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MODERN PATHOLOGY

CK20-negative Merkel cell carcinoma genomics

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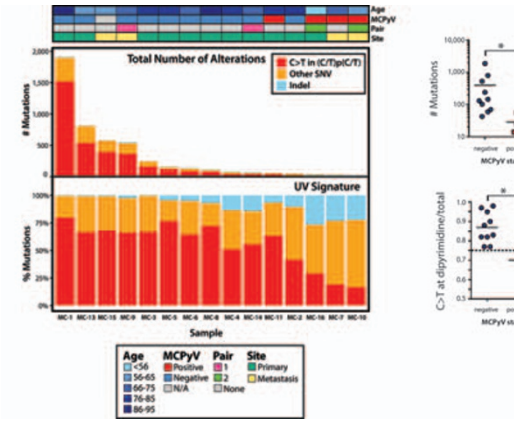

Fully 95% of Merkel cell carcinomas, a primary cutaneous neuroendocrine carcinoma, express cytokeratin-20 (CK20), which distinguishes them from morphologically similar entities such as metastatic small-cell lung carcinoma. Diagnosis of the 5% of Merkel cell carcinomas that are CK20-negative is more challenging. Harms *et al* found that CK20-negative Merkel cell carcinoma has important molecular similarities to CK20-positive Merkel cell carcinoma. Recurrent mutations were identified that were more prevalent in Merkel cell polyomavirus–negative tumors, including several tumor-suppressor genes previously implicated in Merkel cell carcinoma, such as *APC*, *TET2*, and *BAP1*. In addition, the authors identified activating mutations in the PI3K pathway in a subset of tumors. *EZH2*, an oncogenic histone methyltransferase not previously described in Merkel cell carcinoma, is now under investigation as a therapeutic target. Harms and colleagues also showed that Merkel cell polyomavirus–negative tumors display ultraviolet-signature mutation spectra.

Loss of SWI/SNF factors and dedifferentiation in endometrial carcinomas

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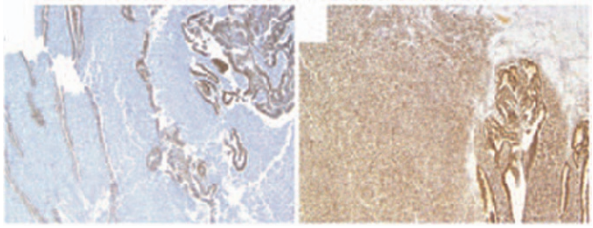
Karnezis *et al* performed targeted sequencing of eight dedifferentiated endometrial carcinomas of the endometrioid type. The sequencing identified somatic frameshift/nonsense mutations in *SMARCA4*, a core ATPase of the switch/sucrose nonfermenting (SWI/SNF) complex. Both the sequencing and immunohistochemistry for SMARCB1 and SMARCA4 showed that the loss of either was mutually exclusive. There was frequent loss of SMARCA2, SMARCA4, and SMARCB1 in the undifferentiated



component of dedifferentiated endometrial carcinomas, whereas none of the 31 International Federation of Gynecology and Obstetrics grade 3 endometrioid carcinomas examined showed such losses. This suggests that the *SMARCA4*-inactivating mutations are relatively common in the undifferentiated tumors but not in the differentiated forms. The presence of either inactivating *SMARCA4* mutations or those in *SMARCB1*, with concurrent loss of SMARCA2, was associated with histologic dedifferentiation in about half of the tumors in the cohort. Thus, in SWI/SNF-deficient cases, co-inactivation of SMARCA4/SMARCA2 or SMARCB1/SMARCA2 may be involved in development of undifferentiated carcinoma from underlying lower-grade endometrioid carcinoma.

Telomeres, macrophages, and glioblastoma prognosis

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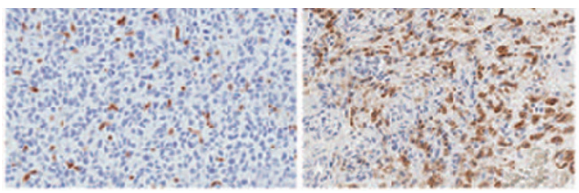

Hung *et al* investigated known mechanisms of telomere maintenance in glioblastomas using RNA-Seq. A connection was noted between glioblastomas lacking a defined telomere-maintenance mechanism and an increased immune signature (CD163-positive tumor-associated macrophages) as compared with the alternative lengthening-of-telomeres pathways common in glioblastoma. Further analysis showed that, in the absence of a defined telomere-maintenance mechanism, there is upregulation of immune-based pathways, including leukocyte transendothelial migration, hematopoietic cell lineage, and complement and coagulation cascades. Increased numbers of tumor-associated macrophages were seen (via CD163 staining) in 80% of tumors with an undefined telomere-maintenance mechanism. Different immunologic pathways tended to drive tumor-associated macrophages, based on the observed telomere-maintenance mechanism. A high number of tumor-associated macrophages was associated

with a poorer prognosis regardless of telomere-maintenance pathway. Whether the tumor-associated macrophages are drivers of poor prognosis or a marker of the telomere-maintenance mechanisms and the mechanistic connection between telomere maintenance and macrophage infiltration remain important areas for further investigation.

LABORATORY INVESTIGATION

Loss of biomarker detection over time in breast cancer samples

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Combs *et al* used specimens from four breast cancer cohorts formatted onto clinically annotated microarrays to investigate the performance of established biomarkers as a function of tissue age. Using automated quantitative analysis (AQUA), the authors observed that scores for estrogen receptor, human epidermal growth receptor 2, and cytokeratin expression levels decreased with age in a linear manner. However, with Ki67, a nonlinear quadratic equation was the best fit, suggesting that greater loss of Ki67 occurs in the initial decades following tissue fixation and plateaus over time. The group used published population data to estimate the expected proportion of positive cases for each cohort. The loss of signal was biomarker-dependent, suggesting that some markers can be readily evaluated in older cohorts without concern for aging bias whereas others require the use of age controls and complex modeling. As tissue repositories age, these degradations of signal should be taken into careful consideration.

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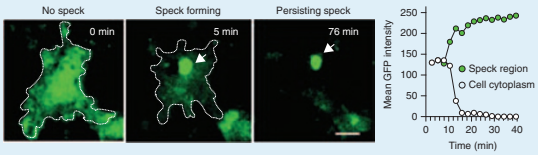
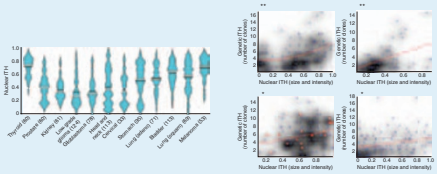
Imaging the inflammasome in immune reactions



Critical in both innate and adaptive immunity, the inflammasome is a large intracellular protein complex assembled to initiate host response to pathogens. In a study reported in *Nature Medicine*, Sagoo *et al* investigated the mechanisms by which the inflammasome promotes an effective immune response using a newly developed *in vivo* two-photon imaging reporter with macrophages labeled with ASC-GFP (apoptosis-associated speck-like protein with a CARD domain–green fluorescent protein). They visualized inflammasome assembly in real time and demonstrated intracellular redistribution of ASC-GFP, speck assembly, and dynamic cellular redistribution, with most cells showing complete ASC oligomerization within 10 minutes. They noted rapid triggering and a transient, spatially confined wave of inflammasome activity spanning 7 hours postinfection. Macrophages in lymph nodes quickly underwent inflammasome activation, immediately followed by pyroptotic cell death. The inflammasome could be a digital switch where pyroptotosis coincides with a release of proinflammatory cytokines. The authors' novel methods facilitate study of inflammasome activity *in vivo* and dissection of related pathways.

Nature Medicine 2016;64–71; doi:10.1038/nm.4016

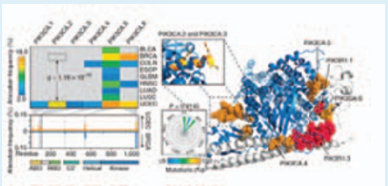
Consequences of intratumoral heterogeneity



As reported in *Nature Medicine*, Andor *et al* used bioinformatics tools to detect clones present in less than 10% of the exome from tumors in The Cancer Genome Atlas. They assessed intratumor heterogeneity (ITH) as a driver of neoplastic progression and mediator of therapeutic resistance. Cross-cancer correlation between genetic ITH and histopathologic ITH (assessed as nuclear variation) indicated that assessment of histologic ITH could provide a proxy for genetic ITH. Intratumoral nuclear diversity correlated with intratumoral genetic diversity. Across tumor types, the presence of more than two clones was associated with inferior survival; however, when more than four clones were present, mortality improved, suggesting a genomic heterogeneity sweet spot. Copy-number variation (CNV) load of the tumor correlated with ITH and outcome, and, again, both low and very high CNV loads were associated with improved survival. The study suggests a trade-off between the cost and benefits of genomic instability in tumor fitness that is applicable across many cancer types.

Nature Medicine 2016;105–113; doi:10.1038/nm.3984

Significantly mutated regions across cancer types



Araya *et al* applied density-based clustering methods to 21 tumor types to detect variably sized significantly mutated regions (SMRs) of genomic DNA. As described in *Nature Genetics*, they used an open approach, including genes encoding proteins and potential noncoding drivers. Their data showed that mutation frequencies in SMRs involved distinct protein regions that are differentially mutated across tumor types, with PIK3CA being a key example. Differences between cancer types in SMR mutation frequencies demonstrated variable spatial clustering of somatic mutations across cancers. A close geometric proximity of alterations was observed in critical protein domains such the PIK3CA helix. Biophysical simulations confirmed increased catalytic activity in mutated PIK3CA. Intriguingly, recurrent alterations in noncoding regions such as transcription factor binding sites and other untranslated regions were present in up to 15% of certain tumor types. This analysis expands the spectrum of recurrent mutations in cancer, but additional functional characterization is needed.

Nature Genetics 2016;48:117–125; doi:10.1038/ng.3471

Emma Judson contributed to this report.