascularization. All ELR-positive CXC chemokine ligands in the mouse signal via the receptor, CXCR2, whereas in humans they signal through CXCR2 and CXCR1. The CXCR2 receptor is the primary receptor for angiogenesis in humans for multiple reasons: First, all the ELR-positive CXC chemokines in humans bind CXCR2 and can mediate neovascularization, whereas only CXCL8 and CXCL6 bind to CXCR1. Second, while CXCR1 and CXCR2 are both expressed by endothelial cells, only CXCR2 is required for endothelial cell chemotaxis (5,6). Third, when the function of CXCR2 is blocked, the response of endothelial cells to CXCL8 is negated (7).

CXCR2 plays an integral role in mediating neovascularization. In one study using the cornea micropocket model in CXCR2+/+ and CXCR2 knockout mice, investigators showed that ELR-positive CXC chemokine-mediated angiogenesis was inhibited in the CXCR2 knockout mice, and in the presence of CXCR2 neutralizing antibodies (5). CXCR2 and the ELR-positive CXC chemokine ligands have also been shown to mediate homing of circulating endothelial progenitor cells to sites of arterial injury (8,9). In addition to CXCR2, the Duffy antigen receptor for chemokines (DARC) has also been shown to modulate the angiogenic effects of the ELR-positive CXC chemokines. This receptor binds chemokines in the absence of detectable signal transduction events (10,11), and acts as a decoy to inhibit angiogenesis by sequestering the ELR-positive CXC chemokines. In one study using transgenic mice expressing mDuffy under the control of the preproendothelin promoter/enhancer which directs expression of the transgene to the endothelium, transgenic expression of DARC resulted in decreased angiogenic response of the animals to ELR-positive CXC chemokines in vivo (12). In a mouse model of prostate cancer, animals on a DARC-deficient background developed larger and more aggressive tumors with greater tumor-associated neovascularization and increased intra-tumor levels of angiogenic ELR-positive CXC chemokines (13). Lastly, in a human non-small cell lung cancer (NSCLC) tumor cell line, over-expression of DARC resulted in binding of angiogenic ELR-positive CXC chemokines by the tumor cells and a marked decrease in tumor-mediated angiogenesis and metastases (10).

The CC chemokines

In addition to the CXC chemokine family, 3 members of the CC chemokine family, CCL2, CCL11 and CCL16, have also been implicated in neovascularization. CCL2 is the best-described CC chemokine mediator of neovascularization. It has been shown that endothelial cells express CCR2, the receptor for CCL2, and demonstrate chemotaxis and tube formation in response to CCL2 *in vitro* (14–16). Moreover, CCL2-mediated neovascularization has also been shown *in vivo* (17–19). CCL2-mediated angiogenesis, however, appears to be dependent on membrane type 1-matrix metalloproteinase (MT1-MMP): *in vivo* and *in vitro* angiogenesis induced by CCL2 is decreased in the absence of MT1-MMP activity (14). The angiogenic effect of CCL2 appears to be independent of its effects on leukocyte chemotaxis and is mediated via direct effects on the vascular endothelium (19). While most of the above mentioned studies have reported the important role CCL2 plays as a mediator of angiogenesis in general, only a few have reported its effect in tumor angiogenesis (15,19). In one study, CCL2 was shown to mediate hemangioma growth and angiogenesis, and

inhibition of CCL2 resulted in markedly reduced hemangioma size (15). In another study, CCL2 supernatant from a human breast cancer cell line demonstrated the production of CCL2, and Treatment of immunodeficient mice bearing human breast carcinoma cells with a neutralizing antibody to MCP-1 resulted in significant increases in survival and inhibition of the growth of lung micrometastases.

Moreover, CCL2 may also mediate homing of endothelial progenitor cells to sites of vascular injury (20). Lastly, *in vivo* CCL2-induced angiogenesis has been associated with both the induction of vascular endothelial growth factor (VEGF)-A gene expression (21), and the transcription factor, MCP-1 induced protein (22). In addition to CCL2, investigators have shown that CCL11 promotes angiogenesis using several different models including a chick chorioallantoic membrane assay, a Matrigel plug assay, and an ex vivo rat aortic ring-sprouting assay (23). Similarly, in a study of human endothelial cells, CCL16 was shown to induce endothelial cell motility by interacting with CCR1, and promoted angiogenic activity in vitro and in vivo (24).

Chemokine ligands that inhibit neovascularization and the CXCR3 receptor

CXCL4, the first chemokine shown to block angiogenesis (25), is a potent inhibitor of endothelial cell chemotaxis and proliferation in vitro, and has been shown to inhibit the angiogenic effect of VEGF and basic fibroblast growth factor (bFGF) (26). In addition, the interferon-inducible ELR-negative CXC chemokines, CXCL9, CXCL10, and CXCL11, are potent inhibitors of angiogenesis in response to the ELR-positive CXC chemokines, VEGF and bFGF. The angiostatic effect of CXCL4, CXCL9, CXCL10, and a CXCL11 appears to be mediated via a common receptor, CXCR3 (27). CXCR3, originally identified on murine endothelial cells (28) exists in three different forms (CXCR3A, CXCR3B, and CXCR3-alt), which are generated from alternative mRNA splicing of a single gene product. The expression of CXCR3A is strongly induced by interleukin (IL)-2, and it is primarily responsible for recruitment of leukocytes (29–35). CXCR3B is the main angiostatic variant of CXCR3 and is expressed on endothelial cells (27,36,37). Lastly, CXCR3-alt, has been shown to have greater affinity for CXCL11 as compared to CXCL9 or CXCL10, but its role in angiogenesis is unclear (38). CXCR3B is the main angiostatic receptor for CXCL4, CXCL9, CXCL10, and CXCL11 (Table) (39,40).

The CXCR3 ligands mediate two distinct functions; they inhibit angiogenesis, and promote Th1-type cell mediated immunity via recruitment of CXCR3-expressing T and NK cells (30,35,41). The local production of IFN-γ at sites of inflammation promotes further expression of CXCL9, CXCL10, and CXCL11, and recruits CXCR3-expressing cells that produce more IFN-γ. These combined effects, which we have described as “immunoangiostasis”, can benefit the host in the context of anti-tumor immunity (42,43). For example, systemic IL-2 therapy in the context of renal cell carcinoma was shown to be effective and dependent on CXCR3: the therapy resulted in up-regulation of CXCR3 on peripheral blood mononuclear cells, but the down-regulation of its ligands within the tumor (42). Moreover, when systemic administration of IL-2 was combined with over-expression of CXCL9 in the tumor, the anti-tumor effects were increased by augmenting the homing of IFN-γ producing leukocytes to the tumor microenvironment, inhibiting tumor-associated angiogenesis, and enhancing immune responses against tumor antigens (42). A similar mechanism has been noted in IL-12-mediated regression of a mouse model of renal cell carcinoma (44), and in NSCLC (45,46). In addition to a reduction in angiogenesis, intratumoral injection of a recombinant CC chemokine, CCL21, induced tumor regression in immunocompetent mice, but not immunosuppressed mice suggesting that T cell immunity was required for the anti-tumor effect of CCL21 (45). Moreover, this was associated with

intra-tumor generation of IFN-γ, CXCL9 and CXCL10, and depletion studies demonstrated that IFN-γ, CXCL9, and CXCL10 attenuated the anti-tumor effects of CCL21.

In addition to binding CXCR3, both CXCL4 and CXCL10 ligands also bind to extra-cellular glycosaminoglycans. To determine whether the angiostatic properties of these ligands were mediated via this mechanism studies were performed using CXCL4 and CXCL10 variants with mutated binding sites for CXCR3 or glycosaminoglycans. The angiostatic activity of CXCL4 was retained in cells that lacked surface heparin sulfate, and the CXCL4 mutants that lacked heparin-affinity were capable of inhibiting angiogenesis indicating that glycosaminoglycans are not essential for angiostasis (47–49). Similarly, when CXCL10 variants with mutated binding sites for CXCR3 or glycosaminoglycans were transfected into a human melanoma cell line, wild-type CXCL10 and CXCL10 mutants with partial or complete loss of glycosaminoglycan binding promoted significant reduction in tumor growth compared to control vector-transfected tumor cells, whereas tranfectants expressing mutants with loss of the CXCR3 binding domain did not inhibit tumor growth (50).

A non-allelic variant of CXCL4 called CXCL4L1, differs from CXCL4 in 3 amino acids in the heparin-binding domain near the carboxy terminus (51). CXCL4L1 protein has been isolated from the α-granules of thrombin-activated human platelets (52), and has been shown to be substantially more potent than CXCL4 in inhibiting human microvascular endothelial cell chemotaxis induced by bFGF and CXCL8, and bFGF- and CXCL8-induced angiogenesis in the rat corneal micropocket model (52,53). This variant has also been shown to be more efficient than CXCL4 in inhibiting tumor-associated angiogenesis in B16 melanoma and A549 lung adenocarcinoma in immunocompromised mice (53).

The CXC chemokine ligand CXCL12 and its receptor, CXCR4, are critical to the homing of progenitor cells in many disease states. In cancer, however, while CXCR4 is expressed by tumor lines and primary cancer cells, CXCL12 is not (54–56). Moreover, depletion of CXCL12 or CXCR4 does not affect tumor size or extent of primary tumor-associated angiogenesis (50,57,58). Nevertheless, depletion of CXCL12 or CXCR4 has been shown to be associated with decreased metastases in animal models of breast and lung cancer (55,56), suggesting that the CXCL12-CXCR4 ligand-receptor pair regulates metastases independent of angiogenesis.

CXC chemokine-mediated angiogenesis and malignancy

CXC chemokine-mediated angiogenesis has been shown to play a critical role in the growth of many malignancies including lung, colorectal, pancreatic, ovarian, prostate, melanoma, brain, and renal cell. It has been shown that NSCLC cell lines that constitutively express high levels of CXCL8 have greater angiogenic activity in mice (58,59); and when pro-angiogenic CXC chemokines are neutralized, angiogenic activity is decreased, and tumor growth and metastases are reduced (57). In a syngeneic tumor model of lung cancer, CXCR2 knockout mice had decreased tumor growth, increased necrosis, and decreased angiogenesis and metastases compared to wildtype mice (11). Similarly, in a $Kras^{LA1}$ mouse model in which mice develop lung adenocarcinoma due to somatic activation of the *KRAS* oncogene, neutralization of the CXCR2 receptor inhibited tumor development and apoptosis within the tumor (60).

In humans, the ELR-positive CXC chemokines CXCL5 and CXCL8 play an important role in NSCLC. In one study using a SCID mouse model, human NSCLC tumor-derived CXCL8 levels were directly related to the extent of angiogenesis; when CXCL8 was depleted, however, there was a significant reduction in tumor size, tumor-induced angiogenesis, and metastases (57). Moreover, a direct relationship between tissue levels of CXCL5 in surgical specimens of NSCLC and the extent of capillary density consistent with tumor angiogenesis

(61), and clinical outcomes, including mortality has been reported (61,62). While a significant correlation exists between CXCL5 and tumor-derived angiogenesis, tumor growth, and metastases, CXCL5 depletion does not completely inhibit tumor growth (57). This is thought to be due to functional redundancy between angiogenic ligands (63). Lastly, in a study evaluating the ELR-negative CXC chemokine CXCL10, investigators used a SCID mouse model of NSCLC and administered continuous intratumor injections of low dose, recombinant human CXCL10 (100ng every other day) which resulted in decreased angiogenesis in the primary tumor, delayed occurrence of metastases, and improved survival (64).

The ELR-positive CXC chemokines have also been studied in human gastrointestinal cancers including pancreatic and colorectal malignancies. In colorectal cancer, in vivo tumor growth is also induced by increased expression of CXCL1 (65). Human pancreatic cancer cell lines secrete the ELR-positive angiogenic CXC chemokines CXCL1 and CXCL8 (66), but their expression differs across the different cell lines (63). When the different cancer cell lines were compared using the corneal micropocket model, tumor-induced angiogenesis was inhibited by blocking the receptor, CXCR2 in one cancer cell line, but not another; supporting the concept of redundancy of angiogenic ligands, even within specific cancers (63).

In one study of human ovarian cancer cell lines, in vitro expression of CXCL8 correlated with increased tumor neovascularization (67). Importantly, when the tumors were implanted into the peritoneum of immunocompromised mice, the mice had increased mortality rates (67). In this same study, the expression of VEGF correlated with ascites production, however, it was not associated with either the extent of angiogenesis or with mortality rates (67). Interestingly, in a separate study, the angiogenic potential of ascites fluid from patients with ovarian cancer was directly correlated with CXCL8 levels (68).

In human prostate cancer, tumorigenesis and metastases correlate with the degree of tumor-associated angiogenesis (69,70). In one study, angiogenesis was measured by quantitating microvessels in 67 patients (23 with non-malignant biopsy specimens, and 34 with malignant specimens) who had undergone prostatectomy (70). Angiogenic activity in prostatic cancer tissue was correlated with pathological staging, and there appeared to be a trend of increasing microvessel count from benign through the advancing stages of prostate cancer.

Moreover, others have shown that the expression of CXCL8 in human prostate cancer cells is associated with tumorigenicity, neovascularization, and lymph node metastases (71). In a SCID mouse model of human prostate cancer, different prostate cancer cell lines were found to use different ELR-positive CXC chemokine ligands: depletion of CXCL1 but not CXCL8 inhibited tumor-related angiogenesis in some cell lines, whereas the depletion of CXCL8 but not CXCL1 inhibited angiogenesis in other lines (72).

Glioblastoma multiforme tumors are also associated with marked angiogenesis (73,74). While the mechanisms responsible with their increased growth and marked angiogenesis remain to be fully defined, a tumor suppressor gene appears to be important and is associated with the expression of angiogenic ELR-positive CXC chemokines in this disease. In one study, a candidate tumor suppressor gene was found to be down-regulated in human glioblastoma specimens compared with normal brain tissue (74). When implanted into immunocompromised mice, the specimens with the lowest expression of the tumor suppressor gene had the largest growth and degree of angiogenesis. The mechanism for this increased tumorigenicity was found to be CXCL8-dependent; inhibition of CXCL8 in vivo markedly reduced their tumor growth and tumor-associated angiogenesis.

The importance of CXCR2 and CXCR2 ligands in tumor-associated angiogenesis and tumorigenesis has been shown in malignant melanoma and renal cell carconoma. In one study, the angiogenic ELR-positive CXC chemokines, CXCL1, CXCL2 and CXCL3 were shown to be highly expressed in patients with malignant melanoma (75). Similarly, in a study of patients with metastatic renal cell carcinoma, plasma samples were assessed for levels of CXCR2 ligands and tumor biopsy samples were assessed for the expression of CXCR2 (76). CXCL1, CXCL3, CXCL5, CXCL8 and VEGF were all elevated in the plasma of the patients, and CXCR2 was expressed on endothelial cells within the tumors. Moreover, using a model of syngeneic renal cell carcinoma, these investigators showed that CXCR2 ligand expression increased in correlation to tumor growth whereas there was a significant reduction in growth in the CXCR2−/− mice which correlated with decreased angiogenesis and necrosis (76). Lastly, in the absence of CXCR2, the orthotopic tumors had a decreased potential to metastasize to the lungs of CXCR2−/− mice.

Conclusion

The angiogenic and angiostatic chemokines are critical mediators of tumor angiogenesis and neovascularization. Future research regarding their role in malignancy may lead to novel therapeutic applications.

Acknowledgments

Sources of funding: This work was supported by the National Institutes of Health [HL97074 to E.C.K., HL73848 to B.M., and CA87879, HL66027 and HL98526 to R.M.S.].

References

1. Silvestre JS, Mallat Z, Tedgui A, Levy BI. Post-ischaemic neovascularization and inflammation. Cardiovasc Res. 2008; 78:242–249. [PubMed: 18252762]
2. Mehrad B, Keane MP, Strieter RM. Chemokines as mediators of angiogenesis. Thromb Haemost. 2007; 97:755–762. [PubMed: 17479186]
3. Keeley EC, Mehrad B, Strieter RM. Chemokines as mediators of neovascularization. Arterioscler Thromb Vasc Biol. 2008; 28:1928–1936. [PubMed: 18757292]
4. Chemokine/chemokine receptor nomenclature. Cytokine. 2003; 21:48–49. [PubMed: 12668160]
5. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, Buechi L, Walz A, Richmond A, Strieter RM. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. J Immunol. 2000; 165:5269–5277. [PubMed: 11046061]
6. Murdoch C, Monk PN, Finn A. Cxc chemokine receptor expression on human endothelial cells. Cytokine. 1999; 11:704–712. [PubMed: 10479407]
7. Heidemann J, Ogawa H, Dwinell MB, Rafiee P, Maaser C, Gockel HR, Otterson MF, Ota DM, Lugering N, Domschke W, Binion DG. Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. J Biol Chem. 2003; 278:8508–8515. [PubMed: 12496258]
8. Hristov M, Zernecke A, Bidzhekov K, Liehn EA, Shagdarsuren E, Ludwig A, Weber C. Importance of CXC chemokine receptor 2 in the homing of human peripheral blood endothelial progenitor cells to sites of arterial injury. Circ Res. 2007; 100:590–597. [PubMed: 17272812]
9. Kocher AA, Schuster MD, Bonaros N, Lietz K, Xiang G, Martens TP, Kurlansky PA, Sondermeijer H, Witkowski P, Boyle A, Homma S, Wang SF, Itescu S. Myocardial homing and neovascularization by human bone marrow angioblasts is regulated by IL-8/Gro CXC chemokines. J Mol Cell Cardiol. 2006; 40:455–464. [PubMed: 16438981]
10. Addison CL, Belperio JA, Burdick MD, Strieter RM. Overexpression of the duffy antigen receptor for chemokines (DARC) by NSCLC tumor cells results in increased tumor necrosis. BMC Cancer. 2004; 4:28. [PubMed: 15214968]

11. Keane MP, Belperio JA, Xue YY, Burdick MD, Strieter RM. Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. J Immunol. 2004; 172:2853–2860. [PubMed: 14978086]
12. Du J, Luan J, Liu H, Daniel TO, Peiper S, Chen TS, Yu Y, Horton LW, Nanney LB, Strieter RM, Richmond A. Potential role for Duffy antigen chemokine-binding protein in angiogenesis and maintenance of homeostasis in response to stress. J Leukoc Biol. 2002; 71:141–153. [PubMed: 11781390]
13. Shen H, Schuster R, Stringer KF, Waltz SE, Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. FASEB J. 2006; 20:59–64. [PubMed: 16394268]
14. Galvez BG, Genis L, Matias-Roman S, Oblander SA, Tryggvason K, Apte SS, Arroyo AG. Membrane type 1-matrix metalloproteinase is regulated by chemokines monocyte-chemoattractant protein-1/ccl2 and interleukin-8/CXCL8 in endothelial cells during angiogenesis. J Biol Chem. 2005; 280:1292–1298. [PubMed: 15516694]
15. Stamatovic SM, Keep RF, Mostarica-Stojkovic M, Andjelkovic AV. CCL2 regulates angiogenesis via activation of Ets-1 transcription factor. J Immunol. 2006; 177:2651–2661. [PubMed: 16888027]
16. Weber KS, Nelson PJ, Grone HJ, Weber C. Expression of CCR2 by endothelial cells : implications for MCP-1 mediated wound injury repair and In vivo inflammatory activation of endothelium. Arterioscler Thromb Vasc Biol. 1999; 19:2085–2093. [PubMed: 10479649]
17. Barcelos LS, Talvani A, Teixeira AS, Cassali GD, Andrade SP, Teixeira MM. Production and in vivo effects of chemokines CXCL1-3/KC and CCL2/JE in a model of inflammatory angiogenesis in mice. Inflamm Res. 2004; 53:576–584. [PubMed: 15597153]
18. Goede V, Brogelli L, Ziche M, Augustin HG. Induction of inflammatory angiogenesis by monocyte chemoattractant protein-1. Int J Cancer. 1999; 82:765–770. [PubMed: 10417778]
19. Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood. 2000; 96:34–40. [PubMed: 10891427]
20. Fujiyama S, Amano K, Uehira K, Yoshida M, Nishiwaki Y, Nozawa Y, Jin D, Takai S, Miyazaki M, Egashira K, Imada T, Iwasaka T, Matsubara H. Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. Circ Res. 2003; 93:980–989. [PubMed: 14525810]
21. Hong KH, Ryu J, Han KH. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood. 2005; 105:1405–1407. [PubMed: 15498848]
22. Niu J, Azfer A, Zhelyabovska O, Fatma S, Kolattukudy PE. Monocyte chemotactic protein (MCP)-1 promotes angiogenesis via a novel transcription factor, MCP-1-induced protein (MCPIP). J Biol Chem. 2008; 283:14542–14551. [PubMed: 18364357]
23. Salcedo R, Young HA, Ponce ML, Ward JM, Kleinman HK, Murphy WJ, Oppenheim JJ. Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3+ endothelial cells. J Immunol. 2001; 166:7571–7578. [PubMed: 11390513]
24. Strasly M, Doronzo G, Cappello P, Valdembri D, Arese M, Mitola S, Moore P, Alessandri G, Giovarelli M, Bussolino F. CCL16 activates an angiogenic program in vascular endothelial cells. Blood. 2004; 103:40–49. [PubMed: 12958070]
25. Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science. 1990; 247:77–79. [PubMed: 1688470]
26. Gupta SK, Singh JP. Inhibition of endothelial cell proliferation by platelet factor-4 involves a unique action on S phase progression. J Cell Biol. 1994; 127:1121–1127. [PubMed: 7962072]
27. Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, Uguccioni M, Galli G, Cosmi L, Maurenzig L, Baggiolini M, Maggi E, Romagnani S, Serio M. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest. 2001; 107:53–63. [PubMed: 11134180]

28. Soto H, Wang W, Strieter RM, Copeland NG, Gilbert DJ, Jenkins NA, Hedrick J, Zlotnik A. The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. Proc Natl Acad Sci U S A. 1998; 95:8205–8210. [PubMed: 9653165]
29. Beider K, Nagler A, Wald O, Franitza S, Dagan-Berger M, Wald H, Giladi H, Brocke S, Hanna J, Mandelboim O, Darash-Yahana M, Galun E, Peled A. Involvement of CXCR4 and IL-2 in the homing and retention of human NK and NK T cells to the bone marrow and spleen of NOD/SCID mice. Blood. 2003; 102:1951–1958. [PubMed: 12730102]
30. Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, Baggiolini M, Moser B. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med. 1996; 184:963–969. [PubMed: 9064356]
31. Loetscher M, Loetscher P, Brass N, Meese E, Moser B. Lymphocyte-specific chemokine receptor CXCR3: regulation, chemokine binding and gene localization. Eur J Immunol. 1998; 28:3696–3705. [PubMed: 9842912]
32. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. N Engl J Med. 1998; 338:436–445. [PubMed: 9459648]
33. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. Nat Immunol. 2001; 2:123–128. [PubMed: 11175804]
34. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest. 1998; 101:746–754. [PubMed: 9466968]
35. Rabin RL, Park MK, Liao F, Swofford R, Stephany D, Farber JM. Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. J Immunol. 1999; 162:3840–3850. [PubMed: 10201901]
36. Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, Orlando C, Maggi E, Marra F, Romagnani S, Serio M, Romagnani P. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. J Exp Med. 2003; 197:1537–1549. [PubMed: 12782716]
37. Salcedo R, Resau JH, Halverson D, Hudson EA, Dambach M, Powell D, Wasserman K, Oppenheim JJ. Differential expression and responsiveness of chemokine receptors (CXCR1-3) by human microvascular endothelial cells and umbilical vein endothelial cells. FASEB J. 2000; 14:2055–2064. [PubMed: 11023990]
38. Ehlert JE, Addison CA, Burdick MD, Kunkel SL, Strieter RM. Identification and partial characterization of a variant of human CXCR3 generated by posttranscriptional exon skipping. J Immunol. 2004; 173:6234–6240. [PubMed: 15528361]
39. Burdick MD, Murray LA, Keane MP, Xue YY, Zisman DA, Belperio JA, Strieter RM. CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodeling. Am J Respir Crit Care Med. 2005; 171:261–268. [PubMed: 15502109]
40. Yang J, Richmond A. The angiostatic activity of interferon-inducible protein-10/CXCL10 in human melanoma depends on binding to CXCR3 but not to glycosaminoglycan. Mol Ther. 2004; 9:846–855. [PubMed: 15194051]
41. Moser M. Regulation of Th1/Th2 development by antigen-presenting cells in vivo. Immunobiology. 2001; 204:551–557. [PubMed: 11846218]
42. Pan J, Burdick MD, Belperio JA, Xue YY, Gerard C, Sharma S, Dubinett SM, Strieter RM. CXCR3/CXCR3 ligand biological axis impairs RENCA tumor growth by a mechanism of immunoangiostasis. J Immunol. 2006; 176:1456–1464. [PubMed: 16424173]
43. Strieter RM, Belperio JA, Burdick MD, Sharma S, Dubinett SM, Keane MP. CXC chemokines: angiogenesis, immunoangiostasis, and metastases in lung cancer. Ann N Y Acad Sci. 2004; 1028:351–360. [PubMed: 15650260]
44. Tannenbaum CS, Tubbs R, Armstrong D, Finke JH, Bukowski RM, Hamilton TA. The CXC chemokines IP-10 and Mig are necessary for IL-12-mediated regression of the mouse RENCA tumor. J Immunol. 1998; 161:927–932. [PubMed: 9670971]

45. Sharma S, Stolina M, Luo J, Strieter RM, Burdick M, Zhu LX, Batra RK, Dubinett SM. Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. J Immunol. 2000; 164:4558–4563. [PubMed: 10779757]
46. Sharma S, Yang SC, Hillinger S, Zhu LX, Huang M, Batra RK, Lin JF, Burdick MD, Strieter RM, Dubinett SM. SLC/CCL21-mediated anti-tumor responses require IFNgamma, MIG/CXCL9 and IP-10/CXCL10. Mol Cancer. 2003; 2:22. [PubMed: 12740040]
47. Bikfalvi A. Platelet factor 4: an inhibitor of angiogenesis. Semin Thromb Hemost. 2004; 30:379–385. [PubMed: 15282661]
48. Bikfalvi A, Gimenez-Gallego G. The control of angiogenesis and tumor invasion by platelet factor-4 and platelet factor-4-derived molecules. Semin Thromb Hemost. 2004; 30:137–144. [PubMed: 15034805]
49. Perollet C, Han ZC, Savona C, Caen JP, Bikfalvi A. Platelet factor 4 modulates fibroblast growth factor 2 (FGF-2) activity and inhibits FGF-2 dimerization. Blood. 1998; 91:3289–3299. [PubMed: 9558385]
50. Arenberg DA, Kunkel SL, Polverini PJ, Morris SB, Burdick MD, Glass MC, Taub DT, Iannettoni MD, Whyte RI, Strieter RM. Interferon-gamma-inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. J Exp Med. 1996; 184:981–992. [PubMed: 9064358]
51. Loscalzo J, Melnick B, Handin RI. The interaction of platelet factor four and glycosaminoglycans. Arch Biochem Biophys. 1985; 240:446–455. [PubMed: 2409923]
52. Struyf S, Burdick MD, Proost P, Van Damme J, Strieter RM. Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis. Circ Res. 2004; 95:855–857. [PubMed: 15459074]
53. Struyf S, Burdick MD, Peeters E, Van den Broeck K, Dillen C, Proost P, Van Damme J, Strieter RM. Platelet factor-4 variant chemokine CXCL4L1 inhibits melanoma and lung carcinoma growth and metastasis by preventing angiogenesis. Cancer Res. 2007; 67:5940–5948. [PubMed: 17575164]
54. Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OM, Anver MR, Kleinman HK, Murphy WJ, Oppenheim JJ. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1alpha. Am J Pathol. 1999; 154:1125–1135. [PubMed: 10233851]
55. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001; 410:50–56. [PubMed: 11242036]
56. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. Am J Respir Crit Care Med. 2003; 167:1676–1686. [PubMed: 12626353]
57. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest. 1996; 97:2792–2802. [PubMed: 8675690]
58. Smith DR, Polverini PJ, Kunkel SL, Orringer MB, Whyte RI, Burdick MD, Wilke CA, Strieter RM. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J Exp Med. 1994; 179:1409–1415. [PubMed: 7513008]
59. Yatsunami J, Tsuruta N, Ogata K, Wakamatsu K, Takayama K, Kawasaki M, Nakanishi Y, Hara N, Hayashi S. Interleukin-8 participates in angiogenesis in non-small cell, but not small cell carcinoma of the lung. Cancer Lett. 1997; 120:101–108. [PubMed: 9570392]
60. Wislez M, Fujimoto N, Izzo JG, Hanna AE, Cody DD, Langley RR, Tang H, Burdick MD, Sato M, Minna JD, Mao L, Wistuba I, Strieter RM, Kurie JM. High expression of ligands for chemokine receptor CXCR2 in alveolar epithelial neoplasia induced by oncogenic kras. Cancer Res. 2006; 66:4198–4207. [PubMed: 16618742]
61. White ES, Flaherty KR, Carskadon S, Brant A, Iannettoni MD, Yee J, Orringer MB, Arenberg DA. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. Clin Cancer Res. 2003; 9:853–860. [PubMed: 12576459]

62. Chen JJ, Yao PL, Yuan A, Hong TM, Shun CT, Kuo ML, Lee YC, Yang PC. Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res. 2003; 9:729–737. [PubMed: 12576442]
63. Wente MN, Keane MP, Burdick MD, Friess H, Buchler MW, Ceyhan GO, Reber HA, Strieter RM, Hines OJ. Blockade of the chemokine receptor CXCR2 inhibits pancreatic cancer cell-induced angiogenesis. Cancer Lett. 2006; 241:221–227. [PubMed: 16458421]
64. Arenberg DA, White ES, Burdick MD, Strom SR, Strieter RM. Improved survival in tumor-bearing SCID mice treated with interferon-gamma-inducible protein 10 (IP-10/CXCL10). Cancer Immunol Immunother. 2001; 50:533–538. [PubMed: 11776375]
65. Wang D, Wang H, Brown J, Daikoku T, Ning W, Shi Q, Richmond A, Strieter R, Dey SK, DuBois RN. CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. J Exp Med. 2006; 203:941–951. [PubMed: 16567391]
66. Takamori H, Oades ZG, Hoch OC, Burger M, Schraufstatter IU. Autocrine growth effect of IL-8 and GROalpha on a human pancreatic cancer cell line, Capan-1. Pancreas. 2000; 21:52–56. [PubMed: 10881932]
67. Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst. 1998; 90:447–454. [PubMed: 9521169]
68. Gawrychowski K, Skopinska-Rozewska E, Barcz E, Sommer E, Szaniawska B, Roszkowska-Purska K, Janik P, Zielinski J. Angiogenic activity and interleukin-8 content of human ovarian cancer ascites. Eur J Gynaecol Oncol. 1998; 19:262–264. [PubMed: 9641227]
69. Bostwick DG, Iczkowski KA. Microvessel density in prostate cancer: prognostic and therapeutic utility. Semin Urol Oncol. 1998; 16:118–123. [PubMed: 9741415]
70. Fregene TA, Khanuja PS, Noto AC, Gehani SK, Van Egmont EM, Luz DA, Pienta KJ. Tumor-associated angiogenesis in prostate cancer. Anticancer Res. 1993; 13:2377–2381. [PubMed: 7510938]
71. Kim SJ, Uehara H, Karashima T, McCarty M, Shih N, Fidler IJ. Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. Neoplasia. 2001; 3:33–42. [PubMed: 11326314]
72. Moore BB, Arenberg DA, Stoy K, Morgan T, Addison CL, Morris SB, Glass M, Wilke C, Xue YY, Sitterding S, Kunkel SL, Burdick MD, Strieter RM. Distinct CXC chemokines mediate tumorigenicity of prostate cancer cells. Am J Pathol. 1999; 154:1503–1512. [PubMed: 10329603]
73. Charalambous C, Chen TC, Hofman FM. Characteristics of tumor-associated endothelial cells derived from glioblastoma multiforme. Neurosurg Focus. 2006; 20:E22. [PubMed: 16709028]
74. Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, Brown E, Barnett GH, Jain RK. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature. 2004; 428:328–332. [PubMed: 15029197]
75. Luan J, Shattuck-Brandt R, Haghnegahdar H, Owen JD, Strieter R, Burdick M, Nirodi C, Beauchamp D, Johnson KN, Richmond A. Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. J Leukoc Biol. 1997; 62:588–597. [PubMed: 9365113]
76. Mestas J, Burdick MD, Reckamp K, Pantuck A, Figlin RA, Strieter RM. The role of CXCR2/CXCR2 ligand biological axis in renal cell carcinoma. J Immunol. 2005; 175:5351–5357. [PubMed: 16210641]

Table

Human chemokine ligands and receptors involved in neovascularization. Modified from reference [3].

Systematic nomenclature	Old nomenclature	Receptor
Angiogenic/arteriogenic		
CXC chemokine family		
CXCL1	Gro-α	CXCR2
CXCL2	Gro-β	CXCR2
CXCL3	Gro-γ	CXCR2
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR2
CC chemokine family		
CCL2	MCP-1	CCR2
CCL11	Eotaxin	CCR3
CCL16	HCC-4/LEC	CCR1
Angiostatic		
CXCL4, CXCL4L1	PF-4, PF-4_{var}	CXCR3B*
CXCL9	Mig	CXCR3B
CXCL10	IP-10	CXCR3B
CXCL11	I-TAC	CXCR3B

* glycosaminoglycan binding may be involved, see text for details