Molecular Pathology Selected Abstracts

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Ability of IHC to detect colon cancer patients with MSI who could benefit from immunotherapy

March 2020—Microsatellite instability status in solid tumors is a critical biomarker for predicting tumor response to an immune checkpoint inhibitor drug. The immune system is more likely to attack those tumors that have a high degree of microsatellite instability, a consequence of the genomic instability that also leads to generation of the neoantigens the immune system is designed to recognize. Patients who are not candidates for immunotherapy may also benefit clinically from knowing the microsatellite status of their tumor. Patients with MSI-high low-stage colorectal cancer (II–III), therefore, will not benefit from additional 5-FU chemotherapy, and those with inherited Lynch syndrome, in whom MSI is a characteristic phenotype, will need additional genetic counseling and familial evaluations. MSI status can be determined by a conventional polymerase chain reaction-based assay that interrogates five mononucleotide microsatellites susceptible to replication “mistakes” mediated by ineffective DNA repair, or it can be determined by a next-generation sequencing (NGS) assay that interrogates a much larger number of these microsatellites. However, instead of using these definitive molecular methods, many laboratories use surrogate IHC methods to assess MSI status by determining tumor-specific loss of expression of the mismatch repair (MMR) proteins MLH1, PMS2, MSH2, and MSH6. IHC has several advantages over molecular methods, including a shorter turnaround time, ability to identify the specific MMR protein that is nonfunctional, consumption of less tissue for testing, and applicability to cases with low tumor purity. Although concordance between these various MSI technical methods is generally high, when discrepancies occur, it can be difficult to determine which method is accurate. The increasing availability of NGS, which can interrogate more of the genome, may help to better identify the subset of patients who will most benefit from immunotherapy. The authors of this study assessed the incidence of mismatch repair-proficient yet microsatellite instability-high cancer cases. They analyzed 443 MSI-high tumors that were concomitantly evaluated by an NGS assay and a matched IHC assay. The NGS assay covered more than 1,000 microsatellite loci and the commonly-targeted MMR genes *EPCAM, MLH1, PMS2, MSH2*, and *MSH6*. Discordance between NGS and IHC results was found in seven percent of cases, representing 21 colorectal cancers, nine endometrial cancers, and one each of cancers of the uterine cervix, small bowel, breast, thyroid, prostate, and uterus. A significant difference between the method-concordant versus discordant groups was a higher proportion of NGS-defined deleterious missense mutations in MMR genes in the discordant cases. Because MMR genes have tumor-suppressing properties, loss-of-function mutations in these genes are a common tumorigenic event. Unlike deletion or truncation mutations, in which the RNA or protein, or both, is typically absent, MMR genes with missense mutations can be transcribed and translated to varying degrees. The functional quantity or quality, or both, of the resulting missense protein may not be “normal,” however, resulting in the same loss-of-function tumorigenic effect. Consistent with this hypothesis, the authors found several recurrent pathogenic missense mutations in key MMR genes in the NGS versus IHC discordant cases. These missense mutations likely created expressed but nonfunctional MMR proteins that led to the initial IHC false-positive determination. Of particular interest were three NGS-determined MSI-high but IHC-expressing discrepant tumors that were clinically responsive to the immune checkpoint inhibitor pembrolizumab, suggesting that the NGS-based result in these discrepant cases may better correlate with clinical outcome. Because a patient’s responsiveness to immune checkpoint inhibitor therapy should never be missed, laboratories performing MSI assays should consider using definitive molecular methods with the highest level of clinical sensitivity.

Hechtman JF, Rana S, Middha S, et al. Retained mismatch repair protein expression occurs in approximately 6% of

microsatellite instability-high cancers and is associated with missense mutations in mismatch repair genes. *Mod Pathol.* 2019. doi:10.1038/s41379-019-0414-6.

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Can our genome predict stroke risk?

Stroke is one of the leading causes of morbidity and mortality worldwide. Although stroke prevention measures can be effective, current methods for identifying high-risk individuals rely primarily on insensitive, nonspecific clinical parameters, such as hypertension, diabetes, hypercoagulability, hypercholesterolemia, and smoking. In comparison, newly available genomic risk score (GRS) testing may improve the prediction of stroke risk based on a person's complex pattern of DNA variants across many different genes. These genomic risk scores, applicable to any common disease, rely on testing a healthy person's DNA, usually from blood or saliva, for thousands of benign genetic variations across the genome. Genetic polymorphisms, when assessed individually, confer a miniscule degree of increased disease risk, but in combination, can be a powerful prognosticator for predicting the development of future disease. The authors conducted a study to improve on an initial 90-gene stroke risk score that had been derived from a 300,000-participant genomewide association study of stroke. They applied this well-validated quantitative stroke risk score to another independent nonoverlapping group of subjects from the UK Biobank, encompassing more than 400,000 white British participants collectively representing more than 3,000 ischemic stroke events. Using 11,995 of these subjects, the authors constructed the metaGRS stroke risk score. They used the remaining subjects (n = 395,000) to validate the mathematical model. This new genomic score was then compared to established clinical risk factors, such as low-density lipoprotein cholesterol, smoking, hypertension, and family history, using an ischemic stroke event as the primary endpoint. The metaGRS score did a better job of predicting ischemic stroke than the genomic risk model alone, demonstrating a twofold increase in stroke predictive power. The top 0.25 percent of at-risk subjects had a threefold excess risk of stroke when compared with the middle decile reference group. The new metaGRS score was also similarly or more predictive of a future stroke event than several traditional clinical risk factors, such as family history of stroke, hypertension, body mass index, and smoking. When conventional clinical parameters and metaGRS were used together, the predictive capability further improved. A limitation of this study was that the cohort used to develop the model, although large, consisted of only British nationals of white ancestry. Therefore, applicability to other ethnicities is unknown. Nevertheless, this metaGRS score alone can identify the 0.25 percent of people with a threefold increased ischemic stroke risk, a risk level and population prevalence comparable to that of familial hypercholesterolemia, an established risk factor for cardiovascular disease. The goal of future efforts will be to target these high-risk individuals for such stroke prevention measures as smoking cessation, education about diet and exercise, glucose control, lipid-lowering lifestyle modifications, and blood pressure and lipid-lowering medication. This multi-pronged approach of using clinical and genomic information will likely be the cornerstone for the future practice of personalized preventative medicine.

Abraham G, Malik R, Yonova-Doing E, et al. Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke. *Nat Commun*. 2019;10. doi:10.1038/s41467-019-13848-1.

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