Sessile Serrated Adenomas With Low- and High-Grade Dysplasia and Early Carcinomas

An Immunohistochemical Study of Serrated Lesions "Caught in the Act"

Todd B. Sheridan, MD,[1] Hubert Fenton, MD,[1] Marc R. Lewin, MD,[1] Ashlie L. Burkart, MD,[1] Christine A. Iacobuzio-Donahue, MD, PhD,[1,2] Wendy L. Frankel, MD,[3] and Elizabeth Montgomery, MD[1]

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Abstract

Sessile serrated adenomas (SSAs) show serrations typical of hyperplastic polyps but display architectural differences and lack traditional dysplasia. SSAs with foci of low- (LGD) or high-grade dysplasia (HGD) or early invasive carcinoma are seldom biopsied and, thus, are not well studied. Immunohistochemical analysis for MLH1, MSH2, MSH6, and PMS2 (mismatch repair gene products) was performed on colon biopsy specimens from 11 patients (age range, 54-87 years; 4 men and 7 women) showing SSA with LGD (n = 1), HGD (n = 5), or focal invasive carcinoma (n = 5). All 11 cases showed intact nuclear staining for MSH2 and MSH6 in the SSA component; in foci of LGD, HGD, or carcinoma; and in background normal mucosa. In contrast, there was tandem loss of MLH1 and PMS2 in zones of LGD (1/1) or HGD (3/5) and early carcinoma (2/4; with concordant loss in associated HGD) but retention in SSA areas (11/11) and normal mucosa (11/11). No patient was known to have hereditary nonpolyposis colorectal cancer/Lynch syndrome. This study offers additional strong evidence that SSA is truly a precursor to at least a subset of sporadic microsatellite-unstable colorectal cancer.

Until recently, colorectal polyps were classified predominantly as hyperplastic or adenomatous. Adenomatous polyps are well-characterized precursor lesions of adenocarcinomas thought to evolve through adenomatous polyposis coli (*APC*) gene mutations,[1] now termed the tumor suppressor (chromosomal instability) pathway[2] or microsatellite-stable pathway. Hyperplastic polyps have long been considered benign, easy to diagnose, and readily separated from other types of colorectal polyps.[3] However, we now recognize a subset of serrated polyps that lacks conventional dysplasia, and the term sessile serrated adenoma (SSA) is rapidly entering our diagnostic lexicon.[2] We now realize that, in all likelihood, lesions interpreted as "mixed hyperplastic and adenomatous polyps" are, in fact, SSAs complicated by conventional dysplasia.[2,4]

Longacre and Fenoglio-Preiser[5] reported other polyps with the architectural features of hyperplastic polyps but with dysplasia, coining the term serrated adenoma. A few years later, Torlakovic and Snover[6] characterized a group of patients with polyposis, which showed similar features to hyperplastic polyps but with a sessile pattern of growth. Despite their lack of conventional dysplasia, these polyps were termed SSAs. This new concept was largely ignored by the pathology community, however, perhaps in part owing to publication in a gastroenterology journal.

In 2003, Torlakovic et al[7] turned their efforts to the pathology literature and reported evidence of abnormal proliferation in some serrated lesions that superficially resembled hyperplastic polyps but that could be distinguished histologically, retaining the term SSA. They also reported reduced expression of MLH1 in a subset of cases, thus linking these lesions to the microsatellite instability

(MSI) pathway. Goldstein et al[8] upheld these findings, linking SSAs to MSI-high adenocarcinomas.

These observations and subsequent supporting molecular and immunohistochemical data have led to the concept of a serrated (mutator) neoplasia pathway.[2] SSAs, particularly those of the right colon, frequently display genetic alterations akin to (but via a different mechanism) those seen in MSI-high adenocarcinomas. MSI-high adenocarcinomas are the hallmark of hereditary nonpolyposis colorectal cancer (HNPCC/Lynch syndrome), a familial syndrome characterized by colonic adenocarcinomas, extracolonic neoplasms, and a germline mutation in one of several mismatch repair (MMR) genes.[9] At least 1 of 4 MMR genes is usually involved, with the majority of mutations seen in *MLH1* or *MSH2*, but *MSH6* or *PMS2* are sometimes involved.[10] MMR genes are involved in correction of errors occurring during cell division, and mutations in these genes are associated with MSI. The syndromic version is characterized by mutations of the MMR genes, whereas the sporadic counterparts have inactivation of the key genes by promoter methylation.[2] It is thought that, in some cases, SSA may develop into aggressive adenocarcinoma more rapidly than traditional adenomatous polyps, paralleling those of HNPCC,[11] and, thus, no precursor lesion is demonstrated in many microsatellite-unstable colorectal cancers. Moreover, the association of SSA and carcinoma has largely been assumed from the presence of these lesions adjacent to MSI-high carcinomas.[8] Evidence of progression through high-grade dysplasia (HGD) had rarely been reported; Goldstein[12] identified HGD and/or focal invasive adenocarcinoma in 8 SSAs with abrupt transition between elements. We report an additional 11 SSAs "caught in the act" with analysis of clinical features and MMR protein expression by immunohistochemical analysis.

Materials and Methods

A computer search of cases between January 1, 1980, and December 5, 2005, at the Johns Hopkins Hospital, Baltimore, MD, identified 16 cases, in house (n = 6) and received in consultation (n = 10), of SSAs with associated low-grade dysplasia (LGD) or HGD and/or invasive carcinoma. Of the consultation cases, paraffin-embedded tissue samples were available in 5 cases, allowing immunohistochemical staining in 11 cases. The diagnosis of SSA and traditional serrated adenoma was agreed on by 3 of us (T.B.S., W.L.F., and E.M.) using criteria set by Torlakovic and Snover[6] and Torlakovic et al.[7] Briefly, to diagnose SSA, we sought lesions that superficially resembled hyperplastic polyps but that displayed a tendency to right-sided location and large size and differed at low magnification in featuring broad-based crypts and often a sessile pattern and at high magnification in displaying subtle alterations in cytologic features and a paucity of endocrine cells. A consensus diagnosis of LGD or HGD and adenocarcinoma also was reached by the same authors. Approval for the study was granted by the institutional review board.

For each case, paraffin-embedded tissue was cut at 4 μm and placed on positively charged slides. Slides with specimens then were placed in a 60°C oven for 1 hour, cooled, and deparaffinized and rehydrated through xylenes and graded ethanol solutions to water. All slides were quenched for 5 minutes in a 3% hydrogen peroxide solution in water to block for endogenous peroxidase.

Antigen retrieval was performed by heat-induced epitope retrieval via a pressure cooker, in which the slides were placed in TRS (DAKO, Carpinteria, CA) for MSH2 and MSH6 and EDTA 9.0 solution for PMS2. Slides then were placed on a DAKO Autostainer immunostaining system, for use with immunohistochemical analysis. Dilutions were as follows: MSH2, 1:200; MSH6, 1:200; and PMS2, 1:50. Primary antibodies incubated for 1 hour at room temperature. The detection system used was a labeled polymer system, EnVision Plus Mouse (DAKO code No. K4001). Staining was visualized with diaminobenzidine (DAB) chromogen. Slides then were counterstained with hematoxylin, dehydrated through graded ethanol solutions, and coverslipped.

In the immunohistochemical staining for MLH1, antigen retrieval was performed by a heat method via a pressure cooker. Slides for MLH1 were placed in an EDTA solution, pH 9.0 (DAKO code No. S2367), for 30 minutes at 94°C using a vegetable steamer and then cooled in solution for 20 minutes. Slides then were placed on a DAKO Autostainer immunostaining system for use with immunohistochemical analysis. Primary antibodies (MLH1, dilution 1:10) were incubated for 1 hour at room temperature. Before the secondary antibodies were added, slides were avidin-biotin blocked for endogenous biotin. The detection system used for the antibodies was a labeled streptavidin-biotin complex. This method is based on the consecutive application of a primary antibody against the antigen to be localized, a biotinylated linking antibody, enzyme-conjugated streptavidin, and substrate chromogen (DAB). Slides then were counterstained with hematoxylin, dehydrated through graded ethanol solutions, and coverslipped.

Results

Colon biopsy specimens from 11 patients (age range, 54-87 years; 4 men and 7 women) showing SSA with LGD (n = 1), HGD (n = 5), or both HGD and focal invasive

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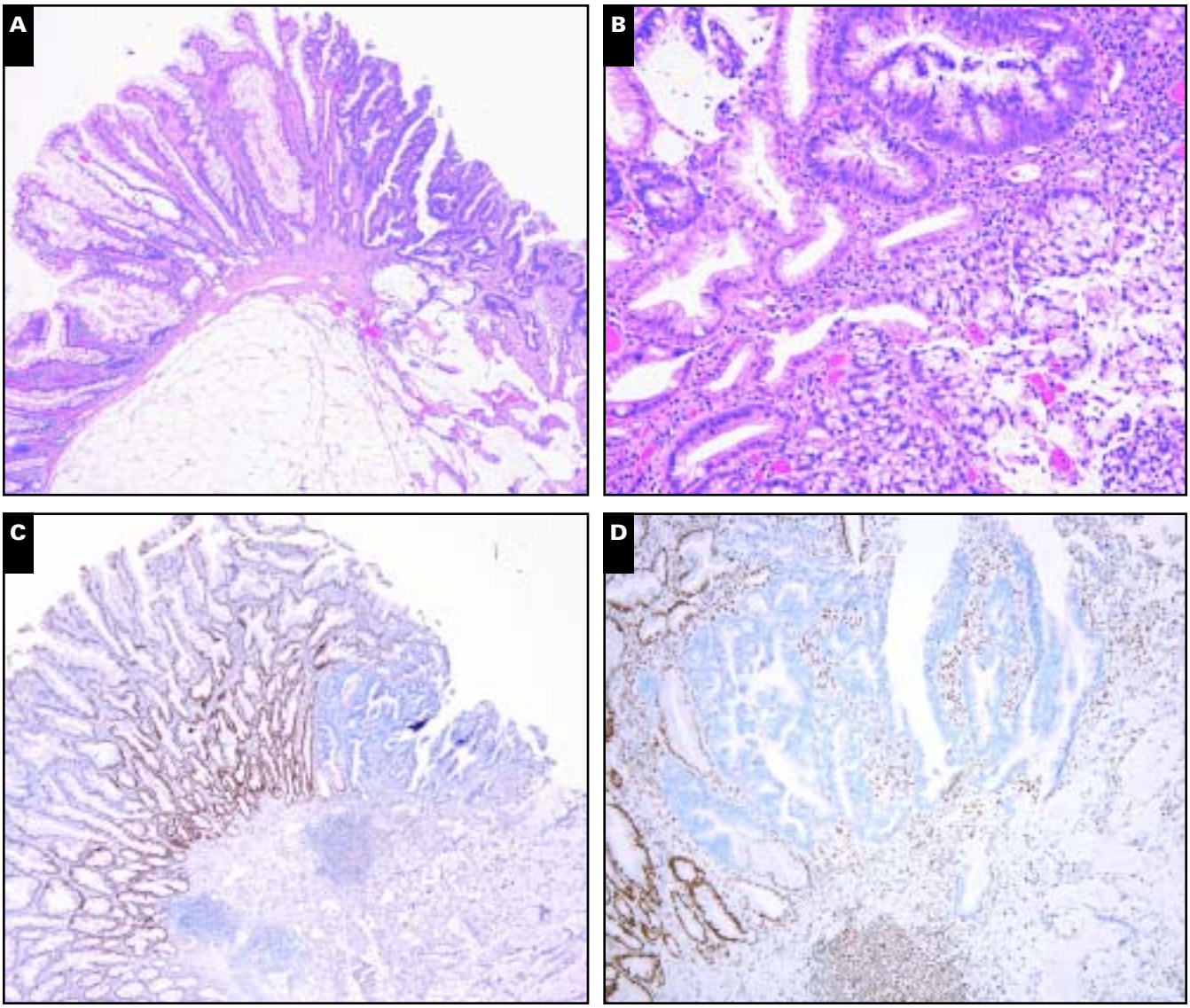

Image 1 **A**, Low magnification of an early carcinoma arising in association with a sessile serrated adenoma (SSA) with dysplasia. The invasive component, seen at the right lower portion of the field, has prominent mucin (H&E, ×2). **B**, Higher magnification of another area of the polyp shown in **A**. There is invasive mucinous/signet cell cancer accompanied by dysplastic glands and glands with more typical features of SSA (H&E, ×20). **C**, MLH1 staining of the lesion depicted in **A** and **B**. There is loss of nuclear staining in the dysplastic and invasive components (×4). **D**, PMS2 staining in the same neoplasm as depicted in **A**-**C** (×10).

carcinoma (n = 5) were analyzed. Lesions were located in the ascending colon (n = 5), transverse colon (n = 1), descending colon (n = 2), and rectosigmoid (n = 3). The size of the polyps ranged from 0.5 to 1.3 cm. Areas of classic hyperplastic polyp were not identified in any of the lesions, and 1 SSA showed a focus of traditional serrated adenoma. None of the patients was known to have HNPCC.

All 11 cases showed nuclear staining for MSH2 and MSH6 in the SSA component; foci of LGD, HGD, and carcinoma; and in background normal mucosa. In contrast, there was tandem loss of MLH1 and PMS2 in zones of LGD (1/1 [100%]), HGD (3/5 [60%]), and early carcinoma (2/4 [50%]) **Image 1**, **Image 2**, and **Image 3**. The cases showing loss of these markers in carcinoma showed concordant loss in associated HGD. All 11 cases showed retention of MLH1 and PMS2 staining in SSA areas and normal mucosa, although reduced labeling was noted at the luminal surface of SSA and normal mucosa (Image 3D). The one focus of traditional serrated adenoma showed retention of all 4 markers. The findings are summarized in **Table 1** and **Table 2**.

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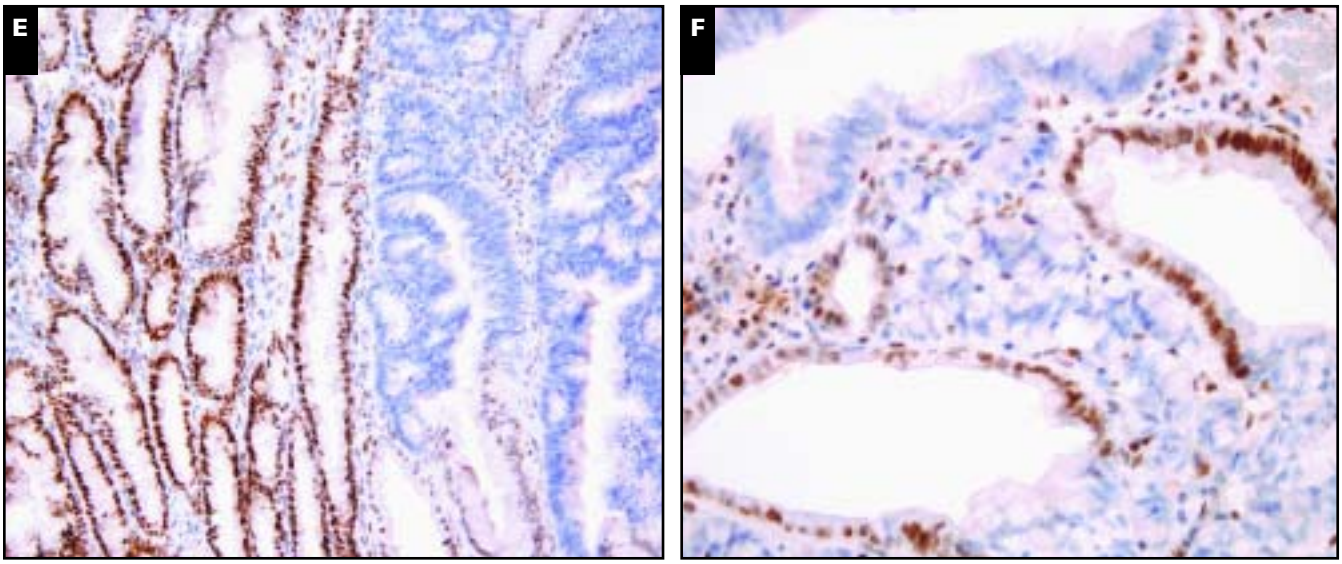

E, Higher magnification of the MLH1 stain seen in **C**. There is loss of nuclear staining restricted to dysplastic zones. Note the gland at the central right portion of the field, which appears to be "in transition" from SSA to conventional dysplasia. There is retention of nuclear staining in the lower nondysplastic portion of the gland (×40). **F**, MLH1 staining of the lesion seen in **A**-**D** showing nuclear loss in the invasive carcinoma component and dysplastic glands but retention in glands with features of SSA (×100).

Table 1
Summary of SSA Staining Pattern and Outcome

Case No./ Sex/Age (y)	Location	Size (cm)	Lesion	MLH1	PMS2	MSH2	MSH6	Outcome
1/M/72	Mid-descending	1.0	HGD and focal invasion	SSA, +; HGD, –; invasive, –	SSA, +; HGD, –; invasive, –	+	+	No follow-up colonoscopies
2/M/56	Rectosigmoid	1.3	HGD	SSA, +; HGD, +	SSA, +; HGD, +	+	+	No follow-up colonoscopies
3/F/60	Ascending	0.3	HGD	SSA, +; HGD, –	SSA, +; HGD, –	+	+	Subsequent B-cell lymphoma; no follow-up colonoscopies
4/M/54	Distal sigmoid	1.0	HGD and invasive	SSA, +; HGD, +; invasive, +	SSA, +; HGD, +; invasive, +	+	+	No residual polyp on follow-up colonoscopy
5/F/82	Ascending	0.5	HGD	SSA, +; HGD, –	SSA, +; HGD, –	+	+	Residual SSA and additional SSAs; later follow-up with hyperplastic polyps
6/F/87	Proximal ascending	0.6	HGD and focal invasion	SSA, +; HGD, –*	SSA, +; HGD, –*	+	+	Died of CHF and COPD; "colon mass," but no autopsy performed
7/F/81	Descending	1.4	HGD	SSA, +; HGD, —	SSA, +; HGD, –	+	+	No follow-up colonoscopies
8/M/71	Rectosigmoid	1.0	HGD	SSA, +; HGD, +	SSA, +; HGD, +	+	+	No follow-up colonoscopies
9/F/80	Transverse	0.3	HGD and invasive	SSA, unavailable; HGD, –; invasive, –	SSA, unavailable; HGD, –; invasive, –	+	+	No follow-up colonoscopies
10/F/67	Ascending	1.0	LGD	SSA, + (weak); LGD, –/weaker	SSA, +; LGD, –	+	+	No follow-up colonoscopies
11/F/72	Ascending	1.4	HGD and invasive	SSA, +; HGD, +; invasive, +	SSA, +; HGD, +; invasive, +	+	+	Right partial colectomy with no residual lesion

CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; HGD, high-grade dysplasia; LGD, low-grade dysplasia; SSA, sessile serrated adenoma; +, positive; –, negative.
* No invasive component present on stained sections.

Discussion

As the histologic characterization of SSA has become better defined, recognition of these lesions has increased, allowing further characterization of their behavior and genetic profile. A model for the stepwise progression of molecular and genetic-epigenetic events has emerged with significant supporting data, but there have been only rare reports of transitional features to study the actual evolution,[12] in part owing to probable rapid overgrowth of SSA by carcinoma. In the present study, we examined 11 cases of SSA with areas of LGD or HGD, some of which had

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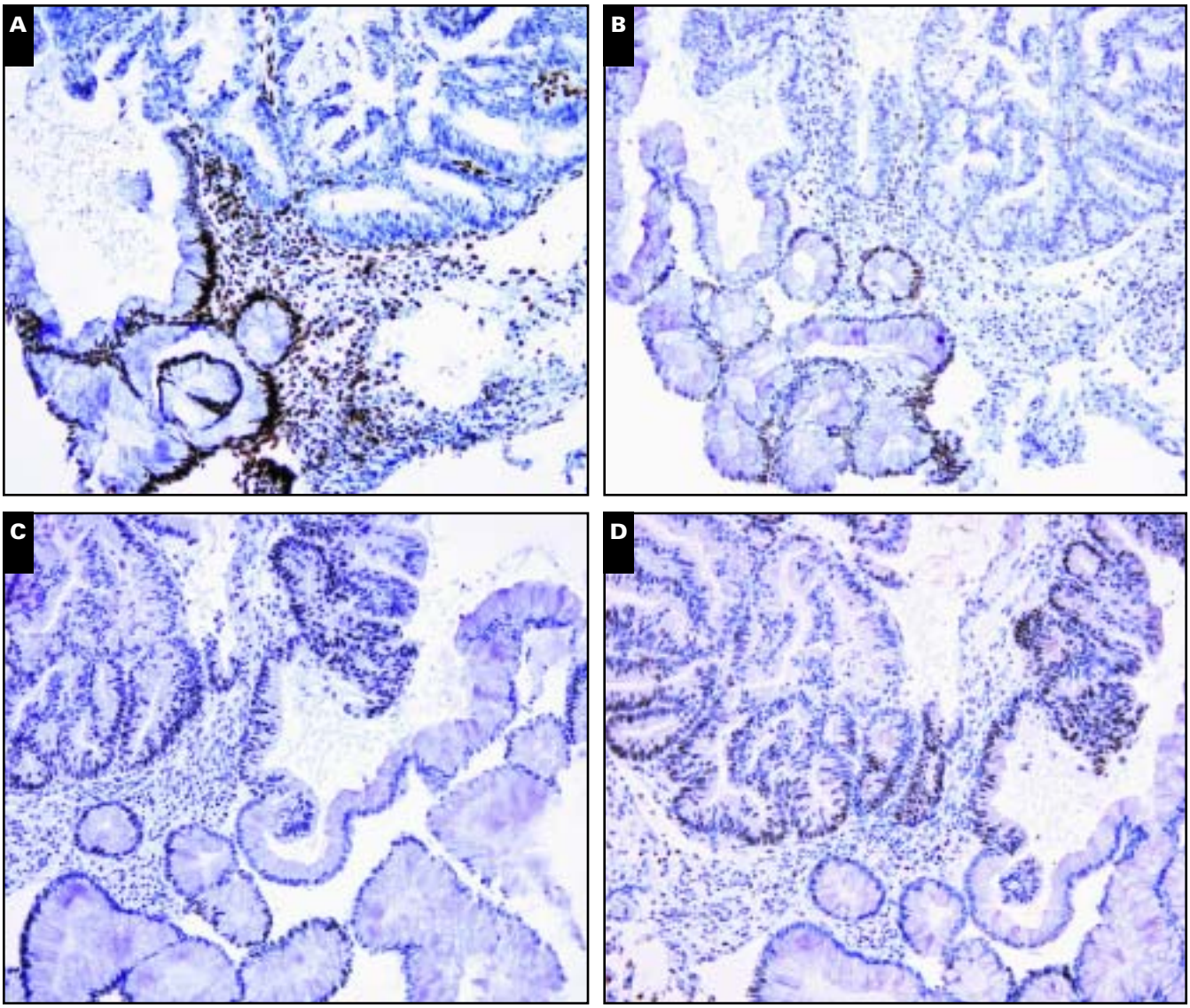

Image 2 **A**, MLH1 staining in sessile serrated adenoma (SSA) with focal high-grade dysplasia. There is nuclear staining in the nondysplastic component, but the dysplastic nuclei show loss of expression (×40). The lamina propria lymphocytes serve as an internal control. **B**, PMS2 staining of the same lesion depicted in **A**. Although the preparation displays overall less intense labeling, there is loss in the dysplastic component of the lesion and retention in the SSA component without dysplasia (×40). **C**, There is retained labeling in the dysplastic and typical SSA component of the lesion seen in **A** with the MSH2 preparation (×40). **D**, MSH6 also shows retention in all components of the lesion (×40).

Table 2
Loss of MLH1/PMS2 in the Dysplasia-Carcinoma Sequence in Sessile Serrated Adenomas

Degree of Conventional-Appearing Dysplasia	MLH1/PMS2 Loss
None	0/11
Low-grade dysplasia	1/1
High-grade dysplasia	3/5
Adenocarcinoma	2/4

progressed to invasive carcinoma. Immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2, a panel to assess MMR protein expression commonly used to suggest a diagnosis of HNPCC, showed loss of both MLH1 and PMS2 in many of these cases. Only the areas of dysplasia or carcinoma showed loss of these markers, and both were lost in tandem.

Loss of MLH1 typically is accompanied by loss of PMS2 staining, probably because the PMS2 gene product is unstable without its heterodimer partner.[13] The primary defect is promoter methylation of *MLH1* and, thus, loss of

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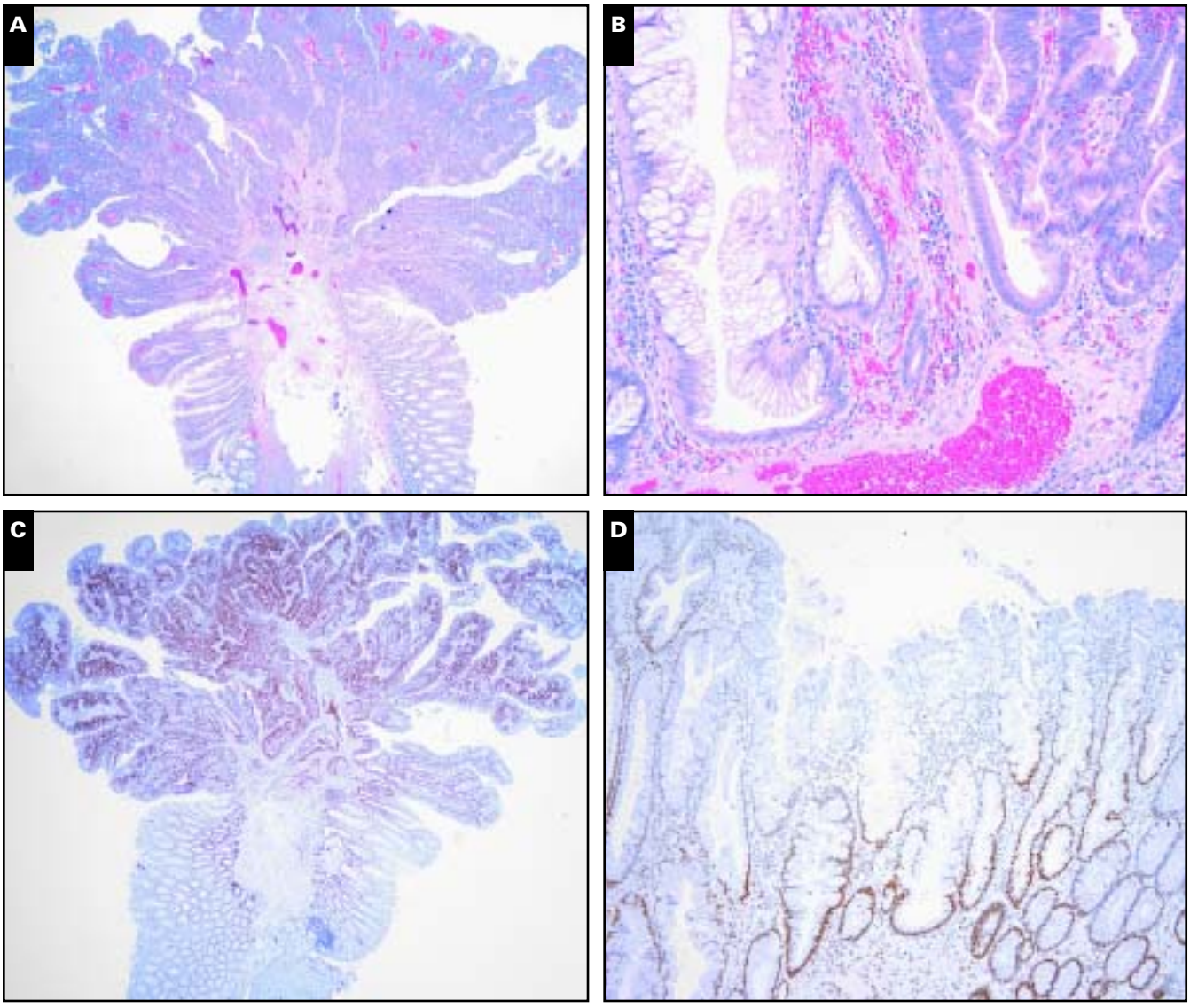

Image 3 **A**, Low magnification of a polypoid carcinoma with an associated sessile serrated adenoma (SSA) (left part of "stalk") (H&E, ×2). **B**, Higher magnification displaying the interface between the carcinoma component and the SSA component. Note the broad-based deep portions of the glands in the SSA component (H&E, ×20). **C**, This carcinoma retained MLH1, in contrast with the other tested cases (×2). **D**, MLH1 stain of the SSA component from the lesion shown in **A**-**C**. Note the relative loss at the surface (×10).

MLH1 staining. The SSA component without traditional dysplasia retained both markers, albeit with reduction of surface labeling, and MSH2 and MSH6 were expressed in the SSA and dysplasia/carcinoma components of all cases. Loss of expression of more than one MMR protein product, including MLH1, demonstrates progression via the MSI-high pathway, and the aforementioned pattern suggests progressive acquisition through an SSA-dysplasia-carcinoma sequence in a subset of the studied cases. We recognize that a host of other molecules might have a role, but the consistent loss of MLH1 and PMS2 is of interest.

The observation that a set of polyps without traditional dysplasia has the capacity to progress to cancers has informed the way we currently practice pathology. The initial work of Torlakovic and Snover[6] has allowed an extensive body of data supporting the concept of a serrated neoplasia pathway. Similar to Goldstein,[12] we found an abrupt transition to conventional-appearing dysplasia and adenocarcinoma, the latter often with mucinous features and direct invasion of the submucosa with minimal or no lateral spread. Also similar to Goldstein,[12] we found loss of MLH1 in areas of dysplasia and carcinoma. The striking loss of MMR gene expression in foci of conventional-appearing dysplasia and

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carcinoma adjacent to SSA with retention of these markers correlates histologic progression with progressive genetic alterations seen in the serrated neoplasia pathway, as noted by Goldstein.[12] On a practical note, however, although there are probably subtle differences between the dysplasia seen as SSA progresses and that in conventional adenomas, we find it difficult to distinguish these 2 processes based on morphologic features. The foci of dysplasia that supervened in our cases were not particularly serrated or eosinophilic but instead looked quite similar to conventional adenomas despite their tendency to have loss of MLH1. Thus, the morphologic features of the dysplasia that supervenes in these cases really do not appear identical to that in "traditional" serrated adenomas, which display eosinophilic cytoplasm, mucin loss, and serrated architecture.

Accumulating molecular data suggest that SSAs, particularly right-sided lesions, develop through MSI, largely due to CpG island hypermethylation (the CpG island methylator phenotype, CIMP).[14-17] Decreased or absent staining for MLH1 has been reported in many SSAs, a consequence of CIMP.[7,16,18-21] *BRAF* mutations also are seen commonly, often correlating with CIMP-high and MSI-high status.[14,17,20,22-24] K-*ras* mutations have been found in some SSAs but are not seen in conjunction with *BRAF* mutations and may be seen more frequently in left-sided lesions or hyperplastic polyps.[15,17,20,22,23] In combination, these features suggest accumulating DNA damage resulting in an MSI-high phenotype. CpG island hypermethylation and *BRAF* mutations have been viewed as an early event in this process, the latter inducing constitutive activation of the RAS-RAF-MEK-ERK-MAP kinase pathway, disrupting apoptosis in crypts, and allowing further accumulation of genetic hits.[22] Some parallels can be drawn to familial adenomatous polyposis, with initiating early events (germline mutation of the *APC* gene) and accumulating DNA damage creating characteristic stages of histologic and genetic signatures. The serrated neoplasia pathway, however, seems more complicated in regard to differences in mutations in hyperplastic polyps and differences in right- and left-sided lesions.

Most important, pathologists should be aware of the histologic features of these lesions and the implications for patient management. The small size of the lesions in this series, including those with coincident carcinoma, suggests that SSAs are precursors of malignancy, and carcinomas may develop despite a relatively small size. The speed of progression of SSA to carcinoma is unknown. One of the cases of early invasive carcinoma in the present study developed within 3 years of the previous colonoscopy. A right-sided polyp was found at that time, diagnosed as a "hyperplastic polyp" at an outside institution. However, it is not known to us whether this lesion actually was an SSA or whether it was completely excised. Given the uncertainty, we support the recommendations by Snover et al,[2] which stress complete removal of the lesions. Finding conventional dysplasia should prompt consideration of surgical management and increased surveillance because these lesions may rapidly progress to carcinoma. Further studies on these transitional lesions may be helpful in determining optimal management.

From the Departments of [1]Pathology and [2]Oncology, the Johns Hopkins Hospital, Baltimore, MD; and [3]Pathology, the Ohio State University, Columbus.

Address correspondence to Dr Montgomery: Surgical Pathology Division, the Johns Hopkins Hospital, Weinberg Bldg, Room 2242, 401 N Broadway, Baltimore, MD 21231-2401.

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