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An Immunohistochemical Study of the Expression of p53 Protein in Colon Cancer

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A total of 471 cases of colonic adenocarcinomas and 28 cases of colonic adenomas were examined immunohistochemically to evaluate the expression of p53 protein in the light of their relationship with various prognostic factors. A monoclonal antibody, p53 DO-7, was used in the study.

Two hundred and fourteen adenocarcinomas(45.5%) showed positive staining for p53, however only three of the adenomas(10.3%) were positive($P<0.05$). p53 was stained to neoplastic nuclei. Adjacent normal mucosal cells were negative.

There were no significant correlations between p53 expression and prognostic parameters such as age, sex, gross configuration, modified Astler-Coller stages, microscopic tumor growth patterns, tumor depth, tumor size and lymph node involvements. However, left sided adenocarcinomas(49.3%) expressed p53 more often than right sided adenocarcinomas(35.6%) ($P=0.01$).

The positive rates were different according to the histologic differentiation; 45.2% in well differentiated, 51.3% in moderately well differentiated, 23.8% in poorly differentiated, and 26.5% in mucinous carcinomas($P=0.011$).

The mean survival periods of the p53 positive and negative groups were 29 months and 32 months, respectively($P=0.385$). However, overall survival for patients with grade one and two positive p53 was better than those of grade three and four positive cases($P=0.028$).

In conclusion, the result of this multivariate analysis suggests that immunohistochemically strong p53 protein expression(more than 30% of tumor cells) has value in estimating a prognosis for patients with colorectal adenocarcinomas.

Key Words : *Colon, Adenocarcinoma, p53 protein, Immunohistochemistry, Prognosis*

INTRODUCTION

p53 is a well-known 53-KDa nulcear phosphoprotein and is located on the chromosome 17p13.1. The p53 protein regulates DNA replication, cell proliferation, and cell death. Although the precise mechanisms by which p53 acts as a tumor suppressor gene are not known, accumulating evidence suggests that normal(wild-type) p53 acts as a molecular policeman

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that prevents propagation of genetically damaged cells(Cotran et al., 1994). It is also known to be mutated in various kinds of human tumors and cell lines. The immunohistochemical detection of p53 is associated with the presence of mutated forms of p53 alleles and several immunohistochemical expressions of p53 protein for human tumors, including carcinomas of the colon, breast, lung, stomach, thyroid, and liver, on frozen and paraffin sections have been recently reported(Iggo et al., 1990 ; Dobashi et al., 1993 ; Martinazzi et al., 1993 ; Collier et al., 1994 ; Hurlimann and Saraga, 1994 ; Bertorelle et al., 1995).

In the case of breast carcinoma, this immunoreactivity has been shown to be correlated with a number of clinical and histopathologic indicators of poor prognosis and may become useful in the future in determining therapeutic strategies(Cattoretti et al., 1988). However findings in colon carcinoma are equivocal(Scott et al., 1991 ; Remvikos et al., 1992 ; Starzynska et al., 1992 ; Sun et al., 1992 ; Yamaguchi et al., 1992 ; Linden et al., 1994).

The objective of this study is to evaluate the value of immunohistochemical detection of p53 protein in routinely formalin-fixed tissue as a prognostic marker in colon adenocarcinoma.

MATERIALS AND METHODS

Patients

Four hundred and seventy one cases of adenocarcinomas and 29 adenomas were included in this study. All 465 patients with colorectal carcinoma underwent resection of the colon and rectum with extensive lymph node dissection from January 1983 to August 1994 at Kyung Hee University Hospital. Six patients had double primary colonic adenocarcinomas. The median age of the 465 carcinoma patients was 54.5 years(range, 20-84 years) and male to female ratio was 1.27 : 1.

Two hundred and nineteen tumors were located in the rectum, 96 in the sigmoid, 18 in the descending colon, 27 in the transverse colon, 87 in the ascending colon, and 23 in the cecum. For further analysis they were divided into right sided colon(132 cases) and left sided colon(338 cases). Grossly adenocarcinoma took one of four forms ; polypoid, fungating, ulcerative, or diffusely infiltrative(Ming and Goldman, 1992). Fifty-seven cases were polypoid, 167 were fungating, 244 were ulcerative, and three were diffusely infiltrative. The authors reviewed all the H-E slides and reclassified histologic types using WHO criteria(Jass and Sobin, 1989). Colorectal adenocarcinomas were predominantly well differentiated(219 cases) or moderately well differentiated(197 cases), 21 tumors were poorly differentiated and 34 were mucinous carcinomas. The histological growth pattern was assessed by the criteria of Ming and Goldman(1992). Number of cases with the infiltrative growth pattern was 282 and the that of expanding pattern was 189. The stages were made for colorectal carcinoma according to the criteria of Astler-Coller(1954) and Turnbull et al.(1967). The numbers of colorectal tumors was 15, 215, 210, and 30 in Astler-Coller and Turnbull et al. stages A-D, respectively.

Immunohistochemistry

Paraffin-embedded sections were deparaffinized in xylene and dehydrated with graded ethanol. After quenching the endogenous peroxidase activity in 0.3% hydrogen peroxide for 10 minutes, nonspecific binding was blocked by treatment with normal horse serum for 10 minutes. Primary anti-p53 antibody DO7, (Novocastra Laboratories Ltd., Newcastle, UK) was applied to the sections at a dilution of 1 : 100 and incubated in a moist chamber for 90 minutes at room temperature. After washing in TRIS buffer saline(Dakopatts, Copenhagen, Denmark), biotinylated horse anti-mouse immunoglobulin(Novocastra Laboratories Ltd., Newcastle, UK) was applied and the sections were incubated for 10 minutes at room temperature. After a thorough washing in TRIS buffer saline, avidin-biotin-peroxidase complex(Novocastra Laboratories Ltd., Newcastle, UK) was applied and the sections were incubated for an additional 10 minutes. After washing out the excess complex, the localization of p53 was visualized by incubating the sections for 10 minutes in 3, 3' - diaminobenzidine tetrahydrochloride (Research Genetics, Huntsville, USA). Negative control sections were made without the primary antiserum to p53.

The pattern of immunoreactivity was considered as "negative" when no positive cells were found, grade one positive when only some isolated positive cells were occasionally identified, grade two positive when wide clusters of positive cells were seen in less than 30% of the area of tumor, grade three positive when sheets of positive cells were found in 30%~60% of the area of the tumor, and grade four positive when sheets of positive cells were in more than 60% of the

area of the tumor.

Statistical analysis

The p53 expression in colorectal carcinomas was compared with clinical and histological features, including age, sex, gross configuration, tumor location, histologic grade, tumor stage, microscopic growth pattern, tumor depth, tumor size, and lymph node involvement. A follow-up of patients whose tumors were examined in this study is currently in progress. Eighty six patients were dead, 381 were alive and 84 were lost to follow-up or dead by other diseases(follow-up rate=82.2%). Statistical analysis was done with chi-square test. By using the proportional hazards model of Cox, multivariate analysis was done of the factors said to affect the prognosis in patients with colorectal carcinomas. Kaplan-Meier survival curve was constructed using the SPSS and assessed using the log rank test to compare the differences of survival among the subgroups.

RESULTS

p53 was detected in 214 out of 471(45.4%) colorectal adenocarcinomas and in 3 out of 28(10.7%) colonic adenomas. The p53 positive staining was localized in the nuclei of carcinoma cells. Staining within the tumor nuclei was granular or reticular in nature. Cytoplasmic staining was not observed. Uniform negative reactivity with anti-p53 antibody was seen in normal adjacent epithelium(Fig. 1). The number of positive cells and staining intensity varied from case to case. All of the three p53 positive adenomas were grade one positive. In the 214 positively stained carcinomas, 53 cases(24.8%) displayed grade one positive pattern of immunoreactivity, 55 cases(25.7%) displayed grade two positive pattern, 48 cases (22.4%) showed grade three positive pattern, and 58 cases(27.1%) showed grade four positive pattern.

The relationship between p53 expression and several clinico-pathological criteria related to prognosis is shown in Table 1. No correlation was found with age, sex, gross configuration, stage, microscopic tumor growth pattern, tumor depth, tumor size and lymph node involvement. Left sided carcinomas expressed 49.3% p53 immunopositivity and right sided carcinomas expressed 35.6%(P=0.01). The percentages of positive cases differed in each histological subtype : 45.2% in well diferentiated, 51.3% in mod-

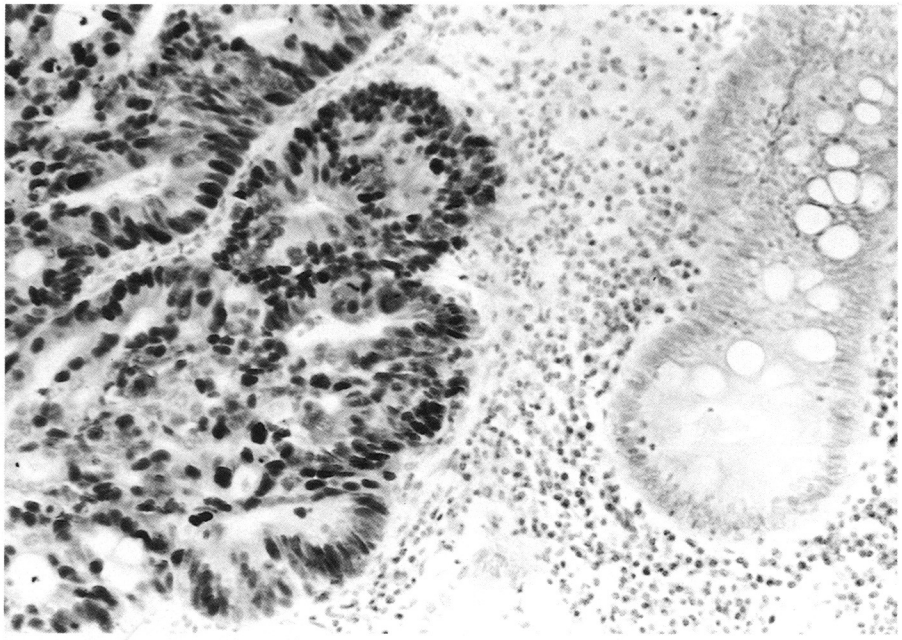

Fig. 1. A colorectal adenocarcinoma showing strong nuclear p53 staining and negative staining in the adjacent normal mucosal gland.

Table 1. p53 Expression and Clinicopathological Features Related to Prognosis

Clinicopathological finding	No. of case	No. of p53 positive	P value
Age			0.065
< 29	18	7(38.8 %)	
30~59	267	112(41.9 %)	
> 60	180	95(52.8 %)	
Sex			0.686
Male	260	117(45.0 %)	
Female	205	97(47.3 %)	
Gross configuration			0.256
Polypoid	57	26(45.6 %)	
Fungating	167	69(41.3 %)	
Ulcerative	244	119(48.7 %)	
Diffusely infiltrative	3	0	
Tumor site			0.01
Right colon	132	47(35.6 %)	
Left colon	339	167(49.3 %)	
Histologic grade			0.011
Well	219	99(45.2 %)	
Moderate	197	101(51.3 %)	
Poor	21	5(23.8 %)	
Mucinous	34	9(26.5 %)	
Modified Astler-Coller's stage			0.165
A	16	4(25.0 %)	
B	215	91(42.3 %)	
C	210	106(50.5 %)	
D	30	13(43.3 %)	
Microscopic growth pattern			0.382
Infiltrative	282	123(43.6 %)	
Expanding	189	91(48.1 %)	
Tumor depth			0.402
Early	23	8(34.8 %)	
Advanced	448	206(46.0 %)	
Tumor size			0.171
< 5cm	192	95(49.8 %)	
> 5cm	279	119(42.7 %)	
Lymph nodes			0.114
Positive	231	114(49.4 %)	
Negative	240	100(41.7 %)	

erately well differentiated, 23.8% in poorly differentiated, and 26.5% in mucinous carcinomas(P=0.011). The percentage of stained nuclei, intensity of staining, and distribution of the stained areas did not correlate with the grade of differentiation and other prognostic factors.

Three hundred and eighty seven instances of follow-up data were analysed(range, 1 month to 121 months). In using the proportional hazards model of Cox, multivariate analysis demonstrated that histologic type, microscopic growth pattern, stage and p53 immunoreactivity were significant prognostic factors(Table 2). Assessed by log rank test, the mean survival periods of the p53 positive group and its negative group were 29 months and 32 months, respectively(P =0.385). According to the stainability in the positive group, mean survival periods were 32 months for negative one, 40 months for grade one positive, 35 months for grade two positive, 22 months for grade three positive, and 21 months for grade four positive(P =0.028) (Fig. 2).

DISCUSSION

In this immunohistochemical study of colorectal cancer, using a monoclonal antibody p53 DO-7 on

Table 2. Multivariate Analysis in 387 Patients with Colonic Adenocarcinomas

Prognostic variables	Categories	DF	Chi-sq	P value
Age	⟨30, 30~60, 60⟩	2	0.01	0.9425
Sex	M, F	1	2.51	0.1129
Site	Rt, Lt	1	3.65	0.0562
Gross	P, F, U, D	3	0.27	0.6063
Tumor size	⟨5cm, 5cm⟩	1	0.27	0.6023
Histological type	Well, Mod, Poor, Muc	3	30.89	0.0000
Micro. growth	Exp, Infilt	1	5.60	0.0180
Tumor depth	Early, Advanced	1	1.64	0.1998
Lymph node	+, −	1	0.21	0.6474
Stage	A, B, C, D	3	5.15	0.0232
p53	+, −	1	4.45	0.0349

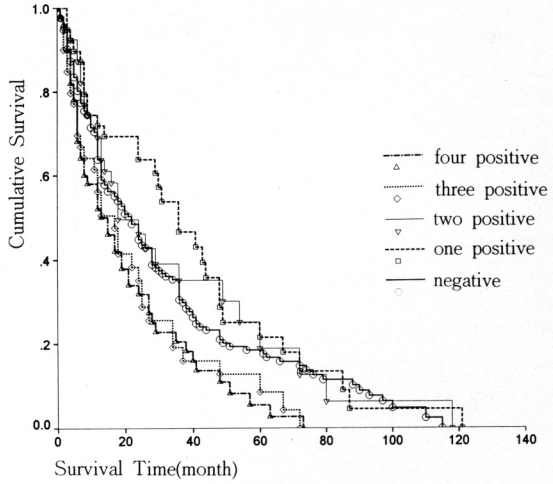

Fig. 2. p53 expression in relation to survival in 387 patients with colonic adenocarcinoma. The curves were calculated by the Kaplan-Meier method and compared by the log rank test.

formalin fixed, paraffin-embedded tissues, there were 10.3% of adenomas and 45.5% of carcinomas with positive evidence of p53 protein in which the reaction was localized to nuclei. Other authors have reported p53 staining of the adenomas which ranged from 0% to 11.0%(Rodrigues et al., 1990 ; Campo et al., 1991 ; Purdie et al., 1991 ; Starzynska et al., 1992 ; Kawasaki et al., 1992 ; Magrisso et al., 1993). Also the values of p53 staining of the carcinomas do not differ much from reports of other studies (Van den Berg et al., 1989 ; Rodriguez et al., 1990 ; Campo et al., 1991 ; Purdie et al., 1991 ; Scott et al., 1991 ; Sun et al., 1992 ; Starzynska et al., 1992 ; Remvikos et al., 1992 ; Kawasaki et al., 1992 ; Yamaguchi et al., 1992 ; Hanski et al., 1992 ; Magrisso et al., 1993 ; Campo et al., 1994 ; Linden et al., 1994 ; Bortorelle et al., 1995). Immunohistochemically, minor differences in the staining positive rate between the studies may be derived from the number of tumors examined or the different kind of material, methods and antibodies used and interpretation. Linden et al.(1994) reported that the number of p53 positive tumors varied for clones PAb 1801(60%), DO-7(82%), and BP53-12(89%).

In this study, positive staining was almost exclusively confined to the nucleus, whereas most non-neoplastic cells remained unstained. Sun et al.(1992) reported that patients whose tumors showed cytoplasmic expression only had more tumor aggressiveness than between patients with tumor positive in both nucleus and cytoplasm and those whose tumors were positive in the nucleus only. Bosari et al.(1995) suggested that cytoplasmic p53 accumulations were associated with wild-type p53 gene and were not related to patient's survival. However our results showed cytoplasmic staining was not seen or not significant. The reason for the occasional cytoplasmic staining was unclear. However, it may reflect cross reaction with similar epitopes in the cytoplasm as must always be considered in immunohistochemistry, or it may reflect a small amount of cytoplasmic synthesis or breakdown products(Thomas et al., 1992).

In this study, the positive rate of p53 expression in colon carcinomas was not affected by age, sex, gross configuration, stage, microscopic growth pattern, tumor depth, tumor size, and lymph node involvement. Similarily in other studies, p53 expression showed no correlation to age, sex, microscopic growth pattern, tumor depth, and tumor size(Purdie et

al., 1991 ; Scott et al., 1991 ; Starzynska et al., 1992 ; Kawasaki et al., 1992 ; Yamaguchi et al., 1992). Starzynska et al.(1992) suggested that the correlation between p53 expression and stage of colorectal tumor is very significant. They described how only 30% of colorectal cancers with localized disease expressed high levels of p53 protein whereas 63% of colorectal tumors which had invaded regional lymph-nodes overexpressed p53. Also Bertonelle et al.(1995) reported that the cases in more advanced stages(C,D) showed a higher incidence of p53 expression than cases in stage A and B. However, most authors, and our own results showed that correlation between the stage and p53 expression was not significant(Campo et al., 1991 ; Purdie et al., 1991 ; Scott et al., 1991 ; Sun et al., 1992 ; Remvikos et al., 1992 ; Kawasaki et al., 1992 ; Yamaguchi et al., 1992). In this study, left sided cancers(49.3%) expressed higher p53 positivity than right sided cancers(36.5%) (P=0.01). Scott et al.(1991), Starzynska et al. (1992), Remvikos et al.(1992), and Bertonelle et al.(1995) reported similar findings. These observations support the hypothesis that adenocarcinomas arising in the colon and in the rectum might have different biological and clinical behavior(Meling et al., 1993). In this study, no significance was seen between p53 expression and histologic subtypes. However mucinous carcinoma(26.5%) showed lower p53 expression than usual adenocarcinoma(46.9%) ($P < 0.05$). Campo et al.(1991) described that mucinous carcinomas, however, were less frequently positive(25%) than the usual adenocarcinomas(73%). And Hanski et al.(1992) reported that usual adenocarcinoma exhibited 72%~76% positivity and mucinous carcinoma showed 36% positivity. The current data on p53 protein overexpression, are compatible with the concept that the mucinous tumors represent a distinct genetic entity differing from nonmucinous carcinomas(Hanski et al., 1992).

It is still controversial whether p53 expressions are associated with prognosis or survival rates. Stazynska et al.(1992) reported that statistical analysis of follow-up data of 80 patients operated on for colorectal cancer(36 with p53 positive and 44 with p53 negative tumor) revealed that p53 expression is significantly associated with early relapse and death. Similarly, in the group of patients with colorectal cancer 69% of p53 positive and only 11% of p53 negative patients developed local recurrence or died during the first year after surgery. Remvikos et al.(1992) investigated 41 colorectal tumors and found a significant association between elevated p53 and the presence of DNA aneuploidy, a factor connected with poor prognosis. Yamaguchi et al.(1992) reported that the 3-year survival rate was 96.7% for the 39 patients with p53-negative tumors, but it was as low as 61.8% for those with p53-positive tumors. The p53-positive tumors recurred at a rate of 23.8% compared with 5.9% for the p53-negative tumors. In addition, the results of multivariate analysis, using the proportional-hazards model of Cox, indicated that the immunoreactivity of p53 was an independent prognostic indicator of colorectal cancer. However, in a study of 52 colorectal carcinomas(Scott et al., 1991) and Sun et al.(1992), no correlation was observed with p53 overexpression.

In our follow-up study, the mean survival time of the p53 positive group and its negative group were 29 months and 32 months, respectively. However it was not statistically significant(P=0.385). When positive groups were divided into four positive grades, mean survival periods showed 32 months in negative, 40 months in grade one positive, 35 months in grade two positive, 22 months in grade three positive and 21 months in grade four positive, which is statistically significant(P=0.028). Linden et al.(1994) showed similar results. The implication of these findings is that, immunohistochemical detection of p53 can be a valuable tool in routine pathology for p53 screening in colorectal cancer, to identify, along with other established prognostic factors, patients with poor short-term prognosis and to decide on optimal treatment for this group.

In conclusion, the result of this multivariate analysis suggests that immunohistochemically detection of strong p53 protein expression(more than 30% of tumor cells) has value in estimating a prognosis for patients with colorectal carcinomas.

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