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The p53 codon 72 polymorphism and susceptibility to colorectal cancer in Korean patients

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TP53 is a major gene involved in the determination of proliferation or growth arrest at the cellular level. The polymorphism of p53 at codon 72 has been widely studied; this variation has been associated with cancer susceptibility and disease outcome. The specific aim of this study was to investigate whether the p53 codon 72 polymorphism is associated with individual susceptibility to colorectal cancer in Korean patients. The frequency of the polymorphism was examined in 156 patients with colorectal cancer and in 293 healthy controls. The polymorphism analysis was performed by amplifying exon 4 of p53 and digesting the products with restriction enzyme. The frequencies of genotypes: Arg/Arg, Arg/Pro and Pro/Pro were 34.6% (54/156), 43.0% (67/156) and 22.4% (35/156), respectively, in the cases with colorectal cancer, and 28.9% (114/293), 47.8% (140/293) and 13.3% (39/293), respectively, in the healthy controls. Statistically, there was a significant difference in the frequency of the genotypes when the healthy controls were compared to the patients with colorectal cancer (p=0.0459). The specific allele frequencies showed borderline significance (p=0.0502). Our findings suggest that the p53 Pro72 variant is associated with an increased risk for colorectal cancer in the Korean population.

Key words: p53, colorectal cancer, polymorphism, susceptibility, metastasis

Colorectal cancer is a common cancer worldwide. Korea has experienced a rapid increase in the incidence of colorectal cancer over the past 10 years [1]. Colorectal carcinogenesis is a multistep process, where genetic changes occur with mutations of several genes, including p*53*, that accumulate with the progression from a normal epithelium to an adenoma to invasive cancer [2, 3]. As resources are increasingly directed towards disease prevention, strategies for identifying and targeting high-risk individuals have become important. Single nucleotide polymorphisms (SNPs) have emerged as important tools for targeting the genes responsible for cancer susceptibility. More than ten million SNPs have been identified; however, the importance of most of the SNPs with regard to cancer has not yet been clarified. The p53 tumor suppressor pathway is well known to be involved in the maintenance of genomic integrity and the prevention of cells from undergoing oncogenic transformation [4]. A SNP, in the *p53* gene, resulting in the substitution of Arginine (Arg) by Proline (Pro) at codon 72 was identified and shown to alter the primary structure of the p53 protein. Though both structural forms are normal in their sequence-specific DNA binding activities, some functional differences have been identified [5]. Recent studies have demonstrated that the p53 codon 72 Arg/Pro polymorphism plays a crucial role in modulating wild-type p53 apoptotic activity. These two variant protein forms have been shown to behave differently; the Arg allele of p53 has been reported to induce apoptosis more effectively than the Pro allele [6–8]. However, its significance with regard to genetic susceptibility to cancer remains a matter of controversy. The association between the p53 Arg72Pro polymorphism and colorectal cancer risk has been examined with inconsistent results. There are three studies that have suggested an elevation in risk associated with Pro carrier genotypes [9–11]. However, other studies reported that the Pro carrier genotypes were associated with a null or had a statistically

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non-significant inverse association [12–16]. Thus, the results reported to date have not been consistent with respect to the association of the codon 72 polymorphism with colorectal cancer susceptibility. In addition, a previous study has shown that the codon 72 polymorphism varies greatly in different ethnic populations [17]; this ethnic difference might have a significant effect on cancer risk in different ethnic groups. However, the association of this polymorphism with colorectal cancer risk, in the Korean population, has not been previously reported.

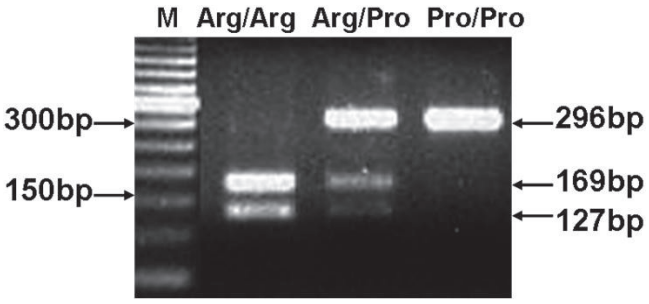
In this study, to understand whether the p53 gene codon 72 polymorphism is a biomarker associated with susceptibility to colorectal cancer in a Korean population, we analyzed the genotype frequencies of the polymorphism in 156 patients with colorectal cancer and 293 healthy controls.

Materials and methods

Tissue samples. One hundred fifty six methacarn-fixed colorectal cancer specimens were evaluated between 2003 and 2004. There was no evidence of familial cancer in any of the patients. The cases included 86 men (55.1%) and 70 women (44.9%) with a median age of 61 (35–87) years at the initial diagnosis. The healthy control population consisted of 159 males and 134 females with a mean age of 51 (23–79). To exclude ethnic differences, only Korean patients were included in this study. Approval was obtained from the institutional review board of The Catholic University of Korea, College of Medicine.

DNA extraction. For the colorectal cancer patients, normal mucosal cells were obtained from the cancer-free colorectal mucosa. DNA extraction was performed by a modified single-step DNA extraction method, as described previously [18]. For the control population, a leukocyte cell pellet from blood samples were obtained from the Buffy coat after centrifugation of 2 ml of whole blood. The cell pellet was used for DNA extraction. The Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) was used according to the manufacturer's instructions to obtain genomic DNA. The DNA purity and concentration were determined by a Nanodrop ND-1000 spectrophotometer (Nanodrop technologies, Wilmington, DE, USA).

PCR-RFLP. A polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP) assay was used to identify the p53 gene codon 72 genotypes with the primers of 5'-ATCTACAGTCCCCCTTGC-3' and 5'-GCAACTGACCGTGCAAGT-3'. The 296 bp target DNA fragment contains the CGC/CCC site of the p53 gene codon 72 located in exon 4. The 10 µl PCR mixture contained 1 µL template DNA, 0.5 µM of each primer, 0.2 µM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Taq polymerase, and 1 µl of 10× buffer. The reaction mixture was denatured for 12 min at 94°C and incubated for 35 cycles (denaturing for 40 s at 94°C, annealing for 40 s at 55°C and extension for 40 s at 72°C). The final extension was continued for 5 min at 72°C. The 296 bp fragment was then digested with 5 units of BstuI (New England Biolabs Inc. Ipswich, MA, USA) for 4 h at 60°C. The digested products were separated on a 2% agarose gel with ethidium bromide and photographed with an Utra Violet Product Image Store system (Fig.1). The Pro/Pro genotype produced a single 296 bp band due to absence of Bstu I restriction site; the wild type Arg/Arg genotype produced two bands (169 bp and 127 bp) and the Arg/Pro genotype produced three bands (296 bp, 169 bp and 127 bp). The results were evaluated by an investigator blinded to the participants' case–control status. More than 10% of the samples were randomly selected for repeated assay and the results were 100% in agreement.



Figur 1. Genotype analysis of the p53 codon 72 polymorphism. RFLP patterns of homozygous Arg/Arg and Pro/Pro, and heterozygous Arg/Pro genotypes.

Statistical analysis. The chi-square test for association was used to test for differences in the genotype or allele frequencies between colorectal cancer patients and the healthy controls. The genotype specific risks were estimated as odds ratios at 95% confidence intervals (CI).

Results

The genotype frequencies of the p53 codon 72 polymorphism in Korean patients with colorectal cancer compared to controls are summarized in Table1. The frequencies of genotypes: Arg/Arg, Arg/Pro and Pro/Pro were 34.6% (54/156), 43.0% (67/156) and 22.4% (35/156), respectively, for the colorectal cancer cases, and 28.9% (114/293), 47.8% (140/293) and 13.3% (39/293), respectively, for the healthy controls. There was a tendency for the Pro allele to be more common in the patients with colorectal cancer than in the controls. Statistically, there was a significant difference in the frequency of genotypes between the healthy controls and the patients with colorectal cancer (p=0.0459). For the frequency of alleles, there was a borderline significance (p=0.0502). When we evaluated the association between the Pro variant and the risk for colorectal cancer, by logistic regression analysis, the Pro/Pro homozygous genotype was associated with a nearly twofold risk (adjusted OR, 1.91; 95% CI, 0.83–4.39) when compared to the Arg/Arg homozygous genotype. However, there was no significant difference between the Pro/Pro

Table 1. Distribution of p53 genotype and frequency in patients with colorectal cancer and controls

P53 genotype	Cases(n=156)		Controls(n=293)		Crude OR (95% CI)	Adjusted[a] OR (95% CI)
	Number	Percent	Number	Percent		
Arg/Arg	54	34.6	114	38.9	1.00	1.00
Arg/Pro	67	43.0	140	47.8	1.01(0.65-1.56)	1.30(0.69-2.44)
Pro/Pro	35	22.4	39	13.3	1.89(1.08-3.32)	1.91(0.83-4.39)
Pro allele frequency[b]	0.439		0.372			
Trend test[c]					p=0.0551	p=0.1326

[a] Adjusted for age (in year) and sex.
[b] Two-sided χ^2-test; for allele frequencies, p=0.0502; for genotype distribution, p=0.0459.
[c] Calculated in the logistic regression model using the number of C alleles in the genotype as a continuous variable.

Table 2. Subgroup analysis of p53 genotype frequency in patients with colorectal cancer and controls

Variable	p53 genotype						Adjusted[a] OR (95% CI)	
	No. of Cases			No. of Controls				
	Arg/Arg	Arg/Pro	Pro/Pro	Arg/Arg	Arg/Pro	Pro/Pro	Arg/Pro vs Arg/Arg	Pro/Pro vs Arg/Arg
Age (in years)								
≤50	9	13	4	94	117	29	1.16(0.48-2.84)	1.38(0.57-3.34)
≥50	45	54	30	20	23	10	1.04(0.51-2.14)	1.38(0.38-4.96)
Sex								
Male	28	39	19	67	79	13	1.33(0.59-3.01)	2.01(0.61-6.67)
Female	26	28	16	47	61	26	1.26(0.46-3.44)	1.89(0.57-6.27)
L/N metastasis								
Positive	27	28	13					
Negative	27	39	22	p=0.4534 (χ^2-test)				

[a] Adjusted for the other covariate [age (in year) as a continuous variable] presented in this table in a logistic regression model for each subgroup.

and Arg/Arg homozygous genotypes. Associations between the p53 gene codon 72 genotype and colorectal cancer stratified by age, gender and lymph node metastasis are shown in Table 2. Because colorectal cancer occurs in Korean patients around 50 years of age, we classified the patients ≤50 years as younger patients. The p53 genotype and allele frequencies were not associated with lymph node metastasis (p=0.4534). When stratified by age and gender, the risk was also not statistically significant (p=0.6340 & 0.7782).

Discussion

The tumor suppressor gene p53 is located on human chromosome 17q3.1 and is composed of 11 exons. It encodes for a 53 KD protein with 393 amino acids, which can be phosphorylated and activated by signals of DNA damage and arrest of the cell cycle in the G1 phase to allow DNA repair or apoptosis [19]. The polymorphisms of codon 72 are located in a proline-rich domain of p53, which plays an important role in p53-mediated apoptosis [20]. Both variants are morphologically considered to be wild-type, and do not differ in their ability to bind to DNA in a sequence-specific manner [21]. The Pro/Pro genotype appears to be a stronger inducer of downstream transcription and less effective at the suppression of cellular transformation. The Arg/Arg genotype is at least five times more efficient at the induction of apoptosis than the Pro/Pro genotype [7]. This region includes five PxxPSH3 (SRC-homology-3) binding motifs, one of which is lost when the proline at codon 72 is replaced by arginine [20]. This may be the biological cause of the change in function.

The association between this polymorphism and cancer risk has been most extensively examined for cancers of the cervix and lung. Some studies showed an increased risk of cervical cancer with the Arg/Arg genotype [22, 23], while for lung cancer, an increased risk has been associated with the Pro carrier genotypes [24]. In addition, the association between the p53 codon 72 polymorphism and the risk of colorectal cancer has been previously examined in several studies [9–16]. Most of the studies have not strongly supported a correlation between the p53 Arg72Pro polymorphism and colorectal cancer [12–16].

In the present study, we found that there was a significant difference in the frequency of the p53 genotype between healthy controls and patients with colorectal cancer (p=0.0459) among Korean patients. Patients with the Pro/Pro homozygous genotype had nearly a twofold increased risk, compared to those with an Arg/Arg homozygous genotype. Therefore, our results suggest that the p53 codon 72 Pro/Pro genotype is correlated

with an increased risk for colorectal cancer in Korean patients and may be a marker for susceptibility to colorectal cancer. Prior studies have demonstrated that genotype distributions corresponded to a significant increase in the risk for colorectal cancer with the Pro genotypes [9–11]. However, another study reported that the Arg homozygous genotype was associated with an increased risk for colon cancer development [25]. These different results might be explained by different ethnic groups studied, environmental factors, lifestyle, and the relatively small number of cases reported in the studies.

The relationship between the p53 codon 72 polymorphism and metastasis has been analyzed in several types of cancer. Hadhri et al. [26] demonstrated that the genotype frequency did not significantly differ between the p53 codon 72 polymorphism and lymph node metastasis in patients with nasopharyngeal carcinoma. Wang et al. [27] found that Arg allele was associated with liver metastasis in colorectal patients. In the present study, genotype frequency was not associated with lymph node metastasis in the patients with colorectal cancer. No prior study has reported on a functional analysis of the p53 codon 72 Arg/Pro polymorphism and metastatic behavior. Additional studies with a large patient cohort are needed to verify these initial observations.

In summary, the results of the present study demonstrated a significant association between colorectal cancer and the p53 codon 72 Arg/Pro polymorphism in Korean population. Further molecular genetic studies should be performed in a large population to identify the mechanisms associated with the p53 codon 72 Arg/Pro polymorphism.

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