MIB-1 and MCM-2 Immunohistochemical Analysis Does Not Aid in Identification of Serrated Colorectal Polyps With Abnormal Proliferation

David Gray, MD,1 Ellen C. Obermann, MD,2 Mark Evans, PhD,1 Arndt Hartmann, MD,2 Kumarasen Cooper, MD,1 and Hagen Blaszyk, MD1

Key Words: Hyperplastic polyp; Serrated polyp; Serrated neoplasia pathway; MIB-1; MCM-2

Abstract We investigated the staining characteristics of serrated polyps with abnormal proliferation (SPAP) using MIB-1 and MCM-2 to determine if they could provide assistance in delineating SPAPs from traditional hyperplastic polyps (HPs). Using published morphologic criteria we reviewed H&E slides of 107 polyps from 80 patients. Thirty-nine (36.4%) polyps met the criteria for SPAP. Within a given region, polyps in the transverse colon had the largest percentage of SPAPs (50.0%) followed by the right colon (40.9%). The majority of SPAPs (82.1%) and HPs (72.1%) showed MIB-1 staining confined to the basal third of the crypts. The majority of SPAPs (59.0%) and HPs (52.9%) showed MCM-2 staining extending into the apical third of the crypts. We do not recommend MIB-1 or MCM-2 staining to differentiate SPAPs from conventional HPs, since staining characteristics are not significantly different between the 2 groups, and frequent variable crypt staining within a given polyp is difficult to interpret.

Although hyperplastic polyps (HPs) of the colorectum share epidemiologic and anatomic distribution characteristics with colorectal adenomas and carcinomas, most investigators consider HPs to be an incidental finding with no potential for neoplastic progression.1 The adenoma-carcinoma sequence has been accepted widely as the evolutionary paradigm for colorectal cancer, recognizing molecular counterparts to a stepwise process. However, numerous studies suggest a morphologic, immunohistochemical, and genetic continuum in the proposed hyperplasia–dysplasia–carcinoma sequence.1-3 A serrated neoplasia precursor pathway has been proposed for some colorectal cancers in which serrated adenomas represent the precursor lesion.4 However, the morphologic complexity of serrated adenomas varies from clearly adenomatous lesions to polyps that closely resemble “conventional” HPs. Recent detailed histologic analyses provided diagnostic criteria to identify such precursor lesions, and the terms sessile serrated polyp and serrated polyp with abnormal proliferation (SPAP) have been proposed.5 6 The diagnostic features generally comprise abnormal proliferation and dysmaturity,6 including expanded crypt proliferation zones, basilar crypt dilatation and serrated architecture, and decreased cell maturation.5 These morphologic criteria seem to be well-defined, but there are few studies on interobserver and intraobserver variability to assess how reproducible the light microscopic recognition of such lesions is. It is far from certain that most general pathologists would be able to consistently distinguish the proposed sessile serrated polyp from a conventional HP, but such distinction may become clinically relevant and crucial for patient management. Ancillary studies, such as demonstration of a distinct immunophenotype, might prove to be helpful tools if morphologic assessment alone is not reliable.
enough in routine pathology practice. Morphologic studies of SPAPs commonly emphasize dysmaturity and an increased proliferation zone extending toward the surface. We assessed the possible usefulness of the proliferation markers MIB-1 and MCM-2 in routine pathology practice.

Materials and Methods

Cases

The institutional review board of the University of Vermont, Burlington, approved the research protocol, and all patients remained anonymous throughout the study. By using the Fletcher Allen Health Care CoPath database, all patients with a diagnosis of colonic HP were identified within a 6-month period (July 1, 1995, to December 31, 1995). Our search found a total of 101 patients who had endoscopic biopsies, resulting in 140 biopsy specimens with a diagnosis of HPs. We excluded 33 polyps and 21 patients owing to missing blocks, insufficient residual tissue, poor orientation, or cautery artifact. Information about the patients’ age, sex, and location of polyps were obtained for the remaining 80 patients. Two polyps were identified in resections for colon cancer. Colon cancer subsequently developed in only 1 patient during a follow-up period of 8 years.

Tissue Samples

Formalin-fixed, paraffin-embedded archival material was used after the original H&E-stained glass slides were reviewed to verify the pathologic diagnoses for each specimen.

Immunohistochemical Staining

All polyps were assessed immunohistochemically using a dextran polymer–peroxidase complex method with 3,3-diaminobenzidine as a chromogen and citrate buffer antigen retrieval.

In brief, 5-µm sections of formalin-fixed, paraffin-embedded polyp tissue were stained using the DAKO Autostainer automated immunostaining system with monoclonal mouse antibodies against the desired antigen and detected with the EnVision+ system (catalog No. K4007, DAKO, Carpinteria, CA). Tissue sections were deparaffinized and rehydrated, and antigen retrieval was performed by pretreatment in Target Retrieval Solution (DAKO) in citrate buffer. Then, 3,3-diaminobenzidine was applied followed by a water rinse. Sections were counterstained with Bennett hematoxylin for 4 minutes, rinsed in 3 changes of buffer, soaked in buffer until blue (1 minute), and rinsed under running tap water. They were dehydrated in alcohol and coverslipped with Permaslip (Alban Scientific, St Louis, MO).

Positive control tissue was normal colonic epithelium. The primary antibody was omitted for negative control experiments.

Histologic and Immunohistochemical Analysis

HPs were classified as normally or abnormally proliferative by 2 pathologists (D.G. and H.B.) based on criteria defined by Goldstein et al.5 and Torlakovic et al.6 Briefly, normal HPs have proliferative zones that are symmetric and basally located, whereas SPAPs resemble traditional HPs but their overall architecture often is distorted by basal crypt dilation and crypts with a horizontal orientation. They exhibit immature epithelial cells that extend beyond the basal third of the crypts and may be distributed asymmetrically, extending higher on one side of the crypt. The abnormal polyps can have segments of immature epithelial cells that are noncontiguous with the basal zones of proliferation. Immature cells are characterized by a lack of mucin production, high nuclear/cytoplasmic ratios, and nuclear crowding.

The normal HPs were subdivided into microvesicular serrated polyps (MVSPs), goblet cell serrated polyp (GCSPs), and mucin-poor serrated polyps (MPSPs) based on mucin content and type. These subtypes have been described in greater detail by Torlakovic et al.6 In brief, MVSPs are characterized by mucin predominantly formed in numerous irregularly sized and shaped vesicles within individual epithelial cells. A few goblet cells may be seen as well. GCSPs form mucin in typical univacuolar goblet cells, which are distributed variably along the middle and distal portions of the crypts. MPSPs have few to no mucin-producing cells and abundant hypereosinophilic cytoplasm. The maximum dimension of all polyps was measured with an ocular micrometer at low power with a 2× objective and a field diameter of 10.9 mm.

Polyps were stained immunohistochemically with MIB-1 and MCM-2 in an attempt to delineate normal from abnormal proliferation. Polyps were classified by the degree of extension of proliferative epithelium from the crypt base. The crypts were divided into basal, middle, and apical thirds. Glands with proliferation confined to the basal third were placed in one group, those with proliferation extending into the middle third were placed in another, and so on. When polyps showed regional variation in staining, categorization was based on the strongest staining portion of the polyp. Polyps with 5 or fewer noncontiguous positive cells per crypt were designated as scant staining.
**Results**

Our database search found 80 patients with 107 HPs suitable for the study. The patients were 53 males (mean age, 58.6 years) and 27 females (mean age, 58.8 years). The polyps were distributed throughout the colon with the majority (53.3%) arising in the rectosigmoid. Thirty-nine polyps (36.4%) met the criteria for SPAP. Within a given region of the colon, the transverse had the highest percentage of SPAPs (50%) followed by the right colon (40.9%). There were roughly equal proportions of GCSPs (20.6%), MVSPs (22.4%), and MPSPs (20.6%). For further details, see Table 1. The mean ± SD size of abnormal polyps was 3.6 ± 1.7 mm and of normal polyps, 3.2 ± 1.2 mm.

The majority of SPAPs (82.1%) and HPs (72.1%) showed MIB-1 staining confined to the basal third of the crypts. HPs showed more polyps with MIB-1 staining extending into the middle portion of the crypts (16.2% vs 2.6% for SPAPs; *P* = .01). None of the polyps had MIB-1 staining extending into the apical third of the crypts. Similar numbers of SPAPs (15.4%) and HPs (11.8%) exhibited scant staining with MIB-1.

MCM-2 demonstrated more robust staining but did not differentiate between polyp types. The majority of SPAPs (59.0%) and HPs (52.9%) showed MCM-2 staining extending into the apical third of crypts. They also had comparable values for extension of staining into the basal third and the middle third.

We found that MIB-1 and MCM-2 exhibited variable staining within a given polyp. Crypt staining was neither uniform nor consistent within the diagnostic groups and added an interpretive step when selecting crypts for grading the staining characteristics.

**Discussion**

There is growing evidence that a subset of HPs may serve as precursor lesions for colorectal cancers resulting from the serrated neoplasia pathway. There are no published reports evaluating the staining characteristics of SPAPs with proliferation markers. Kang et al studied MIB-1 staining in HPs, serrated adenomas, and tubular adenomas. They found that tubular adenomas had the highest rate of positivity in the middle and apical crypt zones followed by serrated adenomas and then HPs. Although MIB-1 seems to be sensitive enough to distinguish adenomas from hyperplastic epithelium, it does not differentiate HPs from SPAPs.

In the present study, we classified 107 HPs as normally or abnormally proliferative based on established morphologic criteria applied to H&E-stained slides. Our series of HPs showed general agreement with the findings of other investigators in the percentage of SPAPs (36.4%). In their series of colon polyps, Lazarus et al identified 41% “atypical HPs” among a sample of 56 polyps. O’Brien et al found 32.9% SPAPs in a series of proximal and distal HPs. When reviewing normal control HPs from the rectosigmoid colon, Goldstein et al found that 22.6% would be reclassified as abnormal based on their criteria. However, this series did not include proximal colon HPs that presumably would increase the total SPAP yield. Torlakovic et al found the least number (18%) of SPAPs in their series of 289 colon biopsy specimens. These differences might reflect variability between observers and/or varied application of criteria.

**Table 1**

<table>
<thead>
<tr>
<th>Type of Polyp</th>
<th>Right (n = 22)</th>
<th>Transverse (n = 14)</th>
<th>Left (n = 14)</th>
<th>Rectosigmoid (n = 57)</th>
<th>Total (N = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAP</td>
<td>9 (40.9)</td>
<td>7 (50.0)</td>
<td>2 (14.3)</td>
<td>21 (36.8)</td>
<td>39 (36.4)</td>
</tr>
<tr>
<td>MVSP</td>
<td>6 (273)</td>
<td>1 (71.1)</td>
<td>4 (28.6)</td>
<td>13 (22.8)</td>
<td>24 (22.4)</td>
</tr>
<tr>
<td>GCSP</td>
<td>3 (13.6)</td>
<td>2 (14.3)</td>
<td>5 (35.7)</td>
<td>12 (21.0)</td>
<td>22 (20.6)</td>
</tr>
<tr>
<td>MPSP</td>
<td>4 (18.2)</td>
<td>4 (28.6)</td>
<td>3 (21.4)</td>
<td>11 (19.3)</td>
<td>22 (20.6)</td>
</tr>
</tbody>
</table>

GCSP, goblet cell serrated polyp; MPSP, mucin-poor serrated polyp; MVSP, microvesicular serrated polyp; SPAP, serrated polyp with abnormal proliferation.

* Data are given as number (percentage of the column total).
In an attempt to assist in the diagnosis of SPAPs, we stained HPs with MIB-1 and MCM-2 to determine whether aberrant morphologic features correlated with the degree and pattern of staining. We do not recommend MIB-1 or MCM-2 staining to differentiate SPAPs from conventional HPs because staining characteristics are not significantly different between the groups and frequent variable crypt staining within a given polyp is difficult to interpret. We did find differences in the number of MPSPs with MIB-1 extension to the middle third relative to SPAPs, but the significance of this observation is unclear.

Adequate evaluation of a SPAP by histologic or immunohistochemical examination ideally requires vertically oriented crypts to assess the described architectural features of SPAPs. Moreover, current morphologic criteria rely in part on cytologic characteristics of crypt epithelial cells as they mature from the basal proliferative zone to the luminal surface. Although routine diagnosis of HPs and tubular adenomas can be made with tangential and even perpendicular sectioning of crypts, confidently making a diagnosis of SPAPs is aided greatly by more precise specimen orientation.

By using published morphologic criteria, we found that SPAPs are located predominantly in the proximal colon, but also can be identified in the rectosigmoid colon. Proliferation markers MIB-1 and MCM-2 do not seem helpful for distinguishing normal HPs from SPAPs. The reliable recognition of SPAPs ideally requires vertically oriented crypts to assess the described architectural features of SPAPs.
Table 2
Staining With MIB-1 of Hyperplastic Polyps by Abnormal Proliferative and Normal Proliferative Subtypes

<table>
<thead>
<tr>
<th>MIB-1 Staining Extension</th>
<th>SPAP (n = 39)</th>
<th>MVSP (n = 24)</th>
<th>GCSP (n = 22)</th>
<th>MPSP (n = 22)</th>
<th>Total (N = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>32 (82.1)</td>
<td>16 (66.7)</td>
<td>18 (81.8)</td>
<td>15 (68.2)</td>
<td>81 (75.7)</td>
</tr>
<tr>
<td>Middle</td>
<td>1 (2.6)</td>
<td>4 (16.7)</td>
<td>1 (4.5)</td>
<td>6 (27.3)</td>
<td>12 (11.2)</td>
</tr>
<tr>
<td>Apical</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Scant staining</td>
<td>6 (15.4)</td>
<td>4 (16.7)</td>
<td>3 (13.6)</td>
<td>1 (4.5)</td>
<td>14 (13.1)</td>
</tr>
</tbody>
</table>

GCSP, goblet cell serrated polyp; MPSP, mucin-poor serrated polyp; MVSP, microvesicular serrated polyp; SPAP, serrated polyp with abnormal proliferation.

* Data are given as number (percentage of the column total).

Table 3
Hyperplastic Polyp Staining With MCM-2 by Abnormal Proliferative and Normal Proliferative Subtypes

<table>
<thead>
<tr>
<th>MCM-2 Staining Extension</th>
<th>SPAP (n = 39)</th>
<th>MVSP (n = 24)</th>
<th>GCSP (n = 22)</th>
<th>MPSP (n = 22)</th>
<th>Total (N = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>9 (23.1)</td>
<td>3 (12.5)</td>
<td>5 (22.7)</td>
<td>5 (22.7)</td>
<td>22 (20.6)</td>
</tr>
<tr>
<td>Middle</td>
<td>7 (17.9)</td>
<td>6 (25.0)</td>
<td>9 (40.9)</td>
<td>4 (18.2)</td>
<td>26 (24.3)</td>
</tr>
<tr>
<td>Apical</td>
<td>23 (59.0)</td>
<td>15 (62.5)</td>
<td>8 (36.4)</td>
<td>13 (59.1)</td>
<td>59 (55.1)</td>
</tr>
</tbody>
</table>

GCSP, goblet cell serrated polyp; MPSP, mucin-poor serrated polyp; MVSP, microvesicular serrated polyp; SPAP, serrated polyp with abnormal proliferation.

* Data are given as number (percentage of the column total).
and diagnosis of SPAPs in pathology practice seems to be important for patient management and surveillance strategies.\textsuperscript{8,9} Thus, results of studies on interobserver and intraobserver variability in the routine histologic diagnosis of SPAPs will help to guide future studies to possibly identify ancillary markers for use by practicing pathologists.

From the \textsuperscript{1}Department of Pathology, University of Vermont College of Medicine, Burlington; and \textsuperscript{2}Institute of Pathology, University of Regensburg, Regensburg, Germany.

Address reprint requests to Dr Blaszyk: Dept of Pathology, University of Vermont College of Medicine, 89 Beaumont Ave, Burlington, VT 05405.

References