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INSIDE LAB INVEST

Angiogenic inhibition prevents breast tumors

Breast cancer is the second leading cause of cancer deaths in US women. Many women have an increased risk of developing this malignancy either because of a genetic predisposition, or due to morphological evidence of proliferative breast pathologies that are statistically correlated with subsequent disease. The work by **Heffelfinger et al**[1] (p. 989) in this issue highlights a novel strategy for preventing breast cancer formation. The authors have previously documented that neovascularization is a feature of hyperplastic breast pathologies and carcinoma *in situ* (CIS) in both human archival tissue[2] and in the well-characterized 7,12-dimethylbenz[a]anthracene (DMBA) mammary carcinogenesis model in rats.[3] Studies published recently in *Laboratory Investigation*[4] showed that inhibition of endothelial proliferation by administration of the angiogenic inhibitor TNP-470 blocked the formation of CIS and invasive disease in the DMBA model. In their latest paper,[1] these investigators demonstrated that the formation of proliferative pathologies and CIS are dependent upon neovascularization, which is at least partly dependent vascular endothelial cell growth factor (VEGF). Administration of the VEGF Receptor 2 inhibitor ZD6474 profoundly inhibited the development of proliferative lesions and CIS, even when administration was started well after the formation of early mammary pathologies. These studies suggest that targeting angiogenesis may be an important strategy for pharmacological prevention of breast cancer.

References

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4 Heffelfinger SC, Gear RB, Schneider J, *et al*. TNP-470 inhibits 7,12-dimethylbenz[a]anthracene-induced mammary tumor formation when administered before the formation of carcinoma *in situ* but is not additive with tamoxifen. Lab Invest 2003;83:1001–1011.

The waxing—and waning—of tissue antigen expression

The late 1970s saw the dawn of routine diagnostic immunohistochemistry, and the number of well-validated diagnostic antibodies numbers in the hundreds. The possibilities for antibody reagents used in the research setting are almost limitless. Pressed by the need for high-throughput evaluation of immunohistochemical reactivity in tissue, tissue microarrays have become increasingly popular. Over a hundred cores of fixed tissue are embedded in one paraffin block. Single sections cut from such a block can be processed for immunohistochemistry; all tissue samples are thus exposed to identical reaction conditions. This has led to the research algorithm of obtaining cut sections from a tissue microarray source—commercial or otherwise—and staining the sections with one's antibodies of interest. The tissue microarray source may be geographically separated from the research laboratory, possibly on a different continent. Hence, there may be significant temporal delay in the processing of tissue sections, particularly if microarray paraffin blocks are batch-cut so as to minimize loss of tissue during refacing. Significant temperature gradients also may be encountered during shipment. Lastly, investigators may choose to delay processing of the tissue sections until a time of convenience. The question must therefore be addressed: how sturdy is this algorithm? In this issue, **DiVito, Charette et al**[1] (p. 1071) provide an alarming result. Using a previously validated tissue microarray of human breast carcinoma,[2] tissue sections containing 200 cores were examined for the antigenic stability of cytokeratin, estrogen receptor, and Ki-67. Immunohistochemical reactivity following up to 3 months of storage was compared in a semiquantitative fashion to that of freshly cut tissue sections. They found that slides stored under ambient conditions (room temperature and air) for 3 months exhibited marked degradation of all three target antigens, sometimes to the point of unreadability. Dip-coating in paraffin and storage in a nitrogen atmosphere at room temperature preserved between 72 and 99% of antigen reactivity, depending upon the marker and detection system used. Use of paraffin coating or nitrogen storage alone protected slides, but to a lesser degree. At the very least, this study provides a major cautionary note for the use of tissue microarrays. It also behooves the source or recipient laboratories to validate the preservation of antigen reactivity for the reagents of interest. On a broader front, one may consider the need for

paraffin dip-coating and nitrogen storage for any delayed use of tissue microarray sections.

References

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Inducible *PAX3–FKHR* expression reveals clues to rhabdomyosarcoma tumorigenesis

Perhaps with the exception of hematologic malignancies, nowhere has the clinical relevance of molecular diagnostics been more clearly demonstrated than in the pediatric 'small blue cell tumors'. For example, the 2;13 chromosomal translocation that is characteristic of alveolar rhabdomyosarcoma (ARMS) results in juxtaposition of the *PAX3* (or *PAX7*) and *FKHR* genes. *PAX–FKHR* fusion transcripts are not only specific for ARMS but expression of *PAX3–FKHR* identifies a high-risk patient subgroup while *PAX7–FKHR* expression correlates with a more favorable prognosis. However, details of the molecular pathways of tumorigenesis in this pediatric soft-tissue malignancy remain to be elucidated. Inside this issue, **Tomescu *et al***[1] (p. 1060) provide new insights into the cellular and molecular pathways that are altered in ARMS. These investigators generated a short-term inducible rhabdomyosarcoma cell line in which *PAX3–FKHR* is fused to a modified estrogen receptor ligand-binding domain such that expression of the chimeric transcription factor is stimulated by 4-hydroxytamoxifen. Using this short-term culture system, along with stably transfected (long-term) rhabdomyosarcoma cell lines, it was determined that induced expression of *PAX3–FKHR* upregulates the *CXCR4* gene, which encodes a G-protein coupled chemokine receptor. Recent studies by this same group showed that treatment of ARMS cells expressing relatively high levels of *CXCR4* with the cognate ligand (*CXCL12/SDF-1*) resulted in their increased migration, adhesion and matrix degradation. In the current study, wild-type *PAX3* was also upregulated while wild-type *PAX7* was downregulated by induced expression of *PAX3–FKHR*. The discovery of genes regulated by *PAX3–FKHR* helps to clarify molecular pathways of ARMS tumorigenesis and progression.

References

1 Tomescu O, Xia S, Strzelecki D, *et al*. Inducible short-term and stable long-term cell culture systems reveal that the PAX3-FKHR fusion oncoprotein regulates CXCR4, PAX3, and PAX7 expression. Lab Invest 2004;84:1060–1070.

Dissecting PTEN and EGFR signaling pathways in gliomas

Diffuse gliomas are the most common primary brain tumors in adults. Glioblastoma multiforme (GBM) is the most malignant (survival usually less than 1 year) and unfortunately the most common of these infiltrating neoplasms. This tumor category also includes diffuse and anaplastic forms of astrocytoma and oligodendroglioma, and the mixed gliomas (oligoastrocytomas). Except for a subtype of anaplastic oligodendrogliomas having losses of chromosomes 1p and 19q, there are no effective therapies at present. Furthermore, a troublesome feature of lower grade gliomas is that they tend to progress to higher-grade neoplasms and there are no proven therapeutic interventions while the tumor is still low grade. While molecular mechanisms of glioma tumorigenesis and progression have begun to emerge, they remain incompletely understood. Inside this issue, **Wang *et al***[1] (p. 941) investigated downstream targets of two known signaling pathways in gliomas: PTEN (phosphatase and tensin homolog) and EGFR (epidermal growth factor receptor). These investigators examined the activation status of Akt, NFκB, and Stat3 using a state-of-the-art tissue microarray containing human tumor samples from 259 diffuse gliomas. They found that the activation state of Akt and NFκB correlated with glioma grade and a correlation between activation of Akt and NFκB was suggested. Next, to explore a possible functional relationship between these two signal transduction molecules, *in vitro* studies were performed using U251MG GBM cells. Stable expression of PTEN by this cell line or exposure to PI3-kinase inhibitors resulted in decreased activation of Akt and a concomitant decrease in NFκB binding activity. These findings suggest that activation of Akt and NFκB plays a role in glioma progression and that Akt activation may regulate NFκB in high-grade gliomas. It will be interesting to now determine whether Akt inhibition can inhibit the aggressive behavior of these neoplasms.

References

1 Wang H, Wang H, Zhang W, *et al*. Analysis of the activation status of Akt, NFκB, and Stat3 in human diffuse gliomas. Lab Invest 2004;84: 941–951.