PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(19):2775-2778
World Journal of Gastroenterology
 ISSN 1007-9327

• ESOPHAGEAL CANCER •

p53 polymorphism in human papillomavirus-associated Kazakh's esophageal cancer in Xinjiang, China

Xiao-Mei Lu, Yue-Ming Zhang, Ren-Yong Lin, Xiao-Hui Liang, Ya-Lou Zhang, Xing Wang, Yan Zhang, Yan Wang, Hao Wen

Xiao-Mei Lu, Ren-Yong Lin, Xiao-Hui Liang, Ya-Lou Zhang, Xing Wang, Yan Zhang, Yan Wang, Hao Wen, Medical Research Center, 1st Teaching Hospital, Xinjiang Medical University, Urumqi 830054, Xinjiang Uygur Autonomous Region, China
Yue-Ming Zhang, Public and Health College, Xinjiang Medical University, Urumqi 830054, Xinjiang Uygur Autonomous Region, China
Supported by Xinjiang Key Laboratory Foundation, No. XJDX0202-2003-05
Correspondence to: Hao Wen, Ph.D., Professor, Medical Research Center, 1st Teaching Hospital, Xinjiang Medical University, No.1 Liyushan Road, Urumqi 830054, Xinjiang Uygur Autonomous Region, China. wenhao2002@hotmail.com
Telephone: +86-991-4362844 **Fax:** +86-991-4324139
Received: 2004-03-26 **Accepted:** 2004-04-05

Abstract

AIM: To investigate the relationship between *p53* codon 72 polymorphism and human papillomavirus (HPV) type 16 infection in Kazakh's esophageal cancer (EC) in Xinjiang, China.

METHODS: Encoding regions of *p53* codon 72 and HPV-16 *E6* were amplified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and polymerase chain reaction (PCR) methods using pairs of primary esophageal squamous cell carcinoma (SCC) tissue and corresponding normal mucosa, which were collected from 104 patients of Kazakh in Xinjiang, China.

RESULTS: Only arginine allele was detected in 70.1% (39/55) of HPV-16-*E6*- positive cases but only in 40.8% (20/49) of HPV-16-*E6*-negative cases ($P<0.05$; *OR*, 3.53; 95% *CI*, 1.57-7.98). In contrast, such a significant correlation between *p53* polymorphism and HPV infection was not evident in corresponding normal mucosae. The allele frequency of *Arg* allele in cancer cases (0.68) was higher than that in normal mucosa samples (0.54) ($P<0.05$; *OR*, 1.80; 95% *CI*, 1.21-2.69).

CONCLUSION: *p53* codon 72 *Arg* homozygous genotype is one of the high-risk genetic factors for HPV-associated SCC of Kazakh. Individuals carrying *Arg* allele compared to those with *Pro* allele have an increased risk for esophageal SCC.

Lu XM, Zhang YM, Lin RY, Liang XH, Zhang YL, Wang X, Zhang Y, Wang Y, Wen H. *p53* polymorphism in human papillomavirus-associated Kazakh's esophageal cancer in Xinjiang, China. *World J Gastroenterol* 2004; 10(19): 2775-2778
http://www.wjgnet.com/1007-9327/10/2775.asp

INTRODUCTION

Esophageal cancer is common in several areas of central Asia, including Xinjiang Uygur Autonomous Region,China. The incidence of Kazakh's EC is the highest among population in Xinjiang and its age-adjusted mortality rate up to 91/100 000 has been reported in Kazakh's population[1]. The population size of Kazakh was estimated to be 13 million around the world and 10 million Kazakh distributed in Kazakhstan and 2 million in Xinjiang, China. The population in the present study was a Kazakh isolated community located at the Northeast of Xinjiang. The genetic homogeneity and geography stability of the population, along with shared exposure to common environmental variables, provide an excellent opportunity for the study of genetic influence on EC. These cancers are mostly SCC , and show a high frequency of mutation in the *p53* tumor suppressor[2]. Epidemiological studies have suggested that a number of risk factors are involved in the carcinogenesis of Kazakh's SCC, including deficiencies in vitamins and minerals, consumption of pickled foods and environmental exposure to specific nitrosamines, *etc*[3,4]. Viral infections, in particular HPV infection, have been reported in esophageal cancers from China, and HPV DNA has been detected in 0-60.0% of cancer tissues by polymerase chain reaction analysis[5,6]. HPV is implicated in the pathogenesis of squamous cell carcinoma of the cervix and esophagus. HPV-16 encodes *E6* protein, which binds to cellular tumor-suppresser protein *p53* and directs degradation through the ubiquitin pathway[7]. This event is mediated by another cellular protein termed *E6*-AP, a component of the ubiquitin pathway[8,9]. The arginine allele at codon 72 of *p53* was found to be more susceptible to degradation by HPV *E6* protein than the proline allele *in vivo*, thus resulting in a high frequency of esophageal SCC in individuals homozygous for arginine at the codon[8]. On the basis of these experiments, it has been widely assumed that *p53* is functionally inactivated by the viral *E6* protein in HPV-associated cancer cells and that infection with high-risk HPV types leads to the same phenotype as a loss of *p53* function because of *p53* gene mutations or direct degradation[9].The association of *p53* codon 72 polymorphism with HPV-16-associated esophagus SCC risk has been studied by several groups but with inconsistent results. Kawaguchi *et al*.[10] reported that the form of *p53* protein carrying an *Arg* residue at this position in HPV-16/18 positive samples was found to be significantly more susceptible to degradation by *E6* protein than that in HPV-16/18 negative samples. There are controversial results from several clinical studies of esophagus SCC[11,12]. A part of Kazakh's esophageal SCC correlated with the presence of HPV-16/18[13]. To our knowledge, *p53* polymorphism in Kazakh's esophageal SCC has apparently not been documented. In this study, we investigated the genotypic frequency of *p53* codon 72 polymorphism and HPV-16 *E6* in Kazakh's esophageal SCC patients in Xinjiang, China. The data we obtained seemed to be the first regarding the association of this polymorphism with HPV-associated risk for cancer of the esophagus.

MATERIALS AND METHODS

Tissue specimens

Pairs of primary Kazakh's esophageal SCC tissue and corresponding normal mucosa were obtained from 63 patients who underwent surgery in the Department of Surgery, 1st Teaching Hospital of Xinjiang Medical University, from 1999 to 2003, and from 41 patients who underwent surgery in

Department of Surgery, the People's Hospital of Xinjiang Uygur Autonomous Region, China, between 1998 and 2000. No patient had been given treatment prior to the study. In all cases the histopathological type of tumors was squamous cell carcinoma. Cancer tissues and well-separated normal esophageal mucosae obtained from surgically resected esophageal SCC patients were fixed in 40 g/L formaldehyde and embedded in paraffin. Genomic DNA was prepared by proteinase K digestion and phenol/ chloroform extraction, followed by ethanol precipitation, as described by Greer *et al*.[14].

HPV detection and identification

First, as a control, purified genomic DNA was successfully amplified by PCR using primers specific for the β-globin gene, indicating a suitable quality and quantity of DNA. PCR analysis was then performed using HPV-16 *E6* oligodeoxynucleotide primers as follows: HPV-16*E6* forward, 5'-GCAAGCAACAGTT ACTGCGA-3' and reverse, 5'-CAACAAGACATACATCGACC -3'. Amplified PCR products were then determined by electrophoresis on 15 g/L agarose gels stained with ethidium bromide. Finally, the gels were analyzed by DC-2000 image system (Bio-Rad, USA).

Analysis of codon 72 polymorphism

p53 exon 4 (codons 33-125) containing codon 72 was amplified by PCR using oligodeoxynucleotide primers 5'-TGAGG*ACC* TGGTCCTCTGAC-3' (forward) and 5'-AGAGGAATCCCAAA GTTCCA-3' (reverse), under the following conditions: denaturation at 94 ℃ for 30 s, primary annealing at 54 ℃ for 30 s, and extension at 72 ℃ for 30 s for 35 cycles. PCR products (412 bp) were digested overnight at 37 ℃ with *Acc* II, which was cut within the sequence corresponding to the *Arg* codon (CGC) at position 72 to generate two fragments of 252 bp and 160 bp[15]. The DNA fragments were then resolved by electrophoresis on 30 g/L agarose gels stained with ethidium bromide. Presence of uncut (412 bp) DNA was indicative of the *Pro* allele and heterozygous for *Arg*/*Pro* genotypes showed three fragments of 412, 252 and 160 bp.

Statistical analysis

Chi-square test was used to examine the correlation between the *p53* codon 72 polymorphism of the esophageal SCC patients and the presence of HPV-16*E6* by SPSS software(12.0).

RESULTS

Frequency of HPV-16E6 among Kazakh's esophageal SCC patients

Pairs of 104 DNA sample from primary Kazakh's esophageal SCC tissues and corresponding normal mucosae were analyzed for the presence of oncogenic HPV-16-*E6* using PCR methods (Figure 1). The frequency of HPV-16-*E6* gene in cancer cases (52.9%) was higher than that in corresponding normal mucosae (39.4%) (Tables 1, 2). These results were similar to previous reports[16].

Table 1 *Arg* and *Pro* alleles of *p53* in SCCs of Kazakh's esophagus (*n*, %)

	Pro	*Pro* / *Arg*	*Arg*
Esophageal SCC (*n* = 104)	21 (20.2)	25 (24.0)	58 (55.8)
HPV16*E6* positive (*n*=55)	8 (14.5)	8 (14.5)	39 (71.0)[a]
HPV16*E6* negative (*n*=49)	13 (26.5)	16(32.7)	20 (40.8)

[a]*P*<0.05 *vs* negative.

Arg allele at the codon 72 in HPV-associated esophageal SCC

PCR-RFLP was carried out on 104 DNA samples from primary Kazakh's esophageal SCC tissues and corresponding normal mucosae to analyze the association between the *p53* codon 72 polymorphism and HPV-associated esophageal SCC by DC-2000 image system (Figure 2). It showed a typical pattern of codon 72 evaluated by restriction analysis. Presence of the *Pro* allele resulted in resistance of the PCR amplified DNA fragment to digestion by *Acc* II. The comparision between the distribution of *p53* alleles at codon 72 in HPV-positive esophageal SCC with the HPV-negative group is shown in Table 1. The frequency of presence of *Arg* allele alone from the Kazakh's esophageal cancer specimens was similar (55.8%, 58 of 104) to other population cases of esophageal cancer[10]. Moreover, there was a marked difference in the frequency of *Pro*/*Arg* alleles between HPV-positive and HPV-negative groups. *p53 Arg* allele alone was detected in 71.0% (39/55) in the HPV-positive group, whereas in 40.8% (20/49) in the HPV-negative group ($P<0.05$; *OR*, 3.53; 95% *CI*, 1.57-7.98) (Table 1). The allele frequency of *Arg* alleles in cancer cases (0.68) was higher than that in normal mucosa samples (0.54) ($P<0.05$; *OR*, 1.80; 95% *CI*, 1.21- 2.69).

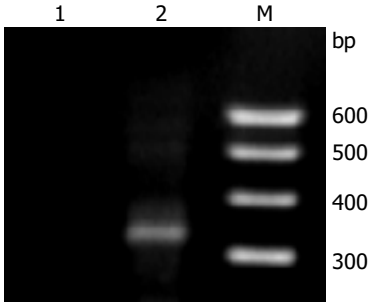

Figure 1 Agarose gel electrophoresis of HPV-16 E6 PCR-amplified fragments. Lane M: 100 bp DNA ladder marker; Lane 1: negative sample; and Lane 2: positive sample.

Table 2 *Arg* and *Pro* alleles of *p53* in normal mucosae of Kazakh's esophagus (*n*, %)

	Pro	*Pro* / *Arg*	*Arg*
Normal mucosa (*n* = 104)	28 (26.9)	40 (38.5)	36 (34.6)
HPV16*E6* positive (*n* = 41)	10 (24.4)	18 (43.9)	13 (31.7)
HPV16*E6* negative (*n* = 63)	10 (15.9)	30 (47.6)	23 (36.5)

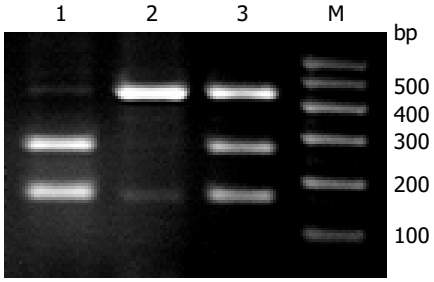

Figure 2 Restriction analysis of *p53* codon 72 polymorphism. The PCR product from the proline alleles had a single band with a fragment of 412 bp in length. The arginine was cleaved by *Acc* II, yielding two small fragments (252 and 160 bp). Lane M: 100 bp DNA ladder marker; Lane 1: homozygous for arginine; Lane 2: homozygous for proline; and Lane 3: digested sample, heterozygous for the polymorphism.

Arg allele at the codon 72 in surrounding normal mucosae in HPV-associated esophageal cancer

Differences in *p53* polymorphism in the corresponding normal mucosae were not significant between HPV-positive and -negative tissues ($P>0.05$; *OR*, 0.81; 95% *CI*, 0.35-1.86) (Table 2).

DISCUSSION

Infection with human papilloma virus is an important etiological factor in the development of SCC and it has been proposed that individuals homozygous for *Arg*/*Arg* at codon 72 of *p53* are several times more susceptible to HPV-mediated cancer[10,17]. In agreement with the result, studies in India and Mexico also found a strong increase in SCC risk associated with *p53* polymorphism and the presence of HPV infection[18,19]. In China similar research has been carried out on esophageal SCC, ovarian carcinoma, and breast carcinoma[20]. However, several studies conducted in different countries failed to reproduce this observation[21-25].This polymorphism has been shown to vary with ethnic and geographical distribution.However, its influence has not been elucidated in the Kazakh population.

In the present study, the frequency of HPV-16-*E6* gene in Kazakh's esophageal SCC cases was higher than that in corresponding normal mucosae, suggesting that there is a trend towards an association between the carcinogenesis of Kazakh's esophageal SCC and the presence of HPV-16 infection. These results were similar to previous report[16], which suggested that infection with HPV-16 might be involved in carcinogenesis of Kazakh's esophageal SCC. In addition, the distribution of *p53* codon 72 *Arg* homozygous genotype in Kazaks's esophageal SCC was significantly higher than that in corresponding normal mucosae, indicating that an individual homozygous for *p53 Arg* would be more likely to develop esophageal SCC than a *Pro*/*Arg* heterozygote or a *Pro* homozygote. Furthermore, it is noteworthy that the distribution of *p53* codon 72 *Arg* homozygous genotype in HPV positive samples of Kazakh's esophageal SCC was at a 3.53-folds higher risk for the development of esophageal SCC compared with HPV negative samples. In contrast, such a significant correlation between *p53* polymorphism and HPV infection was not evident in corresponding normal mucosae. From the above analyses, when stratified with HPV infection, the frequency of *p53* codon 72 *Arg* homozygous genotype was at a 1.48-folds increased risks for developing Kazakh's esophageal SCC compared with *p53 Arg* homozygosity (*Arg*/*Arg*) solely. Therefore, this implied *p53* codon 72 *Arg* polymorphism in combination with HPV infection could increase the risk of development of SCC in Kazakhs.

p53 tumor-suppresser protein accmulates rapidly through post-transcriptional mechanisms and is also activated as a transcriptional factor, thus leading to growth arrest or apoptosis when DNA damage has occurred[26]. The ubiquitin-dependent proteolytic pathway plays a major role in selective protein deregulation. *E6* oncoprotein of oncogenic HPV-16/18 might use this cellular proteolytic system to target *p53* protein[7] and bind to a cellular protein of *E6*-AP, and the *E6-AP* complex might interact with *p53*, resulting in the rapid ubiquitin-dependent degradation of *p53*[27]. The level and half-life of *p53* in *E6* immortalized cell lines or in HPV-positive cervical carcinoma cells have been reported to be generally decreased[28,29]. Certain HPV types such as HPV-16/18 found in SCC of esophagus suggested a model by which *E6* degraded cell growth control by elimination of the *p53* tumor suppresser protein and led to HPV-associated esophageal SCC[10,30].

In conclusion, the current study reveals the potential role of the polymorphism of *p53* at codon 72 in HPV-associated carcinogenesis of esophageal SCC in Kazakh population. Individuals carrying *Arg* alleles compared to those with *Pro* alleles have an increased risk for esophageal SCC.

ACKNOWLEDGMENTS

We thank Professor Zhen-Zhu Sun (Department of Pathology, the People's Hospital of Xinjiang Uygur Autonomous Region, China) for providing the samples of Kazakh's esophageal SCC.

REFERENCES

1 **Zhang YM**. The distribution of esophageal cancer in Xinjiang. *Xinjiang Yixueyuan Xuebao* 1988; **11:** 139-144
2 **Liang YY,** Esteve A, Martel-Planche G, Takahashi S, Lu SH, Montesano R, Hollstein M. *p53* mutations in esophageal tumors from high-incidence areas of China. *Int J Cancer* 1995; **61:** 611-614
3 **Munoz N,** Wahrendorf J, Bang LJ, Crespi M, Thurnham DI, Day NE, Ji ZH, Grassi A, Yan LW, Lin LG. No effect of riboflavine, retinol, and zinc on prevalence of precancerous lesions of oesophagus. Randomised double-blind intervention study in high-risk population of China. *Lancet* 1985; **2:** 111-114
4 **Lin K,** Shen W, Shen Z, Cai S, Wu Y. Estimation of the potential for nitrosation and its inhibition in subjects from high- and low-risk areas for esophageal cancer in southern China. *Int J Cancer* 2003; **10**: 891-895
5 **He D,** Zhang DK, Lam KY, Ma L, Ngan HY, Liu SS, Tsao SW. Prevalence of HPV infection in esophageal squamous cell carcinoma in Chinese patients and its relationship to the p53 gene mutation. *Int J Cancer* 1997; **72:** 959–964
6 **Chen B,** Yin H, Dhurandhar N. Detection of human papilloma-virus DNA in esophageal squamous cell carcinomas by the polymerase chain reaction using general consensus primers. *Hum Pathol* 1994; **25:** 920-923
7 **Scheffner M**, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990; **63:** 1129–1136
8 **Huibregtse JM,** Scheffner M, Howley PM. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. *EMBO J* 1991; **10:** 4129–4135
9 **Huibregtse JM,** Scheffner M, Howley PM. Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. *Mol Cell Biol* 1993; **13:** 4918–4927
10 **Kawaguchi H,** Ohno S, Araki K, Miyazaki M, Saeki H, Watanabe M, Tanaka S, Sugimachi K. p53 polymorphism in human papillomavirus-associated esophageal cance. *Cancer Research* 2000; **60:** 2753-2755
11 **Peixoto Guimaraes D,** Hsin Lu S, Snijders P, Wilmotte R, Herrero R, Lenoir G, Montesano R, Meijer CJ, Walboomers J, Hainaut P. 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
12 **Lu ZM,** Chen KN, Guo M. Detection of HPV in human esophageal cancer in high-incidence area and its correlation with *p53* expression. *Zhonghua Zhongliu Zazhi* 2001; **23**: 220-223
13 **Zou SY,** Si JY, Liu XM, Tang XP. PCR in detdction of human papillomavirus DNA in esophageal carcinoma in Xingjiang. *Aizheng* 1998; **17:** 32-34
14 **Greer CE,** Whee Le CM, Manos MM. PCR Primer A Laboratory Manual. *Cold Spring Harbor Laboratory Press* 1995: 64-69
15 **de la Calle-Martin O**, Fabregat V, Romero M, Soler J, Vives J, Yague J. AccII Polymorphism of the *p53* gene. *Nucleic Acids Res* 1990; **18:** 4963
16 **Chang F,** Syrjanen S, Shen Q, Cintorino M, Santopietro R, Tosi P, Syrjanen K. Human papillomavirus involvement in esophageal carcinogenesis in the high-incidence area of China. A study of 700 cases by screening and type-specific in situ hybridization. *Scand J Gastroenterol* 2000; **35***:* 123-130
17 **Ojeda JM,** Ampuero S, Rojas P, Prado R, Allende JE, Barton SA, Chakraborty R, Rothhammer F. *p53* codon 72 polymorphism and risk of cervical cancer. *Biol Res* 2003; **36:** 279-283
18 **Saranath D,** Khan Z, Tandle AT, Dedhia P, Sharma B, Contractor R, Shrivastava S, Dinshaw K. HPV16/18 prevalence in cervical lesions/cancers and *p53* genotypes in cervical cancer patients from India. *Gynecol Oncol* 2002; **86**: 157-162
19 **Sifuentes Alvarez A,** Reyes Romero M. Risk factors for cervicouterine cancer associated to HPV: *p53* codon 72 polymorphism in women attending hospital care. *Ginecol Obstet Mex* 2003; **71**: 12-15
20 **Li T,** Lu ZM, Guo M, Wu QJ, Chen KN, Xing HP, Mei Q, Ke Y. *p53* codon 72 polymorphism (C/G) and the risk of human papillomavirus-associated carcinomas in China. *Cancer* 2002; **95**: 2571-2576

21 **Humbey O,** Cairey-Remonnay S, Guerrini JS, Algros MP, Mougin C, Bittard H, Aubin F. Detection of the human papillomavirus and analysis of the TP53 polymorphism of exon 4 at codon 72 in penile squamous cell carcinomas. *Eur J Cancer* 2003; **39**: 684-690
22 **Inserra P,** Abrahamsen M, Papenfuss M, Giuliano AR. Ethnic variation of the *P53* codon 72 polymorphism, HPV persistence, and cervical cancer risk. *Int J STD AIDS* 2003; **14**: 800-804
23 **Comar M,** Molin GD, Guaschino S, Campello C. *p53* at codon 72 polymorphism, human papillomavirus infection and cervical lesions: a cross-sectional study from northeastern Italy. *Eur J Obstet Gynecol Reprod Biol* 2004; **114**: 210-214
24 **Tanara G,** Falugi C, Cesario A, Margaritora S, Russo P, Cosimi A. TP53 codon 72 polymorphism does not affect risk of cervical cancer in patients from The Gambia. *Int J Biol Markers* 2003; **18**: 280-283
25 **Ngan HY,** Liu VW, Liu SS, Cheng DK, Ng TY, Wong LC. Homozygous arginine at codon 72 of *p53* has no prognostic significance in cervical cancer. *Tumour Biol* 2000; **21**: 135-138
26 **Levine AJ.** *p53*, the cellular gatekeeper for growth and division. *Cell* 1997; **88:** 323–331
27 **Scheffner M,** Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-Protein ligase in the ubiquitination of *p53*. *Cell* 1993; **75:** 495–505
28 **Scheffner M,** Munger K, Byrne JC, Howley PM. The state of the *p53* and retinoblastoma genes in human cervical carcinoma cell lines. *Proc Natl Acad Sci U S A* 1991; **88:** 5523–5527
29 **Hengstermann A,** Linares LK, Ciechanover A, Whitaker NJ, Scheffner M. Complete switch from Mdm2 to human papillomavirus E6-mediated degradation of *p53* in cervical cancer cells. *Proc Natl Acad Sci U S A* 2001; **98**: 1218-1223
30 **Vos M,** Adams CH, Victor TC, van Helden PD. Polymorphisms and mutations found in the regions flanking exons 5 to 8 of the TP53 gene in a population at high risk for esophageal cancer in South Africa. *Cancer Genet Cytogenet* 2003; **140**: 23-30

Edited by Kumar M and Wang XL **Proofread by** Xu FM