DNA Mismatch Repair Deficiency in Ampullary Carcinoma

A Morphologic and Immunohistochemical Study of 54 Cases

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Key Words: Hereditary nonpolyposis colorectal cancer; Lynch syndrome; Immunohistochemistry; Microsatellite instability (high frequency) histology

Abstract

The significance of DNA mismatch repair (MMR) deficiency or microsatellite instability (MSI) in ampullary carcinomas remains to be defined. This study evaluated the MMR status in 54 consecutive ampullary adenocarcinomas by immunohistochemical and morphologic studies. All tumors were moderately (n = 49) or poorly (n = 5) differentiated, with 7 mucinous and 1 signet-ring cell type. Tumor-infiltrating lymphocytes (TILs) were noted in 36 tumors. Loss of MMR protein by immunohistochemical analysis was identified in 3 (6%), 2 lost MSH6, and 1 lost MLH1/PMS2. One MSH6– case had 3 metachronous colorectal cancers. Five TILs per 10 high-power fields predicted immunohistochemical abnormality in 2 of 3 tumors with a specificity of 80% (41/51); however, none of the 5 tumors that had the highest TIL counts (20-62/10 high-power fields) showed abnormal immunohistochemical results. Thus, MMR deficiency occurs in ampullary carcinoma but appears less frequent than in colorectal carcinoma (CRC). Typical MSI-high histologic features of CRC, such as increased TIL counts, seem to have similar yet subtly different implications in ampullary carcinoma.

Abnormal DNA mismatch repair (MMR) function, secondary to mutation or epigenetic silencing by promoter methylation, has been implicated in the carcinogenesis of a significant subset (up to 15%-20%) of colorectal carcinoma.1-3 Such tumors typically exhibit DNA microsatellite instability (MSI) and often have specific pathologic and clinical characteristics. The role of DNA MMR deficiency in ampullary carcinoma, a tumor that often shares phenotypic characteristics with colorectal carcinoma, is not well defined, however. The few available studies have yielded conflicting results, some reporting MMR abnormalities in as many as 15% of ampullary carcinomas, whereas others found only rare loss of MMR proteins.4-8

DNA MMR abnormalities can be identified by different methods. The DNA can be sequenced, or the presence of MSI can be assessed by polymerase chain reaction using probes for specific microsatellite loci.3 In recent years, however, immunohistochemical analysis has emerged as a specific and simple method for detecting MMR abnormalities. In the case of colorectal carcinoma, immunohistochemical analysis has an overall sensitivity of 92% with a specificity of 99% for predicting MSI; immunohistochemical analysis also has a predictive value in detecting an MMR gene germline mutation that is comparable to MSI.9-11

In addition, in the case of colorectal carcinoma, specific histologic features have been identified that can predict the presence of DNA MMR gene defects.12,13 These features include increased tumor infiltrating lymphocytes (TILs), a Crohn-like peritumoral lymphocytic reaction, morphologic tumor heterogeneity, medullary type, and mucinous differentiation. Similar but somewhat less distinctive associations have been identified in endometrial carcinomas as well.14
In this study, we sought to use immunohistochemical and morphologic assessments to explore the role of DNA MMR abnormality in ampullary carcinoma. Our goal was to evaluate the frequency of abnormal DNA MMR in unselected ampullary carcinomas by immunohistochemical analysis and to assess whether the morphologic features that have been associated with the MSI phenotype in colorectal carcinomas carry the same implication in carcinomas of the ampulla.

Materials and Methods

Patient Data

A consecutive series of 60 ampullary carcinomas resected at Memorial Sloan-Kettering Cancer Center, New York, NY, was selected from the pathology database. Ampullary tumors were defined as tumors grossly appearing to arise from the ampulla or microscopically demonstrating a precursor lesion (adenoma or dysplasia) involving the ampulla. Tumors that involved the periampullary duodenum but whose ampullary origin could not be confirmed were not included. Noninvasive tumors were also excluded. The H&E-stained slides were reviewed in conjunction with the pathology report to confirm the diagnosis. The number of slides of tumor reviewed for each case ranged from 1 to 4. Of the 60 cases that were judged to be ampullary carcinoma, 6 did not have sufficient material for immunohistochemical studies and were thus excluded, and the remaining 54 constituted the study population.

Histologic Assessment

The histologic features assessed included the following: (1) Histologic type: classified as intestinal, pancreatobiliary, poorly differentiated, mucinous, or signet-ring cell. Poorly differentiated tumors were not subtyped into intestinal or pancreatobiliary types. Mucinous or signet-ring cell type was defined as a mucinous or signet-ring cell component representing more than 50% of the tumor. (2) Adenoma or surface dysplasia: classified as present or absent. (3) Tumor differentiation: categorized as well, moderate, or poor. The poorly differentiated tumors consisted of more than 50% of areas with poor gland development and formation of irregular sheets and clusters. (4) Medullary features: characterized by medium-sized cells with variable amounts of eosinophilic cytoplasm growing in solid sheets in association with abundant lymphocytes within the tumor nests and in the stroma and classified as present or absent. (5) Crohn-like reaction: characterized by an abundant lymphoid infiltrate associated with lymphoid follicles at the advancing edge of the tumor and classified as present or absent. (6) TILs: defined as lymphocytes that were infiltrating carcinoma glands, nests, or sheets, exclusive of inflammatory cells associated with stromal tissues or inflammatory debris. TILs were counted using an Olympus BH-2 microscope. Ten high-power fields (HPFs) (total area of 10 fields = 1.96 mm²) were selected randomly from the superficial and deep aspects of the invasive carcinoma, guided solely by the attempt to include the most tumor cells within each field. The lymphocyte counts were recorded as total number of TILs per 10 HPFs and the highest number of TILs per HPF among the 10 fields.

Immunohistochemical Assessment

The tumor sections showing areas of the carcinoma along with adjacent nonneoplastic tissues were identified. Sections from corresponding paraffin blocks were prepared and stained with antibodies against MLH1 (clone G168-728, diluted 1:250; PharMingen, San Diego, CA), MSH2 (clone FE11, diluted 1:50; Oncogene Research Products, Cambridge, MA), MSH6 (clone GRBP.P1/2.D4, diluted 1:200; Serotec, Raleigh, NC), and PMS2 (clone A16-4, diluted 1:200, BD PharMingen, San Diego, CA). Immunohistochemical staining was performed on deparaffinized tissue sections as previously described.10 The immunohistochemical stains were evaluated for the presence or absence of nuclear staining in the invasive carcinoma cells. The adjacent nonneoplastic tissue was used as an internal control for each slide, and stains were interpreted only when normal tissues adjacent to the carcinoma showed distinct nuclear labeling. MMR immunohistochemical staining was defined as normal or abnormal based on the presence or absence of nuclear staining; lack of nuclear staining in the invasive carcinoma cells was defined as abnormal. For evaluation of staining quality, the staining was graded as 1+ or 2+, with 1+ representing positive nuclear staining in 10% or fewer of the tumor cells and 2+ representing staining in more than 10% of the tumor cells.

Results

Patient Data

In the 54 ampullary carcinoma cases that met our inclusion criteria, the patients were 34 men and 20 women, with an age range of 41 to 86 years (mean, 64 years). One patient, a 44-year-old man, had a documented history of familial adenomatous polyposis (FAP). This patient underwent subtotal colectomy at age 27 years and had been followed up yearly since. Colorectal carcinoma never developed. Another patient had a personal history suggestive of hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome with 3 colorectal cancers resected at ages 50, 67, and 85 years. (This patient is referred to as the “clinical HNPCC...
case” in following text.) He was 84 years old at the time the ampullary carcinoma was diagnosed. None of the remaining 52 patients had a known history of FAP or HNPCC.

Morphologic Characteristics

The morphologic characteristics of all 54 tumors are summarized in Table I. The majority of the cases were of the intestinal type (37 [69%]) and moderately differentiated (49 [91%]). An ampullary adenoma or mucosal dysplasia was noted in 31 cases (57%). None of the cases in this series showed a typical Crohn-type lymphocytic reaction or medullary histologic features. The mucinous phenotype was detected in 7 cases (13%) and the signet-ring cell phenotype in 1 (2%). The TIL counts of all 54 tumors ranged from 0 to 62/10 HPFs (mean, 5.5; median, 2); 18 cases showed no TILs; 12 cases (22%) had 5 or more TILs/10 HPFs. The highest number of TILs/HPF among 10 HPFs ranged from 0 to 11 (mean, 1.4; median, 1); 15 cases (28%) had 2 or more highest TILs/HPF of 10 HPFs. Notably, 5 cases had a count of TILs/10 HPFs of 20 or higher (20, 35, 42, 56, and 62), with the highest TIL/HPF among the 10 fields being 4 or higher.

In addition, morphologic assessment of the most recent colonic tumor (only 1 available) from the clinical HNPCC case showed a moderately differentiated mucinous adenocarcinoma with a minor conventional nonmucinous component. In the less mucinous regions, the tumor contained 152 TILs/10 HPFs, with the highest TIL/HPF being 43. Image 1 illustrates the presence of abundant TILs in this tumor, in comparison with this patient’s ampullary tumor that also contained TILs, but less abundantly.

Immunohistochemical Findings

All 54 cases showed positive internal positive control results for all stains. External positive and negative controls also stained appropriately. As outlined in Table 1, 3 cases (6%) showed abnormal MMR immunohistochemical results, 1 lost staining for MLH1 and PMS2, and 2 lost staining for MSH6 alone. All 54 cases showed retained nuclear staining for MSH2. The staining pattern for all 4 markers in 1 MSH6-deficient case, the clinical HNPCC case, is illustrated in Image 2.

In terms of the extent of staining in immunohistochemically normal cases, 1+ staining, defined as less than 10% of tumor staining, was noted in 6 cases for MLH1/PMS2 (5 cases for MLH1 and 1 for PMS2), 3 cases for MSH2, and 2 cases for MSH6. The pathologic characteristics of these cases are listed in Table 1.

In addition, immunohistochemical staining was performed on the most recent colonic carcinoma from the clinical HNPCC case and revealed loss of MSH6 with normal staining for MLH1, PMS2, and MSH2.

Correlation Between Clinicopathologic and Immunohistochemical Findings

The clinical and pathologic features of the 3 immunohistochemically abnormal cases are as follows.

### Table I

Morphologic Characteristics and Mismatch Repair Protein Immunohistochemical Staining Patterns in 54 Cases of Ampullary Carcinoma

<table>
<thead>
<tr>
<th>Morphologic Characteristic</th>
<th>Mismatch Repair Protein Immunohistochemical Staining</th>
<th>MLH1/PMS2</th>
<th>MSH2</th>
<th>MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
<td>Absent</td>
<td>1+</td>
<td>Absent</td>
</tr>
<tr>
<td>Intestinal</td>
<td></td>
<td>37 (69)</td>
<td>1 (3)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Pancreatobiliary</td>
<td></td>
<td>12 (22)</td>
<td>0 (0)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td></td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td>49 (91)</td>
<td>1 (2)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Poor</td>
<td></td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Presence of ampullary adenoma/dysplasia</td>
<td></td>
<td>31 (57)</td>
<td>0 (0)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Crohn-type lymphocytic reaction</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Medullary histologic characteristics</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mucinous type</td>
<td></td>
<td>7 (13)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Signet-ring cell type</td>
<td></td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TIL count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5/10 HPFs</td>
<td></td>
<td>12 (22)</td>
<td>1 (8)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>≥2 highest count/HPF of 10 HPFs</td>
<td></td>
<td>15 (28)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

HPF, high-power field; TIL, tumor-infiltrating lymphocyte; 1+, <10% of tumor staining.
* Data are given as number (percentage).
The 1 case that lost both MLH1 and PMS2 was a 78-year-old woman with an intestinal-type, moderately differentiated adenocarcinoma that had 12 TILs/10 HPFs and a highest TIL/HPF of 2. This patient had no known family or personal history of HNPCC.

Of the 2 cases that lost MSH6 staining alone, one was the clinical HNPCC case whose most recent colonic carcinoma showed mucinous histologic features with high TILs and loss of MSH6 staining by immunohistochemical analysis, as described. His ampullary carcinoma was of the pancreatobiliary type and moderately differentiated, with 14 TILs/10 HPFs and a highest TIL/HPF of 3. The second abnormal MSH6 case was a 52-year-old man with an intestinal-type, moderately differentiated adenocarcinoma that had 4 TILs/10 HPFs with a highest TIL/HPF of 2.

It is interesting that although 2 of the 3 MMR immunohistochemically abnormal ampullary carcinomas showed a TIL count of more than 5 TILs/10 HPFs, none of the 5 cases that had the highest TIL counts (range, 20-62 TILs/10 HPFs) had abnormal immunohistochemical results. With 5 TILs/10 HPFs as a cutoff, TILs had a sensitivity of 67% (2/3) for predicting MMR immunohistochemical abnormality with a specificity of 80% (41/51). Correlation between other morphologic patterns and MMR immunohistochemical results was precluded by the small number of immunohistochemically abnormal cases.

**Discussion**

Significant overlap exists in phenotypic and molecular characteristics between ampullary and colorectal carcinomas; as such, it is plausible to hypothesize that DNA MMR deficiency, a tumorigenesis pathway accounting for up to 15% to 20% of colorectal cancers, may have a role in the pathogenesis of ampullary tumors as well. Thus far, however, there have been only a few pertinent studies on DNA MMR status in ampullary tumors, and the results have not been consistent.

A study by Achille et al noted an MSI rate of 20% in a series of 25 ampullary carcinomas. Similarly, Imai et al reported a high rate of MSI (22%) and deletion or insertion mutations in the poly-p-A microsatellite sequence of the transforming growth factor β receptor-II gene (78%) in their series of 18 ampullary tumors. More recently, Sessa et al found 5 (9%) of 53 ampullary carcinomas with high-frequency MSI (MSI-H). Similarly, Ruemmele et al tested MSI in a series of 140 ampullary adenocarcinomas and found that 10% were MSI-H. Such findings indicate that the MSI phenotype is relatively frequent in ampullary carcinomas. Others, however, disagreed and reported much lower MSI rates. For example, Suto et al and Rashid et al failed to identify any tumor that would qualify for MSI-H. The numbers of ampullary tumors studied in these 2 studies were 16 and 18, respectively. The pertinent literature data are summarized in Table 2.

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*Image 1* Histologic features of an ampullary tumor (A, H&E, ×200) and a colonic tumor (B, H&E, ×200) from a patient with a history suggestive of hereditary nonpolyposis colorectal cancer/Lynch syndrome. Notice that tumor-infiltrating lymphocytes (TILs) (arrows) are present in the pancreatobiliary-type ampullary carcinoma and the colonic carcinoma; however, they are much more abundant and easily discernible in the colonic tumor. Arrows in A mark all discernible TILs in this field; arrow in B marks one of many TILs present in this field.

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There are 3 main reasons that may account for the discrepant prior data. First, classification of ampullary tumors is known to be difficult; misclassifying nonampullary tumors, such as duodenal or pancreatic tumors, as ampullary tumors may, therefore, have contributed to the varied results. Second, as outlined in Table 2, the studies used varied microsatellite markers and inconsistent definitions for the detection of MSI. Notably, the study by Achille et al used 10 markers but did not include BAT25 or BAT 26, the 2 mononucleotide markers that have been shown to be highly sensitive and specific.17,18 Third, the method of case selection in some studies may have introduced a bias in favor of a high MSI rate. In the study in which a 20% rate of MSI was identified,5 2 of the 5 patients whose ampullary tumors showed positive replication error were females, 50 years or younger. These 2 patients also had multiple cancers, one with metachronous endometrial carcinoma and transverse colon carcinoma and the other with synchronous transverse colon colloid carcinoma. The distribution of the cancers and the age and female sex all raise the possibility that these patients were HNPCC probands (these 2 patients had no family history of cancer). In the recent study that found a 9% MSI rate,4 case selection criteria were not specifically detailed, and it is not clear whether consecutive cases were selected.

Reports of immunohistochemical studies of DNA MMR abnormality in ampullary carcinomas are few. Park et al8
studied immunohistochemical expression of MLH1 and MSH2 in 93 tumors and found no loss of either protein in any of the cases. The study by Park et al, however, was performed on tissue microarray sections. Experience from studies on colorectal and endometrial tumors indicates that immunohistochemical staining for DNA MMR proteins, particularly for MLH1, can be technically challenging, and adequate tissue sampling is critical to accurate interpretation of staining results. In our study, heterogeneous staining of MMR proteins was indeed a noticeable phenomenon, suggesting that staining of limited tissue samples may have resulted in false-negative results. Thus, studies using whole tissue sections are desirable. Most recently, Ruemmele et al reported abnormal immunohistochemical staining in 12 (7.7%) of 155 ampullary carcinomas, including 7 with loss of MLH1, 3 with concurrent loss of MSH2 and MSH6, and 2 with isolated loss of MSH6. PMS2 was not included in the antibody panel.

Based on the aforementioned premises and acknowledging the fact that immunohistochemical analysis is an efficient tool for detecting MMR protein abnormality, we designed this study that used immunohistochemical staining with antibodies against all 4 major MMR proteins on whole tissue sections and a study population composed of consecutive patients selected to ensure no overrepresentation of patients with any genetic cancer syndrome such as FAP or HNPCC. We observed loss of MMR protein in 3 (6%) of 54 unselected ampullary carcinomas, and the proteins lost were MLH1 and PMS2 in 1 case and MSH6 alone in 2 cases; one of the 2 MSH6-deficient cases was the case that had a personal history suggestive of HNPCC. Thus, our data suggest that MMR protein abnormality occurs in ampullary carcinomas but appears less frequently than in colorectal carcinoma.

Although the number of MMR immunohistochemically abnormal cases was small in our study, the abnormal patterns we observed (ie, concurrent loss of MLH1 and PMS2 or isolated loss of MSH6) and that observed by Ruemmele et al (ie, concurrent loss of MSH2 and MSH6 or isolated loss of MSH6) were in keeping with patterns observed in endometrial and colorectal tumors. These patterns reflect the biochemical properties of the MMR proteins.

Biliary tract tumors and small bowel tumors are known to be within the HNPCC/Lynch syndrome tumor spectrum. Of our 2 MSH6-deficient cases, 1 indeed had a personal history highly suggestive of HNPCC (specifically, 3 metachronous colorectal carcinomas, with the first diagnosed at age 50 years) and was thus very likely a true Lynch syndrome case. Unfortunately, we did not have germline mutation data or family history for this patient who came to our attention at an old age. Nevertheless, this case implies a few interesting points. First, it implies that ampullary carcinoma should indeed be specifically included in the HNPCC tumor spectrum. Second, HNPCC-related ampullary carcinoma may occur at an old age. We do not have evidence to suggest that any ampullary carcinoma should trigger MMR immunohistochemical/MSI testing or genetic testing, but it appears reasonable that, in families at high risk for HNPCC, when more conventional tumor types are not available, ampullary carcinoma be used as a tumor sample for detection of MMR protein or function.

The 3 MMR immunohistochemically abnormal cases in our series included 2 intestinal-type and 1 pancreatobiliary-type tumors, all moderately differentiated. All 3 tumors had

**Table 2**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Cases</th>
<th>Markers Used</th>
<th>Unstable Cases</th>
</tr>
</thead>
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<tr>
<td>Achille et al, 1997</td>
<td>25</td>
<td>DSX453, MAOB, DXS538, DXS454, APA3, D1S158, RDS, ELN, D7S560, D8S199</td>
<td>5 (20) at ≥6 markers</td>
</tr>
<tr>
<td>Imai et al, 1998</td>
<td>18</td>
<td>BAT26, D2S123, D3S1029, D5S409, TPS3</td>
<td>1 at D3S1029</td>
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<td>Suto et al, 2001</td>
<td>16</td>
<td>BAT25, BAT26, D1S199, D2S123, D3S1611, D10S197, Gli R6, UT762</td>
<td>4 (22) at ≥2 or ≥3 markers; 1 involved BAT26</td>
</tr>
<tr>
<td>Rashid et al, 2002</td>
<td>18</td>
<td>BAT25, BAT26, D1S123, D3S1029, D5S409, TPS3</td>
<td>2; neither involved BAT26</td>
</tr>
<tr>
<td>Park et al, 2003</td>
<td>32</td>
<td>BAT26, BAT25, D1S123, D3S1029, D5S409, IFNα, D10S109, D13S118, TPS3, D7S520, D18S34</td>
<td>5; none involved BAT26</td>
</tr>
<tr>
<td>Sessa et al, 2007</td>
<td>53</td>
<td>BAT25, BAT26, NR21, NR22, NR24</td>
<td>3 at 2 markers; 1 involved BAT26; 1 at ≥3 markers</td>
</tr>
<tr>
<td>Ruemmele et al, 2009</td>
<td>144</td>
<td>BAT25, BAT26, BAT40, D17S250, D2S123, D5S409, D18S61</td>
<td>2 at ≥3 markers; 5 (8) at ≥3 markers</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percentages.
† This study also included additional extrahepatic bile duct tumors; data not shown.
‡ This study also included additional extrahepatic bile duct and gallbladder tumors; data not shown.
§ This study also included additional cases of adenomas and metastases; data not shown.
some TILs, with TILs/10 HPF of 4, 12, and 14 and a highest TIL/HPF of 2 or 3. As such, they suggest that morphologic features, particularly TILs, that are commonly associated with MSI-H colorectal carcinomas may indeed be indicative of MSI-H in ampullary carcinomas as well. This is in agreement with observations by Ruemmele et al16 and with our anecdotal experience. Further supporting this assumption is the occurrence of poorly differentiated tumors with a medullary history and an MSI-H phenotype in this site.5 Indeed, after completion of this study, we encountered an ampullary carcinoma that showed a histologic pattern indistinguishable from that of colonic medullary carcinoma Image 3A and Image 3B and lacked MLH1 immunohistochemical staining Image 3C. The patient was a 63-year-old woman with no known family history of cancer. By immunohistochemical and clinical findings, the possibility of other tumors that may show similar morphologic features, such as pancreatic acinar cell carcinoma and high-grade neuroendocrine carcinoma, was ruled out. Recognizing such a medullary morphologic pattern will allow the correct diagnosis of a tumor that is highly suggestive of the MSI phenotype.

It is to be noted that TILs are more difficult to discern in ampullary carcinomas, especially when the tumor is of the pancreatobiliary type as illustrated in our clinical HNPCC case (Image 1). Thus, identifying TILs in such tumors may be more laborious. Of further interest is our observation that there exist cases in the ampulla that have significantly increased TILs (up to 62/10 HPFs in this series) but normal MMR immunohistochemical results. In fact, all 5 cases in our series that had the highest TIL counts were immunohistochemically normal. On the other hand, the clinical HNPCC case that lost MSH6 in the ampullary and colonic tumors had some TILs, with TILs/10 HPF of 4, 12, and 14 and a highest TIL/HPF of 2 or 3. As such, they suggest that morphologic features, particularly TILs, that are commonly associated with MSI-H colorectal carcinomas may indeed be indicative of MSI-H in ampullary carcinomas as well. This is in agreement with observations by Ruemmele et al16 and with our anecdotal experience. Further supporting this assumption is the occurrence of poorly differentiated tumors with a medullary history and an MSI-H phenotype in this site.5 Indeed, after completion of this study, we encountered an ampullary carcinoma that showed a histologic pattern indistinguishable from that of colonic medullary carcinoma Image 3A and Image 3B and lacked MLH1 immunohistochemical staining Image 3C. The patient was a 63-year-old woman with no known family history of cancer. By immunohistochemical and clinical findings, the possibility of other tumors that may show similar morphologic features, such as pancreatic acinar cell carcinoma and high-grade neuroendocrine carcinoma, was ruled out. Recognizing such a medullary morphologic pattern will allow the correct diagnosis of a tumor that is highly suggestive of the MSI phenotype.

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dramatically elevated TIL counts in his colonic tumor but only modestly increased TIL counts in his ampullary tumor. Such observations seem to suggest that although TILs may be indicative of MSI in ampullary carcinomas as well, their exact implications in ampullary tumors may be somewhat different from those in colorectal tumors. Although MSI is the major molecular mechanism for colorectal tumors with a profound lymphocytic infiltrate, other mechanisms may exist for such tumors in the ampulla. Indeed, we have noted that in the stomach (and occasionally proximal duodenum), morphologically indistinguishable tumors with prominent lymphocytic infiltrates may be related not only to MSI, but also to Epstein-Barr virus (EBV) infection or other unknown etiology; and MSI and EBV infection were mutually exclusive in these tumors. Whether EBV infection is a cause for these MMR immunohistochemically normal ampullary tumors with high TILs remains to be determined.

As has been demonstrated in the case of colorectal cancer, although immunohistochemical analysis is fairly sensitive and specific in detecting abnormal MMR proteins, the stains can be weak and heterogeneous, and some experience with such stains is required to avoid making errors in the interpretation. This is true in ampullary carcinomas as well. In our series, a small but finite subset of tumors had only 1+ staining for some MMR proteins. Thus far, most studies have regarded heterogeneous and/or weak staining as presence of protein expression and have defined “loss of expression” as complete absence of nuclear staining in all tumor cells; although, recently, some authors have speculated about the presence of certain types of mutation or partial methylation as a cause for weak immunohistochemical staining in some cases. Overall, the significance of weak and heterogeneous staining is not certain. Technical factors like improper tissue fixation and suboptimal antigen retrieval may also explain weak and heterogeneous staining.

In conclusion, DNA MMR protein abnormality may occur in ampullary carcinoma, although its frequency seems lower than in colorectal carcinomas. Some MMR-deficient ampullary tumors may be hereditary. Common MSI-H histologic features of colorectal tumors, such as increased lymphocytic infiltration, seem to have similar yet subtly different implications in ampullary carcinomas as a noticeable subset of ampullary carcinoma with high TIL counts may be related to mechanisms other than MSI.

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Agaram et al / Mismatch Repair Deficiency in Ampullary Carcinoma

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