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Clinical Observations

THE SIGNIFICANCE OF P53 GENE MUTATIONS AND EXPRESSIONS IN HUMAN COLORECTAL TUMORS

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Using a polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) approach we analyzed 18 human colorectal adenocarcinomas for mutations in exons 5,6,7,8 of p53 gene. At the same time, p53 gene product expression was studied immunohistochemically in these 18 case in frozen sections. The expression of p53 protein was also immunohistochemically studied in formalin-fixed paraffin embeded specimens of 76 colorectal adenocarcinomas and 112 colorectal polyps. Eight out of 18 cases (44%) showed a variant band indicative of a mutation in exons 5-6 of p53 gene 7 out of 8 cases (88%) with p53 gene mutations were positivelystanined for p53. There was no significant correlation between p53. expression and clinicopathological manifestations and prognosis. but the strongest staining was encountered in those cases with well differentiated and early stage adenocarcinomas, while weaker staining was encountered in poorly differentiated and mucoid adenocarcinomas. p53 expression was not observed in proliferative polyps and adenomas with low grade dysplasia. The frequency of p53 expression reached 88% (P <0.001) when adenoma showed malignant change. Among three types of adenomas, p53 expression was most frequent in villous type (P<0.05). The frequencies of p53 expression in adenoma, adenoma with malignant change and adenocarcinoma were 4%, 88% and 51% respectively. These indicate that genetic changes of p53 gene play an important role in the transformation from benign adenoma to adenocarcinoma. p53 immunohistochemistry can be used as a surrogate marker for p53 gene mutation for early discovery of colorectal adenocarcinomas.

Key words: Colorectal neoplasms, p53 gene, Mutation, Expression, PCR-SSCP

Mutation and Expression of p53 gene is now well described in large bowel cancer. However there are many differences among the results and there are a few reports of p53 gene alternations in the precursor adenoma. We use polymerase chain reation-single strand conformation polymprphism (PCR-SSCP) and immunohistochemstry methods to detect the mutation and expression of p53 gene in colorectal cancers and precursor adenomas, and to assess the relationship between p53 gene alternations and pathological variables of colorectal adenocarcinomas.

MATERIALS AND METHODS

Fresh tissue was obtained from 18 adenocarcinomas of the large bowel cancers from 18

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patients. Archival paraffin-embedded tissue was from 76 adenocarcinomas and 112 polyps.

High-molecular-weith DNA was isolated from surgical tumor specimens and the corresponding normal mucosae by the method of SDS-proteinase K and phenol chloroform treatment.

PCR-SSCP Analysis

Exons 5-8 of the p53 gene were amplified by PCR and mutation was analyzed by SSCP method. The standard reaction mixture (10 ul) consisted of 0.2 mmol/L of each dNTP, (a^{-32} P) dCTP 2.5 uCi, the proper pairs of each 10 pmol/L primer, 1 × PCR buffer, Taq polymerase 1.25 u and 0.2 μg of genomic DNA. The thermal cycles were 30 cycles of 1 min at 94 ℃, 1 min at 60 ℃ and 2 min at 72 ℃. The reaction mixture was diluted 10-fold with formamide-dye solution. A 4 ul sample of diluted mixture was heated for 5 min at 94 ℃ and subjected to electrophoresis in 5% polyacrylamide gel. After electrophoresis at 4 ℃, gel was exposed to X-ray film. Synthetic oligonucleotides used as primers were:

Exons 5-6 (Sense): 5' -TTCCTCTTCCTGCAGTA-CTC -3'

Exons 5-6 (Antisense): 5'-AGTTGCAAACCAG-ACCTCAG-3'

Exons 7-8 (sense): 5' -AGGTTGGCTCTGACTG-TACC-3'

Exons7-8(Antisense): 5'-ATTGTCCTGCTTGCT-TACCTC-3'

Immunohistochemistry

For immunohistochemistry, the streptavidin-biotin-proteinase method was applied using the mouse monoclonal antibody DO-7 recognizing the p53 nuclear protein. The intensity of the p53 nuclear staining on neoplastic cells was scored arbitrarily as follows: very strong+++; strong ++; weak+; and negative−. Negative control studies were carried out in the absence of primary antiserum to p53.

Statistics

Frequency of p53 postive tumor was compared for each variable using Chi square analysis. Kaplan-Meier survival curves were constructed using the BMDP series statistics package.

RESULTS

Analysis of p53 Gene Mutation And Expression

Analysis of p53 gene mutation by PCR-SSCP and p53 expression by immunohistochemistry: Mutation in exons 5-8 of the p53 gene was examined in 18 tumors by PCR-SSCP. When the amplified DNA fregments of 5-6 and 7-8 were separated eletrophoretically in nondenaturing polyacrylamide, some single stranded DNA from tumor specimens showed aberrant mobility distinct from normal strands (Figure 1,2). The frequency of p53 mutation in colorectal cancers was 44% (8/18). 16 T lost 2 bands in SSCP analysis in exons 7-8, which surggested that 16 T was in hemizygosity. p53 immunoreactivity was seen in 88% of tumors showing p53 gene mutation(7/8).

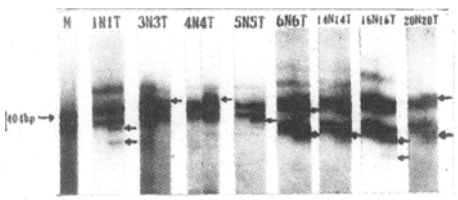

Fig 1. Patterns of SSCP of DNA fregment in exons from normal and tumor specimens

N: normal mucosa T: tumor tissue

Arrowheads: mobility of samples from tumor tissue

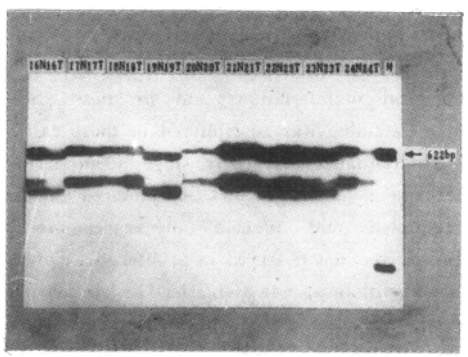

Fig 2. Patterns of SSCP of DNA fregment in exons 7-8 from normal and tumor specimens

N: normal mucosa T: tumor tissue, 16T lost 2 bands.

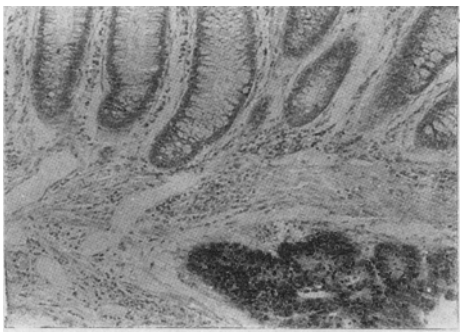

Fig 3. Immunohistochemical staining of p53 using a monoclonal antibody DO-7. Nuclear staining is positive in cancer cells and negative in adjacent normal mucosa.

p53 Expression in Colorectal Cancer

39 out of 76 (51%) colorectal cancers showed nuclear staining (Figure 3). There was no relationship between p53 expression and age, differentiation, lymph matastasis and Dukes' stage. But the p53 expression was high in the Dukes' A stage and highly differentiated cancers (Table 1,2). The 5- year survival rate was 46% for patients with p53-positive tumors and 24% for those with negative ones. The difference between these two groups was statistically significant ($P<0.05$).

p53 Expression in Colorectal Polyps (including Adenomas with Malignant Change)

Table 1. The relationship between p53 exprression and Dukes stage

p53 expression	Dukes A	Dukes B	Dukes C1	Dukes C2	Dukes D
−	4 (29)	12 (46)	12 (57)	4 (57%)	5 (62%)
+	3 (21)	8 (30)	2 (10)	2 (29%)	1 (13%)
++	2 (14)	3 (12)	4 (19)	1 (14%)	2 (25%)
+++	5 (36)	3 (12)	3 (14)	0 (0%)	0 (0%)
	14 (100)	26 (100)	26 (100)	7 (100%)	8 (100%)

Table 2. p53 expression and clinico-pathological variables in colorectal adenomas

Variables	Positive	Negative	%	*P* value
Sex				>0.05
Male	16	54	23	
Female	10	32	24	
location				>0.05
Rectum	19	52	25	
Colon	7	29	19	
Type (including adenomas with malignant change)				<0.05
Proliferative polyp	0	15	0	
Tubular	4	22	15	
Tubular-villous	6	20	23	
Villous	9	16	36	
Dysplasia				<0.001
Mild	0	31	0	
Moderate	2	28	7	
Severe	1	9	10	
Malignant change	23	3	88	

Overall 3 out of 71 adenomas (4%) stained for p53. Expression of this protein was not observed in proliferative polyps and adenomas with low grade dysplasia (Table 2). The frequency of p53 expression reached to 88% ($P<0.001$), when adenomas showed malignant change. Among three kinds of adenomas, p53 expression was most frequent in villous type ($P<0.05$).

DISCUSSION

LOH on 17p has been detected in 75% of colorectal cancer.[1] Baker[2] found p53 gene mutation in 70% of colorectal tumor, except LOH. 40% of invasive sporadic carcinoma had both mutation and LOH.[3] The p53 gene mutation found in this study was in 44% of colorectal cancer, and clustered within exons 5-6, which was familial with the results of others.[4]

We analylzed the p53 expression immuno-histochemically in colorectal tumor using the DO-7 mouse anti-p53 Mab. DO-7 recognizes a denaturation-resistant epitope between the aminoacids 35 and 45. DO-7 is reactive on both the wild type and mutant form of p53. However, the level of wild type p53 is low with a short half life. On the other hand, the level of mutant p53 is 100 fold higher than that of wild type p53, with a long half life. Consequently, the DO-7 detected only mutant p53 protein. p53 gene mutation is consistant with p53 expression in our study. Many authors[5,6] reported p53 immunoreactivity was seen in 42-70% of colorectal cancer, and there was no relationship between p53 expression and tumor grade, Dukes' stage, invasive depth and lymph matastasis. We found p53 expression in 51% of colorectal cancer and no correlation was found within pathological variables. The frequency of p53 expression, however was higher in early stage and well differenciated cancer, which was different from other reports.[5,7]

P53 expression was not correlated with patient survival.[5] Yamaguchi[6] found that p53 positive cancers were associated with liver matastasis and had significantly poor prognosis. We found that patients with p53 positive tumors had higher 5-year survival rate. This might be that patients with early stages received curative operations. We think that p53 expression was not correlated with patient survival.

It is known that villous type of colorectal adenoma are easily changed to malignancy. The high frequency and strongest stainaing was encountered in villous adenoma. This suggest that p53 positive adenoma is easily changed to adenocarcinoma. Our results indicate genetic changes of p53 gene play an important role in the point of transformation from benign adenoma to malignant adenocarcinoma. p53 immuno-histochemistry can act as a surrogate marker for p53 gene mutation. It is helpful for early discovery of colorectal adenocarcinoma.

REFERENCES

1. Vogelstein B, Fearon ER, Stanley BA, et al. Genetic Alternations during Colorectal-Tumor Development. N Engl J Med 1988; 319: 525.
2. Baker SJ, Preisinger AC, Jessup JM, et al. P53 Gene Mutations Occur in Combination with 17p Allelic Deletion as Late Evens in Colorectal Tumorigenesis. Cancer Res 1990; 50: 7717.
3. Kiuchi-Yanoshita R, Konishi M, Ito S, et al. Genetic Changes of Both P53 Alleles Associated with The Conversion from Colorectal Adenoma to Early Carcinoma in Familial Adenomatous Polyposis And Non-Familial Adenomatous Polyposis Patients. Cancer Res 1992; 52: 3965.
4. Shirasawa S, Urabe K, Yanagawa Y, et al. P53 Gene Mutations in Colorectal Tumors from Patients with Familial Polyposis Coli. Cancer Res 1991; 51:.2874.
5. Scott N, sagar P, Stewart J, et al. P53 in Colorectal Cancer: Clinicopathological correlation and Prognostic Significance. Br J Cancer 1991; 63: 317.
6. Yamaguchi A, Nakagawara G, Kurossaka Y, et al. P53 Immunoreaction in Endoscopic Biopsy Specimens of Colorectal Cancer and Its Prognostic Significance. Br J Cancer 1993; 68: 399.
7. Bell SM, scott N, Cross d, et al. Prognostic Value of P53 Overpression And c-Ki-ras Gene Mutation in Colorectal Cancer. Gastroenterology 1993; 104:57.