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Frequency of p53-positive Cells in Differing Histological Phases of Ulcerative Colitis

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Summary. **Several reports attest to the absence of p53 immunoreactive cells within non-neoplastic colorectal mucosa, including histologically normal mucosa and inflamed mucosa from subjects with active ulcerative colitis (UC). Failure to detect p53 in these circumstances has been attributed to the short half-life of wild-type p53. Following our previous demonstration of p53 expression in non-neoplastic mucosa, we have studied specimens from ten subjects with ulcerative colitis with the aim of quantifying and correlating this aim with disease activity. The frequency of p53-positive tubules was 19.0% (245/1292) in active UC, 3.1% (46/1487) in resolving and 0.5% (4/863) in UC in remission and 0.3 % (59/17982) in controls. The number of p53-positive cells per tubule with p53-positive cells was 5.4±4.1 in active, 2.4±3.6 in resolving, and 1.3±0.5 in UC in remission and 1.1±0.3 in controls. Positive cells occurred singly and were limited to the proliferative zone of tubules in UC and controls. p53 labeling index (LI) in the proliferative zone was 9.0±7.7% in active, 1.5±1.4% in resolving, 1.1±0.5% in UC in remission and 1.2±0.3% in controls. Ki-67 LI in corresponding areas was 68.0± 16.4% in active, 63.1±10.2% in resolving, 48.5±14.4% in UC in remission and 60.9±10.9% in controls. p53 LI/ Ki-67 LI percentage which is 100% or more in carcinomas with p53 protein overexpression was 14.3± 13.6% (max 52%) in active, 2.24±1.89% in resolving, 2.14±0.73% in UC in remission and 2.03±0.51% in controls.**

We conclude that p53 immunoreactive cells occur in non-neoplastic conditions and contain wild-type protein. Scattered expression of p53 is not a marker of neoplasia.

If p53 immunostaining is to provide a useful diagnostic tool, then the limits of normal and abnormal expression need to be carefully defined.

Key words—ulcerative colitis, p53 expression, proliferative activity, histological phase.

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INTRODUCTION

p53 mutation is common in human cancers; the overexpression of its product is seen in 60-70% of tumors.[3,5-7] The wild-type p53 gene product is believed to regulate entry into and progression through the normal cell cycle.[8] It is induced during transition from G_0 to G_1 and is present at low levels in most normal fetal and adult tissues.[9] p53-positive cells have been reported as absent in the mucosa in active ulcerative colitis (UC)[1,2] and normal colorectal mucosa.[3] Failure to detect p53 under these circumstances has been attributed to the short half-life of wild-type p53 (20-30 min). The presence of p53 immunoreactive cells is interpreted as overexpression in some papers[9-11] regardless of its extent. We have previously observed p53-positive cells in both normal and UC mucosa[5] and elected in this study to quantify the limits of expression of p53 in subjects with UC but no evidence of dysplasia.

The aim of this study was to elucidate the frequency of p53 positive tubules, the number of p53-positive cells (including index) and their location within tubules, and to correlate these data with disease and proliferative activity.

MATERIALS AND METHODS

Materials

A total of ten patients with UC was used: three men and seven women. The mean age was 31.5 (range 10-61) years (Table 1).

Samples of non-neoplastic(non-dysplastic) mucosa from ten UC surgical specimens (four active, four

Table 1. Cases of ulcerative colitis

No. of patient	Age and sex	Onset	Duration and type of colitis (Month)	Type	Phase of inflammation	p53 positive tubules /tubules examined
1	40M	F	6	Total	Active	43/424 (10.1%)
2	61M	F	4	Total	Active	48/299 (16.1%)
3	21F	F	6	Total	Active	143/467 (30.6%)
4	36F	R	120	Total	Active	11/102 (10.8%)
5	42F	R	24	Total	Resolving	7/167 (4.2%)
6	27F	R	30	Total	Resolving	5/328 (1.5%)
7	25F	R	60	Total	Resolving	28/706 (4.0%)
8	24M	R	120	Total	Resolving	6/286 (2.1%)
9	10F	F	12	Total	Remission	3/540 (0.6%)
10	26F	R	24	Total	Remission	1/323 (0.3%)

F: first attack; R: relapse

Table 2. Frequency of p53-positive tubules

Phase of inflammation	p53 positive tubules/ tubules examined
Active	245/1292 (19.0%) 〈a〉
Resolving	46/1487 (3.1%) 〈b〉
Remission	4/863 (0.5%) 〈c〉
Control (Non-UC case)	59/17982 (0.3%) 〈d〉

$p<0.01$: a-b, a-c, a-d, b-c, b-d; not significant: c-d

resolving and two remission phase) and non-UC, normal mucosa (control) far from the transitional mucosa of 82 early colorectal carcinomas were obtained from the archives of the first Department of Pathology, Niigata University. Three 3 μm-thick sections were cut from formalin-fixed, paraffin-embedded blocks measuring 3.0 cm in length from each case. The first section was stained with hematoxylin and eosin (H•E). The second section was used for p53 immunostaining, and the third for Ki-67 immunostaining.

Immunohistochemical staining

For p53 and Ki-67 immunostaining, paraffin-embedded sections were placed on poly L-lysine coated glass slides, and air-dried at room temperature. Deparaffinized and rehydrated sections were heated by microwave oven (Hitachi, MR-M220, Tokyo, Japan) for seven 3-minute cycles in a citrate-buffer to retrieve antigenic activity, and cooled for 60 min at room temperature. Endogeneous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxidase in methanol for 20 min at room temperature. After blocking non-specific reactions with 10% normal rabbit serum, the sections were first incubated with the p53 antibody (mouse monoclonal antibody PAb1801, Ab-2 Oncogene, Science, Inc. Manhasset, NY, U.S.A.) for one hour at a dilution of 1 : 200 or the Ki-67 antibody (MIB1,[12]) immunotech, SA) for two hours at a dilution of 1 : 50. The sections were then incubated with biotynilated rabbit anti-mouse immunoglobulin for 30 min and next with streptavidin-peroxidase complex (Histofine SAB-PO Kit, Biogenex Laboratories, Tokyo, Japan) for 15 min. Careful rinses were done with several changes of phosphate buffered saline (PBS) between each stage of the procedure. The color was developed with diaminobenzidine. The sections were lightly counterstained with hematoxylin and mounted. Negative controls were carried out by replacing the primary antibody with PBS.

Immunohistochemical analysis

All sections examined showed in-built normal Ki-67 positivity in proliferative cells of the lower crypt and germinal center cells of lymph follicles. This confirmation is necessary for p53 immunostaining study because p53 positivity is lost or markedly decreased in sections with no or poor Ki-67 reactivity.

p53-positive cells were defined as cells with brown staining on the nucleus regardless of staining intensity, but cells with very weak equivocal staining were considered negative. When at least one positive nucleus was visible within a tubule, the tubule was scored as positive. To study the distribution of positive cells in tubules, we selected vertically cut tubules and divided each tubule into 50-μm-long compartments from the base toward the luminal surface.

Table 3. Number of p53-positive cells per tubule cut vertically

Phase of inflammation	No. of p53-positive cells per tubule																Total no. of tubules
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	21	
Active	6	0	1	3	1	1	3	1	0	1	1	1	1	1	1	1	25
Resolving	9	2	1	0	1	0	0	0	0	0	0	1	0	0	0	0	14
Remission	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Control	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10

Table 4. Staining patterns of p53-positive cells in vertically cut tubules of ulcerative colitis

Phase of inflammation	No. of tubules in p53 pattern		
	Scattered	Nested	Diffuse
Active	25	0	0
Resolving	14	0	0
Remission	4	0	0
Control	10	0	0
Total	53	0	0

Rates of Ki-67 positive cells per compartment were measured by counting the number of positive cells against the total number of cells in each compartment. The proliferative zone was defined as the length of basal crypt compartment including cells with a 30% or more Ki-67 rate, which is an average value for the normal proliferative zone in our previous study.[5] Ki-67 LI of a tubule was expressed as a rate of Ki-67 positive cells out of a total number of cells within the proliferative zone. p53 LI of a tubule was measured in the corresponding area to Ki-67 LI in a consecutive p53 stained section.

We used three staining patterns of p53 reactivity which was based upon our previous results on colorectal or gallbladder cancers:[5,13] 1) diffuse pattern—nuclear positive cells diffusely existed in most areas of a tubule; 2) nested pattern—twenty positive cells aggregated circumscribed area of a tubule; 3) scattered pattern—a small number of isolated positive cells scattered in a tubule.

Statistics

Differences in the frequency of p53-positive cases were evaluated by Fisher's exact probability test. Differences in p53 LI, the Ki-67 LI or p53 LI/Ki-67 LI rate were evaluated by *t*-test. Probability values of 0.05 or less were considered statistically significant.

RESULTS

The frequency of p53-positive tubules was significantly higher in active (19.0%) UC than in resolving (3.1%) or remission (0.5%) samples, and controls (0.3%) (Table 2). It was also significantly higher in resolving UC than in the remission ($p<0.01$), and controls ($p<0.01$), but did not differ between UC in remission and controls.

In UC in remission and in controls, one or two p53-positive cells were observed in all tubules (Fig. 1). In the active phase they increased in number, with up to 21 cells per tubule (Fig. 2 and Table 3). p53-positive cells were generally weak in staining intensity, and distributed haphazardly but limited within the proliferative zone in all tubules from controls and colitic mucosa. There was never more than four p53-positive cells within a cluster even in case of active UC with 21 p53-positive cells (Table 4).

p53 LI and Ki-67 LI were significantly higher in active UC than in the other samples (Figs. 3 and 4). In each tubule with p53-positive cell(s), only the tubules in active UC showed a strong correlation between Ki-67 LI and p53 LI (Fig. 5). The rate of p53 LI/Ki-67 LI was also significantly higher in active UC than in the other samples (Fig. 6).

The length of the proliferative zone was increased in active and resolving UC as compared with controls (Fig. 7) ($p<0.01$). No significant difference was observed in the length of proliferative zone between UC in remission and controls.

DISCUSSION

Because of the short half-life of the wild-type p53 gene product, p53 immunoreactive cells were reported as absent in non-neoplastic or non-dysplastic mucosa in active UC[1,2] and normal colorectal mucosa.[3,4,7,9,11,14-16] In the present and previous studies we have demonstrated p53 reactivity within non-neoplastic cells and a correlation between number of p53 immunoreactive cells and severity of inflammation.

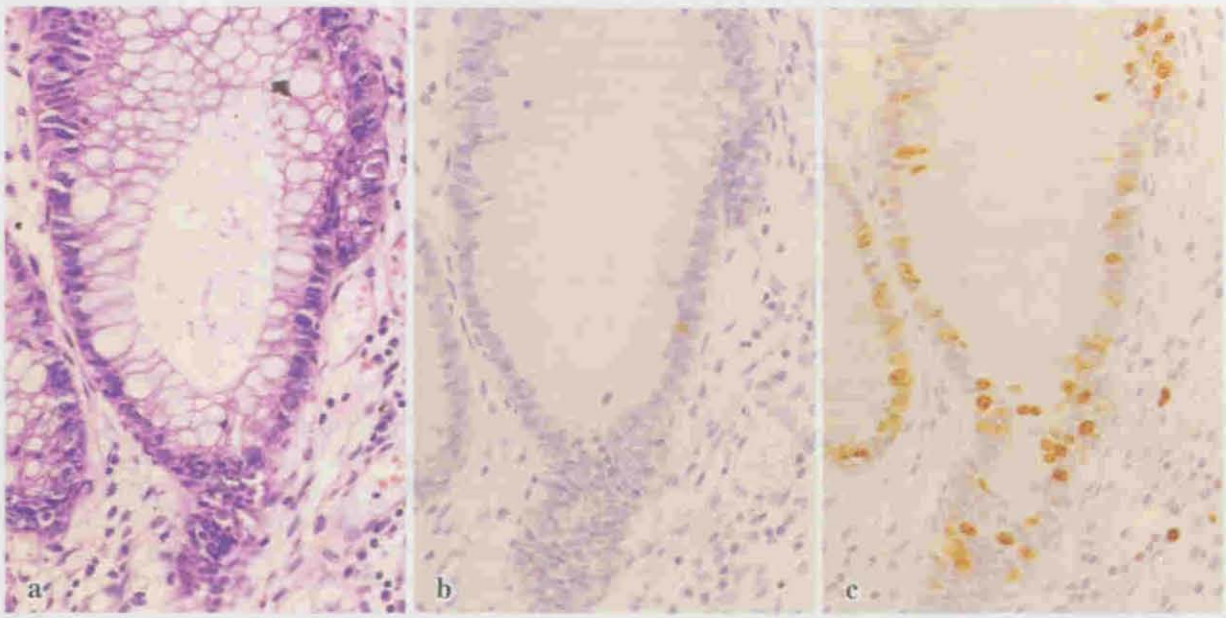

Fig. 1. Ulcerative colitis in remission phase, **b**, **c**. One p53-positive cell is observed within a proliferative zone (NI-2395-51, **a**; H•E staining, **b**; p53 and **c**; Ki-67 immunostaining, each; ×90).

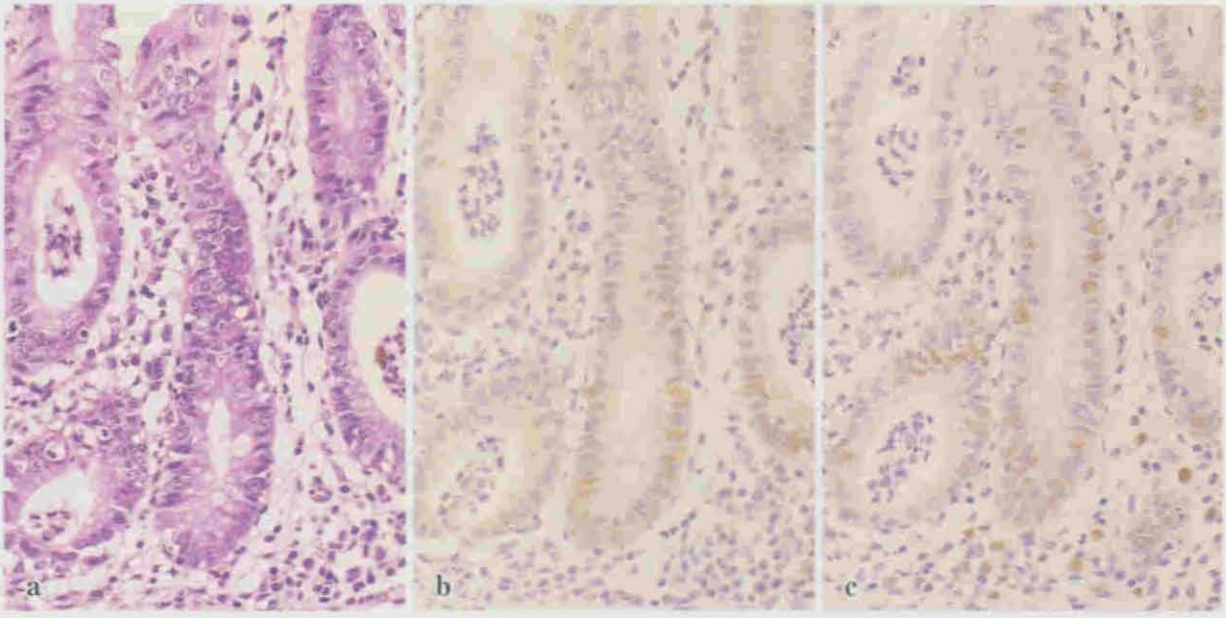

Fig. 2. Ulcerative colitis in active phase, **b**, **c**. Twenty-one p53-positive cells are observed within a proliferative zone (NI-3691-2, **a**; H•E staining, **b**; p53 and **c**; Ki-67 immunostaining, each; ×90).

Overexpression of p53 immunoreactive cells is reported as a useful marker for the diagnosis of carcinoma,[5] because there is a high concordance with p53 mutations.[3,4,7,10,17] If there are no p53-positive cells in non-neoplastic mucosa, even one p53-positive cell would indicate p53 protein overexpression. A few p53 immunoreactive cells do not necessarily indicate mutation, because PAb1801 immunostaining recognizes both the wild-type and mutant forms of p53 protein.[18] Therefore, the definition of p53 protein overexpression needs to be defined. Watanabe et al.[9] reported that overexpression of p53-positive cells,

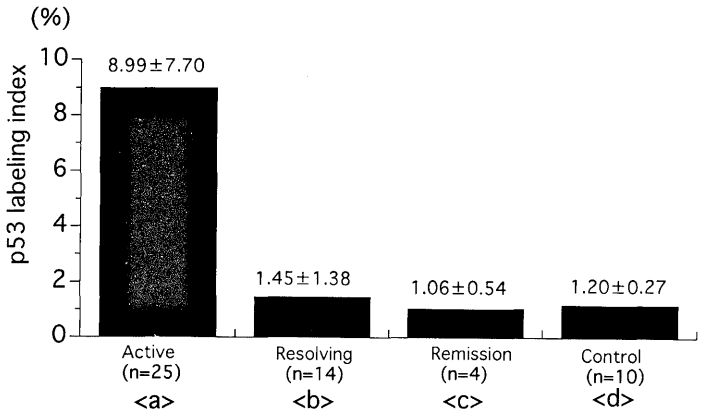

Fig. 3. p53 labeling index in the proliferative zone of tubules in each phase of ulcerative colitis and controls. $p<0.01$: a-b, a-c, a-d; Not significant: b-c, b-d, c-d.

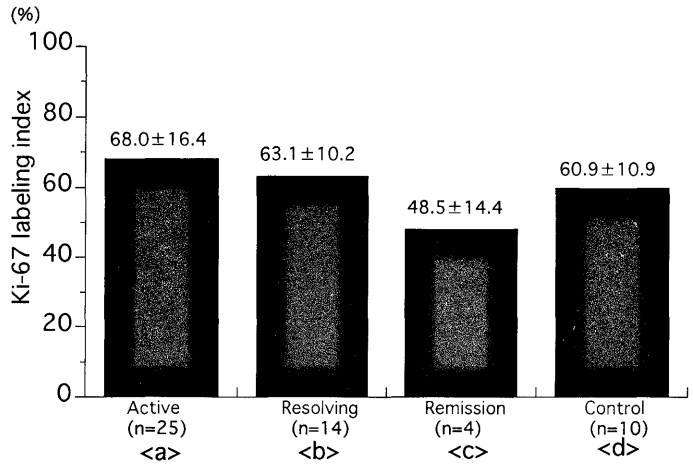

Fig. 4. Ki-67 labeling index in the proliferative zone of tubules in each phase of ulcerative colitis and controls. $p<0.05$: a-c, b-c; Not significant: a-b, a-d, b-d, c-d.

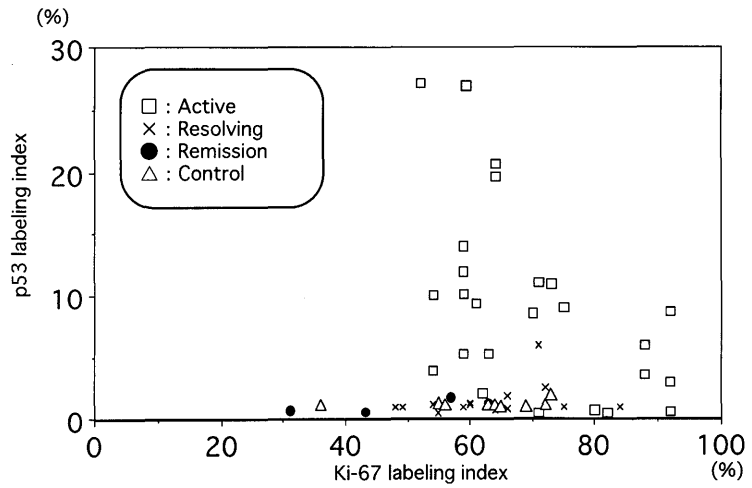

Fig. 5. Correlation between p53 LI and Ki-67 LI in the proliferative zone of tubules with p53-positive cells in ulcerative colitis and controls.

i.e., their nested aggregation or diffuse presence, was not found in non-neoplastic conditions of the colon and rectum. Even within colorectal tubular adenomas, only 4.8% (7/84) of tubules showed a nested p53 positive pattern. Diffuse p53 protein overexpression was detected only in carcinomas. In addition, they reported that the p53 LI/Ki-67 LI percentage was always more than 100% in p53 overexpression.[5,19]

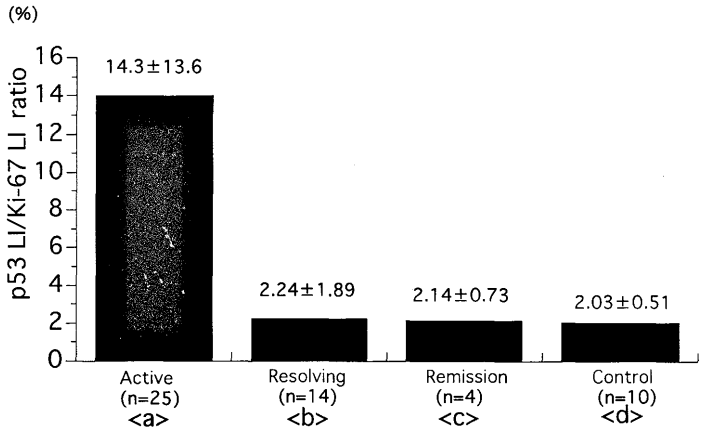

Fig. 6. p53 LI/Ki-67 LI percentage in ulcerative colitis and controls. $p<0.01$: a-b, a-c, a-d; Not significant: b-c, b-d, c-d.

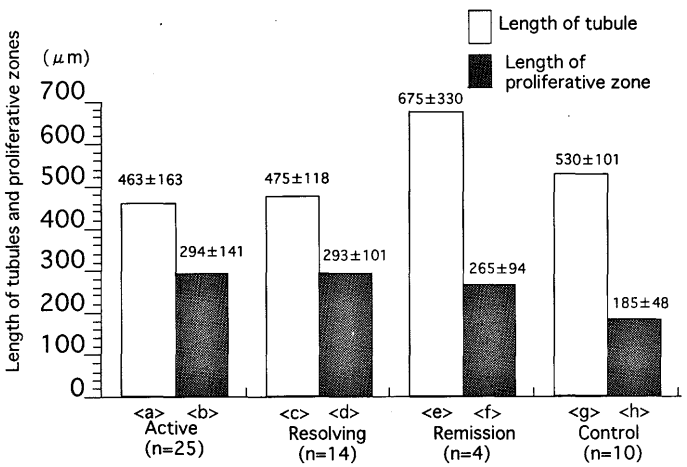

Fig. 7. Length of proliferative zone and tubule in each phase of ulcerative colitis and controls. $p<0.05$: a-e, b-h, d-h; Not significant: a-g, c-e, c-g, e-g, b-d, b-f, d-f, f-h.

From these data, p53 protein overexpression could be defined as a nested pattern or diffuse pattern of p53 reactivity.[5] The scattered pattern should not be regarded as indicative of abnormal p53 expression.

The known function of wild-type p53 includes its action as a G_1 checkpoint of cell cycle, which regulates the proliferative activity of cells.[20-22] In this study, p53-positive cells which occurred in a scattered pattern was greatly affected by the severity of inflammation and limited to the proliferative zone. Therefore, scattered p53-positive cells are likely to contain wild-type p53 protein. This hypothesis on human *in vivo* materials should be explored by immunohistochemistry specific for mutant protein or gene analysis in future studies.

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