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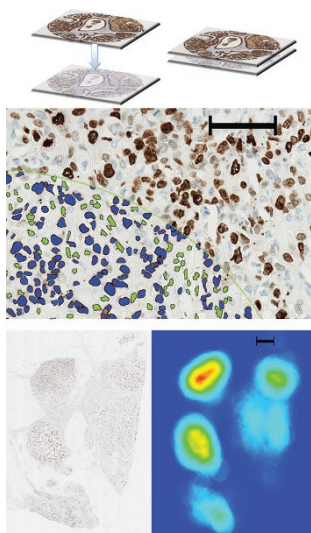
MODERN PATHOLOGY

Computational biomarker analysis versus manual observation
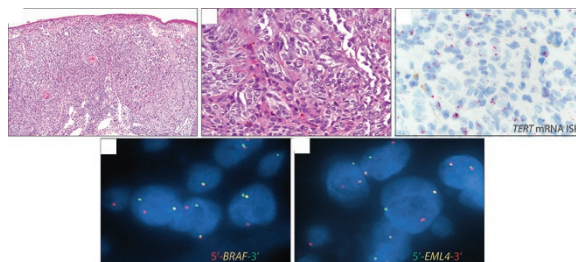See page 318

Stålhammar and colleagues showed that the immunohistochemical use of surrogate markers showed lower sensitivity and specificity compared with digital image analysis in luminal B breast cancer. Lack of consensus in biomarker interpretation can lead to discordant treatment decisions. The goal of developing digital image analysis was to provide biomarker expression consensus (Ki67, ER, PR, HER2) and concordance to the less widely available gene expression assays and increase prognostic power and reproducibility while simultaneously reducing the time required by pathologists. Because manual and digital methods were essentially matched for prognostic value in hazard/mortality prediction, the question became whether the potential improvements justify the investment and training required to introduce this technology. Further study will be required in automation and risk/benefit analysis to evaluate the increased speed of assessment as well as the reduction in sampling bias by pathologists.

Fusion transcripts in heterogeneous spitzoid melanoma
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Wu and colleagues' study was aimed at the wide heterogeneity and lack of objective criteria used in determining malignant potential of spitzoid tumors in pediatric populations. They used whole-transcriptome sequencing of archival fixed tissue of malignant and biologically indeterminate spitzoid tumors to assess fusion transcripts. They also examined *TERT* promoter mutations and messenger RNA in *in situ* hybridization in these tumors. RNA sequence libraries enriched for coding regions were analyzed using a novel assembly-based algorithm designed to identify complex fusions. The authors were able to show diversity in gene fusions that defined the molecular heterogeneity of spitzoid neoplasms. The fixed tissue was used to demonstrate complex and heterogeneous structural rearrangements, and the heterogeneity of fusion transcripts observed via RNA sequencing correlated with morphologic and clinical diversity in this group. *TERT* promoter mutations and telomerase expression were associated with poor outcomes in two patients, but the small sample size calls for further study in larger groups.

LABORATORY INVESTIGATION

### Normalized method for computational pathology
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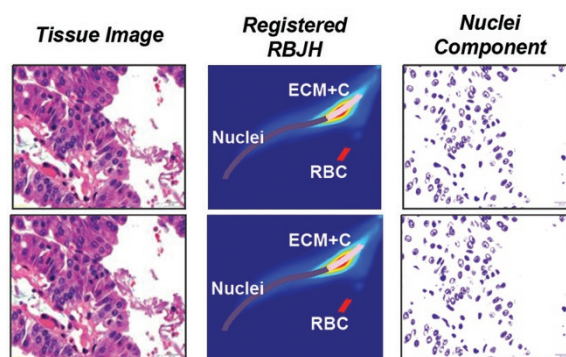
Sarnecki *et al* found that histology slides from different institutions varied in color appearance and that a computational tool for normalizing this noise would increase discriminatory diagnosis of samples. They suggest that computational tools offer cost-effectiveness that will increase accuracy, throughput, and reliability of diagnosis made from these various samples. In a cohort of 45 hematoxylin and eosin images, they found a correlation between blue and green color components, and in a set of 81 immunohistochemically stained (3,3'-diaminobenzidine) images, red and green were highly correlated. For the purpose of identifying tissue components such as nuclei,

existing computational models are routinely limited by differences in dye appearance between images and postprocessing. The nonlinear tissue-component discrimination approach bypassed staining variability without making assumptions and achieved specificity in a wide variety of images. This methodology is a clear advance for computational pathology and could be easy to implement for further testing.

High-resolution microscopy to improve assessment of intraoperative margins in breast cancer

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SECM
500 μm
100 μm
Histology
500 μm
100 μm

Compared with mastectomy, limited breast cancer excision has better cosmetic outcomes as well as lower morbidity. However, it increases the risk of leaving tumor behind. Brachtel *et al* examined 124 pairs of spectrally encoded confocal microscopy (SECM) and histologic images (49 normal and 75 malignant). Rendering diagnoses from either SECM or histology images took 15–20 minutes, and the average sensitivity and specificity of SECM were not higher than those of histology. Intraoperative differences and depth of the tumor below the surface of the specimen provided difficulties. There were areas that will require further investigation to justify a shift in protocol for margin assessment; imaging speed and software updates with automated tissue-type recognition will be investigated further. In the future, the goal is to use SECM to ensure complete tumor extirpation.

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Neutral tumor evolution to reanalyze genomic data

In neutral tumor evolution, all tumor-driving alterations responsible for tumor expansion appear to have been present in the initial cancer cell; subsequent genetic events effectively have no influence on the tumor. Complexity in tumor heterogeneity has long made the vast amount of genomic
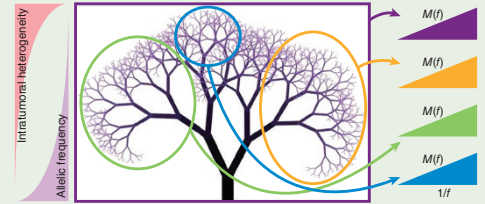

data a challenge for determining tumor evolution in individual cancer profiles. Williams and colleagues showed that neutral tumor evolution results in a power-law distribution of mutant allele frequencies reported by next-generation sequencing. They observed varying, yet significant, rates of neutral evolution across many cancer types, with common occurrence in both colon cancer and gastric cancers. The mutational timelines were free of the cross-sectional bias resulting from extensive heterogeneity and are therefore more accurate; also, they eliminate the extensive analysis required previously for these studies. This power-law distribution technique should improve investigations of cancer evolution and yield clinically relevant insights from commonly available genomic data.

## PARP1 and c-Met inhibition could be synergistic

Data are pervasive about the *BRCA* genes and their role in repairing DNA damage. PARP1 is known to be involved in destabilizing chromatin structure, enabling DNA-repair machinery to access damaged DNA. Therefore, many cancer clinical trials have focused on PARP inhibitors; theoretically, inhibiting PARP1 activity to prevent DNA repair could promote tumor cell death. Reduction in expression of *BRCA* tumor suppressors has been shown to sensitize cells to PARP inhibitors. Du *et al* determined that a combination of c-Met and PARP inhibitors could

c-Met   pY907-PARP1
Case 1   High   High
Case 2   Low   Low

benefit patients whose tumors showed high c-Met expression and who did not respond to PARP inhibition alone. The authors also showed that c-Met interacts with PARP1 at Tyr907 and that PARP1pY907 increases PARP1 enzymatic activity and reduces binding to PARP inhibitors. Their findings suggest that it might be worthwhile to systematically test the synergistic therapeutic effects of PARP and c-Met inhibitors.
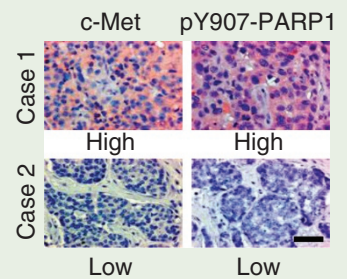
Regional mutations as potential prognostic tool

Historically, cancer sequencing studies have focused on recurrent accumulation of protein-altering mutations to identify cancer driver genes. Araya *et al* proposed a broader search that removes annotation bias and is therefore inclusive of noncoding drivers. Employing a density-based
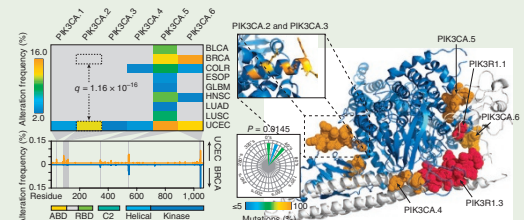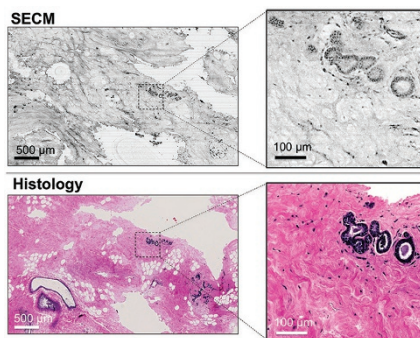

clustering method to search for significantly mutated regions (SMRs), they found that mutation frequencies in SMRs reveal that distinct protein regions are differently mutated across tumor types. The method identified known, canonical oncogenes and suggests previously undescribed candidates as well. Through observations of alterations in PIK3CA substructure they observed that alterations to SMRs within a single gene can be associated with distinct molecular signatures. Indeed, different cancer types often preferentially accumulate mutations in different regions of the same oncogene. This work demonstrates the importance of subgenic functional targeting and the prognostic value of SMRs, improves understanding of the molecular mechanisms driving cancer, and facilitates diagnostic and therapeutic development.