The TP53 Codon 72 Polymorphism and Risk of Sporadic Prostate Cancer among Iranian Patients

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Abstract

Background: The TP53 gene is one of the most frequently mutated genes amongst human malignancies, particularly TP53 codon 72 polymorphism. Furthermore, an association between the TP53 codon 72 variants and prostate cancer has been reported in several studies. Although some studies have indicated an association between the TP53 Arg/Arg variant and an increased risk for prostate cancer, other studies have shown a positive correlation between the TP53 Pro/Pro genotype instead. Therefore, to clarify if this polymorphism is associated with an increased risk of prostate cancer in Iranian men, we conducted a case-control study of 40 sporadic prostate cancer patients and 80 benign prostate hyperplasia cases.

Methods: The TP53 codon 72 was genotyped using an allele specific PCR.

Results: A significant association between the TP53 codon 72 genotype and prostate cancer risk was found (OR = 6.8, 95% CI = [1.8-25.1], P = 0.005). However, the results of this study did not support an association between age, the Gleason score nor TP53 genotype at codon 72 in prostate cancer patients.

Conclusions: TP53 codon 72 polymorphism may have a great impact in the development of prostate cancer.

Keywords: Sporadic prostate cancer, Benign prostate hyperplasia, TP53 codon 72 polymorphism

Introduction

Prostate cancer is the second most frequent cancer in men worldwide, with an estimated 903,500 new cases and 258,400 deaths in 2008 (1, 2). Also, it is the second most common cancer with an estimated age-standardized incidence rate of 11.6 in Iranian men (3). While the natural history of prostate cancer is not recognized very well, several causes point to genetic defects, infection and inflammation (4-9). The TP53 gene, located on chromosome 17p13, is a tumor suppressor gene (10). The p53 is considered as ‘guardian of the genome’ because it plays a crucial role in the cell cycle arrest and inducing of apoptosis (11, 12). This gene is one of the most frequently mutated genes in human malignancies (13, 14). In particular, some studies have been supported a role for TP53 codon 72 polymorphism in development of various cancers (13, 15-17). This mutation is a G-to-C substitution at nucleotide position 313 that results in a change of arginine (CGC) to proline (CCC) (18). An in-vitro study has been shown that the TP53
Arg/Arg variant stimulates apoptosis and prevents transformation properly than the Pro/Pro genotype, indicating individuals with Pro/Pro genotype may be more susceptible for development of cancer (19). Patients with the Pro/Pro variant likely have a poor prognosis and survival, particularly cancers of the ovarian (20), breast (21), thyroid (22), esophageal squamous cell carcinoma (23) and hepatocellular carcinoma (17) or early age onset of cancer such as squamous cell carcinoma of the head and neck (24). An association between TP53 codon 72 variants and prostate cancer have been reported in several studies (15, 25, 26). Although some studies have been indicated an association between the TP53 Arg/Arg variant and an increased risk for prostate cancer (25, 27, 28), others have been shown a correlation between the TP53 Pro/Pro genotype instead (15, 26).

In the only study in North of Iran, no associations have been found between TP53 codon 72 variant and prostate cancer (25, 29). To clarify if this polymorphism is associated with an increased risk of prostate cancer in Iranian men, we conducted a case-control study of 40 sporadic prostate cancer (PCa) and 80 benign prostatic hyperplasia (BPH) cases.

Materials and Methods

Study population and samples

A series of 120 formalin-fixed paraffin-embedded tissue samples including 40 PCa blocks and 80 BPH tissues were retrieved from the archives of the Pathology Laboratory of Sina Hospital in Tehran during 2011. All samples were taken with appropriate local ethical committee approval and informed consent was obtained from recruited patients. None of the patients had received any chemotherapy or radiotherapy before the surgery. All cases were re-examined by pathologist to verify a Gleason score higher than 6.

TP53 codon 72 polymorphism analysis

For each tissue sample, 10-μm sections were cut. Median sections were applied for molecular analysis. First and final sections were stained by hematoxylin–eosin and were re-examined by the pathologist to verify a Gleason score higher than 6. To avoid potential contamination between specimens, the first four sections from each tissue sample were discarded, the microtome was cleaned with 70% ethanol and blades were changed before cutting the next block. Also, an empty block was cut between samples and applied as a PCR control to check potential cross-contamination through microtome use.

Genomic DNA was isolated according to previous published protocols with some modifications (30, 31). In brief, two 10 μm slices of each sample were de-paraffinized with xylene and digested with lysis buffer (50 mM Tris–HCl pH 8.5, 1 mM EDTA, 0.5 % Tween 20) and Proteinase K (200 μg per ml) at 37 °C, overnight. In the next step, DNA purification was performed by phenol–chloroform extraction and ethanol precipitation.

The TP53 codon 72 polymorphism analysis was carried out by using an allele specific PCR amplification. For each sample, PCR was done in two separate reactions by specific primers. In the first reaction that was specific to detect proline allele, p53Pro Plus primer (5'-GCCAGAGGCTGCTCCCCC-) with p53Minus primer was applied. In the second reaction that was specific for arginine allele, p53 Plus primer with p53Arg Minus (5'-CTGGTGCAAGGGCCACGC-) primers was used (32). Amplification reaction was carried out in a 50-μl reaction mixture including 1.5 mM MgCl2, 50 μM of each dNTP, 20 pmol of each primer, 2 μl of Taq DNA polymerase and 100-200 ng of DNA target. PCR performed as follows: an initial 1-min denaturation at 94 °C, followed by 32 cycles of 94 °C for 30 s, 59 °C for 30 s, 72 °C for 45 s and a final elongation at 72 °C for 5 min.

Statistical analysis

The observed and expected TP53 codon 72 allele frequencies for PCa and BPH groups were investigated by the Hardy–Weinberg equilibrium theory. The associations between diseases and variants were evaluated by calculating the Odds Ratio (OR) with 95% Confidence interval (CI). All statistical analysis was conducted using the EPI Info.
version 7. A two-sided \( P \)-value was considered statistically significant when it was < 0.05.

**Results**

This study investigated the \( TP53 \) codon 72 polymorphism on 40 PCa patients and 80 BPH individuals. The mean age (± SD) for patients with PCa and BPH were 69.85 (± 8.58) and 69.91 (± 8.05), respectively. The \( TP53 \) codon 72 allele frequencies were studied using an allele specific PCR to detect arginine or proline (Fig. 1). Table 1 shows the frequencies of \( TP53 \) codon 72 variants in PCa and BPH groups. The frequencies of Arg/Arg, Arg/Pro and Pro/Pro alleles among PCa were 15 (37.5%), 15 (37.5%) and 10 (25%), respectively. In the BPH group, the frequencies of \( TP53 \) codon 72 genotypes were 41 (51.3%), 35 (43.7%) and 4 (5%), respectively. The \( TP53 \) codon 72 allele frequencies were in the Hardy-Weinberg equilibrium among PCa and BPH groups (\( X^2 = 1.02, \) and \( X^2 = 2.27, df = 1; P > 0.05 \)).

As shown in table 1, the \( TP53 \) Pro/Pro genotype was more frequent in PCa cases in comparison to BPH subjects and this difference was statistically significant (\( OR = 6.8, 95\% CI = [1.8-25.1], P = 0.005 \)). When stratifying the PCa and BPH groups by the age ≤ 65 versus > 65, an important association was found between the Pro/Pro variant and an increased risk of prostate cancer in patients older than 65 years old. In the age group > 65, individuals who carried Pro/Pro genotype showed a 9.9-fold higher risk (95% CI= 1.8-54.5, \( P = 0.009 \)) of progression of prostate cancer in comparison to subjects who carried Arg/Arg. However, compared to BPH subjects (Table 2), no statistically significant association was observed between \( TP53 \) alleles and the risk of prostate cancer in PCa patients under the age of 66. Also, as stratifying the PCa group by the age of onset, no important correlation was found between the age of onset and risk of prostate cancer development. Although the Pro allele was more prevalent in age group ≤ 65 years old, this difference was not statistically significant (\( P =0.47 \)).

Table 3 shows the association between \( TP53 \) variants and prostate cancer risk, stratified by the Gleason score among prostate cancer patients. No significant association was found between the Gleason score and \( TP53 \) genotype at codon 72 in these patients.

![Fig. 1: Allele specific PCR /Left to right: Proline/Proline homozygous-Arginine/Proline heterozygous-Proline/Proline homozygous-Arginine homozygous- negative control-Positive Control-1KbSize marker](image-url)
Table 1: Frequency of TP53 codon 72 variants in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) cases

| Genotype       | PCa (n=40) n (%) | BPH (n=80) n (%) | OR (95% CI) | P-value |
|----------------|------------------|------------------|-------------|---------|
| Arg/Arg        | 15 (37.5)        | 41 (51.3)        | 1           |         |
| Arg/Pro        | 15 (37.5)        | 35 (43.7)        | 1.2 (0.5-2.7) | 0.88    |
| Pro/Pro        | 10 (25)          | 4 (5)            | 6.8 (1.8-25.1) | 0.005   |
| Pro/Arg + Pro/Pro | 25 (62.5)   | 39 (48.7)        | 1.7 (0.8-3.8) | 0.21    |
| Arg allele     | 45 (56.3)        | 117 (73.1)       | 1           |         |
| Pro allele     | 35 (43.7)        | 43 (26.9)        | 2.1 (1.2-3.7) | 0.012   |

Table 2: Distribution of TP53 codon 72 variants in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) cases stratified by age

| Age (yr) | ≤ 65 | > 65 | OR (95% CI) | p-value |
|----------|------|------|-------------|---------|
| Genotype | PCa (n=12) n (%) | BPH (n=19) n (%) | OR (95% CI) | p-value |
| Arg/Arg  | 3 (25)          | 8 (42.1)        | 1           |         |
| Arg/Pro  | 6 (50)          | 9 (47.4)        | 2.7 (0.5-13.6) | 0.7   |
| Pro/Pro  | 3 (25)          | 2 (10.5)        | 6 (0.7-53.7) | 0.25   |
| Arg/Pro+ Pro/Pro | 9 (75) | 11 (57.9) | 3.3 (0.7-15.3) | 0.8   |
| Arg allele | 12 (50)        | 25 (65.8)       | 1           |         |
| Pro allele | 12 (50)        | 13 (34.2)       | 1.9 (0.7-5.5) | 0.21   |

Table 3: TP53 codon 72 genotype frequencies in prostate cancer patients stratified by Gleason score

| Genotype       | Gleason score 6-7 n (%) | Gleason score 8-10 n (%) | OR (95% CI) | p-value |
|----------------|-------------------------|--------------------------|-------------|---------|
| Arg/Arg        | 4 (33.3)                | 11 (39.3)                | 1           |         |
| Arg/Pro        | 4 (33.3)                | 11 (39.3)                | 0           | 0.7     |
| Pro/Pro        | 4 (33.3)                | 6 (21.4)                 | 1.8 (0.3-10) | 0.8     |
| Pro/Arg + Pro/Pro | 8 (66.67)            | 17 (60.7)                | 1.3 (0.3-5.3) | 0.9     |
| Arg allele     | 12 (50)                 | 33 (58.9)                | 1           |         |
| Pro allele     | 12 (50)                 | 23 (41.1)                | 1.4 (0.5-3.7) | 0.6     |

Discussion

The tumor suppressor gene TP53 plays a main role in the progression of human cancers (13, 14). The TP53 polymorphism at codon 72 has been found to be associated with susceptibility to cancers in different tumors (13, 15-17, 25, 26). Although several studies have investigated the association between TP53 variant and susceptibility to prostate cancer, their results are conflicting and inconclusive (15, 25, 26, 29, 33).

In this hospital-based case-control study, the association between TP53 gene polymorphism and the risk of prostate cancer was investigated in Iranian men. Our findings revealed that cases with Pro/Pro had a 6.8-fold increased risk of developing prostate cancer in comparison to those with Arg/Arg. Several studies have suggested an important role for TP53 codon 72 variant in the tu-
morgenesis and progression of prostate cancer (15, 25, 26). However, there are inconsistencies regarding the role of TP53 gene polymorphism in the development of prostate cancer. Wu et al. has shown that the proline genotype was 2.6 times more frequent than the arginine variant in prostate cancer patients and this difference was statistically significant (26). In another study, it is revealed that the Pro/Pro allele was correlated with a strikingly lower risk of prostate cancer (25).

This polymorphism happens in a proline-rich region of p53 that is important for the cell cycle arrest and apoptotic activities of this protein (34). It is shown that Arg/Arg variant of TP53 gene was a strikingly efficient suppressor of cellular transformation, an activity normally associated with p53's apoptotic function. Therefore, individuals with Pro/Pro genotype may be more susceptible to the development of cancer (19, 35, 36).

Besides, the findings of this study did not support an association between age or Gleason score with TP53 genotype at codon 72 in prostate cancer patients. These results are consistent to previous studies showing no associations between TP53 codon 72 alleles and these factors in prostate cancer (26, 33, 37). However, an association between the TP53 variant and the age > 65 years was found among PCA cases in comparison to BPH subjects. In other words, the TP53 Pro/Pro genotype was more prevalent in PCA patients compared to BPH subjects in age group > 65 years old. This difference likely arises from the small sample size in age group ≤ 65 years old. It is worth mentioning that this study has some limitations. One is the modest sample size of prostate cancer cases. Also, since the study population was hospital-based, healthy individuals were not investigated as a control group. However, by matching the age of PCA and BPH subjects we try to minimize the potential of this confounding factor.

**Conclusion**

There is an important difference in the frequency of TP53 codon 72 variants between PCa and BPH groups. Our findings suggest that TP53 codon 72 polymorphism may have an impact on the development of prostate cancer. Although the results suggest that the TP53 codon 72 genotype may confer more susceptibility to prostate cancer development, further studies are needed to confirm these results.

**Ethical considerations**

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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