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MODERN PATHOLOGY

ROS1 immunohistochemistry to detect ROS1 rearrangements in lung cancer

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ROS1 gene fusions, recently described in a small subset of lung cancers (1–3%), have therapeutic implications in that small-molecule inhibitors have shown clinical efficacy. These relatively rare cases must be efficiently identified. Fluorescence *in situ* hybridization and sequencing-based techniques can be challenging to apply universally. Yoshida *et al* used an anti-*ROS1* rabbit monoclonal antibody on tissue microarrays containing lung carcinomas for which the *ROS1* rearrangement status was known in order to determine how well it correlated with molecular status. *ROS1*-rearranged tumors ($n = 17$) generally showed diffuse moderate to strong immunoreactivity, whereas 69% of the tumors lacking *ROS1* rearrangements ($n = 253$) were completely negative for the stain. Of the tumors lacking *ROS1* rearrangements, 31% showed primarily weak and focal reactivity. The authors created a scoring system with impressive sensitivity and specificity (94% and 98%, respectively), but its application requires careful observation of the distribution and intensity of the immunoreactivity.

ALDH1A1 reactivity is associated with poor prognosis in esophageal squamous cell carcinoma

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Using increased aldehyde dehydrogenase 1A1 (ALDH1A1) reactivity as a selection criterion, Yang *et al* identified cancer stem-like cells from the esophageal squamous cell carcinoma cell line EC109. These cells showed the cardinal properties of self-renewal, differentiation, and tumor initiation in model systems. They also exhibited augmented invasive and metastatic abilities in a mouse xenograft model. Elevated

expression of other markers associated with stemness and invasion was also noted. To test the relevance of these findings in clinical samples, the authors stained sections with ALDH1A1. The increased levels were significantly associated with histologic grade, depth of invasion, lymph node metastases, and UICC (Union for International Cancer Control) grade. Higher levels of ALDH1A1 were associated with poor patient outcome as assessed by Kaplan–Meier analysis. It is possible that increased content of stem-like cells in esophageal squamous carcinoma is associated with more aggressive tumor biology.

Laboratory Investigation

Monitoring of biomarkers and drugs during surgery

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Bojko and colleagues used porcine lung and liver organ models in applying a novel technology to detect drugs, metabolites, and biomarkers in an organ during surgery. This method, termed solid-phase microextraction, has been used with animal products in other fields, but its application to surgical monitoring is novel. Flexible microprobes are inserted directly into tissue, causing no damage and virtually no disturbance of the organ. Special coatings on the microprobes allow extraction of analytes directly from the tissue. This feasibility study enabled monitoring of multiple endogenous compounds and drugs in addition to metabolic perturbations, perfusion, cold ischemic time, and reperfusion. The virtually real-time data collection is ideal for certain experimental models and may have applications to clinical surgery because of the method's ease of use, capacity for direct monitoring, and low level of invasiveness.

Suppressing the Th17 response in allergic rhinitis patients

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The chronic inflammatory airway disease allergic rhinitis, a common scourge worldwide, is induced by allergens and mediated by T cells. Despite extensive study, its pathogenesis is poorly understood. Recently, increased T helper type 17 (Th17) cells accompanied by interleukin-17 (IL-17) have been associated with allergic rhinitis, and serum IL-17 levels appear to be proportional to severity. The aryl hydrocarbon receptor (AhR) is a transcription factor regulated by ligands with potent immunomodulatory function through regulation of T-cell and dendritic cell (DC) differentiation mediated by ligand activation. Wei *et al* tested *in vitro* the effect and mechanisms of using a nontoxic AhR ligand on human Th17 cells from allergic rhinitis patients. Treatment inhibited the production of proinflammatory cytokines by CD4⁺ T cells and DCs while increasing the anti-inflammatory cytokine IL-10. Differentiation of Th17 cells was also suppressed, suggesting that AhR may be a key regulator of allergic rhinitis and amenable to therapeutic intervention.

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AITL shows recurrent inactivating mutation of *RHOA* GTPase

Using whole-exome sequencing, array comparative hybridization, and gene expression studies, Yoo *et al* studied angioimmunoblastic T-cell lymphoma (AITL) paired with normal tissues. In nine AITL specimens, somatic mutations were noted across 47 func-

tional genes, 29 of which were present in at least two samples. Confirmation in 45 additional AITL cases showed *RHOA* mutations in about 53% of cases. The most commonly encountered mutations were those involving *RHOA* and *CD28*. *RHOA* is a small GTPase protein, and the observed G17V mutation resulted in loss of GTPase activity in biochemical assays. This codon is sometimes mutated in other cancers as well. The loss-of-function *RHOA* mutation results in increased downstream AKT activation. Mutations in *CD28* probably lead to downregulation of T-cell-receptor signaling with transformation of T cells.

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Rejuvenating stem cells in muscles restores strength lost in aging and injury

The local microenvironment is known to be critical for tumor development and progression. Mechanical properties of tissue are involved in developmental processes as well as mature-tissue homeostasis and are also a factor in tumor development. Mouw *et al* recently

reported that tissue mechanics can regulate expression of PTEN (phosphatase and tensin homolog deleted on chromosome 10) via modulation of microRNA (miRNA) expression. Matrix stiffness was strongly associated with an increase in microRNA expression that promoted integrin-mediated activation of β-catenin and MYC. Specifically, miR-18a was induced, which led to a reduction of PTEN expression in both mouse and human breast cancer samples. This appears to be both directly and indirectly mediated by a reduction in the expression levels of homeobox A9 (HOXA9), a known regulator of PTEN. Thus tumor matrix can regulate the PI-3-kinase/AKT pathway through mechanical properties, yet another avenue of tumor promotion.

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Angiosarcoma has recurrent mutations in angiogenesis genes *PTPRB* and *PLCG1*

Some cases of angiosarcoma have been shown to be associated with aberrant activation of angiogenic pathways. Genetic aberrations have been described in angiosarcomas arising in various clinical situations, including spontaneous cases as well as those arising secondary to ionizing radiation or in the setting of chronic lymphedema. Behjati *et al* used whole-genome and whole-exome sequencing approaches to demonstrate novel recurrent mutations in two genes known to regulate angiogenesis: *PTPRB* and *PLCG1*. The mutations were present in 26 and 9% of cases, respectively. *PTPRB*, which encodes an endothelial phosphatase that negatively regulates vascular growth factor tyrosine kinases, was found

to harbor truncating mutations that prevent it from being an effective inhibitor. *PLCG1*, a phospholipase signal transducer downstream of tyrosine kinases that are important in angiogenesis, contained an activating mutation. Additional aberrations were noted such that fully 38% of the tumors showed genetic evidence of angiogenic activation.

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