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Originals

Pathological Approach for Surveillance of Ulcerative Colitis-associated cancer : Usefulness of Immunohistochemistry for p53

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SUMMARY

The patients with long-standing ulcerative colitis (UC) have a high-risk of neoplastic lesions in the colonic mucosa. The UC-associated neoplastic lesion is difficult to detect by endoscopic examination or diagnose histologically. In the present study, we aimed to clarify whether immunohistochemistry for p53 is useful to discriminate the UC-associated neoplasia from inflammed regenerating epithelium. Tissue samples were obtained from colorectomy specimens from 20 patients with long-standing UC (range 6-29 years). The surface of microstructure of the tissues was observed by stereomicroscopy, and the sections were examined using immunohistochemistry for p53. All of T_{2-4} carcinomas were detectable by endoscopic examination before surgery, whereas considerable number of dysplasias (52.5 %), Tis carcinomas (33.3 %), and T_1 carcinomas (60.0 %) were undetectable. Fifty-three of 67 UC-associated neoplastic lesions (79.1 %) were of flat-type macroscopically. The detection rate of flat-type neoplasias (45.3 %) was significantly lower than that of protruding ones (100 %). The positivity of p53 overexpression was 0 % in UC-II, 52.5 % in UC-III, and 70.4 % in UC-IV, respectively. UC-II lesions had lower positivity of p53 overexpression than UC-III ($P=0.027$) or -IV lesions ($P=0.003$). Immunohistochemical analysis of p53 protein is useful to discriminate the UC-associated neoplasia from inflammed regenerating epithelium.

Key Words : ulcerative colitis, cancer, p53, immunohistochemistry

INTRODUCTION

The pathogenesis of ulcerative colitis (UC) is still unclear, but dysregulation of immune system appears to be involved in its chronic inflammatory process. As recent studies have suggested, inflammation plays important roles in the development of various cancers, and indeed, the patients with long-standing UC have a high-risk of its associated colorectal cancer (colitic cancer)[1,2]. The colitic cancer is believed to arise through a chronic inflammation-dysplasia-carcinoma sequence, and therefore, the early detection of precancerous dysplasia is very important to improve the prognosis of long-standing UC patients. In practice, the surveillance of UC patients are performed by the repeated endoscopical examinations[3～5] ; however, UC-associated neoplasia is frequently difficult to detect endoscopically. Moreover, it is difficult to discriminate the UC-associated neoplasia from inflammed regenerating epithelium even by pathological examination.

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Therefore, in the present study we aimed to clarify whether immunohistochemical detection of p53-overexpression is useful to discriminate the UC-associated neoplasia from inflammed regenerating epithelium.

MATERIALS AND METHODS

Patients

Tissue samples were obtained from colorectomy specimens from 20 patients with UC (10 males, 10 females ; mean age 48.4 ± 1.6 years, range 39-64 years ; mean disease duration 17.1 ± 1.1 years, range 6-29 years) who had undergone total coloctomy for UC-associated neoplasia. Endoscopic examinations before surgery were performed by specialists for endoscopy.

Histological assessment

All specimens from the colectomies were opened longitudinally, fixed in 10 % formalin solution and stained with alcian blue (pH 2.5) and Carazzi's haematoxylin or crystal violet. The surface of microstructure of the tissue was observed using stereomicroscopy. The macroscopic appearance of neoplastic lesions was subgrouped into protruding and flat-type. After stereomicroscopic observation, the whole tissues were sectioned at intervals of 5 mm, embedded in paraffin and stained with haematoxylin and eosin.

Each specimen was graded as follows : inflammatory change (UC-I) ; indefinite (UC-II) ; neoplastic but not carcinoma (UC-III) ; carcinoma (UC-IV). This classification was proposed by the Research Committee of Inflammatory Bowels Disease of the Ministry of Health and Welfare of Japan [6]. T grade was evaluated according to the International Union Against Cancer TNM staging system as follows : Tis, intraepithelial or invasion of lamina propria ; T_1, tumor invades submucosa ; T_2, tumor invades muscularis propria ; T_3, tumor invades through muscularis propria into subserosa or into non-peritonealized pericoloic or perirectal tissues ; T_4, tumor directly invades other organs or structures and/or perforates visceral peritoneum.

Immunoshitochemistry

Immunohistochemical staining for p53 was performed with a LSAB-2 kit (DAKO, Marseille, France) as described previously [7]. In brief, the sections (4-μm-thick) placed on silane-coated slides were deparaffinized, rehydrated, and then pretreated with 0.3 % H_2O_2 in methanol for 20 min at room temperature to quench endogenous peroxidase activity. The sections were then placed in 0.01 mol/L citrate buffer (pH 6.0) and treated by microwave heating (MI-77, Azumaya, Tokyo, Japan ; 400 W, 95℃) for 10 min to facilitate antigen retrieval. The sections were incubated with 1 % bovine serum albumin in phosphate-buffered saline (PBS) for 30 min, and then with anti-human p53 antibody (NCL-p53-CM1 ; Novocastra Laboratories, Newcastle, UK ; dilution 1 : 2000) for 1 hour. Thereafter, the sections were incubated with biotinylated secondary antibody for 15 min, washed with PBS, and treated with peroxidase-conjugated streptavidin for 20 min. Finally, the sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride with 0.05 % H_2O_2 for 3 min and then counterstained with Carazzi's hematoxylin. Immunoreactivity was evaluated as positive when a focal nuclear accumulation of p53 protein was detected.

Statistical analysis

Statview 5.0J statistical software (Abacus Concepts Inc., Berkeley, CA) was used for all analyses. Chi-squared analyses were performed to investigate the relationship between p53 expression and clinicopathological features. All values were expressed as the mean ± SEM, and the significance of differences between two groups was assessed using Mann-Whitney U-test. Differences at $P < 0.05$ were considered to be significant.

RESULTS

Macroscopic appearance and detection rate of ulcerative colitis-associated neoplastic lesions

As shown in Table 1, 21 of 40 (52.5 %) dysplasias, 5 of 15 (33.3 %) Tis carcinomas, and 3 of 5 (60.0 %) T_1 carcinomas were undetectable by endoscopic examination before surgery, whereas all of $T_{2\text{-}4}$ carcinomas were detectable. The advanced lesions in T_2-T_4 stage was significantly detectable than dysplasia or T_1 carcinomas ; however, the detection rates of dysplasia, Tis carcinoma, and T_1 carcinoma were not different statistically.

When classified UC-associated neoplastic lesions according to macroscopic appearance, 53 of 67 (79.1 %)

Table 1 Relationship between detection and macroscopic appearance of UC-associated neoplastic lesions

T grade		*P* value	protruding	flat	*P value
Dysplasia (n=40)					
detectable	19		3	16	0.058
undetectable	21		0	21	
Tis (n=15)					
detectable	10	0.205[a]	3	7	0.171
undetectable	5		0	5	
T_1 (n=5)					
detectable	2	0.751[a]	2	0	<0.05
undetectable	3	0.292[b]	0	3	
Advanced (n=7)					
detectable	7	<0.05[a]	6	1	ND
undetectable	0	0.082[b] <0.05[c]	0	0	

[a]Compared with dysplasia, [b]Compared with Tis, [c]Compared with T_1.
*Relationship between detection and macroscopic appearance. ND : not determined.

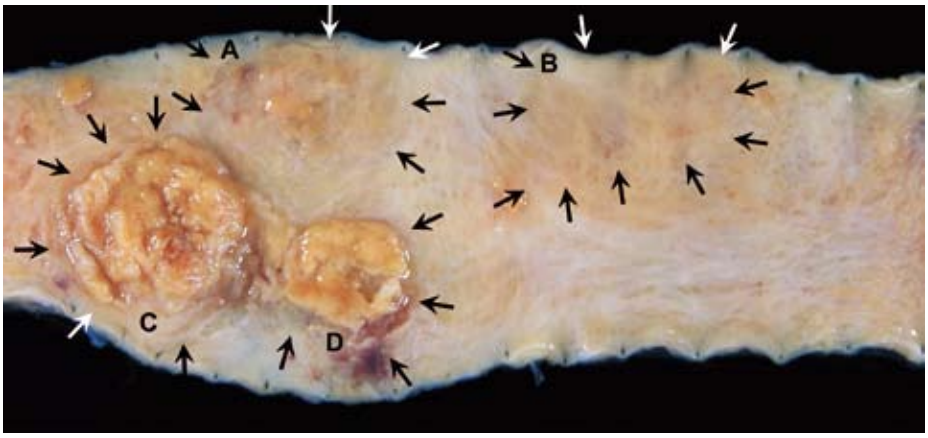

Figure 1 Macroscopic appearance of UC-associated neoplastic lesions. **A**. UC-associated carcinoma (flat-type). The height of lesion is almost same as that of adjacent non-neoplastic colonic mucosa. **B**. Dysplastic lesion (UC-III ; flat-type). The lesion appears slightly reddish but difficult to discriminate from adjacent non-neoplastic colonic mucosa. **C and D**. UC-associated carcinoma (protruding type). The lesions are apparently protruding and possible to discriminate from adjacent non-neoplastic colonic mucosa.

lesions were of flat-type (Figure 1A and B). In detail, 37 of 40 (92.5%) dysplasias, 12 of 15 (80.0%) Tis carcinomas, 3 of 5 (60.0%) T_1 carcinomas were of flat-type, whereas 6 of 7 (85.7%) T_{2-4} carcinomas were protruding (Figure 1C and D). In each T category, the detection rate of lesions tended to be high in the protruding-type. Overall, the detection rate of flat-type neoplasias (45.3%) was significantly lower than that of protruding ones (45.3% vs 100% ; $P=0.0002$).

p53-overexpression in ulcerative colitis and its associated neoplastic lesions

Seventy-two specimens were included in the immunohistochemical analysis for p53. We observed the accumulation of p53 immunoreactivity in the nuclei of tumor cells (Figure 2). The positivity of p53 overex-

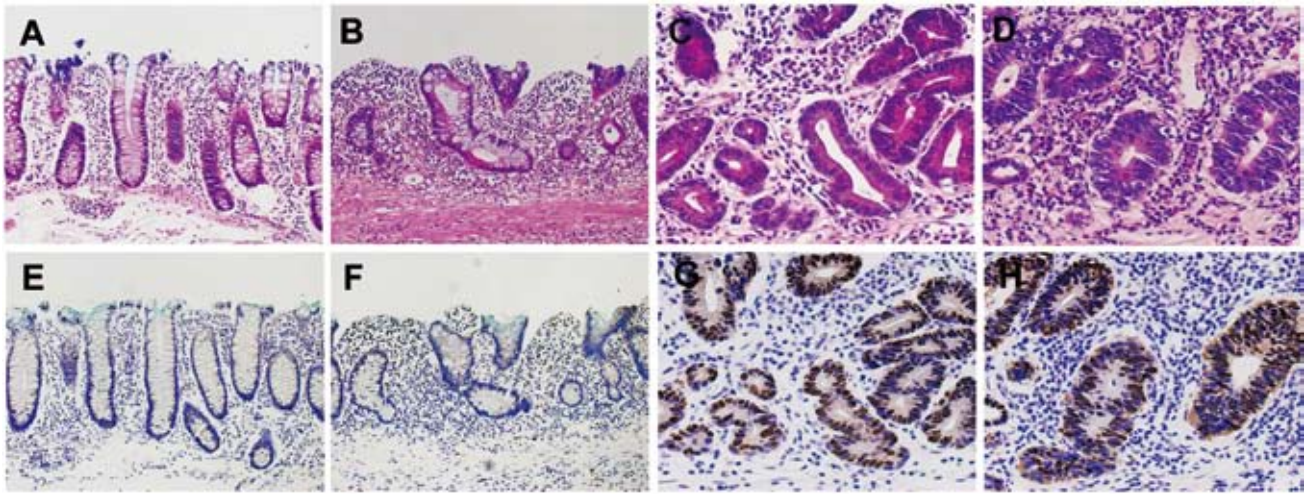

Figure 2 Histology of non-neoplastic epithelium and neoplastic lesions in UC tissues. **A-D**. Haematoxylin-eosin staining. **E-H**. Immunostaining of p53. p53 immunoreactiviy was hardly observed in the inflammed epithelium in lesions classified into UC-I (**E**) and UC-II (**F**). Nuclear accumulation of p53 immmunoreactivity was observed in the dysplastic cells and cancerous cells in lesions classified into UC-III (**G**) and UC-IV (**H**). Magnification, (**A, B, E, and F**) 100×; (**C and G**) 200×; (**D and H**) 300×.

Table 2 Relationship between p53 overexpression and histological diagnosis

	Total number of lesions	Number of positive lesion (%)	P value
Inflammatory change (UC-I)	0	0 (0)	ND
Indifinite (UC-II)	5	0 (0)	
Neoplastic but not carcinoma (UC-III)	40	21 (52.5)	$<0.05^{a}$
Carcinoma (UC-IV)	27	19 (70.4)	$<0.005^{a}$ 0.144^{b}

[a]Compared with UC-II, [b]Compared with UC-III. ND: not determined.

pression was 0% in UC-II, 52.5% in UC-III, and 70.4% in UC-IV, respectively (Table 2). Although statistical analysis showed no difference in the positivity of p53 overexpression between UC-III and -IV, UC-II lesions had lower positivity of p53 overexpression than UC-III ($P=0.027$) or -IV lesions ($P=0.003$).

DISCUSSION

A surveillance colonoscopy has been recommended for patients with long-standing UC to detect their neoplastic lesions at early stage[3~5]. However, the current surveillance still remains unsatisfactory because UC-associated neoplasia is often difficult to detect endoscopically. As shown in Table 1, advanced colitic cancer is usually detectable because great morphological change can be observed in endoscopic examination. However, as we have shown in the present study, the detection rate of early colitic cancer and precancerous dysplasia is largely decreased compared with that of advanced colitic cancer. Although we have no exact answers, the difference of macroscopic finding may affect the detection rate of lesions. Thus, most of early colitic cancer and dysplasia are of flat-type, and such lesions are significantly difficult to detect before surgery when compared with protruding type ones. In this regard, Kiesslich et al. have suggested that magnifying endoscopy makes it possible to observe the surface (known as the "pit pattern") of UC-associated neoplastic lesions more precisely, resulting in early detection of dysplasia or early colitic cancer at about 90% in sensitivity[8]. However, the observation of "pit pattern" by magnifying endoscopy has been suggested to yield false-positive to diagnose of UC-associated neoplastic lesions[9]. Thus, the current surveillance

colonoscopy for UC-associated neoplastic lesions still needs further improvement.

From the view point of pathology, UC-associated neoplasia is also difficult to discriminate from inflammatory regenerating epithelium, and therefore, a useful marker to discriminate them would be advantageous to accurate diagnosis of UC-associated neoplasia. Of note, several studies have shown that p53 alterations are very frequent in UC-associated neoplasia and moreover suggested that p53 alterations is an early event in the development of UC-associated neoplasia [10~13]. Then, we have investigated whether p53 overexpression is useful to discriminate UC-associated neoplasia from inflammatory regenerating epithelium in the colorectum. Interestingly, we found in the present study that p53 overexpression is observed in the lesion classified in UC-III and -IV but not in UC-I and -II, suggesting that p53 overexpression is observed in neoplastic lesions but not in inflammatory regenerating epithelium. In this context, immunohistochemistry of p53 would be a useful marker of UC-associated neoplasia in cases where it is hard to discriminate between neoplasia and regenerative epithelium. On the other hand, it is known that negative staining for p53 protein does not always indicate normality of p53 gene [9]. Indeed, genetic analysis such as PCR-SSCP is supposed to be more accurate than immunohistochemistry to discriminate between UC-associated neoplasia and regenerative epithelium [9]. However, it appears difficult in practice to apply such genetic analyses in the pathological diagnosis of UC tissues, whereas it is easier to apply immunohistochemistry. Accordingly, we consider that immunohistochemistry for p53 is more suitable than genetic analyses for the routine diagnosis of UC tissues.

In conclusion, UC-associated neoplasia, especially precancerous dysplasia and early colitic cancer are difficult not only to detect by endoscopic ecxamination but also to discriminate from inflammed regenerating epithelium. In this regard, immunohistochemical analysis of p53 protein is useful to diagnose UC tissues pathologically.

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