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Role of microsatellite instability-low as a diagnostic biomarker of Lynch syndrome in colorectal cancer

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Lynch syndrome is the most common Mendelian disorder predisposing persons to hereditary colorectal cancer. Carriers of *MSH6* mutations constitute less than 10% of the total of cases with Lynch syndrome and present with a weaker clinical phenotype, including low levels of microsatellite instability (MSI-L) in colorectal tumors. The frequency of *MSH6* mutation carriers among patients presenting with MSI-L colorectal cancer has yet to be determined, as has the appropriate genetic workup in this context. We have reviewed here the clinicopathologic characteristics, immunohistochemistry, and genetic testing results for 71 patients at a single institution diagnosed with MSI-L colorectal cancers. Of 71 patients with MSI-L tumors, 21 underwent genetic testing for *MSH6* mutations, three of whom presented with loss of staining of MSH6 and only one of whom carried a pathogenic germline *MSH6* mutation in exon 4 (c.2677_2678delCT; p.Leu893Alafs*6). This latter patient had a significant family history of cancer and had a rectal primary tumor that showed instability only in mononucleotide markers. In this cohort of MSI-L patients, we detected no notable clinicopathologic or molecular characteristic that would help to distinguish a group most likely to harbor germline *MSH6* mutations. Therefore, we conclude that the prevalence of *MSH6* mutations among patients with MSI-L tumors is very low. Microsatellite instability analysis combined with immunohistochemistry of mismatch repair proteins adequately detects potential *MSH6* mutation carriers among MSI-L colorectal cancers.

Keywords Microsatellite instability-low, Lynch syndrome, colorectal cancer, *MSH6*, mononucleotide markers

Colorectal cancer (CRC) is the second-leading cause of cancer-related mortality in the United States when both sexes are combined (1). It has been estimated that 5–10% of CRC cases are secondary to a genetic condition, with Lynch syndrome (also termed hereditary nonpolyposis colorectal cancer) being the most common Mendelian disorder predisposing persons to CRC and accounting for 3–5% of all CRC cases (2,3).

Lynch syndrome is secondary to the presence of germline mutations in the genes involved in the DNA mismatch repair (MMR) pathway: *MLH1*, *MSH2*, *MSH6*, and *PMS2* (2,4). Mutations in *MLH1* and *MSH2* account for 90% of all

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identified mutations and *MSH6* and *PMS2* for 10% (2). The molecular fingerprint of deficiency in the MMR system is the presence of microsatellite instability (MSI). MSI is detected in the majority of tumors arising in the context of Lynch syndrome by using either immunohistochemical (IHC) analysis of the MMR proteins or a standard PCR-based approach known as MSI analysis (5). The phenotype of families with a diagnosis of Lynch syndrome includes increased lifetime risks for the development of young-onset colorectal and endometrial cancers, as well as tumors of the ovaries, small bowel, urothelium, and other organs (2). It has been observed that carriers of germline mutations in *MSH6* have a distinct clinical phenotype with older age at diagnosis and lower lifetime risk for colorectal and endometrial tumors compared with *MLH1* and *MSH2* mutation carriers (6–8). In addition, it has been reported that tumors arising in the context of *MSH6* mutations can present with intact expression of the MMR proteins and may display low levels of microsatellite instability (MSI-L) or even microsatellite stability (MSS) (6–8).

At present, many academic institutions have established universal screening strategies for Lynch syndrome based on tumor studies with MSI and IHC and thus can systematically detect CRC cases with deficient MMR activity (9). The implementation of such programs results in the subclassification of all CRC in either two (MMR-deficient and MMR-proficient) or three [MSI-high (MSI-H), MSI-L, and MSS] molecular subgroups (10). Traditionally, tumors displaying MSI-L have been grouped along with those with MSS because of their similar clinical and pathological features and absence of a hereditary origin (10,11). However, it is currently unknown whether patients presenting with tumors displaying MSI-L should be considered for genetic testing of Lynch syndrome based on previous studies associating *MSH6* mutations with this tumor subtype (8,12–15). This point is particularly critical in the context of patients with a CRC displaying MSI-L and either young onset at presentation or positive family history of Lynch syndrome–related tumors.

The aim of our current study was to assess the prevalence of *MSH6* mutations in a cohort of patients diagnosed with colorectal tumors displaying MSI-L and to investigate the role of MSI analysis and IHC of the MMR proteins in identifying patients with Lynch syndrome among cases of CRC with MSI-L.

Material and methods

Patients and samples

A total of 71 patients with a diagnosis of CRC displaying MSI-L were included in this study. This cohort of patients was obtained from two prospective institutional databases at The University of Texas MD Anderson Cancer Center (UTMDACC) in the period from 2003 to 2012: (1) a genetic counseling database, which includes all patients undergoing genetic counseling; and (2) a CRC database, which collects clinical, pathologic, and molecular data of patients with CRC undergoing surgical resection. These two databases contained a total of 933 registered cases. Only cases displaying MSI-L by MSI analysis that had results for IHC of MMR proteins were included in the current study. Both the MSI analysis and the IHC results were requested by providers per standard of care and in the context of an institutional initiative, beginning in 2010, to have all surgically resected colorectal tumors undergo universal screening for Lynch syndrome through tumor studies. Clinical, laboratory, and pathology information was retrieved from the electronic medical record. Information regarding the family history was collected by certified genetic counselors at the time of the initial assessment. In those cases not referred for genetic counseling, information on the family history was retrieved directly from the medical record. Personal and family history was assessed for the risk of carrying a germline mutation using the Prediction of Mutations in MLH1 and MSH2 ($PREMM_{1,2,6}$) risk-prediction model (16). Pedigrees made as part of the genetic counseling visit or using the information collected in the chart were assessed for fulfilment of Amsterdam I/II and Bethesda guidelines (17,18). This study was approved by the institutional review board of UTMDACC.

MSI analysis and immunohistochemical examination

For MSI analysis, DNA was extracted from microdissected formalin-fixed, paraffin-embedded tumor and normal tissue sections. The MSI status was ascertained using a panel with seven microsatellite markers (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250, and TGFβRII) and was performed at the Molecular Diagnostic Laboratory in UTMDACC. A microsatellite marker was considered positive when an allelic shift was present in the tumor compared with normal tissue. A case was considered MSI-H when three of the loci (≥30%) tested were positive, MSI-L when one or two (<30%) were positive, and MSS when all loci tested were negative. We included in this study only CRC cases displaying MSI-L (5). To assess the phenotypic differences of MSI-L tumors presenting with mononucleotide compared with dinucleotide allelic changes, we classified patients into two categories: (1) a mononucleotide group, which included patients with tumors with changes in at least one mononucleotide allele in the MSI analysis, and (2) a dinucleotide group, which included patients with changes in dinucleotide alleles only. Immunohistochemical staining was performed on 5-μm sections of formalin-fixed, paraffin-embedded tumor blocks using a panel of four mouse monoclonal antibodies against the mismatch repair proteins MLH1 (550838, clone G168-15, BD Pharmingen; BD Biosciences, San Diego, CA), MSH2 (NA27, clone FE11, Calbiochem, EMD Millipore; Merck, Darmstadt, Germany), MSH6 (610919, clone 44, BD Pharmingen; BD Biosciences), and PMS2 (556415, clone A16-4, BD Pharmingen; BD Biosciences). Loss of expression in the tumor cells was considered solely when there was normal nuclear staining in adjacent nonneoplastic cells, which served as internal controls. The sections stained with these antibodies were reviewed by two expert pathologists (R.R.B. and M.W.T.) to confirm the absence of MSH6 staining.

DNA sequencing

To assess for the presence of germline mutations and large deletions and duplications in the *MSH6* gene, DNA extracted