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p53 Codon 72 Polymorphism and Risk of Cervical Carcinoma in Korean Women

A common polymorphism of the wild type p53 is known at codon 72 of exon 4, with 2 alleles encoding either arginine (CGC, p53Arg) or proline (CCC, p53Pro). A recent study suggested that this polymorphism affects the susceptibility of p53 protein to human papillomavirus E6 oncoprotein mediated degradation and that individuals homozygous for p53Arg are seven times more susceptible to HPV-associated carcinogenesis of the cervix than heterozygotes. To examine whether the p53Arg genotype could be a risk factor for HPV-associated cervical carcinomas in the Korean population, we analyzed the p53 codon 72 polymorphism status of HPV-positive invasive cervical carcinomas from 52 Korean women and 103 healthy control samples. The proportion of individuals homozygous for p53Arg, homozygous for p53Pro, and heterozygous for the two alleles were 40%, 19%, and 41% in normal healthy controls; 42%, 17%, and 40% in women with HPV-positive invasive cervical carcinoma. There were no significant differences in the distribution of p53 genotypes between controls and cervical carcinomas. This finding indicates that the p53Arg genotype is not associated with an increased susceptibility to cervical carcinoma in Korean women.

Key Words: *Genes, p53; Polymorphism (Genetics); Cervix Neoplasms; Papillomavirus*

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INTRODUCTION

Human papillomavirus (HPV) infection has been shown to be an important causative factor in the development of cervical carcinoma. HPV-16 and -18 are the most frequently detected types in cervical carcinomas (1). HPV-16 and -18 encode two major oncoproteins, E6 and E7. It is known that the E7 oncoprotein can bind to and render inactive the retinoblastoma cellular tumor suppressor protein (2). On the other hand, the E6 oncoprotein binds to the p53 cellular tumor suppressor protein and promote its degradation via the ubiquitin dependent proteolysis pathway (3, 4).

A common polymorphism is known at codon 72 of the p53 gene, with two alleles encoding either arginine (p53Arg) or proline (p53Pro) (5). Storey et al. (6) recently investigated the effect of the codon 72 polymorphism of p53 on the susceptibility to E6-mediated degradation. They reported that individuals homozygous for p53Arg are seven times more susceptible to HPV-associated carcinogenesis of the cervix than heterozygotes. Several groups have failed to confirm this result in various ethnic populations (9-15). In these reports no association of the p53Arg genotype with cervical carcinoma was found. To investigate whether the p53Arg genotype could be a risk factor for HPV-associated invasive cervical carcinomas in the Korean population, we examined the p53 codon 72 genotypes of HPV-positive invasive cervical carcinomas from 52 Korean women and 103 healthy control samples.

MATERIALS AND METHODS

Tissue specimens and DNA extraction

Snap-frozen tumor samples from Korean women who had undergone surgery for cervical carcinoma at Keimyung University Dongsan Medical Center in Taegu city between 1993 and 1999 were used. DNA of tissue samples were extracted by QIAamp tissue kit (QIAGEN Inc., Valencia, U.S.A.). DNA of healthy controls were extracted from EDTA-treated whole blood by Dr. GenTLE kit (Takara Shuzo Co., Otsu, Japan).

Detection and typing of HPV

Detection and typing of HPV were done by a PCR analysis described by Fujinaga et al. (7). Briefly, the E6/E7 ORF region was amplified using consensus primers (5'-TGTCAAAAACCGTTGTGTCC-3' and 5'-GAGCTGTCGCTTAATTGCTC-3'). PCR was done in 40 μL, containing about 200 ng DNA, 0.2 unit TaKaRa Taq DNA polymerase (Takara Shuzo Co., Otsu, Japan) and 1.5 mM $MgCl_2$ for 34 cycles (1 min at 95℃, 2 min at 55℃, and 2 min at 72℃). The products were electrophoresed on 1.5% NuSieve 3:1 agarose (FMC BioProducts Co., Rockland, U.S.A.) gel. HPV types were identified by sizes of the PCR products and further confirmed by restriction enzyme analysis using *Ava*II, *Rsa*I, *Bgl*II, or *Acc*I (TaKaRa Shuzo Co.).

Genotyping of p53 gene at codon 72 by PCR

The genotypes of p53 gene at codon 72 were detected by PCR with allele specific primers (p53Pro+/p53− for Pro sequence; p53+/p53Arg− for Arg sequence) as described by Storey et al. (6) with slight modification. Briefly, PCR with allele specific primers was done in 20 μL, containing about 100 ng DNA, 0.1 unit TaKaRa Taq DNA polymerase (TaKaRa Shuzo Co.) and 1.5 mM $MgCl_2$ for 30 cycles. The products were electrophoresed on 2% agarose gel. The arginine specific PCR results in a 141 bp product and the proline specific PCR results in a 177 bp product (Fig. 1A). The genotypes were confirmed by *Acc*II restriction enzyme digestion, which discriminates between the polymorphic sites (8). PCR for restriction enzyme analysis was done with p53+ and p53− primers. The PCR-amplified DNA products were digested with 4 units of *Acc*II enzyme (TaKaRa Shuzo Co.) for 3 hr at 37℃, and the products were subjected to electrophoresis in 1.5% NuSieve 3:1 agarose (FMC BioProducts Co., Rockland, U.S.A.) gel. Since the Arg allele, but not the Pro allele, has a single *Acc*II recognition site (CGCG), the former was determined as two bands of 159 and 124 bp, and the latter as a single band of 283 bp (Fig. 1B).

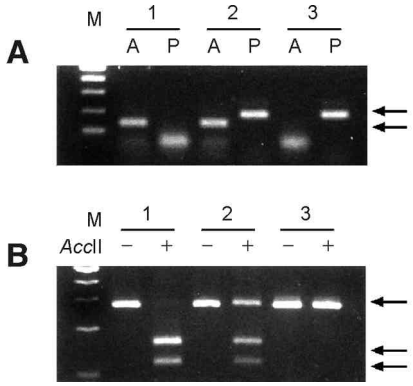

Fig. 1. PCR analysis of the polymorphism at codon 72 of the p53 gene. **A:** PCR products primed with allele specific primers (see Materials and Methods) are shown by the arrows in the lanes A (Arg allele specific primers, 141 bp) and P (Pro allele specific primers, 177 bp). **B:** PCR products primed with p53+ and p53− primers (see Materials and Methods) digested (+) or undigested (−) by AccII. The Arg and Pro alleles are determined as the 159+124 bp and 283 bp bands respectively, which are indicated by the arrows. 1, example of Arg/Arg homozygote; 2, example of Arg/Pro heterozygote; 3, example of Pro/Pro homozygote; M, 100 bp ladder-size marker.

RESULTS

The PCR-based analysis used for this study specifically detected the genotypes of polymorphic alleles (Arg or Pro allele) as shown in Fig. 1. The frequencies of the p53 codon 72 genotypes are summarized in Table 1. There were no significant differences in the proportion of the different p53 codon 72 genotypes between individuals with HPV-16/18 positive cervical carcinomas and controls.

DISCUSSION

In a previous report, Storey et al. (6) showed that the codon 72 Arg/Pro polymorphism of p53 gene affects the susceptibility of p53 to E6-mediated degradation in vitro, with p53Arg more susceptible than p53Pro. They also suggested that a seven-fold increase in the risk of human papillomavirus-associated cervical carcinoma in Caucasian women homozygous for the arginine form at the codon 72 polymorphism of p53.

In this study, we examined this hypothesis in the

Table 1. Distribution of p53 codon 72 genotypes in patients and controls

	Arg/Arg (%)	Pro/Pro (%)	Arg/Pro (%)
HPV-positive invasive cervical carcinomas (N=52)	22 (42.3)	21 (40.4)	9 (17.3)
HPV-16/18-positive invasive cervical carcinomas (N=46)	18 (39.1)	19 (41.3)	9 (19.6)
Controls (N=103)	41 (39.8)	42 (40.8)	20 (19.4)

Numbers in parenthesis represent relative frequencies

Korean population by analyzing p53 genotypes of HPV-positive invasive cervical carcinomas isolated from 52 Korean patients as well as blood samples from 103 healthy controls. We did not detect significant differences in the frequencies of Arg versus Pro alleles between individuals with HPV-16/18-positive invasive cervical carcinomas and healthy controls. Our results provide evidence that the p53Arg genotype is not a risk factor for the development of HPV-associated cervical carcinoma. It is unlikely that the discrepancy between our results and those of Storey et al. (6) could be attributed to differences in methods, because we used similar techniques, which were further confirmed by the use of *Acc*II restriction enzyme analysis.

Consistent with our results, other groups (9-15) found no statistically significant differences in the distribution of p53 codon 72 genotypes between the control women and cervical cancer patients. The results of Storey et al. could be due to chance because it was based on a comparison between a smaller group of cancer patients and a small control group. As it is known that significant differences exist in p53 genotypes frequencies between different populations (16), the genotypes of the controls in the report of Storey et al. may also reflect a mixed ethnic origin.

Since our study was done in a population with a relatively high prevalence of arginine homozygotes, the possibility that this study in populations with a high frequency proline allele, such as black Africans (16) or Brazillians (17), might yield significant results can not be totally ruled out. However, at least in Korean women, it seems that the p53Arg genotype is not associated with an increase in susceptibility to HPV-associated cervical carcinoma.

REFERENCES

1. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. *Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992; 79: 328-37.*
2. Dyson N, Howley PM, Munger K, Harlow E. *The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989; 243: 934-6.*
3. Scheffner M, Werness BA, Huibergste JM, Levine AJ, Howley PM. *The E6 oncoprotein encoded by human papillomavirus type 16 and 18 promotes the degradation of p53. Cell 1990; 63: 1129-36.*
4. Werness BA, Levine AJ, Howley PM. *Association of human papillomavirus type 16 and 18 E6 proteins with p53. Science 1990; 248: 76-9.*
5. Matlashewski GJ, Tuck S, Pim D, Lamb P, Scheneider J, Crawford LV. *Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol 1987; 7: 961-3.*
6. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. *Role of p53 polymorphism in the development of human papillmavirus-associated cancer. Nature 1998; 393: 229-34.*
7. Fujinaga Y, Shimada M, Okazawa K, Fukushima M, Kato I, Fujinaga K. *Simultaneous detection and typing of genital human papillomavirus DNA using the polymerase chain reaction. J Gen Virol 1991; 72: 1039-44.*
8. de la Calle-Martin O, Fabregat V, Romero M, Soler M, Vives J, Yague J. *AccII polymorphism of the p53 gene. Nucleic Acids Res 1990; 18: 4963.*
9. Rosenthal AN, Ryan DA, Ai-Jehani RM, Storey A, Harwood CA, Jacobs IJ. *p53 codon 72 polymorphism and risk of cervical cancer in UK. Lancet 1998; 352: 871-2.*
10. Minaguchi T, Kanamori Y, Matsushima M, Yoshikawa H, Taketani Y, Nakamura Y. *No evidence of correlation between polymorphism at codon 72 of p53 and risk of cervical cancer in Japanese patients with human papillomavirus 16/18 infection. Cancer Res 1998; 58: 4585-6.*
11. Lanham S, Campbell I, Watt P, Gornall R. *p53 polymorphism and risk of cervival cancer. Lancet 1998; 352: 1631.*
12. Hayes VM, Hofsta RM, Buys CHCM, Hollema H, van der Zee AGJ. *Homozygous arginine-72 in wild type p53 and risk of cervical cancer. Lancet 1998; 352: 1756.*
13. Helland A, Langerod A, Johnsen H, Olsen AO, Skovlund E, Borresen-Dale A. *p53 polymorphism and risk of cervical cancer. Nature 1998; 396: 530-1.*
14. Josefsson AM, Magnuson PKE, Ylitalo N, Quarforth-tubbin P, Ponten J, Adami HO, Gyllensten UB. *p53 polymorphism and risk of cervical cancer. Nature 1998; 396: 531.*
15. Hildesheim A, Schiffman M, Brinton LA, Fraumeni Jr JF, Herrero R, Bratti MC, Schwartz P, Mortel R, Barnes W, Greenberg M, McGowan L, Scott DR, Martin M, Herrera JE, Carrington M. *p53 polymorphism and risk of cervical cancer. Nature 1998; 396: 531-2.*
16. Beckman G, Birgander R, Sjalander A, Saha N, Holmberg PA, Kivela A, Beckman L. *Is p53 polymorphism maintained by natural selection? Hum Hered 1994; 44: 266-70.*
17. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. *p53 polymorphism and risk of cervical cancer. Nature 1998; 396: 532.*