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Screening for Lynch Syndrome by Microsatellite Instability Analysis and Immunohistochemistry

Jason D. Merker, MD, PhD,
CAP Molecular Oncology Committee

Hereditary nonpolyposis colorectal cancer (HNPCC) is an inherited cancer syndrome characterized by early onset colorectal cancer as well as endometrial, urinary tract, small bowel, ovarian, gastric, pancreatic, hepatobiliary, brain, and skin tumors. HNPCC is an autosomal dominant syndrome defined by family history and clinical criteria[1], and it accounts for approximately 2% of all colorectal cancers. In a subset of HNPCC patients, as well as in some colorectal cancer patients that do not meet the formal criteria for HNPCC, the cancer predisposition is caused by germline mutations in DNA mismatch repair (MMR) genes. The identification of inherited MMR mutations by genetic testing impacts the care and surveillance of the patient[2] and enables predictive testing of family members.

Individuals with germline mutations in one of the MMR genes are defined as having Lynch syndrome. The revised Bethesda Guidelines[3] established criteria to identify individuals at increased risk for Lynch syndrome based on clinical information, family history, or histology; however, most individuals identified by the revised guidelines do not have Lynch syndrome. Furthermore, genetic testing for germline MMR mutations is complicated, time-consuming, and expensive. Consequently, it is generally recommended that patients at increased risk for Lynch syndrome undergo pre-screening with microsatellite instability (MSI) analysis and immunohistochemistry (IHC). Individuals with tumors that display high levels of microsatellite instability or loss of expression of MMR proteins by immunohistochemistry are then referred for germline mutation testing. Use of this step-wise approach detects virtually all cases of Lynch syndrome in a cost-effective manner.

MSI refers to a type of genomic instability in tumor tissue caused by the failure of the DNA mismatch repair system to correct errors that are introduced during normal DNA replication. This process results in the accumulation of a variety of mutations throughout the genome of the tumor cells, including alterations in the length of small repetitive microsatellite sequences that are scattered throughout the genome. For MSI analysis, DNA is usually extracted from paraffin-embedded tumor tissue and normal tissue or peripheral blood. Reliable demonstration of MSI requires that at least 30% of the tumor specimen is composed of tumor cells.

Polymerase chain reaction is used to amplify the region containing the microsatellite, and the products are then separated on the basis of size. A microsatellite is considered unstable if the distribution of the fragments from the tumor sample differs from that of the normal tissue (Figure 1).

To determine if a tumor has a high-frequency MSI (MSI-H) phenotype, a panel of five or more microsatellite markers is examined.[3, 4] MSI-H is defined as instability in ≥30% of the examined microsatellites. A tumor is designated as low-frequency MSI (MSI-L) or microsatellite stable (MSS) if <30% of the markers show instability. Reliably distinguishing between MSS and MSI-L requires analysis of more than five markers, but this distinction is not necessary in the clinical setting at the current time since MSS and MSI-L tumors display similar phenotypes. Virtually all tumors from individuals with Lynch syndrome demonstrate MSI-H, but MSI-H is also observed in at least 10% of sporadic colorectal cancers. Therefore, MSI testing is a useful screening test for Lynch syndrome. However, diagnosis of Lynch syndrome requires further molecular testing to directly identify the MMR gene mutation.

Using IHC to detect loss of MMR protein expression is a complementary method to screen for individuals with Lynch syndrome. IHC analysis for the DNA MMR proteins MLH1, MSH2, MSH6, and PMS2 is readily available on a clinical basis. The vast majority of colorectal tumors that demonstrate an MSI-H phenotype is found to show loss of expression of one or more of the DNA MMR proteins,[5] although occasional tumors with an MSI-H phenotype that do not demonstrate loss of MMR protein expression have been described. Loss of MMR protein expression is detected by the absence of nuclear staining in the tumor cells and the presence of nuclear staining in lymphocytes and normal colon epithelial cells (Figure 2). Since these proteins are present in complexes, loss of expression of one MMR protein is often associated with the loss of the partner MMR protein. Loss of expression of MLH1 is almost always accompanied by loss of PMS2 expression, and loss of expression of MSH2 is almost always accompanied by loss of MSH6 expression. In contrast, loss of MSH6 expression and loss of PMS2 expression can sometimes be seen without accompanying loss of MSH2 or MLH1 expression. Demonstration of the loss of particular MMR proteins can then direct which genes should be examined by germline mutation analysis. For example, loss of MSH2 and MSH6 expression generally indicates the presence of a germline *MSH2* mutation. Loss of MSH6 only or PMS2 only generally indicates the presence of a germline *MSH6* or *PMS2* mutation, respectively. The implications are a little more complicated for tumors with loss of MLH1 and PMS2 expression. This pattern can indicate the presence of a germline mutation but can also occur in approximately 15% of sporadic colorectal cancer due to non-heritable *MLH1* promoter hypermethylation. Since DNA MMR IHC results can strongly suggest the presence of a germline DNA MMR mutation, many institutions require the patient be provided with genetic counseling by an appropriate health care provider prior to initiating IHC studies.

Concomitant MSI and IHC analysis effectively identifies colorectal cancer patients at increased risk for Lynch syndrome who should be offered germline mutation analysis. The decision to perform MSI analysis, IHC, or both is dependent on institutional strengths and resources. Once a tumor is determined to be MSI-H and/or demonstrates loss of MMR protein expression by IHC, the individual can, after appropriate genetic counseling, elect to have molecular genetic testing to identify a germline mutation in one of the MMR genes. The order of genes to be tested is influenced by the IHC results and that approximately 80% of identified germline mutations in the MMR genes of Lynch syndrome families are detected in *MLH1* and *MSH2*. The identification of germline MMR gene mutations significantly impacts the care of colorectal cancer patients and their families, and a step-wise approach allows the efficient and cost-effective identification of Lynch syndrome cases.

References:

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Figure 1. Capillary electropherograms from MSI analysis of normal (A) and tumor (B) tissue from a young patient with colorectal cancer. MSI is observed at the three mononucleotide repeat loci (*NR21*, *BAT25*, *MONO27*) shown. This case demonstrated MSI in all five mononucleotide markers tested and was

classified as MSI-H. Immunohistochemical analysis of this tumor is shown in Figure 2.

Figure 2. Immunohistochemical analysis of a colorectal tumor with antibodies directed against MLH1 (A), PMS2 (B), MSH2 (C), and MSH6 (D) at low- and high-power magnification. This tumor demonstrates loss of MLH1 and PMS2 staining and intact MSH2 and MSH6 staining. The tumor has a MSI-H phenotype (see Figure 1) and demonstrates loss of MLH1 and PMS2 protein expression; therefore, the clinical team recommended that the patient undergo germline *MLH1* mutation testing. Photos courtesy of Dr. Matthew Anderson.