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MODERN PATHOLOGY

Mutational patterns and significance in acute myelogenous leukemia

See page 706

Using target-capture enrichment followed by next-generation sequencing, Ohgami *et al* characterized mutations in 19 genes from 93 patients with acute myelogenous leukemia (AML). Of these, 79% showed at least one mutation. AML patients with recurrent genetic abnormalities were less likely to show mutations than those with myelodysplasia-related changes. Complex karyotype AML showed a higher prevalence of *TP53* mutations, consistent with the abrogation of apoptosis expected with genomic insults. *ASXL1* and *U2AF1* mutations were associated with AML with myelodysplasia-related changes, and *U2AF1* was specifically enriched for trilineage morphologic dysplasia. In multivariate analysis, *U2AF1* and *TP53* mutations were independent predictors of overall survival and disease-free survival, respectively. This study shows that mutations in specific genes segregate with morphologic and cytogenetic classifications of AML and provides further insight into clinical behavior and molecular pathogenesis.

Ki67 index methods in pancreatic neuroendocrine tumors

See page 686

An essential component of pancreatic neuroendocrine tumor classification is assessment of Ki67 proliferation

rates. Reid *et al* examined four methods of measurement for the index: (i) “eyeball” estimation, (ii) an automated count, (iii) a manual eye count, and (iv) a camera-captured/printed image. Eyeballing was fast and cheap but ultimately inaccurate and unreliable. Surprisingly, automated counting was not only the most expensive method but was also operator-dependent, posing a significant risk of variably overcounting non-tumor cells. The manual eye count was time-consuming and had poor reproducibility. Camera-captured/printed image counting took an intermediate length of time as compared with the other methods, and it was highly reproducible and cost-effective because it used standard imaging equipment readily available to most pathologists. The authors suggest that this is the most suitable method for clinical applications. Clinical validation of this method would seem to be in order to ensure that its measurements recapitulate the expected differences in the clinical behavior of pancreatic neuroendocrine tumors.

Laboratory Investigation

Phosphoprotein detection in breast cancer tissue

See page 561

Assessment of phosphoproteins can yield important indicators of pathway activation. Gündisch *et al* examined the preanalytical parameters of cold ischemia time, temperature before and during tissue fixation, and sample type. Using quantitative reverse-phase protein arrays, the authors evaluated 9 proteins and 16 phosphoproteins in formalin-fixed paraffin-embedded breast cancer tissue from a mouse model and human clinical samples. Cold temperatures before and during fixation produced markedly superior reactivity preservation of phosphoproteins. Some phosphoproteins were more sensitive than others to cold ischemia time. Core

biopsies resulted in improved retention of phosphoprotein reactivity compared with their cognate resection specimens. Rapidly fixing small biopsy specimens (less than 1 mm) at a cold temperature is the preferred modality for analyte preservation. Consistent with previous studies, some phosphoproteins were much more labile than others. This knowledge can be used to choose the markers best suited to the handling of tissue specimens as well as to determine how to modify the way in which tissues are processed for such studies.

VEGF signaling in compensatory lung growth

See page 456

Using a mouse model of compensatory lung growth following unilateral pneumonectomy, Matsui *et al* investigated the role of vascular endothelial growth factor (VEGF) signaling in both new hyplastic growth and hypertrophy of existing alveoli. In adult mice, this compensatory growth led to restoration of original volume, surface area, alveolar count, and protein content within two weeks. Compensatory growth was significantly suppressed by both VEGF neutralizing antibody treatment and in VEGF receptor-1 tyrosine kinase-deficient mice. This was partially mediated by reduced recruitment of VEGFR1-positive cells from the bone marrow compartment. The recruited cells expressed surfactant proteins confirming pneumocyte differentiation. This study confirms the importance of VEGF signaling in adult lung growth and suggests mobilization of bone marrow progenitors as a mechanism.

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Genomic heterogeneity of prostate cancer

In a study published in *Nature Genetics*, Cooper *et al* colleagues performed genome-wide DNA sequencing of distinct areas of prostatic tissue from three men with multifocal prostate cancer. Prostate cancer is known to be multifocal in some cases, and previous allele-loss findings have suggested that the foci are independent, although other studies support relatedness. The study included areas that were morphologically compatible with nonneoplastic prostate and were found to have levels of mutations as high as in the malignant areas. The findings suggest the presence of overlapping fields of mutations in prostatic carcinogenesis. Within single cancer nodules, they showed clones coexisting with distinct *ERG* translocations consistent with multiple independent translocation events. The presence of numerous subclones and overlapping clonal fields suggests an ongoing mutational milieu. This genetic heterogeneity helps explain the lack of agreement among previous studies and has important implications for single-agent targeted therapies because it suggests that selection for resistant clones could be quite rapid.

Nature Genetics 2015; 47:367–372; doi:10.1038/ng.3221

Mass-spec imaging of the proteome of tissue samples

As reported in *Nature Medicine*, Guo and colleagues used small tissue biopsy specimens to rapidly produce a permanent digital file, cataloging and quantifying more than 2,000 proteins with a high degree of reproducibility. They applied this method to samples of renal cell carcinoma. Not surprisingly, unsupervised clustering not only produced clear distinctions between normal kidney and renal cell carcinomas but also clearly distinguished the papillary and clear cell subtypes. This analytic methodology provides a layer of proteome information to complement and correlate with DNA genotyping, messenger RNA, microRNA, and metabolic expression studies and helps to both support and extend the traditional histologic classification scheme for renal cell carcinoma. This technology will have broad application for study of other tissue and cancer types.

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Inhibiting the NLRP3 inflammasome

During periods of glucose restriction, ketosis supplies β -hydroxybutyrate (BHB) and acetoacetate to allow production of adenosine triphosphate. Prolonged fasting, which leads to ketosis, is known to reduce inflammation. In a study reported in *Nature Medicine*, Youm *et al.* demonstrated that BHB inhibits the NOD-like receptor (NLR) family, pyrin domain containing protein 3 (NLRP3) inflammasome. NLRP3 senses many pathogen-derived, environmental, and host-derived factors and ultimately ignites an inflammatory cascade leading to cell death known as pyroptosis. This discovery provides a mechanistic link between ketosis and innate immune suppression. *NLRP3*-mediated processes are also implicated in a variety of common diseases, such as atherosclerosis, type 2 diabetes, and gout. Gain-of-function mutations in *NLRP3* are seen in inherited cryopyrin-associated periodic syndromes. In another paper, Coll *et al* describe a small-molecule inhibitor of the NLRP3 inflammasome that might have important clinical applications in these diseases.

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