Molecular pathology selected abstracts

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Isoform switching: a mechanism of acquired resistance to isocitrate dehydrogenase inhibition

December 2018—The use of small-molecule inhibitors to treat solid tumors, particularly nonsmall cell lung cancer, has provided insight into the varied mechanisms of resistance to these drugs. The paradigm for developing resistance to tyrosine kinase inhibitors for targets such as epidermal growth factor receptor *(EGFR)* alterations in the management of nonsmall cell lung cancer includes mechanisms ranging from alternate signaling pathways to secondary mutations and amplification of the oncogene. Small-molecule inhibitors have been used widely for inhibiting receptor tyrosine kinases, but their use in targeting oncometabolites has been limited. One such application involves the isocitrate dehydrogenase (IDH) enzymes, which occur as two isoforms: cytoplasmic IDH1 and mitochondrial IDH2. Conserved alterations of IDH1 (R132) and IDH2 (R140 and R172) are seen in solid tumors, such as gliomas, chondrosarcomas, and intrahepatic cholangiocarcinomas, as well as in myeloid hematologic malignancies. Altered catalytic function of the IDH1/2 isoforms leads to the accumulation of the oncometabolite 2-hydroxyglutarate (2HG). 2HG build-up results in the development of a repressive chromatin landscape secondary to increased methylation, which contributes to oncogenesis. Small-molecule inhibitors that reverse 2HG production and its effect on the chromatin landscape are in various phases of clinical trials. These inhibitors include ivosidenib (IDH1 inhibitor) and enasidenib (IDH2 inhibitor). The authors conducted a study in which they documented resistance to IDH inhibitors in solid tumors and myeloid malignancies through isoform switching. For instance, a patient with acute myeloid leukemia that harbored an *IDH1* alteration (R132C) initially responded to the IDH1 inhibitor ivosidenib. This patient subsequently relapsed. Increased blast counts and 2HG levels were associated with a pathogenic alteration of the alternate isoform (*IDH2* R140Q). Bidirectional isoform switching was observed for *IDH1* alterations treated with ivosidenib and *IDH2* alterations treated with enasidenib. This study also highlights the use of digital droplet PCR to serially monitor variant allele frequencies in this setting. The authors acknowledge that the frequency of isoform switching as a mechanism of resistance to IDH inhibition is unknown. However, this study documents isoform switching as a resistance mechanism, highlights the use of molecular techniques such as digital droplet PCR to monitor for relapses, and suggests the need for future clinical trials that evaluate the targeting of both IDH isoforms using the dual IDH1/2 inhibitor AG-881.

Harding JJ, Lowery MA, Shih AH, et al. Isoform switching as a mechanism of acquired resistance to mutant isocitrate dehydrogenase inhibition. *Cancer Discov.* 2018. doi:10.1158/2159-8290.

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Profiling of chromatin-accessibility landscape of primary cancers

December 2018—The chromatin-accessibility profiles generated in this study by The Cancer Genome Atlas represent the largest pan-cancer effort to characterize the regulatory landscape of human cancers. The study primarily used an assay for transposase-accessible chromatin using sequencing (ATAC-seq). This assay utilizes hyperactive transposase enzymes that cut DNA at highly accessible sites while simultaneously ligating specific adapter sequences at these sites. Next-generation sequencing-based reads from these adapter sequences are used to characterize these genomic regions. A limited number of tumors were also profiled using whole-genome bisulfite sequencing (WGBS). This latter methodology helps to determine patterns of methylation at CpG islands that are implicated in repression of transcriptional activity. Large-scale chromatin accessibility has been determined using techniques such as DNase-seq and CHIP-seq. DNase-seq involves sequencing regulatory DNA regions that form complexes with proteins and are, therefore, protected from cleavage by DNase I. Similarly, CHIP-

seq involves the sequencing of regulatory DNA fragments that form complexes with transcription factors. The ATAC-seq approach to identifying sites of chromatin accessibility was validated by finding significant overlap with regions defined by the Roadmap Epigenomics Project that involved DNase-seq, as well as CHIP-seq–defined regulatory elements. WGBS revealed reduced methylation at sites of chromatin accessibility identified by ATAC-seq. Novel regulatory regions identified in this manner were targeted using CRISPR interference in an in vitro setting, which led to downregulation of linked genes, providing proof of principle of utilizing this approach. This led to the identification of cancer type-specific areas of chromatin accessibility for specific oncogenes. For instance, 5' and 3' elements were identified for *MYC* in colorectal carcinomas while only 3' elements were identified for clear cell renal cell carcinomas. Interestingly, distal 3' elements showed a stronger cancer type-specific association than 5' elements. In certain tumor types, these distal elements helped with additional molecular subclassification, such as the clustering of breast carcinomas into basal and luminal subtypes. In addition, many of these distal regulatory regions were enriched for motifs of transcription factors, such as *AR* in prostate cancer and *MITF* in melanoma. Other interesting correlations included the analysis of transcription factor footprints. A combination of low read depth at a transcription factor motif (referred to as footprint depth), increased accessibility of flanking areas, and reduced methylation at these sites was used as a surrogate of transcription factor occupancy at a given site. As proof of principle, such signatures were found to be enriched for the *NKX2-1* transcription factor in lung adenocarcinomas but not in squamous cell carcinomas. One of the better characterized alterations involving noncoding DNA in cancer involves *TERT* promoter mutations that lead to the generation of ETS transcription factor binding motifs. In this study, *TERT* promoter mutations at known hotspots led to increased promoter accessibility and associated increases in gene expression. These results can be used in assessing noncoding somatic alterations that exhibit allele-specific regulatory effects. In summary, this study provides a rich foundation for studying the accessible genome of diverse human cancers.

Corces MR, Granja JM, Shams S, et al. The chromatin accessibility landscape of primary human cancers. *Science.* 2018;362. doi:10.1126/science.aav1898.

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