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INSIDE LAB INVEST

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Measuring molecular heterogeneity in human cervical cancer

See page 1206

Immunohistochemistry is playing an increasing role in establishing prognosis and guiding therapeutic options for human cancers. A key issue, however, is tumor heterogeneity in the expression of antigenic proteins. In this era of research using 'tissue microarrays' (TMAs), sampling error becomes an even greater concern because of the small samples. In this issue, Iakovlev *et al* sought to optimize tissue sampling to compensate for tumor heterogeneity. Their test system was the enzymatic marker of intrinsic tissue hypoxia, carbonic anhydrase IX (CAIX), as a surrogate marker for global tumor oxygenation status in human invasive cervical carcinoma. This is relevant because tissue oxygenation may affect the responsiveness of many cancers to radiotherapy and novel biological therapies.

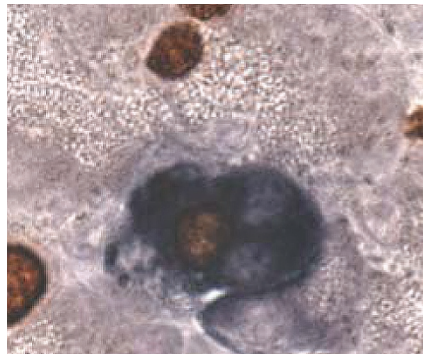
Using direct *in situ* pO_2 measurements, the authors demonstrated that the size of the tissue sample (either by direct biopsy or via simulated TMA core sampling) was the major factor affecting the accuracy of using CAIX immunohistochemistry as a surrogate marker of tissue hypoxia. Multiple biopsies or cores also improved global tumor assessment, provided that the samples were sufficiently separated in space.

The authors conclude that rigorous attention to sampling and improved analytical methods will improve the reliability of measuring molecular markers by immunohistochemistry. However, immunohistochemistry for CAIX was inferior to pO_2 measurements as a tool for assessment of tumor oxygenation status.

Evaluating β-cell expansion in the pre-diabetic state

See page 1240

A long-standing tenet of the pathogenesis of type 1 diabetes (T1D) is that the majority of β-cells are lost prior to the initial diagnosis. This notion has been challenged, as histological analysis of pancreata obtained from long-standing T1D diabetes patients identified continued chronic insulitis and evidence of pancreatic β-cell



replication. Data on β-cell destruction from the pre-diabetic state have been limited. In this issue, Kauri *et al* describe the dynamics of β-cell development prior to overt insulitis in a diabetes-prone rat strain, BBdp.

These authors have previously shown increased numbers of extra-islet insulin-positive clusters (EICs) in these animals. EICs have been considered an indicator of islet neogenesis and are thought to originate from extra-islet progenitor cells, possibly from ducts. In the current study, the authors tested whether there is a defect in islet expansion in BBdp rats due to innate islet cell abnormalities. Islet development, proliferation, and apoptosis were measured in pre-diabetic animals and controls. Their data suggest that β-cell defects that precede the immune attack not only may attract immune cells into the islet but could reduce the capacity of adult β cells. Partial blockage at the G1/S cell cycle boundary may contribute to the limited capacity for β-cell expansion in the pre-diabetic state.

A key question remains whether similar β-cell abnormalities occur in T1D patients. Since determination of T1D susceptibility remains a formidable task even within families with genetic predisposition, experimental studies such as that presented by Kauri *et al* are critical for uncovering the β-cell defects present in the pre-diabetic state, and therefore interventional windows in patients at risk for T1D.

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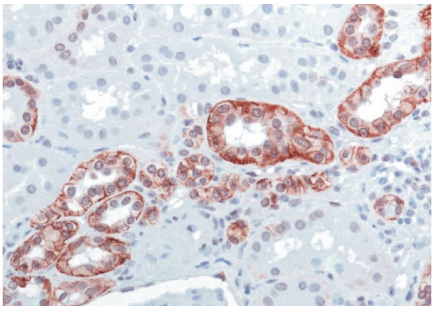
Nuclear E-cadherin and mutated VHL in clear-cell renal cell carcinoma

See page 1252

Neoplasia in von Hippel-Lindau (VHL) disease results from germ-line VHL mutation and subsequent somatic inactivation of the remaining allele, thus fulfilling Knudson's 'two-hit' tumor suppression model. The loss of functional VHL tumor suppressor gene is a feature of both syndrome-associated and sporadic neoplasms such as renal cell carcinoma, pheochromocytoma, and

capillary hemangioblastoma. Biallelic loss of VHL is associated with sporadic clear-cell renal cell carcinoma (CC-RCC). Among numerous cellular functions, the protein acts as a substrate recognition element of the E3 ubiquitin-protein ligase complex, which targets proteins for proteolytic degradation.

Under normoxic conditions, pVHL binds to hypoxia-inducible factor alpha (HIFα) and targets this transcription factor for ubiquitin-mediated proteolysis. With hypoxia, stabilization of HIFα results in the activation of vascular endothelial growth factor and a host of other HIF-regulated genes, which are believed to promote tumorigenesis. VHL loss and HIF activation have an established role in renal epithelial oncogenesis, and intense



attention is being given to the involvement of downstream HIF-target genes and proteins. One of these is E-cadherin, a transmembrane constituent of adherent cell–cell junctions whose loss is a key feature of many epithelial cancers.

In this issue, Gervais *et al* demonstrate that VHL mutation may lead to an abnormal cellular distribution of E-cadherin. Specifically, a subset of CC-RCC with relatively good prognosis demonstrated positive VHL immunoreactivity and nuclear localization of E-cadherin. DNA sequencing revealed subtle mutations in the VHL gene in this subset of CC-RCC. In CC-RCC xenografts grown in severe combined immunodeficient mice, nuclear E-cadherin localization also occurred in tumors without functional VHL. The results suggest that regulation of E-cadherin pathways by VHL plays a major role in the biologic behavior of human CC-RCC and that these two antigens may be clinically useful prognostic markers.

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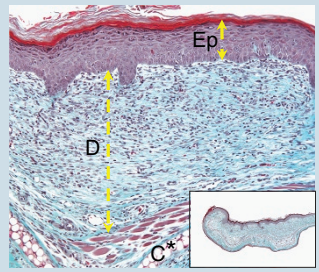
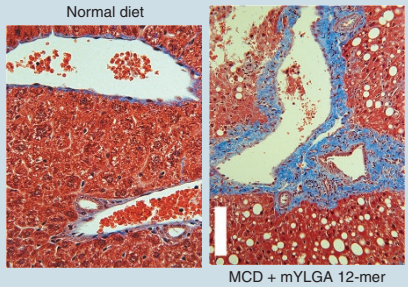


Limiting dermatitis by mast (cell) action Factors that contribute to acquired and innate immunity are well studied, but the mechanisms that limit the extent and duration of inflammatory processes are less clear. A study by pathologist Stephen Galli and colleagues examined the role of mast cells in contact hypersensitivity dermatitis (CHS). Using genetically engineered mice they show that mast cell deficiency exacerbates experimental CHS induced by urushiol, the allergen-containing sap of poison ivy and poison oak. Engraftment of the mice with bone marrow–derived cultured mast cells limited the extent of CHS, but not if the donor mast cells were harvested from mice deficient in interleukin-10 (IL-10). The study also shows that mast cell–derived IL-10 also limits the extent and duration of hapten and UVB damage–induced dermatitis. These data identify a previously unrecognized function for mast cells and mast cell–derived IL-10 in limiting leukocyte infiltration, inflammation, and tissue damage.
Nature Immunology 2007;8:1095–1104; doi:10.1038/ni1503

For whom the bug tolls: TLR4 and septic shock Sepsis claims more than 200,000 lives each year in the United States. Endotoxin, or lipopolysaccharide, is a component of gram-negative bacteria that triggers this inflammatory response. The toxin binds to a lipopolysaccharide-binding complex and induces a signaling cascade characterized by the activation of the transcription factor NF-κB. This transcription factor then turns on genes encoding certain phagocytic cytokines and chemokines. Ligands for Toll-like receptor 4 are thought to be involved in this cascade, but the details are unclear. A study from *Nature Medicine* reports that myeloid-related protein-8 and -14 (Mrp8 and Mrp14) jointly contribute to sepsis pathogenesis and its lethal effects. These proteins are released after phagocyte activation, and mice lacking the Mrp8-Mrp14 complex are protected from the endotoxin-induced shock. Because the Mrp8-Mrp14 complex amplifies phagocyte activation, it may be a promising therapeutic candidate.
Nature Medicine 2007;13:1042–1049; doi:10.1038/nm1638

Scavengers can be prothrombotic! Dyslipidemia is a dominant risk factor for atherothrombotic disorders, but how lipid abnormalities contribute to enhanced platelet reactivity and clotting is unknown. Unraveling this relationship is considered critical to the prevention of coronary artery disease. A recent study in *Nature Medicine* has identified a crucial role of the CD36 scavenger receptor in dyslipidemic thrombosis. The fact that mice lacking CD36 are protected from dyslipidemic thrombosis highlights the role of CD36 as a receptor for oxidized LDL products and as an oxidative stress sensor that activates platelets. The interaction between these oxidized lipids and platelet CD36 explains the link between dyslipidemia, oxidant stress, and thrombosis.
Nature Medicine 2007;13:1086–1095; doi:10.1038/nm1626



When Fas met fat Abnormal Fas activation is a key component of hepatocyte death and the development of both alcoholic and nonalcoholic steatohepatitis. Met protects against this mechanism of apoptosis by competing with Fas ligand binding to Fas. A recent study from the University of Pittsburgh Department of Pathology shows that the excess lipids involved in human fatty liver disease disturb the association of Met with Fas, thus sensitizing hepatocytes to Fas ligand. Molecular analysis showed a specific YLGA motif in Met to be the primary Fas binding site. This was confirmed *in vivo* by treating mice with a YLGA-containing peptide that binds the extracellular portion of Fas, antagonizes Fas ligand-mediated hepatocyte death, and protects mice from nonalcoholic steatohepatitis. This offers a potential therapy for nonalcoholic steatohepatitis, which currently has limited treatment options.
Nature Medicine 2007;13:1078–1085; doi:10.1038/nm1625