Molecular pathology selected abstracts

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Role of methylation profiling in subclassification and treatment of metastatic prostate cancer

September 2020—Whole genome methylation profiling is used to subclassify neuroepithelial tumors and soft tissue sarcomas. Extending its use to much more common cancers, such as prostate cancer, has the potential to benefit a large number of patients. Metastatic castration-resistant prostate cancer (mCRPC) is the incurable and lethal form of prostate cancer and consists of different subgroups with variable morphologies and genomic alterations. The emergence of distinct subtypes of mCRPC likely represents adaption of the cancer cells to treatment and the microenvironment. The authors conducted a study that integrated methylation profiling with genomic sequencing and RNA transcriptome analysis in 100 mCRPC tumors, yielding a comprehensive molecular profile of these metastatic tumors. They identified two distinct methylation subgroups: one associated with the development of treatment-emergent small-cell neuroendocrine prostate cancer (t-SCNC) and another with high-level methylation similar to the CpG island methylator phenotype (CMP) found in other tumors. T-SCNC is a subgroup of mCRPC characterized by transition from adenocarcinoma to neuroendocrine (small-cell) phenotype that occurs under the pressure of such androgen-signaling inhibitors as abiraterone or enzalutamide. As expected, this subgroup showed decreased androgen-receptor signaling and elevated expression of neuroendocrine markers. At the same time, the androgen-receptor locus was the most differentially hypomethylated locus in this t-SCNC subgroup, suggesting that alterations in the androgen-receptor pathway play a vital role in driving the cancer phenotype in this subgroup. On the other hand, the CMP methylation subgroup (22 percent in this cohort) was enriched for tumors with *TET2, IDH1, DNMT3B,* and *BRAF* mutations, with less frequent *ETS* family-associated fusion and biallelic *TP53* inactivation. However, there was still a large portion of tumors in this CMP group that did not harbor somatic mutations in genes involved in methylation pathways. No somatic mutations were observed in any *DNMT* or *TET* family genes, other than in *DNMT3B* and *TET2*, suggesting that these genes might selectively influence the methylation mechanism in prostate cancer. In addition to shedding light on methylation subgrouping, the authors focused on the mechanism of mCRPC tumorigenesis, particularly on pathways involving the androgen receptor. Androgen receptor is a dominant driver and therapeutic target for prostate cancer as a whole. The authors found that several genes that respond to androgen-receptor signaling—for example, *KLK3, NKX3-1, FOLH1, SCHLAP1,* and *PIK3CA*—were more hypomethylated in mCRPC than in benign prostate or adjacent non-neoplastic tissue. Prior studies have shown that t-SCNC is enriched for *TP53* or *RB1* loss-of-function mutations, or both. Neither gene was hypermethylated, suggesting that methylation is unlikely to contribute to inactivation of these tumor-suppressor genes. Additionally, in mCRPC with *TMPRSS2-ERG* fusion, methylation of the upstream promoter region of *TMPRSS2* was predictive of *ERG* expression in the same tumor, suggesting that methylation of this *TMPRSS2* promoter contributed to the tumor phenotype. Although the authors did not assess the direct clinical implications of their data, many of their findings could serve as the basis of future studies that contribute to developing improved diagnostic/prognostic biomarkers, disease classifications, and possible therapeutic targets, as both methylation pathways and genes regulated by these methylations can be targeted. Therefore, combining methylomics with the standards of genomics/transcriptomics and routine histopathologic diagnoses may become a pathology practice standard of care.

Zhao SG, Chen WS, Li H, et al. The DNA methylation landscape of advanced prostate cancer. *Nat Genet.* 2020;52:778–789.

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Application of genomic and phenotypic information from a national database to rare inherited diseases

There are thousands of rare diseases known to the medical world, which, in specific combinations, can pose a more significant health threat than they create individually. Even with widespread use of whole exome sequencing, more than half of patients with rare diseases are still without definite genetic etiologies. The authors demonstrated diagnostic advantages of using whole genome sequencing (WGS) in a large unified study cohort centralized within the United Kingdom National Health Service (NHS). They performed WGS on 9,802 people who had a rare disease or extreme quantitative trait, of which 9,024 were probands and 778 affected relatives. The authors grouped the patients' diagnoses into disease domains for expert review and compiled the diagnostic-grade gene list from literature reviews of the relevant disease phenotypes. Sixteen percent (1,138) of 7,065 patients who had extensively phenotyped disease were identified as harboring causal gene variants, including patients missed by previous exome studies. In some difficult cases, WGS revealed a causative intronic variant or large tandem repeat that could not be captured by exome sequencing. By incorporating open chromatin data and histone modification data in the WGS data analysis, the authors identified novel variants in the regulatory noncoding regions. Functional studies confirmed the impact of those variants on the protein. This result showed that regulatory regions contribute to disease phenotypes and, therefore, are a promising area for future research and treatment strategy. The authors also employed additional new approaches to analyze the WGS data. Leveraging the statistical power of this large dataset, including comprehensive clinical and phenotypic information, and internal control samples, they applied a Bayesian model for genotype-phenotype association analysis and identified 95 genes that showed strong evidence of causing rare diseases. At least 79 were established disease-associated genes, demonstrating a high positive predictive value for this model. Using this model in tandem with a genome-wide association study (GWAS) in a subset of people with extreme RBC indices, the authors identified variants in known causal genes or plausible candidate genes. These causative genes were not previously identified using conventional GWAS analysis, showing the practical clinical utility of the large sample size and new statistical methodology. Overall, this study demonstrates the usefulness of a large comprehensive genotype-phenotype dataset combined with WGS for identifying causative rare disease variants, especially those in noncoding regions. The practical lesson for non-nationalized health systems is to recognize the inherent value in integrating phenotypic information, often gleaned from the electronic medical record, with comprehensive genomic data to improve rare disease diagnosis and subsequent patient care.

Turro E, Astle WJ, Megy K, et al. Whole-genome sequencing of patients with rare diseases in a national health system. *Nature*. 2020;583:96–102.

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