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Link between epidermal growth factor receptor and NF-κB in human diffuse gliomas

Gliomas are the most common primary neoplasm of the brain, to include astrocytic, oligodendroglial and ependymal tumors. Owing to their infiltrative growth patterns, astrocytomas and oligodendrogliomas are referred to as diffuse gliomas. As such, they are often refractory to current therapeutic modalities, such as surgery, radiotherapy and chemotherapy. Recent advances in the identification of molecular and genetic alterations associated with the formation and progression of gliomas have shed light on new therapeutic approaches. For example, epidermal growth factor receptor (EGFR) is a member of the type 1-receptor tyrosine kinase family. These kinases can induce expression of NF-κB, an important molecule in cell survival and malignant transformation in addition to its function in inflammatory responses. In brain tumors, amplification of EGFR has been observed in 50% of glioblastoma multiforme (GBM) and in a smaller percentage of anaplastic astrocytomas. Activation of EGFR suppresses apoptosis and mediates resistance to radiation and chemotherapy, but the specific signaling pathways of EGFR involved in oncogenic transformation have not been well characterized. **Kapoor *et al***[1] dissected the key steps of EGFR signaling pathway in a human GBM cell line. A robust increase of NF-κB (p65/p50 heterodimer)–DNA complex was observed by electrophoretic mobility shift assays in the nuclear extract of GBM cells treated with epidermal growth factor (EGF), indicating nuclear translocation and activation of NF-κB. It was demonstrated that the phosphokinase B or Akt, not p42/44MAPK, was required for EGFR-mediated NF-κB activation. Overexpression of Akt in GBM cells increased activation of NF-κB. The activation of both Akt and NF-κB was reduced in T691 cells, a GBM cell line with a truncated and inactivated EGFR. It was further demonstrated that adaptor protein (Gab1) and relay molecules (SHP2 and PI3-kinase) were sequentially recruited to the EGFR after EGF stimulation. Association of Gab1/SHP-2 and PI3-kinase induced the phosphorylation of Akt, which in turn activated NF-κB. This *in vitro* study has carefully dissected the EGFR-mediated intracellular signaling pathway, showing that NF-κB activation in human GBM with abnormal expression of EGFR is through overexpression of Akt. **Wang *et al***[2] in this issue of *Lab Invest* provided further evidence for activation of NF-κB and Akt in 259 human diffuse gliomas in a high-throughput tissue microarray and fresh-frozen GBM tissues. There was a significant positive correlation between activation of Akt and NF-κB with histological grades of human diffuse gliomas. These results are in concordance with the *in vitro* findings of Kapoor *et al*[1] and support the role of Akt as an upstream regulator for NF-κB activation in human gliomas. Targeting the EGFR-mediated signaling pathway may therefore hold promise in the treatment of these otherwise treatment-resistant tumors.

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VEGFR in breast carcinoma

Vascular endothelial growth factor (VEGF) is a potent endothelial cell mitogen and angiogenic growth factor that enhances endothelial cell invasion through the extracellular matrix (ECM), and hence development of new vasculature. Enhanced VEGF secretion occurs in human cancers, and correlates with increased tumor neovascularization and progression. This suggests that inhibiting angiogenesis may prevent invasive cancer. While various cell types other than endothelial cells express VEGF receptors (VEGFR), little is known about the biological actions of VEGF on nonendothelial cells. The *in vitro* effect of VEGF on nonendothelial cell proliferation and invasiveness was studied by **Fitzpatrick *et al***[1] using human MDA-MB-231 breast carcinoma cells and human HTR-8/SV-neotrophoblast cells. mRNA transcripts for VEGFR-2 and VEGF coreceptors neuropilins-1 and -2 were demonstrable in these breast carcinoma cells. mRNA transcripts for VEGFR-1, -2, and -3 and for neuropilins-1 and -2 were present in the trophoblast cells. Both cell lines expressed transcripts for VEGF-A, -B, -C, and -D. Incubation with exogenous VEGF-A did not affect the proliferation rate of the breast carcinoma or trophoblast cells. However, incubation with VEGF-A reduced the ability of both cell lines to invade through a reconstituted extra cellular matrix (Matrigel), and decreased the cell surface expression of the

urokinase-type plasminogen activator, a molecule required for invasion. Inhibition of VEFG action by inclusion of a VEFG-neutralizing antibody abrogated the VEGF-effect on invasive behavior. They conclude from their study that biological actions of VEGF on nonendothelial cells may differ from the effects of this molecule on endothelial cells. In particular, the authors speculate that VEGF inhibition of the motility and invasive behavior of nonendothelial cells enables them to stay in close proximity to the neovasculature, thereby enhancing tumor cell or trophoblast survival while not inhibiting their proliferation. Regardless of whether this speculation is borne out, this study makes clear that VEGF effects on nonendothelial cells must be taken into account when studying the growth of tumors. This study also dovetails nicely with the report in this *Lab Invest* issue by **Heffelfinger *et al***,[2] in which a VEGFR tyrosine kinase inhibitor, ZD6474, is shown to inhibit the progression of preinvasive mammary lesions in a rat model of mammary tumors. This includes atypical hyperplasia, which is a microscopic lesion not predicted to be dependent upon angiogenesis. VEGF-modulation of both endothelial and nonendothelial cells thus appears to occur, a finding that may provide significant opportunity for exploring new approaches to prevention and therapy in breast cancer.

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Aging decreases susceptibility to acute silicosis

Silicosis is an interstitial lung disease, which results from exposure to silica. Both acute and chronic exposure to silica particles lead to the formation of silicotic nodules in the lung parenchyma, which are composed of macrophages, fibroblasts and abundant collagen. Several studies have suggested that tumor necrosis factor-alpha (TNF-α) that is produced by macrophages appears to play a critical role in the pathogenesis of silicosis.

The aging process, which is associated with a progressive decline in both immune and pulmonary functions, depresses chemotaxis and phagocytosis activity of the macrophage. **Corsini *et al***,[1] a group of researchers from Italy, conducted a study on the immunotoxicological consequences of the defective activation of alveolar macrophages with aging in an experimental model of acute silicosis. It was based on their previous finding that aging was associated with a progressive decline in the ability of rat alveolar macrophages to produce TNF-α in response to lipopolysaccharide. The study employed young (3 months old) and old (>18 months old) rats that were intrathecally instilled with silica. In young rats, silica instillation induced a significant increase in TNF-α, lactate dehydrogenase and cellularity of the bronchoalveolar lavage fluid. In contrast, old rats showed no changes in the bronchoalveolar lavage fluid. These results were confirmed by in vitro studies, where silica failed to induce TNF-α release in alveolar macrophages obtained from old rats. Furthermore, silica exposure in young adult rats resulted in an increase in lung hydroxyproline content, which correlated with increased collagen deposition. Histologic changes were observed only in young adult rats, consisting of reactive inflammation and silicotic nodules in the interstitium. In addition, there was a significant reduction in Fas-mediated apoptosis activity of alveolar macrophages in old rats, which leads to a significant reduction in silica-induced apoptosis of macrophages. A decrease in silica-induced TNF-α production, Fas-mediated apoptosis, and the lack of neutrophils accumulation contributes to the lack of response in aged animals. This study provides a biological explanation for epidemiological findings that the risk of contracting silicosis is higher at younger age. 'Our data suggest that old individuals are not necessarily more sensitive to immunotoxic compounds, and that it is critical to define the impact of age on the toxic response and progression of the disease', concluded Emanuela Corsini, first author of this study. **Blanco *et al***[2] in this issue of *Lab Invest* used a rat model to study the altered expression of adhesion molecules and epithelial-mesenchymal transition in lung carcinomas, which developed following silica-induced chronic inflammation. These studies suggest that inhibition of TNF-α release by macrophages in silica-induced lung injury may prevent silicosis and lung carcinoma formation.

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