Molecular Testing of Colorectal Cancer in the Modern Era

What Are We Doing and Why?

Efsevia Vakiani, MD, PhD

KEYWORDS

• Molecular testing • Colorectal cancer • Next-generation sequencing

Key points

- Microsatellite instability testing is recommended for all patients with colorectal cancer. It is important in identifying patients with Lynch syndrome in addition to guiding therapeutic decisions.
- Extended RAS gene mutation testing is recommended for patients who are being considered for therapy with inhibitors of the epidermal growth factor receptor.
- Next-generation sequencing assays are increasingly used in clinical molecular pathology laboratories; in addition to detecting specific genetic events they can provide information about microsatellite instability status.

ABSTRACT

A plethora of tests are routinely ordered and interpreted by pathologists to assist the management of colorectal cancer patients. Many of these tests are immunohistochemistry assays using antibodies against prognostically relevant proteins, some of which predict therapeutic response. This review focuses on tissue DNA-based tests. It presents novel methodologies for assessing well-established biomarkers, updates the expanding spectrum of genetic alterations that are associated with resistance to inhibition of epidermal growth factor receptor signaling, and briefly discusses emerging actionable alterations that may translate into new therapeutic options for colorectal cancer patients. The utility of next-generation sequencing is emphasized.

OVERVIEW

Colorectal cancer is the fourth most frequently diagnosed cancer in the United States, affecting approximately 8% of women and men.[1] A majority of adenocarcinomas arise from conventional adenomas; histologic progression is accompanied by accumulation of molecular changes.[2] Most commonly, the initiating event is a mutation in *APC*, which results in activation of the WNT/β-catenin pathway. Mutations in *RAS/RAF* constitutively activate the mitogen-activated protein kinase (MAPK) pathway to promote tumor growth. Subsequent clonal expansion results from alterations in several genes, including *TP53* and *SMAD4*, and is usually associated with chromosomal instability.[3] A minority of cancers develop through a different pathway characterized by microsatellite instability (MSI); these tumors may be sporadic or associated with Lynch syndrome.[4]

Despite improvements in detection, prevention, and management, colorectal carcinoma remains the second leading cause of cancer-related death with more than 50,000 deaths reported in 2014.[5] Targeted therapies for patients with unresectable metastatic colorectal cancer consist of bevacizumab, a monoclonal antibody against vascular

The author has no conflicts of interest to disclose.
Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA
E-mail address: vakianie@mskcc.org

Surgical Pathology ■ (2017) ■–■
http://dx.doi.org/10.1016/j.path.2017.07.013

endothelial growth factor A, as well as cetuximab and panitumumab, 2 monoclonal antibodies against the epidermal growth factor receptor (EGFR).[6] Clinical trials assessing novel molecules and drugs that are effective in other tumor types are currently under way.

ESTABLISHED BIOMARKERS

MICROSATELLITE INSTABILITY

Microsatellites are short sequences of genomic DNA containing repeats that are prone to mistakes during replication. Defective DNA mismatch repair mechanisms result in alterations of microsatellite length, which can be used to assess for mismatch repair proficiency. Approximately 15% of colorectal cancers show MSI, and most of these arise sporadically through hypermethylation of the *MLH1* promoter. Approximately 3% of microsatellite unstable colon cancers occur in patients with deleterious germline alterations in *MLH1*, *MSH2*, *MSH6* and *PMS2*, or *EPCAM*. *EPCAM* is located immediately upstream of *MSH2*; deletions in its terminal region result in *MSH2* promoter hypermethylation and inactivation.[7]

MSI can be assessed in several ways. The approach most familiar to surgical pathologists is immunohistochemical staining with antibodies directed against MLH1, MSH2, MSH6, and PMS2, as detailed in Michael Markow and colleagues' article, "Immunohistochemical Pitfalls: Common Mistakes in the Evaluation of Lynch Syndrome," in this issue. Polymerase chain reaction (PCR) using fluorescently labeled primers against select microsatellites is the principal DNA-based method used when testing for MSI. The currently recommended panel of microsatellites consists of 5 mononucleotide repeats. Both tumor and normal DNA are required for analysis (**Fig. 1**). Microsatellite stable tumors show similar peak profiles when tumor DNA is compared with that of non-neoplastic tissue from the same patient, whereas unstable tumors show expansion or contraction of greater than or equal to 30% of examined loci ($\geq$2 when a panel of 5 microsatellites is used). Instability at only 1 locus is classified as indeterminate, because this finding may be seen in some patients with Lynch syndrome; low-frequency MSI has been used to describe instability at 1 locus but is appropriate terminology only when the Bethesda panel (ie, 2 dinucleotide and 3 mononucleotide repeats) is used.

Next-generation sequencing (NGS) is increasingly used to assess for MSI. Stadler and colleagues[8] evaluated 224 tumors sequenced with a 341-gene NGS panel and found that all tumors with less than 20 mutations were mismatch repair proficient, whereas those with 20 mutations to 150 mutations were mismatch repair deficient. Three cases with greater than 150 mutations were microsatellite stable but had hotspot mutations in the central catalytic subunit of the DNA polymerase epsilon (*POLE*) gene, consistent with the ultramutator phenotype described in both colorectal and endometrial carcinomas.[8–10] Bioinformatics tools can also be used to analyze NGS data and predict microsatellite status. Niu and colleagues[11] developed MSIsensor, a software program that uses paired tumor-normal NGS data to predict MSI and found an excellent correlation between results obtained from PCR using 5 microsatellite sequences. Salipante and colleagues[12] developed a method (mSINGS) to infer microsatellite status

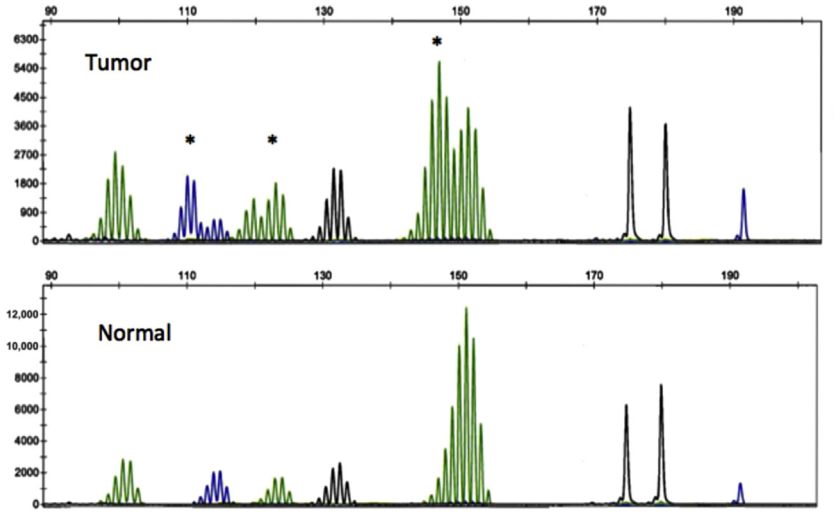

Fig. 1. Example of a tumor showing microsatellite instability using the PCR method. Capillary electrophoresis tracings afer PCR of 5 microsatellites markers show that 3 of the 5 markers (*asterisks*) show variability in their length in the tumor DNA compared with what is seen in normal DNA.