The Relationship between Genetic Polymorphism Of p53 Codon72 and Susceptibility to Cervical Cancer

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Abstract **Objective** To explore the relationship between genetic polymorphism of p53 codon72 and the susceptibility to cervical cancer. **Methods** The cervical tissues were from 33 women with squamous cervical carcinoma, in addition, the periphery blood was obtained from 51 healthy women as control. DNA samples from the peripheral blood and pathological tissue were evaluated by PCR with allele specific primers. **Results** The rates of homozygosity for the arginine (Arg) allele, homozygosity for the proline (Pro) allele and heterozygosity for the Arg/Pro alleles were 33.3%, 15.2% and 51.5% in cervicl cancerous tissues, respectively; 21.6%, 17.6% and 60.8% in control group, respectively. The OR and 95% CI for the genotype frequency of the Pro/Pro alleles or the Arg/Pro alleles was 1.80 (0.68~2.62) compared with the Arg/Arg alleles. The result of Chi-square analysis showed no significant differences between two groups, but OR value suggested a weak correlation between the Arg/Arg genotype and SCC. **Conclusion** In Han population in China, although a possible susceptibility of p53 codon72 polymorphism to SCC cannot be ruled out, but this risk was quite weak.

Key words p53; codon 72; polymorphism; squamous cervical cancer;

Cervical carcinoma is the third malignant tumor of women, which leads to about 200,000 women's death each year over the world. In our country each year about 131500 new cases emerge, and mortality is 3.89 per hundred thousand.

The population distribution of cervical carcinoma has an obvious regional phenomenon in China, and the incidence of cervical carcinoma in countryside or mountain area is higher than that in cities or plain area. The incidence is quite high in Shan´xi proince, in where Lueyang county exhibits predominant position as the highest spot. It stretches down toward northwest and northeast geographically, which forms the high incidence in Qinling Mountain area.

Carcinogenesis is a complex process involving a number of genetic and epigenetic events. Human papillomavirus (HPV) is considered the mainly etiologic factor for cervical high-grade precursor lesions and cervical cancer [1].

HPV16 and HPV18 are the most frequent types in cervical carcinoma development [2, 3]. HPV16 is mostly associated with squamous cervical carcinoma (SCC) and HPV18 with adenocarcinomas (AC). E6 and E7 gene of HPV early region encoded have function of transformation, which are carcinogenic key genes of HPV.

p53, an important tumor-suppressor gene of human, is located in chromosome 17P13 and encodes a 53kDa protein[4]. Deficiency of p53 function is a critical step in tumor development. However, variation and loss of heterozygosity of p53 was seldom found in development of cervical cancer [5]. The reason for p53 mutation is abided by HPV E6 protein and degraded through mediated ubiquitin [6].

Recent years, a common polymorphisms of p53 exon 4 at codon72 is regarded as a cofactors in carcinogenic transformation of HPV induced tumor. Wild-type p53 protein exhibits a common polymorphism at amino acid 72, at where either a praline or an arginine amino acid can be encoded. Story A *et al.* (1998) [7]

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noted that women with the Arg/Arg genotype were more susceptible to HPV−associated tumorigenesis than the women with the Arg/Pro genotype in the United Kingdom. Furthermore, Thomas M *et al.*[8]showed that the arginine form of p53 protein was significantly more susceptible to high−risk HPV E6 mediated degradation than the praline form.

Some studies supported the hypothesis of Story A [9, 10], however, many studies failed to confirm the hypothesis [11, 12], even several studies for the same population had obtained inconsistent results.

The reason of the difference has two aspects to explain: first, the content of samples was different, credibility with large sample studies is not the same as that with small samples. Secondly, Hardy−Weinberg equilibrium is unbalance in different population. It has been reported that p53 codon72 polymorphism varies between the different ethnic populations [13, 14].

Up to now, it had only one report on the relationship between p53 codon72 and cervical cancer in Han population in China [15]. The samples were only 15 cervical cancer patients. The Arg/Arg genotype of the cases vs. corresponding genotype of controls, $\chi^2 = 6.45$, $P < 0.05$, Chi−square analysis showed significant differences in two groups. But other studies on Asian women did not achieve similar results. So we conducted a new investigation to study whether the p53 codon72 arginine alleles confer a risk factor for cervical carcinoma in Han population.

To elucidate the association betweem p53 codon72 and cervical cancer is better to screen the women who have high−risk to develop cervical cancer. Assessing the risk degree is beneficial to improve the efficiency of follow−up examination for HPV infectors.

MATERIALS AND METHODS

Subject Samples

The cervical tissues were from 33 women with squamous cervical carcinoma in the Tumor Hospital of Shan′xi Province, in where all cervical specimens were confirmed in cytological diagnosis. The periphery blood was obtained from 51 women who attended routine healthcare examination and no cervical lesions were observed. All ethical identification of those who were involved in the study was confirmed. Finally, a standardized questionnaire was canvassed on attendees to obtain demographic and other relevant information.

DNA extraction

DNA was isolated from the malignant cervical tissues or the peripheral leukocytes according to the manufacture manual of Extract−DNA Animal PCR Kits (Figure 1). All DNA samples were dissolved in water and stored at −20℃. The quality of DNA was checked by $OD_{260/280}$ measurement.

PCR Amplification of p53 Polymorphic Sequences

The polymorphic regional sequences of the p53 gene was amplified from DNA of the cervical tissues and blood samples. The primer p53 Pro +/p53 Pro − (Table 1) were used for the Pro allele PCR and the primer p53 Arg +/Arg −(Table 1) for the Arg allele

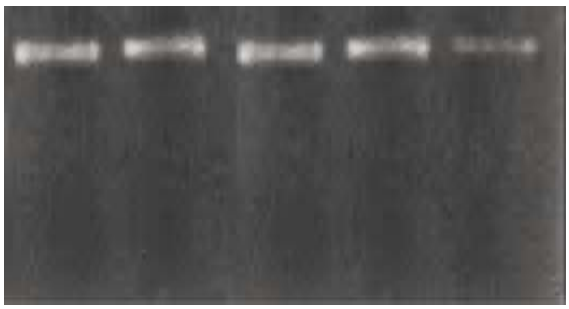
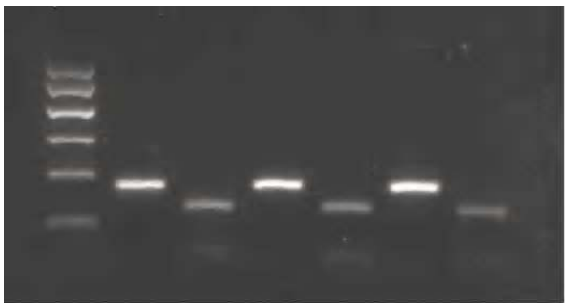


Fig. 1 0.8% Agarose gel analysis DNA isolation of cervical samples



1 2 3 4 5 6 7

Fig. 2 Representative agarose gel analysis of p53 polymorphism at codon72. Line1 from top to bottom respectively showed marker 100bp, 200bp, 300bp, 400bp, 500bp, line 2, 4, 6 were Pro product 177bp, line 3, 5, 7 were Arg product 141bp.

Table 1 Nucleotide sequence of primers p53 codon 72 allele

Primers	Sequence
p53 Arg^+	(codon 33−38) 5′ TCCCCCTTGCCGTCCCAA 3′
p53 Arg^-	(codon 72−78)5′ CTGGTGCAGGGGCCACGC 3′
p53 pro^+	(codon 66−72) 5′ GCCAGAGGCTGCTCCCCC 3′
p53 pro^-	(codon 120−126) 5′ CGTGCAAGTCACAGACTT 3′

Table 2 Frequencies of Codon 72 Polymorphism

p53 codon72	Cases with SCC (%)	control (%)	OR (95%CI)
alleles			
Arg	39 (59.1)	53 (52.0)	1.34 (0.68~2.62)
Pro	27 (40.9)	49 (48.0)	1 (reference)
Genotypes			
Arg/Arg	11 (33.3)	11 (21.6)	1.80 (0.37~8.97)
Arg/Pro	17 (51.5)	31 (60.8)	0.99 (0.25~4.08)
Pro/Pro	5 (15.2)	9 (17.6)	1 (reference)
Total	33 (100.0)	51 (100.0)	
Arg/Arg	11 (33.3)	11 (21.6)	1.82 (0.82~2.41)
Arg/Pro or Pro/Pro	22 (66.7)	40 (78.4)	1 (reference)

PCR. In each reaction a blank sample was employed as negative control to ensure no sample contamination. The PCR mixture was heated at 94° C for 4 minutes with denaturation and in PCR with the primer p53Pro+/p53 Pro−followed by 35 cycles of 40 seconds at 94° C, at 59° C for 40 seconds and at 72° C for 30 seconds, with the primer p53 Arg +/Arg−followed by 35 cycles of 40 seconds at 94°C, at 59°C for 40 seconds and at 72°C for 30 seconds. Final elongation was at 72° C for 10 min. PCR products were analyzed on 2.5% agarose gels and visualized under UV light transilluminator.

Statistical methods

SPSS10. 0 was conducted to analyze and process all data materials. X2 was used to find association. OR and 95% CI were calculated to estimate the risk ratio of Arg/Arg vs. Pro/Arg and Pro/Pro groups.

RESULTS

Analysis of p53 codon72 polymorphism indicated that the frequencies of the Arg allele and the Pro allele were 59.1% and 40.9% in the cases with squamous cervical carcinoma, 52.0% and 48.0% in control group, respectively. The Arg allele vs. the Pro allele, OR=1.34 (95%CI=0.68~2.62). The rates of the Arg and the Pro homozygosity and the Arg/Pro heterozygosity were 33.3%, 15.2% and 51.5% in cases with squamous cervical carcinoma, 21.6% , 17.6% and 60.8% in control group, respectively. OR and 95% CI of the Arg/Arg

genotype was 1.82 (0.82~2.41) compared with the Pro/Pro and the Arg/Pro genotypes. Chi-square analysis showed no significant differences between two groups (Table 2).

DISCUSSION

p53 protein displays polymorphism at amino acid 72, which resulted in either a Pro or an Arg residue at this position. Storey A et al. concluded that the HPV-associated cervical cancer patients with two copies of the Arg form have a sevenfold higher risk of developing cervical cancer than those with one arginine form. However, other studies failed to confirm that results. Nowadays, relevant studies generally regarded that p53 codon72 polymorphism was associated with region/ethnic crowd differences.

Our research on Hans population in Shan′xi Province showed that Arg/Arg vs. Arg/Pro and Pro/Pro co-genotype between two groups, OR =1.82 (95%CI =0.82-2.41), χ^2 =1.435, P =0.231, the difference did not have statistical significant, which revealed no association between the Arg/Arg genotype and cervical cancer and no evidence to support the hypothesis of Storey *et al.*

The results of this study failed to confirm those of the previous Xi MR et al.′s study[15], which showed that the Arg/Arg genotype of cases vs. that of controls, χ^2= 6.45, $P < 0.05$. Chi-square analysis showed significant differences in the proportions. There should be some aspects to be taken into account. First, the sample size of Xi MR *et al.*′s was relatively small, 15 cases and 20 controls. The small sample size has a tendency to induce sampling error. Secondly, 38% Arg/Arg genotype of controls in Xi MR′s report was higher than other studies on the association between p53 codon72 gene and risk of tumor development in our country. The Arg/Arg genotype frequencies of the healthy Han ethical women in Henan and Shandong province [16,17]were all less than 30%, comparatively close with our result, 21.6% Arg/Arg in control population. Based on the above reasons our study suggests that our control population be more representative of Han population.

Although Chi-square analysis showed no significant differences between two groups, OR value suggested a weak correlation between Arg/Arg genotype and cervical cancer. The number of our samples may be the reason that conclusion didn′t have statistical significance. The OR was so close to one, it need more samples to draw a statistical significant conclusion.

Some studies indicated that p53 Arg homozygosity was a possible risk factor of HPV associated cervical tumorigenesis. Greece and South Africa etc.[9,10], in their race/ethical control population, the frequencies of the Arg/Arg genotype were less than 20%. However, some studies in American Caucasian[11,12] and in some countries in Europe [18~20] showed that the frequencies of the Arg/Arg genotype in those control population were more than 47%. Our result showed that p53 Arg homozygosity was 21.6%. Therefore, in Han population in China, a possible susceptibility of p53 codon72 polymorphism to cervical cancer at a late carcinogenetic stage cannot be ruled out, but this risk was quite weak.

Furthermore, the association between p53 codon72 polymorphism and squamous cervical cancer in Han population was not only correlated with the frequencies of the Arg homozygosity of controls, but also be involved in other factors, such as HPV types or HPV variables etc. Duin MV [21]suggested that in p53 codon72 Arg/Arg women with the infection of HPV-16 350T variants rendered a higher risk of cervical cancer. The association between p53 codon72 polymorphism and cervical cancer should be further investigated on HPV variables and host polymorphisms to identify the populations with high risk to develop cervical cancer.

In p53 analyses, we used clinical specimens instead of blood samples. This approach likely results in an overestimation of homozygous p53 Arg/Arg or p53 Pro/Pro women, due to the possible occurrence of allelic loss at the p53 locus. Loss of heterozygosity at this locus has been reported in 8~15% of cervical carcinomas [16,22]. However, it was known that in abnormal cervical tissues the dyskaryotic cells were admixed with normal cells. The carcinoma samples contained at least 5% stroma. Therefore, the fraction of normal cells in samples should be certained to obtain right p53 genotyping. Since the methodology for p53 genotype detection was based on two separate PCR reactions, the detection of low copy

number of one p53 allele is not affected by the possible presence of high copy number of the other allele. This was supported by reconstruction experiments[21]. In addition, a study [23]showed that amongst the same women the allele frequencies were well approximate whether DNA samples extracted from either cervical tissue or peripheral blood. Therefore, we believe that samples have not been misinterpreted due to a possible allelic loss at the p53 locus.

REFERENCES

1.Munoz N. Human papillomavirus and can: the epidemiological evidence. J Clin Virol, 2000, 19: 1−5.

2. Rapp L, Chen JJ. The papillomavirus E6 proteins. Biochim Biophys Acta,1998, 1378: 1−19.

3. Koromilas AE, Li S, Matlashewski G. Control of interferon signaling in human papillomavirus infection. Cytokine Growth Factor Rev, 2001, 12: 157−170.

4.Levine AJ, Momand J, Finlay AF, *et al.* The p53 tumour suppressor gene. Nature, 1991, 351: 453−456.

5.Busby−Earle RM, Steel CM, Williams AR, *et al.* p53 mutations in cervical carcinogenesis−low frequency and lack of correlation with human papillomavirus status. Br J Cancer, 1994, 69: 732−737.

6.Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science, 1990, 248: 76−79.

7.Storey A, Thomas M, Kalita A, *et al.* Role of p53 polymorphism in the development of human papillomavirus−associated cancer. Nature, 1998, 393: 229−234.

8.Thomas M, Kalita A, Labrecque S, *et al.* Two polymorphic variants of wild−type p53 differ biochemically and biologically. Mol Cell Biol, 1999, 19: 1092−1100.

9.Agorastos T, Lambropoulos AF, Constantinidis TC, *et al.* p53 codon 72 polymorphism and risk of intra−epithelial and invasive cervical neoplasia in Greek women. Eur J Cancer Prev, 2000, 9: 113−118.

10.Pegoraro RJ, Rom I, Lanning PA, *et al.* p53 codon 72 polymorphism and human papillomavirus type in relation to cervical cancer in South African women. Int J Gynecol Cancer, 2002, 12: 383−388.

11.Calhoun ES, McGovern RM, Janney CA, *et al.* Host Genetic Polymorphism Analysis in Cervical Cancer. Clin Chem, 2002, 48: 1218−1224.

12.Madeleine MM, Shera K, Schwartz SM, *et al.* The p53 Arg72Pro Polymorphism, Human Papillomavirus, and Invasive Squamous Cell Cervical Cancer. Cancer Epidemiol Biomarkers Prev, 2000, 9: 225−227.

13.Beckman G, Birgander R, Sjalander A, *et al.* Is p53 polymorphism maintained by natural selection? Hum. Hered, 1994, 44: 266−270.

14.Ojeda JM, Ampuero S, Rojas P, *et al.* p53 codon 72 polymorphism and risk of cervical cancer, Biol Res, 2003, 36: 279−283.

15.Mingrong Qie, Yanhua Zhang, Junmei Wu, *et al.* Study on the Relationship Between Cervical Cancer and p53 Codon 72 Polymorphism. Journal of Sichuan University (Medical Science Edition), 2002, 33: 274−275.

16.Peixoto Guimaraes D, Hsin Lu S, Snijders P, *et al.*Absence of association between HPV DNA, TP53 codon 72 polymorphism, and risk of oesophageal cancer in a high−risk area of China. Cancer Lett. 2001, 162: 231−235.

17.Lina Mu, Xuefu Zhou, Baoguo Ding, *et al.* Genetic polymorphisms of p53 codon72 and the risk of gastric cancer−case−control study, China Oncology, 2003, 13: 1−4.

18.Cenci M, French D, Pisani T, *et al.* p53 polymorphism at codon 72 is not a risk factor for cervical carcinogenesis in central Italy. Anticancer Res, 2003, 23:1385−1387.

19.Dybikowska A, Dettlaff A, Konopa K. p53 codon 72 polymorphism in cervical cancer patients and healthy women from Poland. Acta Biochim Pol, 2000, 47:1179−1182.

20.Rosenthal AN, Ryan A, Al−Jehani RM, *et al.* p53 codon 72 polymorphism and risk of cervical cancer in UK. Lancet, 1998, 352: 871−872.

21.Duin MV, Snijders PJ, Vossen MT, *et al.* Analysis of human papillomavirus type 16 E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. J Gen Virol, 2000, 81: 317−325.

22.Mullokandov MR, Kholodilov NG, Atkin NB, *et al.* Genomic alterations in cervical carcinoma: losses of chromosome heterozygosity and human papillomavirus tumor status. Cancer Research, 1996, 56: 197−205.

23.Saranath D, Khan Z, Tandle AT, *et al.* HPV16/18 Prevalence in Cervical Lesions/Cancers and p53 Genotypes in Cervical Cancer Patients from India. Gynecol Oncol, 2002, 86: 157−162.