Evaluation of the frequency of TP53 gene codon 72 polymorphisms in Iranian patients with endometrial cancer

Nasrin Ghasemi a,*, Mojgan Karimi-Zarchi b, Mohammad Reza Mortazavi-Zadeh b, Ali Atash-Afza b

a Research and Clinical Center for Infertility, Yazd Shahid Sadoughi Medical Sciences University, Safayeh, Bouali St, 8916877391, Yazd, Iran
b Yazd Shahid Sadoughi Medical Sciences University, Yazd, Iran

Received 27 June 2009; received in revised form 14 September 2009; accepted 20 September 2009

Abstract
Polymorphisms of the TP53 gene codon 72 exhibit less effective function in tumor suppression and usually are associated with human cancer. To investigate the frequency of proline and arginine alleles of TP53 codon 72, the present study analyzed the DNA from blood samples of 30 Iranian women with endometrial cancer, in comparison with 32 healthy women. A Pro/Pro genotype was associated with increased endometrial cancer risk (odds ratio OR = 3.7, 95% confidence interval CI = 0.539–25.59). In patients, Pro allele frequency (68%) was higher than Arg frequency (32%), and higher also than Pro frequency in healthy control subjects (55%) (OR = 1.9, 95% CI = 0.903–3.893). It could be that the Pro allele is less apoptotic than the Arg allele, and that the Arg allele most probably activates transcription factors more efficiently than the Pro variant. These novel findings on the frequency of TP53 gene codon 72 polymorphism in endometrial cancer in Iranian women indicate that in this population the Pro allele might be associated with increased risk of endometrial cancer.

1. Introduction

The TP53 tumor suppressor gene is a target of mutation in human malignancy, and loss of TP53 function is usually linked with cancer development. The TP53 tumor suppressor gene induces apoptosis, senescence, or temporary cell cycle arrest under a variety of circumstances and mechanisms, including genotoxic stresses, oncogenic signaling, and hypoxia.

The most important biochemical function of the TP53 protein is the stimulation of the transcription of a large group of genes involved in growth arrest or apoptosis. TP53 binds to promoters and introns of genes and involves many proteins, including components of the basal transcriptional apparatus, histone acetyltransferases, and other transcriptional cofactors essential for transcriptional initiation [1–3]. The TP53 protein increases the transcriptional expression of several genes involved in the response to genotoxic agents, such as ionizing radiation and certain chemicals, including chemical therapeutic drugs [4]. TP53 facilitates initiation of the cell cycle arrest and DNA damage repair. If cells are not reparable for this DNA damage, TP53 turns on cell death programs and the cells then run through apoptosis [2]. TP53 gene inactivation occurs frequently in a variety of cancers [5]. Therefore, TP53 is a key tumor suppressor against cancer development, and most human tumors carry inactivated TP53 mutations [6].

In addition to the many mechanisms that alter TP53 function, it has been suggested that genetic polymorphisms in the TP53 gene could also affect some of its functions. In TP53, the single nucleotide polymorphism (SNP) at codon 72 of exon 4 has a variety of potentially oncogenic properties [7]. This SNP changes amino acid residue 72 from arginine to proline, which can be easily detected by polymerase chain reaction. These two alleles of TP53 exhibit different biochemical functions. They bind to components of the transcriptional cofactors, which could differentially affect their transcriptional abilities [6].

The Arg allele has been found to be preferentially expressed in breast cancer patients [8], and many reports have indicated that the Arg allele is associated with cancer predisposition [9]. Nonetheless, several studies of the TP53 codon 72 polymorphism have yielded varied results [10–15]. The TP53 proline homozygosity could be part of a common causal pathway for cervical cancer in Indian women.
women with HPV infection [16]. Saranath et al. [17], however, argued that the Arg genotype has higher effects on developing cancer in HPV16/18 infected women.

The extent to which SNPs are associated with increased cancer risk could differ among ethnic groups [18,19]. Few reported case–control studies have addressed the association of polymorphism of TP53 codon 72 with endometrial cancer (EC). There was a significant association between TP53 gene polymorphism and EC in some studies [20–25], and a relation between TP53 gene polymorphism and the type of EC has been suggested [26,27]. In other studies, however, no association of these SNPs was found with EC [28]. Most recent studies have determined the relationship between inherited DNA changes of TP53 and the type of EC has been suggested [26,27]. In other studies, however, no association of these SNPs was found with EC [28]. Most recent studies have determined the relationship between inherited DNA changes of TP53 and the type of EC has been suggested [26,27].

Given that TP53 gene polymorphism may be associated with risk of EC, the objective of the present study was to investigate the differential frequency of two TP53 gene variants in EC patients, compared with a control group of healthy women.

2. Materials and methods

In this retrospective study, the surgical records of 30 patients with EC in the Department of Oncology, Shahid Sadoughi Medical sciences University Hospital were reviewed. After contacting the patients, a 5-mL blood sample was taken from each of them. Their records were used to complete a questionnaire for each participant. They did not have signs or symptoms of other diseases or cancers of other organs. The control group (n = 32) consisted of healthy women volunteers (with no signs or symptoms of disease or cancer) with at least two live births and no history of abnormal uterine bleeding. Formal written consent was obtained from the participating women. This study was approved by the Research Committee of Yazd University of Medical Sciences.

2.1. Histology

Hysterectomy specimens were used for detailed histopathologic analysis. All available microscopic slides for each case were reviewed by at least two pathologists for histological classification, tumor grade, and staging. The endometrial tissues were not available for genetic analysis of loss of heterozygosity.

2.2. Genetic study

Blood samples were taken from patients and control subjects through peripheral venous tapping into a tube containing ethylene-diamine-tetraacetic acid. DNA was extracted from peripheral blood using a standard salt-put method. Analysis of the TP53 genotype at codon 72 was performed using the primers F,5’-GCCAGAGGCT GCTCCCCC-3’ and R,5’-CGTGAAGTCACAGACTT-3’ for amplification of the Pro codon and primers F,5’-TCCC CCTTGCGC-3’ and R,5’-CTGGTGCAAGGGCC ACGC-3’ for amplification of the Arg codon.

The polymerase chain reaction (PCR) conditions comprised an initial denaturing step at 94°C for 5 minutes, followed by 45 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. Reaction products were fractioned on a 2% agarose gel and visualized after ethidium bromide staining with a digital camera system (Gel Digidoc II; Qiagen, Valencia, CA; Tehran, Iran).

2.3. Statistical analysis

The SPSS v. 15.0 software package was used (SPSS, Chicago, IL) was used for data analysis. Differences in the frequencies of the TP53 alleles in the case and control groups were analyzed using a chi-square test. A P-value of ≤0.05 was considered statistically significant. The odds ratio (OR) was calculated with 95% confidence interval (CI).

3. Results

The TP53 polymorphism of 72 codon was evaluated in 30 patients with EC (mean age 53.16 years) and was compared with 32 age-matched healthy control subjects (P = 0.12). The number of cases with history of contraceptive use did not differ significantly between groups (9 of 30 in patients vs. 11 of 32 in the control group) (P = 0.71).

The histological results of their endometrial tissues showed that 25 patients had endometrioid carcinoma and...
5 patients had non-endometrioid carcinoma. According to the FIGO classification, most were grade I (68%) and in stage III (47%) (Table 1).

The Arg/Arg genotype was present in 2 of the 30 patients (frequency 7%) and in 4 of the 32 control subjects (frequency 12%) (Table 2). The Arg/Pro genotype was present in 15 patients (50%) and in 21 control subjects (66%) of the control women (P = 0.41). The homozygous Pro/Pro genotype was present in 13 patients (43%), compared with 7 control subjects (22%) (P = 0.04). The Pro/Pro was associated with an increased risk of EC (OR = 3.7, 95% CI = 0.539–25.59).

The Pro allele frequency was 68% in the 30 patients and 55% in the 32 control subjects (Table 3). The Arg allele frequency was 32% in patients and 45% in control subjects. The higher Pro allele frequency in patients than in control subjects was not significant, perhaps because of the limited sample sizes. The Pro allele was associated with EC risk (OR = 1.9, 95% CI = 0.903–3.893). The frequency of alleles was not significantly different across stages and grades of the cancer (P = 0.38).

4. Discussion

There is strong evidence that allele variants in some oncogenes and tumor-suppressor genes are genetic risk factors for various cancers. In the present study, the frequency of TP53 polymorphism codon 72 was evaluated in patients with EC. The TP53 72Pro allele was more frequent in patients than in control subjects. These findings are in agreement with previous reports that the Pro allele has less apoptotic ability than the Arg variant [10]. The two alleles of codon 72 TP53 gene exhibit different oncogenic properties, but contradictory outcomes have been reported in various type of cancers [12,13,21,22]. Similarly, contradictory findings are reported for TP53 codon 72 polymorphism in breast cancer [10–15]; this is one of the hormone-related cancers, which could have similar risk factors as EC.

The Arg allele most probably activates transcription more efficiently than the proline variant [10]; however, it allele is more sensitive to degradation by HPV viruses than is the Pro allele [29]. The Arg allele is more effective in causing cell death than the Pro allele; that is, it has greater ability to provoke apoptosis [30,31]. Recently, the Pro allele was shown to induce cell-cycle arrest better than the Arg allele [32]. These data indicate that polymorphic variants of TP53 might have different functions in cell-cycle regulation. Furthermore, failure in DNA repair leads to genomic instability, which could cause cancer [33]. Healthy Asian heterozygotic subjects (Arg/Pro) express the Pro allele largely at the RNA level [8], but codominant segregation of these alleles has been observed [7].

In the present study of Iranian women, increased risk of EC was associated with the Pro allele at codon 72 of TP53. An odds ratio of 3.7 shows a high risk of EC for the Pro/Pro genotype, but with a wide confidence interval (0.539–25.59), perhaps due to the limited sample size. However, controversial results have reported the relation between both alleles of TP53 codon 72 and EC [24,25]. The difference between previous and present results may be due to several different factors, such as ethnic differences, sample size, the techniques used, and variations in genetic susceptibility.

Loss of heterozygosity on chromosome 17 p, where the TP53 gene is located, has been found in nearly 50% of patients with EC, so the frequency of homozygosity for Pro or Arg might be increased in studies using samples obtained from cancer tissues. Loss of heterozygosity can be identified in cancers by as the presence of heterozygosity at a genetic locus in germline DNA and the absence of heterozygosity at that locus in the cancer cells. Loss of heterozygosity is not seen usually in endometrioid carcinoma, however, but is found more frequently in nonendometrioid carcinoma [28]. In the present study, most of the cases endometrioid carcinoma. Race- or ethnicity-specific variations in the frequency of the genotypes have also been documented [34,35].

In conclusion, the proportion of codon 72 Pro alleles was higher in cancer patients, and the present findings suggest that a loss of TP53 function may be linked to the Pro allele. Nonetheless, large, population-based studies of EC patients should be conducted, with larger sample sizes, for confirmation of the association of the Pro allele of TP53 codon 72 as a general risk factor for EC. In addition, loss of

---

Table 2

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sample size</th>
<th>Genotype, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pro/Pro</td>
</tr>
<tr>
<td>Cases</td>
<td>n = 30</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Controls</td>
<td>n = 32</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>n = 62</td>
<td>20 (32)</td>
</tr>
</tbody>
</table>

The homozygous Pro/Pro genotype was significantly more common in patients than in control subjects (P = 0.041). The Pro/Pro genotype was associated with an increased risk of endometrial cancer (OR = 3.7, 95% CI = 0.539–25.59).

Table 3

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Alleles</th>
<th>Allele, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pro</td>
</tr>
<tr>
<td>Cases</td>
<td>n = 60</td>
<td>41 (68)</td>
</tr>
<tr>
<td>Controls</td>
<td>n = 64</td>
<td>35 (55)</td>
</tr>
<tr>
<td>Total</td>
<td>n = 124</td>
<td>76 (61)</td>
</tr>
</tbody>
</table>

Frequency of the proline allele was higher in patients than in control subjects, but not significantly. The proline allele was associated with risk of endometrial cancer (odds ratio = 1.9; 95% confidence interval = 0.903–3.893).
heterozygosity could be tested in DNA of the tumor cells using SNP polymorphism array CGH and compared with germline DNA.

References


