INSIDE THE USCAP JOURNALS

## MODERN PATHOLOGY

MDM2 levels in dedifferentiated liposarcoma
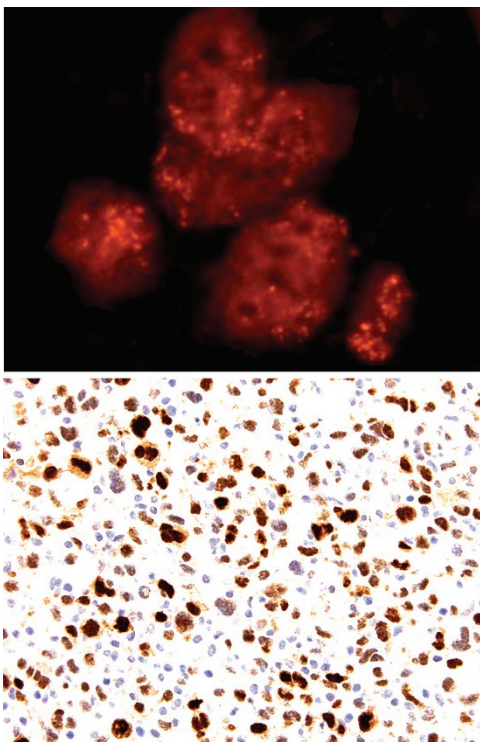
Amplification of the 12q13–15 chromosomal region involving the murine double minute-2 (*MDM2*) locus is characteristic of both well-differentiated and dedifferentiated liposarcoma. Dedifferentiated liposarcoma, the more aggressive form, can be graded using the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC, or "French") system. The system has three grades—low (1), intermediate (2), and high (3)—based on combined scores for tumor differentiation, mitotic rate, and level of tumor necrosis. The fold amplification of *MDM2* is high but variable between cases. Jour and colleagues used fluorescence *in situ* hybridization to characterize the amplification level of *MDM2*. The technique is very helpful diagnostically in cases when the biopsy lacks the characteristic lower-grade adipocytic component. Although the grade 3 dedifferentiated liposarcomas behaved more aggressively in terms of local recurrence than lower-grade liposarcomas on multivariate analysis, higher *MDM2* amplification levels did not correlate with local recurrence independently.

### Grading consequences of PTEN loss in prostate biopsies
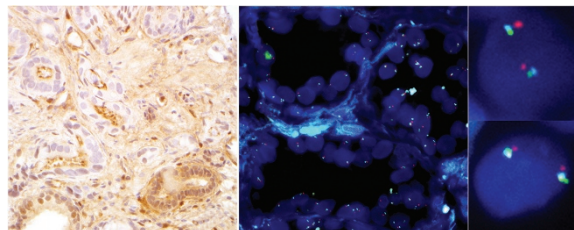
PTEN is a classic tumor suppressor gene encoding a protein that dampens signaling through the PI-3-kinase/AKT pathway. It is the causative gene in Cowden syndrome and is somatically inactivated in several types of cancer. In a study reported in this issue, Lotan *et al* assessed PTEN protein loss in patients whose tumors were given a Gleason score of 6 on biopsy but were upgraded to 7 or more at prostatectomy. Immunohistochemistry of prostate cores revealed PTEN loss in 18%, and examination of the *PTEN* locus using fluorescence *in situ* hybridization showed high concordance of gene deletion and protein loss. Logistic regression analysis indicated that loss of PTEN correlated with an increase to a Gleason score of 7 or greater upon examination of the prostatectomy specimen even when adjusting for all other applicable parameters. Thus, assessment of additional biomarkers could augment and improve the accuracy of Gleason grading in prostate core biopsies.

Laboratory Investigation

### Circulating cancer stem cells and colon cancer diagnosis
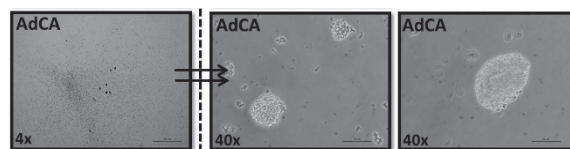
Assessment of circulating tumor cells (CTCs) in the blood is emerging as a potentially important component of cancer surveillance. CTCs can be identified before it is possible to detect metastatic disease radiologically. Kantara *et al* focused on a subset of CTCs with properties of cancer stem cells (CSCs) believed by some to be responsible for distant failure due in part to their therapeutic resistance. They isolated a relatively pure cellular fraction of CTCs both from

their xenograft mouse model using HCT-166 cells and from colon cancer patients who expressed keratin 19 and characteristic CSC markers. These cells were capable of spheroid cell growth, a functional property of CSCs, and could not be isolated from subjects without colon cancer. Some CTCs have marker expression and functional properties of CSCs, and markers of CSCs can be used to isolate these cells from the blood.

Molecular subclassification of diffuse large B-cell lymphoma from FFPE samples

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Dataset by qRT-PCR using FFPE specimens
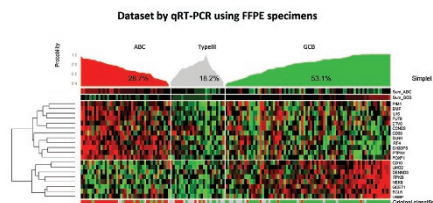
Gene expression profiling, which has been successfully applied to cases of diffuse large B-cell lymphoma (DLBCL), distinguishes three subtypes: activated B cell–like (ABC), germinal center B cell–like (GCB), and type III. The ABC subtype has an inferior prognosis. Such cell-of-origin characterization creates groups with more uniform outcomes than when disease is treated as a homogeneous cohort. Xue *et al* demonstrate that these profiles can be determined from fresh-frozen and formalin-fixed paraffin-embedded (FFPE) samples using massively parallel reverse transcription-PCR using large training and validation sets. Their methodology creates cohorts with expected rates of response to standard DLBCL therapies and confirms that the ABC subgroup is associated with constitutive nuclear factor-κB pathway activation and poor prognosis. This methodology could be applied in a clinical setting using FFPE samples when nonfixed samples are unavailable.

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Predicting the effects of noncoding somatic mutations in cancer

Somatic mutations in noncoding DNA are commonly encountered in cancer, but their effects on gene expression and function are poorly understood. In a study reported in *Nature Genetics*, Fredriksson and colleagues examined noncoding somatic mutations in 505 cancer cases across 14 types and their correlation with RNA expression levels. The results confirm the strong association of the recurrent

mutations in the *TERT* promoter region first recognized in melanoma, and increasingly in many other cancers, with increased TERT RNA expression. They further demonstrate that these *TERT* promoter mutations are associated with increased expression of the nearby gene *CLPTM1L*. This relationship holds across many cancer types. However, the authors failed to find a strong correlation between any other noncoding somatic mutation and cognate gene expression. It therefore seems that the recurrent *TERT* promoter mutations are exceptional rather than the rule, and that most noncoding somatic mutations do not affect the expression levels of nearby genes.
Nature Genetics 2014;46:1258–1263; doi:10.1038/ng.3141
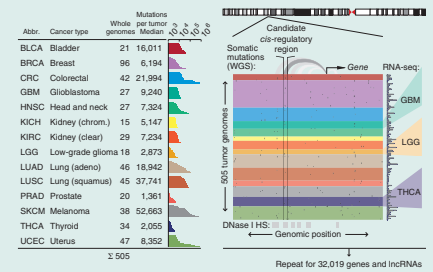
Hepatocellular carcinoma genomes across ancestral populations

Hepatocellular carcinoma (HCC) is the third most common cause of cancer deaths worldwide. Etiologies are varied and segregate with ancestry. Hepatitis B virus infection predominates in East Asia and Africa, whereas hepatitis C virus is prevalent in Japan. Aflatoxin B1 exposure is a strong risk factor in China and Africa, and alcohol-related liver disease is prominent in Western societies. Examining hundreds of HCC genomes from various regions, Totoki *et al* identi-

fied some 30 candidate driver genes in 11 core pathways. Hotspot *TERT* promoter mutations (or focal *TERT* amplification) and integration of viral genome were present in 68% of cases, suggesting that TERT activation is a critical ancestry-independent driver. Activation of the PI3K-mTOR pathway was common, as were alterations in the β-catenin, p53-Rb, chromatin remodeling, and Nrf2-Keap1 pathways. Although a unique mutational signature was noted in Asian cases, there was much commonality of ultimate pathway effects despite variable etiology and ancestry.
Nature Genetics 2014;46:1267–1273; doi:10.1038/ng.3126
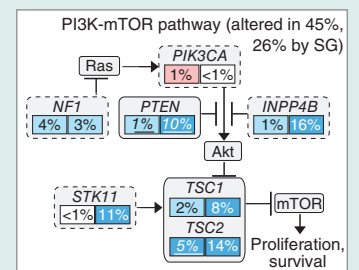
In silico assembly of cancer protein networks

Although we have identified a considerable number of cancer genomic mutational landscapes, we lack experimental confirmation of specific effects of mutations on protein-interaction networks and phenotypes. AlQuraishi and colleagues used a multistate statistical mechanics

model incorporating both genomic and biophysical data sets to predict SH2-phosphoprotein network function in normal and human cancer cells. Using the Cancer Genome Atlas data sets and biochemistry experimental data, they found that mutations involving phosphoproteins often create new protein–protein interactions but mutations involving SH2 domains virtually invariably result in loss-of-protein interactions. Some mutations are catastrophic and eliminate all interactions; others are more selective, with variable effects on protein networks. Somewhat surprisingly, rare or idiosyncratic mutations are often as functionally consequential as recurrent mutations. These efforts expand our understanding of mutations' effects on protein networks and can be used to focus experiments. They also suggest potential therapeutic implications of novel or unusual mutations.
Nature Genetics 2014;46:1363–1371; doi:10.1038/ng.3138