p53 Codon 72 Polymorphism Does Not Affect the Risk of Cervical Cancer in Patients from Northern Italy


Department of Human and Hereditary Pathology, Anatomic Pathology Section, University of Pavia and Instituto di Ricovery e Cura a Carattere Scientifico Policlinico San Matteo [P. T., N. V., R. Z., P. M., L. C.], and Department of Genetics and Microbiology, University of Pavia, [M. R. S., G. N. R.], 27100 Pavia, Italy

Abstract

A case-control study was performed to investigate the risk of cervical cancer associated with p53 polymorphism at codon 72, encoding either arginine or proline. It has been recently suggested that the arginine isoform increases the susceptibility to invasive cervical cancer; however, data remain controversial. The polymorphism was examined by both allele-specific PCR and RFLP analysis in 101 patients with primary cervical cancer and in 140 healthy women of the same age and from the same geographical area. The distribution of p53 genotypes in cervical cancer patients and in controls was not significantly different (P = 0.445), and homozgyosity for arginine at residue 72 was not associated with an increased risk for cervical cancer (odds ratio, 0.81; 95% confidence interval, 0.47–1.42; P = 0.52). Similarly, different genotype distribution and increased risk were not observed when patients versus controls were analyzed according to human papillomavirus status and cancer histotype. Therefore, no evidence of association between homozygosity for p53 arginine and cervical cancer was found in our population sample.

Introduction

Certain HPV3 genotypes are involved in the development of cervical cancer (1). HPV16 and HPV18 are the most frequently detected genotypes in invasive cervical carcinoma. Oncoprotein E6 of tumor-associated HPVs binds to tumor suppressor protein p53, inducing its degradation through the ubiquitin pathway (2, 3). A common p53 polymorphism at codon 72, encoding either arginine or proline, has been shown to affect E6-mediated degradation of p53 in vitro (4). Moreover the arginine form of p53 has been associated with an increased risk of HPV-related cancer in humans by Storey et al. (4) and Zehbe et al. (5). This observation, however, remains controversial because it has not been confirmed in other studies on different populations (6–10).

To examine whether the arginine polymorphic variant of p53 could represent a risk factor for cervical carcinoma in the Northern Italy population, we performed a retrospective study using both ASP and RFLP analysis.

Materials and Methods

Cases and Specimens

A total of 241 women, all born in the same geographical area, were investigated for the p53 polymorphism at codon 72:101 subjects with primary cervical cancer (median age, 49.0 years; range, 32–67) and 140 healthy women of similar age (median age, 47.0 years: range, 30–64). The patients included 64 cases with primary cervical SCCs, and 37 cases with primary cervical AdCs. Forty-six patients (75 cases with HPV-positive and 11 cases with HPV-negative tumors) were consecutively observed in a single gynecological unit between January 1995 and May 1998. The remaining 15 patients were retrieved from cases with HPV-negative tumors observed between 1990 and 1994. All of the patients underwent surgery, and the treatment consisted in type III hysterectomy of Piver with lymph node dissections. The p53 polymorphism analysis was performed on formalin-fixed, paraffin-embedded normal lymph nodes. The controls, all of whom were women with normal Pap smear and no previous history of cervical dysplasia, were retrieved from 734 subjects who attended consecutively a family planning service between January 1995 and August 1996. Frozen cervicovaginal lavages were available for the p53 polymorphism analysis of the controls. The HPV status of the patients and controls, ascertained at observation, was realanalyzed for the present study.

Analysis of p53 Codon 72 Polymorphic Alleles

DNA Extraction. DNA was extracted from formalin-fixed, paraffin-embedded nontumoral tissues (lymph nodes) of patients and from frozen cervicovaginal lavages of controls. One to three sections (depending on sample size) of formalin-fixed, paraffin-embedded tissue were incubated at 58°C overnight in 150 µl of extraction buffer [50 mM KCl, 10 mM Tris-HCl (pH 7.5), 2.5 mM MgCl2, 0.1 mg/ml gelatine, 0.45% NP40, 0.45% TWEEN 20, and 0.5 mg/ml proteinase K]. Exfoliated cervicovaginal cells suspended in PBS solution were collected by low-speed centrifugation and incubated for 1 h at 56°C in 100 µl of extraction buffer. The solutions were heated at 95°C for 15 min to inactivate proteinase K and were subsequently centrifuged. One to 5 µl of digested material were used directly for enzymatic amplification by PCR.
p53 Polymorphism and Risk of Cervical Cancer

Polymorphism and Risk of Cervical Cancer

Cycler, Perkin Elmer). Reaction products (10 µl each) were incubated overnight with 1 unit of Taq polymerase (Dynazyme II, Finnzymes). Cycling parameters were: 95°C for 2 min, 65°C for 2 min, 72°C for 2 min for 33 cycles with a final elongation at 72°C for 10 min (PTC-200, MJ Research). Twenty µl of the PCR product (199-bp fragment) were incubated overnight with 1 unit of AccI restriction enzyme (Dynazyme II, Finnzymes) and then were analyzed by electrophoresis on a 4% Nusieve gel.

RFLP Analysis. Target sequences were amplified with the primers described by Ara et al. (11) in a 25-µl volume containing 30 ng of template DNA, 0.4 µM each primer, 200 µM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1.5 units of Taq polymerase (Dynazyme II, Finnzymes). Cycling parameters were: 95°C for 2 min, 65°C for 2 min, 72°C for 2 min for 33 cycles with a final elongation at 72°C for 10 min (PTC-200, MJ Research). Twenty µl of the PCR product (199-bp fragment) were incubated overnight with 1 unit of AccI restriction enzyme (Dynazyme II, Finnzymes) and then were analyzed by electrophoresis on a 4% Nusieve gel.

The base substitution causing modification of amino acid residue 72 from proline to arginine generates an AccI cutting site; therefore, the enzymatic digestion of the arginine corresponding sequence yields two DNA fragments of 113 and 86 bp, respectively.

HPV DNA Analysis

All of the cases and controls were reanalyzed for the identification of HPV infection. DNA extraction, primers (consensus MY09/MY11 and type specific for HPV16 and -18), and PCR amplification procedures for the detection of HPV in formalin-fixed, paraffin-embedded cervical cancers and in cervicovaginal lavages were described in previous studies, in which the choice of primers and the strategy of amplification were also discussed (12, 13). In addition, the tumors that were HPV-negative and all of the cervicovaginal lavages were tested by using HPV general primers GP5+/GP6+ (14) not previously utilized.

Statistical Analysis

χ² analysis was used to examine differences in the proportions of the three p53 codon 72 genotypes between cervical cancer patients and controls. Fisher correction was applied when appropriate. OR and 95% CI were used to compare categorical variables.

Results

The analysis of HPV infection from patients and controls confirmed previously obtained results (12, 13). In the cancer group, HPV16 or HPV18 DNA sequences were present in 75 cases (45 SCCs and 30 AdCs); HPV16 was found in 60 tumors (42 SCCs and 18 AdCs), and HPV18 was found in 15 tumors (3 SCCs and 12 AdCs). Twenty-six tumors (19 SCCs and 7 AdCs) were HPV-negative. In the control group, no HPV DNA sequences were detected by using consensus primers MY09/MY11. The amplification of negative tumors and cervicovaginal lavages with general primers GP5+/GP6+ failed to yield any additional HPV-positive cases.

The analysis of p53 polymorphism was performed in both ASP and RFLP analysis. The amplification of the arginine allele with primer pair p53⁰/Arg⁻ gives a PCR product of 177 bp; the primer pair Pro⁰/p53⁻ amplifies a 177-bp fragment corresponding to the proline allele. AccI digestion of the PCR products, obtained with primers described by Ara et al. (11), identifies a fragment of 199 bp (proline) or two fragments of 113 bp + 86 bp (arginine). Case 1, Pro/Pro; cases 2, 4, and 5, Arg/Arg; and case 3, Arg/Pro.
region, harboring p53, has been reported in 15–22% of cervical cancer (16). Therefore, the genotype analysis was performed on normal tissues from patients and not on tumor samples to avoid the risk of overestimating the p53 homozygosity. Moreover, two different methods were employed to test p53 genotypes, because allele-specific assays may perform differently according to the source of DNA (formalin-fixed paraffin-embedded tissue or frozen cells).

Our data are comparable to those of most studies as to p53 allele frequencies. We found no statistically significant differences in the distribution of p53 genotypes between controls and patients, and homozygosity for arginine at residue 72 was not associated with an increased risk for cervical cancer. Similarly, a different genotype distribution and cancer risk were not observed when patients and controls were analyzed according to HPV status and cancer histotype. Therefore, we can conclude that no evidence of association between homozygosity for p53 arginine and cervical cancer was present in our population sample.

The discrepancy between our results and those of Storey et al. (4) and Zehbe et al. (5) might be explained either by the small sample size or by the design of those studies. It should be noted that Joseffson et al. (see Ref. 10), by comparing the control group of Zehbe et al. (5) with a higher number of cases with cervical cancer, did not demonstrate any significant association.

It is well known that p53 allele frequencies vary among ethnic groups (17). Because consistent epidemiological relationships between HPV infection and cervical cancer have been reported worldwide, it is crucial that investigations of p53 polymorphism and risk of cancer should be extended to populations living at different latitudes to verify the reported association. In particular, epidemiological studies in populations living near the equator, who have a higher frequency of the proline variant (17), could help to identify subgroups of subjects at higher risk for cervical cancer.

**References**


**Table 1**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total</th>
<th>Genotypes</th>
<th>F-proline allele</th>
<th>HW (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>A/P</td>
<td>P/P</td>
</tr>
<tr>
<td>Controls</td>
<td>140</td>
<td>86</td>
<td>47</td>
<td>7</td>
</tr>
<tr>
<td>Patients</td>
<td>101</td>
<td>57</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>HPV+</td>
<td>75</td>
<td>39</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>HPV−</td>
<td>26</td>
<td>18</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>SCC</td>
<td>64</td>
<td>34</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>AdC</td>
<td>37</td>
<td>23</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>

F, Frequency; A/A, Arg/Arg; A/P, Arg/Pro; P/P, Pro/Pro.

a No deviation from Hardy-Weinberg (HW) equilibrium was observed for any group.

0.143; AC, P = 0.833). Similarly, homozygosity for arginine at residue 72 was not associated with an increased risk for cervical cancer (OR, 0.81; 95% CI, 0.47–1.42; P = 0.52) also for groups with different HPV-status (HPV-positive: OR, 0.68; 95% CI, 0.37–1.25; P = 0.23; HPV-negative: OR, 1.41; 95% CI, 0.54–4.02; P = 0.6) and cancer histotype (SCC: OR, 0.71; 95% CI, 0.38–1.35; P = 0.36; AdC: OR, 1.03; 95% CI, 0.46–2.37; P = 1.0).

**Discussion**

Storey et al. (4) showed that the codon 72 arginine variant of p53 codes for a protein that is more sensitive to HPV16 and HPV18 degradation than the proline variant. These data were further supported by genotype analysis in a group of cervical cancer patients who showed a 7-fold enrichment of the arginine allele over the proline allele as compared to healthy controls (4). Moreover, Thomas et al. (15) have recently presented evidence that, in vitro, the wild-type p53 arginine variant induces apoptosis with faster kinetics and suppresses transformation more efficiently than the wild-type p53 proline variant. These observations may have implications for the development of cancer in subjects harboring wild-type p53 sequences and for the responsiveness of tumors to therapy.

The functional differences between the two p53 isoforms described by Storey et al. (4) provoked a series of epidemiological studies to verify whether homozygous arginine at codon 72 was a candidate risk factor for cervical HPV-related cancer. In a recent study, Zehbe et al. (5) obtained results consistent with those of Storey et al. (4); however, the data from the original report were not confirmed by several other authors (6–10).

Our study was based on a large sample so as to give a reliable estimate of p53 polymorphism and cervical cancer association, and it was designed to minimize sources of bias. Patients and controls were of similar age and were recruited from a homogeneous population born in a restricted geographical area. This population is characterized by a low prevalence of genital HPV infection, which is higher among younger women (13). Most of our controls were older than 35 years and without history of cervical dysplasia or genital warts, accounting for their HPV-negative status. Both cervical SCC and AdC were investigated as well as tumors with different HPV status. Most of the cases were consecutively observed in a single institution, and, among them, the prevalence of HPV-negative tumors was about 13%. Fifteen HPV-negative tumors were retrieved from a previous series; however, we believe that this potential bias should not have hampered the main conclusion of the study. Loss of homozygosity at the 17p13 chromosomal...
p53 Codon 72 Polymorphism Does Not Affect the Risk of Cervical Cancer in Patients from Northern Italy

P. Tenti, N. Vesentini, M. Rondo Spaudo, et al.


Updated version  Access the most recent version of this article at: http://cebp.aacrjournals.org/content/9/4/435

Cited articles  This article cites 17 articles, 5 of which you can access for free at: http://cebp.aacrjournals.org/content/9/4/435.full#ref-list-1

Citing articles  This article has been cited by 6 HighWire-hosted articles. Access the articles at: http://cebp.aacrjournals.org/content/9/4/435.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.