Pathology Elsewhere

Laboratory Investigation (2004) 84, 1402–1403. doi:10.1038/labinvest.3700179

Endostatin and its inhibitory effect on endothelial progenitor and circulating endothelial cells

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is essential for the growth of solid tumors and facilitates their metastases. Recently, a number of angiogenesis inhibitors have undergone clinical testing as either a single therapy or in combination with other agents. One of them is endostatin, a cleavage product of collagen XVIII, that has been shown to inhibit the growth of primary tumors and metastases in mouse models and has no known toxic side effects thus far. However, the exact mechanism by which endostatin exerts its antiangiogenic activity remains unclear.

A recently published study by **Schuch *et al*¹** showed that endostatin inhibited vascular endothelial growth factor (VEGF)-induced circulating endothelial cells (CEC). They found that endostatin significantly reduced the number of VEGF-induced peripheral blood mononuclear cells. Furthermore, endostatin antagonized the induction of endothelial progenitor cell mobilization by VEGF and reduced CEC numbers by inducing their apoptosis. The study suggests that CECs are a novel target for endostatin and its inhibition may contribute to endostatin antitumor activity *in vivo*. In addition, CEC relative numbers may serve as a surrogate marker for the biological activity in patients undergoing antiangiogenic therapies.

In this issue, **Van Wijngaarden *et al*²** demonstrated disintegration of blood vessels and hypoxia and anoxia with subsequent tumor necrosis, and that several target genes were induced or suppressed after endostatin treatment. In particular, core binding factor a-1/osteoblast-specific factor-2 (cbfa1/osf2) gene, which was specifically expressed in granulocytes, was suppressed by endostatin treatment. These two papers raised the possibility of inhibiting circulating cells in antiangiogenic therapy, in these instances, inhibition of CEC or circulating granulocytes.

Further studies on endostatin may lead to a better understanding of its mechanism of action and may lead to other, quite possibly more effective anti-tumor therapeutic approaches.

Arief Suriawinata, MD

References

- 1 Schuch G, Heymach JV, Nomi M, *et al*. Endostatin inhibits the vascular endothelial growth factor-

induced mobilization of endothelial progenitor cells. *Cancer Res* 2003;63:8345–8350.

- 2 Van Wijngaarden J, de Rooij K, Van Beek E, *et al*. Identification of differentially expressed genes in a renal cell carcinoma tumor model after endostatin-treatment. *Lab Invest* 2004;84:1472–1483.

Keratinocyte growth factor (fibrinogen growth factor 7) is a paracrine growth factor for normal and neoplastic breast epithelium

Breast cancer is a leading cause of mortality for women in Western nations. An understanding of breast cancer development and progression has enabled major treatment advancements, especially including selective estrogen receptor modulation. Estrogen, or a related ligand, acts via the phosphoprotein estrogen receptors (ER), which are present on cytoplasmic and nuclear membranes. Once the ligand is bound, the receptor undergoes a conformational structural change that permits dimerization and interaction with coactivator molecules. These ER complexes can then interact with nuclear gene transcription sites to promote cellular proliferation; directly with estrogen-responsive elements (ERE), or via a tethered interaction to proteins at AP-1 or SP-1 sites. Peptide growth factors, such as epidermal growth factor (EGF), transforming growth factor (TGF)- α , heregulin, insulin, insulin-like growth factor (IGF)-I and dopamine may independently promote activation of ER. An additional growth factor involved in mammary epithelial proliferation is keratinocyte growth factor/fibroblast growth factor (FGF) 7. It is one of the family of FGFs, which comprises over 22 members.

While it has been shown by others that FGF7 is a potent growth factor for mammary cells, the exact cellular localization of FGF7 and its receptor in normal and neoplastic breast epithelial cells and the potential functions during normal mammary growth and breast cancer development are not known. **Palmieri *et al*¹** elucidated and characterized the role of FGF7 in normal breast development and neoplastic breast cancer growth. Using Western blot analysis, they first determined the specificity of antibodies against recombinant FGF7 and FGF10, determining that antibodies H-73 and C-19 detected FGF7 with no significant crossreactivity with FGF10, and that H-73 binding, in particular, was not affected by the presence of heparin or heparan sulphate. Using H-73 against normal, benign and malignant specimens, they immunolocalized FGF7 to the epithelial and myoepithelial cells of all three. In addition, they found minimal stromal staining in lobular carcinoma *in situ* and invasive ductal carcinoma. These results were similar for the FGF7-receptor (using antibody C-19), except no

stromal staining was noted. They next noted that fibroblast culture medium induced dose-dependent increases in the growth of primary mammary epithelial cells and normal breast epithelial cell lines. This growth was abrogated by the addition of an anti-FGF7 antibody (MAB251), proving that FGF7 is the mitogenic component in breast fibroblast-culture medium. In addition, FGF7 added to a myoepithelial cell line and to malignant breast cell lines of epithelial and myoepithelial origin demonstrated a measurable increase in the rate of cell proliferation. Cell numbers and size were also increased in epithelial–myoepithelial organoid clusters after addition of FGF7. The stimulus for FGF7 release from fibroblasts was tested using various cytokines and estrogen receptor ligands. Of those tested, only $\text{IL-1}\beta$ showed modest, but significant, stimulation of FGF7 release.

These results tie in nicely with the work by **Tamaru *et al***² in this issue of *Lab Invest* who provide further evidence for the relationship between FGF7 (KGF) and breast cancer. Using several malignant breast cancer cell lines, KGF was found to be tightly coexpressed with ER α . The expression of KGF in these cells was not affected by ER ligands such as estrogen and tamoxifen. Furthermore, the presence of KGF and KGF receptor was significantly

correlated with a lower frequency of apoptotic cells, even after the addition of anticancer drugs, suggesting an antiapoptotic, rather than mitogenic role of KGF in human breast cancer. These results highlight KGF as an additional growth factor that can modulate ER function in malignant breast epithelium independent of ER ligands. Targeting of growth factors and growth factor receptors may become an important component along with selective estrogen receptor modulation in the treatment of breast cancer and allow continuing progress in improving the survival curve of breast cancer patients.

Gabriel S Levi, MD

References

- 1 Palmieri C, Robert-Clark D, Assadi-Sabet A, *et al*. Fibroblast growth factor 7, secreted by breast fibroblasts, is an interleukin- 1β -induced paracrine growth factor for human breast cells. *J Endocrinol* 2003;177:65–81.
- 2 Tamaru N, Hishikawa Y, Ejima K, *et al*. Estrogen receptor-associated expression of keratinocyte growth factor and its possible role in the inhibition of apoptosis in human breast cancer. *Lab Invest* 2004;84:1460–1471.