Microsatellite Testing in Colon Cancer

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Significant progress has been made in understanding the molecular genetic basis of colorectal cancer (CRC). That information is paving the way to understanding the genetic basis of other tumors, as well. Oncology nurses should anticipate the routine integration of this information and testing of CRC tumors to understand the molecular basis of the disease in clinical practice. Molecular testing can lead to the identification of families at risk for hereditary cancer syndromes, particularly Lynch syndrome, which sometimes is referred to as hereditary nonpolyposis colorectal cancer. Knowledge of the genetic basis of CRC also contributes valuable information aimed at selecting appropriate and effective targeted therapy.

Three pathogenetic pathways have been identified and implicated in the development of CRC: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). The characteristics of those molecular pathways are shown in Table 1.

Microsatellite Instability Defined

MSI is the condition in which genetic hypermutability (i.e., a state in which mutations are abnormally frequent) exists. MSI results from defective DNA mismatch repair (MMR) genes. Defective MMR, which can lead to MSI, occurs in two main situations: (a) an individual with Lynch syndrome who has a germline MMR mutation develops an acquired mutation in his or her working allele of the MMR gene, or (b) when an individual has acquired *MLH1* promoter hypermethylation of one MMR gene and develops an acquired mutation of the other allele. Screening a colorectal tumor for MSI provides phenotypic evidence that MMR is not functioning properly but does not identify the underlying pathology.

MMR genes correct errors that spontaneously occur during DNA replication, including single-base mismatches or short insertions and deletions (Matloff, Lucas, Polydorides, & Itzkowitz, 2013). The proteins involved in MMR form a complex that binds to the mismatch, identifies the correct strand of DNA, and then subsequently excises the error and repairs the mismatch. Cells with abnormally functioning MMR tend to accumulate errors rather than correct them. As a result, gene sequences are not preserved consistently through DNA replication, and new microsatellite fragments are created. That repair system is mainly composed of four proteins (MLH1, MSH2, MSH6, and PMS2) interacting together to recognize mismatches and remove them (Buecher et al., 2013).

Laboratory Techniques

MSI detects that MMR is defective, but does not imply the mechanism by which it is impaired. PCR technology can be used as a cost-effective screening tool for MMR gene mutations that can be confirmed by gene sequencing. MSI testing can be performed on fresh, frozen, or paraffin-embedded tumor material. PCR-based assays reveal defective microsatellites.

Five markers (often called Bethesda markers) have been recommended by the National Cancer Institute to screen for MSI in CRC tumors (Weissman et al., 2012). The Bethesda panel includes two mononucleotide repeats (BAT-25 and BAT-26) and three dinucleotide repeats (D2S123, D5S346, and D17S250). If the tumor has no instability (i.e., none of the repeat lengths has changed), the tumor is considered microsatellite stable. MSI detection in two of the markers (or 30% or more of unstable markers if a larger panel is used) is considered a positive result (Buecher et al., 2013). The MSI-low phenotype occurs with instability in only one marker, or 10%–30% of markers in larger panels.

The PCR method does not detect which protein in the MMR is deficient (Gibson, Lacy, Matloff, & Robert, 2014). PCR technology cannot distinguish between sporadic cancers or Lynch syndrome in MSI-high tumors. Immunohistochemical (IHC) analysis of MMR proteins is an alternative method to detect MSI and primarily is used to complement MSI genetic testing when Lynch syndrome is suspected (Buecher et al., 2013). The loss of expression of one or more of those proteins indicates an MMR defect and determines which gene is most likely to have a germline mutation. The interpretation of IHC must consider the dependent expression of specific MMR protein heterodimers: MSH2/MSH6 and MLH1/PMS2. PMS2 and MSH6 are considered minor MMR proteins that work with the two major MMR proteins, MLH1 and MSH2, respectively, and whose expression is dependent on their binding to the major partner. Therefore, the loss of expression of MSH2 is frequently associated with the loss of expression of MSH6, and this pattern is highly suggestive of an MSH2 germline mutation. In addition, loss of expression of MLH1 is frequently associated with loss of expression of PMS2, and this pattern may result either from MLH1 germline mutation or from acquired somatic hypermethylation of the MLH1 gene promoter (Power, Gloglowski, & Lipkin, 2010). Loss of MSH2/MSH6 suggests Lynch syndrome, whereas loss of MLH1/PMS2, although seen in Lynch syndrome, is characteristic