

CXC Chemokines in Angiogenesis Relevant to Chronic Fibroproliferation

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Abstract: The CXC chemokines are a unique family of cytokines known for their ability to behave in a disparate manner in the regulation of angiogenesis. The mechanisms for the different activity in regulating angiogenesis by members of this chemokine family is related to the following: 1) the presence or absence of the structural/functional motif (Glutamic acid-Leucine-Arginine; 'ELR' motif) that immediately precedes the first cysteine amino acid residue in the primary structure of these cytokines; 2) interferon-inducible gene expression; and 3) receptors that these chemokines use to mediate their biological activity. Members that contain the 'ELR' motif (ELR⁺) are potent promoters of angiogenesis, and mediate their angiogenic activity via binding and activating CXCR2 on endothelium. In contrast, members that are inducible by interferons and lack the ELR motif (ELR⁻) are potent inhibitors of angiogenesis, and bind to the alternatively splice variant of CXCR3, CXCR3B on endothelium. This review will discuss the biology of these angiogenic and angiostatic CXC chemokines, and discuss their disparate angiogenic activity in the context of a variety of chronic fibroproliferative disorders.

Key words: Cytokines, Angiogenesis, Neovascularization, chronic fibroproliferative disorders.

INTRODUCTION

Angiogenesis, defined as new blood vessel growth, is a critical biological process in both physiologic and pathologic conditions. Angiogenesis can occur under physiologic conditions that include embryogenesis and the ovarian/menstrual cycle. In contrast, pathological angiogenesis is associated with chronic inflammation/chronic fibroproliferative disorders and tumorigenesis of cancer. For purposes of this review, angiogenesis and neovascularization will be used interchangeably in the context of pathological or aberrant angiogenesis.

Inflammation and angiogenesis, while distinct and separable processes, are closely related events and often temporally overlap in nature [1]. Chronic inflammation associated with chronic fibroproliferation histologically appears as granulation-like tissue, a prominent feature of which is neovascularization. The metabolic demands of tissue undergoing proliferative, reparative, and hyperplastic changes are extremely high and require a proportionally greater capillary blood supply, as compared to normal tissue. Thus, aberrant angiogenesis associated with chronic inflammation/fibroproliferative disorders is analogous to neovascularization of tumorigenesis of cancer, and provides a therapeutic target for novel intervention in these difficult to treat disorders.

A variety of factors have been described that either promote or inhibit angiogenesis [2-16]. In the local microenvironment, net angiogenesis is determined by a balance in the expression of angiogenic, as compared to angiostatic factors. CXC chemokines are heparin-binding proteins that display unique disparate roles in the regulation of angiogenesis. The family has four highly conserved cysteine amino acid residues, with the first two cysteines separated by a non-conserved amino acid residue [17-19]. A second structural domain dictates their functional activity. The NH₂-terminus of several CXC chemokines contains three amino acid residues (Glu-Leu-Arg; 'ELR' motif) which immediately precedes the first cysteine amino acid residue [17-19]. The CXC chemokines with the 'ELR' motif (ELR⁺) promote angiogenesis [19]. In contrast, CXC chemokines that are interferon-inducible and lack the ELR motif (ELR⁻) inhibit angiogenesis [19]. The dissimilarity in structure dictates the use of different CXC chemokine receptors on endothelial cells, which ultimately leads to signal-coupling and either promotion or inhibition of angiogenesis.

ANGIOGENIC ELR+ CXC CHEMOKINES

The CXC chemokine family members that promote angiogenesis are CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 (Table I) [19]. Angiogenic factors in a local microenvironment can function in a direct, parallel, or serial manner to promote angiogenesis. For example, a serial mechanism for the maintenance of an angiogenic microenvironment is the following: 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 endothelial cell-derived CXCL8 [20]; the up-regulated expression of CXCL8 functions in an autocrine and paracrine manner to maintain the angiogenic phenotype of the endothelial cell [20]. The ability of CXCL8 to promote enhanced endothelial cell survival and proliferation has been substantiated by other studies [21]. Moreover, this pathway *in vitro* can be reproduced *in vivo* using human tumor cells, that normally do not form tumors, mixed with endothelial cells over-expressing Bcl-2 lead to CXCL8-dependent tumorigenesis [20]. Furthermore, other serial pathways that promote CXC chemokine mediated angiogenesis have been found that include epidermal growth factor- and hepatocyte growth/scatter factor-induced activation of signal pathways that contribute to the expression of CXCL8 in cancer cells and subsequent tumor-associated angiogenesis [22, 23]. These results demonstrate the existence of novel paracrine and autocrine serial signal pathways in cells that leads to enhanced microvascular angiogenesis that is related to ELR⁺ CXC chemokines.

Table I. 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
<i>CXCR2 is the receptor for angiogenic CXC chemokines</i>	
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
<i>CXCR3B is the Receptor for Angiostatic CXC Chemokines</i>	

While CXCL12 is not an ELR⁺ CXC chemokine, CXCL12 via CXCR4 had been implicated in mediating angiogenesis [24-27]. This in turn has led to speculation that the predominant function of this ligand/receptor pair in tumorigenesis results from this perceived angiogenic capability. However, in a recent model of tumorigenesis and metastases of human non-small cell lung cancer (NSCLC), studies demonstrated that CXCR4 was predominately expressed on the tumor cells, and did not mediate angiogenesis in an *in vivo* model system of heterotopic or orthotopic human NSCLC tumor growth and metastasis [28]. 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 [28]. However, there was a marked attenuation of metastases of these tumors, suggesting that CXCL12/CXCR4 biological axis mediates metastases of the tumor cells in an angiogenesis-independent manner [28]. A possible

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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 "out compete" tumor associated endothelial cells for CXCL12. In contrast, known angiogenic factors (i.e., CXCL5, CXCL8, and VEGF) are found to be elevated in human NSCLC, as compared with normal lung tissue [18, 29, 30]. 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 [18, 29, 30]. Therefore these findings suggest a dichotomy in the function for CXCL12 versus the other angiogenic factors, such that ELR+ CXC chemokines and VEGF promote metastasis through their stimulatory effects on angiogenesis, whereas CXCL12 mediates metastasis through direct effects on tumor cell migration. These same effects are relevant to aberrant angiogenesis associated with chronic inflammatory/fibroproliferative disorders where surrounding parenchymal or other stromal cells may express CXCR4 and out compete endothelial cells for its ligand, CXCL12.

ANGIOGENIC (ELR+) CXC CHEMOKINE MEDIATED ANGIOGENESIS IS THROUGH BINDING AND ACTIVATION OF THEIR PUTATIVE RECEPTOR, CXCR2, NOT CXCR1

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 [31]. 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 [31-33], the expression of CXCR2 not CXCR1, has been found to be the primary functional chemokine receptor in mediating endothelial cell chemotaxis [31, 32, 34]. Heidemann and associates [21], 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 [21]. 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 [21]. Recently Schraufstatter and colleagues [34], have confirmed that microvascular endothelial cells express both CXCR1 and CXCR2, but migration of these cells in response to ELR+ CXC chemokines is mediated only by CXCR2. These studies demonstrated the following: 1) CXCR2, not CXCR1, is necessary to mediate migration of endothelial cells in response to ELR+ CXC chemokines; 2) inhibition of epidermal growth factor receptor blocked CXCL8 mediated migration of microvascular endothelial cells; 3) CXCL8 stimulation of CXCR2 on these cells resulted in phosphorylation of epidermal growth factor receptor; and 4) that the effect of CXCL8 mediated migration was inhibited by Cathepsin B [34]. These studies highlight the importance of CXCR2 in mediating the angiogenic activity of ELR+ CXC chemokines, and cross-talk exists between a G-protein couple receptor, CXCR2, and a protein tyrosine kinase receptor, epidermal growth factor receptor, that ultimately may be necessary to fully orchestrate the effects of ELR+ CXC chemokines in promoting angiogenesis.

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 [31]. 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^{-/-} demonstrate reduced growth, increased tumor-associated necrosis, inhibited tumor-associated angiogenesis and metastatic potential [35]. These *in vitro* and *in vivo* studies establish that CXCR2 is an important receptor that mediates ELR+ CXC chemokine-dependent angiogenic activity, and future studies will address whether therapeutically targeting CXCR2 will impact on aberrant angiogenesis associated with chronic inflammation/fibroproliferative disorders.

INTERFERON (IFN)-INDUCIBLE (ELR-) CXC CHEMOKINES ARE INHIBITORS OF ANGIOGENESIS

The angiostatic members of the CXC chemokine family include CXCL4, CXCL9, and CXCL10, CXCL11 [18, 19, 36] (Table I). CXCL10 can be induced by all three interferons (IFN- α , β , and γ) [17, 37, 38]. CXCL9 and CXCL11, other IFN-inducible ELR- members of the CXC chemokine family, similar to CXCL10 and MIG/CXCL9, inhibits neovascularization in response to either ELR+ CXC chemokines, basic fibroblast growth factor (bFGF), and VEGF [36]. These findings suggest that all IFN-inducible ELR- CXC chemokines are potent inhibitors of angiogenesis. Moreover, this interrelationship of interferons and IFN-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-18, IL-15, IL-12, and IL-2 and 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 with angiostasis, and creates the concept of "immunoangiostasis".

The concept of immunoangiostasis is related to the fact that IFN-inducible ELR- CXC chemokines are potent inhibitors of angiogenesis, and at the same time play a critical role in orchestrating Th1/type 1 cytokine-induced cell-mediated immunity. CXCR3 (see below) is found on endothelial cells, as well as Th1 T, B, and NK cells [17]. The CXCR3 ligands represent the major chemoattractants for the recruitment of Th1 cells during cell-mediated immunity. On the basis of the ability of IFN-inducible CXC chemokines to promote Th1 immunity and at the same time inhibit angiogenesis, a term has been coined, "immunoangiostasis", for their potential biological role in promoting inhibition of aberrant angiogenesis. 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 intradermal purified protein derivative (PPD) 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*, and with further fibrotic organization of the granuloma the microbe is contained. Therefore the immunoangiostatic host response to this microorganism has provided an optimal response to promote dormancy and potential eradication of an aerobic microbe. This same biology may be exploited to attenuate aberrant angiogenesis in chronic inflammation/fibroproliferative disorders.

CXCR3 IS THE PUTATIVE RECEPTOR FOR ANGIOSTATIC INTERFERON-INDUCIBLE (ELR-) CXC CHEMOKINE INHIBITION OF ANGIOGENESIS.

All three IFN-inducible ELR- CXC chemokines specifically bind to the CXC chemokine receptor, CXCR3 [17, 37, 38]. The original observation that CXCR3 was found on endothelium was shown in murine endothelial cells [39]. Romagnani and colleagues subsequently identified CXCR3 expression on human endothelium [40]. CXCR3 is expressed by a small percentage of endothelial cells in human tissues, as well as endothelial cells *in vitro* [40]. CXCL9, CXCL10, and CXCL11 all attenuate endothelial cell proliferation in a CXCR3-dependent manner [40]. 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 [33].

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 [41]. These variants have been termed, CXCR3A and CXCR3B, and appear to be significantly different in the NH₂-terminus of the receptor [41]. CXCR3B, in contrast to CXCR3A, mediated the angiostatic activity of IFN-induced ELR- CXC chemokines [41]. In addition, the first CXC chemokine determined to have angiostatic activity, CXCL4, also binds to CXCR3B [41]. Primary cultures of human endothelial cells, whose growth is inhibited by CXCL9, CXCL10, CXCL11, and CXCL4, express CXCR3B, 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 by targeting CXCR3.

THE ROLE OF ANGIOGENIC (ELR+) AND ANGIOSTATIC INTERFERON-INDUCIBLE (ELR-) CXC CHEMOKINES IN THE REGULATION OF ANGIOGENESIS ASSOCIATED WITH CHRONIC INFLAMMATORY/FIBROPROLIFERATIVE DISORDERS

Angiogenesis is increasingly being recognized for its role in promoting the pathogenesis of chronic inflammatory/fibroproliferative disorders. For example, rheumatoid arthritis is associated with the unrestrained proliferation of fibroblasts and capillary blood vessels that leads to the formation of the pannus and destruction of joint spaces. Macrophages isolated from rheumatoid synovium produce pro-angiogenic factors [42]. Recently, Koch and associates have found that CXCL8 and CXCL5 represent major angiogenic factors in the synovium of rheumatoid arthritis [43]. Psoriasis, is a well known angiogenesis-dependent skin disorder that is characterized by marked dermal neovascularization. Keratinocytes isolated from psoriatic plaques demonstrate a greater production of angiogenic activity. Interestingly, this angiogenic phenotype is due, in part, to a combined defect in the over-expression of the angiogenic cytokine CXCL8 resulting in a pro-angiogenic environment [44].

Coronary artery atherosclerosis continues to be the leading cause of morbidity and mortality in the United States [45]. The pathogenesis of coronary atherosclerotic plaque formation is a complex process that demonstrates features of exaggerated injury and repair including recruitment of mononuclear cells, fibroproliferation, deposition of extracellular matrix, and angiogenesis, which lead to progressive fibrosis, calcification, and eventual luminal occlusion [46-48]. Aberrant angiogenesis has also been demonstrated in atherosclerosis [42, 49-51]. Simonini and colleagues have demonstrated that CXCL8 is a significant angiogenic factor in coronary atherectomy specimens [52]. These investigators found CXCL8 levels to be markedly greater in coronary atherectomy specimens, as compared to control samples from the internal mammary arteries [52]. CXCL8 expression by immunohistochemistry was highly correlated with localization of Factor VIII-related antigen expression on endothelial cells in the coronary atherectomy specimens. The contribution of CXCL8 to net angiogenic activity from coronary atherectomy specimens was assessed using the rat cornea micropocket assay, and was found to represent the majority of the angiogenic activity. These findings suggest that, in human coronary atherosclerosis, CXCL8 is an important mediator of angiogenesis and may be contributory to plaque formation via its angiogenic properties.

Evidence exists for angiogenesis in the lung. The angiogenic response of the bronchial circulation/systemic circulation in the lung is a fundamental response related to alterations in the pulmonary vascular resistance [53-57]. Compensatory neovascularization of up to 30% of the original pulmonary blood flow can occur in the bronchial circulation in all mammals in response to marked increases in pulmonary vascular resistance [57]. In fact, Mitzner and colleagues [53], have demonstrated in the mouse that neovascularization from the systemic circulation can supply up to 15% of the normal pulmonary flow within 5 to 6 days post-pulmonary artery ligation. This response is an attempt to maintain blood flow to the metabolic pulmonary tissue, especially in reaction to injury and repair. To extend these studies and to determine the angiogenic factors that were instrumental in mediating angiogenesis under these conditions, these same investigators have found that ELR+ CXC chemokines, not VEGF, are markedly elevated during this process [58]. They found that ligation of the left pulmonary artery in the mouse consistently induced the

formation of a new vasculature, which developed from the visceral pleura and entered the upper left lung directly within 5-6 days after ligation [58]. They found a temporal pattern of differential gene expression from two independent regions of the same lung: one where angiogenesis is induced, and the other where angiogenesis does not occur. Gene microarray analysis confirmed by quantitative real-time RT-PCR showed that ELR+ CXC chemokines, not VEGF, were the predominant proangiogenic signals expressed early to promote neovascularization in the lung [58]. These results support the notion that under ischemic/hypoxic conditions, ELR+ CXC chemokines are involved in promoting angiogenesis.

Idiopathic pulmonary fibrosis (IPF) is a chronic and often fatal pulmonary fibroproliferative disorder. The pathogenesis of IPF that ultimately leads to end-stage fibrosis demonstrates features of dysregulated/abnormal repair with exaggerated neovascularization/vascular remodeling, fibroproliferation, and deposition of extracellular matrix, leading to progressive fibrosis and loss of lung function. While numerous eloquent studies have examined the biology of fibroblast proliferation and deposition of ECM in interstitial lung disease, few studies have examined the role of angiogenesis/vascular remodeling that promotes fibrogenesis in these disorders.

The existence of neovascularization in IPF was originally identified by Turner-Warwick, who examined the lungs of patients with widespread interstitial fibrosis and demonstrated neovascularization leading to anastomoses between the systemic and pulmonary microvasculatures and evidence of extensive vascular remodeling in areas of fibrosis [59]. These findings have been further substantiated with evidence of extensive neovascularization during the pathogenesis of pulmonary fibrosis in bleomycin-induced pulmonary fibrosis [60].

Studies have corroborated the findings of Turner-Warwick, and have shown that the BALF and lung tissue from patients with IPF have marked angiogenic activity that is almost entirely attributable to the imbalance in the over-expression of the angiogenic ELR+ CXC chemokine, CXCL8, as compared to the relative down-regulation of the angiostatic interferon-inducible ELR- CXC chemokine, CXCL10 [61]. To determine whether the imbalance in the expression of these CXC chemokines were relevant to the pathogenesis of pulmonary fibrosis, studies were extended to a murine model system of bleomycin-induced pulmonary fibrosis. In this model system, the expression and biological activity of murine macrophage inflammatory protein-2 (MIP-2/CXCL2/3 an angiogenic ELR+ CXC chemokine homologous to human GRO- β/γ /CXCL2/3) and the angiostatic CXC chemokine, CXCL10, were correlated to the magnitude of lung fibrosis during bleomycin-induced pulmonary fibrosis [62, 63]. CXCL2/3 and CXCL10 were measured during bleomycin-induced pulmonary fibrosis from BAL and whole lung tissue homogenates, and were found to be directly and inversely correlated, respectively, with total lung hydroxyproline levels, a measure of lung collagen deposition [62, 63]. Moreover, if either endogenous CXCL2/3 was depleted or exogenous CXCL10 was administered to the animals during bleomycin exposure, both treatment strategies resulted in marked attenuation of pulmonary fibrosis that was entirely attributable to a reduction in angiogenesis in the lung [62, 63].

The organizing phase of acute respiratory distress syndrome (ARDS) is associated with fibroproliferation. Recently Keane and associates [64] demonstrated that this phase is related to marked angiogenesis related to ELR+ CXC chemokines. These investigators obtained bronchoalveolar lavage fluid (BALF) from patients with ARDS or ventilated control patients and assessed angiogenic and angiostatic CXC chemokine levels [64]. They determined that there was an imbalance in the expression of angiogenic as compared to angiostatic CXC chemokines from BALF of patients with ARDS as compared to controls [64]. This imbalance in angiogenic and angiostatic CXC chemokines correlated with both angiogenic activity and levels of pro-collagen I and pro-collagen III in BALF of ARDS patients. In contrast, while BALF levels of VEGF were elevated in ARDS patients, VEGF did not appear to be a predominant factor in contributing to the overall angiogenic activity. Taken together, these findings support the notion that angiogenesis is critical to promote fibroplasia and deposition of ECM associated with chronic fibroproliferation, and that angiogenic and angiostatic factors, such as CXC chemokines play an important role in the pathogenesis of this process. On this basis, they represent potential therapeutic targets for the treatment of chronic inflammatory/fibroproliferative disorders associated with aberrant angiogenesis.

CONCLUSION

Net angiogenesis is regulated by an opposing balance of angiogenic and angiostatic factors. CXC chemokines are a unique cytokine family

that exhibit on both a structural/functional basis and receptor binding/activation either angiogenic or angiostatic biological activity. CXC chemokines appear to be important in the regulation of angiogenesis associated with both tumorigenesis and the pathogenesis of chronic inflammatory/fibroproliferative disorders. 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 disorders associated with aberrant angiogenesis.

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REFERENCES

- [1] Jackson, J. R.; Seed, M. P.; Kircher, C. H.; Willoughby, D. A.; Winkler, J. D. *FASEB J.* **1997**, *11*(6), 457-65.
- [2] Auerbach, W.; Auerbach, R. *Pharmacol. Ther.* **1994**, *63*(3), 265-311.
- [3] Auerbach, R.; Auerbach, W.; Polakowski, I. *Pharmacol. Ther.* **1991**, *51*(1), 1-11.
- [4] Ziche, M.; Morbidelli, L.; Donnini, S. *Exp. Nephrol.* **1996**, *4*, 1-14.
- [5] Pluda, J. M. *Semin. Oncol.* **1997**, *24*(2), 203-18.
- [6] Pluda, J. M.; Parkinson, D. R. *Cancer* **1996**, *78*(3 Suppl.), 680-7.
- [7] Gastl, G.; Hermann, T.; Steurer, M.; Zmija, J.; Gunsilius, E.; Unger, C.; Kraft, A. *Oncology* **1997**, *54*(3), 177-84.
- [8] Risau, W. *Circ. Res.* **1998**, *82*(8), 926-8.
- [9] Risau, W. *Nature* **1997**, *386*(6626), 671-4.
- [10] Hotfilder, M.; Nowak-Gottl, U.; Wolff, J. E. *Klin. Padiatr.* **1997**, *209*(4), 265-70.
- [11] Hui, Y. F.; Ignoffo, R. J. *Cancer Pract.* **1998**, *6*(1), 60-2.
- [12] Kumar, R.; Fidler, I. J. *In Vivo* **1998**, *12*(1), 27-34.
- [13] Zetter, B. R. *Chest* **1988**, *93*(3 Suppl.), 159S-166S.
- [14] Zetter, B. R. *Annu. Rev. Med.* **1998**, *49*, 407-24.
- [15] Lund, E. L.; Spang-Thomsen, M.; Skovgaard-Poulsen, H.; Kristjansen, P. E. *Acta Neurol. Scand.* **1998**, *97*(1), 52-62.
- [16] Lund, E. L.; Kristjansen, P. E. *Ugeskr. Laeger* **1999**, *161*(20), 2929-33.
- [17] Luster, A. D. N. *Engl. J. Med.* **1998**, *338*(7), 436-45.
- [18] Belperio, J. A.; Keane, M. P.; Arenberg, D. A.; Addison, C. L.; Ehlert, J. E.; Burdick, M. D.; Strieter, R. M. *J. Leukoc. Biol.* **2000**, *68*(1), 1-8.
- [19] Strieter, R. M.; Polverini, P. J.; Kunkel, S. L.; Arenberg, D. A.; Burdick, M. D.; Kasper, J.; Dzuiba, J.; Van Damme, J.; Walz, A.; Marriot, D.; et al. *J. Biol. Chem.* **1995**, *270*(45), 27348-57.
- [20] Nor, J. E.; Christensen, J.; Liu, J.; Peters, M.; Mooney, D. J.; Strieter, R. M.; Polverini, P. J. *Cancer Res.* **2001**, *61*(5), 2183-8.
- [21] Heidemann, J.; Ogawa, H.; Dwinell, M. B.; Rafiee, P.; Maaser, C.; Gockel, H. R.; Otterson, M. F.; Ota, D. M.; Lugerling, N.; Domschke, W.; Binion, D. G. *J. Biol. Chem.* **2003**, *278*(10), 8508-15.
- [22] Dong, G.; Chen, Z.; Li, Z. Y.; Yeh, N. T.; Bancroft, C. C.; Van Waes, C. *Cancer Res.* **2001**, *61*(15), 5911-8.
- [23] Hirata, A.; Ogawa, S.; Kometani, T.; Kuwano, T.; Naito, S.; Kuwano, M.; Ono, M. *Cancer Res.* **2002**, *62*(9), 2554-60.
- [24] Bachelder, R. E.; Wendt, M. A.; Mercurio, A. M. *Cancer Res.* **2002**, *62*(24), 7203-6.
- [25] Salcedo, R.; Oppenheim, J. J. *Microcirculation* **2003**, *10*(3-4), 359-70.
- [26] Kijowski, J.; Baj-Krzyworzeka, M.; Majka, M.; Reza, R.; Marquez, L. A.; Christofidou-Solomidou, M.; Janowska-Wieczorek, A.; Ratajczak, M. Z. *Stem. Cells* **2001**, *19*(5), 453-66.
- [27] Salcedo, R.; Wasserman, K.; Young, H. A.; Grimm, M. C.; Howard, O. M.; Anver, M. R.; Kleinman, H. K.; Murphy, W. J.; Oppenheim, J. J. *Am. J. Pathol.* **1999**, *154*(4), 1125-35.
- [28] Phillips, R. J.; Burdick, M. D.; Lutz, M.; Belperio, J. A.; Keane, M. P.; Strieter, R. M. *Am. J. Respir. Crit. Care Med.* **2003**, *167*(12), 1676-86.
- [29] Smith, D. R.; Polverini, P. J.; Kunkel, S. L.; Orringer, M. B.; Whyte, R. I.; Burdick, M. D.; Wilke, C. A.; Strieter, R. M. *J. Exp. Med.* **1994**, *179*(5), 1409-15.
- [30] Arenberg, D. A.; Kunkel, S. L.; Polverini, P. J.; Glass, M.; Burdick, M. D.; Strieter, R. M. *J. Clin. Invest.* **1996**, *97*(12), 2792-802.
- [31] Addison, C. L.; Daniel, T. O.; Burdick, M. D.; Liu, H.; Ehlert, J. E.; Xue, Y. Y.; Buechi, L.; Walz, A.; Richmond, A.; Strieter, R. M. *J. Immunol.* **2000**, *165*(9), 5269-5277.
- [32] Murdoch, C.; Monk, P. N.; Finn, A. *Cytokine* **1999**, *11*(9), 704-12.
- [33] Salcedo, R.; Resau, J. H.; Halverson, D.; Hudson, E. A.; Dambach, M.; Powell, D.; Wasserman, K.; Oppenheim, J. J. *FASEB J.* **2000**, *14*(13), 2055-64.
- [34] Schraufstatter, I. U.; Trieu, K.; Zhao, M.; Rose, D. M.; Terkeltaub, R. A.; Burger, M. *J. Immunol.* **2003**, *171*(12), 6714-22.
- [35] Keane, M. P.; Belperio, J. A.; Xue, Y.-Y.; Burdick, M. D.; Strieter, R. M. *J. Immunol.* in press.
- [36] Strieter, R. M.; Belperio, J. A.; Arenberg, D. A.; Smith, M. I.; Burdick, M. D.; Keane, M. P. In *Universities in delicate balance: Chemokines and the nervous system*, ed; Ransohoff, R. M.; Suzuki, K.; Proudfoot, A. E. I.; Hickey, W. F., 'Ed.' Elsevier Science B.V.: Amsterdam, The Netherlands, 2002; 'Vol.' pth pp. 129-148.
- [37] Rollins, B. J. *Blood* **1997**, *90*(3), 909-28.
- [38] Balkwill, F. J. *Viral Hepat.* **1998**, *5*(1), 1-14.
- [39] Soto, H.; Wang, W.; Strieter, R. M.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Hedrick, J.; Zlotnik, A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*(14), 8205-10.
- [40] Romagnani, P.; Annunziato, F.; Lasagni, L.; Lazzeri, E.; Beltrame, C.; Francalanci, M.; Uguccioni, M.; Galli, G.; Cosmi, L.; Maurenzig, L.; Baggolini, M.; Maggi, E.; Romagnani, S.; Serio, M. *J. Clin. Invest.* **2001**, *107*(1), 53-63.
- [41] 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. *J. Exp. Med.* **2003**, *197*(11), 1537-49.
- [42] Koch, A. E.; Leibovich, S. J.; Polverini, P. J. *Arthritis Rheum.* **1989**, *29*(4), 471-479.
- [43] Koch, A. E.; Volin, M. V.; Woods, J. M.; Kunkel, S. L.; Connors, M. A.; Harlow, L. A.; Woodruff, D. C.; Burdick, M. D.; Strieter, R. M. *Arthritis Rheum.* **2001**, *44*(1), 31-40.
- [44] Nickoloff, B. J.; Mitra, R. S.; Varani, J.; Dixit, V. M.; Polverini, P. J. *Am. J. Pathol.* **1994**, *144*(4), 820-8.
- [45] Nicholls, E. S.; Peruga, A.; Restrepo, H. E. *World Health Stat. Q* **1993**, *46*(2), 134-50.
- [46] Fowler, S.; Berberian, P. A.; Shio, H.; Goldfischer, S.; Wolinsky, H. *Circ. Res.* **1980**, *46*(4), 520-30.
- [47] Joris, I.; Zand, T.; Nunnari, J. J.; Krolkowski, F. J.; Majno, G. *Am. J. Pathol.* **1983**, *113*(3), 341-58.
- [48] Munro, J. M.; Cotran, R. S. *Lab. Invest.* **1988**, *58*(3), 249-61.
- [49] Folkman, J. *Nat. Med.* **1995**, *1*(1), 27-31.
- [50] Folkman, J.; Shing, Y. *J. Biol. Chem.* **1992**, *267*(16), 10931-4.
- [51] Clark, R. A. *J. Dermatol. Surg. Oncol.* **1993**, *19*(8), 693-706.
- [52] Simonini, A.; Moscucci, M.; Muller, D. W.; Bates, E. R.; Pagani, F. D.; Burdick, M. D.; Strieter, R. M. *Circulation* **2000**, *101*(13), 1519-26.
- [53] Mitzner, W.; Lee, W.; Georgakopoulos, D.; Wagner, E. *Am. J. Pathol.* **2000**, *157*(1), 93-101.
- [54] Charan, N. B.; Carvalho, P. *J. Appl. Physiol.* **1997**, *82*(1), 284-91.
- [55] Charan, N. B.; Baile, E. M.; Pare, P. D. *Eur. Respir. J.* **1997**, *10*(5), 1173-80.
- [56] Schlaepfer, K. *Arch Surg.* **1924**, *9*, 25-94.
- [57] Michel, R. P.; Hakim, T. S.; Petsikas, D. *J. Appl. Physiol.* **1990**, *69*(3), 1022-32.
- [58] Srisuma, S.; Biswal, S. S.; Mitzner, W. A.; Gallagher, S. J.; Mai, K. H.; Wagner, E. M. *Am. J. Respir. Cell Mol. Biol.* **2003**, *29*(2), 172-9.
- [59] Turner-Warwick, M. *Thorax*. **1963**, *18*, 225-237.
- [60] Peao, M. N. D.; Aguas, A. P.; DeSa, C. M.; Grande, N. R. *Anat. Rec.* **1994**, *238*, 57-67.
- [61] Keane, M. P.; Arenberg, D. A.; Lynch, J. P., 3rd; Whyte, R. I.; Iannettoni, M. D.; Burdick, M. D.; Wilke, C. A.; Morris, S. B.; Glass, M. C.; DiGiovine, B.; Kunkel, S. L.; Strieter, R. M. *J. Immunol.* **1997**, *159*(3), 1437-43.
- [62] Keane, M. P.; Belperio, J. A.; Arenberg, D. A.; Burdick, M. D.; Xu, Z. J.; Xue, Y. Y.; Strieter, R. M. *J. Immunol.* **1999**, *163*(10), 5686-92.
- [63] Keane, M. P.; Belperio, J. A.; Moore, T. A.; Moore, B. B.; Arenberg, D. A.; Smith, R. E.; Burdick, M. D.; Kunkel, S. L.; Strieter, R. M. *J. Immunol.* **1999**, *162*(9), 5511-8.
- [64] Keane, M. P.; Donnelly, S. C.; Belperio, J. A.; Goodman, R. B.; Dy, M.; Burdick, M. D.; Fishbein, M. C.; Strieter, R. M. *J. Immunol.* **2002**, *169*(11), 6515-21.