Molecular Evaluation of Colorectal Adenocarcinoma

Current Practice and Emerging Concepts

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KEYWORDS

• Colorectal cancer • Targeted therapy • Lynch syndrome • Microsatellite instability
• Mismatch repair • EGFR pathway • Immune checkpoint blockade • PD-L1

Key points

- Molecular testing is a standard component of the routine pathologic evaluation of colorectal carcinoma.
- Assessment of mismatch repair pathway status in colorectal carcinoma provides prognostic information, can guide therapeutic decisions, and serves as an effective method of identifying patients with Lynch syndrome.
- Multiple assays, including immunohistochemistry, microsatellite instability testing, promoter methylation, and sequencing are used to assess mismatch repair pathway status.
- Evaluation of mutations in downstream components of the epidermal growth factor receptor (EGFR) signaling pathway is required to determine which patients with metastatic disease will benefit from targeted anti-EGFR therapy.
- Advances in colorectal carcinoma molecular diagnostics will help refine patient selection for targeted therapies and may enable better disease monitoring.

ABSTRACT

Molecular testing in colorectal cancer helps to address multiple clinical needs. Evaluating the mismatch repair pathway status is the most common use for molecular diagnostics and this testing provides prognostic information, guides therapeutic decisions and helps identify Lynch syndrome patients. For patients with metastatic colorectal cancer, testing for activating mutations in downstream components of the EGFR signaling pathway can identify patients who will benefit from anti-EGFR therapy. Emerging molecular tests for colorectal cancer will help further refine patient selection for targeted therapies and may provide new options for monitoring disease recurrence and the development of treatment resistance.

OVERVIEW

Colorectal carcinoma (CRC) is one of the single most common cancers in both men and women, and is also one of the largest overall contributors to cancer-associated mortality.[1] After lung cancer, it is the single most common solid tumor for which molecular testing is routinely used in clinical practice. CRC has one of the best understood mechanisms of molecular pathogenesis of all solid tumors. In the 25 years since the original stepwise model of CRC pathogenesis was proposed, the

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comprehensive genetic landscape of colorectal cancer has come into focus, greatly aided by modern molecular techniques.[2–4] In this context, molecular evaluation of CRC has been a part of routine pathology practice for nearly 15 years, and addresses multiple clinical needs, including prognostication, therapeutic guidance, and identification of inherited cancer predisposition syndromes. This review focuses on currently accepted molecular testing for CRC, while also briefly highlighting future areas of testing.

As a framework for understanding molecular testing in CRC, it is helpful to recognize that there are 2 generally separate genetic pathways to colorectal carcinogenesis.[5] The classic stepwise sequence, known as the chromosomal instability pathway, is characterized by frequent mutations in the *KRAS*, *APC*, and *TP53* genes, and is typically associated with chromosomal aneuploidy. In contrast, tumors that arise from the hypermutable pathway, characterized by deficient mismatch repair (MMR) activity, do not have gross chromosomal rearrangements and losses, but rather accumulate many missense mutations and relatively small deletions and insertions in specific types of DNA sequences. Determining which mechanism of genetic instability gave rise to a CRC has inherent prognostic value and also provides the necessary context for interpreting additional molecular alterations.

MISMATCH REPAIR–DEFICIENT TUMORS AND LYNCH SYNDROME

Overall, approximately 15% of CRCs arise from the MMR-deficient hypermutable pathway. These cancers arise in 2 different settings. Approximately 3% to 4% of CRCs arise in the context of Lynch syndrome, an autosomal dominant inherited cancer predisposition syndrome, whereas the remaining 12% arise through sporadic inactivation of the MMR pathway via somatic mutations or epigenetic mechanisms.[6] The MMR pathway primarily functions to repair errors introduced during DNA replication. Four core proteins, MSH2, MSH6, MLH1, and PMS2, are essential for the proper function of this pathway, and germline mutations in any of the 4 corresponding genes can give rise to Lynch syndrome. Additionally, germline deletions involving the *EPCAM* gene, which is located upstream of the *MSH2* gene, can lead to methylation of the *MSH2* promoter and subsequent silencing of gene expression. Patients with Lynch syndrome inherit only a single functional copy of 1 of the 4 core MMR genes. Subsequent inactivation of the remaining functional allele can therefore lead to aberrant accumulation of somatic pathogenic mutations and drive carcinogenesis.

Although Lynch syndrome is most commonly associated with a predisposition for developing colorectal carcinoma, it actually represents a predisposition to develop a wide variety of cancers, including endometrial and ovarian, gastric, small bowel, and urinary tract cancers.[7] Patients with Lynch syndrome also have an elevated risk of developing glioblastomas, an association recognized as Turcot syndrome, and sebaceous neoplasms, referred to as Muir-Torre syndrome. Less robust associations have also been reported with pancreatic, prostate, and breast cancer. Because of these elevated risks, patients with Lynch syndrome should undergo enhanced cancer screening and the relatives of patients with Lynch syndrome also should be tested to determine if they are germline mutation carriers as well.

Rarely, patients may inherit 2 defective copies of a single MMR gene, resulting in Constitutional Mismatch Repair Deficiency syndrome.[8,9] Individuals with this condition are predisposed to develop CRC at a much younger age than patients with Lynch syndrome, frequently develop café-au-lait macules, and are at risk for a slightly different spectrum of cancers than patients with Lynch syndrome.

Since the molecular basis for Lynch syndrome was elucidated in the early 1990s, several systems have been developed to identify patients with Lynch syndrome. The Amsterdam criteria were developed primarily for research use and are based solely on clinical criteria.[10] Although assessment of these criteria may help raise suspicion for Lynch syndrome, they are insufficiently sensitive to serve as a reliable method for identifying all patients with Lynch syndrome. The Bethesda guidelines were developed to help identify which cases of CRC should be evaluated for MMR deficiency and rely on both clinical and histologic data.[11] Notably, cancers that arise due to MMR pathway deficiency often have characteristic pathologic features (**Fig. 1**) and often induce a prominent lymphocytic inflammatory response that occurs in several distinctive patterns (**Fig. 2**). Although the presence of these histologic features may be suggestive of MMR deficiency, histology alone is neither specific nor sensitive enough to be used for Lynch syndrome identification. Furthermore, reliance on clinical criteria to identify patients with Lynch syndrome can be challenging in everyday practice due to incomplete knowledge of family pedigrees. Even when the criteria are appropriately evaluated using complete data, the Revised Bethesda Guidelines may fail to detect up to 25% of patients with Lynch syndrome. In