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Modern Pathology

Retinoblastoma gene mutations in MCC

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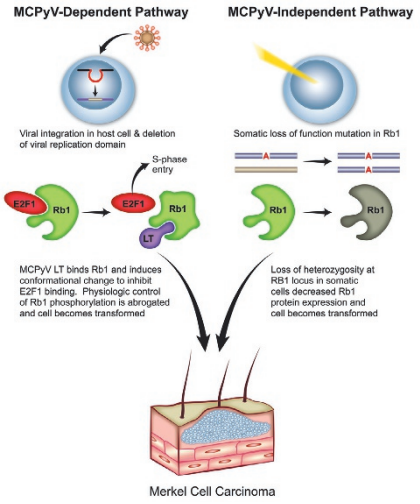

Approximately 80% of Merkel cell carcinoma (MCC) cases are associated with integration of the Merkel cell polyoma virus. This leads to expression of the large viral T antigen that binds to the retinoblastoma protein and presumably disrupts its function. Using whole-exome sequencing, Cimino and colleagues characterized cases of MCC that were either positive or negative for the polyoma virus. Overall, the profiles were very similar, but the polyoma virus–negative cases showed nonsense truncating in the retinoblastoma gene (*RB1*). Immunohistochemical studies confirmed universal disruption of the retinoblastoma pathway in both the viral and nonviral cases. Therefore, inhibiting the retinoblastoma pathway in MCC via either viral interruption or somatic mutation is probably necessary for pathogenesis. This suggests that the critical function of the polyoma virus may be to abrogate this pathway.

Tyrosine kinase receptors in pheochromocytoma and paraganglioma

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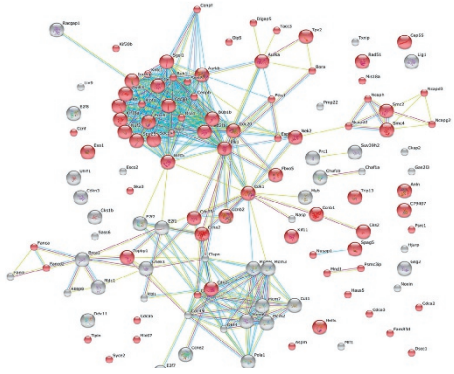
Neuroendocrine tumors such as pheochromocytomas and paragangliomas, which show somewhat unpredictable behavior clinically, can be responsive to tyrosine kinase receptor inhibitors. However, little is known about the basis of this response. Using a pheochromocytoma cell line, Cassol *et al* modeled the response to the tyrosine kinase receptor inhibitor sunitinib. Sunitinib targets such as vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, and KIT receptors were explored on tissue microarrays. The



expression patterns of these factors in pheochromocytoma differed from those for paraganglioma. Certain combinations of these receptors in various cellular compartments along with a high MIB-1 proliferation index were associated with metastatic risk. Sunitinib treatment of the mouse pheochromocytoma cell line inhibited replication and affected pathways involved in cell cycle regulation and DNA metabolism, among other functions. The results demonstrate that tyrosine kinase inhibitor targets are expressed and elucidate some of the pathway network effects to be expected when these inhibitors are employed.

Laboratory Investigation

Targeting adenoviral vectors to endothelium

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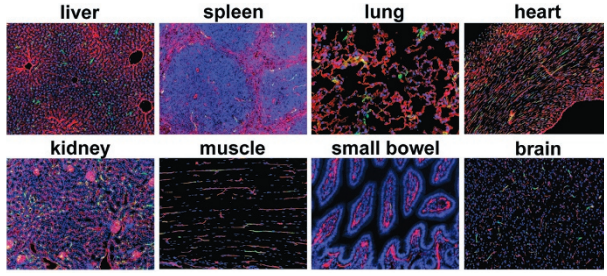

Human recombinant adenovirus serotype 5 (Ad5) is frequently used as a gene transfer vector because of its payload-delivery properties and minimal risk of somatic-mutation induction. However, Ad5 predominantly transduces hepatocytes, limiting its effective scope. Lu and colleagues report that Ad5 can target vascular endothelium with incorporation of a myeloid cell–binding peptide. Transduction of vascular endothelium cells is an attractive tropic target because there is good exposure upon intravenous delivery of vector. This modification allows Ad5 to target vascular endothelial cells and transduce cells in numerous organs,

including the heart, lung, brain, kidney, pancreas, and large and small bowel, and in skeletal muscle. This suggests that these organs could be targets for the delivery of genetic material for treatment of a wide variety of both benign and malignant diseases.

HOPE for improved methylome analysis from paraffin-embedded tissues

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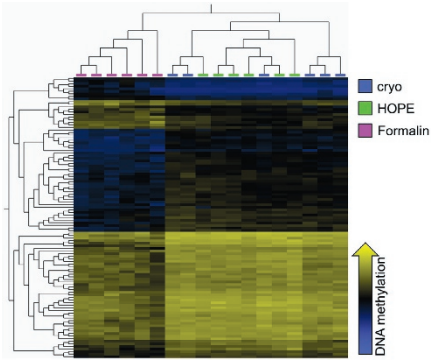

Structural genomics and mutations are critical events in cancer, but alterations of the DNA methylome also have a broad and dramatic influence on gene expression patterns. Thorough characterization of the methylome is important for research and is likely to lead to clinical applications. Robust technologies to detect DNA methylation in semiquantitative arrays are emerging. However, formalin fixation is known to introduce numerous crosslinking artifacts. Marwitz *et al* compared equivalent and adjacent portions of tumor that were either fresh-frozen, fixed in formalin, or fixed by the non-crosslinking HOPE technique (HEPES glutamic acid buffer–mediated organic solvent protection effect)—the latter two groups of samples were embedded in paraffin. The fresh-frozen and HOPE samples yielded equivalent results in methylome analysis, whereas formalin treatment was significantly inferior. Although HOPE will not be universally substituted for formalin, these results demonstrate that the technique can improve histologic visualization of tissue sections prior to methalome analysis.

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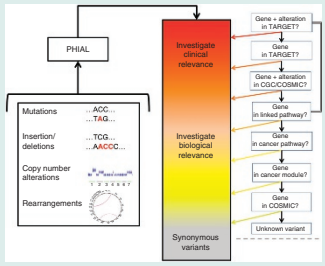
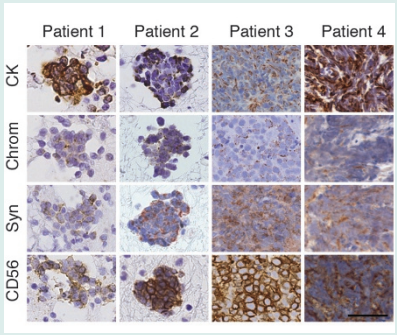
Clinical whole-exome sequencing from FFPE



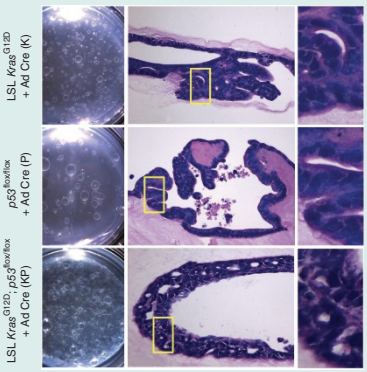
As recently reported in *Nature Medicine*, Van Allen and colleagues obtained roughly equivalent results for whole-exome sequencing of formalin-fixed paraffin-embedded (FFPE) tumor samples and cognate frozen samples, including mutational analysis and global chromosomal copy-number data. Drawing from the primary literature, manual curation, and expert opinion, the authors compiled a list of genes and alterations with theragnostic, prognostic, and diagnostic implications (the TARGET database). They applied these data, with rules governing significance, to create an analytic algorithm they termed "precision heuristics" for interpreting the alteration landscape (PHIAL). Results could be obtained from FFPE samples in less than 3 weeks. When the authors applied this approach across 511 previously analyzed exomes, they found that 39% of cases formed a long tail of clinically actionable genes found in less than 2% of all the cases. This suggests the potential superiority of casting a broad discovery net versus more focused approaches, but improvements in patient outcomes based on this enriched information remain to be demonstrated.
Nature Medicine 2014;682–688; doi:10.1038/nm.3559*

Profiling circulating tumor cells in small-cell lung carcinoma



Viable samples of the aggressive neuroendocrine tumor small-cell lung carcinoma are difficult to obtain because most cases are inoperable. Hodgkinson *et al* recently reported in *Nature Medicine* that they could secure circulating tumor cells from the bloodstream of these patients to serve as a readily obtainable "liquid biopsy." The isolated cells are tumorigenic and can be grown as xenografts in immune-compromised mice. In addition, these patient-derived explants show response profiles similar to those of platinum and etoposide therapies as seen in the corresponding patients, many of whom develop resistance to these therapies. Genetic profiling showed strong similarities between the circulating tumor cells and the explanted samples grown in mice. The findings suggest that such explant models could be used not only to model clinical challenges in this disease, such as drug resistance, but also to assess personalized approaches to treatment.
Nature Medicine, published online 1 June 2014; doi:10.1038/nm.3600

Organoid culture of GI tissues yields oncogenic insights



In a study published in *Nature Medicine*, Li *et al* used a single air–liquid interface culture to obtain organoid cultures of mouse colon, stomach, and pancreas. Organoid cultures from mice with floxed alleles allowing controlled expression of *Kras*, *Apc*, *Smad4*, and *p53* mutations enable examination of the precise effects of these genes in the various tissues. Pancreatic and gastric organoids carrying *Kras* and/or *p53* mutations led to organoid dysplasia that progressed to invasive adenocarcinoma after *in vivo* transplantation back into mice. By contrast, the colon organoids required a combination of *Apc*, *p53*, *Kras* and *Smad4* to achieve the same results. The authors also demonstrated that the microRNA miR-483 drives colon organoid dysplasia and induces tumorigenicity in combination with *Apc* mutation. This system allows detailed analysis of the requirements for malignant transformation of epithelial cells in organoid environments that better model *in vivo* environments.
Nature Medicine 2014;20:769–777; doi:10.1038/nm.3585*

*These papers include USCAP members as authors.