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Original contribution

Challenging cases encountered in colorectal cancer screening for Lynch syndrome reveal novel findings: nucleolar MSH6 staining and impact of prior chemoradiation therapy

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Summary Many pathology laboratories have developed specific screening protocols to detect patients with Lynch syndrome. With recent recommendations to test all patients with newly diagnosed colorectal cancer for Lynch syndrome, the volume of testing will increase, and the most economic and reliable screening test will prevail. Although the detection of microsatellite instability by polymerase chain reaction and the detection of loss of the mismatch repair proteins by immunohistochemistry can each be used as a screening tool, each methodology has its strengths and weaknesses. During the time of our study, we used both polymerase chain reaction and immunohistochemistry to screen for Lynch syndrome in colorectal cancer specimens. We encountered 21 cases that posed significant interpretive challenges. A previously unpublished pattern of nucleolar MSH6 staining and potential spurious results induced by chemoradiation therapy are described. We feel that it is important to report these cases so that potential pitfalls in screening for Lynch syndrome can be avoided.

1. Introduction

Lynch syndrome, an autosomal-dominant cancer-susceptibility syndrome, is the most common cause of inherited colorectal cancer (CRC). The estimated incidence is 2.8% among all patients with newly diagnosed CRC [1,2]. Individuals with Lynch syndrome are at high risk for developing additional primary cancers including colorectal, endometrial, gastric, ovarian, and urothelial cancers. Relatives of individuals with Lynch syndrome are also at risk for developing Lynch syndrome–associated cancers and could benefit from genetic testing to determine if they require heightened cancer surveillance.

Many guidelines have been created over the years to detect Lynch syndrome families. The original Amsterdam criteria attempted to identify patients with hereditary nonpolyposis CRC (HNPCC) by taking personal and familial histories of CRC into account [3]. Over time, these guidelines evolved, and additional prediction models have been developed. Currently, one of the most widely-used systems is the revised Bethesda guidelines that uses a

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combination of clinical, familial, and histologic features to help determine who should receive genetic testing for Lynch syndrome [4,5]. One limitation of these and other guidelines is the use of age as a decision point. Recent studies have shown that the Bethesda criteria fails to identify a significant number (25%-28%) of patients with Lynch syndrome [1,6].

Based on these and other studies, there is currently an initiative to screen all patients with newly diagnosed CRC for Lynch syndrome. One question that has arisen, regardless if we test all or a subset of patients with CRC, is which methodology should we use. Lynch syndrome–associated tumors arise because of germline mutations in one of the mismatch repair (MMR) genes, typically *MLH1*, *MSH2*, *MSH6*, or *PMS2*, which leads to microsatellite instability (MSI). Hence, either molecular detection of MSI by polymerase chain reaction (PCR) or immunohistochemical detection of loss of the MMR proteins can be used as the initial screening test for Lynch syndrome. Each methodology has its strengths and weaknesses.

In this study, we report a series of cases that posed significant interpretive challenges when both MSI and immunohistochemistry (IHC) were used. A previously unpublished pattern of nucleolar MSH6 staining created the most difficulty. We feel that it is important to report these cases to demonstrate the challenges and potential pitfalls that can be encountered in daily practice when assessing CRC specimens for Lynch syndrome.

2. Materials and methods

In this retrospective study, we examined the MMR protein staining patterns and MSI results of a series of CRCs that were tested between January 2006 and May 2010. During this period, our laboratory was performing both MSI testing by PCR and MMR protein staining by IHC on all CRC resections that met the revised Bethesda criteria [5] and on additional biopsies and resections at the clinician's request. Clinical information such as patient age, prior malignancies, prior exposure to chemotherapy and/or radiation therapy, and results of germline mutational analysis for potential Lynch syndrome was obtained from the electronic medical record. The University of Pittsburgh Institutional Review Board approved this study (IRB no. PRO10050383).

2.1. Immunohistochemistry

Standard automated IHC was performed for MLH1, MSH2, MSH6, and PMS2 (Table 1) on formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections. After deparaffinization in xylene and rehydration in ethanol, antigen retrieval was performed (Table 1). The Leica Bond-III stainer (Leica Microsystems, Bannockburn, IL) was used for MLH1 and PMS2; the enzymatic reactivity was visualized with the Bond Polymer Refine Detection Kit (Leica Microsystems). The Ventana BenchMark XT staining system (Ventana Medical Systems, Inc, Tucson, AZ) was used for MSH2 and MSH6; the enzymatic reactivity was visualized with the iVIEW DAB Detection Kit (Ventana Medical Systems).

Table 1 Antibodies used in this study

Antibody	Clone/ company	Dilution	Stainer	Epitope retrieval
MLH1	ES05/ Novocastra	1:250	Leica Bond-III	Heat-induced/ Soln 2/pH 9.0/ 20 min
PMS2	A16-4/ Ventana	Predilute	Leica Bond-III	Heat-induced/ Soln 2/pH 9.0/ 30 min
MSH2	G219-1129/ Ventana	Predilute	Ventana Benchmark XT	Heat-induced/ CC1/pH 9.0/60 min
MSH6	44/BD	1:200	Ventana Benchmark XT	Heat-induced/ CC1/pH 9.0/60 min

Novocastra, Leica Microsystems Inc; BD Transduction Laboratories, Franklin Lakes, NJ; Soln 2; Bond epitope retrieval solution 2, Leica Microsystems Inc; CC1; Cell Conditioning Solution 1, Ventana Medical Systems.

All immunohistochemical stains for the MMR proteins were independently reviewed by 2 of the authors (O. R. and A. K.). *Positive staining* was defined as unequivocal nuclear staining in the neoplastic cells. *Loss of expression* was defined as a tumor cell population without any nuclear staining in the presence of staining in normal epithelial, stromal, and/or lymphoid cells. Four variable patterns of protein expression were observed: (1) *patchy staining/focal loss* - variable nuclear intensity from area to area, alternating positive and negative nuclei, and areas of tumor with complete loss in less than 50% of the tumor (often <25%); (2) *near complete loss (focally intact)* - large areas with complete uniform loss; focal areas of intact staining comprised 50% or less (often <10%) of the tumor; (3) *nucleolar staining* - nuclei were uniformly negative with uniform bright staining of nucleoli; and (4) *weak/indistinct staining* - weak nuclear staining often accompanied by weak cytoplasmic staining of similar intensity to the adjacent stromal nuclei and cytoplasm.

2.2. Microsatellite instability testing

Formalin-fixed, paraffin-embedded tissue specimens were used for all analyses. Tumor targets were manually microdissected from 4-μm unstained histologic sections under the guidance of a hematoxylin and eosin–stained slide using an Olympus SZ61 stereo microscope (Olympus, Hamburg, Germany). DNA was isolated from each target with the DNeasy Blood and Tissue kit on the automated