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The Preliminary Study on the Relationship between HPV-associated Cervical Cancer and p53 Codon 72 Polymorphism in Sichuan Province

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Abstract. *Objective:* To study the relationship between HPV-associated cervical cancer and p53 codon 72 polymorphism in Sichuan Province. *Methods:* Three groups of women were studied: 30 women for normal control; 30 women with ovarian cancer; 50 women with cervical cancer. DNA from peripheral blood samples and from pathologic tissue sections was examined by PCR with allele-specific primers. *Results:* The proportions of individuals homozygous for the arginine allele, homozygous for the proline allele and heterozygous for the two alleles were 33.3%, 6.7% and 60% respectively among normal women; 40%, 6.7% and 53.3% in women with ovarian cancer respectively; 80%, 6% and 14% in women with cervical cancer respectively. χ^2 analysis showed significant differences in the proportions. *Conclusion:* In this population, individuals homozygous for the arginine variant of codon 72 of the p53 gene were at increased risk of cervical cancer.

Key words: p53 codon 72; polymorphism; homozygote; heterozygote; HPV-associated cervical cancer

A polymorphism at codon 72 of the human tumor-suppressor gene, p53, results in translation to either arginine or proline. Reports abroad suggested that the risk of HPV-associated cervical cancer in white women is higher for those homozygous for the p53Arg than those who are p53Arg/p53Pro heterozygous and p53Pro homozygous. Because Arg replaces Pro in the genetic products of p53 codon 72 polymorphism, increasing the sensitivity for p53 degraded by E6 protein from HPV, p53Arg homozygous can be regarded as a factor to predict the occurrence of cervical cancer. But report on the contrary suggested that p53Arg homozygous doesn't increase the risk of cervical cancer, and it differs from the human race. Up to now there are still no reports in our country about the relationship between p53 codon polymorphism and cervical cancer. The purpose of this study is to discuss the change of p53 codon 72 polymorphism in cervical cancer in our country, and provide the theoretical basement for early diagnosis, prediction and pathogenesis of cervical cancer.

Materials and methods

Specimens and preparation of DNA samples

We selected 30 pathologic tissue sections of ovarian cystadenocarcinoma, 50 of cervical squamous cell cancer, and 30 blood samples of normal control women in our hospital from August, 1996 to December, 2001. All the sections were checked by the professional pathologist. 1-2 mm^3 pieces from the clotted blood samples were excised and incubated over-night in 380 μl of SET (150 mmol/L sodium chloride, 10 mmol/L tris-HCl, 10 mmol/L edetic acid), containing 0.5% sodium dodecyl sulphate and 250 μg/L proteinase K (Bo-ehringer Mannheim, Lewes, UK). DNA was extracted with phenol-chloroform. For the tissue surrounded with wax, a 10 μm section was cut. The remaining tissue was scraped off and put in a sterile 2 5 ml screw-capped plastic tube containing 100 μl 10% chelating resin (Sigma, Saint Louis, MS,USA) in distilled water and 1 μl 20 mg/ml proteinase K. The mixture was incubated at 56 ℃ for 30 min, with shaking, boiled for 8 min, and centrifuged at 10000 g for 10 min. The supernatant was used directly in PCR[1].

PCR amplification of p53 codon 72 polymorphic alleles

P53 arginine and proline sequences were amplified with the allelic-specific primers individually, as shown in Table 1. PCR was done in a volume of 20 μl, containing 100 ng DNA, 80 μmol/L ATP, 0.1 U Red Hot DNA polymerase (Ad-vanced Biotechnologies, Epson, UK), 1 × buffer and 1.5 mmol/L magnesium. PCR was done for 35 cycles. 20 μl products from each of the PCRs were used for electrophoresis on a 3% agarose gel. The arginine-specific PCR

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resulted in a product of 141bp and the proline-specific PCR a product of 177 bp[2]. The results were shown in Fig.1.

Statistics

95% CIs for the genotype frequencies was calculated. χ^2 analysis was used to examine differences between the cervical-cancer group, the ovarian-cancer group and the control group.

Results

The distribution of the frequencies of the p53 codon 72 genotypes in the three groups was shown in Table 2 and Fig.2. There was no significant difference in the proportions of the different p53 codon 72 genotypes between the normal control group and the ovarian-cancer group (χ^2=0.02, P>0.05); While there were significant differences in the p53Arg homozygous between the cervical-cancer group and the ovarian-cancer group (χ^2=14.61, P<0.005), and between the cervical-cancer group and the normal control group (χ^2=19.36, P<0.005).

Discussion

The previous study suggested that a subgroup of women may have a genetic susceptibility to cervical cancer once infected by oncogenic HPV types. The study of Storey and his colleagues[1] confirmed that E6 from HPV-16 and HPV-18 is more effective at degrading p53 codon 72 Arg than p53 condon 72 Pro *in vivo*, while E6 from HPV-11 is less active towards p53Arg and inactive with p53Pro. The marked change in structure conferred on p53 by the Pro/Arg polymorphism could alter the binding of E6, E6AP or other components of the ubiquitin pathway to p53 *in vivo*. When the sequence between residues 66 and 100 of p53 is lost, it is no longer susceptible to p53 degradation, indicating that the polymorphic sequences of p53 are needed for E6 to target p53 for degradation. This polymorphism lies in a region of p53 that involved in the induction of apoptosis, and which shares marked similarity to an SH3 (SRC-homology-3) - binding domain[2-5]. Storey compared the cervical cancer group with the normal group, finding that there was significant difference (P<0.005) for p53 homozygous between the cervical-cancer group (N=30) and the control group (N=41), the proportions were 76.7% and 36.6% respectively. The result indicates that p53Arg homozygous may represent a risk factor of cervical cancer[1]. Our results showed that the proportions of p53Arg homozygous, p53Pro homozygous and p53Arg/p53Pro heterozygous were 33.3%, 6.7% and 60% among the normal control group; 40%, 6.7% and 53.3% among the ovarian-cancer group; and 80%, 6% and 14% among the cervical-cancer group, respectively. χ^2 analysis showed significant differences (P<0.005) for p53Arg homozygous between the cervical-cancer group and the other two groups and no significant difference (χ^2=0.02, P>0.05) for p53Arg

Table 1 Nucleotide sequence of primers p53 codon 72 allele

Primer	Sequence
P53Arg+	5'T C C C C C T T G C C G T C C C A A
P53Arg−	5'C T G G T G C A G G G G C C A C G C
P53Pro+	5'G C C A G A G G C T G C T C C C C C
P53Pro−	5'C G T G C A A G T C A C A G A C T T

Table 2 Frequency of Arg and Pro p53 alleles in normal and ovarian cancer and cervical cancer tissue

Group	Pro	Arg	Pro/Arg
Control group N=30	2(6.7%)	10(33.3%)	18(60%)
Ovarian cancer group N=30	2(6.7%)	12(40%)	16(53.3%)
Cervical cancer group N=50	3(6%)	40(80%)	7(14%)

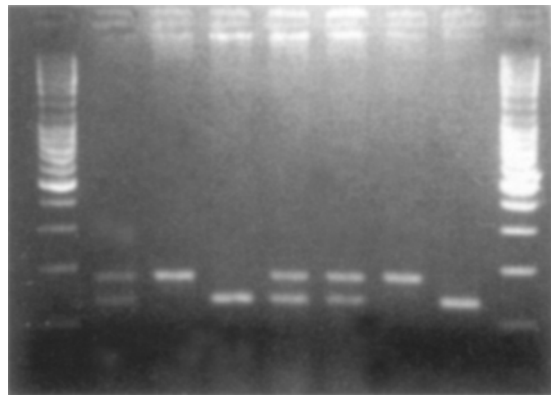

Fig. 1 Allele specific PCR amplification of p53 codon 72 polymorphism. Lane 1 and 9: Marker (100 base-pair DNA ladder; GIBCO BRL). Lane 2, 5, and 6: p53 heterozygotes. Lane 3 and 7: $p53_{Pro}$ homozygotes. Lane 4 and 8: $p53_{Arg}$ homozygotes

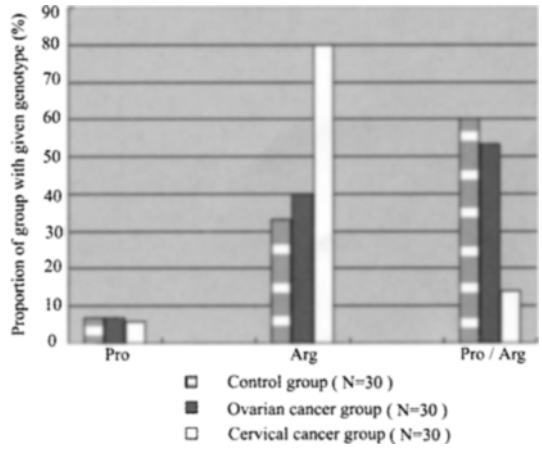

Fig. 2 Distribution of p53 condon 72 alleles in women with or without cervical cancer

homozygous between the ovarian-cancer group and the control group, indicating that homozygous for p53Arg can represent an important factor for predicating the risk of cervical cancer.

In cervical cancer, whose etiology is linked with certain types of HPV, there was an over-expression of p53 codon 72 Arg homozygous compared with heterozygous or homozygous p53 ccodon 72 Pro alleles. As to whether there is a relationship between p53 codon 72 polymorphism and the human race, different conclusions may be achieved because the samples of all current studies are limited, so epidemiological survey should be taken in larger populations and in different geographical regions to detect the change of p53 polymorphism. Our study is to inquire into the condition of the expression of p53Arg homozygous in cervical cancer in Chinese women, and conclusion can be made only if enlarging the samples in the future's study.

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