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p53 Genetic Polymorphism of Gastric cancer in Korea

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Background : Deletion or functional loss of the *p53* tumor suppression gene plays a role in oncogenic transformation. The codon 72 polymorphism on exon 4 in the *p53* gene produces variant proteins with either arginine (Arg) or proline (Pro), and is associated with an increased susceptibility of cancers of the lung, esophagus, breast, cervix and nasopharynx on a genetic basis. We designed this study to evaluate the influence of the *p53* codon 72 polymorphism on gastric cancer in Korea.

Methods : We extracted the peripheral blood samples in 84 patients with gastric cancer, 66 patients with *H. pylori*-associated chronic gastritis and 43 controls without *H. pylori* infection. PCR-RFLP analysis was performed to detect *p53* codon 72 polymorphism in these patients.

Results : There was no specific genotype of *p53* polymorphism in the gastric cancer group compared to the other groups and no difference in genotypes by histologic subtypes. Classified by tumor location, Pro/Pro genotype was associated with an increase in proximal cancer and Arg/Arg genotype with distal cancer. As the frequency of *p53* Arg allele increased, the cancer was of a more poorly differentiated type.

Conclusions : The specific genotype of *p53* polymorphism seems to correlate with tumor location. Increased frequency of *p53* Arg allele is associated with more poorly differentiated cancers.

Key Words : *p53*, Polymorphism, Gastric cancer

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is an established risk factor for the development of gastric cancer, and other environmental factors such as dietary habits[1] and smoking[2] are known to play a role in gastric carcinogenesis. A multifactorial model of human gastric carcinogenesis is currently accepted.

Recent studies have shown that particular genetic changes may influence the susceptibility to cancer. Individual variation in cancer rates has been associated with specific variant alleles (polymorphism) of different genes that are present in a significant proportion of the normal population. Polymorphisms in a wide variety of genes may modify the effect of environmental exposures[3].

The *p53* gene, located on the short arm of chromosome 17, encodes a protein that plays a critical role in DNA transcription, cell cycle regulation, and tumor suppression. The *p53* expression is stimulated in certain cellular environments such as DNA damage, hypoxia, irradiation, and other cellular stress[4]. Mutation of the *p53* gene represents one of the most common genetic alternations in human cancers, and the acquisition of such defects is strongly related to cancer formation and progression[5].

The codon 72 polymorphism on exon 4 of the *p53* gene,which produces variant proteins with arginine (Arg) or proline (Pro), has been reported to be associated with the risk

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of certain cancers - lung, esophagus, breast, cervix and nasopharynx[6-8]. The specific genotype of the *p53* codon 72 polymorphism could be a risk factor for certain tumors and by making an environment favorable for tumor formation. In Japanese patients, the Pro/Pro genotype contributes to susceptibility for diffuse types of gastric cancer[9]. However, the genotypes of the *p53* codon 72 polymorphism varied significantly with race[10]. There has been no report on the *p53* codon 72 polymorphism of gastric cancer patients in Korea.

In the present study, we assessed the role of the *p53* codon 72 polymorphism of gastric cancer in Korea. We also examined the *p53* polymorphism according to the histologic classification of gastric cancer as defined by Lauren including: tumor location, stage and tumor grading.

MATERIALS AND METHODS

Patients

We studied sera from 193 patients who had undergone upper endoscopy at the Catholic University St. Vincent hospital, Suwon, Korea from Feb. 2002 through Dec. 2003. Eighty-four patients with gastric cancer, 66 *H. pylori* associated chronic gastritis patients with dyspeptic symptoms and 43 *H. pylori* negative healthy individuals as normal controls were enrolled in this study. None of the patients had other chronic illness or genetic disorders. Each patient was classified as *H. pylori* positive or negative according to the serologic and histologic results. Both tests were positive in *H. pylori* positive patients and negative in *H. pylori* negative patients. Sixty- four patients with gastric cancer underwent surgery. The surgical specimens of gastric cancer were classified as 40 intestinal type and 24 diffuse type as defined by Lauren[11]. Tumor stage was based on the TNM tumor classification system[12]. We determined the tumor stage by surgical pathologic findings in operated patients and by radiologic findings in unoperated patients.

p53 codon 72 polymorphism

DNA was extracted from 200 L of buffy coat preserved at -40℃ by the use of QIAamp DNA blood mini kit (QIAGEN Inc., Valencia, CA, USA). PCR- restriction fragment length polymorphism (RFLP) analysis of codon 72 of the p53 was used to identify the p53 BstUI genotypes12). The two primers were 5′-TTGCCGTCCCAAGCAATGGATGA-3′ and 5′-TCTGGGAA GGGACAGAAGATGAC-3′. Each PCR reaction mixture contained 10 pmol of each primer and genomic DNA. The reaction mixtures were preincubated for 10 minutes at 94℃. The PCR conditions were 94℃ for 30 seconds and 55℃ for 1 minute, followed by 72℃ for 1 minute for 40 rounds. After confirmation of an amplified fragment of the expected size, 199 bp, on agarose gel, the PCR products were digested with 2 units of restriction enzyme BstUI (New England Biolabs, Beverly, MA, USA) at 60℃ for 16 hours. The DNA fragments were electrophoresed through a 2% agarose gel and stained with ethidium bromide. Most significantly,the Pro allele is not cleaved by BstUI at codon 72 and has a single band with a fragment length of 199 bp. The Arg allele is cleaved by BstUI and yields 2 small fragments, 113 bp and 86 bp. The heterozygote has 3 bands, 199, 113 and 86 bp (Figure 1).

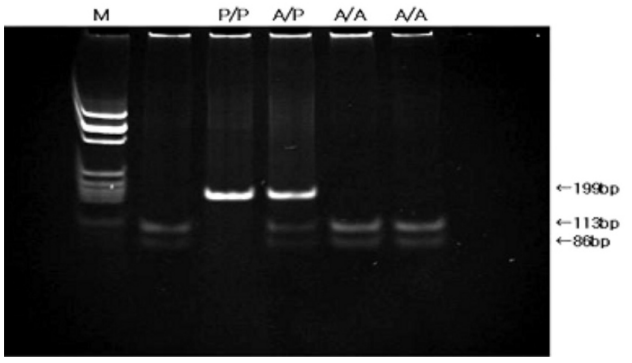

Figure 1. Detection of *p53* codon 72 polymorphism by BstUI digestion. The Pro allele is not cleaved and has a single band with a fragment length of 199 bp. The Arg allele is cleaved and has two fragments, 113 and 86 bp. The heterozygote has three bands. (A/A; Arg-Arg, A/P; Arg-Pro, P/P; Pro-Pro genotype, M; molecular marker)

Statistical analysis

The Chi-square test for association was used to test the difference of genotype frequencies between normal controls and gastric cancer patients, and between *H. pylori* associated chronic gastritis and gastric cancer patients. The other frequency tables were constructed using the SPSS statistical package with statistical significance using Chi-square test.

RESULTS

Eighty-four patients with gastric cancer, 66 *H. pylori* associated chronic gastritis patients and 43 *H. pylori* negative healthy controls were analyzed (Table 1). According to the *p53* codon 72 genotypes, the age for gastric cancer patients was 58.4±14.1 years (Arg/Arg), 61.5±10.5 years (Arg/Pro) and 64.3 ±14.0 years (Pro/Pro) respectively. There was no significant age related difference (p=0.30). The genotype frequencies of *p53* codon 72 polymorphism in Korean gastric cancer cases and controls were summarized in Table 2. There was not a specific genotype in the gastric cancer group when compared with the other groups. When gastric cancers were classified by histological subtype, there were no statistical differences in the

Table 1. Demographic characteristics of patients

Groups	No.	Sex (M:F)	Age (yr)
Gastric cancer	84	61 : 23	61.1±12.3
H. pylori associated chronic gastritis	66	39 : 27	56.8±11.0
H. pylori negative control	43	18 : 25	54.9±12.7

M, male; F, female

Table 2. Frequency of *p53* codon 72 genotypes in patients with *H. pylori* associated chronic gastritis and gastric cancer

Groups	*p53* codon 72 genotype			
	Arg-Arg	Arg-Pro	Pro-Pro	
Gastric cancer	30 (35.7 %)	42 (50.0%)	12 (14.3%)	NS
H. pylori associated chronic gastritis	24 (36.3%)	32 (48.5%)	10 (15.2%)	
Control	17 (39.5%)	18 (41.9%)	8 (18.6%)	NS

* NS, not significant

Table 3. Frequency of *p53* codon 72 genotypes according to the Lauren's classification

Lauren's classification (n)	Age (yr)	*p53* codon 72 genotype		
		Arg-Arg	Arg-Pro	Pro-Pro
Intestinal (40)	61.3±10.1	9 (22.5%)	26 (65.0%)	5 (12.5%)
Diffuse (24)	55.5±10.8	11 (45.8 %)	10 (41.7%)	3 (12.5%)

* operation : 64 patients p=0.13

Table 4. Frequency of *p53* codon 72 genotypes according to the location of tumors

Location of tumor	M : F	Age (yr)	*p53* codon 72 genotype		
			Arg-Arg	Arg-Pro	Pro-Pro
Proximal (high body & cardia)	19 : 4	61.2±12.4	4 (17.4%)	12 (52.2%)	7 (30.4%)*
Distal (antrum & lower body)	42 : 19	60.6±12.5	26 (42.6%)*	30 (49.2%)	5 (8.2%)

M, male; F, female

* p=0.01

Table 5. TNM stage of gastric cancer according to the *p53* codon 72 genotypes

Genotype (n)	TNM stage	
	I/II (48)	III/IV (36)
Arg-Arg (30)	15(50.0%)	15(50.0%)
Arg-Pro (42)	25(59.5%)	17(40.5%)
Pro-Pro (12)	8(66.7%)	4(33.3%)
Arg allelic frequency	55/96(57.3%)	47/72 (65.3%)

p=0.29

Table 6. Histologic differentiation according to the *p53* codon 72 genotypes

Genotype (n)	Histologic differentiation		
	Well	Moderate	Poor
Arg-Arg (30)	1 (3.3%)	11(36.7%)	18(60.0%)
Arg-Pro (42)	12(28.6%)	10(23.8%)	20 (47.6%)
Pro-Pro (12)	1(8.3%)	11(91.7%)	0(0.0%)
Arg allelic frequency	14/28(50.0%)	32/64(50.0%)	56/76(73.4%)*

*p=0.007

genotypic distribution between the intestinal and diffuse type (Table 3).

We also examined the differences in genotype by tumor location, stage and tumor grading. In gastric cancer patients with a proximal location (cardia and high body of stomach), the frequencies of the 3 genotypes, Arg-Arg, Arg-Pro and Pro-Pro, were 17.4, 52.2 and 30.4%, respectively. In distal cancer (lower body and antrum), the frequencies of the genotypes were 42.6, 49.2 and 8.2%. The Pro/Pro genotype was associated with proximal location and the Arg/Arg was associated with distal location (p=0.01) (Table 4). In gastric cancer patients who had more than stage III tumor by TNM status, the frequencies of the

3 genotypes, Arg-Arg, Arg-Pro and Pro-Pro, were 50.0, 40.5 and 33.3%. The Arg allelic frequency is 57.3% in stage I/II and 65.3% in stage III/IV. An increased frequency of the Arg allele in *p53* codon 72 with advanced stage was observed, but there was no statistical significance (p=0.29) (Table 5). In poorly differentiated tumors, the Arg/Arg genotype was more frequently observed, and the Arg allelic frequency was significantly increased (p=0.007) (Table 6).

DISCUSSION

The association of *p53* with tumorgienesis is mediated by the production of a 21kDa protein (p21/cip1/waf1) that inhibits cyclin dependent kinase (CDK) activity and blocks the normal cell cycle. It plays a rolein apoptosis in repairing damaged DNA[14]. The polymorphic variants of codon 72 differ in their specific DNA binding affinity, transcriptional activity and induction of apoptosis[15].

There is a discrepancy between the p53 immunoreactivity and *p53* gene mutation in gastric cancer. Most of the overexpressed p53 protein detected by immunohistochemical stain is a mutant form exhibiting an increased half-life when compared to the wild form. The *p53* gene contains 11 exons. Almost all mutations of the *p53* gene occur in exon 5~8. Several studies have shown that p53 overexpression was observed in approximately 75% of gastric cancers, while the gene was mutated in only 20% of cases[16]. To understand this disparity, it is helpful to examine exon 4 polymorphism.

The results of this study revealed that the genotypic frequencies were Arg/Arg (35.7%), Arg/Pro (50.0%), and Pro/Pro (14.3%) in treated patients, and Arg/Arg (36.3%), Arg/Pro (48.5%), and Pro/Pro (15.2%) in controls respectively. There was no specific genotype of *p53* polymorphism in the gastric cancer patients. Several studies have reported roles for *p53* polymorphic variants in modulating environmental risk factors for cancer. Patients who are smokers and have the Pro/Pro genotype are more likely to develop lung cancer[17]. In contrast, nonsmokers with lung cancer have an increased frequency of the Arg/Arg genotype[18]. In women, the Arg/Arg genotype results in a 7-fold increased risk for the development of cervical cancer associated with human papilloma virus (HPV) when compared with other genotypes[6]. We investigated the distribution of the *p53* codon 72 genotypes in controls and gastric cancer patients by *H. pylori* state, histologic subtypes and tumor locations. There were no significant relationships between *H. pylori* state, histologic subtypes and the *p53* codon 72 genotypes. In the matter of tumor locations, cancers with Pro/Pro genotype were more likely to be located in the proximal part of the stomach. In contrast, cancers with Arg allele had an increased frequency in the distal part. The distal cancers have several different risk factors when compared with the proximal cancers. The distal cancers are associated with *H. pylori* associated gastritis, atrophy and intestinal metaplasia[19] and are more frequently found in patients with high salt and nitrate diets[20]. Our results do not show a statistically significant association between *H. pylori* state and the *p53* codon 72 genotypes. However, we cannot determine the relationship because the degree of inflammation or atrophy induced by *H. pylori* infection is not included as one of the variables in our study.

In general, the patient's age, cancer location, histologic subtypes, tumor grading and TNM status could be associated with the prognosis of advanced cancer[21, 22]. The *p53* codon 72 genotypes may affect the prognosis with the protective effects of *p53* playing a more important role in early stagedisease[23]. Miller et al. insisted that the cancer patients with the Pro/Pro allele in *p53* had a significantly worse prognosis than others because of the poorer response to treatment[24]. However, Zhang et al. reported that the frequency of the Arg allele was positively correlated with patient's age at baseline, but that the age-related increase in the percentage of Arg allele was not associated with the prognosis of advanced gastric cancer[25]. However this assertion is somewhat controvertial. In our study, an increase in the Arg allele was negatively correlated with patient's age, but did not reach it is not statistical significance. Cancers with Arg allele have an increased frequency of the poorly differentiated type. These results stand in contrast to reports from the West[24] and China[25].

In conclusion, there is acorrelation between the *p53* codon 72 genotypes and location of the gastric cancer. Although we have not found a specific genotype of *p53* codon 72 to be a significant cause of gastric cancer in Korean patients, we suggest that an increase in Arg allele of *p53* codon 72 is correlated with poor tumor differentiation.

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