Molecular pathology selected abstracts

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Evaluation of mutational processes and somatic driver mutations in cancer exomes

February 2019—As next-generation sequencing-based tumor profiling gains popularity, multiple informative variables other than single-gene alterations will continue to be added to clinical reports. Examples of metrics that are being incorporated into such reports are tumor mutational burden and parameters to assess microsatellite instability. Other important parameters include various mutational signatures. In this context, the authors conducted a study in which they evaluated somatic alterations from 7,815 cancer exomes from The Cancer Genome Atlas across 26 cancer types for oncogenic driver alterations and mutational signatures. Driver alterations are thought to confer a selective growth advantage to clonal tumor populations. For instance, a *BRAF* p.V600E alteration in a cutaneous melanoma is often considered to be a driver alteration when compared with passenger alterations that do not confer a relative selective advantage. In contrast to driver alterations, mutational signatures are rarely clinically reported. The Catalogue of Somatic Mutations in Cancer (COSMIC) database lists at least 30 curated signatures. Common environmental factors associated with specific signatures include smoking (signature four) and exposure to ultraviolet radiation (signature seven). On the other hand, mutations in various enzymes are associated with specific mutational signatures. For instance, deficient mismatch repair is characterized by fewer than 3bp insertions and deletions at mono/polynucleotide repeats (signatures six and 26). Some associations emphasized in this study include polymerase epsilon (*POLE*) exonuclease domain mutations, which are commonly encountered in a subset of endometrial carcinomas. These *POLE* proofreading domain mutations lead to hypermutated cancers that respond well to immunotherapeutic agents. For instance, mutations occurring in a T[C>T]G context (signature 10) were causally linked to downstream alterations in multiple oncogenes (*PIK3CA* p.R88Q) and tumor-suppressor genes (*PTEN* p.R130Q, *ARID1A* p.R1989*, and *TP53* p.R213*). Similarly, *BRAF* p.V600E alterations were associated with only somatic (not germline) mismatch repair deficiency in colorectal carcinomas, supporting the hypothesis that this *BRAF* alteration might promote susceptibility to developing mismatch repair deficiency. Other interesting observations included tumors with isocitrate dehydrogenase 1 (*IDH1* p.R132H) alterations that are known to lead to a repressive chromatin landscape secondary to increased methylation. These tumors showed an inverse correlation with signature one, thought to occur secondary to spontaneous deamination of 5-methylcytosine. In summary, this study identified 39 statistically significant associations between driver alterations and mutational signatures from nearly 8,000 tumor samples across 26 cancer types. These findings increase understanding of the mutational pathogenesis of varied tumor types.

Poulos RC, Wong YT, Ryan R, et al. Analysis of 7,815 cancer exomes reveals associations between mutational processes and somatic driver mutations. *PLOS Genet.* 2018;14(11):e1007779. doi:10.1371/journal.pgen.1007779.

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Next-generation sequencing of circulating cell-free DNA during pregnancy

Non-invasive, next-generation sequencing-based testing of cell-free DNA derived from maternal plasma for fetal chromosomal anomalies is increasingly being adopted in medical practice. In most cases, plasma cell-free DNA (cfDNA) obtained from pregnant women originates in the placenta or is derived from maternal cells. The fetal fraction (placental origin cfDNA:total cfDNA) reaches a threshold for effective analysis at approximately 10 weeks of gestation. Sequencing information, most commonly pertaining to chromosomal copy number changes, is compared to reference datasets derived from profiling pregnant women carrying euploid fetuses to screen for chromosomal aneuploidy. Prenatal cfDNA testing has gone from a research setting to transforming prenatal care

worldwide in less than a decade. As of late 2017, between 4 and 6 million pregnant women had undergone analysis of DNA from their plasma to screen for fetal aneuploidy. CfDNA testing is primarily used to detect trisomy 13, 18, and 21. In some studies, estimates of analytical sensitivity and specificity in high-risk women range from 93 to 99.9 percent, which is superior to traditional testing involving a combination of serum biochemical assays and sonographic metrics. Importantly, low false-positive rates have contributed to reducing the number of invasive confirmatory procedures, such as amniocentesis and chorionic villus sampling, by as much as 40 to 76 percent, according to some studies. Increased testing volume has expanded the medical community's understanding of the limitations of such testing. For instance, documented causes of false-positive results in cfDNA aneuploidy testing have been found in maternally derived cfDNA (chromosomal abnormality, cancer), placentally derived cfDNA (confined placental mosaicism, death of a twin in utero), and allograft derived cfDNA (bone marrow transplant). Furthermore, medical conditions such as obesity and thromboembolic disorders, which are associated with a low fetal fraction, can lead to false-negative test results. While cfDNA aneuploidy screening has been adopted as a first-tier test for all pregnant women in some European countries, numerous barriers have prevented more widespread adoption in the United States. These barriers include variable reimbursement by insurers and public payers, an education gap among health care providers, and a lack of resources for required pretest counseling. The screening is monitored by the Clinical Laboratory Improvement Amendments (CLIA) program as a laboratory-developed test validated by independent laboratories. No FDA-approved cfDNA test is available. In addition to aneuploidy screening, cfDNA testing is clinically available for evaluating microdeletion and microduplication syndromes, such as terminal 5p deletion in Cri du chat syndrome. Avenues for expanding such testing include single-gene assessments, such as for rhesus D genotyping in rhesus D-negative women, skeletal dysplasias, beta-thalassemia, sickle cell anemia, and hemophilia.

Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. *N Engl J Med.* 2018;379(5):464–473.

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