Abnormal Change of p53 Gene in Gastric and Precancerous Lesions and APC Gene Deletion in Gastric Carcinoma and Near Tissues*

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Summary: p53 gene mutation (exon4, 5, 6, 7, 8 and intron6) in gastric cancer and precancerous lesions and p53 gene(exon4 and ontron6), APC gene deletion in gastric carcinomas were studied by PCR/SSCP and PCR/RFLP. Results showed mutation rate of p53 in metaplasia, dysplasia and gastric carcinoma was 37.5 % (3/8), 42.11 % (8/19), 53.33 (16/30) respectively. There was significant difference among groups of metaplasia, dysplasia, cancer and normal controls. No exon8 mutation was found in metaplasia and dysplasia, but 4 cases were found to have exon8 mutation in cancer group. It is suggested that exon8 mutation occurs at the late stage of gastric cancer, but exon 5,6,7 mutation occur in the course of precancerous lesion to cancer. Loss of heterozygosity (LOH) of exon4, intron6, APC was 47,37 % (9/19), 8.73% (2/23), 16.67 % (3/18) respectively. LOH of exon4 had something to do with poor differentiation, lymph node metastasis, depth of invasion. LOH of exon4 may be one of prognostic marker of gastric cancer. We are led to conclude that p53 gene mutation is an early event and perhaps work together with ras oncogene in gastric carcinogenesis

Key words: p53 gene mutation; p53 gene deletion; APC gene deletion; gastric cancer; precancerous lesion

Hypothesis of tumor suppressor gene had been proposed twenty years ago[1], but the first suppressor gene Rb (retinoblastoma) was separated and cloned until 1986[2]. Suppressor gene, including p53 and APC genes, now has become new "hot" subject in the area of tumor research. p53 gene mutation, deletion and APC gene deletion occurred in some human tumors and there may be some relation between APC gene and p53 gene deletion[3-7]. In this paper, PCR-RFLP, PCR-SSCP methods were used to analyze p53, APC gene alterations in gastric cancer and precancerous lesions in order to investigate the role of p53 and APC gene in gastric carcinogenesis.

1 MATERIALS AND METHODS

1.1 Subjects

Eighty-seven paraffin-embedded samples from gastrectomy and endoscopic biopsy were selected, which included normal gastric mucosa, superficial gastritis, intestinal metaplasia, dysplasia and intestinal gastric cancer. In addition, 25 pairs of fresh gastric cancer and neighboring tissues from gastrectomy were obtained. All samples were identified histologically.

1.2 Methods

1.2.1 DNA Extraction High-molecular-weight DNA was prepared from paraffin-embedded tissues and fresh tissues. DNA was extracted by phenol and chloride, and then precipitated with absolute ethanol. DNA was dissolved in TE buffer.

1.2.2 PCR-SSCP PCR was performed by using Saiki's method. Total volume of each tube is 10 μl. PCR mixture of each tube contained 0.2 mM dNTP (dATP, dCTP, dGTP, dTTP), 2.5 mM $MgCl_2$, 0.25 μM primer kindly provided by Dr. Shiao (NIH, USA), Taq polymerase (Sino-American Biotechnical Co.) 1.5 U, 0.1 μg/μl DNA template. The reactions were carried out in PE 9600 thermocycler, DNA was denatured at 94℃ for 4 min and then denatured in 94℃ for 15 s, annealed at 60℃ for 15 s, extended at 72℃, for 15 s, reaction for 35 cycles. Finally, they were extended at 72℃

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for 10 min to ensure complete extension. A negative control containing no DNA template was run in parallel for each amplification reaction. Before carrying out SSCP. 5 μl of PCR product was electrophoresed in 2% agarose and visualized with EB stain (0.5 μg/ml) to confirm the absence of contamination and to ensure that PCR product was mixed with 4 μl of a denaturing solution of 95 %(V/V) formamide, 20 mmol/L disodium EDTA, 0.05 %(W/V) xylene cyanole, and 0.05 %(W/V) bromophenol blue. Before electrophoresis, the samples were heated to 95℃ for 5 min in a water bath. After heat denaturation, the tubes were immediately placed on ice to prevent renaturation.

Five μl of each denatured sample was loaded onto 12 %(W/V) polyacrylamide gel (ratio of acrylamide to bisacrylamide 29 : 1). Electrophoresis of gel was carried out at 4℃ (cold water cycle) at 10 mA constant current for 2 — 3 h.

Single strands of DNA were visualized with silver staining. The band pattern of each sample was compared to that of histologically normal mucous samples. Any extra bands present in the sample was considered as positive for a mutation.

1.2.3 PCR-RFLP Same PCR method was used. Primer was p53 exon4 and APC exon11. 5 U restriction enzyme BstUI 10 U/μl for p53 exon4 detection or RsaI 10 U/μl for APC gene detection and buffer in a total volume of 10 μl were added to 5 μl of PCR product, incubated at 37 ℃ overnight. 5 μl of the mixture was electrophoresed in 2 % agarose gel and observed under ultroviolet lamp. 176 bp PCR product of p53 exon4 contained BstUI site, which could generate 100 bp, 58 bp and 18 bp by BstUI cleavage, and 133 bp PCR product of APC exon11 can generate 86 bp, 47 bp by RsaI. According to these, the deletion can be analyzed.

2 RESULTS

2.1 p53 Gene Mutation Analysis

p53 exon4, 5, 6, 7 and 8, intron6 mutation in 15 cases of normal mucosa, 15 cases of superficial gastritis, 8 cases of intestinal metaplasia, 19 cases of dysplasia and 30 cases of intestinal gastric cancer were analyzed by using PCR-SSCP. No exon4, intron6 mutation was found in all cases, and no exon8 mutation was found in metaplasia and dysplasia. 4 exon8 were found in gastric cancer patients. In metaplasia, 1 case was found to have exon5, 6, 7 mutation respectively. 2 cases of exon5 mutation and 3 of exon6, 7 mutation were found in dysplasia. 7 cases of exon5 mutation, 2 of exon6, 3 of exon7 and 4 of exon8 mutation were found in gastric cancer. p53 mutation rates in metaplasia, dysplasia and gastric cancer were 37.50 %, 42.11 %, 53.33 % respectively. p53 mutations rates in groups of metaplasia, dysplasia and gastric cancer are significantly higher than control group ($P<0.05$, $P<0.01$, $P<0.01$), but no statistical difference was found among these three groups as well as mild, moderate and severe dysplasia (table 1).

Table 1 p53 mutation in gastric cancer and precancerous lesions

Histologic type (*n*)	Exon 4	5	6	7	8	Intron6	Positive rate of mutation
Normal control(15)	0	0	0	0	0	0	
Superficial gastritis(15)	0	0	0	0	0	0	
Metaplasia(8)	0	1	1	1	0	0	37.50%*
Dysplasia(19)	0	2	3	3	0	0	42.11%**
Intestinal gastric cancer(30)	0	7	2	3	4	0	53.33%**

*$P<0.05$, **$P<0.01$ comparing to normal control

2.2 p53 APC Gene Deletion Analysis

p53 exon4, intron6 and APC exon11 gene deletions were studied in 25 pairs of gastric cancer and neighboring tissues by PCR-RFLP. The results showed there were 19, 23 and 18 pairs of exon4, intron6 and APC gene information individuals respectively. LOH was found in 9 pairs of exon4, 2 pairs of intron6 and 3 pairs of APC gene with the LOH rates being 47.37 % (9/19), 8.70 % (2/23), and 16.7% respectively. There are some relations between P53 ex-

on4 LOH and prognosis of gastric cancer. In 9 cases of LOH of p53 exon4, 6 cases had poor differentiation, 8 cases metastasis, 8 cases invasion into muscle or plasma layer. Statistically, the lower the differentiation, the higher LOH rate ($P<0.05$), and LOH was associated with lymph node metastasis and invasion ($P<0.05$). No relationship was found between LOH of p53 intron6, APC and these prognostic factors, but all patients with APC gene deletion had p53 gene deletion (table 2).

Table 2 Relationship between p53 exon4 LOH and prognosis of gastric cancer

LOH of informative individuls	Differentiation			Lymphonode metastasis		Tumor thrombosis		Invasion degree			
	P	M	W	Y	N	Y	N	T_1	T_2	T_3	T_4
Y(9)	6	2	1	8	1	5	4	0	1	2	6
N(10)	1	3	6	3	7	6	4	2	4	3	1

T_1 mucous layer; T_2 superficial muscle layer; T_3 muscle layer; T_4 plasma layer

3 DISCUSSION

p53 gene had been known as oncogene before 1988[8]. Recently, studies showed that wild type p53 gene is a kind of tumor suppressor gene[9]. p53 gene localizes to chromosome 17P. 11 exons in 20 kb DNA region transcripts into 2.5 kb mRNA and codes nuclear phosphate protein which includes 393 amino acids. p53 gene is associated with many tumors such as hepatic carcinoma, breast tumor colon cancer[9]. It is reported that p53 exon5 to 8 mutation rate in gastric cancer reach 27%—60%[10, 11, 16]. Uchino found p53 mutation rate was about 83 % in early gastric cancer, 52% in advanced cancer[15]. There are G : C—A : T transition transversion, missense mutation types. Though there were some reports about p53 gene mutation in gastric cancer, few reports were about p53 gene mutation law from precancerous lesions to advanced gastric cancer. In this article, p53 gene mutation in single lesion of different stage of precancerous lesions and gastric cancer were studied by PCR-SSCP method. Our results showed p53 mutation rate is 37.50 % (3/8) in metaplasia, 42.11 % (8/19) in dysplasia, 53.33 % (16/30) in cancer respectively and no mutation was found in superficial gastritis. It's consistent with Shiao's results[13, 14]. But difference was that exon8 mutation was found in various lesions in his study. In our study exon 8 mutation only occurred in gastric cancer. Exon5, 6, 7 mutations can be found in precancerous lesions, but exon5 mutation is more frequent in gastric cancer. Exon6, 7 mutation occurred in precancerous stage and over canceration course. It is suggested that exon5, exon8 mutation is late event of carcinogenesis, while, exon6, 7 mutations participate in early change of gastric cancer.

It is well known that gastric cancer is associated with environmental factors and diet. "Hot spot" of exon8 mutation existing in Chinese people may be related with specific pathogenetic factors of gastric cancer in China. In addition, our studies showed no exon4 mutation. But LOH rate of exon4 reached 47.37 %, and related with low differentiation, lymph node metastasis and invasion degree. It may be a prognostic factor of gastric cancer. APC gene deletion rate was lower than other countries[7], but all patients with APC gene deletion also had p53 deletion and mechanism of this phenomenon remain to be investigated. Possibly, it is also one of the factors in carcinogenesis.

Our results suggested that p53 mutation is an early event in carcinogenesis of gastric cancer and p53 gene plays an important role in pathogenesis of gastric cancer.

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