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p53 protein expression in colorectal tumors in terms of PCNA and DNA ploidy analysis

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Summary: The expression of a p53 protein was studied by using immunohistochemical technique in 44 patients with colorectal adenoma and 139 patients with colorectal cancer in comparison with the proliferating cell nuclear antigen (PCNA), one of antigens associated with cell proliferation, as well as the DNA ploidy.

The positive p53 protein expression was seen in 15.9 and 61.5% of patients with colorectal adenoma and cancer, respectively. The positive rate of carcinoma was significantly higher than that of adenoma. In adenomas, the p53 expression was not associated with their histological types. However, it had become significantly increased when the degree of the atypia was moderate/severe or more advanced and in tumors as large as 10 mm or greater in diameter. These findings suggest that p53 may play an important role in the process of malignant transformation of colorectal adenoma.

Furthermore, the expression of p53 protein was evaluated in comparison with clinicopathological findings, PCNA, DNA ploidy pattern in colorectal cancer. As a result, the positive p53 protein indicated a trend toward high lymphnode metastasis, a high PCNA labeling index and aneuploidy pattern of DNA analysis showed biologically malignant behavior of malignant cells, demontrating that the p53 portein was not in association with the progression of cancer.

Introduction

The p53 protein was found by Lane et al.[1] in 1979 as a protein bound specifically to SV40 T antigen in cells which had undergone neoplastic transformation caused by oncogenic DNA virus SV40. The protein, derived from the cells, has a molecular weight of 53kd. Early after the detection, it has been clarified that the p53 gene is able to transform cells[2-4] and make them immortal[4,5] if cells are transfected with it. It is widely accepted that a nuclear oncogene, coded by cells, has the same function as *myc, myb, fos*. All these p53 genes of which the presence was confirmed have proved to be mutants.[6] It is impossible that all cells are transformed even if they are transfected with the wild-type p53 gene. In addition, the wild type p53 gene has been reported to inhibit transformation.[7] Consequently, the wild type gene is regarded as an anti-oncogene. It acts as an anti-oncogene, whereas mutant p53 genes are produced by a point mutation or an allelic deletion, as dominant oncogenes. This inconsistency has been explained by inactivation of the wild-type protein derived from the endogenous p53 gene by mutant p53 proteins.[8]

The wild-type p53 protein, which is unstable owing to its short half-life, fails to be identified immunohistochemically. Those, which can immunohistochemically be identified may be stable mutant p53 proteins, are produced by a point mutation and bound to other proteins, mostly the heat shock protein family.[9-11]

The expression of p53 protein as demonstrated immunohistochemically has been reported in many human malignancies including colon cancer,[12] breast cancer,[13] and lung cancer.[14] However, only few studies on p53 proteins in colorectal adenoma and the association of their expression with clinicopathological findings in colorectal cancer have been performed.

The expression of p53 protein in colorectal adenoma and cancer was studied immunohistochemically in an attempt to provide insights into their role in the process of malignant transformation of colorectal adenoma. Then, the correlations among p53 protein expression, clinicopathological findings, PCNA, and the DNA ploidy was investigated in order to clarify their role in the progression of cancer.

Materials and Methods

1) Subjects

Patients who underwent surgical resection for colorectal cancer (n=139) and endoscopic surgery for colorectal adenoma (n=44) at the First Department of Surgery, Nagasaki University, and its affiliated hospitals were included in this study.

2) Immunohistochemical staining of p53 protein

A surgically resected tissue was frozen at − 80 ℃ and sectioned with a cryostat into thin slices of 5 *um* in thickness. Each section was mounted on a poly-L-lysine coated slide glass, air-dried, and fixed for 30 minutes in 4% paraformaldehyde solution. It was treated with PAb 1801, a monoclonal antibody to p53 protein, to stain p53 protein

immunohistochemically by the labeled streptavidine biotin method (LSAB method).

3) Immunohistochemical staining of proliferating cell nuclear antigen (PCNA)

Surgical specimens of colorectal cancer fixed in formalin were embedded in paraffin and cut into slices of 5 um thick. The slices were deparaffinized, and PCNA was stained histologically by the method of LSAB with PC-10, a monoclonal antibody to PCNA. The labeling index (LI) was determined by counting 1000 nuclei.

4) Determination of DNA contents

Fresh tissues of colorectal cancer were cut into pieces with scissors and treated with 0.1% Triton-X 100 for stripping nuclei. The stripped nuclei were treated with RNase, and after adding propidium iodide (PI), DNA contents in nuclei were determined by FACScan. When a fresh tissue was not available, 3 or 4 slices of 50 um thick were cut from a paraffin embedded block, and processed by the method of B. Schutte et al.,[15] and the preparation was stained by PI by the method of Vindelφv.[16]

The DNA index (DI) was determined based on the resultant histogram. Diploid was defined as DI=1.0, whereas aneuploid, as DI ≒ 1.0.

Results

1) Histological features of p53 protein

Tissues of colorectal cancer and adenoma expressing the p53 protein are presented in Fig. 1 and 2. In colorectal cancer, the p53 protein was demonstrated in the nuclei of malignant cells whereas none was found in the nuclei in the normal mucosal layer. In colorectal adenoma, the p53 protein was found in some of the nuclei.

2) Expression of p53 protein in colorectal adenoma

The expression of p53 protein was observed in 7 (15.9%) of 44 patients with colorectal adenoma, and the rates were fluctuated with varying variety, depending on histological types (Table 1). The positive p53 protein expression was not necessarily noted in patients with mild to mild/moderate degrees of atypia. A moderate or more advanced degrees of atypia revealed positive p53 expession with exception. In patients who belong to the two groups that demonstrate either a moderate or less advanced atypia or, a moderate/severe or more advanced atypia, there was a statistically significant difference in frequency of p53 protein expression between the two groups (Table 2). The frequency of p53 protein expression of the tumor of 10 mm or less in diameter was different from that of the tumor of

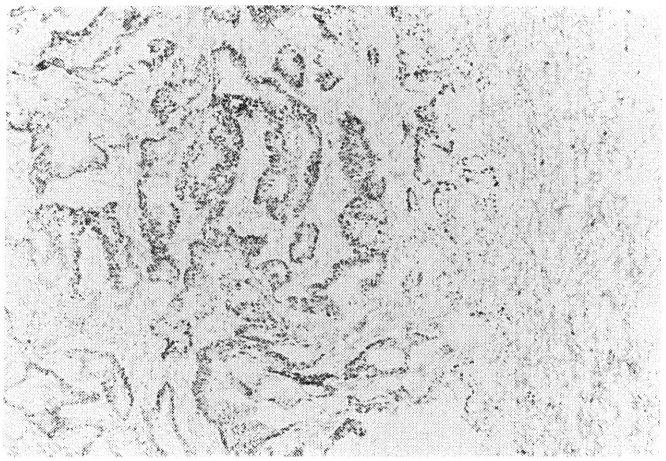

Fig. 1. Expression of the p53 protein in moderately differentiated adenocarcinoma of the colon

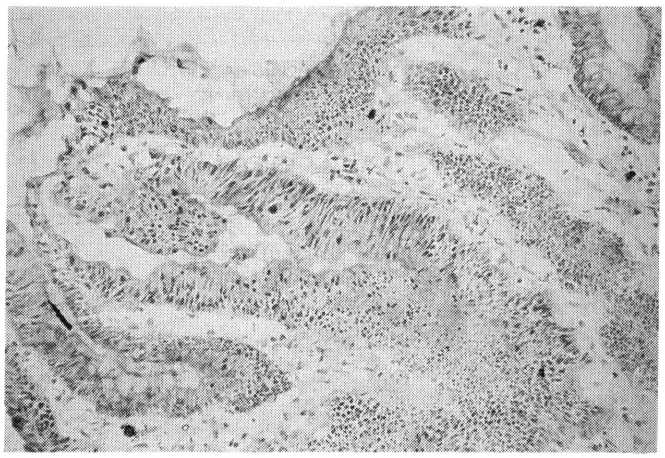

Fig. 2. Expression of the p53 protein in tubulo-villous adenoma of the colon

Table 1. Expression of p53 protein in colorectal adenoma

histological type	No. of cases	expression of p53 protein
tubular adenoma	37	3 (8.1%)
tubulo-villous adenoma	6	4 (66.7%)
villous adenoma	1	0
Total	44	7 (15.9%)

Table 2. Association of expression of p53 protein with degree of atypia in colorectal adenoma

degree of atypia	No. of cases	expression of p53 protein	
mild	1	0	*
mild/moderate	7	0	
moderate	23	1 (4.3%)	
moderate/severe	12	6 (50.0%)	
severe	1	0	

* $p < 0.05$

more than 10 mm, with statistically significant difference (Table 3).

Table 3. Association of expression of p53 protein with tomor size in colorectal adenoma

size (mm)	No. of cases	expression of p53 protein
< 10	31	3 (9.7%)
10 ≦ < 20	12	3 (25.0%) *
≧ 20	1	1 (100%)

* $p < 0.05$

3) Expression of p53 protein in terms of clinicopathological findings, DNA ploidy, and PCNA (LI)

The expression of p53 protein was positive in 83 (61.5%) of 135 patients with colorectal adenocarcinoma, but negative in all patients with mucinous carcinoma or adenosquamous carcinoma (Table 4).

Table 4. Expression of p53 protein in colorectal cancer

histological type	No. of cases	expression of p53 protein
adenocarcinoma	135	83 (61.5%)
mucinous carcinoma	2	0
adenosquamous carcinoma	2	0

The p53 expression in colorectal adenocarcinoma did not correlate with the degree of histological differentiation, the depth of the intestinal wall, the presence of liver metastasis, peritoneal dissemination, and/or distant metastasis. The p53 expression tended to increase in patients with lymphnode metastasis, but the rise was not statistically significant (Table 7). In patients with advanced adenocarcinomas of stage Ⅲ or Dukes'C, the p53 expression was obviously increased without statistically significant difference (Table 8 and 9).

The incidence of DNA aneuploidy pattern was higher than that of diploidy in analysis of DNA ploidy patterns, but the difference was not statistically significant (Table 5).

In analysis of PCNA, the values of LI for the positive cases were liable to be greater than those for negative cases, but the difference was not statistically significant (Table 6).

Table 5. Association of expression of p53 protein with DNA ploidy in colorectal adenocarcinoma

DNA ploidy	No. of cases	expression of p53 protein
diploid	51	28 (54.9%)
aneuploid	71	46 (64.8%)

Table 6. Association of expression of p53 protein with labeling index (LI) of proliferating cell nuclear antigen (PCNA) in colorectal adenocarcinoma

expression of p53 protein	No. of cases	LI of PCNA
negative	37	52.7 ± 9.66
positive	59	56.2 ± 11.5

Table 7. Association of expression of p53 protein with clinicopathological findings in colorectal adenocarcinoma

	No.of cases	expression of p53 protein
histological differentiation		
well	40	22 (55.0%)
moderately	89	57 (64.0%)
poorly	6	4 (66.7%)
depth the intestinal wall		
≦ sm	5	3 (60.0%)
≧ pm	130	80 (61.5%)
lymphatic invasion		
negative	13	8 (61.5%)
positive	122	75 (61.5%)
venous invasion		
negative	83	50 (60.2%)
positive	52	33 (63.5%)
liver metastasis		
negative	127	78 (61.4%)
positive	8	5 (62.5%)
peritoneal dissemination		
negative	129	79 (61.2%)
positive	6	4 (66.7%)
lymphnode metastasis		
negative	77	43 (55.8%) *
positive	58	40 (69.0%) *
distant metastasis		
negative	133	81 (60.9%)
positive	2	2 (100 %)

* $p < 0.1$

Table 8. Association of expression of p53 protein with stage classification in colorectal adenocarcinoma

stage	No. of cases	expression of p53 protein
I	12	6 (50.0%) *
II	59	33 (55.9%) *
III	25	18 (72.0%) *
IV	21	14 (66.7%) *
V	18	12 (66.7%) *

* $p < 0.1$

Table 9. Association of expression of p53 protein with Dukes' stage in colorectal adenoma

Dukes' stage	No. of cases	expression of p53 protein
A	12	6 (50.0%)
B	65	37 (56.9%) *
C	58	40 (69.0%) *

* $p < 0.1$

Discussion

Tissues of colorectal adenomas and carcinomas were immunohistochemically searched with PAb 1801 for identifying p53 protein. In this series, this protein was expressed in 15.9 and 61.5% of patients with colorectal adenoma and cancer, respectively.

The agent PAb 1801 used in this study is a monoclonal antibody which can recognize both wild-type and mutant p53 proteins. The detection of the p53 protein in the surgical specimens with this agent enabled us to demonstrate exclusively in the nuclei of malignant cells and adenoma. None was found in normal cells. It is accepted that the wild-type p53 protein is unstable, due to its short half-life. As a result, it is defined that the wild type p53 protein fails to be detected by the conventional immunohistochemical staining technique. On the basis of this fact, all detected in this study must be mutant p53 proteins. It is concluded that this simple immunohistochemical testing with PAb 1801 is of great value to exclusively demonstrate individual mutants and reveal their histological distributions.

It is well known that gene alterations in colorectal cancer include point mutations of the ras gene and allelic deletions in chromosome 5q, 17p and 18q, which have been reported by Vogelstein et al. The allelic deletion in 17p was demonstrated in 70% or more of patients with colorectal cancer.[17] The gene for the p53 protein is located on the short arm of NO.17 chromosome or 17p. Baker et al. reported an allelic deletion in association with malignant transformation in adenoma and point mutations of the remaining alleles, suggesting the possibility that the gene is strongly deranged in the course of malignant transformation of adenoma.[18]

According to Van Den Berg et al.,[12] the p53 protein is increasingly expressed as malignancy of lesions progressing from a normal epithelium to dysplastic adenoma, and further progressing to cancer. This suggests a possibility that the p53 protein can be used as a marker for tracing the adenoma-carcinoma sequence in the colorectum. The present study indicated that the p53 protein expression increased as the dysplasia of colorectal adenoma progressed in grade and as the tumors increased in size. In addition, it was expressed in about 60% of patients with colorectal cancer. Consequently, it is assumed that p53 is greatly influenced by the process of malignant transformation of colorectal tumors.

Colorectal cancer was defined by many factors such as the depth of the intestinal wall, lymphnode metastasis, liver metastasis, peritoneal dissemination, disease stages of colorectal cancer. In particular, the disease stage by Dukes' classification correlates well with the prognosis in the clinical use. The DNA ploidy analysis also contributes to prediction of the prognosis.[19] Only a few studies have been performed to evaluate the prognostic value of p53 protein. Rembicos et al. who used FCM to monitor the p53 protein and DNA content simultaneously demonstrated the p53 protein in 54% of patients with colorectal cancer. They also reported that it was expressed at a significantly higher rate in cases of aneuploid than diploid, but the frequency did not vary significantly depending on stages of Astler and Collor.[20] Cattoretti et al. examined 200 patients with breast cancer for the p53 protein, and found that there is a correlation between the expression and estrogen receptor-negative, growth factor receptor-positive, high-grade tumors. They stated that the p53 protein could be a new parameter for assessing cellular biology and prognosis in breast cancer.[13]

In the present study, however, the expression of p53 protein tended to increase when lymphnode metastasis is positive or carcinomas progress in Stage III or more advansed and Dukes'C classification. Furthermore, it is more likely to increase when the DNA ploidy analysis shows aneuploid. There is no correlation each other with statistically significant difference. With respect of the value of LI of PCNA, there was a tendency that this index is greater in case of that the p53 protein is positive than negative although the tendency was not statistically significant. Thus, this study clarified that p53 protein expression did not relate to any prognostic factor including cytologic and histologic malignant findings in colorectal cancer. Consequently, it is obvious that this result does not indicate the fact that p53 protein plays a key role in the progression of cancer.

In colorectal adenoma, the p53 protein expression was revealed in 15.9% of the entire patients and in 9.7% of the patients with the tumors of less than 10 mm in diameter. It is indicated that there is a good possibility to become carcinoma in a tumor of less than 10 mm in diameter if the p53 protein is expressed even if the precise diagnosis of adenoma had been made pathologically. In conclusion, the p53 protein expression is of great help to predict the possibility of transformation from adenoma to carcinoma in a follow-up study, warning how much a malignant potential of adenoma should be taken into consideration.

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