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Point/Counterpoint

Immunohistochemistry versus Microsatellite Instability Testing for Screening Colorectal Cancer Patients at Risk for Hereditary Nonpolyposis Colorectal Cancer Syndrome

Part II. The Utility of Microsatellite Instability Testing

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Germline mutations in the mismatch repair genes mutL homolog 1 (*MLH1*) and mutS homolog 2 (*MSH2*), *MSH6*, and postmeiotic segregation increased 2 (*PMS2*) lead to the development of hereditary nonpolyposis colorectal cancer (HNPCC). Diagnosis of HNPCC relies on the compilation of a thorough family history of cancer, documentation of pathological findings, tumor testing for microsatellite instability (MSI) and immunohistochemistry (IHC), and germline mutation analysis of the suspected genes. As a hallmark of HNPCC, microsatellite instability is widely accepted as a primary method for identifying individuals at risk for HNPCC. It serves as an excellent, easy-to-evaluate marker of mismatch repair deficiency. Recent improvements in MSI testing have significantly enhanced the accuracy and reduced its cost. Proficiency testing for MSI is available, and laboratory-to-laboratory reproducibility of such testing can be easily evaluated. In addition, the combination of microsatellite instability testing, *MLH1* promoter methylation analysis, and *BRAF* (V600E) mutation analysis can distinguish a sporadic colorectal cancer from one associated with HNPCC, helping to avoid costly molecular genetic testing for germline mutations in mismatch repair genes. In this article, we discuss the development of MSI markers used for HNPCC screening and focus on the advantages and disadvantages of MSI testing in screening for HNPCC patients. We conclude that MSI is as sensitive and specific as IHC, given its excellent reproducibility and its potential capability to indicate mutations not be detected by IHC. MSI has been used and will continue to prevail as the primary screening tool for identifying HNPCC patients. ***(J Mol Diagn 2008, 10:301–307; DOI: 10.2353/jmoldx.2008.080062)***

The diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) at the molecular level relies on the presence of a deleterious germline mutation in one of the mismatch repair (MMR) genes. Because cancer morbidity and mortality can be dramatically reduced by colonoscopic screening of individuals with the HNPCC syndrome and by prophylactic surgeries, molecular screening of colorectal cancer patients for HNPCC is now feasible.[1–4] The challenge is to establish a strategy that is able to screen effectively for HNPCC. Microsatellite instability (MSI) in colorectal cancer was discovered in 1993 and was subsequently found to be present in colon cancer tissue from most HNPCC patients.[5–8]

Genotyping for microsatellite instability was initially used to screen for HNPCC,[1,3] while immunohistochemistry (IHC) analysis of the MMR proteins has been more recently proposed as an alternative method for screening HNPCC.[2] Two recent studies have indicated that microsatellite instability testing and immunohistochemistry are both highly effective strategies for selecting patients for molecular genetic testing (germline mutation analysis).[2,9] However, it is unclear which approach should be used as the primary method for screening HNPCC. Here, we summarize both the early and more recent literature data on the use of MSI, discuss the

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molecular basis of microsatellite instability in MMR-deficient tumors, and outline the advantages and limitations of this methodology. Our analysis indicates that given several merits of MSI that IHC does not have (see Advantages of MSI, below), MSI is an excellent, easy to use marker for identifying HNPCC. Therefore, it is important that clinicians are aware of the pros and cons of these two tests as both are widely used in screening HNPCC cases.

Literature Review

Microsatellite Instability

Microsatellites are short, tandemly repeated DNA sequences of 1 to 6 bases scattered throughout the human genome;[10,11] they can be affected by a form of genomic instability called microsatellite instability.[5,6,8,12] MSI is a change in length of a microsatellite allele due to either insertion or deletion of repeating units during DNA replication and failure of the DNA mismatch repair system to correct these errors. MSI analysis has been used as a screening method to identify HNPCC patients and a subgroup of colorectal cancer patients for further genetic testing.

The DNA Mismatch Repair System and HNPCC

DNA MMR is an effective post-replication mechanism. Most errors that occur during DNA replication are immediately corrected by the 3′ to 5′ exonuclease activity of DNA polymerase. It is estimated that 99.9% of the mutations that escape the proofreading activity of DNA polymerase (DNA polymerase slippage) are repaired by the DNA MMR system, particularly single-bp mismatches and "loop outs" of unpaired bases.[13] The replication machinery slips more frequently on repetitive sequences than on non-repetitive sequences, so microsatellite instability occurs in the repetitive sequences in MMR-deficient cells. The causes of MMR defects are: i) germline mutations in any one of the five DNA MMR genes—mutS homolog 2 (*MSH2*), mutL homolog 1 (*MLH1*), *MSH6*, and, infrequently, postmeiotic segregation increased 2 or 1 (*PMS2* or *PMS1*), causing HNPCC[14]; and ii) somatic inactivation of *MLH1* caused by promoter hypermethylation in approximately 15% of sporadic colorectal cancer.[5,8,15]

In MMR-deficient cells, genes that contain a microsatellite in their coding regions are more prone to frameshift mutations. Mutations in key genes that regulate cell growth and apoptosis ultimately lead to dysregulated cell proliferation and/or cell death, which further speeds the evolution of colorectal cancer.[16] One example is the well studied frameshift mutations in the *TGF-βRII* gene, which commonly occurs in colorectal cancer but not in endometrial cancer. In most colorectal cancers, the polyadenine tract mutations affect both alleles of *TGF-βRII*, suggesting that *TGF-βRII* functions as a tumor suppressor during colorectal cancer development and is a critical target of inactivation in mismatch repair-deficient tumors.[17–19] Similar frameshift mutations in coding microsatellites also occur in other genes involved in growth control and apoptosis (*TCF4*, *IGFIIR*, *BAX*, and *RIZ*), as well as in genes involved in DNA mismatch repair itself (*MSH6*, *MSH3*, and *MSH2*).[14]

MSI as a Marker for HNPCC Screening

The original (1997) Bethesda guidelines[20,21] proposed a panel of five microsatellite markers for the uniform analysis of MSI in HNPCC. This panel, which is referred to as the Bethesda panel, included two mononucleotide (BAT-25 and BAT-26) and three dinucleotide (D5S346, D2S123, and D17S250) repeats. Samples with instability in two or more of these markers are defined as MSI-High (MSI-H), whereas those with one unstable marker are designated as MSI-Low (MSI-L). Samples with no detectable alterations are MSI-stable (MSS). Because mononucleotide markers appear to be more sensitive than dinucleotide markers for the detection of MSI-H, limitations in the original panel resulting from inclusion of dinucleotide repeats were addressed at a 2002 National Cancer Institute workshop, and revised recommendations for MSI detection were proposed. The revision mainly recommends testing a secondary panel of mononucleotide markers, such as BAT-40, to exclude MSI-L in cases in which only the dinucleotide repeats are mutated.[22] According to the revised Bethesda guidelines, strategies based on MSI testing were effective in identifying *MLH1/MSH2* mutation carriers (sensitivity 81.8% and specificity 98.0%).[9]

Advantages of MSI

Microsatellite Instability Serves as an Excellent, Easy-to-Evaluate Marker of MMR Deficiency, and Recent Improvements in MSI Testing Significantly Enhance Accuracy and Reduce Cost

A hallmark of tumors in HNPCC is microsatellite instability. Typically half or more of all microsatellites have mutations (contraction or elongation) in the tumor cells; therefore, microsatellite instability serves as an excellent, easy-to-evaluate marker of mismatch repair deficiency. Since both HNPCC and MSI are caused by MMR defects, MSI can be used as a surrogate marker of HNPCC and has been widely accepted as a primary method for identifying individuals at risk for HNPCC.

As mentioned under Literature Review, a recent follow-up NCI workshop recognized the limitations of the original Bethesda panel[20,21] due to the inclusion of dinucleotide repeats, which are less sensitive and less specific than mononucleotide repeats for identification of cancers with MMR deficiency.[22] To improve the accuracy of MSI testing using the Bethesda panel of MSI markers, a panel of five mononucleotide markers was developed and incorporated into a multiplex fluorescence assay: the Promega (Madison, WI) MSI Analysis System.[23] These mononucleotide repeat markers are quasi-monomorphic; that is, almost all individuals are homozygous for the