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Proliferative activity of mixed hyperplastic adenomatous polyp/serrated adenoma in the large intestine, measured by PCNA (proliferating cell nuclear antigen)

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Abstract: The proliferative activity of serrated adenomas of the large intestine was determined by examining proliferating cell nuclear antigen (PCNA). The PCNA labeling index, determined by visual inspection, and the PCNA area rate, determined with the newly developed image processor for analytical pathology (IPAP), of serrated adenoma were found to be similar to the values for tubular adenoma, and indicated the presence of high proliferative activity in the bottoms of crypts. Determination of the pattern of distribution of PCNA-positive cells indicated the presence of a proliferative zone in the lower region or bottom of the serrated adenoma. However, 5 of the 20 serrated adenomas exhibited an irregular on widely extended proliferative zone, and 2 were complicated by cancer. These findings indicated that serrated adenoma is also a highly proliferative tumor and that it may be complicated by cancer if atypia is increased and disturbance of the proliferative zone is present.

Key words: serrated adenoma, PCNA, image analyzer, colon

Introduction

Serrated adenoma is the name proposed by Longacre and Fenoglio-Preiser[1] in 1990 for the lesion previously known as mixed hyperplastic adenomatous polyp (MHAP), since Urbanski et al.[2] reported it in 1984; the lesion has now been classified as a specific type of colon adenoma by Fenoglio-Preiser.[3] Serrated adenomas are composed of serrated glands resembling hyperplastic polyps on low-power microscopic examination. They are often lined by a homogeneous cell population that contains more mucus than most adenomas, but when examined at higher magnification, they exhibit fewer mature cells than typical hyperplastic polyps.

Longacre and Fenoglio-Preiser[1] found that serrated adenoma occurred at ages ranging from 15 to 88 years, measured 0.2–7.5 cm in diameter, and was a slight preponderance of large lesions in the cecum. They found that serrated adenomas exhibited higher surface mitotic activity and nuclear pseudostratification than hyperplastic polyps, but slightly less than conventional adenomas. Thirty-seven percent of serrated adenomas contained foci of significant dysplasia and 11% contained regions of intramucosal carcinoma.[1] These investigators[1] therefore suggested that serrated adenoma was a neoplastic lesion.

In this study, we used immunohistochemical staining for proliferating cell nuclear antigen (PCNA) to compare the proliferative activity of serrated adenomas with that of other colonic epithelial lesions.

Materials and methods

Tissue samples were obtained by biopsy and polypectomy under endoscopic observation at the Hyogo College of Medicine Hospital and institutions affiliated with the First Department of Pathology of Hyogo College of Medicine. Samples from 20 patients with serrated adenoma, 10 subjects with normal mucosa of the large intestine, 9 patients with hyperplastic polyp, 11 with tubular adenoma with low-grade atypia, 15 with tubular adenoma with high-grade atypia, and 15 with well differentiated adenocarcinoma were studied.

In the samples of 2 of the 20 patients with serrated adenoma, obvious complication by cancer was observed.

The tissue samples were fixed in 10% formalin in the usual way, embedded in paraffin within 3 days of

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fixation, and sectioned. PCNA immunohistochemical staining of tissue sections was performed by the ABC method, using PC-10 (DAKO).

The maximum diameters of polypectomized specimens were measured and the results for the various lesions were compared.

To ensure objectivity, reproducibility, and simplicity, measurements of the PCNA area rate were performed with an image analyzing apparatus, the image processor for analytical pathology (IPAP), which was newly developed cooperatively by the Environmental Health Science Laboratories, Sumitomo Chemical Co. Ltd. (Japan) and Sumitomo Metal Co. Ltd. (Japan).

With the IPAP, the PCNA area rate (percentage of total PCNA-positive area to total nuclear area, 30-fold magnification) was measured. Then, for cases other than serrated adenoma, microscopic fields were examined. Measurement was made from the surface to the bottom of a random sampling of tubules in tumorous and non-tumorous lesions.

For cases of serrated adenoma, the crypts were divided into thirds, the upper, middle, and lower zones, and the microscopic fields for each zone were examined.

For determination of the PCNA labeling index by visual inspection, 1000 cells were examined and the rate of positivity (percent) was obtained. Measurement was made from the surface to the bottom of a random sampling of tubules in tumorous and non-tumorous lesions.

For cases of serrated adenoma, the PCNA labeling index was determined for each zone.

To determine the pattern of distribution of PCNA-positive cells, the upper and lower halves of crypts were observed separately. The pattern of distribution was classified as upper crypt type, lower crypt type, or total crypt type, including or not including the surface layer, as defined by Wada et al.[4] (Fig. 1).

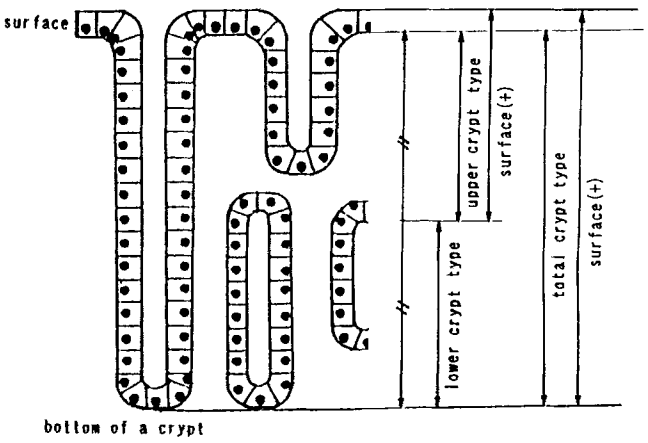

Fig. 1. Classification of the pattern of distribution of proliferating cell nuclear antigen (*PCNA*)-positive cells in colonic epithelial lesions

For statistical comparison of findings for two groups, variance was analyzed by the F test, and then Student's *t*-test was applied.

Results

Maximum diameter of polypectomized specimens

As the severity of atypism increased, lesions increased in size (Table 1). The difference in maximum diameter between tubular adenoma with low-grade atypia and hyperplastic polyp was significant ($P < 0.01$), as were the differences between tubular adenoma with high-grade atypia and tubular adenoma with low-grade atypia ($P < 0.01$), and between adenocarcinoma and serrated adenoma ($P < 0.01$).

However, the difference in maximum diameter between serrated adenoma and tubular adenoma with high-grade atypia was not significant.

PCNA area rate and PCNA labeling index

The PCNA area rate and PCNA labeling index increased in the following order, normal mucosa of the large intestine, hyperplastic polyp, tubular adenoma with low-grade atypia, and tubular adenoma with high-grade atypia and adenocarcinoma, in proportion to the degree of atypia (Table 2, Fig. 2). The difference in both PCNA area rate and PCNA labeling index between tubular adenoma with low-grade atypia and hyperplastic polyp was significant ($P < 0.01$), as were the differences between tubular adenoma with high-grade atypia and tubular adenoma with low-grade atypia ($P < 0.01$), and between adenocarcinoma and tubular adenoma with high-grade atypia ($P < 0.01$).

Comparing the upper, middle, and lower zones of serrated adenomas, both the PCNA area rate and PCNA labeling index increased from the upper zone to the lower zone. The difference in both the PCNA area

Table 1. Mean maximum diameter of polypectomized specimens of colonic epithelial lesions

	Mean maximum diameter of polypectomized specimens
Hyperplastic polyp ($n = 9$)	3.4 ± 1.0 mm
Tubular adenoma with low-grade atypia ($n = 11$)	5.6 ± 3.5 mm
Tubular adenoma with high-grade atypia ($n = 15$)	7.8 ± 2.2 mm
Serrated adenoma ($n = 20$)	8.2 ± 6.4 mm
Adenocarcinoma ($n = 15$)	12.0 ± 5.5 mm

Table 2. Proliferating cell nuclear antigen (*PCNA*) area rate, determined with image processor for analytical pathology (*IPAP*), and PCNA labeling index determined by visual inspection, for the surface, middle, and lower region of serrated adenomas and other colonic epithelial lesions

	PCNA-area rate (%)	PCNA-labeling index (%)
Normal colonic mucosa	12.8 ± 2.5	23.8 ± 5.8
Hyperplastic polyp	18.2 ± 5.7	27.6 ± 10.3
Tubular adenoma of low-grade atypia	31.0 ± 8.1	40.9 ± 11.8
Tubular adenoma of high-grade atypia	44.6 ± 10.1	59.2 ± 9.8
Adenocarcinoma	69.2 ± 11.8	74.3 ± 13.6
Serrated adenoma (upper zone)	16.4 ± 7.5	25.6 ± 6.1
Serrated adenoma (middle zone)	31.9 ± 9.9	40.4 ± 8.9
Serrated adenoma (lower zone)	49.6 ± 11.6	60.2 ± 10.1

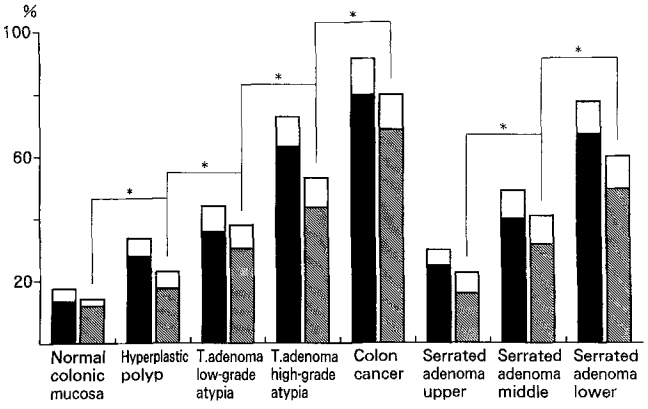

Fig. 2. PCNA area rate, determined with image processor for analytical pathology (*IPAP*; *hatched columns*) and PCNA labeling index (*black columns*), determined by visual inspection, for the surface, middle, and lower regions of serrated adenomas and other epithelial lesions of the large intestine. *Outlined white area*, SD. *$P < 0.01$

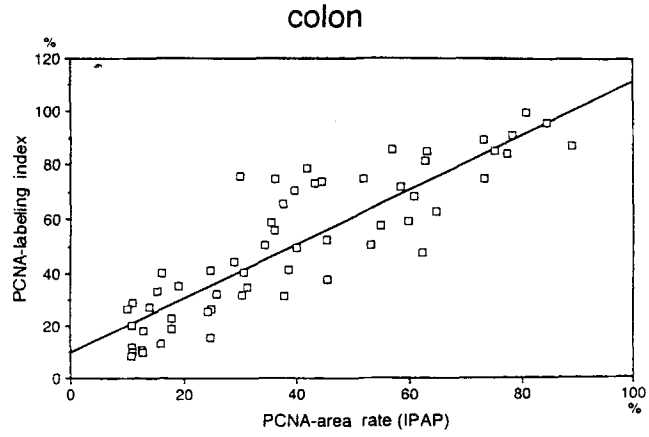

Fig. 3. Correlation between PCNA area rate and PCNA labeling index. $r = 0.760$ ($P < 0.01$)

rate and the PCNA labeling index between the upper zone and the middle zone was significant ($P < 0.01$), as were the differences between the lower and middle zones ($P < 0.01$).

Values in the upper zone of serrated adenomas were higher than those in the normal mucosa of large intestine, and those in the middle zone and lower zone were similar to those in tubular adenomas with low-grade atypia and tubular adenomas with high-grade atypia, respectively (Table 2, Fig. 2).

The PCNA area rates obtained with IPAP and the labeling indices judged by visual inspection were well correlated, the correlation coefficient being 0.76 ($P < 0.01$, Fig. 3).

Pattern of distribution of PCNA-positive cells

In the distribution pattern of PCNA-positive cells, the lower crypt type was predominant in the normal mucosa of the large intestine and in the hyperplastic polyps, whereas 75% of cases of tubular adenoma

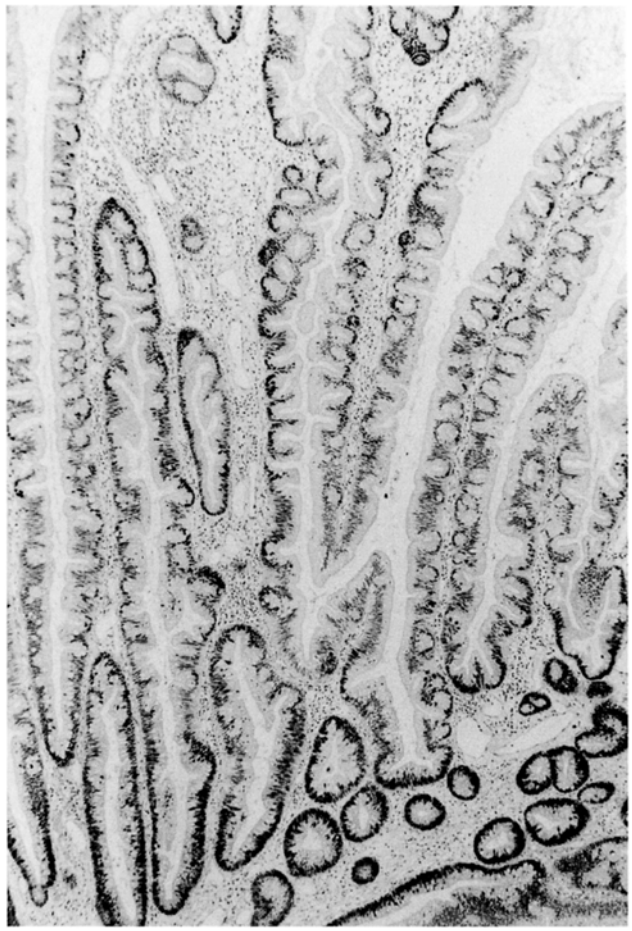

Fig. 4. PCNA immunohistochemical staining of serrated adenoma; PCNA-positive cells are prominent in the lower and bottom region of the crypt

Table 3. Pattern of distribution of PCNA-positive cells in colonic epithelial lesions

		Upper crypt type		Total crypt type	
	Lower crypt type	Surface (−)	Surface (+)	Surface (−)	Surface (+)
Normal colonic mucosa	100%				
Hyperplastic polyp	100%				
Tubular adenoma with low-grade atypia	16%	71%	4%	8%	
Tubular adenoma with high-grade atypia		4%	25%	19%	52%
Adenocarcinoma					100%
Serrated adenoma	75%			25%	

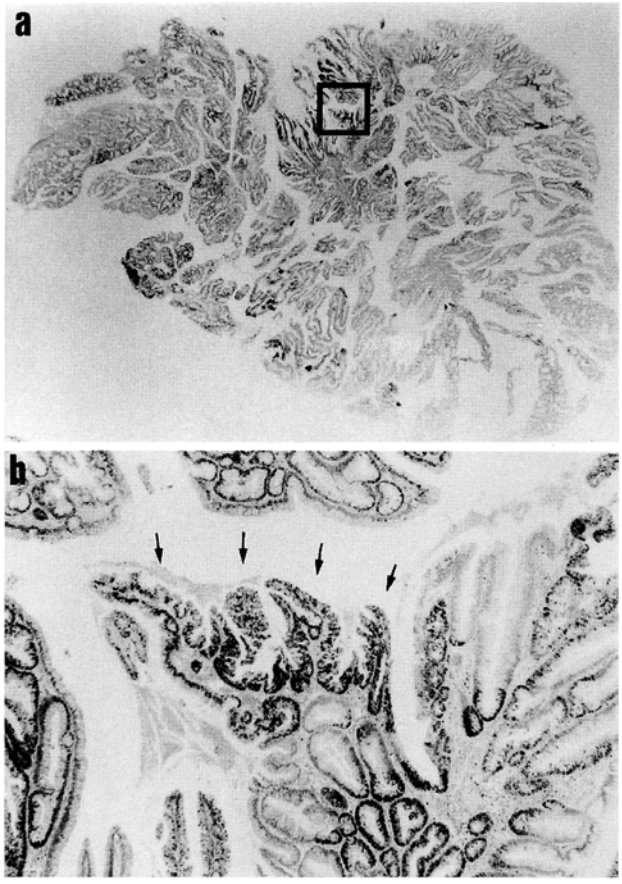

Fig. 5a,b. PCNA immunohistochemical staining of serrated adenoma complicated by cancer. Cancer is seen in the mucosa (**b**, *arrow*)

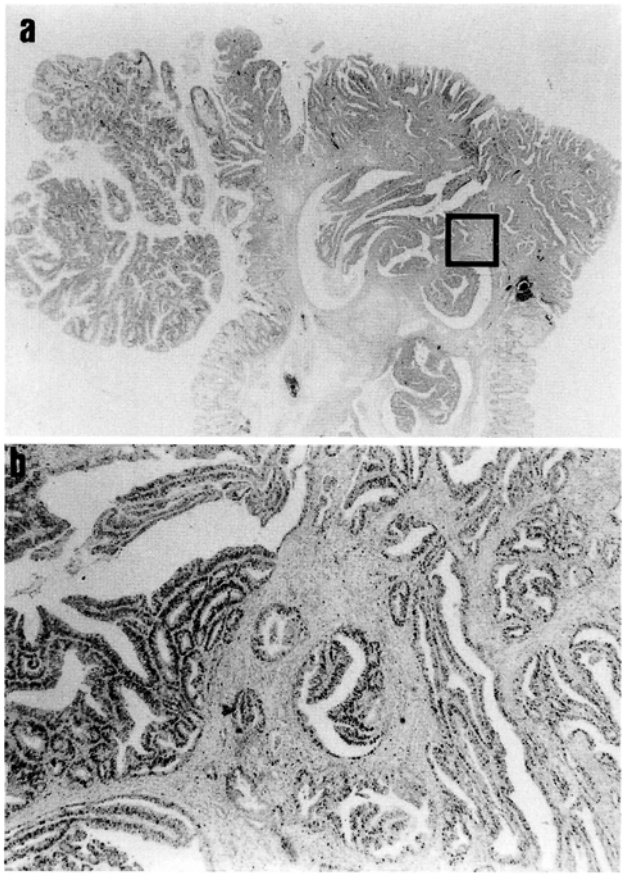

Fig. 6a,b. PCNA immunohistochemical staining of serrated adenoma complicated by cancer. **b** Submucosal (*sm*) infiltrating cancer is present

with low-grade atypia were upper crypt type; 71% of the cases of tubular adenoma with high-grade atypia had labelled cells distributed in all the layers, and 100% of adenocarcinomas had labeled cells distributed in all layers.

Of the serrated adenomas, 75% were of the lower crypt type, with PCNA-positive cells being distributed in the lower to the bottom regions of the crypt. The remaining 25% were of the total crypt type. Thus, labeled cells were predominantly located in the lower regions of serrated adenomas, although some were distributed throughout the crypts (Table 3, Fig. 4).

Two cases of total crypt type of serrated adenoma were complicated by cancer (Figs. 5, 6).

Discussion

PCNA is an accessory protein of DNA polymeras δ and is thought to play an important role in the elongation or replication of the DNA chain. Its accumulation in the nucleus during the G_1 and S stages of the cell cycle has been reported,[5–12] and the labeling index, which is the percentage of PCNA-positive cells, has been reported to be correlated with the proliferative activity and the prognosis of various malignant tumors.[13–20]

In the present study, we used PCNA immunohistochemical staining in routine paraffin sections to examine serrated adenoma and epithelial lesions of the large intestine as representative lesions containing proliferating cells. For simplicity, objectivity, and reproducibility of data, these being indispensable features of pathological diagnosis, we used a newly developed image analyzing apparatus, the image processor for analytical pathology (IPAP). Unlike other existing image analyzing systems, this system is equipped with an automatic data processor and a menu for nuclear and tissue analysis. With this system, automatic or semi-automatic imaging of various tissue specimens stained by conventional or immunohistological procedures is possible, and quantitative analysis of images can be performed.[21] The IPAP was very useful in this study, and objective data were obtained (Fig. 7). The PCNA area rate was used because frequent overlapping of nuclei is observed in tumors of the digestive tract. The PCNA area rates obtained by IPAP correlated well with the PCNA labeling indices obtained by visual inspection.

Haapasalo et al.[22] reported that quantitative evaluation, using the PCNA area rate, was excellent for the evaluation of astrocytic neoplasms. We have confirmed their findings in this study.

In 1970, the existence of hyperplastic glands in polyps was first described by Goldman et al.[23] in adenomas of villus architectural subtype. In 1984. Urbanski et al.[2] proposed the term, "mixed hyperplastic adenomatous polyp (MHAP)," for this lesion. Recently, Longacre and Fenoglio-Preiser[1] reported 110 cases of serrated adenoma collectively, and evaluated their mitotic activity by counting mitoses.

In their study, the incidence of serrated adenoma was 110 cases out of 18000 cases of polyps in the large intestine. They also reported that, unlike hyperplastic polyps, serrated adenomas were usually 5mm or larger, 46.6% were in the cecum or descending colon, they exhibited a histologically serrated pattern, exhibited mitosis in the surface layer, had a prominent nucleolus, exhibited pseudostratification of nuclei, had an increased nuclear to cytoplasmic ratio (Figs. 8, 9, 10), and exhibited atypia in 37% of cases; 11% were complicated by cancer. We also observed obvious complication by cancer in 2 of 20 cases of serrated adenomas (10%; Figs. 5, 6). We used PCNA to determine the proliferative activity of serrated adenomas.

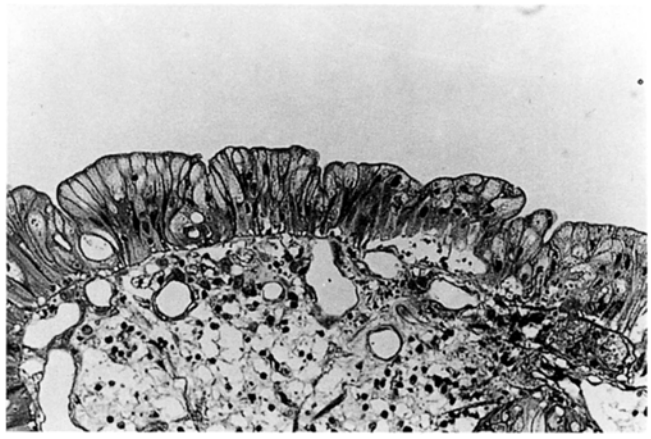

Fig. 8. Histologic picture. Serrated adenoma, upper zone, Disturbance of nulear polarity is observed.

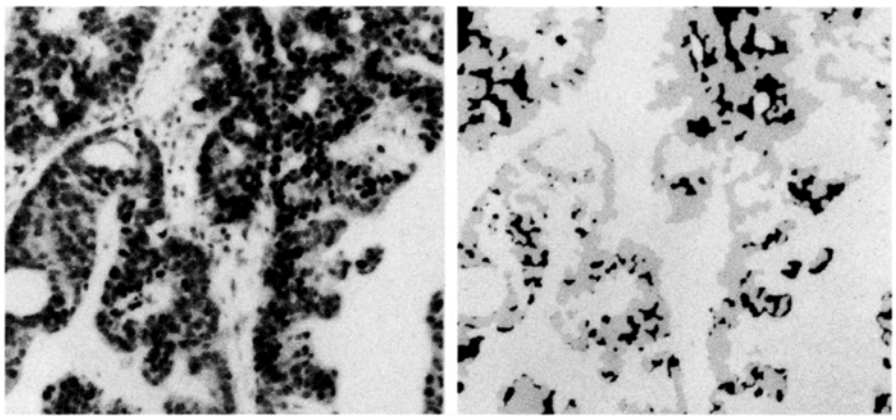

Fig. 7. PCNA area rate measured with IPAP. Region with cancer, IPAP image on the *right* and PCNA immunohistochemical stain on the *left*. Faintly stained regions are PCNA-positive nuclei, and dark regions are PCNA-negative nuclei

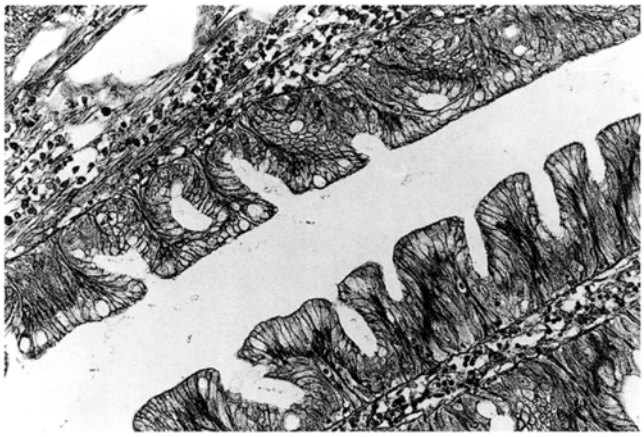

Fig. 9. Histologic picture. Serrated adenoma; middle zone. Immature goblet cells and nuclear pseudstratification are observed

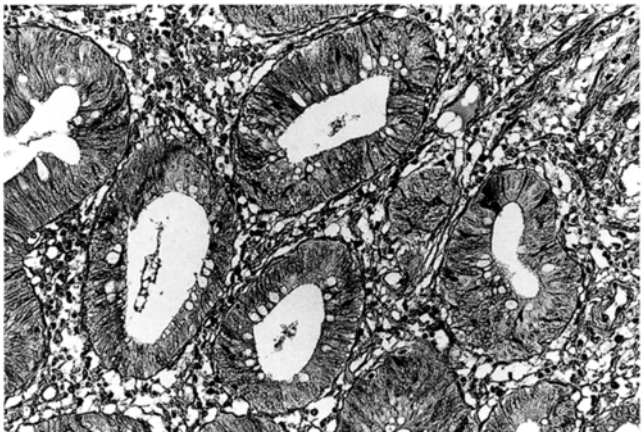

Fig. 10. Histologic picture. Serrated adenoma, lower zone, Tubular adenoma is present

We found that the PCNA area rate, determined with IPAP, and the PCNA labeling index, determined by visual inspection, increased from the surface to the middle to the lower zone, indicating increasing proliferative activity toward the lower regions of the crypt, with the highest activity being observed in the bottom, the adenoma region. Our findings for proliferative activity corresponded well to the mitotic index described by Longacre and Fenoglio-Preiser.[1] This adenoma region exhibited high activity, almost the same as or slightly higher than that of tubular adenoma with high-grade atypia, and the middle zone exhibited activity similar to that of tubular adenoma with low-grade atypia, indicating that serrated adenoma is an adenomatous lesion with high proliferative activity.

Examination of the distribution pattern of PCNA-positive cells showed that, in 75% of serrated adenomas, PCNA-positive cells were distributed in the lower or bottom region of crypts, while in 25% in they were distributed throughout all the zones, but predominantly in the lower regions, suggesting disturbance of the proliferative zone. In addition, 2 cases of complication by cancer were observed among the 20 cases of serrated adenoma examined.

Serrated adenoma thus appears to be a tumorous lesion with very high proliferative activity, and its pattern of proliferation, determined by PCNA immunohistochemical staining, indicated that it was a tumorous lesion with proliferative potential which originated in the lower or bottom region of the mucosa in the large intestine. Tumor cell differentiated to adenoma or immature goblet cells, and then cause disturbance in the proliferative zone and possible complication by cancer. Our findings thus suggest that it is important to distinguish serrated adenoma and hyperplastic polyps in biopsies during routine examination, and, further, when the diagnosis of serrated adenoma is made it is important that polypectomy be recommended to the physician in charge.

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