Molecular Differences between Sporadic Serrated and Conventional Colorectal Adenomas

Kazuo Konishi,1 Toshiko Yamochi,2 Reiko Makino,1,3 Kazuhiro Kaneko,1 Taikan Yamamoto,1 Hisako Nozawa,1 Atsushi Katagiri,1 Hiroaki Ito,1 Kentarou Nakayama,2 Hidekazu Ota,2 Keiji Mitamura,1 and Michio Imawari1

1Second Department of Internal Medicine, 2Second Department of Pathology, and 3Clinical Laboratory, Showa University School of Medicine, Tokyo, and 4Department of Obstetrics and Gynecology, Shimane Medical University, Izumo, Japan

ABSTRACT

Purpose: The purpose is to compare the molecular characteristics of serrated adenomas (SAs) with those of conventional adenomas (CADs) and hyperplastic polyps (HPs).

Experimental Design: We evaluated the proliferative activity and molecular alterations in 47 SAs (25 pure-type and 22 mixed-type), 71 CADs, and 23 HPs.

Results: The proliferative activity of SAs, as evaluated by Ki-67 expression, was intermediate between CADs and HPs. There was no significant difference in the incidence of KRAS or p53 mutations between the three histological groups. In the microsatellite instability (MSI) analysis, 21% of SAs (9 of 43) showed MSI at two or more loci (MSI-H); corresponding values were 5% of CADs (3 of 64) and 8% of HPs (1 of 13; SAs versus CADs, P = 0.0125). MSI-H was more likely to be found in pure-type SAs (36%; 8 of 22) than in mixed-type SAs (5%; 1 of 21; P = 0.0212). Loss of hMLH-1 expression was found in 8 of 9 SAs with MSI-H. The incidence of BRAF or KRAS mutations was 36% (15 of 41) in SAs, whereas loss of p53 (8–10) are later events.

Conclusions: Genetic instability is more frequently implicated in the tumorigenesis of SAs, especially pure-type SAs, than in that of CADs. In contrast, activation of the Ras/Raf/MEK/MAP kinase cascade by BRAF or KRAS mutation, independently of the genetic instability, may be associated with the progression of about half of SAs.

INTRODUCTION

Over the last two decades, it has been accepted that the majority of colorectal cancers develop through a well-defined adenoma-carcinoma sequence (1). Preexisting tubular or villous adenomas have been recognized as precursors to colorectal carcinomas. Genetic alteration in the adenoma-carcinoma sequence comprises two concepts, which have been well described in the literature (2). The first concept is chromosomal instability, a mechanism associated with loss of heterozygosity, which accounts for a significant proportion of tumor suppressor gene inactivation. In most colorectal tumors, alteration of the adenomatous polyposis coli gene (3) and KRAS mutations (4, 5) are genetic events observed early in the adenoma, whereas loss of chromosome 18q (6, 7) and alterations in p53 (8–10) are later events.

The second concept is DNA microsatellite instability (MSI) caused by mutations of DNA mismatch repair genes such as hMLH1, hMSH2, hMSH6, PMS1, and PMS2 (11–14). Germline mutations of these genes are implicated in hereditary nonpolyposis colorectal cancer (15, 16). In sporadic colorectal cancers, however, mutations of the mismatch repair genes are relatively infrequent, whereas biallelic hypermethylation of the promoter of hMLH1 appears to be the most important mechanism for the inactivation of mismatch repair genes (17, 18). Genetic alterations of these genes lead to destabilization of tracts of simple repetitive DNA in colorectal tumors. Tumor suppressor gene inactivation occurs as a consequence of the state of genetic instability.

Hyperplastic polyps (HPs) are common lesions in the colorectum, especially in the distal bowel (19, 20). Typical HPs seldom exceed 5 mm in size and are considered to be nonneoplastic lesions without malignant potential. However, recent studies have suggested that HPs may be neoplastic lesions that are precursors of colorectal cancers because genetic changes such as KRAS mutations (21), BRAF mutations (22), MSI (23, 24), and loss of chromosome 1p (25) have been observed in these polyps. In contrast, serrated adenomas (SAs), first described by Longacre and Fenoglio-Preiser in 1990 (26), are recognized as a new subtype of colorectal adenoma. SAs are histologically defined as adenomas that have the morphological features of HPs but also contain cytological features of conventional adenomas (CADs). Because some cases of carcinoma have been reported to be associated with SA, SAs are considered to be precursors of colorectal carcinomas, similar to CADs (26, 27). Moreover, HPs and SAs have been proposed to represent a pathogenesis continuum based on the presence of their mixed polyps and similar mucin-production profiles (28, 29).
formed consent was obtained from all patients. The study was approved by the ethics committee of our university, and investigations included patients with familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or hyperplastic polyposis. We excluded patients who had familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or hyperplastic polyposis (32). Specimen collection procedures were approved by the ethics committee of our university, and informed consent was obtained from all patients.

MATERIALS AND METHODS

Subjects. A total of 47 SAs from 46 patients who underwent endoscopic (n = 46) or transanal resection (n = 1) at Showa University Hospital between September 1997 and December 2001 was prospectively collected in this study. A series of 71 CADs and 23 HPs that were resected endoscopically between September 1997 and December 2001 were analyzed as controls. We excluded patients who had familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or hyperplastic polyposis (32). Specimen collection procedures were approved by the ethics committee of our university, and informed consent was obtained from all patients.

Histological Evaluation. Serial sections (3 μm) were cut from the paraffin block and were prepared for H&E staining and immunostaining. All H&E-stained sections were reviewed by a single pathologist (T. Yamochi), who was blinded to the colonoscopic findings. The histological diagnosis of SAs was based on the definition of Longacre and Fenoglio-Preiser (26). Benign dysplastic lesions without serrated architecture were classified as CADs, and benign nondysplastic lesions with serrated architecture were classified as HPs. SAs were subclassified as mixed or pure depending upon the presence or absence of a histological component other than SA. Lesions exhibiting the features of either an adenoma with severe atypia or intramucosal carcinoma were categorized as high-grade dysplasia (HGD). Lesions having the features of adenoma with mild or moderate atypia were categorized as low-grade dysplasia (LGD). Tumor location was classified into three groups: rectum; left-colon; and right-colon (33). Other histopathological features were determined according to the general rules of the Japanese Research Society for Cancer of the Colon and Rectum (34).

Immunohistochemical Staining and Evaluation of Ki-67 and p53. Deparaffinized and rehydrated sections were heated in a microwave oven in 0.01 M sodium citrate buffer (pH 6.0) for 20 min to retrieve antigens. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide for 5 min. Sections were incubated with anti-hMLH1 monoclonal antibody (1:25; Becton Dickinson, Franklin Lakes, NJ) overnight at 4°C. Biotinylated rabbit antimouse immunoglobulin (DAKO Cytomation) was used as the secondary antibody, followed by peroxidase-conjugated streptavidin (DAKO). Staining was considered assessable when unclear staining was seen in either stromal or germinal follicle lymphocytes or in normal epithelial cells located in the base of crypts. The hMLH1 loss varied in extent from focal areas to entire lesions (35). Loss of nuclear expression by tumor cells was classified as complete absence, focal absence, or presence of staining of tumor cells.

DNA Preparation from Pure-Type SAs, CADs, and HPs. To extract genomic DNA, 5-μm thick sections were obtained from an archival block of formalin-fixed, paraffin-embedded tumor tissue for each histological type, as described previously (31). DNA from normal colonic mucosa (frozen or formalin-fixed tissue) or peripheral blood was used as the normal control for analysis.

Laser-Capture Microdissection for Mixed-Type SAs. To exclude contamination between tumor types, separate samples were obtained from mixed-type SAs for serrated and non-serrated adenomatous areas (i.e., hyperplastic tissue or conventionally adenomatous tissue) using laser-capture microdissection (Fig. 1), as described previously (31).

Mutations of KRAS and p53. Mutations of the KRAS codon 12 and p53 exons 5–8 were evaluated by fluorescence-based PCR-single-strand conformation polymorphism, using previously described primers (36, 37). After amplification, the PCR products from each sample were used to detect KRAS or p53 mutations by fluorescence-based single-strand conformation polymorphism analysis using an automated DNA sequencer (ALF Express; Amersham Pharmacia Biotech, Uppsala, Sweden) with an external cooling bath. Peak patterns were analyzed using the ALFwin Fragment Analyzer program (Amersham Pharmacia Biotech), and shifted peaks were defined as mutations in DNA fragments. The nucleotide sequences of the DNA fragments with shifted peaks were determined as described previously (38).

Mutations of BRAF. The primers for exons 11 and 15 were used to evaluate BRAF mutations (22, 39). These primers covered most of the gene’s previously identified mutation hot spots. Mutational screening of the BRAF gene was performed by direct sequencing methods, as reported previously (38). All mutations were reconfirmed by independent PCR reactions and sequencing.

Analysis of MSI. PCR was performed to amplify DNA samples from the tumors and the adjacent normal tissue at five
microsatellite loci. The primers used in this study were BAT25, BAT26, D2S123, D5S346, and D17S250 (40). The PCR was performed in a volume of 10 μl, with one fluorescently labeled oligonucleotide. The reactions were incubated at 94°C for 15 sec followed by the appropriate annealing temperature and 1 min at 72°C. PCR products were electrophoresed on an automated DNA sequencer (Amersham Pharmacia Biotech) using polyacrylamide gel containing urea, and analyzed using the ALFwin Fragment Analyzer program. Tumors showing novel peak patterns were evaluated as MSI positive. A single observer (H. Nozawa) performed the MSI analysis, and positive or equivocal samples were reevaluated by a second observer (K. Nakayama). A tumor sample was considered to contain high-frequency MSI (MSI-H) if two or more of the five informative markers demonstrated instability and was considered to have low-frequency MSI (MSI-L) when only one marker was unstable (40). All PCR reactions were repeated on the same sample and only consistent changes in the duplicate reactions were scored as abnormalities.

**Statistical Analysis.** Values related to Ki-67-positive rates are shown as means ± SE. Data for patients’ ages and Ki-67-positive rates were analyzed by the Mann-Whitney U test; molecular or pathological data were analyzed using the chi² test, and by Fisher’s exact test when testing small samples. A value of P < 0.05 was considered significant.

**RESULTS**

Table 1 shows the pathological characteristics of patients with SAs, CADs, and HPs. There were no significant differences in gender, age, and location between the three histological types, and there were no significant differences in the incidence of HGD or tumor size between SAs and CADs. Twenty-five pure-type SAs and 22 mixed-type SAs were histologically identified in this series. The 22 mixed-type SAs tumors included 14 mixed-SA/HP lesions and 8 mixed-SA/CAD lesions.

Table 2 shows the comparison of Ki-67 immunostaining in SAs, CADs, and HPs. In the lower crypt zone, similar values for the cell proliferative index were observed for SAs and HPs. However, this value was significantly lower for CADs than for SAs (P < 0.001) and HPs (P < 0.001). In the upper crypt zone, the cell proliferative indices for SAs were significantly higher.
than those for HPs \((P < 0.0001)\) but significantly lower than those for CADs \((P < 0.0001)\). The overall cell proliferative indices did not significantly differ between SAs and CADs \((P = 0.3060)\), whereas these indices for SAs were significantly higher than those for HPs \((P = 0.0082)\). The ratios of the cell proliferation indices in the upper to lower zones for SAs, CADs, and HPs were 0.82, 2.47, and 0.01, respectively.

Seventy-seven percent of cases of SAs \((36 of 47)\) showed an alteration in one or more genetic marker. In current study, the incidence of genetic alterations was shown by the ratio to the number of cases with a genetic alteration to the total number of informative cases for each histological type.

\textbf{KRAS} mutations were observed in 15% of SAs \((7 of 47)\), 15% of CADs \((11 of 71)\), and 13% of HPs \((3 of 23)\). There was no significant difference in the incidence of \textit{KRAS} mutations between the three histological types. The 7 SAs with \textit{KRAS} mutations were 2 pure-type and 5 mixed-type SAs. All of these mixed-type SAs with \textit{KRAS} mutations were mixed-SA/CAD lesions. Of these 5 mixed-type SAs, 3 samples showed \textit{KRAS} mutations in both the serrated and nonserrated adenomatous epithelium, whereas the remaining 2 showed mutations in either the serrated or nonserrated adenomatous epithelium.

Nine percent of SAs \((4 of 43)\), 11% of CADs \((7 of 63)\), and 0% of HPs \((0 of 20)\) had \textit{p53} mutations. There were no significant differences in the incidence of \textit{p53} mutations between the three histological types. Of the 4 SAs with \textit{p53} mutations, 2 were pure-type SAs, and the rest were mixed-type SAs. In the 2 mixed-type SAs with \textit{p53} mutations, only 1 mixed-SA/HP sample showed the same \textit{p53} mutations in both the serrated and nonserrated epithelium. We compared the incidence of \textit{KRAS} or \textit{p53} mutations between SAs and CADs on the basis of location, macroscopic type, size, or histological grade (Table 3) and found no significant differences between SAs and CADs.

Nuclear expression of \textit{p53} was demonstrated in 17% of SAs \((8 of 47)\), 23% of CADs \((16 of 71)\), and 0% of HPs \((0 of 23)\). There was a significant difference in nuclear immunostaining of \textit{p53} between HPs and SAs \((P = 0.0461)\) or CADs \((P = 0.0097)\). Moreover, overexpression of the \textit{p53} gene product was present in 2% of SAs \((1 of 47)\) and 3% of CADs \((2 of 71)\). All overexpressed cells were detected in the high-grade dysplastic epithelium of both SAs and CADs.

In the MSI analysis (Fig. 2), MSI-H was shown in 21% of SAs \((9 of 43)\), 5% of CADs \((3 of 64)\), and 8% of HPs \((1 of 13)\). The incidence of MSI-H differed significantly between SAs and CADs \((P = 0.0125)\). MSI-L was shown in 30% of SAs \((12 of 43)\), 11% of CADs \((7 of 64)\), and 8% of HPs \((1 of 13)\). The incidence of MSI-H was higher in SAs with HGD than in CADs with HGD \((2 of 7 and 0 of 20, respectively; P = 0.0598)\). The 2 SAs with both HGD and MSI-H were of pure-type histology. Of the 22 mixed-type SAs, MSI was present in 6 of 13 mixed-SA/HP samples and 2 of 8 mixed-SA/CAD samples. Five of 6 mixed-SA/HP samples showed MSI-L and 1 showed MSI-H. Of the 6 mixed-SA/HP lesions containing SA with MSI, 3 showed the same mutation within the contiguous HP. Two mixed-SA/CADs with MSI had MSI-L. In these 2 mixed-SA/CAD lesions, conventional adenomatous glands showed MSI, but no detectable MSI appeared in the serrated adenomatous glands.

\textbf{BRAF} mutations were detected in 36% of SAs \((17 of 47)\). All of the \textbf{BRAF} mutations were found in exon 15; the most common mutation observed in SAs \((13 of 17)\) was the conversion of valine to glutamic acid at codon 599 (Fig. 3). Only 1 SA sample with \textbf{BRAF} mutations contained \textit{KRAS} mutations. The combined incidence of \textit{BRAF} and \textit{KRAS} mutations occurred in 49% of SAs \((23 of 47)\). \textbf{BRAF} mutations were found in 33% of SAs with MSI-H \((3 of 9)\) and in 38% of SAs without MSI-H \((13 of 34)\). One SA with \textbf{BRAF} mutation was not informative for MSI analysis. Similarly, \textit{KRAS} mutations were observed in 11% of SAs.
of SAs with MSI-H (1 of 9) and 18% of SAs without MSI-H (6 of 34). These differences were not statistically significant.

We compared the clinicopathological or molecular characteristics between pure-type and mixed-type SAs (Table 4). Macroscopically, sessile SAs were more frequently observed in the mixed-type than in the pure-type SAs (32%: 7 of 22; and 8%: 2 of 25, respectively). This difference was nearly significant \( P = 0.063 \). Of the 9 sessile-type SAs, 5 were located in the proximal colon and 7 were >10 mm in size. MSI-H was more likely to be found in the pure-type SAs than in the mixed-type SAs (36%: 8 of 22; and 5%: 1 of 21, respectively); this difference was significant \( P = 0.0212 \). The incidence of MSI-H was higher in the proximal than in the distal pure-type SAs (2 of 3 and 6 of 22, respectively), although the difference was not significant \( P = 0.2313 \). In contrast, there was no significant difference in the incidence of \( BRAF \) or \( KRAS \) mutations between the pure-type and the mixed-type SAs.

Nine SAs with MSI-H, 3 CADs with MSI-H, and 1 HP with MSI-H were examined immunohistochemically by using antibodies to hMLH1 to determine the relationship between hMLH-1 expression and MSI-H status. Loss of hMLH-1 expression was seen in 8 of 9 SAs with MSI-H (4 complete and 4 focal absence (Fig. 4), 3 CADs with MSI-H (3 focal absence), and 1 HP with MSI-H (1 complete absence.).

**DISCUSSION**

A monoclonal antibody to Ki-67 stains the nuclei of proliferating cells in all cell cycle phases except G0 (41, 42). The proliferating cells of HPs were located in the lower zone of the crypts, similar to those of normal mucosa. However, cellular proliferative activity in the upper zone of SAs was significantly higher than that of HPs and was intermediate between that of HPs and CADs. We observed no significant difference between SAs and CADs in the overall proliferative activity in the crypts. However, the ratio of upper crypt to lower crypt for SAs was closer to that for HPs than for CADs. These immunohistochemical findings suggest that SAs represent a different biological feature from HPs or CADs.

Although several studies have analyzed the molecular defects in SAs, the genetic alterations of \( KRAS \) or \( p53 \) are controversial. Hiyama et al. (43) reported mutations of adenomatous polyposis coli, \( KRAS \), and \( p53 \) in 40, 50, and 47% of SAs, respectively. Yao et al. (28) and Rubio and Rodensjo (44) reported \( p53 \) expression in 62 and 50% of SAs, respectively. However, other authors have been unable to confirm an increased prevalence of adenomatous polyposis coli, \( KRAS \), and \( p53 \) mutations and \( p53 \) expression (45–49). The reasons for these differences in the prevalence of molecular markers are unclear because similar molecular markers were used to evaluate the tumors. The limited number of samples and different methods used in each study may explain some of the differences. In our study, we observed \( KRAS \), \( p53 \) mutations, and \( p53 \) expression in 15%, 9%, and 19% of SAs, respectively. On the basis of the clinicopathological characteristics, the alteration of \( KRAS \) and \( p53 \) did not differ significantly between SAs and CADs.
Table 4  Comparison of differences in a range of clinicopathological or molecular characteristics between pure-type and mixed-type serrated adenomas

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<th>Pure-type SAs&lt;sup&gt;a&lt;/sup&gt; (n = 25)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mixed-type SAs (n = 22)&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> SAs, serrated adenomas; CRC, colorectal cancers; left-c, sigmoid and descending colon; right-c, transverse, ascending colon and cecum; LGD, low-grade dysplasia; HGD, high-grade dysplasia; MSS, microsatellite stable; MSI-L, low-frequency microsatellite instability; MSI-H, high-frequency of microsatellite instability; mut +, presence of mutation; mut −, absence of mutation; IHC, immunohistochemistry.

<sup>b</sup> Twenty-five pure-type SAs from 24 patients and 22 mixed-type SAs from 22 patients.

<sup>c</sup> The numbers for p53 mutations or MSI reflect the numbers of cases among the informative cases.

5 mixed-SA/CAD tumors with KRAS mutation, 3 tumors showed the same mutation in both the serrated and conventional adenomatous epithelium. These observations suggest that some SAs may be a biologically similar, morphologically variant form of CADs.

Jass et al. (29, 50) proposed that MSI drives the evolution of some HPs to SAs and colorectal cancers. Alternatively, others have reported that ~5% of SAs showed MSI (43, 49). To explain the difference between these results, Sawyer et al. (49) suggested that detection of MSI can be problematic in DNA from paraffin-embedded tissue and its prevalence may have been overestimated. Different microsatellite markers were used in previous studies. Thus, we repeated all PCR reactions on the same sample, and only changes that were consistent between the duplicated reactions were scored as abnormal. The MSI status of our study was confirmed using the microsatellite markers recommended by the National Cancer Institute workshop on MSI for Cancer Detection and Familial Predisposition (40). In our study, a relatively high percentage of SAs (21%) showed MSI-H, in contrast to a lower percentage of CADs (5%) and HPs (8%). Thus, SAs are more likely to arise through the development of genetic instability than CADs are.

In a recent study of hyperplastic polyposis by Jass et al. (50), MSI-H was commonly demonstrated both in the dysplastic epithelium of serrated polyps and in synchronous cancers. They proposed that serrated polyps would be the precursors of MSI-H colorectal cancers. Makinen et al. (27) observed that the incidence of MSI-H was higher in carcinomas with SA than in other carcinomas (37.5 and 11.0%, respectively). In our series, MSI-H was found in 21% of SAs (9 of 43) but only in 5% of CADs (3 of 64). Moreover, the incidence of MSI-H was significantly higher in pure-type SAs than in mixed-type SAs. However, two studies from Western countries (29, 51) demonstrated that mixed-type SAs were more likely to be MSI-H than pure-type SAs, although these differences were not statistically significant. The molecular pathogenesis of SAs in Japanese patients might be different from that in Western patients, although a large study is needed to confirm this suggestion. On the other hand, 7 SAs (6 pure-type and 1 mixed-type) were HGD in our study. MSI-H was found in 2 of 7 SAs with HGD, whereas neither KRAS nor p53 mutation was detected. These 2 SAs with MSI-H were pure-type. However, MSI-H, KRAS mutations, and p53 mutations were shown in 0, 30, and 24% of CADs with HGD, respectively. Thus, we suggest that pure-type SAs may serve as a precursor lesion of sporadic MSI-H cancers.

In the previous study (52), sporadic MSI-H cancers have been reported to occur more often in the proximal colon. Our current study identified the distal colon as the most common site for SAs, as reported in the previous studies (26, 53). The pure-type SAs were mainly located in the distal colon. However, 2 of 3 proximal pure-type SAs showed MSI-H, whereas 6 of 22 pure-type SAs in the distal colon were MSI-H. Although the difference was not statistically significant because of a small number of tumors studied, proximal pure-type SAs may have a high incidence of MSI-H. We may speculate that right-sided pure-type SAs are more likely to be a precursor of MSI-H cancer than left-sided ones are.

Proximal hereditary nonpolyposis colorectal cancer adenomas that characterized by the presence of defective mismatch repair are prone to rapid malignant transformation (54). Jass et al. (55) suggested that sporadic MSI-H adenomas evolve faster than non-MSI-H adenomas. If some pure-type SAs give rise to MSI-H cancers, they might be relatively aggressive compared with CADs. As suggested in a previous study (56), this might explain many of the so-called missed carcinomas in which a recent colonoscopy failed to detect the carcinomas.

Recently, Hawkins et al. (35) demonstrated that all of the serrated polyps detected in patients with sporadic MSI-H cancers showed hMLH1 promoter methylation. Methylation of the hMLH1 promoter is correlated with decreased expression of the hMLH1 protein (17). In our immunohistochemical analysis, all but 1 SA with MSI-H showed a loss of hMLH1 expression. Park et al. (51) reported that CpG island methylator phenotype-high status, which implies methylation in two or more MINTs or
target genes such as \textit{hMLH-1} or \textit{p16}, is significantly higher in sporadic SAs than in CADs. Epigenetic alterations such as methylation of tumor suppressor or mismatch repair genes may play an important role in the pathogenesis of sporadic SAs.

The Ras/Raf/MEK/MAP kinase cascade is an important pathway of intracellular signaling from activated cell surface receptors to transcription factors in the cell nucleus (57). A Ras protein known as \textit{KRAS} participates in this cascade. Davies \textit{et al.} (39) have reported the presence of \textit{BRAF} mutations in a small fraction of colorectal cancers. In our current study, \textit{BRAF} mutations were found in 36% of SAs in contrast to the low incidence (2.8%) previously reported in CADs (58). The combined incidence of \textit{BRAF} and \textit{KRAS} mutations was found in 49% of SAs. Rajagopalan \textit{et al.} (59) and Wang \textit{et al.} (60) have suggested that \textit{BRAF} mutations occur more frequently in microsatellite-unstable cancers than in microsatellite-stable cancers. Our data pointed to the possibility that the pure-type SAs are the precursors of MSI-H cancers. However, Chan \textit{et al.} (22) recently reported the lack of MSI in their series of SAs, although there was the high frequency of \textit{BRAF} mutations in SAs (100%, 9 of 9). We observed no significant correlation between \textit{BRAF} or \textit{KRAS} mutations and MSI-H status in SAs. Therefore, these

\textbf{Fig. 4} Immunohistochemical staining of \textit{hMLH1}. \textit{A}, loss of nuclear expression in a serrated adenoma. \textit{B}, nuclear expression in the normal mucosa adjacent to the serrated adenoma.
observations suggest that, independently of genetic instability, activation of the Ras/Raf/MKK/MAP kinase pathway led by \textit{BRAF} or \textit{KRAS} mutations may be linked with the pathogenesis of about half SAs.

A recent study (61) proposed that an atypical HP with sessile configuration located in the right colon and/or >10 mm in diameter should be diagnosed as sessile serrated adenoma and that this type of lesion may be associated with the development of colorectal carcinoma through the MSI pathway. In our series, 7 mixed-type SAs had the features of sessile serrated adenoma. However, these mixed-type SAs had no detectable MSI-H (data not shown). The clinicopathological significance of sessile serrated adenoma is still controversial. Additional analysis is needed to examine whether the sessile serrated adenoma is of a premalignant nature in the serrated neoplasia pathway.

Recently, SAs with admixed hyperplastic and/or adenomatous glands have been reported but not fully explored. Our study separated mixed-type from pure-type SAs. In a recent study (29), it was suggested that a proportion of MSI-L cancers arise through the morphogenetic sequence hyperplastic polypl-serrated adenoma. In our study, 5 of 13 mixed-SA/HYP lesions and 5 of 25 pure-type SAs showed MSI-L. Two of these 5 mixed-type SAs showed MSI-L in both the serrated adenomato-s and hyperplastic epithelium, whereas the remaining had MSI-L in the serrated adenomatous epithelium. Hawkins \textit{et al.} (35) suggested that MSI is an early event in the neoplastic process of the serrated neoplasia pathway. This suggestion fits with our result of MSI-L in mixed-SA/HP lesions. However, \textit{Jass et al.} (62) have reported the higher frequency of \textit{KRAS} mutation in MSI-L cancers than in other cancers. They also suggested that MSI-L cancers should be distinguished as a subset combining features of the suppressor and mild mutator pathways. In our study, however, all of the mixed-type SAs with \textit{KRAS} mutations was mixed-SA/CAD lesions. Only 1 of 5 pure-type SAs with MSI-L showed \textit{KRAS} mutation. Thus, it still remains unclear whether or not serrated polyps are associated with pathogenesis of sporadic MSI-L cancers.

In summary, the combined approaches of Ki-67, p53, and hMLH1 immunohistochemistry; \textit{KRAS}, \textit{BRAF}, and p53 mutations; and MSI allow us to better understand the tumorigenesis of SAs. Our data suggest that SAs should be considered a biologically distinct subtype of colorectal adenomas and that genetic instability is much more frequently implicated in the tumorigenesis of SAs than of CADs. Moreover, pure-type SAs may play an important role in the pathogenesis of sporadic MSI-H cancer. In contrast, independently of the genetic instability, activation of the Ras/Raf/MEK/MAP kinase cascade led by \textit{BRAF} or \textit{KRAS} mutation may be associated with the pathogenesis of about half SAs.

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Molecular Differences between Sporadic Serrated and Conventional Colorectal Adenomas

Kazuo Konishi, Toshiko Yamochi, Reiko Makino, et al.