Short Communication

Role of \(p53\) and \(p21\) polymorphisms in the risk of cervical cancer among Chinese women

Pei Jiang\(^1,2\), Jianxin Liu\(^1\), Wen Li\(^1\), Xiaoxi Zeng\(^2\), and Jianxin Tang\(^1,2\)*

\(^1\)School of Info-physics and Geomatics Engineering, Central South University, Changsha 410083, China
\(^2\)Key Laboratory of Green Packaging and Application of Biological Nanotechnology of Hunan Province, Hunan University of Technology, Zhuzhou 412007, China

*Correspondence address. Tel: +86-731-22182107; Fax: +86-731-22182095; E-mail: jy1046@yahoo.com.cn

The objective of this study was to identify whether polymorphic variants of \(p53\) at codon 72 and \(p21\) at codon 31 were associated with increased risk for cervical cancer, either independently or jointly, among Chinese women from southern Han. We genotyped \(p53\) codon 72 and \(p21\) codon 31 polymorphisms of peripheral blood DNA from 104 cervical cancer patients and 160 controls. Genotyping was confirmed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and direct DNA sequencing. We observed an increased risk of cervical cancer associated with the \(p53\) Arg/Arg (OR, 2.25; 95% CI, 1.11–4.54) or \(p21\) Ser/Ser (OR, 2.09; 95% CI, 1.11–4.54) genotype, compared with the \(p53\) Pro/Pro or \(p21\) Arg/Arg genotype, respectively. In addition, interaction between these \(p53\) and \(p21\) polymorphisms increased the risk of cervical cancer in a multiplicative manner, with the OR being 3.96 (95% CI, 1.51–10.41) for subjects carrying both \(p53\) Arg/Arg and \(p21\) Ser/Ser genotypes. These findings suggest that there is a significant association between the genetic polymorphism of \(p53\), \(p21\), and the risk of cervical cancer among Chinese southern women, and there is a possible gene–gene interaction in the incidence of cervical cancer.

Keywords \(p53\); \(p21\); genetic polymorphism; cervical cancer

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Introduction

Cervical cancer is the second most common type of cancer worldwide in women. Although the role of high-risk human papillomavirus (HPV) in cervical carcinogenesis is evident [1], only a small proportion of such HPV-infected cases progresses to cervical cancer. This indicates that the development of cervical cancer may be influenced by genetic factors. Identification of genetic variants that are associated with cervical cancer will contribute to understanding of underlying mechanisms behind its development and potentially provide therapeutic targets.

Disruption of cell cycle control, which is crucial for normal growth and differentiation and is regulated by cycling-dependent kinase, can lead to tumor growth and progression. \(p21\) (Waf-1) is a universal inhibitor of cyclin kinase [2], and upregulation of \(p21\), which inactivate G1-associated cyclin-cdk complexes, is associated with the \(p53\)-mediated G1/S cell cycle arrest. \(p53\) is a tumor suppressor gene, which maintains homeostasis through \(p21\)-mediated induction of G1 arrest or Bax-mediated apoptosis [3]. After carcinogen exposure, upregulation of \(p21\) and \(p53\) can delay progression past the G1 point [4]. Mutation in either \(p53\) or \(p21\) may lead to loss of homeostatic control during human carcinogenesis.

Polymorphisms in these cell cycle regulation genes have been reported, and their frequencies are depended on race. These include \(p53\) codon 72 polymorphism, which produces variant proteins with arginine (CGC; Arg) or proline (CCC; Pro), and \(p21\) codon 31 polymorphism, which produces variant proteins with serine (AGC; Ser) or arginine (AGA; Arg) [5,6]. Storey et al. reported a higher susceptibility of the \(p53\) Arg allele at codon 72 to HPV-E6-mediated degradation, which could translate it into a new important cervical tumorigenesis risk factor [7]. However, this finding was refuted by others [8–10]. A series of epidemiological studies also found that the \(p21\) codon 31 polymorphism was associated with increased risks of lung cancer, cervical cancer, and gastric cancer [11–13], although conflicting findings were still reported [14,15].

To investigate the crucial role that \(p53–p21\) interaction plays in the cell cycle control, DNA repair, apoptotic cell death, and the reported functional significant of variants, we conducted a case–control study to investigate the relationship between \(p53\) or/and \(p21\) polymorphism(s), and cervical cancer risk among Chinese southern women.
Materials and Methods

Study subjects and samples
This study includes 104 newly diagnosed patients with cervical cancer and 160 cancer-free controls. Patients were recruited between January 2007 and December 2008 at the No. 1 Hospital of Zhuzhou (Zhuzhou, China). Final diagnoses of cases were confirmed by routine histopathological examination. Control subjects were cancer-free individuals randomly selected from a cancer screening program for early detection of cancer conducted in the same regions during the same period when the case patients were recruited. The selection of criteria for the controls included no individual history of cancer and frequency, matched to case patients based on age. All subjects were unrelated Chinese women from southern Han in Zhuzhou. At recruitment, written informed consents about the study were obtained from all the patients and controls. Each participant was then interviewed to collect information on demographic characteristics. The research protocol was approved by the Institutional Review Board of the hospital.

The samples used for genotype analysis were obtained from peripheral blood lymphocytes. DNA was extracted by phenol–chloroform extraction.

p53 codon 72 polymorphism
p53 codon 72 genotypes were detected using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique as previously described by Xu et al. [16]. PCR conditions used were initial denaturation at 94°C for 2 min, followed by 35-step cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min, and final extension at 72°C for 10 min. Ten microliters of PCR products were digested with BstUI restriction enzyme (New England Biolabs, Beijing, China) for 2 h at 60°C. When BstUI restriction site (Arg allele) was present, the 279-bp fragment was digested into two small fragments (160 and 119 bp). The Pro allele was not cleaved by BstUI, having a single 279 bp band. Heterozygote contained three bands, corresponding to 279, 160, and 119 bp [Fig. 1(A)].

p21 codon 31 polymorphism
Polymorphism in p21 codon 31 was also determined by PCR–RFLP [17]. Amplification was performed by initial denaturation at 94°C for 5 min, followed by 35-step cycles of 94°C for 40 s, 60.3°C for 30 s, 72°C for 1 min, and final extension at 72°C for 10 min. Final PCR product was digested by BlpI. The Ser alleles with BlpI site generated two fragments (89 and 183 bp). The Arg allele which lacks a BlpI site yielded a 272-bp fragment. Heterozygote contained three bands, corresponding to 272, 183, and 89 bp [Fig. 1(B)].

The genotypes within 10% of each sample group were confirmed by sequencing the PCR products using an automated sequencing system (ABI Prism 3730 Genetic Analyzer). To test the reliability of the assay, 50 randomly selected samples were re-tested with identical results.

Statistical analysis
Differences in age, cigarette smoking, alcohol consumption, and family history between cervical cancer patients and
controls were evaluated using the $\chi^2$ test. The associations between the polymorphisms and risk of development of cervical cancer were estimated by odds ratio (OR) and their 95% confidence intervals (CIs) calculated by unconditional logistic regression models. $P < 0.05$ was considered statistically significant. All analyses were done using Statistical Package for the Social Sciences 13.0 (SPSS, Inc.).

Results

Characteristics of study subjects

The frequency distribution of general characteristics of the patients and controls is presented in Table 1. The cases and controls seemed to be adequately matched in terms of age. The median age was 46.5 years (28–69 years) for the patients and 45.3 years (26–71 years) for the controls ($\chi^2 = 0.571$). There was no significant difference between patients and controls in alcohol consumption and family history. However, significantly more smokers were presented among cases than among controls (14.4 vs. 5.6%; $\chi^2 = 5.904$), which indicated that smoking was a risk factor for cervical cancer in our study population.

Distributions of $p53$ and $p21$ polymorphisms

The allele and genotype frequencies of $p53$ and $p21$ polymorphisms are summarized in Table 2. The observed genotype frequencies of $p53$ and $p21$ polymorphisms in controls did not deviate significantly from those expected from the Hardy–Weinberg equilibrium. The $p53$ 72 Arg and $p21$ 31 Ser allele frequencies were 54.8 and 54.3% among patients and 44.7 and 45.0% among controls, respectively. The frequencies of $p53$ Pro/Pro, Arg/Pro, and Arg/Arg genotypes among patients were significantly different from those among controls, with the Arg/Arg variant being more frequent among cases than among controls (31.7 vs. 18.7%; $P < 0.05$). Similarly, the frequencies of $p21$ Arg/Arg, Ser/Arg, and Ser/Ser genotypes among patients were significantly different from those among controls, with the Ser/Ser homozygotes being overrepresented among patients compared with controls (30.8 vs. 20.0%; $P < 0.05$).

The association between $p53$ and $p21$ polymorphisms and cervical cancer risk

An unconditional logistic regression model was used to estimate the association between genotypes and the risk of cervical cancer (Table 2). The $p53$ Arg/Arg genotype was
Table 3 Risk of cervical cancer associated with p21 genotypes by p53 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>p53 codon72</th>
<th>p21 codon31</th>
<th>Cases (n = 104)</th>
<th>Controls (n = 160)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Pro/Pro + Arg/Pro</td>
<td>Arg/Arg + Ser/Arg</td>
<td>53</td>
<td>51</td>
<td>105</td>
<td>65.6</td>
</tr>
<tr>
<td>Pro/Pro + Arg/Pro</td>
<td>Ser/Ser</td>
<td>18</td>
<td>17.3</td>
<td>25</td>
<td>15.6</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Arg/Arg + Ser/Arg</td>
<td>19</td>
<td>18.2</td>
<td>23</td>
<td>14.4</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Ser/Ser</td>
<td>14</td>
<td>13.5</td>
<td>7</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*ORs were adjusted for age, cigarette smoking, alcohol consumption and family history.

*p < 0.05.

associated with an increased risk for the development of cervical cancer (OR, 2.25; 95% CI, 1.11–4.54), compared with the Pro/Pro genotype. Similarly, the p21 Ser/Ser genotype was also associated with an increased risk of developing cervical cancer (OR, 2.09; 95% CI, 1.04–4.19), compared with the Arg/Arg genotype. However, the heterozygous genotypes for both polymorphisms (p53 Arg/Pro or p21 Ser/Arg) were not associated with the risk, suggesting a possible recessive effect of these polymorphisms on cervical cancer. Because of this observation, we combined the p53 Pro/Pro and Arg/Pro or p21 Arg/Arg and Ser/Arg into one group for subsequent analysis. We examined whether there was a statistical joint effect between the p53 and p21 polymorphisms (Table 3). We found that patients carrying the p53 Arg/Arg genotype were also more likely to carry the p21 Ser/Ser genotype than the controls (13.5 vs. 4.4%; P < 0.05). Furthermore, the OR increased to 3.96 (95% CI, 1.51–10.41) among subjects carrying both p53 Arg/Arg and p21 Ser/Ser genotypes. These results clearly indicated a more than multiplicative joint effect between the p53 Arg/Arg and p21 Ser/Ser genotype on the risk of developing cervical cancer according to the statistical model.

Discussion

In the present study, we examined the association between the p53 codon 72 and p21 codon 31 polymorphisms and the risk of cervical cancer. In this hospital-based case–control analysis among southern Chinese women, we observed that both p53 codon 72 and p21 codon 31 polymorphisms were associated with an increased risk for development of cervical cancer. Furthermore, an increased multiplicative interaction between p53 Arg/Arg and p21 Ser/Ser genotypes was also detected.

The p53 codon 72 polymorphism has received considerable attention in the last few years after the initial report [7], which described that the p53–72Arg was more susceptible to degradation by HPV-E6 protein than the p53–72Pro and was associated with an increased risk of cervical cancer. Extensive studies attempted to reproduce this study, with inconsistent results. Zehbe et al. [18] studied Swedish and Italian women with cervical cancer, finding that in both groups Arg homozygotes were enriched in cancer and precursor lesions compared with controls. Arbel et al. [19] were able to find the low incidence of cervical cancer in Israeli Jewish women, which may be related to the low frequency pattern of the homozygous Arg p53 polymorphism. Our findings also confirm the initial findings of Storey et al. [7], as well as the data of those that detected a higher prevalence of homozygosity for Arg in patient with cervical cancer, as reported previously [20,21]. However, some reports failed to demonstrate a potential role of the p53 codon 72 polymorphism in cervical carcinogenesis. There was no association between the Arg/Arg genotype and cervical carcinomas in patients from Italy, Portugal, and Korea [22–24]. Differences among reports may be the results of variation in allele frequency among different ethnic populations [25], inter-laboratory variation in the methods used to determine allele frequencies [26], or differences in the frequencies of HPV types represented in the cervical tumor tested [7]. According to Storey et al., the Arg allele is more susceptible to degradation by the HPV E6 protein, with individuals homozygous for Arg being about 7-fold more susceptible to HPV-associated tumorigenesis than heterozygote [7]. Nagpal et al. [27] and Hou et al. [28] also reported a correlation between HPV associated cervical cancer and Arg homozygosity of p53 codon 72. Contrary to these findings, some studies revealed an increased risk for the development of cervical cancer of Arg homozygosity of p53 codon 72 seemed to be unrelated to the HPV infection [29,30]. Therefore, asynergist effect between high-risk HPVs and Arg homozygosity in cervical cancer needed further evidence from the epidemiological observation.

The p21 codon 31 polymorphism has been linked to an increased risk of cancer in some but not all previous studies. Wu et al. [31] found the Ser allele of p21 codon 31 might be associated with an increased risk of esophageal cancer. Shih et al. [32] reported no association between p21 codon 31 genotypes and lung cancer. Harima et al. [33] and Bhattacharya et al. [34] reported an association between the
Arg allele of the p21 codon 31 polymorphism and cervical cancer in Japanese women and in Indian women; however, Lee et al. [35] reported no such significant association. On the contrary, Roh et al. [12] observed that the Ser/Ser homoyogte could be a risk factor for the development of cervical adenocarcinoma associated with high-risk HPV. Our data also provide evidence that Ser/Ser is a risk factor for development of cervical cancer among Chinese southern women. There is a clear discrepancy between these results and those of Harima et al. [33] and Bhattacharya et al. [34]. There are several potential reasons for the discrepancy among these reports. Allele frequency among different ethnic groups was different, the frequency of the Arg allele showed highly significant variations ranging from 4% in Caucasians (Swedes) to 50% in Chinese [36]. On the other hand, in these studies, different methodologies were used, such as reverse transcription-PCR and DNA sequencing in Harima’s study and RFLP and DNA sequencing in our study. Analytic samples used in the genotyping were another positive aspect of these studies. All of the samples used in our study consisted of DNA extracted from peripheral white blood cells, whereas those used in Bhattacharya’s study were from tumor tissues. Nevertheless, whether the p21 codon 31 polymorphism had effect on cervical cancer needed to be validated in future studies with larger sample sizes.

In conclusion, our study showed a significant association between p53 and p21 polymorphisms and an increased risk of developing cervical cancer. The association of p53 and p21 polymorphisms with the risk of cervical cancer displayed a multiplicative gene–gene interaction. This is the first study in Chinese population to identify such a relationship between p53 and p21 genes, and these findings suggested that the p53 tumor suppressor pathway plays an important role in the development of cervical cancer.

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References