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Histology of colorectal adenocarcinoma with double somatic mismatch-repair mutations is indistinguishable from those caused by Lynch syndrome

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Summary

Lynch syndrome (LS) is the most common form of hereditary colon cancer. Germline mutations in the mismatch-repair (MMR) genes MLH1, MSH2 (EPCAM), MSH6, and PMS2, followed by a second hit to the remaining allele, lead to cancer development. Universal tumor screening for LS is routinely performed on colon cancer, and screening has identified patients with unexplained MMR deficiency that lack MLH1 methylation and a germline mutation. Tumor sequencing has since identified double somatic (DS) mutations in the MMR gene corresponding with the absent protein in 69% of these patients. We assessed whether histomorphology could distinguish patients with DS mutations from those with LS. Colorectal cancer patients with DS mutations were identified from population-based cohorts from Iceland (2000–2009); Columbus, Ohio (1999–2005); and the state of Ohio (2013–2016). Next-generation sequencing was performed on tumors with unexplained MMR deficiency. Patients with LS from Ohio cohorts were the comparison group. The histologic features associated with MMR deficiency (tumor-infiltrating lymphocytes, Crohn-like reaction, histologic subtype, necrosis) were evaluated. We identified 43 tumors with DS mutations and 48 from patients with LS. There was no significant difference in histologic features between tumors in LS patients and tumors with DS mutations. Because histology of tumors with DS mutations is indistinguishable from those caused by LS, tumor sequencing for evaluation of DS mutations should be considered to help clarify sporadic versus hereditary causes of unexplained MMR deficiency.

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Keywords

Double somatic mutations; Biallelic mutations; Lynch syndrome; MMR deficiency; Lynch-like syndrome

Introduction

Colorectal carcinoma (CRC) is a common cancer affecting both men and women with approximately 140 000 individuals diagnosed each year in the United States [1]. Lynch syndrome is an autosomal dominant cancer predisposition syndrome that accounts for 2% to 4% of all CRCs [1,2]. Lynch syndrome is defined by the presence of an inactivating germline mutation in one of the DNA mismatch-repair (MMR) genes MLH1,MSH2, MSH6, or PMS2 or deletions in the EPCAM gene that leads to methylation and silencing of the adjacent MSH2 gene. Loss of the remaining functional allele results in defective MMR activity predisposing to tumor development [3–5]. The most common tumor in individuals with Lynch syndrome is CRC; however, there is also an increased risk of developing cancers of the endometrium, ovary, stomach, small bowel, pancreas, hepatobiliary, urinary tract, brain, and sebaceous neoplasms, among others [4,5].

Identifying individuals with Lynch syndrome is important because these patients and their affected family members are at high risk for developing Lynch syndrome–related neoplasms at young ages. These individuals can benefit from heightened cancer surveillance or prevention options, which enable cancer prevention or early cancer detection and intervention [6]. Universal screening of all CRCs and endometrial cancers to identify individuals with Lynch syndrome has been endorsed by multiple major professional medical organizations [7]. Studies have identified certain histopathologic features that are associated with defective MMR, namely, increased tumor-infiltrating lymphocytes (TILs), a Crohn-like peritumoral lymphocytic reaction (CLR), mucinous/signet ring cell differentiation, and medullary differentiation [7–10]. In addition, CRC in individuals with Lynch syndrome more frequently involves the right or transverse colon than the left colon and typically lacks dirty necrosis. Unfortunately, these features are not specific or sensitive enough to be used alone for screening purposes [10].

Currently, the most common approach to universal screening for Lynch syndrome uses immunohistochemistry (IHC) to assess for absent expression of MMR proteins [7,10]. Universal screening with IHC using antibodies directed against MLH1, MSH2, MSH6, and PMS2 has a high sensitivity (approximately 93%) for detecting defective MMR and for predicting MMR gene mutation [3,10–13]. Alternatively, because defective MMR activity is associated with microsatellite instability (MSI), polymerase chain reaction techniques detecting MSI can be used as an initial screening test for Lynch syndrome [7,10].

Although loss of MMR protein expression by IHC and/or a high level of MSI by PCR is concerning for Lynch syndrome, these findings are not diagnostic. Sporadic MMR deficiency is relatively common and most frequently involves loss of MLH1 and PMS2 expression due to MLH1 promoter hypermethylation [7]. Even after excluding MLH1 promoter hypermethylation, there remain cases of unexplained MMR deficiency that lack

detectable germline mutations in the MMR genes. The frequency of unexplained MMR deficiency is variable in the literature, ranging from 32% to 72% of patients with MMR deficiency or 2% to 5% of all screened patients [3,7,14–17]. More recent studies on tumors with unexplained MMR deficiency have reported that up to about 70% of these tumors harbor double somatic mutations in the affected MMR gene leading to loss of MMR protein expression and MSI [17–20]. A population-based study from Iceland found double somatic MMR mutations in 1.4% of all CRC cases diagnosed over a 10-year period [21].

We evaluated whether or not histologic features typically associated with MMR deficiency could be used to distinguish CRCs with acquired double somatic MMR mutations from CRCs in patients with Lynch syndrome.

Materials and Methods

Patients with CRC harboring double somatic mutations involving the MMR genes were previously identified [17]. These patients were enrolled in the previously published Columbus-Area HNPCC study (1999–2005) [3,13] and the statewide prospective Ohio Colorectal Cancer Prevention Initiative (2013–2016) [22,23]. In addition, a patient cohort with CRC was obtained from the Icelandic Cancer Registry (2000–2009) [21]. Institutional review board (IRB) approval for the Columbus-area HNPCC Study was obtained by Ohio State University (OSU) IRB (1999C0051) and the other 2 participating health systems' IRBs (OhioHealth and Mount Carmel). IRB approval for the Ohio Colorectal Cancer Prevention Initiative was obtained by the individual participating hospitals, community oncology programs, or by ceding review to the OSU IRB (2012C0123). Regarding the Icelandic cohort, the study was approved by the Icelandic National Bioethics Committee (VSNb2013010033/03.15), the Icelandic Data Protection Authority (2013010109TS), and the OSU IRB (2013C0144). Essentially, all patients with MSI-high and/or abnormal IHC without detectable germline mutations or MLH1 hypermethylation were selected for somatic MMR gene mutation analysis using ColoSeq Tumor next-generation sequencing method (University of Washington), as described by Haraldsdottir et al [17,24]. Histologic features of CRC harboring double somatic mutations were then compared with CRC from patients with Lynch syndrome from the Ohio cohorts.

Histologic features associated with high-level MSI were evaluated for each CRC. The degree of TIL was scored as 0 (b1 per high-power field [HPF]), 1+ (1–2/HPF), and 2+ (≥3/HPF). The degree of CLR was also scored as 0 (none), 1+ (mild/moderate), and 2+ (marked). Histologic subtypes associated with MSI (mucinous, signet ring, poorly differentiated, and medullary) as well as the presence and extent of necrosis were also evaluated. The histologic features were evaluated on at least one whole representative section and were scored by 2 pathologists (J. A. H. and W. F.) who were blinded to germline MMR mutational status (Lynch syndrome versus double somatic mutations). The mutated MMR gene was noted for each CRC within the 2 groups.

Results

From the 3 population-based cohorts, we included 91 cases of CRC with MMR deficiency that had available slides for histologic evaluation. Forty-three CRCs were negative for germline mutations in MMR genes but had somatic mutations involving both copies of the defective MMR gene, referred to as double somatic tumors. Sixteen double somatic tumors were from the Iceland cohort and 27 were from the Ohio cohorts. For comparison, 48 CRCs from patients with Lynch syndrome were identified from the Ohio cohorts.

CRC harboring double somatic mutations and those from patients with Lynch syndrome were evaluated for histologic features typically associated with MMR deficiency (Table 1). No significant difference was identified regarding the presence of necrosis between groups. Necrosis was more frequently focal than extensive in both double somatic and Lynch syndrome tumors. Histologic features associated with MSI, such as TIL, CLR, and histologic subtypes (mucinous, signet ring, poorly differentiated, and medullary), were common in both double somatic and Lynch syndrome tumors (Fig. 1). TIL and CLR were present in 81% (35/43) and 88% (37/42) of double somatic tumors and 75% (36/48) and 73% (30/41) of Lynch syndrome tumors, respectively. The presence or degree of TIL and CLR did not significantly differ between Lynch syndrome and double somatic tumors. Mucinous and/or signet ring histology was noted in 53% (23/43) of double somatic and 48% (23/48) of Lynch syndrome tumors, and poorly differentiated and/or medullary histology was seen in 19% (8/43) of double somatic and 15% (7/48) of Lynch syndrome tumors. One tumor in each group had both mucinous/signet ring and poorly differentiated components.

The most common MMR gene mutated in double somatic tumors was MLH1 (n = 25; 58%) followed by MSH2 (n =15; 35%) (Table 2). In Lynch syndrome tumors, the most commonly mutated MMR gene was MSH2 (n = 24; 50%) followed by MLH1 (n = 10; 21%). MSH6 and PMS2 mutations were more commonly seen in Lynch syndrome tumors than in double somatic tumors. Fig. 2 shows MMR IHC in representative cases of MSH2-deficient Lynch syndrome and double somatic CRC.

Discussion

Universal screening of CRC has identified patients with MMR defects that lack identifiable germline mutations in MMR genes and lack acquired hypermethylation of the MLH1 gene. Some groups have labeled these cases of unexplained MMR deficiency as “Lynch-like.” This term is misleading because Lynch-like tumors actually have a variety of underlying genetic abnormalities that result in defective MMR. Furthermore, it seems that most of these cases represent sporadic CRC (ie, nonsyndromic); thus, the clinical course and predisposition to additional neoplasms also differ from Lynch syndrome. The 2 most common explanations for “unexplained” MMR deficiency, which together account for up to 90% of cases, are acquired double somatic mutations in an MMR gene and erroneous interpretation of MMR protein IHC resulting in a false positive for MMR deficiency [7,17]. It is important to identify these patients because they do not require intensive cancer surveillance among the patients or their at-risk relatives that is typically performed in patients with Lynch syndrome. The remaining cases of unexplained MMR deficiency may

be related to a heritable defect such as germline MMR gene mutations or translocations that are not detectable by current testing methods, somatic MMR gene mutations that are not detectable by current testing methods, other germline gene defects such as biallelic MUTYH mutations, or somatic mosaicism (although very unlikely with next-generation sequencing methodologies) [7]. Of note, rarely MMR-deficient tumors with double somatic MMR mutations may also have germline mutations in other DNA repair genes, such as germline biallelic MUTYH mutations [23,25].

We evaluated histologic features that are associated with MSI and Lynch syndrome to determine whether or not such features could be used to distinguish MMR-deficient CRC due to underlying double somatic mutations from those in patients with Lynch syndrome. Tumor histology was not significantly different between patients with double somatic tumors and patients with Lynch syndrome. This is probably due to similar underlying oncogenesis involving defective MMR function that is present in both Lynch syndrome and double somatic tumors. Haraldsdottir et al [17] reported that tumors with double somatic MMR mutations have a hypermutated phenotype, which is characteristic of microsatellite-unstable, MMR-deficient tumors, supporting the hypothesis that double somatic MMR mutations result in loss of MMR function. It is likely that tumors with double somatic mutations develop through the conventional adenoma pathway and not the serrated pathway [7,26]. Thus, these CRCs likely arise from conventional tubular or tubulovillous adenomas that acquire biallelic somatic MMR gene mutations.

Our study results are similar to a prior study that compared clinicopathological features of CRC and endometrial carcinoma in patients with Lynch-like tumors and Lynch syndrome [26]. Of note, this study did not specifically evaluate for the presence of double somatic mutations in the Lynch-like tumors; however, their Lynch-like group likely included tumors with double somatic MMR mutations. In this study, Mas-Moya et al [26] noted a predilection of Lynch-like tumors to involve the right colon, and conversely, tumors in the left colon/rectum were more likely to be in patients with Lynch syndrome. They also noted that synchronous or metachronous carcinomas were more frequently identified in patients with Lynch syndrome compared with patients with Lynch-like tumors. This was also reported by Haraldsdottir et al [17]. Mas-Moya et al noted that isolated loss of MSH6 expression was more likely in carcinomas associated with Lynch syndrome. They found no significant difference between Lynch syndrome and Lynch-like cases with regard to tumor stage, tumor grade, tumor size, TIL, CLR, mucinous differentiation, signet ring cell differentiation, or medullary differentiation. Approximately 80% of their Lynch-like CRC had histopathology suggestive of high-level MSI, which is similar to the tumors with confirmed double somatic mutations in our study. Of note, studies evaluating Lynch-like endometrial cancers also indicate that histomorphology is likely of little value in distinguishing Lynch syndrome cases from Lynch-like tumors [26,27].

In conclusion, many of the unexplained MMR-deficient tumors are caused by acquired double somatic mutations within the tumor. There are no reliable histologic features that can be used to distinguish CRC harboring double somatic mutations from those in patients with Lynch syndrome. Thus, tumor sequencing for evaluation of double somatic mutations should

be considered in patients with unexplained MMR deficiency to clarify sporadic versus hereditary CRC allowing for proper genetic counseling and screening.

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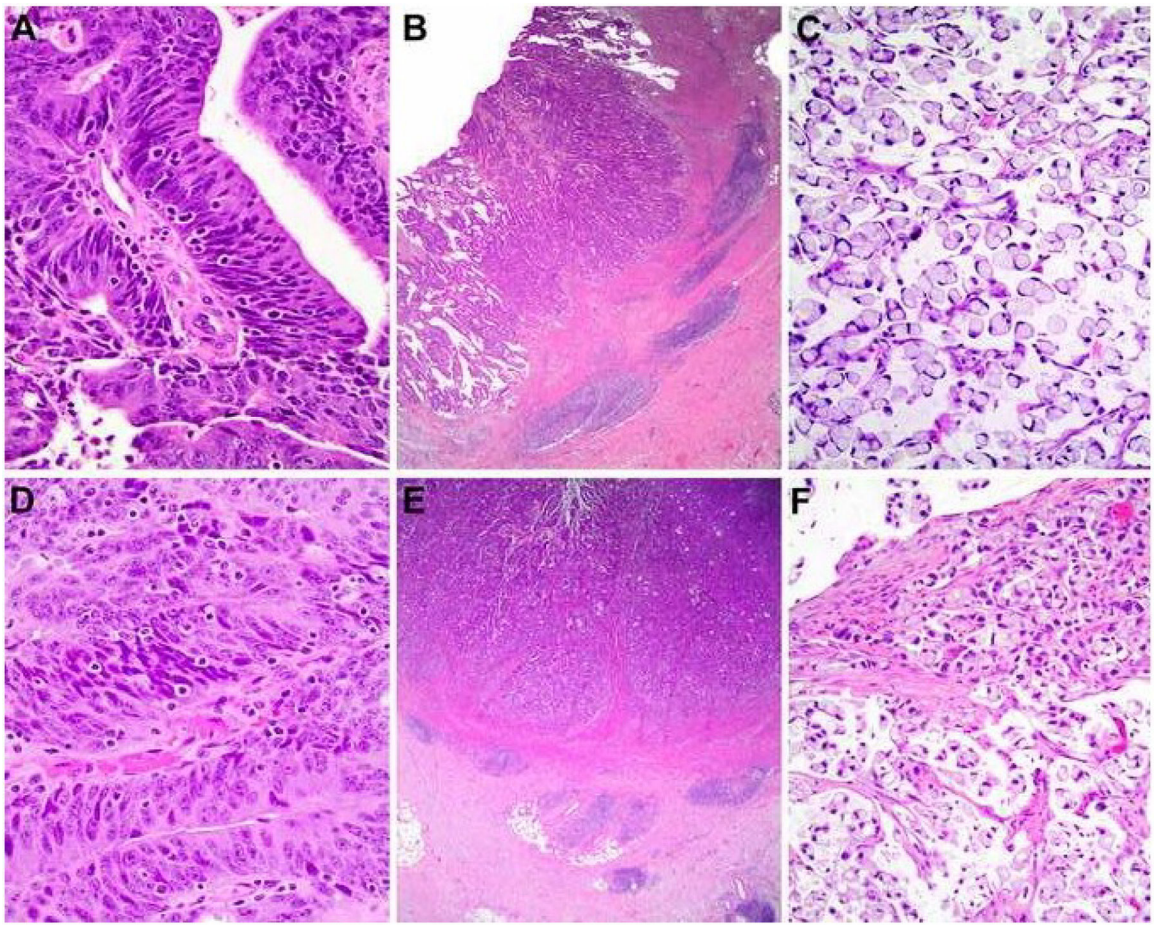

Figure 1.

Morphologic features in double somatic (DS) and Lynch syndrome (LS) tumors. Tumors from DS: (A) TILs (original magnification x400), (B) Crohn-like reaction (CLR; x20), and (C) signet ring (x200). Tumors from LS: (D) TIL (x400), (E) CLR (x20), and (F) signet ring (x200). Hematoxylin and eosin.

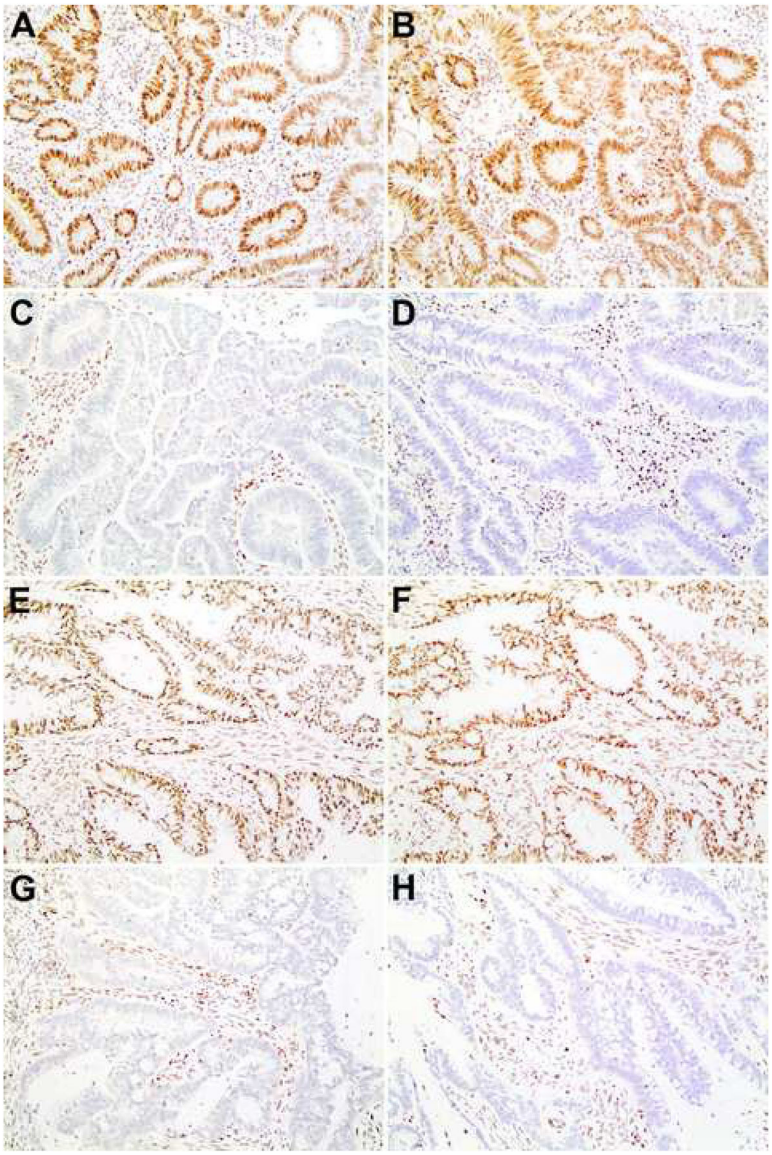

Figure 2.

IHC of representative cases of double somatic (DS) and Lynch syndrome (LS) tumors with defective MSH2. DS tumor: MLH1 (A) and PMS2 (B) present with loss of MSH2 (C) and MSH6 (D; original magnification x200). LS tumor: MLH1 (E) and PMS2 (F) present with loss of MSH2 (G) and MSH6 (H; x200).

Table 1.

Histologic comparison of double somatic and Lynch syndrome colorectal carcinomas

	Double Somatic (n=43)	Lynch Syndrome (n=48)	*p* value
TIL (%)			0.6956
1+	26%	17%	
2+	56%	58%	
CLR (%)			0.2641
1+	43%	32%	
2+	45%	41%	
Necrosis	37%	33%	0.7029
MSI Histology *	70%	60%	0.3565

TIL = Tumor Infiltrating Lymphocytes

CRL = Crohn's-like Reaction

MSI = Microsatellite Instability

* MSI histology defined as mucinous, signet ring, poorly differentiated, or medullary histology

Table 2.

Comparison of MMR gene mutations in double somatic and Lynch syndrome colorectal carcinomas

	MLHI	MSH2	MSH6	PM52
Double Somatic (n=43)	25 (58%)	15 (35%)	1 (2%)	2 (5%)
Lynch Syndrome (n=48)	10 (21%)	24 (50%)	7 (15%)	7 (15%)