Out of the Shadows: CXC Chemokines in Promoting Aberrant Lung Cancer Angiogenesis

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Angiogenesis is a pervasive, normal biological event related to critical new blood vessel growth under both physiologic and pathologic conditions. The absolute dependence of tumorigenesis on angiogenesis has taught us several important things about aberrant angiogenesis involving neoplasia, including premalignant lesions. First, the tumor vascular system must rapidly respond to increased tissue metabolic cues by generating tumor microvasculature. Second and in contrast to normally tightly controlled angiogenesis (e.g., during the menstrual cycle or wound repair), successful tumor-associated angiogenesis has adapted multiple strategies to perpetuate itself. Appreciating the importance of tumor-associated angiogenesis and recognizing its complexity highlights why therapeutic targeting of only one factor, such as vascular endothelial growth factor (VEGF), within a tumor may not produce a dramatic outcome. To better understand aberrant angiogenesis within and near the tumor, it will be necessary to appreciate the temporal and spatial signals that control specific gene products that promote and perpetuate aberrant tumor-associated angiogenesis, especially under conditions where microenvironmental cues change over time or change in response to therapeutic intervention.

How do tumor cells respond to their microenvironment? What signals regulate factors that promote angiogenesis and are used by a tumor to preserve aberrant angiogenesis? Although several transcriptional factors and their gene products may be operative, hypoxia-inducible factor-1α (HIF-1α) and the nuclear factor-κB (NF-κB) are two major transcriptional factors that can sense microenvironmental cues relevant to the change in metabolic needs and redox potential of tumor cells and are activated in several tumors. HIF-1α acts as a master transcriptional switch for regulating oxygen homeostasis (1). HIF-1 consists of a heterodimer of HIF-1α and HIF-1β subunits (1), and HIF-1α expression normally is under strict regulation. The von Hippel-Lindau tumor suppressor protein targets HIF-1α for rapid ubiquitination under nonhypoxic conditions (1, 2), whereas activation of phosphoinositide 3-kinase/AKT/mammalian target of rapamycin and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways is involved in activating HIF-1α under both normoxic and hypoxic conditions (1, 3, 4). In contrast, HIF-1β is constitutively expressed (1, 2). The HIF-1 heterodimer recognizes and binds to specific cis-elements in the promoter of specific genes (e.g., VEGF) that are necessary for promoting glycolysis and angiogenesis (1, 2, 5).

NF-κB proteins are members of a heterodimeric family of transcription factors and are classically formed by p50/p65 proteins (6, 7). Normally, NF-κB is under strict regulation by its sequestration in the cytoplasm in association with a heterotrimeric complex of NF-κB with the inhibitor of κB (IκB). Activation of NF-κB is related to phosphorylation of IκB by IκB kinases followed by IκB ubiquitination and degradation by the proteasome and release of NF-κB for intranuclear localization and transactivation of a specific cis-element (6, 7).

Change in the redox potential of the cell (due to generation of reactive oxygen species) and activation of phosphoinositide 3-kinase/AKT and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways are involved in activation of NF-κB. NF-κB activates several genes that are relevant to innate and adaptive immunity, cellular proliferation, cell survival, and angiogenesis [e.g., Glu-Leu-Arg (ELR+) Cys-X-Cys (CXC) chemokines; refs. 7, 8].

Cross-talk between the above-described signal pathways of angiogenesis maintains the proangiogenic microenvironment of the tumor. For example, knockdown of the expression of tumor cell–derived HIF-1α in a preclinical model of a human cancer xenograft in immunocompetent mice markedly inhibited the expression of VEGF in tumors (9); the magnitude of tumor-associated angiogenesis, however, was still preserved in this model. Maintenance of tumor-associated angiogenesis in this system was due to the production of reactive oxygen species and subsequent activation of NF-κB leading to the induction of the proangiogenic ELR+ CXC chemokine CXCL8 (9). This compensatory pathway preserved tumor-associated angiogenesis. These findings support the notion that targeting one angiogenesis-related transcriptional pathway and its downstream gene product(s) may only partially attenuate tumor-associated angiogenesis, and full attenuation may require simultaneously targeting two or more angiogenesis-related pathways and their downstream mediators.

In this issue of the journal, Sun and colleagues (10) report a study that adds to the complexity of targeting angiogenesis and to the list of critical transcriptional factors for inducing proangiogenic mediators in non–small cell lung cancer (NSCLC). They used a cellular model system to assess the expression and function of angiogenic factors in tumor progression related to stimulation with interleukin-1β (IL-1β), which is associated with tumor development and production of tumor-derived angiogenic factors. Inflammatory cells, especially the tumor-associated macrophages (which produce proinflammatory and proangiogenic factors), can infiltrate into and influence angiogenesis in tumors (Fig. 1). Macrophages and monocytes are the major source of cells producing the proinflammatory cytokine IL-1β. These investigators found that IL-1β dramatically induced the expression of an array of proangiogenic ELR+ CXC
chemokines that augmented the angiogenic activity of NSCLC cell lines. Induction by NSCLC cells of the expression of ELR+ CXC chemokines was directly related to transcriptional activation not only by NF-κB but also by cyclic AMP–responsive element binding protein (CREB). Initial microarray analyses (confirmed by quantitative PCR) of mRNA from NSCLC cells treated with IL-1β showed marked induction of several proangiogenic factors, including VEGF, and proangiogenic ELR+ CXC chemokines, such as CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL8 (10). Furthermore, under unstimulated conditions, levels of certain ELR+ CXC chemokines were higher in NSCLC cell lines than in normal bronchial epithelial cells (10), suggesting that the genes for these chemokines were trans-activated even under unstimulated conditions.

Sun and colleagues (10) addressed whether induction of proangiogenic ELR+ CXC chemokine expression by IL-1β–treated NSCLC cells was associated with enhanced angiogenic activity, which they assessed by endothelial chemotaxis in the presence of IL-1β.

Fig. 1. Representative microphotographs (×40 magnification) of a lung adenocarcinoma (H&E staining) and of immunohistochemical expression of the markers phosphorylated CREB (p-CREB), CXCL5, and NF-κB in malignant lung epithelial cells; CD68 (marking macrophages) and CD31 (marking endothelial cells, or blood vessels) within the microenvironment; and CXCR2 in malignant lung epithelial cells and in endothelial cells within the microenvironment. The red arrows (CXCR2 and CD31) point to representative vascular structures within the tumor.
or absence of neutralizing antibodies to CXCR2 (the putative receptor on endothelial cells for proangiogenic ELR\(^+\) CXC chemokines) in IL-1\(\beta\)-stimulated NSCLC cell–conditioned medium (11). They found that endothelial migration in response to the medium was markedly attenuated in the presence of specific neutralizing antibodies to CXCR2; however, they did not find a reduction in endothelial chemotaxis in the presence of neutralizing antibodies to VEGF. These findings suggested that IL-1\(\beta\)-induced angiogenic activity in NSCLC was mainly attributable to the expression of CXCR2-dependent proangiogenic ELR\(^+\) CXC chemokines and not to VEGF.

To determine the mechanism for IL-1\(\beta\)-induced proangiogenic ELR\(^+\) CXC chemokine expression in NSCLC cells, Sun and colleagues (10) assessed whether NF-\(\kappa\)B and CREB were necessary for promoting the expression of proangiogenic ELR\(^+\) CXC chemokines in these cells. Using knockdown strategies targeting a \(>80\%\) reduction in NF-\(\kappa\)B (p65) or CREB expression, they found that knockdown of either transcription factor significantly reduced expression of ELR\(^+\) CXC chemokines in response to IL-1\(\beta\) stimulation (10). These collective findings supported the concept that IL-1\(\beta\) signaling, activation of NF-\(\kappa\)B and CREB, and transactivation of proangiogenic ELR\(^+\) CXC chemokine genes mediated the major part of the proangiogenic response of NSCLC cells.

To determine whether the expression of proangiogenic ELR\(^+\) CXC chemokines in the absence of IL-1\(\beta\) stimulation was associated with lung cancer development, Sun and colleagues (10) used an \textit{in vitro} model system of lung carcinogenesis that included normal and immortalized bronchial epithelial cells, as well transformed and highly tumorigenic NSCLC cell lines. They found that the expression of proangiogenic ELR\(^+\) CXC chemokine genes directly correlated with progression from normal to premalignant to a highly invasive phenotype; this process was directly associated with inherent activities of the transcription factors NF-\(\kappa\)B and CREB.

The small-molecule inhibitor KG-501 binds to the transcriptional coactivator CBP, blocks the interaction of CBP with the active form of CREB, and inhibits the interaction of CBP with NF-\(\kappa\)B (12, 13). Sun and colleagues (10) showed that KG-501 significantly attenuated IL-1\(\beta\)-induced expression of proangiogenic ELR\(^+\) CXC chemokines and that this attenuation directly correlated with reduced angiogenic activity in NSCLC cells.

Important questions remain. Are the findings of Sun and colleagues (10) on the significance of proangiogenic ELR\(^+\) CXC chemokines unique to NSCLC? Despite tremendous efforts to target VEGF in cancer, trials of a monoclonal antibody against VEGF in patients with renal cell carcinoma, colon cancer, and NSCLC have not been overwhelmingly positive (14–16). Does this mean that we should be more thorough in evaluating the tumor microenvironment for additional tumor-associated angiogenic factors to design optimal therapeutic strategies that target multiple angiogenic factors rather than only one within the tumor microenvironment, or that tumor-associated angiogenesis is not important, or that VEGF is not as important as we think it is? Do these studies, together with the findings of Sun and colleagues (10), highlight the importance of other angiogenic pathways that show the importance of ELR\(^+\) CXC chemokines in the promotion of tumor-associated angiogenesis? The activation of the transcription factors CREB and NF-\(\kappa\)B and the induction of proangiogenic ELR\(^+\) CXC chemokines play important roles that cannot be discounted in preserving aberrant angiogenesis in lung and other cancers. Recent studies in murine models of lung tumorigenesis found that high expression of CXCR2 ligands contributed to inflammation, neovascularization, and neoplasia; that a CXCR2-neutralizing antibody was active in reducing established tumors (17); and that a CXCR2-neutralizing antibody (in Kras\(^{\mathrm{A1}}\) mice) inhibited the progression of premalignant alveolar lesions and induced apoptosis of vascular endothelial cells within alveolar lesions (18). The microenvironment was required for sensitivity to the antibody in both the malignant and premalignant preclinical settings, and these studies provide compelling evidence in support of targeting the ELR\(^+\) CXC chemokine-CXCR2 axis for treatment or prevention. The work of Sun and colleagues (10) will help in developing future clinical studies of strategies designed to impair neoplasia-associated angiogenesis via targeting multiple tumor-derived signals that promote angiogenesis, including ELR\(^+\) CXC chemokines.

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**References**

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