

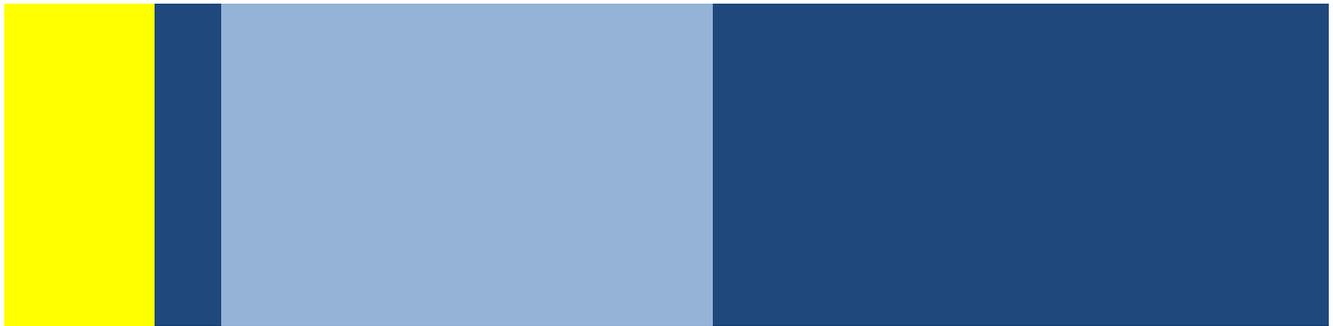


POET REPORT

Perspectives on Emerging Technology

Prognostic Uses of MSI Testing

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PATHOLOGIST'S MESSAGE

The classification of colon cancer is evolving as our understanding of molecular pathogenesis improves. A combination of genomic status (microsatellite and chromosomal instability) and epigenomic status (CpG island methylation) can now define colon carcinogenesis. While histopathologic features of colon cancer such as location, morphology, lymphocyte response, mucinous change, and border status strongly predict pathogenesis, they are not robust enough for reliable establishment of the pathogenesis of a particular colon cancer.

Microsatellite instability (MSI) is the hallmark of a defective mismatch repair (MMR) system and generally occurs because of a germline mutation in one of the MMR genes or methylation of the MLH1 promoter. Microsatellites are repetitive sequences distributed throughout the genome that consist of mono-, di-, or higher order nucleotide repeats such as (A)_n or (CA)_n which are more frequently copied incorrectly when DNA polymerases cannot bind efficiently. The MMR system, consisting of several proteins including MLH1, MSH2, MSH6 and PMS2, is responsible for the surveillance and correction of these errors. Polymerase chain reaction (PCR) amplifies specific microsatellite repeats, and detects MSI through the comparison of the length of nucleotide repeats in tumor cells and normal cells. If the length of repeat sequences from tumor vs. normal cells differs in more than 30% of the tested regions, microsatellite instability is said to be present.

Alternatively, immunohistochemistry (IHC) can confirm the presence or absence of MMR proteins. In general, MMR defects are the result of a germline mutation in one of the MMR genes followed by a hit on the second allele of that gene, or methylation of the promoter of a MMR gene (usually MLH-1) resulting in loss of protein function. There are three reasons why a pathologist may be interested in assessing MMR status:

- Prognosis – Several studies have shown that MSI tumors have a more favorable prognosis and are less prone to lymph node and systemic metastasis.
- Prediction of response to 5-FU and irinotecan therapy – Current data suggests that stage II MSI tumors do not benefit (and might actually be harmed by) 5-FU therapy and MSI tumors may be more responsive to irinotecan than microsatellite stable (MSS) tumors.
- Detection of Lynch Syndrome - The role of MSI as a genetic marker of Lynch Syndrome is well established. Both MSI detection and IHC are highly sensitive methods for the identification of a defective MMR system and guide clinicians towards informative, cost-effective genetic testing.

For all of these reasons, our clinical colleagues are requesting MSI testing with increasing frequency. The pathologist must select the best testing method based on the sample and clinical question. IHC can detect approximately 95% of tumors with a defective MMR system (those showing MSI), but will miss tumors containing mutations that produce a defective but still detectable protein by IHC (missense

mutations). MSI is a functional assay that will identify all tumors with a deficient MMR system and most cases of Lynch Syndrome; however, isolated defects in MSH6 or PMS2 do not always produce MSI.

CLINICAL CONTEXT

Colorectal carcinoma (CRC) is the third most common cancer of both men and women in the United States, with projections of 146,970 new cases and 49,920 deaths from CRC in 2009, accounting for almost 9% of all cancer deaths. (1) Molecular analysis has suggested that colorectal carcinoma is not a single disease but can be classified into three broad groups that exhibit chromosomal instability (CIN), microsatellite instability (MSI), or CpG island methylation. (Ogino and Goel 2008) with overlap between the groups.

The MMR system is responsible for the detection and correction of insertion/deletion mutations of short tandem repeat sequences and single base pair mismatches that occur during cell division. Failure of the MMR system may result from an inherited mutation in an MMR gene, a somatic mutation in an MMR gene, epigenetic suppression of MMR gene function, or a combination of the above any of which leads to microsatellite instability (MSI-H). All cases of hereditary nonpolyposis colorectal carcinoma (HNPCC) or Lynch Syndrome and approximately 15% of sporadic CRC exhibit defective MMR. Sporadic colon carcinomas displaying MSI-H are most commonly the result of MLH1 gene silencing by DNA methylation while Lynch Syndrome results from a germline mutation in an MMR gene. In contrast to Lynch Syndrome, sporadic MSI-H tumors are associated with smoking, female sex, and older age. Irrespective of the cause of the MMR deficiency, most DNA copy errors occur in non-coding regions of the genome. However more than 30 mutated genes have been identified in MMR deficient tumors, including those coding for DNA repair proteins MRE11A and hRAD50, growth factors TGF- β RII and IGFRII, pro-apoptotic factor BAX, mismatch repair proteins MSH3 and MSH6, and the histone modifier HDAC2. Although not specific, most MSI-H tumors show a characteristic phenotype including right sided location, low pathologic stage, mucinous differentiation, lymphocytic infiltration, and pushing margins.

MSI-H status has been shown to correlate with CRC patient survival. A meta-analysis of 7642 CRC patients from 32 studies, including 1277 MSI-H patients, revealed that MSI-H tumors were associated with better prognosis than MSS tumors (hazard ratio for overall survival was 0.65). MSI-H was an independent prognostic marker associated with a favorable outcome in CRC and these tumors were less prone to lymph node involvement and systemic metastases. Several studies support this further by confirming that only 4% of MSI-H tumors exhibit metastatic disease vs. 15-17% of MSS tumors. Yet, despite the consistency of this finding, clinical practice has not routinely incorporated MSI testing. Interestingly, CRC tumors harboring a V600E BRAF mutation are associated with an unfavorable outcome. While MSI-H CRC without an associated BRAF mutation has a favorable prognosis, patients showing MSI-H and a BRAF mutation have a prognosis similar to MSS CRC.

The value of MSI-H as a predictive marker for responsiveness to 5-FU, irinotecan, and other chemotherapeutic agents is controversial. Publication of conflicting results with respect to 5-FU effects over the past decade ranges from beneficial to no significant effect to detrimental, using overall survival and disease free survival as end-points. Most of these studies were retrospective, single institutional studies. These studies used inconsistent methods to identify MSI-H tumors and varied with respect to the stage of CRC included in the analysis. Emerging data suggests that irinotecan may be particularly beneficial in MSI-H CRC. Several preclinical models have suggested that MSI-H cell lines are sensitive to irinotecan, most likely related to mutations in the DNA repair proteins MRE11A and hRAD50. Similar to 5-FU responsiveness, the limited clinical data available suggest a trend toward benefit in MSI-H CRC but some studies show no effect.

The most common use of MSI testing in the clinic is the detection of Lynch Syndrome, where the role of MSI as an effective marker of Lynch Syndrome is well established.

TECHNOLOGY OVERVIEW

MSI Testing

MSI is tested through PCR amplification of a set of mono-and/or di-nucleotide repeats on tumor and normal DNA, followed by comparison of the peak patterns by capillary electrophoresis. If there are differences in the peak pattern for a specific microsatellite marker, the tumor is considered to show MSI at that marker. In 1998, an National Cancer Institute (NCI) consensus panel recommended analyzing two mononucleotide markers (BAT25 and BAT26) and three dinucleotide markers (D2S123, D5S346 and D17S250). With this panel of markers, the tumor is classified as MSI-H if size alterations or shifts are observed in two or more of the five markers. If only one marker shows instability, the tumor is said to show a low microsatellite instability (MSI-L) phenotype. If none of the markers show instability then the tumor exhibits a microsatellite stable (MSS) phenotype. While some labs use a different or an extended panel of markers, CAP survey data indicates that most labs are using the NCI panel or a commercially available kit from Promega. The Promega kit uses only mononucleotide markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24) since recent studies have shown that mononucleotide markers have higher specificity and similar or better sensitivity than dinucleotide markers for the detection of an MSI-H phenotype. The kit also contains two pentanucleotide markers (Penta C and Penta D) that are used as specimen identification markers to insure that the tumor and normal DNA are derived from the same patient (i.e., to detect specimen mix-ups). With this set of markers tumors are considered as MSI-H if 2 or more of the 5 markers show instability.

As of yet, there is no FDA-approved MSI test. Consequently, MSI testing is considered a laboratory-developed test (LDT), and the lab must conduct the appropriate analytic validation testing required by

CLIA'88 regulations. Since 2004, the CAP Molecular Oncology Committee has been offering MSI proficiency testing with two mailings a year. In 2010, the IHC Committee will offer proficiency testing for DNA MMR IHC testing. In 2011, the two committees will offer a joint survey.

DNA MMR Immunohistochemistry

IHC with antibodies to MLH1, MSH2, PMS2, and MSH6 proteins is commonly performed. The DNA MMR proteins are DNA repair enzymes that exhibit nuclear expression when present. Normal lymphocytes and colonic epithelium generally exhibit moderate to strong nuclear staining and should be used as internal controls to validate pre-analytic and analytic performance of each IHC assay. An absence of staining within the tumor, with normal expression in the internal control cells indicates a loss of expression for that protein. These four stains show a number of expression patterns in CRC. The most common pattern is for all stains to show normal expression. This strongly suggests that the tumor has normal DNA MMR and that the patient is unlikely to have hereditary nonpolyposis colorectal cancer (Lynch Syndrome). However, about 5% of CRC with defective DNA MMR (MSI-H tumors) show normal expression; thus this pattern does not absolutely exclude the possibility of HNPCC. Possible reasons for this include an unevaluated DNA MMR gene product is defective (e.g., MSH3) or that one of these genes is expressed but not functioning normally, perhaps due to a missense mutation. The second most common pattern shows loss of MLH1 and PMS2 but normal staining for MSH2 and MSH6, indicating that the tumor has defective DNA MMR (invariably an MSI-H phenotype). These patients may or may not have HNPCC. Approximately two-thirds of these cases represent sporadic tumors that have MLH1 inactivation due to MLH1 promoter hypermethylation with the other third representing HNPCC patients with germline MLH1 mutations. The remaining three patterns show a loss of MSH2 and MSH6, MSH6 alone, or PMS2 alone frequently resulting in an MSI-H phenotype. Loss of MSH2 and MSH6, and PMS2 alone, has a high positive predictive value for HNPCC. Most patients whose CRC show loss of MSH2 and MSH6 are found to have a germline MSH2 mutation.

Pathologists should be aware that performing IHC for prognostic or predictive reasons may have legal genetic implications since it can inadvertently identify patients that have HNPCC who may have not wanted to know (particularly patients that show loss of MSH2+MSH6, MSH6 alone, or PMS2 alone). Consequently, pathologists should determine if state-specific guidelines require informed consent due to the consideration that IHC testing may be a form of "genetic" testing.

IMPACT ON CURRENT PATHOLOGY PRACTICE

Pathologists need to recognize the histologic and clinical features that should prompt at least a recommendation for MMR testing. For anatomic pathology laboratories utilizing immunohistochemistry, becoming proficient in the technical aspects of this testing, interpretation of immunoreactivity and

clinical significance of results will provide important prognostic and predictive information on this common tumor. In the short term, most practices will outsource MSI testing by PCR to a reference laboratory.

ACCELERATION/DECELERATION TRIGGERS TO ADOPTION

An increased understanding by clinicians, pathologists, and patients of the value of MMR testing for prognosis, selection of therapy and identifying Lynch Syndrome patients will accelerate the adoption of this testing. Pathologist will play a central role and this can serve as an opportunity to advance the role of the pathologist in active patient management. Additionally, an understanding of the clinical benefit of identifying HNPCC patients before they have developed tumors will also accelerate the adoption of this test. A report from the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) working group found moderate evidence that universal screening of all colorectal cancer patients is the most sensitive approach for identifying Lynch Syndrome patients and the related carriers. Next-generation sequencing technologies, that can rapidly and inexpensively sequence all of the DNA MMR genes, might obviate the need for MSI and IHC testing for HNPCC screening in the future. However, these technologies are not likely to be widely available for several years. Additionally, MSI and IHC testing offers useful information, not provided by simply doing germline sequencing of the DNA MMR genes. An increased understanding of the value of this testing for guiding clinical management of patients with specific widely accepted recommendations by oncologists will increase adoption of this testing. However, we need further studies to clarify the role of MSI and IHC testing for altering patient management.

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