

VALUE OF P53 PROTEIN EXPRESSION AND ITS RELATIONSHIP WITH SHORT-TERM PROGNOSIS IN COLORECTAL CANCER

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P53 mutations are the most frequently detected genetic alteration in human cancer.^{1,2} The p53 gene is located on the short (p) arm of chromosome 17, and 17p deletions are found in 6%-25% of colonic adenomas and in as many as 75% of colonic carcinomas.^{3,4} Mutations of p53, often accompanied by loss of the wild-type allele, typically occur when an *in situ* neoplasm develops into invasive malignancy.³ Wild-type p53 acts to regulate transcription, and when cells are damaged by UV light, irradiation or chemotherapeutic agents, p53 accumulates and helps to stop cells in the G1-phase of cell cycle.⁵ Wild-type p53 binds to double-stranded DNA at specific sequences as a tetramer. In some cases where the presence of a mutant p53 allele and loss of the wild-type allele result in tumor formation due to the loss of the growth-suppressive function of p53, it acts as a tumor suppressor gene. Consistent with this finding, the growth of some cancer cell lines having only a mutant p53 allele can be suppressed by transfection of the wild-type p53 gene into these cells.⁶

Mutant p53 protein could act in a dominant-negative fashion to inhibit the function of wild-type p53, resulting in a growth advantage for these clones of cells. Once point mutation occurs on one allele, the other allele is rapidly lost, resulting in the high incidence of concomitant p53 mutation plus chromosomal loss.⁶ Mutant p53 expression is found in as many as 70% of colorectal carcinomas.⁷ Previous studies have shown that allelic loss of 17p, indicative of p53 mutation, is associated with a likelihood of distant metastasis, poor prognosis, and DNA aneuploidy.⁸

The mutant p53 gene product has a prolonged half life and is, therefore, detectable by immunohistochemistry which seems to be a valid test for p53 mutation in colorectal cancer.⁹ Immunohistochemical studies have also demonstrated that nuclear p53 protein over-expression in the primary colorectal carcinoma is associated with decreased survival in both patients with positive lymph

FIGURE 1. A case of moderately differentiated adenocarcinoma of the colon showing p53 positivity diffusely present in the nuclei of the pleomorphic tumor cells (anti p53, 100x).

FIGURE 2. A case of well-differentiated adenocarcinoma of the colon showing p53 positivity in tumor cells. p53 staining is not seen in normal cells (anti p53, 200x).

nodes¹⁰ and those associated with advanced hepatic metastasis.¹¹ This study investigates the relation between expression and percentage of p53 positive cells and eight clinicopathologic variables, and the potential role of p53 molecule as a valuable prognostic marker in colorectal cancer.

Materials and Methods

Twenty-eight patients, comprising 12 women and 16 men (aged 43-84, median 65 years) with colorectal cancer were included in this study which took place from May to November 1997. Surgical resection was performed in all patients. Samples of tumor and adjacent normal mucosa after surgical resection were removed from the specimen and later used for immunohistochemical examination.

Dukes' stage was used for staging these patients at the time of resection and specimens were reviewed by the same pathologist for histological grade, lymph node metastasis and immunohistochemistry. Postoperative Dukes' stage distribution was: 1 patient (4%) with Dukes' A; 13 patients (46%) with Dukes' B; 13 patients (46%) with Dukes' C; and 1 patient (4%) with Dukes' D. Fifteen (54%), 6 (21%), 6 (21%), and 1 (4%) tumors were located in the rectum, ascending colon, descending colon and transverse colon, respectively. Continuous follow-up was done for 24 of 28 cases (mean follow-up = 32.9 months, range 10-43 months). Data on degree of differentiations, tumor volume, lymph node metastasis, recurrence or metastasis were evaluated.

Immunohistochemical Method for p53 Expression

Twenty-eight consecutive colorectal cancer resection specimens obtained from 28 patients were examined. Samples of tumor and adjacent normal mucosa were fixed in 10% neutral-buffered formalin embedded in paraffin, and 5 µm consecutive sections were cut. The avidin-biotin

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peroxidase complex (ABC) method was used for immunostaining. Sections obtained from formalin-fixed material were cut, dewaxed and treated with 0.3% hydrogen peroxide (H₂O₂) in methanol for 30 minutes to quench the endogenous peroxidase activity. Slides were rinsed in distilled water and incubated for 10 minutes at 750W in 10 mM citrate buffer in a thermoresistant container. Distilled water and buffer were added periodically to the container to prevent drying during the incubation process. The slides were cooled in buffer for 20 minutes to room temperature, washed in distilled water and rinsed in phosphate-buffered saline (PBS). The primary antibody was applied to the sections and incubated for 30 minutes at room temperature. This was followed by incubation with a 1/100 dilution of biotin-labeled anti-mouse secondary antibody for 15 minutes and ABC for 15 minutes. Careful rinses with PBS between each step of the procedure were performed. The color was developed with diaminobenzidine (DAB) solution and the sections were lightly counterstained with hematoxylin, dehydrated, and mounted. For immunohistochemical staining of p53, the primary antibody used was p53 protein clone-7, diluted 1:100 (Dako, Copenhagen, Denmark). Protein expression was determined by nuclear staining of tumor cells. In each tumor, percentage of p53 positive cells was calculated. A tumor

TABLE 1. The distribution of p53 expression (%), the percentage of p53 (+), cells (%) and their relations with common clinicopathologic variables.

Clinicopathologic variables	Cases (n)	p53 positivity (%)	p53(+) cells (%)
Sex			
Female	12	75	34
Male	16	87	35
Age			
<65 (years)	13	69	28
>65 (years)	15	93	50
Tumor location			
Ascendant	6	67	22
Descendant	6	50	31
Rectum, transverse	15	100*	40
Tumor size			
<4 cm	16	87	
>4 cm	12	75	
Tumor grade differentiated			
Poorly	7	71	30
Other	21	86	37
Stage			
Dukes A + B	14	82	39
Dukes C + D	14	88	45
Lymph node metastasis			
Negative	18	89	36
Positive	10	70	33
Recurrence and/or metastasis			
Negative	14	77	26
Positive	10	90	54*
Out of follow-up	4		19

*p<0.05.

TABLE 2. Actual number of cells used for calculating the p53 percentage in each patient.

Patient no.	Number cells (%)
1	80
2	18
3	40
4	–
5	80
6	61
7	30
8	55
9	74
10	8
11	20
12	24
13	–
14	20
15	20
16	15
17	20
18	50
19	48
20	40
21	–
22	70
23	80
24	–
25	–
26	15
27	20
28	70

with less than 5% tumor cell nuclei showing p53 staining were scored as p53 negative. All other tumors showing p53 immunoreactivity were considered to be positive.

Statistical Analysis

The frequency of p53 positive tumors was compared for each variable by using chi-squared analysis and Fisher's exact test. The method of *One-way Anova* was used for percentage of p53 positive cells. Values of $P < 0.05$ were considered to be significant in all analyses.

Results

P53 staining was positive in 23 of 28 patients (82.1%) with adenocarcinomas. Staining was confined to malignant nuclei and was never found in adjacent normal mucosa. The relationship between p53 expression and several clinicopathological variables are summarized in Table 1. No correlation was found between p53 staining with sex, age, tumor volume, tumor grade, tumor stage, lymph node metastasis and recurrence and/or metastatic cancer.

Interestingly, carcinoma of the rectum showed much more p53 over-expression than carcinomas of the colon ($P < 0.05$).

In the study, the percentages of p53 positive cells were also compared with several clinicopathologic variables. The results are summarized in Tables 1 and 2. There was no correlation between the percentage of p53 positive cells and sex, age, tumor site, tumor volume, tumor grade, tumor stage and lymph node metastasis. However, recurrent and/or metastatic cancer showed higher percentage of p53

positive cells than non-metastatic or non-recurrent cancers ($P<0.05$)

Discussion

The disease stage which was assessed by serosal invasion and metastases of lymph node, liver and peritoneum, is the most important prognostic factor reflecting a patient's five-year survival. These parameters predicting the disease stage as well as other parameters such as DNA ploidy, cell proliferative activity and protooncogene products have been studied frequently. In colorectal carcinoma, there are many reports that couple protooncogene p53 products with the malignant potential.^{12,13} Alteration of this gene has been associated with postoperative outcome and poor prognosis.¹⁴ This may be due to loss of wild type p53 tumor suppressor function, radioresistance or chemoresistance, or acquisition of oncogenic properties.

A review of the literature shows that over-expression of p53 can be an independent significant predictor for survival,¹⁵ but some authors failed to show the independent prognostic value of p53 in colorectal cancer.^{16,17} In this study, the relationship between p53 over-expression and several clinicopathologic variables in 28 colorectal cancer patients were examined. Regarding the relationship between p53 immunoreactivity and some clinicopathologic variables, no significant correlation was found between p53 expression and sex, age, tumor volume, tumor grade, tumor stage, lymph node metastasis, recurrence and or metastatic cancer. But there was a significant correlation between p53 expression and rectal carcinomas ($P<0.05$). The percentage of p53 positive cells were also compared with these clinicopathologic variables in this study. No correlation were found between the percentage of p53 positive cells and sex, age, tumor site, tumor volume, tumor grade, tumor stage and lymph node metastasis. However, there was significant correlation between the percentage of p53 positive cells and recurrent and/or metastatic cancer ($P<0.05$).

Although the present study had a small number of cases with short-term follow-up, the immunohistochemical assessment of p53 and, more importantly, the percentage of p53 positive cells may be valuable in predicting the risk of recurrence and metastasis after curative surgery of colorectal cancer. Since the conventional clinicopathological prognostic factors cannot be assessed in the preoperative evaluation of colorectal carcinoma, assessment of the percentage of p53 positive cells on samples obtained during diagnostic endoscopic biopsies can be important in the preoperative evaluation of colorectal carcinomas.

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