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Clinical significance of immunohistochemical study of p53 protein in colorectal carcinoma

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Abstract

AIM: To determine the clinical significance of p53 protein expression in colorectal carcinoma.

METHODS: The expression of p53 protein was examined in 92 colorectal carcinomas using the monoclonal antibody PAb 1801. Correlation between p53 protein expression and prognosis in colorectal carcinoma was analyzed using the log-rank test.

RESULTS: The frequency of p53 protein expression was 57.61%, corresponding with Dukes' stage of bowel cancer. Analysis of survivor data demonstrated that the survival rate of the colorectal carcinoma with positive staining for p53 protein was lower than that of group with negative staining.

CONCLUSION: Expression of p53 protein is correlated with poor prognosis in colorectal carcinoma.

Key words: Colorectal neoplasms; Oncogenes; Protein p53; Immunohistochemistry

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INTRODUCTION

p53 gene mutations and differential p53 protein expression occurs in most human tumors[1-5]. In the case of breast carcinoma, the immunoreactivity of p53 protein has been shown to correlate with poor prognosis[5,6]; however, whether p53 protein expression is correlated with prognosis of colorectal carcinoma remains controversial.

MATERIALS AND METHODS

Clinical data

Ninety-two patients with colorectal carcinoma were included in this study. The mean age at operation was 55.53 ± 13.10 (range: 23-82)-year-old, and the cohort consisted of 49 males and 43 females. The distribution of these tumors according to the Dukes' staging system was 17 cases of stage A, 33 cases of stage B, and 42 cases of stage C. The histological classification of tumors was based on previous criteria[7], and consisted of 9 well-differentiated adenocarcinoma, 62 moderately-differentiated, and 21 poorly-differentiated. Sixty-three of the patients were followed up for 4 years after operation.

Immunohistochemistry

Immunoperoxidase staining was performed on sections of formalin-fixed paraffin-embedded tumor blocks using monoclonal antibody derived from fusion of BALB/C splenocytes with NB-1 mouse myeloma cells, specific for human p53 protein with binding near the N terminals (clone PAb 1801; Oncogene Science Inc, United States). For the staining procedure, 5-micron sections were deparaffinized, rehydrated and placed in a solution of 1% H_2O_2 in methanol to inactivate endogenous peroxidase. After washing with tap water and PBS (2 × 5 min each), the sections were incubated with 10% normal goat serum for 30 min to reduce nonspecific staining. The specimens were then incubated with 1:50 PAb 1801 in a moist chamber overnight at 4 ℃. The sections were washed with PBS, incubated with a 1:400 dilution of biotinylated rabbit anti-mouse IgG (Dako A/S, Denmark) at 37 ℃ for 30 min, and covered with a 1:400 dilution of avidin-HRP (Dako A/S) at 37 ℃ for 30 min. After a final wash with PBS, the antibody staining was visualized by exposure to diaminobenzidine in TBS containing fresh hydrogen peroxide (0.01% H_2O_2). As a last step, the slides were lightly stained in Mayer's hematoxylin. Negative controls were prepared by replacing the primary antibody with PBS.

Statistical analysis

Correlations between the p53 protein expression and clinico-pathologic features were determined by using the chi-square test with Yates' correction. Survival data was analyzed by using the log-rank test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The p53 immunostaining was localized to the nuclei of neoplastic

Table 1 p53 protein expression in colorectal cancer

Dukes' stage	*n*	p53 (+), *n* (%)	Differentiation	*n*	p53 (+), *n* (%)
A	17	6 (35.30)	well	9	4 (44.44)
B	33	19 (57.58)	moderate	62	37 (59.67)
C	42	28 (66.67)	poor	21	12 (57.14)

$P < 0.05$ *vs* Dukes' stage C. Survival rate of patients with p53 positive staining was compared with that of patients with p53 negative staining. Patients with p53 positive colorectal carcinomas showed a significantly shorter survival period as compared with those without p53 ($P < 0.05$).

cells. Normal colorectal mucosa and the control group were completely negative for p53 protein. The majority of p53 protein in colorectal carcinomas showed a uniform immunostaining pattern throughout the carcinomatous tissues for all or nearly all malignant cells. In 92 patients with colorectal carcinoma, 53 (57.6%) had positive immunostaining for p53 protein. Positive staining for p53 protein was found in 35.3% (6/17) of patients with Dukes' A, 57.6% (19/33) of Dukes' B and 66.7% (28/42) of Dukes' C. The proportion of positively reacting colorectal tumors increased as the Dukes' staging progressed, and the incidence of p53 expression in Dukes' stage C was significantly higher than that in Dukes' stages A and B ($P < 0.05$). However, no relationship between p53 protein expression and tumor differentiation was found ($P > 0.05$).

DISCUSSION

The *p53* gene mapped on chromosome 17 short arm contains 11 exons and 10 introns[8,9]. Among these, exons 5 through 8 are considered a mutant hot spot due to the inclination of those sequences towards mutation[9]. The *p53* gene is an important tumor suppressor gene. The wild-type *p53* gene can suppress tumor cell growth and reverse the transformed phenotype[10,11]. Baker *et al*[12] reported that when cells were transfected with the wild-type *p53* gene they formed colonies 5- to 10-fold less efficiently than cells transfected with the mutant *p53* gene. The p53 protein, encoded by the *p53* gene, functions in negative growth regulation to control cell proliferation[13]. This is role is based in part on its ability to bind DNA[14]. Most mutations that occur in any of the four evolutionarily conserved domains alter the conformation of the p53 protein[15], which result in DNA binding that is weaker than that of the wild-type p53. Mutant p53 proteins have an increased half-life, and they are more stable than the wild-type p53 in tissue and can be more readily detected by immunohistochemistry. Expression of the p53 protein has been demonstrated immunohistochemically in a number of human malignancies, including carcinomas of colon[1], breast[2] and lung[3]. In breast cancer, p53 expression is reportedly related to estrogen, growth factor receptor status and tumor infiltration, as well as to poor prognosis[5,6]. Therefore, p53 expression in breast cancer represents as a new marker for judging prognosis. However, opinions regarding its precise role in determining prognosis are varied among the authors in the literature.

Using the log-rank test to analyze the follow-up data of 64 cases, we found that survival rate of patients with colorectal carcinoma who showed positive staining for p53 protein was lower than the group of patients showing negative staining. The question that comes to the forefront then is: why does p53 expression correlate to poor prognosis in colorectal carcinoma? We believe that the p53 protein detected by immunohistochemistry may include almost all mutant forms of the p53 protein, and that the overall ability of this detected p53 profile to bind DNA and suppress cell proliferation was decreased compared with a profile of primarily or exclusively the wild –type form. Therefore, the colorectal carcinoma showing positive p53 protein staining has more malignant potential and a related shorter survival period than that with negative p53 protein staining.

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