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

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of the more common sporadic MMR/MSI CRC. A 5%–10% false-negative rate exists with both MSI and IHC testing (Burt et al., 2013).

Clinical Implications of Microsatellite Instability Testing

MSI is found in about 15% of CRCs and has a key role in the diagnostic strategy of identifying individuals with Lynch syndrome, whose tumors are characterized by the presence of this phenotype (Buecher et al., 2013). About 25% of individuals with Lynch syndrome do not meet traditional clinical Amsterdam or Bethesda criteria for germline testing (Weissman et al., 2012) (see Figure 1). Lynch syndrome, which is an autosomal dominant condition caused by an inactivating germline mutation of one of the four genes involved in the MMR system (*MLH1*, *MSH2*, *MSH6*, *PMS2*), is the most frequent form of hereditary CRC and accounts for about 5% of all cases of CRC. The identification of individuals with Lynch syndrome is critical because the application of an early and intensive surveillance program combined with prophylactic surgery significantly reduces the incidence of colorectal and gynecologic cancers and other malignancies, as well as improves mortality rates, for both the patient and affected relatives. Therefore, accurate identification is beneficial in terms of cost-effectiveness and improved quality of life (Serrano et al., 2012).

Knowledge of MSI also provides important prognostic information. MSI tumors are associated with a good prognosis and are known to have a resistance to 5-fluorouracil (5-FU)–based adjuvant chemotherapy, which has a clinical application when selecting therapy (Kloor, Staffa, Ahadova, & von Knebel Doeberitz, 2014; Meguerditchian & Bullard Dunn, 2013). Patients with MSI-high CRC who receive 5-FU treatment do not have an advantage over those not receiving it, and this treatment might be harmful in MSI stage II CRC (Buecher et al., 2013; Hampel, 2010). Given the observation that MSI-high tumors display less aggressive behavior, in addition to the fact that MSI tumors respond poorly to 5-FU–based chemotherapy, MSI testing for stage II CRCs is becoming more routine (Gala & Chung, 2011) and is recommended by the National Comprehensive Cancer Network (Kelley, Van Bebber, Phillips, & Venook, 2011). A better understanding of somatic genetics and molecular pathways involved in MMR CRC is guiding continued research toward novel and tailored therapeutic strategies for this disease.

The trend toward universal MSI screening for all CRC tumors is not without controversy (Senter, 2012; Weissman et al., 2012). Screening for MSI does not replace a thorough cancer risk assessment by a qualified genetics professional. Because testing can be complex, tumor and molecular results may not be straightforward, and psychosocial issues may arise, all of which necessitate involvement of a trained genetics professional. Genetic cancer risk assessment and counseling are important components of a Lynch syndrome evaluation (Power et al., 2010). In some institutions, universal tumor testing has been implemented on all newly diagnosed patients with CRC and more recently—to a lesser extent—all newly diagnosed endometrial cancers, regardless of age at diagnosis or family history (Weissman et al., 2012). Before such policies are implemented, clear protocols need to be established to ensure families at risk for Lynch syndrome receive an appropriate evaluation and psychosocial support.

Universal molecular testing of CRC tumors to identify individuals with Lynch syndrome is gaining popularity, largely because of the unreliability of traditional clinical diagnostic criteria, but it is not without limitations. An estimated 15%–20% of patients with CRC have an autosomal dominant form

Table 1. Characteristics of the Molecular Pathways in CRC

Characteristic	CIN	MSI	CIMP
Definition	Loss or gain of chromosome arms, translocations, or gene amplifications	MSI CRCs have a better prognosis in general and a different response to the chemotherapeutic agent 5-FU. Sporadic MSI CRC usually occurs at an older age, and in these cases, no family history of cancer typically exists. Genetic MSI CRC usually occurs at a younger age (younger than 50 years) and with a family history of cancer.	Sometimes referred to as the serrated pathway
Precursor lesions	Tubular adenoma polyp or villous adenoma polyp	Polyps at an early age	Serrated adenoma
Mutation	Acquired somatic mutations in *APC*, *KRAS*, *TP53*, *SMAD4*, *PI3KCA*, *SOX9*, *ARID1A*, and *FAM123B*	Two-thirds of MSI tumors (10% of all CRCs) are sporadic and caused by somatic biallelic hypermethylation of the MLH1 promoter. One-third of MSI tumors (5% of all CRCs) are germline Lynch syndrome. MMR genes, *MSH2*, *MSH6*, *MLH1*, and *PMS2*, are involved in tumor initiation and progression.	DNA hypermethylation at specific regulatory sites, enriched in CpG islands in the promoter regions of tumor suppressor Genes mainly in the BRAF mutation
Affected side of colon	Primarily left-sided	Primarily right-sided	Tend to be right-sided, but can be found throughout the colon

CIMP—CpG island methylator phenotype; CIN—chromosomal instability; CRC—colorectal cancer; 5-FU—5-fluorouracil; MMR—mismatch repair; MSI—microsatellite instability

Note. Percentage of CRCs is 50%–75% for CIN,15% for MSI, and 20% for CIMP.

Note. Based on information from Gibson et al., 2014; Kalady, 2013; Rosner & Strul, 2014.

Amsterdam II Criteria

- At least three relatives with a Lynch syndrome cancer (e.g., colorectal, endometrial, small bowel, ureter, renal pelvis cancer) *and*
- One of these relatives is a first-degree relative[a] of the other two *and*
- Two successive generations are affected *and*
- At least one diagnosis is at age 50 years or younger *and*
- Familial adenomatous polyposis is excluded *and*
- Tumors should be verified pathologically and histologically.

Bethesda Criteria

- Colorectal cancer diagnosed in a patient aged younger than 50 years
- Regardless of age, the presence of synchronous or metachronous colorectal or other Lynch syndrome–related tumors.
- Colorectal cancer diagnosed in a patient with one or more first-degree relatives with a Lynch syndrome–related cancer, with one of the cancers being diagnosed at younger than 50 years of age.
- Colorectal cancer diagnosed in a patient with two first- or second-degree relatives[b] with Lynch syndrome cancers, regardless of age.

[a] First-degree relatives include siblings, offspring, and parents.

[b] Second-degree relatives include grandparents, aunts, and uncles.

Figure 1. Amsterdam II and Bethesda Criteria

Note. Based on information from Burt et al., 2013; Umar et al., 2004; Weissman et al., 2012.

of inheritance but not MSI, and will not be identified with universal screening (Hall, 2010; Matloff et al., 2013). The advent of next-generation sequencing may help further identify families with hereditary predisposition and MSI tumors. Patients who receive care in an institution that performs universal MSI testing on CRC tumors do not necessarily have to give informed consent for MSI testing and may not realize that they might learn that they have Lynch syndrome and are at risk for the hereditary predisposition to colorectal and other cancers, which could be accompanied by negative psychosocial consequences (Hall, 2010). The ordering healthcare provider needs to ascertain that those with MSI-high tumors are referred to genetics professionals for complete evaluation and realize the clinical implications and potential risks of MSI testing (Weissman et al., 2012).

The MSI phenotype was first discovered in CRC, but since then, its detection has been regarded as indicative of a defective MMR system. MSI is not unique to CRCs, but also is observed in other tumor types, including gastric cancer, endometrial cancer, ovarian cancer, sebaceous carcinomas, glioblastoma, and lymphomas (Rosner & Strul, 2014). Oncology professionals should continue to expect more knowledge and research to emerge, which will provide insight into the best way to use the information gleaned from molecular testing, such as screening for hereditary predisposition syndromes and improved tailored treatment for malignancy.

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References

Buecher, B., Cacheux, W., Rouleau, E., Dieumegard, B., Mitry, E., & Lièvre, A. (2013). Role of microsatellite instability in the management of colorectal cancers. *Digestive and Liver Disease, 45,* 441–449. doi:10.1016/j.dld.2012.10.006

Burt, R.W., Cannon, J.A., David, D.S., Early, D.S., Ford, J.M., Giardiello, F.M., . . . Freedman-Cass, D. (2013). Colorectal cancer screening. *Journal of the National Comprehensive Cancer Network, 11,* 1538–1575.

Gala, M., & Chung, D.C. (2011). Hereditary colon cancer syndromes. *Seminars in Oncology, 38,* 490–499.

Gibson, J., Lacy, J., Matloff, E., & Robert, M. (2014). Microsatellite instability testing in colorectal carcinoma: A practical guide. *Clinical Gastroenterology and Hepatology, 12,* 171–176.

Hall, M.J. (2010). Counterpoint: Implementing population genetic screening for Lynch Syndrome among newly diagnosed colorectal cancer patients—Will the ends justify the means? *Journal of the National Comprehensive Cancer Network, 8,* 606–611.

Hampel, H. (2010). Point: Justification for Lynch Syndrome screening among all patients with newly diagnosed colorectal cancer. *Journal of the National Comprehensive Cancer Network, 8,* 597–601.

Kalady, M.F. (2013). Sessile serrated polyps: An important route to colorectal cancer. *Journal of the National Comprehensive Cancer Network, 11,* 1585–1594.

Kelley, R.K., Van Bebber, S.L., Phillips, K.A., & Venook, A.P. (2011). Personalized medicine and oncology practice guidelines: A case study of contemporary biomarkers in colorectal cancer. *Journal of the National Comprehensive Cancer Network, 9,* 13–25.

Kloor, M., Staffa, L., Ahadova, A., & von Knebel Doeberitz, M. (2014). Clinical significance of microsatellite instability in colorectal cancer. *Langenbeck's Archives of Surgery, 399*(1), 23–31. doi:10.1007/s00423-013-1112-3

Matloff, J., Lucas, A., Polydorides, A.D., & Itzkowitz, S.H. (2013). Molecular tumor testing for Lynch syndrome in patients with colorectal cancer. *Journal of the National Comprehensive Cancer Network, 11,* 1380–1385.

Meguerditchian, A.N., & Bullard Dunn, K. (2013). Biomarkers and targeted therapeutics in colorectal cancer. *Surgical Oncology Clinics of North America, 22,* 841–855. doi:10.1016/j.soc.2013.07.002

Power, D.G., Gloglowski, E., & Lipkin, S.M. (2010). Clinical genetics of hereditary colorectal cancer. *Hematology/Oncology Clinics of North America, 24,* 837–859.

Rosner, G., & Strul, H. (2014). Should microsatellite instability be tested in all cases of colorectal cancer? *Current Colorectal Cancer Reports, 10,* 27–35. doi:10.1007/s11888-013-0204-3

Senter, L. (2012). Genetic testing by cancer site: Colon (nonpolyposis syndromes). *Cancer Journal, 18,* 334–337.

Serrano, M., Lage, P., Belga, S., Filipe, B., Francisco, I., Rodrigues, P., . . . Pereira, A.D. (2012). Bethesda criteria for microsatellite instability testing: Impact on the detection of new cases of Lynch syndrome. *Familial Cancer, 11,* 571–578.

Umar, A., Boland, C.R., Terdiman, J.P., Syngal, S., de la Chapelle, A., Rüschoff, J., . . . Srivastava, S. (2004). Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *Journal of the National Cancer Institute, 96,* 261–268.

Weissman, S.M., Burt, R., Church, J., Erdman, S., Hampel, H., Holter, S., . . . Senter, L. (2012). Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. *Journal of Genetic Counseling, 21,* 484–493. doi:10.1007/s10897-011-9465-7