John I. Allen, Section Editor

Microsatellite Instability Testing in Colorectal Carcinoma: A Practical Guide

Joanna Gibson,[*] Jill Lacy,[‡] Ellen Matloff,[§] and Marie Robert[*]

[*]Department of Pathology, [‡]Department of Internal Medicine (Medical Oncology), [§]Department of Genetics, Yale School of Medicine, New Haven, Connecticut

Roadmap to the FUTURE of PRACTICE

In this month's "Road Ahead" article, we continue last month's theme that focused on the role of pathology in the care of our GI patients. Many gastroenterologists have successfully incorporated pathology into their core practices either within a business infrastructure or as part of a larger health care system. Dr Gibson and her colleagues at Yale University School of Medicine have helped inform us about a particularly difficult management problem: how to handle the genetically high-risk patients we see frequently in our hospitals and endoscopy units. New comprehensive practice guidelines concerning management of hereditary colon cancer syndromes are in development, but this short article provides a clear and concise guide for the practicing gastroenterologist.

John I. Allen, MD, MBA, AGAF
Special Section Editor

As health care reform progresses, the pressure to more closely integrate clinical service lines such as colorectal cancer (CRC) management has intensified. The practicing gastroenterologist may find that they are not equipped to understand pathology information required for coordinated team-based care of their patients. This is especially true in the case of molecular classification of colorectal cancer, something that has become a standard component of comprehensive oncologic care and has been incorporated into many gastroenterology (GI) practice pathology services. Molecular characterization and classification of colorectal cancer not only provides insight into the pathogenesis of cancer but has prognostic and therapeutic implications and is important for the gastroenterologist to understand and manage well. This review is a practical guide to the most common molecular tests used in what has become standard GI practice.

Molecular Classification of Colorectal Cancer

The molecular classification of colon cancer is based on the cumulative study of precursor lesions (such as adenomas and sessile serrated polyps), inherited colon cancer syndromes (such as familial adenomatous polyposis syndrome and Lynch syndrome/hereditary nonpolyposis colon cancer), and molecular profiling of colorectal cancers. Broadly, colorectal cancers are divided into 2 general groups based on genomic differences: chromosomal instability, accounting for 75% to 80% of all colorectal cancers, and microsatellite instability (MSI), accounting for 15% to 20% of all colorectal cancers.[1,2] Inherited colorectal susceptibility syndromes are estimated to account for approximately 1% to 2% of the MSI cancers and less than 1% of chromosomal instability cancers.

Microsatellite Instability Pathway

MSI is defined by changes of microsatellite length (repetitive noncoding DNA sequences) resulting from deficient mismatch repair (dMMR) during DNA replication.[1,3,4] The protein complex responsible for mismatch repair function is a tetramer composed of 2 heterodimers: MLH1/PMS2 and MSH2/MSH6.[4] The expression of each protein in a heterodimer is dependent on

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Abbreviations used in this paper: **CRC, colorectal cancer; dMMR, deficient mismatch repair; GI, gastroenterology; IHC, immunohistochemistry; MSI, microsatellite instability; MSS, microsatellite stable; PCR, polymerase chain reaction.**

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its partner, such that if one protein is absent, the partner protein consequently is degraded. When this occurs, the heterodimer is not available to form a functional tetramer and dMMR, as manifested by MSI, is the result.

Most dMMR/MSI cancers occur sporadically and are associated with the loss of MLH1 expression owing to epigenetic silencing of the MLH1 gene promoter via CpG island methylation.[1,2] The precursor lesion of sporadic dMMR/MSI cancers is believed to be the sessile serrated polyp, an epithelial proliferation characterized by the V600E BRAF mutation. Therefore, sporadic dMMR/MSI cancers also frequently harbor the V600E BRAF mutation.[5]

Approximately 1% to 2% of dMMR/MSI cancers occur in the setting of Lynch syndrome as a result of a hereditary gene defect in 1 of the 4 MMR genes.[6,7] The most frequently mutated gene in Lynch syndrome patients is MSH2 (40%), followed by MLH1 (30%). MSH6 and PMS2 are mutated at lesser frequencies, approximately 15% each. In contrast to the sporadic setting, dMMR/MSI cancer in Lynch syndrome patients arises from adenomas without BRAF mutations. Therefore, cancers in Lynch syndrome patients will have a wild-type BRAF gene.[5]

Technical Aspects of Testing

Methods of Deficient Mismatch Repair/ Microsatellite Instability Detection

dMMR is detected by immunohistochemistry (IHC) and MSI is detected by polymerase chain reaction (PCR).[3,8] PCR involves extraction of DNA from a tumor followed by DNA amplification of microsatellite markers, and determination of the amplified microsatellite lengths as compared with nontumor DNA from the same patient. Although laboratories vary with regard to the number of microsatellites tested, most use a standard set of 5 microsatellite markers. A tumor is classified as MSI-high if 2 or more of the 5 microsatellite markers show instability, as MSI-low if only 1 of 5 markers is unstable, and as microsatellite stable (MSS) if the microsatellite markers show no expansion.[3,8]

The IHC method uses antibodies directed against each MMR protein to detect the expression of the proteins in the tumor cells.[3] In cancers with dMMR/MSI, loss of nuclear expression of MMR proteins is seen in the cancer cells. In contrast, non-neoplastic cells, such as lymphocytes or adjacent colonic mucosa, show preserved nuclear expression of the MMR proteins, irrespective of the hereditary or sporadic setting. The non-neoplastic cells therefore serve as an important internal control for the IHC procedure. Most laboratories test each of the 4 MMR proteins. The majority of dMMR/MSI cancers show loss of expression of both MMR proteins in a heterodimer (either MLH1/PMS2 or MSH2/MSH6) in the cancer cells, with preserved expression of the other heterodimer. In sporadic dMMR/MSI cancers, loss of MLH1/PMS2 expression is characteristic, whereas in Lynch syndrome either heterodimer may be lost.[3,6] Occasionally, unusual IHC patterns exist, usually in the setting of Lynch syndrome, such as isolated loss of MSH6 in 10% of cancers or isolated loss of PMS2 in approximately 5% of cancers.[6]

Polymerase Chain Reaction vs Immunohistochemistry

The results obtained from PCR and IHC studies are complementary but provide different information.[3,7] The PCR method does not detect which protein in the mismatch repair tetramer is deficient. Therefore, PCR cannot distinguish between sporadic or Lynch syndrome associated dMMR/MSI cancer. IHC, on the other hand, provides specific mismatch repair protein expression data and can suggest etiology. Loss of MSH2/MSH6 suggests Lynch syndrome, whereas loss of MLH1/PMS2, although seen in Lynch syndrome, is characteristic of the more common sporadic dMMR/MSI cancer.[6] When present, abnormal IHC results also can be used to guide gene sequencing in patients with a high risk of Lynch syndrome. If the nuclear protein expression of all 4 MMR proteins is intact, the tumor is assumed to be MSS, with rare exceptions, and PCR may not be needed except in patients at high risk for Lynch syndrome.

IHC is inexpensive, is widely available in most pathology laboratories, and can be performed on both biopsy specimens and resection specimens, usually within 1 to 2 days. In the majority of cases, interpretation of IHC expression is straightforward and requires little training, with false-negative results rarely occurring. The latter occurs in less than 10% of Lynch syndrome patients with mutations that lead to protein dysfunction with preserved immunoreactivity.[4] PCR analysis is performed on tissue removed from a tissue block containing an adequate tumor sample (at least 30% of the tissue within the block consisting of tumor) for DNA extraction, as well as accompanying normal tissue for comparison. Biopsy samples may not contain sufficient tumor volume for PCR, whereas most resections are sufficient. The turnaround time for PCR is 5 days to 2 weeks.