

CXC Chemokines

Angiogenesis, Immunoangiostasis, and Metastases in Lung Cancer

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ABSTRACT: CXC chemokines have been found to be important in the regulation of angiogenesis and tumor-related immunity, and in promoting organ-specific metastases. This review highlights the importance of CXC chemokine ligands and receptors in mediating non-small cell lung cancer tumor-associated angiogenesis, “immunoangiostasis,” and organ-specific metastases. These findings may ultimately lead to clinical strategies to target CXC chemokines in non-small cell lung cancer.

KEYWORDS: CXC chemokines; angiogenesis; tumor growth; tumor metastases

INTRODUCTION

Lung cancer is the leading cause of malignancy-related mortality.¹ The biology of lung cancer is complex, and a variety of biomarkers have been suggested to account for the virulence of lung cancer. Clearly, understanding the biology of factors that contribute to lung cancer tumorigenicity, avoidance of host immunity, and metastases will lead to novel strategies for therapeutic intervention of this devastating disease. In the last decade, studies have demonstrated that CXC chemokines/chemokine receptors can directly enhance or inhibit tumor-associated angiogenesis, promote tumor-related immunity, and enhance organ-specific metastases.

CXC CHEMOKINES REGULATE ANGIOGENESIS

Preneoplastic to neoplastic transformation, tumor growth, invasion, and metastases are dependent on the establishment of a proangiogenic environment. Net angiogenesis in the local microenvironment is determined by an imbalance in the

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overexpression of angiogenic, as compared to angiostatic factors. CXC chemokines are heparin-binding proteins that display disparate roles in the regulation of angiogenesis. They have four highly conserved cysteine amino acid residues, with the first two cysteines separated by a non-conserved amino acid residue.²⁻⁴ A second structural domain within CXC chemokine family also dictates their functional activity. The NH₂ terminus of several CXC chemokines contains three amino acid residues (Glu-Leu-Arg; "ELR" motif), which precede the first cysteine amino acid residue of the primary structure of these cytokines.²⁻⁴ Members that contain the ELR motif (ELR+) are angiogenic factors.⁴ Members that lack the ELR motif (ELR-) and are interferon-inducible inhibit angiogenesis.⁴ In terms of structure and function, the CXC chemokine family behaves in various ways in the promotion or inhibition of angiogenesis relevant to lung cancer.

ELR+ CXC Chemokines Promote Angiogenesis

The angiogenic members of the CXC chemokine family include CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 (TABLE 1).⁴ While angiogenic factors can behave in a redundant manner, recent studies have shown that serial interactions may ultimately contribute to perpetuation of the angiogenic phenotype of the endothelium. For example, vascular endothelial cell growth factor (VEGF) activation of endothelial cells leads to upregulation of the anti-apoptotic molecule, Bcl-2, which in turn is associated with the expression of endothelial cell CXCL8, which functions in an autocrine and paracrine manner to maintain the angiogenic phenotype of the endothelial cell.⁵ The ability of CXCL8 to promote enhanced endothelial cell survival and proliferation has been substantiated by other studies.⁶ Moreover, this pathway *in vitro* can be reproduced *in vivo* using human tumor cells, which normally do not form tumors, mixed with endothelial cells overexpressing Bcl-2, leading to CXCL8-dependent tumorigenesis.⁵ Furthermore, other serial pathways that promote CXC chemokine-mediated angiogenesis have been found that include activation of signal pathways induced by epidermal growth factor and hepatocyte growth/scatter factor, which that contribute to the expression of CXCL8 in cancer cells and subsequent tumor-associated angiogenesis.^{7,8} These results demonstrate the existence of novel paracrine and autocrine serial signal pathways in cells that lead to enhanced intra-tumor microvascular survival and density and tumor growth related to ELR+ CXC chemokines.

While CXCL12 is not an ELR+ CXC chemokine, CXCL12 via CXCR4 had been implicated in mediating angiogenesis.⁹⁻¹² This in turn has led to speculation that the predominant function of this ligand-receptor pair in tumorigenesis results from this perceived angiogenic capability rather than an ability to mediate metastasis. However, in a recent model of tumorigenesis and metastases of human non-small cell lung cancer (NSCLC), Phillips and colleagues¹³ demonstrated that CXCR4 was predominantly expressed on the tumor cells and did not mediate angiogenesis in an *in vivo* model system of heterotopic or orthotopic human non-small cell carcinoma (NSCLC) tumor growth and metastasis. In this study, when CXCL12 was depleted *in vivo* during tumorigenesis and metastases, there was no change in the size of the primary tumor, nor was there any evidence for a decline in primary tumor-associated angiogenesis.¹³ However, there was a marked attenuation of metastases of these tumors, suggesting that the CXCL12/CXCR4 biological axis mediates metastases of

TABLE 1. Structural/functional differences of CXC chemokines in the regulation of angiogenesis

Angiogenic	E-L-R-C-X-C Motif
CXCL8	A-K-E-L-R-C-Q-C
CXCL5	L-R-E-L-R-C-V-C
CXCL1	A-T-E-L-R-C-Q-C
CXCL2	A-T-E-L-R-C-Q-C
CXCL3	V-T-E-L-R-C-Q-C
CXCR2	Receptor for angiogenic CXC chemokines
Angiostatic	Non-E-L-R-C-X-C motif
PF4/CXCL4	D-G-D-L-Q-C-L-C
IP-10/CXCL10	S-R-T-V-R-C-T-C
MIG/CXCL9	V-R-K-G-R-C-S-C
I-TAC/CXCL11	F-K-R-G-R-C-L-C
CXCR3B	Receptor for angiostatic CXC chemokines

the tumor cells in an angiogenesis-independent manner.¹³ A possible explanation for the disparity of the *in vivo* studies (i.e., not demonstrating the importance of CXCL12/CXCR4 in mediating angiogenesis of tumor growth) from other *in vitro* studies of CXCL12/CXCR4-mediated angiogenesis, is that tumor cells expressing CXCR4 are able to “outcompete” tumor-associated endothelial cells for CXCL12. In contrast, known angiogenic factors (i.e., CXCL5, CXCL8, and VEGF, vascular endothelial growth factor) are found to be elevated in human NSCLC, as compared with normal lung tissue.^{3,14–16} The depletion of these angiogenic factors *in vivo* results in a net reduction of angiogenesis and a consequent reduction in tumor size and metastatic propensity.^{3,14–16} Therefore, these findings suggest a dichotomy in the function for CXCL12 versus the other angiogenic factors, such that CXCL5, CXCL8, and VEGF promote metastasis through their stimulatory effects on angiogenesis, whereas CXCL12 mediates metastasis through direct effects on tumor cell migration.

CXCR2 Is the Putative Receptor for Angiogenic CXC Chemokine-Mediated Angiogenesis

The fact that all ELR+ CXC chemokines mediate angiogenesis highlights the importance of identifying a common receptor that mediates their biological function in promoting angiogenesis. The candidate CXC chemokine receptors are CXCR1 and/or CXCR2. Only CXCL8 and CXCL6 specifically bind to CXCR1, whereas all ELR+ CXC chemokines bind to CXCR2.¹⁷ The ability of all ELR+ CXC chemokine ligands to bind to CXCR2 supports the notion that this receptor mediates the angiogenic activity of ELR+ CXC chemokines.

While CXCR1 and CXCR2 are detected in endothelial cells,^{17–19} the expression of CXCR2, not CXCR1, has been found to be the primary functional chemokine re-

ceptor in mediating endothelial cell chemotaxis.^{17,18} Heidemann and associates²⁰ have further confirmed the importance of CXCR2 in mediating the effects of angiogenesis in human microvascular endothelial cells. They found that endothelial cells respond to CXCL8 with rapid stress fiber assembly, chemotaxis, enhanced proliferation, and phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK 1/2) related to activation of CXCR2.²⁰ Blocking the function of CXCR2 by either specific neutralizing antibodies or inhibiting downstream signaling using specific inhibitors of ERK1/2 and PI3 kinase impaired CXCL8-induced stress fiber assembly, chemotaxis, and endothelial tube formation in endothelial cells.²⁰

The importance of CXCR2 in mediating ELR+ CXC chemokine-induced angiogenesis has been shown *in vivo* using the cornea micropocket assay of angiogenesis in CXCR2+/+ and -/- animals. ELR+ CXC chemokine-mediated angiogenesis was inhibited in the corneas of CXCR2-/- mice and in the presence of neutralizing antibodies to CXCR2 in the rat corneal micropocket assay.¹⁷ These studies have now been extended to a lung cancer syngeneic tumor model system in CXCR2-/-, as compared to CXCR2+/+, mice. Lung cancer in CXCR2-/- mice demonstrates reduced growth, increased tumor-associated necrosis, inhibited tumor-associated angiogenesis, and metastatic potential.²¹ These *in vitro* and *in vivo* studies establish that CXCR2 is an important receptor that mediates ELR+ CXC chemokine-dependent angiogenic activity.

ELR+ CXC Chemokines Promote Angiogenesis Associated with Tumorigenesis

The ELR+ CXC chemokines are important mediators of angiogenesis during tumorigenesis of NSCLC. CXCL5, as compared to CXCL8, has been determined to have a higher degree of correlation with NSCLC-derived angiogenesis.²² Surgical specimens of NSCLC tumors demonstrate a direct correlation of CXCL8 with tumor angiogenesis. In model systems of human NSCLC tumorigenesis in SCID mice, CXCL5 expression was directly correlated with tumor growth, tumor-derived angiogenesis, and metastatic potential. Depletion of CXCL5 in this model system resulted in attenuation of both tumor growth and spontaneous metastases related to attenuation of angiogenesis.²² Furthermore, two recent studies have supported observation that the presence of ELR+ CXC chemokines in NSCLC are correlated with both angiogenesis and patient prognosis.^{23,24}

Interferon-Inducible CXC Chemokines Are Inhibitors of Angiogenesis

The angiostatic members of the CXC chemokine family include CXCL4, CXCL9, and CXCL10, CXCL11 (TABLE 1).^{3,4,25} CXCL10 can be induced by all three interferons (IFN- α , - β , and - γ).^{2,26,27} CXCL9 and CXCL11, other IFN-inducible ELR- members of the CXC chemokine family, which are similar to CXCL10 and MIG/CXCL9, inhibit neovascularization in response to ELR+ CXC chemokines, basic fibroblast growth factor (bFGF), and VEGF.²⁵ These findings suggest that all interferon (IFN)-inducible ELR- CXC chemokines are potent inhibitors of angiogenesis. Moreover, this interrelationship of interferons and IFN-inducible CXC chemokines and their biological function is directly relevant to the function of other cytokines, such as Th1/type 1 cytokines that stimulate the expression of interferons. Therefore cytokines, such as IL-23, IL-18, IL-15, IL-12, and IL-2, and

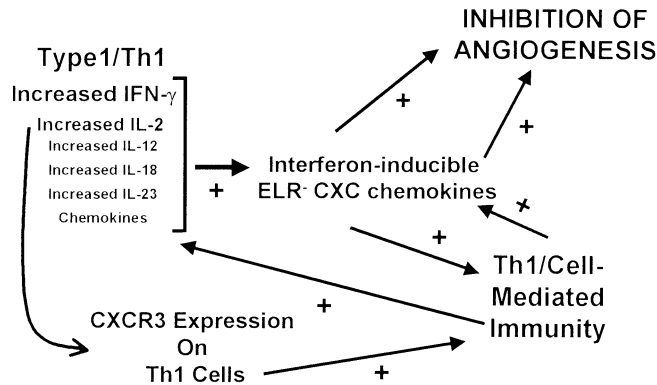


FIGURE 1. The interrelationship of Th1 cytokine/cell-mediated immunity and interferon-inducible ELR–CXC chemokines. The biology of interferon-inducible ELR–CXC chemokines in the inhibition of angiogenesis is linked to cell-mediated immunity. This highlights the importance of an appropriate immune response to tumor-associated antigens and the subsequent generation of angiostatic factors that can further modify local tumor-associated angiogenesis leading to the concept of immunoangiostasis.

chemokines, such as CCL19 and CCL21, via the induction of IFN- γ will have profound effects on the production of CXCL9, CXCL10, and CXCL11. The subsequent expression of IFN-inducible CXC chemokines represents the final common pathway and may, in part, explain the mechanisms for the attenuation of angiogenesis related to interferons. Furthermore, this cytokine cascade interconnects with Th1/type1 cytokine-mediated immunity toward tumor-associated antigens and creates the concept of “immunoangiostasis” (Fig. 1).

CXCR3 Is the Putative Receptor for Angiostatic Interferon-Inducible CXC Chemokine Inhibition of Angiogenesis

All three IFN-inducible ELR–CXC chemokines specifically bind to the CXC chemokine receptor CXCR3.^{2,26,27} The original observation that CXCR3 was found on endothelium was shown in murine endothelial cells.²⁸ Romagnani and colleagues subsequently identified CXCR3 expression on human endothelium.²⁹ CXCR3 is expressed by a small percentage of endothelial cells in human normal and pathological tissues as well as endothelial cells *in vitro*.²⁹ CXCL9, CXCL10, and CXCL11 all block endothelial cell proliferation in a CXCR3-dependent manner.²⁹ These data provide definitive evidence of CXCR3 expression by endothelial cells. Salcedo and associates have substantiated these findings for the expression of CXCR3 on endothelial cells and determined that CXCL9, CXCL10, and CXCL11 could inhibit the endothelial cell chemotactic response to CXCL8.¹⁹

The role of CXCR3 in mediating the angiostatic activity of IFN-inducible ELR–CXC chemokines has been further clarified with the finding that CXCR3 exists as two alternatively spliced variants.³⁰ These variants have been termed CXCR3A and CXCR3B and appear to be significantly different in the NH₂-terminus of the receptor.³⁰ CXCR3B, in contrast to CXCR3A, mediated the angiostatic activity of IFN-

induced ELR– CXC chemokines.³⁰ In addition, the first CXC chemokine determined to have angiostatic activity, CXCL4, also binds to CXCR3B.³⁰ Primary cultures of human endothelial cells, whose growth is inhibited by CXCL9, CXCL10, CXCL11, and CXCL4, express CXCR3B, but not CXCR3A. Moreover, specific neutralizing antibodies to CXCR3B react with endothelial cells from neoplastic tissues, providing evidence that CXCR3B is also expressed *in vivo* and may account for the angiostatic effects of these CXC chemokines. While it remains to be determined whether alternatively spliced variants of CXCR3 exist in rodents, we have found that neutralizing CXCR3 *in vivo* blocks the angiostatic effects of CXCL10 in the rat cornea micropocket assay. These findings open new avenues for consideration of therapeutic interventions in the treatment of aberrant angiogenesis associated with cancer by targeting CXCR3.

Interferon-Inducible CXC Chemokines Attenuate Angiogenesis Associated with Tumorigenesis

IFN-inducible ELR– CXC chemokines have been shown to inhibit angiogenesis in tumor model systems of NSCLC. Overexpression of CXCL9 by three different strategies including gene transfer resulted in the inhibition of NSCLC tumor growth and metastasis via a decrease in tumor-derived angiogenesis.³¹ Similar strategies have been found for CXCL10 in melanoma.³² These findings support the importance of the interferon-inducible ELR– CXC chemokines in inhibiting NSCLC growth by attenuation of tumor-derived angiogenesis and further support the need to generate agonists to CXCR3B.

THE COMBINED ROLE OF CXC CHEMOKINES IN MEDIATING TH1 CELL–MEDIATED IMMUNITY AND ANGIOSTASIS

The Concept of “Immunoangiostasis”

While IFN-inducible ELR– CXC chemokines are potent inhibitors of angiogenesis, these same chemokines play a critical role in orchestrating Th1/type I cytokine–induced cell-mediated immunity. CXCR3 is also found on Th1 T, B, and, natural killer (NK) cells.² The CXCR3 ligands represent the major chemoattractants for the recruitment of Th1 cells during cell-mediated immunity. Recently Sharma and associates found that CCL21 has the ability to suppress tumor growth by recruitment of mononuclear cells.³³ Intratumoral injection of recombinant CCL21 in syngeneic murine lung tumors induced potent antitumor responses and tumor eradication in several of the treated mice.³³ CCL21-mediated antitumor responses were lymphocyte dependent, as evidenced by the fact that this therapy did not alter tumor growth in SCID mice.³³ Studies performed in CD4- and CD8-knockout mice also revealed a requirement for both CD4 and CD8 lymphocyte subsets for CCL21-mediated tumor regression.³³ In immunocompetent mice, intratumoral CCL21 injection led to a significant increase in CD4 and CD8 T lymphocytes and dendritic cells, infiltrating both the tumor and the draining lymph nodes.³³ These cell infiltrates were accompanied by the enhanced elaboration of Th1 cytokines and the IFN-inducible ELR– CXC chemokines CXCL9 and CXCL10.³³ After stimulation with irradiated autolo-

gous tumor, lymph node–derived lymphocytes from CCL21-treated tumor-bearing mice demonstrated enhanced cytolytic capacity, suggesting the generation of systemic immune responses.

To extend these studies and to ascertain whether IFN- γ and the IFN-inducible ELR– CXC chemokines mediated the antitumor effect of CCL21, depletion of CXCL10, CXCL9, or IFN- γ during CCL21 treatment of tumors resulted in a marked impairment of CCL21-mediated antitumor effect.³⁴ Moreover, there was an interdependence of IFN- γ , CXCL9, and CXCL10—as neutralization of any one of these cytokines caused a concomitant decrease in the expression of all three cytokines.³⁴ Furthermore, neutralization of either IFN- γ , CXCL9, or CXCL10 led to a decrease in the presence of CXCR3-positive T cells and dendritic cells at the tumor site.³⁴ These findings provide evidence that IFN-inducible ELR– CXC chemokines may be important in the development of antitumor immunity.

On the basis of the ability of IFN-inducible CXC chemokines to promote Th1 immunity and at the same time inhibit angiogenesis, we have coined the term “immunoangiostasis” for their potential biological role in promoting tumor regression (FIG. 1). Perhaps the concept of immunoangiostasis-promoting Th1 immunity via Th1 mononuclear cell recruitment and at the same time inducing angiostasis may seem a paradox. However, the precedent for this concept already exists related to host response to *Mycobacterium tuberculosis*. *M. tuberculosis* is an aerobic bacillus that is the prototypic microbe that requires the full development of Th1-induced cell-mediated immunity to contain the infection. The response is characterized by granuloma formation with a rim of mononuclear cells, epithelioid cells, giant cells, fibroblasts, and endothelial cells surrounding caseating necrosis, which is acellular and devoid of vasculature. The mononuclear cellular response in the rim of the granuloma together with subsequent processing of *M. tuberculosis* antigen in the secondary lymphoid tissue leads to adaptive immunity that can be recapitulated as the response to the intradermal purified protein derivative of *M. tuberculosis*. Concomitant with the development of the immune response to *M. tuberculosis* antigen, the host further responds with robust inhibition of angiogenesis in the center of the granuloma, which leads to caseating necrosis. The microenvironment within caseating necrosis is both acidic and hypoxic in nature, which induces dormancy of *M. tuberculosis*. With further fibrotic organization of the granuloma, the microbe is contained. Therefore, the host response of immunoangiostasis to this microorganism has provided an optimal response to promote dormancy and potential eradication of an aerobic microbe.

The paradigm for immunoangiostasis can also be employed as a therapeutic strategy for the treatment of cancer. The effect of this therapy would enhance selective and specific extravasation of Th1 cells into the tumor; enhance Th1-mediated immunity *in situ* (i.e., increase *in situ* immunity to tumor-associated antigens); increase the expression of local IFN- γ ; further augment the local tumor microenvironment expression of CXCR3 ligands; amplify further *in situ* Th1-mediated immunity; and at the same time promote CXCR3B ligand–mediated angiostasis analogous to the pathogenesis of caseating necrosis seen associated with *M. tuberculosis*. This biology is relevant to the concept of “*in situ* vaccination.” The analogy of host defense in *M. tuberculosis* to immunoangiostasis of the tumor is ideal, because the cancer cell, analogous to the *M. tuberculosis* bacillus, will go dormant in an “anaerobic” avascular environment.

EVIDENCE THAT CHEMOKINES REGULATE THE PATTERN OF ORGAN-SPECIFIC METASTASIS OF LUNG CANCER

Paget in 1889 was the first to demonstrate that carcinoma has a distinct metastatic pattern preferentially involving the regional lymph nodes, bone marrow, lung, and liver.³⁵ Recently, Müller and colleagues³⁶ have provided new insights into potential mechanisms related to organ-specific metastasis of breast cancer cells directly related to a CXC chemokine and its receptor. They found that CXCR4 was the most highly expressed chemokine receptor in human breast cancer. The ligand for CXCR4, SDF-1/CXCL12, exhibited peak mRNA levels in organs that are preferential destinations of breast cancer metastasis. Moreover, *in vivo* neutralization of SDF-1/CXCL12/CXCR4 interactions with specific anti-CXCR4 antibodies resulted in significant inhibition of metastasis of breast cancer in an organ-specific manner.

Although the above study has provided evidence that the predominant function of SDF-1/CXCL12/CXCR4 in tumorigenesis is one of inducing metastasis, studies have also indicated that this chemokine receptor/ligand pair are potent angiogenic factors.^{11,12} Evidence suggests that SDF-1/CXCL12 α may be involved in upregulating levels of VEGF and bFGF, and that subcutaneous injection of SDF-1/CXCL12 α into mice induces formation of local small blood vessels.^{11,12} However, it has yet to be demonstrated in an *in vivo* tumor model system that endogenous SDF-1/CXCL12 binding to CXCR4 mediates a significant portion of primary tumor angiogenesis and angiogenesis-dependent tumor growth.

To address the role of the CXCR4/SDF-1/CXCL12 axis in NSCLC, we developed both a tissue culture-based model system and an *in vivo* mouse paradigm to characterize the activity of this receptor/ligand pair. Our results indicate that NSCLC cells express CXCR4 but not its ligand SDF-1/CXCL12. SDF-1/CXCL12 stimulation of CXCR4 on NSCLC cells leads to chemotaxis, calcium mobilization, and activation of mitogen-activated protein kinase p42/44. In addition, SDF-1/CXCL12 protein levels are significantly higher in those organs known to be highly susceptible for NSCLC metastasis, as compared to the primary tumor and plasma levels, suggesting that a chemotactic gradient could be established between the site of the primary tumor and those organs that develop NSCLC tumor metastases. To determine whether CXCR4-expressing tumor cells have a selective advantage for metastases, primary tumor and metastases were assessed for expression of CXCR4. A majority of the tumor cells in the primary tumor were found to express CXCR4, suggesting that a portion were actually CXCR4 negative. However, when the metastases were isolated from the adrenal glands, liver, lungs, and bone marrow of tumor-bearing SCID mice, more than 99% of the NSCLC tumor cells expressed CXCR4. Moreover, analysis of the heart and kidney revealed few, if any, metastases. Therefore, it seems likely that, in common with breast cancer cells, CXCR4 plays an important role in NSCLC tumor metastasis. Furthermore, *in vivo* neutralization of SDF-1/CXCL12 in a SCID mouse system of spontaneous metastasis of human NSCLC resulted in marked attenuation of NSCLC metastases to several organs including the adrenal glands, liver, lung, and bone marrow. At the same time, SDF-1/CXCL12/CXCR4 does not promote tumor-associated angiogenesis or tumor growth *in vivo* in primary tumors. Thus these findings provide strong evidence to suggest that the SDF-1/CXCL12/CXCR4 axis may be important in orchestrating the metastasis of NSCLC cells to a number of organs throughout the host in an angiogenesis-independent manner.

CONCLUSION

Although chemokine biology was originally felt to be restricted to only recruitment of populations of leukocytes, it has become increasingly clear that these cytokines can display pleiotropic effects in mediating biology that go beyond their originally described function. There is no better human disease to study diversity of chemokine function than tumor biology. Chemokines have autocrine, paracrine, and hormonal roles related to tumor growth and to metastasis to distant preferential organs. Moreover, CXC chemokines can play a critical role in mediating the full development of immunity to tumor-associated antigens. Understanding this expanded role of chemokines in tumor biology and using strategies to augment their expression to mediate immunoangiostasis will provide novel therapeutic intervention.

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REFERENCES

1. AMERICAN CANCER SOCIETY. 2000. Cancer Facts and Figures 2000: Selected Cancers. American Cancer Society. Atlanta, GA.
2. LUSTER, A.D. 1998. Chemokines—chemotactic cytokines that mediate inflammation. *N. Engl. J. Med.* **338**: 436–445.
3. BELPERIO, J.A. *et al.* 2000. CXC chemokines in angiogenesis. *J. Leukocyte Biol.* **68**: 1–8.
4. STRIETER, R.M. *et al.* 1995. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J. Biol. Chem.* **270**: 27348–27357.
5. NOR, J.E. *et al.* 2001. Up-regulation of Bcl-2 in microvascular endothelial cells enhances intratumoral angiogenesis and accelerates tumor growth. *Cancer Res.* **61**: 2183–2188.
6. LI, A. *et al.* 2003. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J. Immunol.* **170**: 3369–3376.
7. DONG, G. *et al.* 2001. 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.* **61**: 5911–5918.
8. HIRATA, A. *et al.* 2002. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. *Cancer Res.* **62**: 2554–2560.
9. BACHELDER, R.E., M.A. WENDT & A.M. MERCURIO. 2002. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res.* **62**: 7203–7206.
10. SALCEDO, R. & J.J. OPPENHEIM. 2003. Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. *Microcirculation* **10**: 359–370.
11. KJOWSKI, J. *et al.* 2001. The SDF-1-CXCR4 axis stimulates VEGF secretion and activates integrins but does not affect proliferation and survival in lymphohematopoietic cells. *Stem Cells* **19**: 453–466.
12. SALCEDO, R. *et al.* 1999. 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.* **154**: 1125–1135.

13. PHILLIPS, R.J. *et al.* 2003. 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.* **167**: 1676–1686.
14. SMITH, D.R. *et al.* 1994. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. *J. Exp. Med.* **179**: 1409–1415.
15. ARENBERG, D.A. *et al.* 1996. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. *J. Clin. Invest.* **97**: 2792–2802.
16. ARENBERG, D.A. *et al.* 1996. 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.* **184**: 981–992.
17. ADDISON, C.L. *et al.* 2000. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. *J. Immunol.* **165**: 5269–5277.
18. MURDOCH, C., P.N. MONK & A. FINN. 1999. Cxc chemokine receptor expression on human endothelial cells. *Cytokine* **11**: 704–712.
19. SALCEDO, R. *et al.* 2000. Differential expression and responsiveness of chemokine receptors (CXCR1-3) by human microvascular endothelial cells and umbilical vein endothelial cells. *FASEB J.* **14**: 2055–2064.
20. HEIDEMANN, J. *et al.* 2003. Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *J. Biol. Chem.* **278**: 8508–8515.
21. KEANE, M.P. *et al.* 2004. Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. *J. Immunol.* **172**: 2853–2860.
22. ARENBERG, D.A. *et al.* 1998. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. *J. Clin. Invest.* **102**: 465–472.
23. CHEN, J.J. *et al.* 2003. 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.* **9**: 729–737.
24. WHITE, E.S. *et al.* 2003. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. *Clin. Cancer Res.* **9**: 853–860.
25. STRIETER, R.M. *et al.* 2002. CXC chemokine in angiogenesis. *In* *Universes in Delicate Balance: Chemokines and the Nervous System*. R.M. Ransohoff *et al.*, Eds.: 129–148. Elsevier Science B.V. Amsterdam.
26. ROLLINS, B.J. 1997. Chemokines. *Blood* **90**: 909–928.
27. BALKWILL, F. 1998. The molecular and cellular biology of the chemokines. *J. Viral Hepat.* **5**: 1–14.
28. SOTO, H. *et al.* 1998. The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. *Proc. Natl. Acad. Sci. USA* **95**: 8205–8210.
29. ROMAGNANI, P. *et al.* 2001. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J. Clin. Invest.* **107**: 53–63.
30. LASAGNI, L. *et al.* 2003. 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.* **197**: 1537–1549.
31. ADDISON, C.L. *et al.* 2000. The CXC chemokine, monokine induced by interferon-gamma, inhibits non-small cell lung carcinoma tumor growth and metastasis. *Hum. Gene Ther.* **11**: 247–261.
32. FELDMAN, A.L. *et al.* 2002. Retroviral gene transfer of interferon-inducible protein 10 inhibits growth of human melanoma xenografts. *Int. J. Cancer* **99**: 149–153.
33. SHARMA, S. *et al.* 2000. Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. *J. Immunol.* **164**: 4558–4563.
34. SHARMA, S. *et al.* 2003. SLC/CCL21-mediated anti-tumor responses require IFN-gamma, MIG/CXCL9 and IP-10/CXCL10. *Mol. Cancer* **2**: 22.
35. PAGET, S.L. 1889. The distribution of secondary growths in cancer of the breast. *Lancet* **1**: 571.
36. MULLER, A. *et al.* 2001. Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**: 50–56.