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*p*53 Codon 72 Polymorphism in the Patients with Breast Cancer

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Abstract We investigated the association of the Arg/Pro polymorphism at codon 72 of *p*53 gene with breast cancer risk in women of D.P.R. Korea. Tumor tissue samples from 97 women with primary breast carcinoma and radix pili tissue samples from 96 healthy females were collected and then analyzed through polymerase chain reaction. As results, in dominant genetic model of Pro allele, a statistically significant association was found between the *p*53 codon 72 polymorphism and breast cancer risk, but no correlation between the genotype distribution and clinicopathologic features was observed. This suggests that Arg/Pro & Pro/Pro has higher risk of breast cancer than Arg/Arg. (Odds ratio=2.0, 95% Confidential Interval=1.0～4.0, p=0.039)

Key words breast cancer, risk factor, *p*53 codon 72(Arg72Pro) polymorphism

Introduction

The great leader Comrade **Kim Jong Il** said as follows.

"The most important factor in the current development of medical science and technology is to concentrate on solving the pressing scientific and technological problems in the health service."("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 18)

The breast cancer is the most common malignancy and the leading cause of cancer mortality among women in the worldwide [1]. Although many risk factors of the development of breast cancer such as the inherited genetic predisposition have been identified, the molecular mechanisms related to breast carcinogenesis remain under investigation. Different types of genetic and epigenetic alterations are significant in the stepwise development of breast neoplasms.

The *p*53 is one of the most well established tumor suppressor genes and is related to the pathogenesis of various human cancers. The *p*53 plays a key role in preservation of genome integrity. This tumor suppressor gene is a multifunctional tetrameric transcription factor involved in the control of cell cycle progression, DNA repair, apoptosis and senescence. The *p*53 gene is the most frequently altered gene in human cancer including breast cancer. Germline mutation carriers of *p*53 are at very high risk of developing breast cancer, and in addition, about 40% of sporadic breast cancers show somatic mutation of *p*53, indicating its importance in the pathogenesis of breast cancer, acting as a G_1/S checkpoint control for recognizing damaged DNA and inducing DNA repair in genetically damaged cell or driving apoptosis if being impossible to repair [2].

In the world, there are many studies on the association between *p*53 mutations and breast cancer. Apart from the studies that have been carried out on the mutational status of *p*53,

there are many researches regarding the importance of the genetic polymorphisms of this gene [3－5]. In addition to genetic mutations, the pathogenesis and prognosis of breast cancer may also be affected by *p*53 single nucleotide polymorphisms (SNPs) that are involved in repair of DNA damage. In 1987, a new *p*53 mutation structure was discovered based on morbility differences when performing P53 protein electrophoresis, resulting in a new concept of SNP and carcinogenesis. The morbility difference was due to a polymorphism located at *p*53 codon 72 [6]. This polymorphism encodes either arginine (Arg) or praline (Pro) leading to three variant forms; Arg/Arg, Arg/Pro and Pro/Pro. The two polymorphic variants have been shown to have not only structural differences, but also different biological properties [6].

The codon 72 polymorphism, the most common SNP in the *p*53 gene, is located in the non-conserved prolin-rich region of P53 protein and affects the structure of the putative SH3-binding domain; therefore this region is important in apoptosis function of *p*53. In 2003, it is demonstrated that *p*53 with Pro allele at codon 72 exhibits a lower ability to induce apoptosis in vitro than *p*53 with Arg allele at codon 72 [7], suggesting that this polymorphism is associated with breast cancer risk.

The current hypothesis that attempt to explain why Arg72 has a higher *p*53-mediated apoptosis induction capability can be divided into two major categories.

First, the Arg72 variant retains a higher potential to localize itself to mitochondria. Hence, this cellular activity might provide an opportunity to enhance interaction between *p*53 and *MDM2*. MDM2 is an ubiquitin ligase (E3) that binds to *p*53 gene and triggers its ubiquitination towards nuclear exportation, which accompanies cytochrome C release.

Second, the Arg72 variant was found to interact with the pro-apoptotic gene product “PREP” more intensively, when compared to the Pro72 variant. This interaction would result in enhancing apoptosis activity more than 10-fold. To sum up, these studies fully illustrate that the *p*53 codon 72 polymorphism leads to amino acid changes, and its variant also acts differently [8－10]; However, more precise functional studies of codon 72 variants should be carried out in order to uncover the in-depth molecular mechanism that is associated with different diseases.

1. Materials and Methods

1.1. Controls and cases

Eligible cases were 97 women who were treated with mastectomy or breast conserving surgery due to primary breast cancer at Breast Oncology Institution in Pyongyang Maternity Hospital during the period of November 2011 to May 2012 and they were consecutively recruited as cases in this study.

96 healthy (free history of breast diseases) females who attended the breast cancer screening program at Breast Oncology Institution in Pyongyang Maternity Hospital were consecutively recruited as controls during the same period. All these women were confirmed as free from breast diseases by physical examination and mammography.

1.2. Genotype analysis

DNAs were extracted from the tumor tissue of cases and the radix pili tissue of controls. Each of genomic DNA was used for PCR amplifications using two pairs of primers designed for *p*53 Arg72Pro polymorphism.

The sequence of primers is as follows. The forward primer used for the amplification of Arg variant was 5′–TCC CCC TTG CCG TCC CAA–3′ and reverse was 5′–CTG GTG CAG GGG CCA CGC–3′. The forward primer set used for the amplification of Pro variant was: forward, 5′–GCC AGA GGG TGC TCC CCC–3′ and reverse was 5′–CTG GCA AGT CAC AGA CTT–3′.

Each PCR reaction was preformed in a total volume of 10μL. (Containing 50ng genomic DNA, 0.5units Taq DNA polymerase, 10mmol/L dNTPs 0.5μL, 1mmol/L primers 1μL and 10×PCR buffer solution 1.0μL with containing 2.5mmol/L $MgCl_2$)

The PCR conditions are as follows. The reaction was carried out in a condition of 5 min initial denaturation at 94℃ followed by 35 cycles of amplification at 94℃ for 30s, 62℃ on Arg variant and 54℃ on Pro variant for 30s, and 72℃ for 40s, and at last followed by a final elongation cycle at 72℃ for 5min on the GeneAmp PCR system (“peQSTAR”, Deutschland & Osterreich, United Kingdom). A 1μL of PCR product was diluted with the same volume of loading buffer followed by 8% polyacrylamide gel electrophoresis, along with a 100-bp DNA ladder.

Arg/Arg homozygote produced a single band of 141bp, Pro/Pro homozygote was identified by a single band of 177bp and Arg/Pro heterozygote displayed two bans of 141 and 177bp.

1.3. Statistical analysis

All data were analyzed using SPSS Version 16.0 software (Statistical Package for the Social Sciences). The relationship between a genotype and breast cancer risk was determined to obtain the odds ratio (OR) and 95% confidential interval (CI). The two-sided Pearson χ^2 test method was used to compare the clinicopathologic parameters with *p*53 codon 72 genotypes. A probability of less than 0.05 was considered to be statistically significant.

2. Results and Discussion

2.1. The association between p53 codon 72 genotype and breast cancer risk

The frequencies of *p*53 codon 72 genotype was determined by genotyping from 97 breast cancers tissue from cases and 96 radix pili tissues from controls (table 1.)

Table 1. Frequencies of the *p*53 codon 72 genotype

Sample type	Total cases	Arg/Arg	Arg/Pro	Pro/Pro	*P*-value
Control	96	29(30.2%)	64(66.7|%)	3(3.1%)	0.053
Case	97	17(17.5%)	79(81.4%)	1(1.0%)	

As shown in table 1, the proportion of *p*53 codon 72 genotype in our country of the patients with breast cancer were 17.5% in Arg/Arg, 81.4% in Arg/Pro and 1.0% in Pro/Pro. The frequencies in healthy females were 30.2%, 66.7% and 3.1%, respectively.

The *p*53 codon 72 allele frequencies were estimated by controls and cases (table 2).

Table 2. *p*53 codon 72 allele frequencies

Sample type	Total cases	Arg allele/%	Pro allele/%	OR	95% CI	P-value
Control	96	63.5	36.5	1.2	0.8～1.9	0.287
Case	97	58.2	41.8			

As shown in table 2, allele frequencies did not differ significantly between the controls and the cases (p=0.287). The Arg allele frequencies were 63.5% and 58.2% in the controls and the cases, respectively and the Pro allele frequencies were 36.5% and 41.8%, respectively.

And then, the frequencies of *p*53 codon 72 genotype were observed in recessive and dominant genetic model of Pro allele (table 3).

Table 3. Frequencies of the *p*53 codon 72 genotype in recessive and dominant genetic model of Pro allele among cases and controls

Sample type	Total cases	Recessive genetic model		Dominant genetic model	
		Arg/Arg & Arg/Pro	Pro/Pro	Arg/Arg	Arg/Pro & Pro/Pro
Control	96	93(96.9%)	3(3.1%)	29(30.2%)	67(69.8)
Case	97	96(99.0%)	1(1.0%)	17(17.5%)	80(82.5%)
OR, 95% CI, P-value		3.1, 0.3～30.3, 0.307		2.0, 1.0～4.0, 0.039	

In recessive genetic model of Pro allele, frequencies of the *p*53 codon 72 genotype did not differ significantly between the controls and the cases (p=0.307). However, in dominant genetic model of Pro allele, a statistically significant association was found between the *p*53 codon 72 genotype and breast cancer risk, suggesting that non-Arg/Arg has higher risk of breast cancer than Arg/Arg. (OR=2.0, 95% CI=1.0～4.0, p =0.039)

Some studies supported the finding that *p*53 codon 72 Arg allele represents a significant risk in breast carcinogenesis [11, 12], but it has been reported that patients with the Pro allele are more susceptible to cancer development and a poor clinical outcome [13, 14]. Recently, a novel molecular difference underlying the tumor-suppressing function of the *p*53 codon 72 genotype has been proved [15] and this may at least partially explain why Pro allele is associated with breast cancer risk.

Although it has been proposed that there are inherent differences in the relative prevalence of the polymorphism in various populations [4, 5], it is interesting to find a discrepancy in the distributions of the *p*53 codon 72 polymorphism between Asian and Caucasian populations. There was more Pro allele (40.6% in Asians vs. 26.4% in Caucasians) and there was twice the incidence of Pro/Pro homozygotes (18% vs. 8%) among the Asian population [3]. Compatible with the *p*53 codon 72 polymorphism locating at the proline-rich domain which is responsible for the growth suppression and apoptosis activity, Pro allele was less efficient in suppressing cell transformation, slower to induce apoptosis, and less efficient at binding and inactivating P73 protein, which is a tumor suppressor protein responsible for apoptosis. The reason for the greater frequency of the Pro allele in Asian patients with breast cancer remains unclear [3].

2.2. Association between *p*53 codon 72 polymorphism and the clinicopathologic features of breast cancer

Previous studies reported that *p*53 codon 72 polymorphism is associated with Age of patients [12], with tumor size [16], with menopausal status, nodal status, tumor stage and histopathological tumor grade [13], and with ER positive breast cancer risk [14]. We analyzed whether the *p*53 codon 72 polymorphism is associated with some clinicopathologic features of breast cancer including age at diagnosis, age at menarche, menopausal status, age at frist live birth, family history of breast cancer, body mass index (BMI), pT stage, pN stage and pTNM stage (table 4).

Table 4. Association between *p*53 codon 72 polymorphism and the clinicopathologic features of breast cancer

Features		Total cases	Arg/Arg	Arg/Pro & Pro/Pro	*p*-value
Age at diagnosis	≤39	21	3(14.3%)	18(85.7%)	0.663
	40～49	45	7(15.6%)	38(84.4%)	
	50≤	31	7(22.6%)	24(77.4%)	
Age at menarche	≤13	16	2(12.5%)	14(87.5%)	0.563
	14≤	81	15(18.5%)	66(81.5%)	
Menopausal status	Post-	35	7(20.0%)	28(80.0%)	0.554
	Pre-	59	9(15.3%)	50(84.7%)	
Age at frist live birth	≤29	80	15(18.8%)	65(81.3%)	0.964
	30≤	11	2(18.2%)	9(81.8%)	
Family history of breast cancer	Yes	6	0(0.0%)	6(100.0%)	0.244
	No	91	17(18.7%)	74(81.3%)	
Body mass index (BMI)	23.0	37	5(13.5%)	32(86.5%)	0.414
	<23.0	60	12(20.0%)	48(80.0%)	
pT stage	T1	37	4(10.8%)	33(89.2%)	0.861
	T2	24	3(12.5%)	21(87.5%)	
	T3	2	0(0.0%)	2(100.0%)	
pN stage	N0	32	3(9.4%)	29(90.6%)	0.690
	N1	23	3(13.0%)	20(87.0%)	
	N2	4	0(0.0%)	4(100.0%)	
	N3	4	1(25.0%)	3(75.0%)	
pTNM stage	Ⅰ	23	1(4.3%)	22(95.7%)	0.419
	Ⅱ	32	5(15.6%)	27(84.4%)	
	Ⅲ	8	1(12.5%)	7(87.5%)	

As shown in table 4, the unlucky fact is that no significant differences were found in the distribution of these clinicopathological parameters between *p*53 codon 72 polymorphism.

Our result is consistent with ref [11]. Because of this, codon 72 polmorphism does interfere with development of breast cancer, but not with the progression of the disease. In contrast, this association is inconsistent as previous results [12－14, 16]. However, a more definitive conclusion regarding the influence of this polymorphism on breast cancer outcome will depend on the longterm analysis of our patients's clinical evolution and survival.

Because the biological behavior of breast cancer is characterized by a long natural history of breast diseases and late development of metastases, follow-up periods longer than 10 years are usually required to properlye valuate prognostic factors in this malignancy. The reason whether *p*53 codon 72 polymorphism is associated with specific clinicopathologic features of breast cancer is currently unknown.

Conclusion

In summary, our results indicate that Pro allele at codon 72 of the *p*53 gene is associated with a higher susceptibility to breast cancer development. Therefore, it may be hypothesized, that the high prevalence of the Arg/Pro & Pro/Pro genotype in our country may be somehow contributing to the high incidence of breast cancer.

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