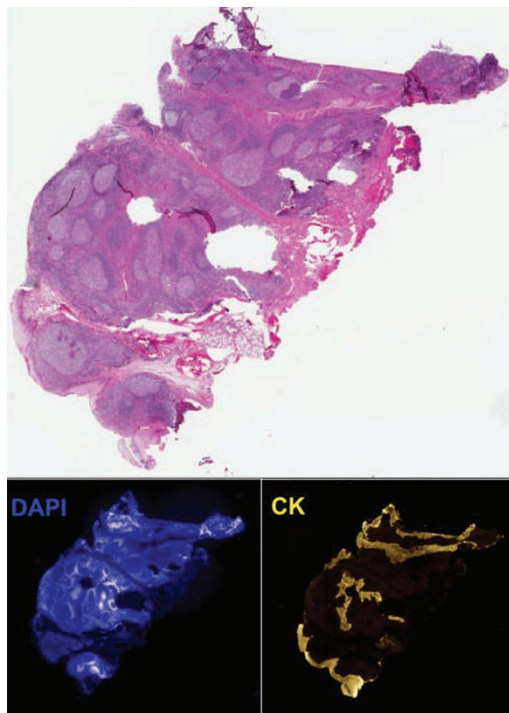


INSIDE THE USCAP JOURNALS

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Imaging advances and applications in medical diagnostics and discovery



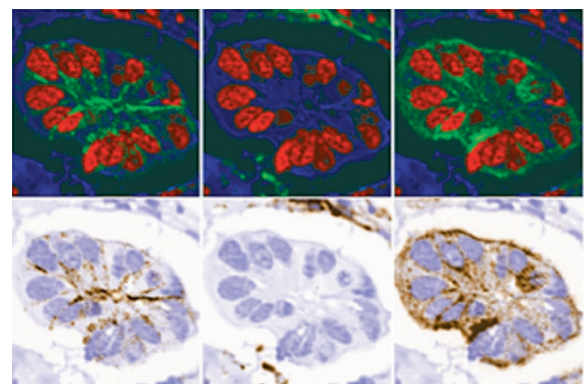
Laboratory Investigation's themed issue this month focuses on recent advances related to cellular imaging techniques in cancer research, including the fields of tumor biology, biomarker discovery, objectification of biomarker expression, and quantification of disease heterogeneity. A primary focus of the issue is the morphological analysis of tissue sections, one of the foundations of translational research and diagnostic surgical pathology. In addition to their longstanding roles in research and diagnosis, morphological techniques such as immunohistochemistry, immunofluorescence, and *in situ* hybridization enable pathologists to make better prognostic and predictive evaluations. Diagnostic pathology still relies heavily on subjective morphological evaluation via classic light microscopy techniques that have not evolved significantly for generations. With the advent of computerized image analysis, morphological analysis can now be quantified, resulting in increased precision and consistency. We can now objectively measure novel parameters; for example, we can simultaneously detect multiple markers, spatial cellular relationships, and tumor heterogeneity. These developments will most likely lead to a more precise personalized approach to cancer therapy.

The first review, by Cooper *et al*, focuses on developments in quantitative imaging technologies that enable the integration of image features with genomic and clinical data. One of the exciting breakthroughs discussed relates to molecular and clinical associations that may be revealed through quantitative nuclear morphology. This technology has led to more comprehensive and objective classification of tumor subtypes. Many of the developments reviewed were based on public data from the Cancer Genome Atlas, which can serve as an open platform for other groups performing similar *in silico* tissue-based studies.

The review by Heindl *et al* focuses on the localization and quantification of the elements that compose the tumor microenvironment. A better understanding of the complex spatial cellular associations of the tumor microenvironment is essential to our understanding of cancer development and drug resistance. The authors discuss techniques for mapping individual cellular components, including the infiltrating immune cell population, fibroblasts, and the vascular infrastructure.

Qualitative evaluation of immunohistochemistry and *in situ* hybridization of nucleic acids are associated with substantial assay variability owing to both a lack of rigorous method validation and subjective interpretation of results. Carvajal-Hausdorf *et al* provide a broad review of more precise quantitative platforms for the *in situ* measurement of protein and RNA. They also describe the challenges of transitioning these methods from the research lab to use by surgical pathologists as companion diagnostics.

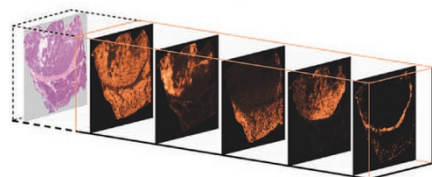
Levenson *et al* describe a novel technique that allows multiplex evaluation of up to a hundred analytes per tissue section. This technology will be especially useful in probing multiple prognostic and predictive biomarkers when limited tissue is available, as well as helping to elucidate the



interplay of intracellular signaling pathways and cell–cell interactions in phenotypically distinct cell populations.

As alternatives to standard histopathology, where tissue sections are stained with histochemical stains, Akalin *et al* examine advances in the measurement of cellular composition using molecular fingerprint techniques, such as infrared and Raman microspectroscopy. A major focus is the utility of spectral histopathology in the classification of lung cancer histopathological sections.

Imaging mass spectrometry is a novel, tissue-based research tool that measures spatial molecular arrangements in histological sections in exceptional detail. This enables the mapping of multiple analytes and direct correlation of their distribution with histological features, creating a power tool for the discovery of new biomarkers of disease. Aichler and Walch describe several powerful clinicopathological applications, including prediction and prognosis of therapy response, classification of tissue-based diseases, and inter- and intratumoral heterogeneity.



Finally, the review by Hoffman centers on noninvasive imaging of cancer cells expressing fluorescent proteins, a technique that bridges cellular and *in vivo* biology. This technology has many applications, including real-time following of metastases in individual animals and determination of the efficacy of antitumor therapies in murine models. At the cellular level, the differential labeling of the nucleus and cytoplasm makes it possible to visualize the nuclear-cytoplasmic dynamics throughout the life cycle of the cell.

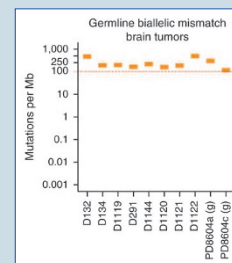
nature.com/pathology

Genomics of a mismatch-repair cancer

By sequencing the cancer genomes of high-grade brain tumors with inherited biallelic mismatch-repair deficiency (bMMRD), Shlien *et al* found an extraordinary rate of substitution mutations (more than 250/Mb), surpassing that in virtually all cancers (more than 7,000 genomes). All bMMRD tumors acquired early driver mutations in DNA polymerase ϵ or δ , creating a unique mutational signature.

Analysis of sequential tumor biopsy specimens demonstrated rapid accumulation of simultaneous mutations (approximately 600 mutations per cell division) that plateaued at approximately 20,000 exonic mutations in less than 6 months. Colonic polyps with bMMRD retained DNA polymerase ϵ and δ genes and lacked massive mutational loads. This study suggests that these two types of deficient DNA repair are highly cooperative to allow mutations, but that the genome of the resulting cancer may be limited in its ability to sustain viability beyond a certain, albeit substantial, accrued number of exonic mutation events.

Nature Genetics 2015;47:257–262; doi:10.1038/ng.3202

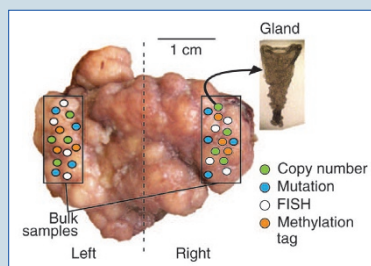


Colorectal carcinoma gets a big bang

Having carefully acquired and genomically profiled 349 individual glands from 15 colorectal carcinomas, Sottoriva *et al* describe their findings of colonic carcinoma growing as a single expansion composed of numerous intermixed subclones. These were not significantly culled by selective pressure, and both the clonal and subclonal events arose early in tumor development. No selective sweeps were in evidence, and the intratumoral heterogeneity was uniformly high. The authors propose a “big bang” model in which accumulation of numerous mutations and the intratumoral heterogeneity are established early and main-

tained rather than resulting from later clonal drift. Transformation to an advanced tumor is not followed by subsequent clonal expansions. Thus, early mutation of certain subsets of genes would make some tumors “born to be bad,” and certain clinical outcomes with genetic determinants could be assessed relatively early in a disease course.

Nature Genetics 2015;47:209–216; doi:10.1038/ng.3214



Genetics of body mass index

Body mass index (BMI) is a common assessment tool for obesity, and, for the most part, interindividual variability in BMI is believed to be genetically determined. Previous genome-wide association studies (GWAS) have identified 77 genetic loci as being associated with some measure of obesity. In *Nature*, Locke *et al* present the results of their meta-analysis of approximately 339,000 individuals from 125 studies with GWAS or MetaboChip data.

They found 97 loci associated with BMI, 56 of which are novel. Many affect other metabolic pathways, reflecting the systemic nature of obesity. It is estimated that more than 20% of BMI variation in individuals may be accounted for by these loci. Further pathway analysis suggests that the central nervous system may be found to have a genetically determined role in obesity susceptibility as genes involving synaptic function and glutamate signaling are identified. Not surprisingly, additional pathways include insulin regulation, energy metabolism, lipid biology, and adipogenesis.

Nature 2015;518:197–206; doi:10.1038/nature14177

