Sessile Serrated Adenomas of the Large Bowel. Clinicopathologic and Immunohistochemical Study Including Comparison with Common Hyperplastic Polyps and Adenomas

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Summary

Sessile serrated adenoma (SSA) is a newly characterized type of the large bowel adenoma. It arises in hyperplastic polyp (HP) and represents a precursor lesion of colorectal carcinoma with microsatellite instability. SSAs differ from common HPs by abnormal proliferation of the crypt epithelium and by nuclear atypia. We examined 15 SSAs from 15 patients. The age range was 25-80 years (average 60 years). Six patients were females and 9 were males. For comparison, we examined 10 conventional tubular adenomas and 10 common HPs with vesicular cells. The sites of SSAs were as follows: 8 in rectum, 4 in rectosigmoid colon, 1 in transverse colon, 1 next to mucinous carcinoma of ascending colon, 1 in anastomosis after resection of the transverse colon adenocarcinoma. The diameter of the lesions ranged from 5 to 12 mm. Histologically, SSAs showed asymmetrical proliferation of the epithelium, irregular shape of the crypts with their branching and some crypt dilatations especially in the basal parts of the crypts. Cellular atypia (dysplasia) was usually low. In 5 cases the nuclei were focally stratified and localized in the lower part of the cells. High-grade dysplasia was found only in SSA adjacent to mucinous adenocarcinoma. Immunohistochemically, SSAs showed secretion of gastrointestinal mucin expressing MUC2 and MUC5A. Both MUC2 and MUC5A were also positive in mucinous carcinoma. In previous studies these expressions were considered specific for serrated type of carcinogenesis. However, our study found positivity of MUC2 and MUC5A also in conventional adenomas. Expression of p53 in SSAs was minimal. SSAs have malignant potential comparable with conventional adenomas and for this reason they must be distinguished from HPs.

Key words: sessile serrated adenoma - tubular adenoma - colon - hyperplastic polyp - MUC2 - MUC5A

Souhrn

Sesilní „serrated“ adenomy tlustého střeva

Sesilní „serrated“ adenomy (SSA, adenomy se „zoubkovaným” uspořádáním epitelu krypt) představují nově vymezený typ adenomů tlustého střeva, které vznikají v hyperplastických polypech a jsou prekurzorem kolorektálních karcinomů s mikrosatelitní nestabilitou. Od klasických hyperplastických polypů se liší abnormální proliferací epitelu krypt a buněčnými atypiemi. V poslední době jsme vyšetřili 15 „serrated” adenomů tlustého střeva u 9 mužů a 6 žen ve věku od 25–80 let (průměrný věk 60). Kontrolní skupinu tvořilo 10 běžných tubulárních adenomů tlustého střeva a 10 klasických hyperplastických polypů z vezikulárních buněk. SSA byly 8krát lokalizovány v rektu, 4krát v rektosigmoideu a jednou v příčném tračníku. Jejich velikost kolísala od 5–12 mm. U jednoho nemocného byl SSA nalezen v okraji mucinózního adenokarcinomu vzestupného tračníku a u dalšího v anastomóze tlustého střeva po resekci karcinomu transverza. Histologicky se SSA vyznačovaly asymetrickou proliferací epitelu, nepravidelným průběhem, nápadným větvením a dilatací krypt, zvláště v bazálních úsecích. Buněčné atypie (dysplazie) většinou dosahovaly mírného stupně, v 5 adenomech byla jádra v malém rozsahu stratifikovaná, lokalizovaná v dolní polovině buněk. Těžká dysplazie epitelu byla nalezena pouze v SSA v okraji mucinózního adenokarcinomu. Imunohistochemicky vykazovaly SSA sekreci gastrointestinálního typu hlenu (reakce s MUC2 a MUC 5A), který byl pozitivní také v mucinózním adenokarcinomu. Přestože se tento typ hlenu považuje za specifický pro „serrated” typ kancerogeneze, byl prokázán i v kontrolní skupině konvenčních adenomů. Pozitivita p53 byla v SSA minimální. Sesilní „serrated” adenomy tlustého střeva mají maligní potenciál srovnatelný s běžnými adenomy a z tohoto důvodu musí být odlišeny od klasických hyperplastických polypů.

Klíčová slova: sesilní „serrated” adenom - tubulární adenom - tlusté střevo - hyperplastický polyp - MUC2 - MUC5A

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Hyperplastic (serrated) polyps (HPs) of the large bowel are traditionally regarded as benign non-neoplastic lesions without any significant risk of malignization (14, 17, 19–21). They usually occur in the left-sided large bowel, their size rarely exceeding 5 mm. The epithelium of the crypts is serrated near the surface and its proliferation is limited to the basal zone of the crypt. According to the amount of intracytoplasmic mucin HPs can be classified into three types: HPs with vesicular cells, HPs with goblet cells, and HPs with mucin depletion (14, 17, 18). Although these types differ one from another by frequency of MSI and mutations of k-ras and BRAF (18), the clinical significance of the typization still remains unclear (17).

In recent years, a novel type of the serrated polyp was characterized, and it was termed "serrated adenoma" (SA) (2, 3, 5, 9, 10, 13, 15, 18, 20, 21). However, it is currently recommended to prefer the term "sessile serrated adenoma" (SSA) to "serrated adenoma". Term SSA was used for the first time by Torlakovic et al. (17) who differentiated this adenoma type from the conventional adenoma with serrated structures (4, 6, 7, 16). SSAs differ from common HPs by abnormal proliferation of the crypt epithelium and cellular atypia. They occur as isolated lesions or as multiple polyps in so-called hyperplastic polyposis (4, 14, 16, 17, 20). Importantly, these SSAs represent precursor lesion for the colorectal carcinoma with microsatellite instability (MSI) (1–10, 13–18, 20, 21).

Here we would like to present our experience with 15 cases of SSAs. We studied morphological features of SSAs and the immunohistochemical expressions of mucin. For comparison we performed also a similar study in a series of conventional tubular adenomas (TAs) and common HPs.

Clinical Data

The series includes 15 SSAs from 15 patients, with the age range 25–80 (average 60.0 years). With an exception of one 25-year-old woman, the age ranged from 40 to 80 years. Six patients were females and 9 were males. The locations of the lesions were as follows: 8 in the rectum, 4 in the rectosigmoid colon, 1 in the transverse colon, 1 next to a mucinous carcinoma of the ascending colon, 1 in the anastomosis after a resection of the transverse colon adenocarcinoma. In three patients one or two common HPs of the vesicular type were removed in addition to SSA. Further three patients had a tubular adenoma in addition to SSA, and one of the TAs showed signs of initial malignization. The comparison series included 10 consecutive 3–5mm HPs of the vesicular cell type and 10 conventional left-sided TAs with the diameter 5–10 mm and with low-grade dysplasia.

Materials and Methods

Formalin-fixed paraffin-embedded tissues from endoscopic specimens and from resectate specimens were sectioned and stained with hematoxylin and eosin and PAS stain. For immunohistochemistry, following antibodies were used: MUC 2 (Cep58, MW 1:400, Novocastra, Newcastle upon Tyne, U.K.), MUC 5AC (CLH2, MW 1:400, Novocastra), MUC 6 (CLH5, MW 1:400, Novocastra), CK 20 (KS20.8, MW, 1:100, DakoCytomation, Glostrup, Denmark), Ki 67 (MIB1, MW, 1:1000, DakoCytomation) and p53 (DO-7; DakoCytomation). The primary antibodies were visualized using streptavidin-biotin-peroxidase complex (DakoCytomation).

Results

The diameter of SSA ranged from 5 to 12 mm. Two SSAs had short stalk and all others were sessile. **Histologically**, all SSAs showed an irregular arrangement of the crypts, abnormal proliferation of the epithelium and nuclear atypia. Crypts were often elongated and/or showed an irregular dilatation especially in their basal parts (figure 1). The basal parts of some crypts were parallel to the lamina muscularis mucosae (figure 2), and in one case the crypts "penetrated" into hyperplastic lamina muscularis mucosae and submucosa. The superficial parts of the crypts showed crypt branching, and the "serrated" pattern (figure 3) was also seen in the middle and basal zone of the crypts. The epithelium included atypical cells and mature appearing goblet and columnar cells. Atypical cells had enlarged vesicular nuclei with an irregular nuclear margin and a well-discernible nucleolus. The nuclei were in the basal parts of the cells. In 5 lesions some nuclear stratification was visible, but the nuclei were never seen in the upper half of the cell (figure 4). Atypical cells were localized mainly in the basal crypt zone from which they proliferated toward the superficial part of the crypts. They proliferated focally or continuously in the middle crypt zone. Only rarely they reached the surface of the crypts (abnormal proliferation of the epithelium). These cells showed occasional mitotic figures. In all zones of the crypts mature appearing

goblet and columnar cells were seen among the atypical cells. These more differentiated cells even predominated in dilated basal parts of some crypts. Dysplasia of high-grade was found only in SSA next to mucinous adenocarcinoma. In the superficial epithelium of SSAs there were usually seen well-differentiated columnar cells with elongated nuclei and eosinophilic cytoplasm.

Immunohistochemically, the cell proliferation evaluated with MIB1 antibody reached in various crypts a various proximal expansion. The cell proliferation was usually intense in the lower half to the lower three fourths of the crypt and occasionally it reached the superficial epithelium. The MIB1 positivity was in an inverse relationship with positivities for CK20, MUC2 and MUC5A (which all highlighted more differentiated cells). Goblet cells and differentiated columnar cells were positive diffusely for MUC2 in all SSAs. MUC5A was positive diffusely in 14 SSAs and focally positive in one SSA. This positivity was not limited to superficial parts of the adenomas but instead, it was apparent in all crypt zones with an equal intensity (figure 5). A strong MUC5A reactivity was also seen in mucinous adenocarcinoma. MUC6 was negative in all SSAs. p53 was slightly positive in rare nuclei of the basal crypt zone in 14 SSAs.

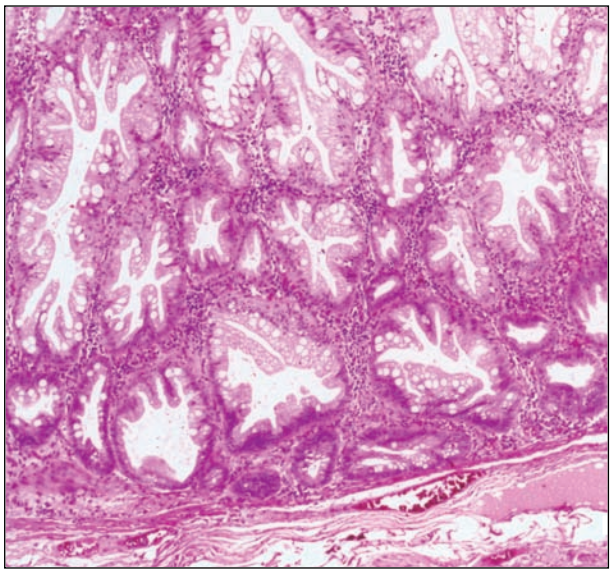

Fig. 1. Dilatation and epithelial serration in the basal part of the crypts in SSA. HE, x300

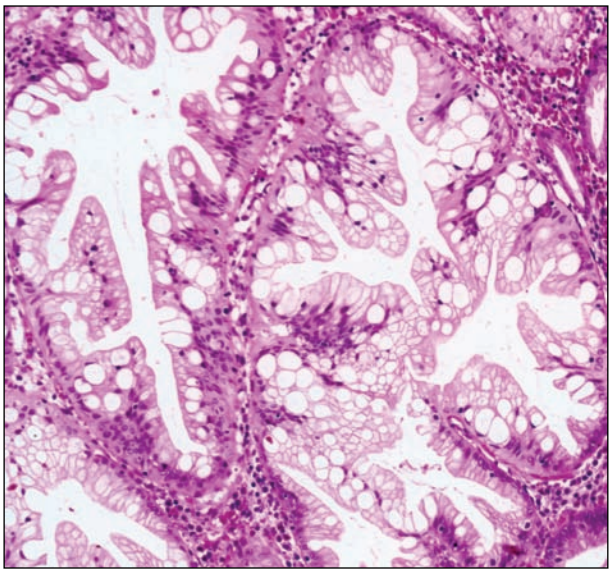

Fig. 3. Well apparent crypt serration in the superficial part of SSA. The crypts contain well-differentiated goblet and columnar cells. HE, x500

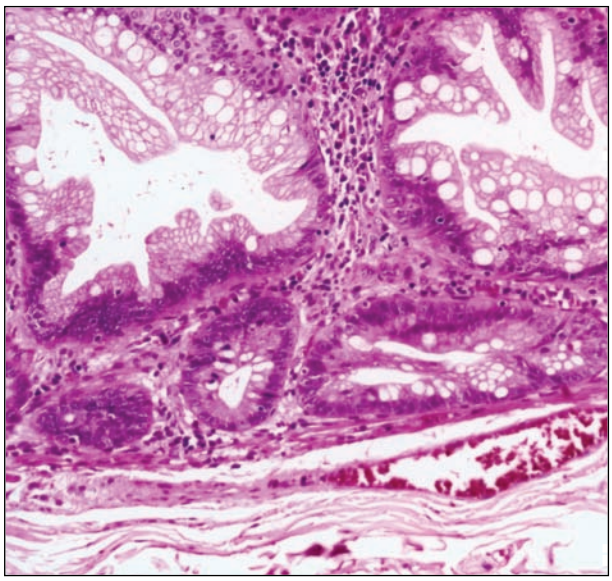

Fig. 2. Horizontal growth (parallel to muscularis mucosae) of the basal part of the crypts. The serrated epithelium contains atypical cells and differentiated goblet and columnar cells. HE, x500

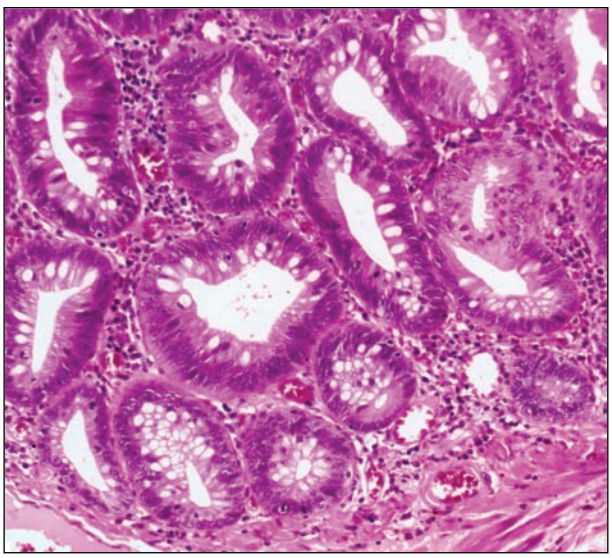

Fig. 4. Basal part of the crypts is lined with atypical cells. Focally, a nuclear stratification is present. Nuclei are localized in lower portion of the cells. Among atypical cells, the differentiated goblet and columnar cells are seen. HE, x500

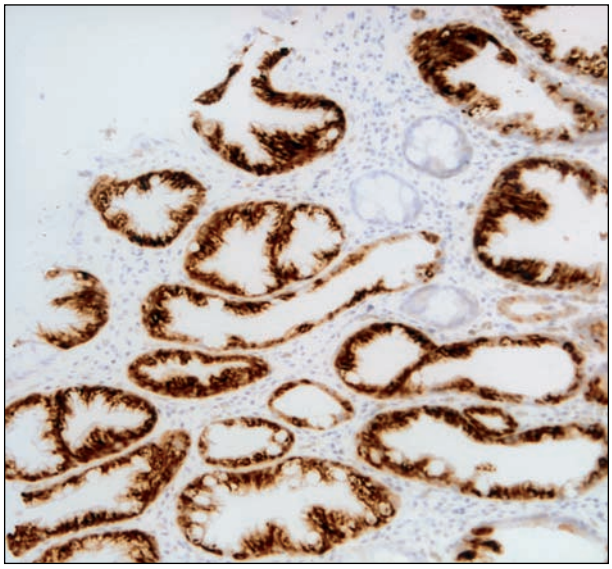

Fig. 5. Strong immunoreactivity for gastric mucin MUC5A in the entire length of the crypts was seen in SSAs. ABC technique, x250

In all 10 common HPs of the vesicular cell type, the proliferation was limited to the basal third of the crypts. The nuclear atypia was not observed. The mucin cells in the superficial crypt zone expressed both MUC2 and MUC5A, and p53 was negative in all polyps.

In conventional TAs the proliferation was diffuse or even more intense in the superficial and middle crypt zones. p53 was negative in all cases. MUC2 was positive in all TAs, but it was not so strong as seen in SSAs. MUC5A was positive diffusely in 5 TAs (figure 6), focally in 4 TAs, and it was negative in one TA. MUC6 was negative in all TAs.

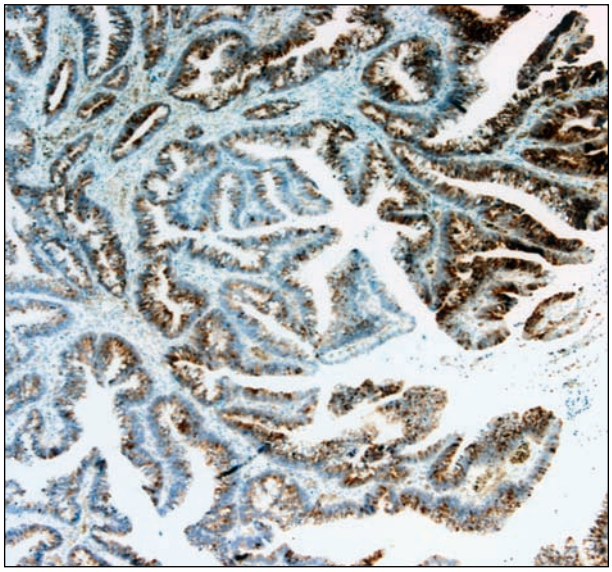

Fig. 6. Immunoreactivity for gastric mucin MUC5A in conventional TA. ABC technique, x100

Discussion

Sessile serrated adenoma was described by Longacre and Fenoglio-Preiser in 1990 as a distinctive polypous lesion that occurs more frequently in the right side of the colon and that usually measures more than 1cm in diameter (11). They arise in HPs and can progress to carcinoma (the so-called serrated type of carcinogenesis) (1–10, 13–18, 20, 21). Histologically, SSAs show abnormal proliferation of the crypt epithelium and nuclear atypia (dysplasia). The abnormal proliferation is characterized by irregular elongation of the proliferative zone of the crypt epithelium (so-called asymmetrical proliferation), and by irregular architecture of the crypts with their frequent branching and dilatation especially in the basal crypt parts. These basal parts of the crypt are often parallel to the lamina muscularis mucosae and they occasionally "penetrate" into the submucosa (inverted serrated polyps) (19). Besides the abnormal proliferation already mentioned, a suppression of apoptosis also plays a role in pathogenesis of the lesion (16). The nuclear atypia in SSAs is usually mild. The nuclei are enlarged and round, with well apparent nucleoli, localized in the basal part of the cells. The stratification and loss of polarity of the nuclei typical for common TAs are not a frequent finding in SSAs (10). The cellular atypia should not be confused with a mature columnar epithelium with a nuclear pseudostratification and an eosinophilic cytoplasm. Such an epithelium occurs typically on the surface of SSAs.

An increased proliferation without nuclear atypia can occur in HPs, probably as an initial feature of the development of SSA. In such cases, the differentiation between SSAs and HPs can be difficult. Moreover, the classification of HPs with a mild atypia is still not consensual. Originally such lesions were termed "serrated polyps with abnormal proliferation" (17, 18), "serrated polyps with immature crypts" or "serrated polyps with dysmaturational crypts" (5). Recently Lazarus et al. called them "atypical serrated polyps" (10). However, according to Snover et al. the diagnosis of SSA depends mainly on structural features that include alteration of the crypt architecture and the presence of mature goblet cells and gastric type foveolar cells, whereas cellular atypia is very mild (16). The nature of so-called mixed hyperplastic-adenomatous polyps is still under discussion as well. Most authors consider them a distinctive entity (6, 7, 9, 10, 15, 17). Fenoglio-Preiser et al. (11) and Iino et al. (8) include them among SSAs, and Snover et al. regards them as a combination of serrated adenoma and conventional adenoma (16).

In the largest series of colorectal polyps with a long-term follow-up, Lazarus et al. (10) found SSAs in 16% of polyps. This frequency of SSAs is substantially higher than that in previous studies in which SSAs had been found in 0.6–3.5% of polyps (2, 11, 17). This difference can be explained by different criteria for the diagnosis or by a frequent underestimation of histological features in HPs (4-6, 10). In the study of Lazarus et al. (10), SSAs were found in older patients (above age 48 years) and mostly in the sigmoid colon and the rectum. SSAs were larger than HPs in general, but still more than one third of them did not exceed 5mm. The growth rate in more dysplastic SSAs was higher than that in conventional adenomas (10, 16). Thus, these findings are in contrast with the results of the previous studies (5, 11, 17). The existence of small and left-sided SSAs is also supported by the findings of other authors (7, 13, 14, 16, 20) as well as by our present observations. In our series SSAs were located mostly in the rectosigmoideum and their diameter was 5-12 mm. The age ranged from 40 to 80 years (with the exception of a 25-year-old woman who had one larger SSA with a stalk). In a majority of SSAs the atypia was low and focal. In 5 SSAs, the stratification of enlarged nuclei was present, consistent with a low-grade dysplasia of the conventional adenoma. In our series, dysplasia of a high-grade, observed in 11-45% of SSAs in the previous studies (10, 11), was detected only in one SSA adjacent to the mucinous carcinoma. Parts of SSA with a high-grade dysplasia were found in the marginal zone of the colorectal carcinoma in 5.8% cases by Mäkinen et al. (13).

On immunohistochemistry, SSAs were diffusely positive for MUC2 and a majority of them also for MUC5A. This reactivity was seen along the lenght of the crypts, thus being not restricted to the surface of the lesion, in contrast with previous observation (9). The gastric type of mucin was strongly expressed also by mucinous carcinoma with structures of SSA at the tumor margin as well as by the cells in superficial parts of HPs. The gastrointestinal genotype of mucin should not be considered specific for "serrated" type of carcinogenesis as it was supposed by Jass (9), as this type was found (albeit with a lesser intense immunoreactivity) in 9 of 10 conventional TAs of our series. Additional gastric type mucin MUC6 was negative in all SSAs, HPs and conventional adenomas.

p53 is one of the genes that play a substantial role in the colorectal carcinogenesis (15, 20, 21). The immunohistochemical nuclear positivity of p53 protein (interpreted as a sign of mutation of p53 gene) was observed in 17–50% SSAs with a low-grade dysplasia and in 60-67% SSAs with a high-grade dysplasia (20, 21). In contrast, Oh et al. (15) like ourselves found only a slight p53 protein positivity in rare cells of SSAs. It was suggested that p53 mutation is less important in the genesis of the carcinomas with MSI. In our control series HPs and conventional TAs were p53 negative. The discrepancies in p53 protein status among various studies are difficult to explain. It is well known that immunohistochemical positivity for p53 protein does not always reflect the presence of the mutation (12), and, in our opinion, this may be the case of p53 expression reported in many SSAs. In addition, the immunohistochemical results of p53 protein vary considerably depending on the antibody used and on other technical factors. Therefore further study using molecular genetic techniques (instead of immunohistochemistry) is needed.

Various published results indicated HPs as the most probable precursor lesions for SSAs (1, 3, 4, 7–10, 14–16, 20). This opinion is supported by molecular genetic alterations similar both in SSAs and HPs. The "serrated" type of carcinogenesis is characterized by MSI and mutations of protooncogenes k-ras and BRAF, both playing an important role in cellular proliferation, differentiation and apoptosis (3, 9, 14, 18). Errors in DNA replication are controlled and repaired by DNA mismatch repair genes that include MLH1 and hMSH2. The methylation and loss of function of MLH1 and hMSH2 were found in 15% and 20% of HPs with vesicular and goblet cell type, respectively, and in 86% of SSAs (17). BRAF mutation occurred more frequently in HP with vesicular cells than in SSAs, whereas k-ras mutation was more frequent in HPs with goblet cells than in SSAs and HPs with vesicular cells (17).

In accordance with previous studies our findings show that histologic features can differentiate well between SSAs and HPs (1, 2, 4-7, 10, 14, 16, 17). These features include elongations and dilatations of the crypts that contain irregular serrated epithelium. The presence of mature appearing goblet and gastric foveolar type cells also in basal segments of the crypts is an additional feature occurring typically in SSA, yet not in HP. Cellular atypia was generally of a mild grade except of an area of a high-grade dysplasia found next to the margin of mucinous carcinoma. Immunohistochemically, SSAs contained mucin of the gastrointestinal type. However, in contrast with previous study of Jass (9), this mucin type is not specific for SSA as it is present also in many conventional TAs as proved in our comparative series. Regarding p53, our results did not confirm the frequent p53 positivity in SSAs published previously (20, 21).

In conclusion, SSAs have a malignant potential comparable with that of conventional adenomas. When SSAs are more dysplastic, they grow probably even faster than conventional adenomas (10), and have a tendency to recur. These are

the reasons for a higher frequency of endoscopic examinations in patients with SSA.

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