Molecular pathology selected abstracts

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Extrachromosomal DNA: link to oncogene amplification and cancer outcomes

October 2020—An increase in the number of copies of a gene, or amplification, is regarded as the most common gain-of-function alteration across various cancer types. The authors developed a bioinformatics tool (Amplicon Architect) to identify extrachromosomal oncogene (ecDNA) amplification from whole genome sequencing (WGS) data based on three characteristic features: circularity of ecDNA, absence of a centromere, and high levels of amplification. The tool was validated in 44 cancer-derived cell lines known to have ecDNA. A combination of centromeric and noncentromeric FISH probes was used to identify extrachromosomal DNA, and the tool was able to classify 83 percent of these signals as representing circular ecDNA amplicons. Interestingly, some of these cases revealed the presence of concurrent extrachromosomal and intrachromosomal signals, suggesting that some ecDNA had reintegrated into the genome. The Amplicon Architect tool was used to analyze WGS data from The Cancer Genome Atlas (n = 3,731) and Pan-Cancer Analysis of Whole Genomes (n = 1,291). Amplicons that had a copy number greater than four and were at least 10 kilobases in size were classified into the broad categories of circular ecDNA and noncircular DNA for the study. The latter included linear amplifications, with small deletions of less than one megabase; heavily rearranged segments of DNA, including deletions of more than one megabase; and DNA that exhibited a molecular signature of having been generated secondary to breakage-fusion-bridge mechanisms. WGS revealed that ecDNA was identified in up to 14 percent of 3,212 tumors versus less than 0.4 percent of 1,810 matched normal specimens. Circular ecDNA represented a common mechanism of amplification of such genes as *CDK4* and *MDM2* and was found to be enriched in aggressive tumor types, such as glioblastomas, sarcomas, and esophageal cancer. When the analysis was restricted to highly amplified oncogenes with a copy number greater than eight, circular ecDNA was found to account for 53.5 percent of these amplifications. With regard to the characterization of circular amplicons, no specific patterns of breakpoints were identified, and the latter exhibited minimal sequence homology. This suggested a random distribution of breakpoints, likely secondary to nonhomologous end joining contributing to ecDNA breakpoint repair. Furthermore, a subset of breakpoints likely occurs due to chromothripsis events, which involve a large number of clustered chromosomal rearrangements in cancer cells. Other interesting observations included an increased amount of amplicon-derived gene fusions and significantly higher gene expression in circular compared with noncircular amplicons. The authors showed that the latter finding is likely secondary to epigenetic events. Finally, the authors demonstrated the clinical significance of their findings by showing that circular ecDNA was significantly associated with lymph node metastasis, higher cellular proliferation scores, and reduced levels of immune infiltrates, suggestive of more aggressive disease. Analysis of five-year survival associated with circular ecDNA revealed significantly poorer outcomes. Because circular ecDNA was frequently identified in aggressive tumors, additional statistical analysis using a Cox proportional hazards model was conducted to test for five-year survival outcomes after controlling for disease subtype. This revealed significantly higher hazard ratios (1.48; $p<0.001$) in these patients compared with patients without focal somatic copy number amplification, suggesting an adverse prognostic impact. The authors concluded that circular ecDNA is frequently identified in human tumors and associated with poor clinical outcomes.

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Utility of molecular methods in identifying unexplained causes of stillbirth

Stillbirths are frequently attributed to obstetrical conditions, placental or umbilical cord abnormalities, fetal structural abnormalities, gross chromosomal abnormalities, and maternal medical conditions. However, in up to 60 percent of stillbirths, which are defined as fetal death in utero at 20 or more weeks of gestation, the underlying cause is unknown. Whole exome sequencing (WES) approaches have been used extensively to investigate the underlying cause of fetal structural anomalies and unexplained childhood disorders, but such approaches have been used on only a limited basis in evaluating stillbirths. The authors conducted a study that involved performing WES to identify single-nucleotide variants and small insertions and deletions in a cohort of 246 stillbirth cases and a control group composed of healthy relatives of probands of mixed ancestry. The analysis involved evaluating the protein-coding regions, or exons, of 18,653 genes and a small segment of the adjacent nonprotein-coding, or intronic, sequences to identify splicing variants. Disease-causing mutations traditionally have been identified in DNA sequences that are conserved across species. However, such an approach has significant limitations in identifying mutations in nonconserved DNA sequences that may represent the evolution of novel function. Many such genes show minimal variation within the human population and are referred to as “intolerant” to variation. Using a computational cutoff to identify loss-of-function variants in intolerant genes, the authors identified a disease-causing alteration in 14.5 percent (35 of 241) of cases compared to only 7.3 percent (531 of 7,239) of controls. Surprisingly, the alterations identified in cases of stillbirth were enriched in disease-causing genes listed in the Online Mendelian Inheritance in Man (OMIM) database (odds ratio, 2.22) and genes not associated with disease (non-OMIM genes; odds ratio, 2.02). Additional evidence supporting the disease-causing role of the latter group of novel genes involved observing that close to half (11 of 25; 44 percent) of the non-OMIM genes had an embryonic lethal phenotype in mouse models in which the corresponding gene had been knocked out. In contrast to the cases of stillbirth, a similar analysis of 589 cases of postnatal disease and 251 cases of live births with a fetal anomaly found that loss-of-function alterations in intolerant genes were primarily enriched in known disease-causing (OMIM) genes. A potential limitation of this study involved an absence of genomic data from the parents. For instance, it was not possible to establish whether heterozygous alterations in a subset of cases represented a de novo alteration or a compound heterozygous alteration, in part because many of these were identified in genes known to be associated with stillbirth. Therefore, combined family (trio) sequencing has the potential to further increase the diagnostic yield of such a WES testing approach. The authors concluded that this study supports the role of clinical WES in identifying disease-causing alterations in a subset of stillbirth cases. The frequency of putative stillbirth-causing mutations in disease-causing (OMIM) genes was similar to that for novel non-OMIM genes.

Stanley KE, Giordano J, Thorsten V, et al. Causal genetic variants in stillbirth [published online ahead of print August 12, 2020]. *N Engl J Med*. doi:10.1056/NEJMoa1908753.

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