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# Cancer CXC chemokine networks and tumour angiogenesis

*Robert M. Strieter*[a,b,c,*], *Marie D. Burdick*[a], *Javier Mestas*[a], *Brigitte Gomperts*[c], *Michael P. Keane*[a], *John A. Belperio*[a]

[a]*Department of Medicine, Division of Pulmonary and Critical Care Medicine, David Geffen School of Medicine at the University of California, Pathology and Pediatrics, 900 Veteran Ave., 14-154 Warren hall, Los Angeles, CA 90095-1786, USA*
[b]*Department of Pathology, Division of Pulmonary and Critical Care Medicine, David Geffen School of Medicine at the University of California, Los Angeles, CA 90095-1786, USA*
[c]*Department of Pediatrics, Division of Pulmonary and Critical Care Medicine, David Geffen School of Medicine at the University of California, Los Angeles, CA 90095-1786, USA*

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ABSTRACT

Chemokines have pleiotropic effects in regulating immunity, angiogenesis, stem cell trafficking, and mediating organ-specific metastases of cancer. In the context of angiogenesis, the CXC chemokine family is a unique group of cytokines known for their ability to behave in a disparate manner in the regulation of angiogenesis. The glutamic acid–leucine–arginine (ELR+) CXC chemokines are potent promoters of angiogenesis, and mediate their angiogenic activity via signal-coupling of CXCR2 on endothelium. By contrast, members of the CXC chemokine family, such as platelet factor-4 (PF4; CXCL4) and interferon-inducible CXC chemokines are potent inhibitors of angiogenesis, and use CXCR3 on endothelium to mediate their angiostatic activity. This review will discuss the biology of CXC chemokines in the context of angiogenesis related to cancer.



## 1. Introduction

Angiogenesis is a critical biological process under both physiological and pathological conditions. Angiogenesis can occur under physiological conditions that include embryogenesis and the ovarian/menstrual cycle. By contrast, pathological angiogenesis has been most studied in tumour biology related to cancer. A variety of factors have been described that either promote (angiogenic) or inhibit (angiostatic) angiogenesis.[1–14] In the local tumour micro-environment, net angiogenesis is determined by an imbalance in the over-abundance of angiogenic, compared with relative under-expression of angiostatic factors. The CXC chemokine family contains members that can function as either promoters or inhibitors of angiogenesis. CXC chemokines are heparin-binding proteins, with their heparin-binding domain in the C-terminus of the molecule. The family has four highly conserved cysteine amino acid residues, with the first two cysteines separated by a non-conserved amino acid residue.[15–17] The N-terminus of CXC chemokines dictates their specificity for binding to their cognate receptors. For example, CXC chemokines that contain a glutamic acid–leucine–arginine motif ('ELR' motif; ELR+) in the N-terminus of the molecule that immediately precedes the first cysteine amino acid residue are potent promoters of angiogenesis.[15–17] By contrast, platelet factor-4 and other interferon-inducible CXC chemokines that lack the conserved ELR motif are potent inhibitors of angiogenesis.[17] The dissimilarity in structure in the N-terminus of these CXC chemokines plays an important role in receptor specificity on endothelial cells. On the basis of the unique functional differences of the CXC chemokines, there has been increasing interest in their importance in regulating angiogenesis relevant to cancer.

* *Corresponding author*: Tel.: +1 310 794 1999; fax: +1 310 794 1998.
E-mail address: rstrieter@mednet.ucla.edu (R.M. Strieter).
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## 2. Angiogenic ELR+ CXC chemokines

The angiogenic CXC chemokine family members include CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8 (Table 1).[17] The angiogenic CXC chemokines interacting alone or with other angiogenic factors can function in a direct, parallel, or serial manner to promote angiogenesis. For example, vascular endothelial cell growth factor (VEGF) activation of endothelial cells can lead to up-regulation of the anti-apoptotic molecule, Bcl-2, that in turn promotes the expression of endothelial cell-derived CXCL8;[18] CXCL8 then functions in an autocrine and paracrine manner to maintain the angiogenic phenotype of the endothelial cell.[18,19] The autocrine and paracrine effect of VEGF in promoting the expression of CXCL8 has been further substantiated in a recent finding for neutrophils in the promotion of angiogenesis by a paracrine feed-forward mechanism involving endothelial cell-derived CXCL8.[20] Activation of neutrophils with N-formyl-Met–Leu–Phe (fMLP) results in the generation of pro-angiogenic activity related to both VEGF and CXCL8.[20] Moreover, in a feed-forward manner neutrophil-derived VEGF induces the expression of endothelial cell-derived CXCL8, which in turn maintains the formation of CXCL8-dependent capillary-like structures. Other examples of serial pathways that promote CXC chemokine mediated angiogenesis are operative and include receptor activation of seven transmembrane G protein-coupled receptors (7TM-GPCR) and tyrosine kinase receptors (TKRs). The activation of 7TM-GPCR and TKRs lead to downstream signalling and activation of NF-κB that can contribute to the expression of angiogenic CXC chemokines in cancer cells and enhanced tumour-associated angiogenesis.[21–24] These results demonstrate the existence of novel paracrine and autocrine signal pathways that lead to enhanced ELR+ CXC chemokine-dependent angiogenesis.

**Table 1 – CXC chemokine family members that regulate angiogenesis**

| Chemokine family |
|---|
| *Angiogenic ELR+ CXC chemokines* |
| IL-8 (CXCL8) |
| ENA-78 (CXCL5) |
| GRO-α(CXCL1) |
| GRO-β(CXCL2) |
| GRO-γ(CXCL3) |
| GCP-2 (CXCL6) |
| PBP |
| CTAP-III |
| β-TG |
| NAP-2 (CXCL7) |
| *Angiostatic non-ELR+ CXC chemokines* |
| PF-4 (CXCL4 and CXCL4L1) |
| CXCL14 |
| *Interferon-inducible non-ELR+ CXC chemokines* |
| IP-10 (CXCL10) |
| MIG (CXCL9) |
| ITAC (CXCL11) |

CXCR2 is the putative receptor for their biological activity.
CXCR3 is the putative receptor for their biological activity with the exception of CXCL14.

## 3. Role of CXCR2 in mediating the angiogenic response of ELR+ CXC chemokines

All angiogenic ELR+ CXC chemokines mediate their angiogenic activity through CXCR2.[25] Initial candidate CXC chemokine receptors for the angiogenic activity of ELR+ CXC chemokines were CXCR1 and/or CXCR2. However, only CXCL8 and CXCL6 specifically bind and activate CXCR1, whereas, all ELR+ CXC chemokines activate CXCR2.[25] Subsequent studies have confirmed the expression of CXCR2, not CXCR1, to be the primary functional chemokine receptor in mediating endothelial cell chemotaxis.[25,26] Endothelial cells respond to CXCL8 with rapid stress fibre assembly, chemotaxis, enhanced proliferation, and phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK 1/2) related to activation of CXCR2.[19] Blocking the function of CXCR2 by either specific neutralising antibodies or inhibiting downstream signalling using specific inhibitors of ERK1/2 and PI3kinase impaired CXCL8-induced stress fibre assembly, chemotaxis, and tube formation by endothelial cells.[19]

CXCR2 activation leads to dissociation of the hetero-trimeric protein complex ($G_{\alpha\beta\gamma}$) to α and βγ subunits that mediate downstream regulation of several intracellular signalling pathways (i.e., cAMP/protein kinase A (PKA), protein kinase C (PKC), phospholipase C (PLC), phosphoinositide 3-kinase (PI3Kinase)/AKT/mTOR, Ras/Raf/MEK/JNK/p38/ERK1/ERK2, and activates NF-kB pathways.[27–31] Many of these signalling pathways are identical to signal transduction by TKRs that are important for cellular proliferation, migration, and inhibition of apoptosis.[27–29,31]

While CXCR2 expression on endothelial cells in vitro has been shown to be important in signalling of ELR+ CXC chemokines, the importance of CXCR2 in mediating ELR+ CXC chemokine-induced angiogenesis in vivo has been shown in the cornea micropocket assay of angiogenesis in CXCR2+/+ and CXCR2−/− animals. Angiogenesis mediated by ELR+ CXC chemokines is inhibited in either the cornea micropocket assay of CXCR2–/– mice or in CXCR2+/+ mice in the presence of neutralising antibodies to CXCR2.[25] The finding that CXCR2 is both necessary and sufficient to mediate the angiogenic effects of ELR+ CXC chemokines has led to pre-clinical studies relevant to cancer. Using a murine model of lung cancer in CXCR2–/– versus CXCR2+/+ mice, tumours in both heterotopic or orthotopic positions in CXCR2–/– mice demonstrated reduced growth, increased tumour-associated necrosis, inhibited tumour-associated angiogenesis, and reduced metastatic potential in an angiogenesis-dependent manner.[32] The above findings for the role of CXCR2 in promoting tumour-associated angiogenesis were confirmed in CXCR2+/+ mice bearing tumours treated with specific neutralising anti-CXCR2 antibodies.[32] These studies established that CXCR2 is a critical receptor that mediates ELR+ CXC chemokine-dependent angiogenesis.

Other receptors that may modulate the angiogenic effects of ELR+ CXC chemokines exist. The Duffy antigen receptor for chemokines (DARC) is known to be a promiscuous chemokine receptor that binds chemokines in the absence of any detectable signal transduction events.[32] DARC binds the angiogenic CXC chemokines including CXCL8, CXCL1 and CXCL5.[34–36] Stable transfection and over-expression of

DARC in a non-small cell lung cancer (NSCLC) tumour cell line resulted in the binding of the angiogenic ELR+ CXC chemokines by the tumour cells.[33] The binding of tumour cell-derived ELR+ CXC chemokines to the tumour cells themselves interfered with the local tumour paracrine microenvironment of tumour cell interaction with host responding endothelial cells, and prevented the ability of these angiogenic factors to stimulate endothelial cells and promote tumour-associated angiogenesis.[33] Expression of DARC by NSCLC cells was associated with a marked decrease in tumour-associated vasculature and a reduction in metastatic potential. These findings suggested that competitive binding of ELR+ CXC chemokines by tumour cells expressing a 'decoy-like' receptor can prevent paracrine activation of endothelial cells in the tumour microenvironment and reduce tumour-associated angiogenesis.

## 4. Mechanisms that contribute to the over-expression of angiogenic CXC chemokines during aberrant angiogenesis

Specific stimuli within the microenvironment or epigenetic cellular events contribute to the expression of angiogenic CXC chemokines during aberrant angiogenesis. All of the angiogenic CXC chemokine promoters contain a putative *cis*-element that recognises the nuclear factor-κB (NF-κB) family of transcriptional factors[17,37]. Therefore, NF-κB plays an important role as a 'master switch' in the transactivation of angiogenic CXC chemokines.[38] To highlight the importance of NF-κB in the transactivation of angiogenic CXC chemokine promoters and expression of these cytokines relevant to aberrant angiogenesis, several studies have found that this biology is critical to angiogenesis relevant to tumour growth and potential metastases.

Elevated tumour cyclo-oxygenase (COX)-2 activity plays a multifaceted role in NSCLC and other cancers. Pold and colleagues[39] recently linked COX-2 expression in NSCLC, over-expression of ELR+ CXC chemokines in a NF-κB-dependent manner and ELR+ CXC chemokine-dependent tumour associated angiogenesis. Two COX-2 gene-modified NSCLC cell lines were studied and it was found that COX-2 over-expression enhanced the in vitro expression of both CXCL8 and CXCL5.[39] By contrast, specific COX-2 inhibition decreased the production of both CXC chemokines as well as nuclear translocation of NF-κB.[39] In an immunodeficient mouse model of human NSCLC, depletion of CXCL5 and CXCL8 inhibited the enhanced tumour growth of COX-2-over-expressing tumours.[39] These findings support the notion of a novel pathway of activation of ELR+ CXC chemokines via COX-2 over-expression leading to NF-κB activation, which resulted in CXCL5 and CXCL8-dependent tumour-associated angiogenesis.

Glioblastoma is a devastating tumour of the central nervous system, with lethality approaching 80% in the first year post-diagnosis.[40] The hallmark of these tumours is the marked presence of angiogenesis.[41] However, the precise molecular mechanisms underlying the regulation of glioblastoma growth and angiogenesis remain to be elucidated. Recently, a candidate tumour suppressor gene, *ING4*, has been identified that is involved in regulating glioblastoma tumour growth and angiogenesis.[41] The expression of *ING4* was found reduced in glioblastomas, compared with normal human brain tissue; and the extent of reduction correlated with the progression from lower to higher grade of tumour.[41] Human glioblastomas that exhibited decreased expression of *ING4* when engrafted into immuno-incompetent mice grew faster and displayed greater angiogenesis than control tumours.[41] The mechanism for increased tumourigenicity in glioblastomas that expressed lower levels of *ING4* was related to *ING4*'s physical ability to bind the p65 (RelA) subunit of NF-κB, impair its nuclear translocation, and subsequently inhibit transactivation of NF-κB-dependent genes.[41] In fact, the mechanism for the angiogenic activity of glioblastomas that expressed low levels of *ING4* was CXCL8-dependent, as inhibition of CXCL8 markedly reduced their tumour growth and tumour-associated angiogenesis.[41] These results indicate that *ING4* has an important role in glioblastoma pathogenesis related to ELR+ CXC chemokine expression in response to NF-κB transactivation. Furthermore, this finding links a tumour suppressor gene to NF-κB function and control of the expression of angiogenic ELR+ CXC chemokines in human tumours.

The importance of targeting NF-κB to attenuate the expression of angiogenic CXC chemokines has been further demonstrated using a strategy to block activation of NF-κB. Wu and colleagues have transfected glioblastoma cells with a mutant IkappaBalpha (IkappaBalphaM) that is resistant to phosphorylation and degradation, and hence blocks NF-κB activity to demonstrate decreased expression of CXCL8 and angiogenesis.[42] In this model system, an epidermal growth factor receptor (EGFR) mutant leading to activation of NF-κB constitutively activated glioblastoma cells and subsequent enhanced tumourigenicity related to augmented tumour-associated angiogenesis. [42] CXCL8 was found to play a significant role in mediating the angiogenic activity, however, when these cells were transfected with IkappaBalphaM, there was a profound reduction in expression of CXCL8 that correlated with reduced tumour-associated angiogenesis. Xiong and colleagues, using a similar strategy to block NF-κB in a model system of human pancreatic carcinoma, were able to inhibit tumour growth, tumour-associated angiogenesis, and metastases of these tumours[43] The studies by Wu and colleagues[42] highlight that the signalling pathway of EGFR activation can lead to activation of NF-κB and expression of angiogenic CXC chemokines, such as CXCL8. In addition, in the context of cancer, this pathway of activation may be further enhanced in the presence of mutationally activated forms of the Ras proto-oncogene, which is often associated with promoting cellular transformation. Sparmann and Bar-Sagi[44] have recently linked the interactions between tumour cells and host responding cells within the tumour microenvironment. They found that CXCL8 is a transcriptional target of Ras signalling, and using a tumour model system demonstrated that Ras-dependent CXCL8 was required for tumour-associated angiogenesis and tumour growth.

The ELR+ CXC chemokines are important mediators of tumourigenesis related to melanoma. For example, CXCL1, CXCL2 and CXCL3 are highly expressed in human melanoma.[45] To determine the biological significance of the presence of these ELR+ CXC chemokines in melanoma, human CXCL1, CXCL2 and CXCL3 genes were transfected into immortalised murine melanocytes that otherwise by

themselves do not form tumours.[45,46] The persistent expression of CXCL1, 2 or 3 in these cells transformed their phenotype to one with anchorage-independent growth in vitro and the ability to form tumours in vivo in immuno-incompetent mice.[45,46] The tumours were highly vascular and similar to the vascularity of B16 melanoma controls.[45,46] When tumours were depleted of CXCL1, 2 or 3 there was a marked reduction of tumour-derived angiogenesis directly related to inhibition of tumour growth.[45,46] These findings support the notion that the ELR+ CXC chemokines have the ability to act both as autocrine growth factors for melanoma and as potent paracrine mediators of angiogenesis to promote tumourigenesis and metastases.

The progression and growth of ovarian carcinoma is also dependent on successful angiogenesis, and CXCL8 has been determined to play a significant role in mediating human ovarian carcinoma-derived angiogenesis and tumourigenesis.[47] The expression of CXCL8, bFGF, and VEGF was examined in five different human ovarian carcinoma cell lines.[47] All cell lines in vitro expressed similar levels of bFGF, however, these cells expressed either high or low levels of CXCL8 or VEGF. When implanted into the peritoneum of immuno-incompetent mice, the high expressing CXCL8 tumours were associated with all animals dying <51 d.[47] The expression of CXCL8 was directly correlated with neovascularisation and inversely correlated with survival, whereas VEGF expression was only correlated with production of ascites.[47] No correlation was found for bFGF with either tumour neovascularisation or survival.[47] This study has been substantiated in patients with ovarian cancer, where ascites fluid demonstrates angiogenic activity directly correlated to CXCL8.[48] These findings support the notion that angiogenic ELR+ CXC chemokines play a greater role than bFGF and VEGF in mediating angiogenesis associated with ovarian cancer.

CXCL8 is markedly elevated and contributes to the overall angiogenic activity of NSCLC.[49] Extending these studies to an in vivo model system of human tumourigenesis (i.e. human non-small cell lung cancer/severe combined immunodeficient mouse (NSCLC/SCID) mouse chimera), [50] tumour-derived CXCL8 directly correlates with tumourigenesis.[50] Tumour bearing animals depleted of IL-8/CXCL8 demonstrated a >40% reduction in tumour growth and a reduction in spontaneous metastases [50] The attenuation of tumour growth and metastases was directly correlated to reduced angiogenesis.[50] These findings have been further corroborated using several human NSCLC cell lines grown in immuno-incompetent mice. NSCLC cell lines that constitutively express CXCL8 display greater tumourigenicity that is directly correlated to angiogenesis.[51]

While CXCL8 was the first angiogenic ELR+ CXC chemokine to be discovered in NSCLC, CXCL5 has now been determined to have a higher degree of correlation with NSCLC-derived angiogenesis.[35] Surgical specimens of NSCLC tumours demonstrate a direct correlation of CXCL5 with tumour angiogenesis. These studies were extended to a SCID mouse model of human NSCLC tumourigenesis. CXCL5 expression was directly correlated with tumour growth.[35] Moreover, when NSCLC tumour-bearing animals were depleted of CXCL5, both tumour growth and spontaneous metastases were markedly attenuated.[35] Furthermore, when all ELR+ CXC chemokines were evaluated in human NSCLC, it appears that they correlate with patient mortality.[52,53]

Prostate cancer tumourigenesis and metastases is dependent on angiogenesis.[54,55] Serum levels of CXCL8 have been found to be markedly elevated in patients with prostate cancer. These levels are highly correlated with the stage of the disease and have been determined to be an independent variable from the ratio of free/total prostate-specific antigen (PSA).[55] In fact, the combined use of f/t PSA and CXCL8 levels were more effective in distinguishing prostate cancer from benign prostatic hypertrophy.[55] This suggests that ELR+ CXC chemokines play an important role in mediating prostate cancer-derived angiogenesis in support of tumourigenesis and metastases. The observation in patients has been substantiated in human/SCID mice chimeras of human prostate cancer tumourigenesis.[36] Three human prostate cancer cell lines were examined for constitutive production of angiogenic ELR+ CXC chemokines.[36] Tumourigenesis of the human prostate cancer cell line, PC-3, was shown to be attributable, in part, to the production of the angiogenic CXC chemokine, CXCL8. Depletion of endogenous CXCL8 inhibited PC-3 tumour growth in SCID mice, which was entirely attributable to inhibition of PC-3 tumour-derived angiogenesis [36] By contrast, the human prostate cancer cell line, Du145, was found to utilise a different angiogenic CXC chemokine, CXCL1, to mediate tumour-derived angiogenesis.[36] Depletion of endogenous CXCL1, but not CXCL8, reduced tumour growth that was directly related to attenuated angiogenic activity.[36] Thus, prostate cancer cell lines can utilise distinct CXC chemokines to mediate their tumourigenic potential. Other studies have confirmed this observation in prostate cancer models.[56]; and similar findings have been shown in gastric carcinoma, breast, and head and neck cancer.[57–62]

## 5. Angiostatic CXC chemokines

The angiostatic members of the CXC chemokine family include CXCL4, CXCL4L1, CXCL9, CXCL10, CXCL11 and CXCL14.[16,17,37,63,64] (Table 1). Platelet factor-4 (PF-4)/CXCL4 was the first chemokine described to inhibit neovascularisation.[65] However, the product of the non-allelic variant gene of CXCL4, PF-4var1/PF-4alt, designated CXCL4L1, was recently isolated from thrombin-stimulated human platelets and purified to homogeneity.[64] Although secreted CXCL4 and CXCL4L1 differ in only three amino acid residues, CXCL4L1 is more potent for inhibiting angiogenesis in response to angiogenic factors in both in vitro and in vivo models of angiogenesis.[64] By contrast, CXCL9, CXCL10 and CXCL11 are induced by both type I and II interferons.[15,66–69] The interferon-inducible non-ELR+ members of the CXC chemokine family inhibit angiogenesis in response to ELR+ CXC chemokines, bFGF or VEGF.[37] These findings suggest that all interferon-inducible ELR- CXC chemokines are potent inhibitors of angiogenesis. Moreover, the inter-relationship of interferons and interferon-inducible CXC chemokines and their biological function are directly relevant to the function of other cytokines, such as Th1/type1 cytokines that stimulate the expression of interferons. Therefore, cytokines such as IL-23, IL-21, IL-18, IL-15, IL-12 and IL-2, and chemokines such as CCL19 and CCL21 via the induction of interferons[70,71] will have profound effects

on the production of CXCL9, CXCL10 and CXCL11. The subsequent expression of interferon-inducible CXC chemokines represents the final common pathway for the attenuation of angiogenesis related to interferons. Furthermore, this cytokine cascade interconnects with Th1/type1 cytokine-mediated immunity toward tumour-associated antigens and creates the concept of 'immuno-angiostasis'.

Breast and kidney-expressed chemokine (BRAK)/CXCL14 is another non-ELR+ CXC chemokine, which has been recently identified to inhibit angiogenesis.[63] CXCL14 was first identified by differential display of normal oral epithelial cells and head and neck squamous cell carcinoma.[72] CXCL14 was down-regulated in tumour specimens, compared with normal adjacent tissue.[72] The biological significance of the absence of CXCL14 in these tumours remained to be elucidated until Shellenberger and colleagues.[63] discovered that CXCL14 inhibited microvascular endothelial cell chemotaxis in vitro in response to CXCL8, bFGF and VEGF; and inhibited neovascularisation in vivo in response to the same angiogenic agonists. Schwarze and colleagues.[73] have found that CXCL14 expression is observed in normal and tumour prostate epithelium and focally in stromal cells adjacent to prostate cancer. Interestingly, CXCL14 was found to be significantly upregulated in localised prostate cancer and positively correlated with Gleason score.[73] By contrast, CXCL14 levels were unchanged in benign prostate hypertrophy (BPH) specimens.[73] Using a model of human prostate cancer in immunodeficient mice, prostate cancer cells transfected with CXCL14 were found to have a 43% reduction tumour growth, compared with controls.[73] The above studies support the notion that the loss or inadequate expression of CXCL14 is associated with the transformation of normal epithelial cells to cancer and the promotion of a pro-angiogenic microenvironment suitable for tumour growth.

While CXCL12 is a non-ELR+ CXC chemokine, CXCL12 via CXCR4 has been implicated in promoting angiogenesis.[74–77] This has lead to the speculation that the predominant function of this ligand/receptor pair in tumourigenesis is due to its angiogenic effect, not necessarily due to its potential for mediating organ-specific metastases. However, in order for the biological axis of CXCL12/CXCR4 to mediate tumour-associated angiogenesis, then both the ligand and receptor should be temporally and spatially present within the tumour. Schrader and colleagues.[78] demonstrated in both renal cell carcinoma cell lines and actual patient specimens that CXCR4 is expressed predominately by the tumour cells, and its ligand CXCL12 is essentially absent within the tumour. These findings have been further substantiated in human breast cancer and NSCLC specimens, in which CXCR4 was found expressed on the tumour cells, and did not mediate tumour-associated angiogenesis.[79,80] The studies demonstrated that when animals bearing breast or NSCLC tumours were treated with either neutralising anti-CXCL12 or anti-CXCR4 antibodies, there was no change in the size of the primary tumour nor was there any evidence for a decline in primary tumour-associated angiogenesis.[79,80], however, there was a marked attenuation of tumour metastases in an organ-specific manner.[79,80] These studies support the notion that CXCL12/CXCR4 biology mediates metastases of the tumour cells in an angiogenesis-independent manner.

## 6. Angiostatic CXC chemokines mediate inhibition of angiogenesis through binding and activation of their putative receptor, CXCR3

The major receptor that has been identified for angiostatic CXC chemokines is CXCR3. CXCR3 exists as alternative splice forms (i.e. CXCR3A, CXCR3B and CXCR3-alt) that are involved in mediating recruitment of Th1 cells and acts as the receptor for inhibition of angiogenesis[15,66,67,81,82] CXCR3A is the major chemokine receptor found on Th1 effector T cells, cytotoxic CD8 T cells, activated B cells and NK cells[15,81,83–87] IL-2 is a major agonist for the expression of CXCR3A on these cells.[81,84,87] CXCR3 was originally identified on murine endothelial cells[88], subsequent studies demonstrated that CXCR3 ligands could block both human microvascular endothelial cell migration and proliferation in response to a variety of angiogenic factors.[89,90] Further clarification of the role of CXCR3 in mediating angiostatic activity has come from the discovery that CXCR3 exists as two alternative splice forms.[91] These variants have been termed CXCR3A and CXCR3B.[91] CXCR3A mediates the CXCR3 ligand-dependent chemotactic activity of mononuclear cells.[91] CXCR3B mediates the angiostatic activity of CXCL4, CXCL9, CXCL10 and CXCL11 on human microvascular endothelial cells.[91] Moreover, specific antibodies to CXCR3B immunolocalise to endothelial cells within neoplastic tissues.[91] To add to the complexity of CXCR3 biology, a variant of human CXCR3 has been recently identified, which post-transcriptional exon skipping generates CXCR3-alt.[82] This receptor is expressed and responds to CXCL11 $\ggg$ CXCL9 and CXCL10.[82]

While the above studies have supported that CXCR3 is the putative receptor for CXCL4, CXCL9, CXCL10 and CXCL11, it has remained unclear in vivo whether these CXCR3 ligands use CXCR3 on endothelium to mediate their angiostatic effect. Yang and Richmond[92] have recently demonstrated that CXCL10 mediates its angiostatic activity in vivo by binding to CXCR3, and not via binding to glycosaminoglycans. To clarify this issue, they created expression constructs for mutants of CXCL10 that exhibit partial or total loss of binding to CXCR3 or loss of binding to glycosaminoglycans. They transfected a human melanoma cell line with these expression vectors, and stable clones were selected and inoculated into immuno-incompetent mice.[92] Tumour cells expressing wild-type CXCL10 showed remarkable reduction in tumour growth compared with control vector-transfected tumour cells, which was due to reduction in tumour-associated angiogenesis. Mutation of CXCL10 resulting in partial loss of receptor binding or loss of glycosaminoglycans binding did not significantly alter the ability to inhibit tumour growth. By contrast, expression of the CXCL10 mutant that failed to bind to CXCR3, also failed to inhibit tumour growth.[92] The above study has been confirmed in another in vivo angiogenesis assay using the cornea micropocket model (Fig. 1). Furthermore, Burdick and colleagues have found that CXCL11 in a CXCR3-dependent manner inhibits angiogenesis in a murine model of pulmonary fibrosis.[93] These data suggest that CXCR3 receptor binding, but not glycosaminoglycan binding, is essential for the tumour angiostatic activity of CXCR3 ligands.

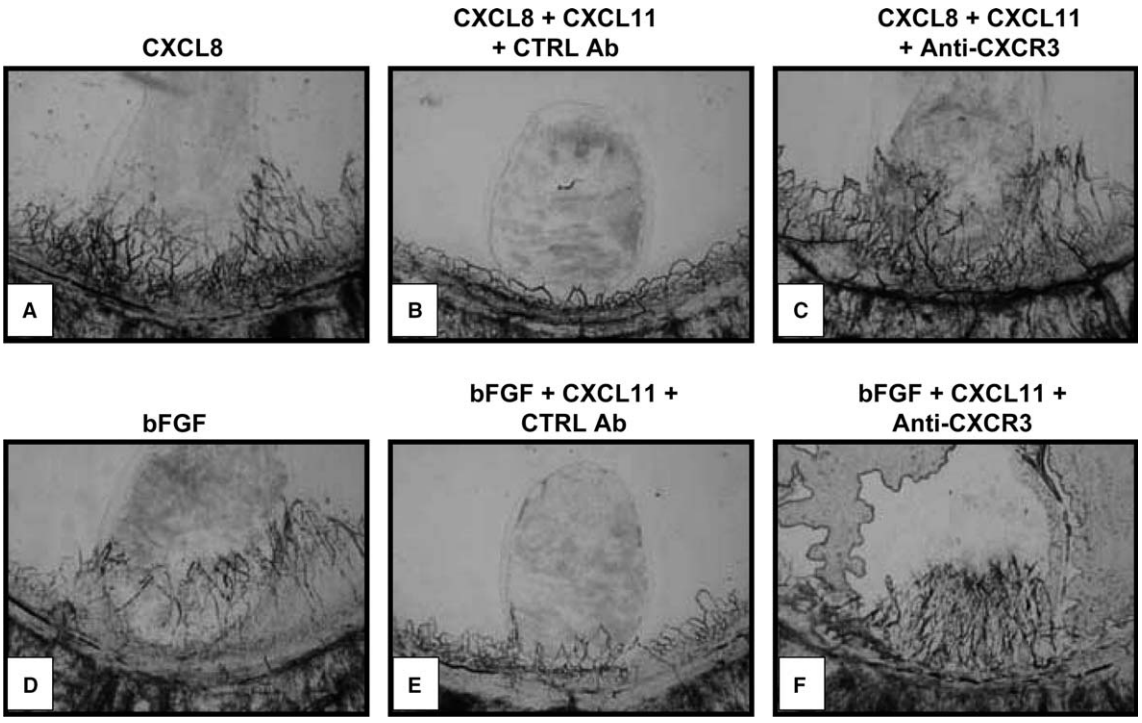


**Fig. 1 – CXCR3 is the putative receptor for mediating the angiostatic activity of CXCR3 ligands in the rat corneal micropocket assay of angiogenesis. (A) CXCL8 and (D) bFGF are shown to promote angiogenesis. The CXCR3 ligand, CXCL11, is shown to inhibit both (B) CXCL8 and (E) bFGF. Inhibition of CXCR3 results in reconstitution of (C) CXCL8 and (F) bFGF mediated angiogenesis in the presence of CXCL11.**

## 7. Angiostatic CXC chemokines attenuate angiogenesis associated with tumourigenesis

Angiostatic CXC chemokines have been shown to inhibit angiogenesis in several model systems. To examine the role of CXCL10 in the regulation of angiogenesis in carcinoma, the level of CXCL10 from human surgical NSCLC tumour specimens was examined, and found to be significantly higher in the tumour specimens than in normal adjacent lung tissue.[94] The increase in CXCL10 from human NSCLC tissue was entirely attributable to the higher levels of CXCL10 present in squamous cell carcinoma (SCCA), compared with adenocarcinoma.[94] Moreover, depletion of CXCL10 from SCCA surgical specimens resulted in augmented angiogenic activity.[94] The marked difference in the levels and bioactivity of CXCL10 in SCCA and adenocarcinoma is clinically and pathophysiologically relevant, and represents a possible mechanism for the biological differences of these two cell-types of NSCLC. Patient survival is lower, metastatic potential is higher, and evidence of angiogenesis is greater for adenocarcinoma, compared with SCCA of the lung.[95–97] These studies were extended to a SCID mouse system to examine the effect of CXCL10 on human NSCLC tumourigenesis. SCID mice were inoculated with either adenocarcinoma or SCCA cell lines.[34,94] The production of CXCL10 from adenocarcinoma and SCCA tumours was inversely correlated with tumour growth.[94] However, CXCL10 levels were significantly higher in the SCCA, compared with adenocarcinoma tumours.[94] The appearance of spontaneous lung metastases in SCID mice bearing adenocarcinoma tumours occurred after CXCL10 levels from either the primary tumour or plasma had reached a nadir.[94] In subsequent experiments, SCID mice bearing SCCA tumours were depleted of CXCL10, whereas, animals bearing adenocarcinoma tumours were treated with intra-tumour CXCL10.[94] Depletion of CXCL10 in SCCA tumours resulted in an increase in their size.[94] By contrast, reconstitution of intra-tumour CXCL10 in adenocarcinoma tumours reduced both their size and metastatic potential, that was unrelated to infiltrating neutrophils or mononuclear cells (i.e. macrophages or natural killer (NK) cells) and directly attributable to a reduction in tumour-associated angiogenesis.[94] Similar strategies have been found for CXCL10 in melanoma using a gene therapeutic strategy.[98]

Similar to CXCL10, CXCL9 also plays a significant role in regulating angiogenesis of NSCLC. CXCL9 levels in human specimens of NSCLC were not significantly different from that found in normal lung tissue.[99] However, these results suggested that the increased expression of ELR+ CXC chemokines and other angiogenic factors found in these tumours were not counter-regulated by a concomitant increase in the expression of the angiostatic CXC chemokine, CXCL9. Thus, this imbalance could promote a microenvironment that promotes angiogenesis. To alter this imbalance, studies were performed to over-express CXCL9 by three different strategies including gene transfer.[99] These experiments resulted in the inhibition of NSCLC tumour growth and metastasis via a decrease in tumour-associated angiogenesis.[99] These findings support the

importance of the interferon-inducible non-ELR+ CXC chemokines in inhibiting NSCLC tumour growth by attenuation of tumour-derived angiogenesis.

## 8. Immuno-angiostasis: the role of CXCR3/CXCR3 ligand biological axis in mediating Th1 cell-mediated immunity and angiostasis

While CXCR3/CXCR3 ligands inhibit angiogenesis, CXCR3 ligands represent the major chemo-attractants for the recruitment of Th1 cells expressing CXCR3 during cell-mediated immunity.[15,83–86] Thus, CXCR3 ligands play a critical role in orchestrating Th1 cytokine-induced cell-mediated immunity via the recruitment of mononuclear cells expressing CXCR3.[15,83–86] We have linked CXCR3/CXCR3 ligands to Th1 cytokine-induced cell-mediated immunity and inhibition of angiogenesis leading to suppression of tumour growth in pre-clinical models of NSCLC.[70,71,100] Intra-tumoural injection of a recombinant CC chemokine, CCL21, in tumours induced complete tumour eradication in several of the treated mice.[70,71,100] While CCL21 mediated anti-tumour responses were lymphocyte-dependent, its biological effect was entirely dependent on the spatial generation of intra-tumour IFN-γ and CXCR3 ligands.[70,71,100] In immunocompetent mice, intra-tumoural CCL21 injection led to significant increases in mononuclear cells infiltrating both the tumour and the draining lymph nodes, and a reduction in angiogenesis.[70,71,100] CCL21-treated tumour-bearing mice demonstrated enhanced cytolytic capacity, suggesting the generation of a systemic immune response to tumour-associated antigens (TAA). The mononuclear cell infiltration into the tumour was associated with enhanced production of Th1 cytokines and CXCR3 ligands.[70,71,100] To assess the importance of Th1 cytokines and CXCR3 ligands in mediating the effects of CCL21, depletion studies of IFN-γ or CXCR3 ligands in the presence of CCL21 treatment demonstrated that CXCL9 > CXCL10 ⩾ IFN-γ attenuated the anti-tumour effects of CCL21.[70,71] These findings support the notion that CCL21 mediated anti-tumour response was CXCR3 ligand-dependent. These findings are similar to the previously reported study of IL-12-mediated regression of renal cell carcinoma in a murine model, where the anti-tumour effect of IL-12 was lost when CXCR3 ligands

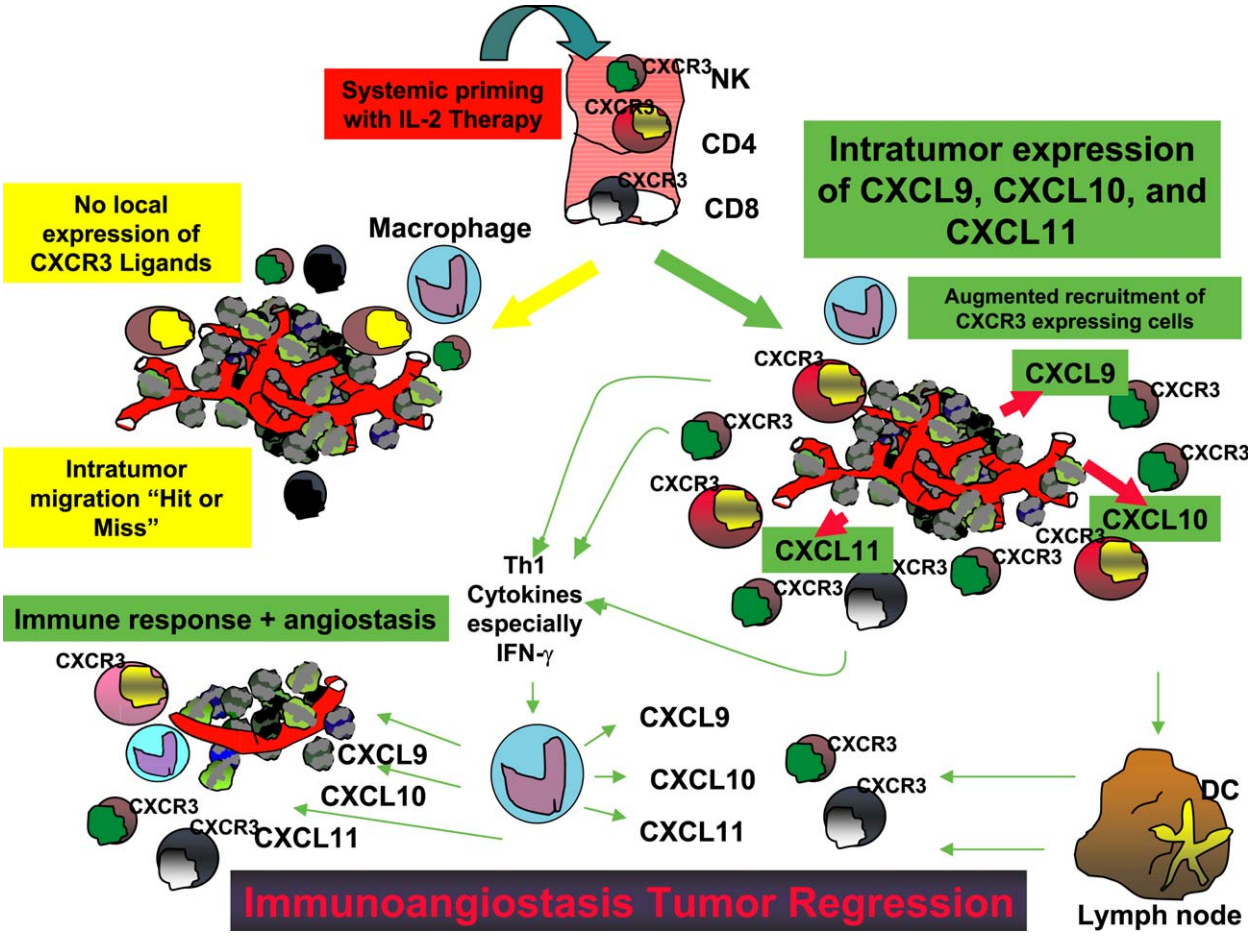


**Fig. 2 – The priming and induction steps are necessary for the full induction of the concept of immuno-angiostasis. Systemic IL-2 promotes the expression of CXCR3 on mononuclear cells, however, without the establishment of a chemotactic gradient from the circulation to within the tumour, mononuclear cells have no chemotactic gradient to extravasate into the tumour-concept of 'hit or miss'. Induction of the local expression of CXCR3 ligands within the tumour leads to the establishment of a chemotactic gradient for mononuclear cells expressing CXCR3, and concomitantly promotes angiostasis. The combined two steps of systemic priming for mononuclear cell expression of CXCR3 and local induction of CXCR3 ligand expression fully establishes immuno-angiostasis.**

were depleted.[101] These findings provide evidence that CXCR3/CXCR3 ligand biology is critical for the development of anti-tumour immunity and inhibition of angiogenesis relevant to a variety of tumours.

On the basis of the ability of CXCR3/CXCR3 ligands to promote Th1-dependent immunity and at the same time inhibit angiogenesis, we have coined the term 'immuno-angiostasis' for their biological role in promoting tumour regression. Although the concept of immuno-angiostasis (i.e. promoting Th1 immunity via Th1 mononuclear cell recruitment and at the same time inducing angiostasis) may seem a paradox, the precedent for this concept exists related to the host defence of *Mycobacterium tuberculosis*. *M. tuberculosis* is an aerobic bacillus that requires the full development of host mediated Th1-induced cell-mediated immunity to contain the micro-organism. The response is characterised by granulomas with a rim of mononuclear cells, epithelioid cells, giant cells, fibroblasts and endothelial cells surrounding a central area of caseating necrosis, which is devoid of vasculature – the concept of angiostasis. The mononuclear cell response in the rim of the granuloma together with subsequent processing of *M. tuberculosis* antigen leads to adaptive immunity – the concept of immunity. The microenvironment within avascular caseating necrosis is angiostatic and hypoxic in nature, which induces dormancy of *M. tuberculosis*. Further organising fibrosis of the granuloma contains the microbe in a dormant state. Therefore, the full development of immuno-angiostasis provides an optimal host response to promote eradication and/or dormancy of this aerobic microbe, which is analogous to promoting tumour-related immuno-angiostasis (Fig. 2).

## 9. Conclusion

Although CXC chemokine biology was originally felt to be restricted to recruitment of subpopulations of leukocytes, it has become increasingly clear that these cytokines can display pleiotropic effects in mediating biology that goes beyond their originally described function. CXC chemokines are a unique cytokine family that exhibit, on the basis of structure/function and receptor binding/activation, either angiogenic or angiostatic biological activity in the regulation of angiogenesis. CXC chemokines appear to be important in the regulation of angiogenesis associated with tumourigenesis relevant to cancer. These findings support the notion that therapy directed at either inhibition of angiogenic or augmentation of angiostatic CXC chemokines may be a novel approach in the treatment of a variety of cancers.

## Conflict of interest statement

None declared.

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## REFERENCES

1. Auerbach W, Auerbach R. Angiogenesis inhibition: a review. *Pharmacol Ther* 1994;**63**(3):265–311.
2. Auerbach R, Auerbach W, Polakowski I. Assays for angiogenesis: a review. *Pharmacol Ther* 1991;**51**(1):1–11.
3. Ziche M, Morbidelli L, Donnini S. Angiogenesis. *Exp Nephrol* 1996;**4**:1–14.
4. Pluda JM. Tumor-associated angiogenesis: mechanisms, clinical implications, and therapeutic strategies. *Semin Oncol* 1997;**24**(2):203–18.
5. Pluda JM, Parkinson DR. Clinical implications of tumor-associated neovascularization and current antiangiogenic strategies for the treatment of malignancies of pancreas. *Cancer* 1996;**78**(suppl 3):680–7.
6. Gastl G, Hermann T, Steurer M, et al. Angiogenesis as a target for tumor treatment. *Oncology* 1997;**54**(3):177–84.
7. Risau W. Angiogenesis is coming of age [Editorial; Comment]. *Circ Res* 1998;**82**(8):926–8.
8. Risau W. Mechanisms of angiogenesis. *Nature* 1997;**386**(6626):671–4.
9. Hotfilder M, Nowak-Gottl U, Wolff JE. Tumorangiogenesis: a network of cytokines. *Klin Padiatr* 1997;**209**(4):265–70.
10. Hui YF, Ignoffo RJ. Angiogenesis inhibitors. A promising role in cancer therapy. *Cancer Pract* 1998;**6**(1):60–2.
11. Kumar R, Fidler IJ. Angiogenic molecules and cancer metastasis. *In Vivo* 1998;**12**(1):27–34.
12. Zetter BR. Angiogenesis. State of the art. *Chest* 1988;**93**(suppl 3):159S–66S.
13. Zetter BR. Angiogenesis and tumor metastasis. *Annu Rev Med* 1998;**49**:407–24.
14. Lund EL, Spang-Thomsen M, Skovgaard-Poulsen H, Kristjansen PE. Tumor angiogenesis – a new therapeutic target in gliomas. *Acta Neurol Scand* 1998;**97**(1):52–62.
15. Luster AD. Chemokines–chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;**338**(7):436–45.
16. Belperio JA, Keane MP, Arenberg DA, et al. CXC chemokines in angiogenesis. *J Leukoc Biol* 2000;**68**(1):1–8.
17. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995;**270**(45):27348–57.
18. Nor JE, Christensen J, Liu J, et al. Up-regulation of Bcl-2 in microvascular endothelial cells enhances intratumoral angiogenesis and accelerates tumor growth. *Cancer Res* 2001;**61**(5):2183–8.
19. Heidemann J, Ogawa H, Dwinell MB, et al. Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *J Biol Chem* 2003;**278**(10):8508–15.
20. Schruefer R, Lutze N, Schymeinsky J, Walzog B. Human neutrophils promote angiogenesis by a paracrine feedforward mechanism involving endothelial interleukin-8. *Am J Physiol Heart Circ Physiol*.
21. Dong G, Chen Z, Li ZY, Yeh NT, Bancroft CC, Van Waes C. Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma. *Cancer Res* 2001;**61**(15):5911–8.
22. Hirata A, Ogawa S, Kometani T, et al. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. *Cancer Res* 2002;**62**(9):2554–60.
23. Levine L, Lucci 3rd JA, Pazdrak B, et al. Bombesin stimulates nuclear factor kappa B activation and expression of proangiogenic factors in prostate cancer cells. *Cancer Res* 2003;**63**(13):3495–502.

24. Richmond A, NF-kappa B. chemokine gene transcription and tumour growth. *Nat Rev Immunol* 2002;**2**(9):664–74.
25. Addison CL, Daniel TO, Burdick MD, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR(+) CXC chemokine-induced angiogenic activity. *J Immunol* 2000;**165**(9):5269–77.
26. Murdoch C, Monk PN, Finn A. CXC chemokine receptor expression on human endothelial cells. *Cytokine* 1999;**11**(9):704–12.
27. Sugden PH, Clerk A. Regulation of the ERK subgroup of MAP kinase cascades through G protein- coupled receptors. *Cell Signal* 1997;**9**(5):337–51.
28. Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. *Science* 1997;**278**(5346):2075–80.
29. Shyamala V, Khoja H. Interleukin-8 receptors R1 and R2 activate mitogen-activated protein kinases and induce c-fos, independent of Ras and Raf-1 in Chinese hamster ovary cells. *Biochemistry* 1998;**37**(45):15918–24.
30. Couty JP, Gershengorn MC. Insights into the viral G protein-coupled receptor encoded by human herpesvirus type 8 (HHV-8. *Biol Cell* 2004;**96**(5):349–54.
31. Manna SK, Ramesh GT. Interleukin-8 induces nuclear transcription factor-kappaB through a TRAF6-dependent pathway. *J Biol Chem* 2005;**280**(8):7010–21.
32. 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**(5):2853–60.
33. 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**(1):28.
34. 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**(12):2792–802.
35. Arenberg DA, Keane MP, DiGiovine B, et al. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. *J Clin Invest* 1998;**102**(3):465–72.
36. Moore BB, Arenberg DA, Stoy K, et al. Distinct CXC chemokines mediate tumorigenicity of prostate cancer cells. *Am J Pathol* 1999;**154**(5):1503–12.
37. Strieter RM, Belperio JA, Arenberg DA, Smith MI, Burdick MP, Keane MP. CXC chemokine in angiogenesis. In: Ransohoff RM, Suzuki K, Proudfoot AEI, Hickey WF, editors. *Universes in delicate balance: chemokines and the nervous system*. Amsterdam, The Netherlands: Elsevier Science BV; 2002. p. 129–48.
38. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998;**16**:225–60.
39. Pold M, Zhu LX, Sharma S, et al. Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. *Cancer Res* 2004;**64**(5):1853–60.
40. American Cancer Society. Cancer Facts and Figures 2004: Selected Cancers. American Cancer Society, 2004.
41. Garkavtsev I, Kozin SV, Chernova O, et al. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature* 2004;**428**(6980):328–32.
42. Wu JL, Abe T, Inoue R, Fujiki M, Kobayashi H. IkappaBalphaM suppresses angiogenesis and tumorigenesis promoted by a constitutively active mutant EGFR in human glioma cells. *Neurol Res* 2004;**26**(7):785–91.
43. Xiong HQ, Abbruzzese JL, Lin E, Wang L, Zheng L, Xie K. NF-kappaB activity blockade impairs the angiogenic potential of human pancreatic cancer cells. *Int J Cancer* 2004;**108**(2):181–8.
44. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* 2004;**6**(5):447–58.
45. Luan J, Shattuck-Brandt R, Haghnegahdar H, et al. Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. *J Leukoc Biol* 1997;**62**(5):588–97.
46. Owen JD, Strieter R, Burdick M, et al. Enhanced tumor-forming capacity for immortalized melanocytes expressing melanoma growth stimulatory activity/ growth-regulated cytokine beta and gamma proteins. *Int J Cancer* 1997;**73**(1):94–103.
47. 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**(6):447–54.
48. Gawrychowski K, Skopinska-Rozewska E, Barcz E, et al. Angiogenic activity and interleukin-8 content of human ovarian cancer ascites. *Eur J Gynaecol Oncol* 1998;**19**(3):262–4.
49. Smith DR, Polverini PJ, et al. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. *J Exp Med* 1994;**179**(5):1409–15.
50. Arenberg DA, Kunkel SL, Burdick MD, Polverini PJ, Strieter RM. Treatment with anti-IL-8 inhibits non-small cell lung cancer tumor growth. *J Investig Med* 1995;**43**(suppl 3): 479A.
51. Yatsunami J, Tsuruta N, Ogata K, et al. Interleukin-8 participates in angiogenesis in non-small cell, but not small cell carcinoma of the lung. *Cancer Lett* 1997;**120**(1):101–8.
52. White ES, Flaherty KR, Carskadon S, et al. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. *Clin Cancer Res* 2003;**9**(2):853–60.
53. Chen JJ, Yao PL, Yuan A, et al. 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**(2):729–37.
54. Bostwick DG, Iczkowski KA. Microvessel density in prostate cancer: prognostic and therapeutic utility. *Semin Urol Oncol* 1998;**16**(3):118–23.
55. Fregene TA, Khanuja PS, Noto AC, et al. Tumor-associated angiogenesis in prostate cancer. *Anticancer Res* 1993;**13**(6B):2377–81.
56. 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**(1):33–42.
57. Miller LJ, Kurtzman SH, Wang Y, Anderson KH, Lindquist RR, Kreutzer DL. Expression of interleukin-8 receptors on tumor cells and vascular endothelial cells in human breast cancer tissue. *Anticancer Res* 1998;**18**(1A):77–81.
58. Richards BL, Eisma RJ, Spiro JD, Lindquist RL, Kreutzer DL. Coexpression of interleukin-8 receptors in head and neck squamous cell carcinoma. *Am J Surg* 1997;**174**(5):507–12.
59. Kitadai Y, Haruma K, Sumii K, et al. Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. *Am J Pathol* 1998;**152**(1):93–100.
60. Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ. Expression of interleukin 8 correlates with the metastatic

potential of human melanoma cells in nude mice. *Cancer Res* 1994;**54**(12):3242–7.
61. Cohen RF, Contrino J, Spiro JD, Mann EA, Chen LL, Kreutzer DL. Interleukin-8 expression by head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 1995;**121**(2):202–9.
62. Chen Z, Malhotra PS, Thomas GR, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res* 1999;**5**(6):1369–79.
63. Shellenberger TD, Wang M, Gujrati M, et al. BRAK/CXCL14 is a potent inhibitor of angiogenesis and is a chemotactic factor for immature dendritic cells. *Cancer Res* 2004;**64**:8262–70.
64. 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**(9):855–7.
65. Maione TE, Gray GS, Petro J, et al. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. *Science* 1990;**247**(4938):77–9.
66. Rollins BJ. Chemokines. *Blood* 1997;**90**(3):909–28.
67. Balkwill F. The molecular and cellular biology of the chemokines. *J Viral Hepat* 1998;**5**(1):1–14.
68. Strieter RM, Belperio JA, Phillips RJ, Keane MP. CXC chemokines in angiogenesis of cancer. *Semin Cancer Biol* 2004;**14**(3):195–200.
69. Strieter RM, Belperio JA, Phillips RJ, Keane MP. Chemokines: angiogenesis and metastases in lung cancer. *Novartis Found Symp* 2004;**256**: 173–84.. discussion 184–188, 259–269.
70. Sharma S, Yang SC, Hillinger S, et al. SLC/CCL21-mediated anti-tumor responses require IFNgamma, MIG/CXCL9 and IP-10/CXCL10. *Mol Cancer* 2003;**2**(1):22.
71. Hillinger S, Yang SC, Zhu L, et al. EBV-induced molecule1 ligand chemokine (ELC/CCL19) promotes IFN-gamma-dependent antitumor responses in a lung cancer model. *J Immunol* 2003;**171**(12):6457–65.
72. Frederick MJ, Henderson Y, Xu X, et al. In vivo expression of the novel CXC chemokine BRAK in normal and cancerous human tissue. *Am J Pathol* 2000;**156**(6):1937–50.
73. Schwarze SR, Luo J, Isaacs WB, Jarrard DF. Modulation of CXCL14 (BRAK) expression in prostate cancer. *Prostate* 2005;**13**:13.
74. Bachelder RE, Wendt MA, Mercurio AM. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res* 2002;**62**(24):7203–6.
75. Salcedo R, Oppenheim JJ. Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. *Microcirculation* 2003;**10**(3–4): 359–70.
76. Kijowski J, Baj-Krzyworzeka M, Majka M, et al. The SDF-1-CXCR4 axis stimulates VEGF secretion and activates integrins but does not affect proliferation and survival in lymphohematopoietic cells. *Stem Cells* 2001;**19**(5):453–66.
77. Salcedo R, Wasserman K, Young HA, et al. 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**(4):1125–35.
78. Schrader AJ, Lechner O, Templin M, et al. CXCR4/CXCL12 expression and signalling in kidney cancer. *Br J Cancer* 2002;**86**(8):1250–6.
79. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;**410**(6824):50–6.
80. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor4 biological axis in non-small cell lung cancer metastases. *Am J Respir Crit Care Med* 2003;**167**(12):1676–86.
81. 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**(11):3696–705.
82. 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**(10):6234–40.
83. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001;**2**(2):123–8.
84. Loetscher M, Gerber B, Loetscher P, et al. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. *J Exp Med* 1996;**184**(3):963–9.
85. 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**(7):3840–50.
86. Qin S, Rottman JB, Myers P, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998;**101**(4):746–54.
87. Beider K, Nagler A, Wald O, et al. 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**(6):1951–8.
88. Soto H, Wang W, Strieter RM, et al. The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. *Proc Natl Acad Sci USA* 1998;**95**(14):8205–10.
89. Romagnani P, Annunziato F, Lasagni L, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest* 2001;**107**(1):53–63.
90. Salcedo R, Resau JH, Halverson D, et al. Differential expression and responsiveness of chemokine receptors (CXCR1-3) by human microvascular endothelial cells and umbilical vein endothelial cells. *FASEB J* 2000;**14**(13):2055–64.
91. Lasagni L, Francalanci M, Annunziato F, et al. 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**(11):1537–49.
92. 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**(6):846–55.
93. Burdick MD, Murray LA, Keane MP, et al. CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodeling. *Am J Respir Crit Care Med* 2005;**171**(3):261–8.
94. Arenberg DA, Kunkel SL, Polverini PJ, et al. 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**(3):981–92.
95. Minna JD. Neoplasms if the lung. In: Isselbacher KJ, editor. *Principles of internal medicine*. 12th ed. New York: McGraw-Hill; 1991. p. 1102–10.
96. Carney DN. Cancers of the lungs. In: Carney Fishman AP, editor. *Pulmonary diseases and disorders*. 2nd ed. New York: McGraw-Hill; 1988. p. 1885–2068.

97. Yuan A, Pan-Chyr Y, Chong-Jen Y, et al. Tumor angiogenesis correlates with histologic type and metastasis in non-small cell lung cancer. *Am J Resp Crit Care Med* 1995;**152**:2157–62.
98. Feldman AL, Friedl J, Lans TE, et al. Retroviral gene transfer of interferon-inducible protein 10 inhibits growth of human melanoma xenografts. *Int J Cancer* 2002;**99**(1):149–53.
99. Addison CL, Arenberg DA, Morris SB, et al. The CXC chemokine, monokine induced by interferon-gamma, inhibits non-small cell lung carcinoma tumor growth and metastasis. *Hum Gene Ther* 2000;**11**(2):247–61.
100. Sharma S, Stolina M, Luo J, et al. Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. *J Immunol* 2000;**164**(9):4558–63.
101. Tannenbaum CS, Tubbs R, Armstrong D, Finke JH, Bukowski TA, 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**(2):927–32.