Original Article

No evidence of correlation between p53 codon 72 polymorphism and risk of bladder cancer in Moroccan patients

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ABSTRACT

Background: The p53 codon 72 polymorphism has been investigated extensively for its association with various cancers around the world. Several studies have investigated the association between p53 polymorphism at codon 72 and risk of developing bladder cancer, but the results are still controversial. **Aim:** The aim of our study was to evaluate the association between the p53 polymorphism and the bladder cancer risk among Moroccan patients. **Materials and Methods:** This study was carried out on fresh biopsies from 41 patients with bladder cancer confirmed and 38 blood samples from control donors. Deoxyribonucleic acid was genotyped by "Allele-specific polymerase chain reaction" using specific primers to each polymorphic variant. **Results:** Frequencies of Arg/Arg, Arg/Pro and Pro/Pro genotypes among cases were 17%, 66%, and 17%, while in controls the frequencies of Arg/Arg, Arg/Pro and Pro/Pro were 15.8%, 63.2%, and 21%, respectively. The difference between cases and controls was not statistically significant. An increased risk of bladder cancer development was not clearly related to any polymorphic variant of the p53 Arg72Pro in our study group from Moroccan population. Moreover, the frequency of the Arg allele was higher (71.45%) than the Pro allele (28.55%) in high stage of bladder tumors, but this difference was statistically not significant. **Conclusion:** This study suggests that Arg allele could be more involved in developing bladder tumor in Moroccan population than the Pro allele. Therefore, enlarging the sampling will be necessary to confirm this association in Moroccan population.

Key words: Bladder cancer, ethnicity, p53 polymorphism

INTRODUCTION

Worldwide, bladder cancer is the 7[th] most common cancer, approximately accounting 336,000 new cases each year.[1,2] In Morocco, according to the regional cancer registers, bladder cancer is the 6[th] most common cancer with an incidence of 5.8 and 11.3 per 100,000 persons in Casablanca and Rabat, respectively.[3,4] The mean age of bladder cancer occurrence was 62.9 years in women and 63.8 years in men. Urothelial carcinoma (UC) was by far the most frequent histological type (70% in women and 82% in men), while squamous cell carcinoma accounted 10% of cases in women and 4.8% in men.[3] Tobacco smoking, specific industrial chemicals, dietary nitrates, and arsenic represent the most important exogenous risk factors.[5,6]

Exposure to these carcinogens may cause deoxyribonucleic acid (DNA) damage and alterations. Indeed, several DNA alterations have been described in bladder cancer and have shown promising results in terms of molecular markers, such as allele losses or deletions,[7] gene amplifications,[8] DNA mutations,[9] microsatellite instabilities,[10] gene promoters methylation,[11,12] and genes polymorphism.[13]

TP53 is a tumor suppressor gene which is mapped on chromosome 17p13 and is one of the most frequently mutated genes in most types of human cancer, including bladder

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cancer.[14,15] It encodes p53 tumor suppressor protein which has a key role in cell cycle control. Indeed, p53 is involved in different pathways in the cell process. It is responsible for the transcription of a site-specific DNA-binding protein and acts as a transcription factor of cell growth regulator genes.[16] In response to genotoxic insults from endogenous or environmental agents, p53 inhibits cell growth by inducing apoptosis or cell cycle arrest in either the G1 or G2 phase.[17] Although *TP53* somatic mutations are detected primarily in high grade and invasive bladder tumors,[18,19] polymorphisms in p53 seem to have a modest effect on cell phenotype, leading to different patterns of cancer susceptibility.[20]

In the *TP53* gene, several polymorphisms have been identified, both in noncoding and coding regions.[21-24] Most of these polymorphisms are single-nucleotide polymorphisms (SNPs) affecting a single base. Within the coding regions of *TP53*, only two important polymorphisms are present and alter the amino acid sequence of their products;[21,25] these polymorphisms are located at codon 47 and codon 72 in exon 4. Codon 72 (Arg72Pro) is a frequent functional SNP that leads to an arginine-proline amino acid change, which has been widely studied indifferent cancers,[26-28] such as cancer of the stomach,[29] esophagus,[30] colorectum,[31] lung,[32] breast,[33] and cervix.[34]

Numerous studies have been conducted to investigate the potential association between p53 Arg72Pro polymorphism and bladder cancer in humans.[35-44] However, the role of Arg72Pro polymorphism in bladder carcinogenesis remains controversial. It seems that the distribution of this polymorphism (Arg/Arg and Pro/Pro homozygotes and Arg/Pro heterozygotes) varies ethnically. According to a meta-analysis accomplished by Jiang *et al.*,[45] a decreased risk of bladder cancer was observed among Asians in homozygote comparison (Arg/Arg vs. Pro/Pro) and dominant model (Arg/Arg plus Arg/Pro vs. Pro/Pro). However, a high risk was revealed among whites in all genetic models, except for the recessive model (Arg/Arg vs. Arg/Pro plus Pro/Pro). In contrast, no correlation between bladder cancer risk and Arg72Pro polymorphism was found among Africans and Turk population in any genetic model.[45] Another meta-analysis study[46] supports conclusions that p53 codon 72 polymorphism may be associated with bladder cancer and that the difference in genotypes distribution may be associated with the stage of bladder cancer. These findings warrant larger studies to clarify the p53 codon 72 polymorphism role of and to evaluate p53 gene-environment interactions. Furthermore, only one similar study was performed among Africans.[39] Thus, it would be necessary to extend this study in other African populations.

In the present study, we examined the genotype distribution of *TP53* Arg72Pro SNP, using polymerase chain reaction (PCR) with allele-specific primers, to evaluate its possible relevance in susceptibility to bladder cancer in Morocco and to study the correlation of this SNP with the clinicopathological variables of bladder cancer cases.

MATERIALS AND METHODS

Cases and specimens

DNA from 46 patients with bladder disease was available from our laboratory DNA bank. The study design and population has been previously described.[47]

As p53 allele frequencies have been shown to vary according to ethnic group, controls and patients were from the same ethnic background.

Genotyping of p53 gene at codon 72

p53 Arg72Pro polymorphism was determined by PCR with allele-specific primers "Allele-specific polymerase chain reaction" as described in our previous study realized in cervical cancer[48] This PCR based-technique was realized using specific primers designed especially to amplify either the p53 Pro or the p53 Arg allele. DNA was amplified in separate reactions with p53 Pro and p53 Arg primers.[48] p53 Pro sequences were detected by PCR using the primer pair p53 $Pro^+/p53^-$: (p53 Pro^+: 5′GCCAGAGGCTGCTCCCCC; $p53^-$: 5′CGTGCAAGTCACAGACTT, 177 bp) and p53 Arg by the primer pair: $p53^+/Arg^-$ ($p53^+$: 5′TCCCCCTTGCCGTCCCAA; p53 Arg^-: 5′CTGGTGCAGGGGCCACGC, 141 bp).

PCR was carried out in a final volume of 25 μL containing 50 ng of genomic DNA, 1X PCR buffer, 2 mM MgCl2, 0.25 mM of each dNTP, 1 μM of each primer, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA).

DNA was amplified as follows: one initial denaturation step at 95°C for 10 min; followed by 35 (Arg) or 40 (Pro) cycles of 30 s at 95°C, 30 s of annealing at 59°C (Arg) and 58°C (Pro) and 30 s at 72°C, followed by a final elongation cycle at 72°C for 7 min. PCR products were analyzed by electrophoresis on a 2% agarose gel. The gels were then stained with ethidium bromide and visualized under ultraviolet illumination.

Statistical analysis

Frequencies for codon Arg72Pro polymorphism of p53 in cases and controls were compared using the Chi-square test. The correlation between the polymorphism and clinicopathological parameters was evaluated statistically using Chi-square test by comparison between proportions. All these tests were performed by MedCalc software version 9. A goodness-of-fit Chi-square test was used to determine whether the polymorphisms were in Hardy-Weinberg equilibrium between cases and controls.

RESULTS

DNAs from 46 patients with bladder disease were analyzed for the polymorphism of p53 at codon 72. The histological analysis has shown that the majority of cases (41 of 46) were bladder cancer confirmed and were classified as follows: 40 had transitional cell carcinomas and one had an adenocarcinoma. Then five others patients had inflammatory urinary disease (cystitis). The tumor staging revealed that among 40 UC cases, 9 were classified as pTa (22.5%), 24 as pT1 (60%), 6 as pT2 (15%), and only 1 case was staged as pT4 (2.5%). The tumor grading showed that among 40 UC cases, 13 cases were classified as low grade (32.5%), whereas 27 were high grade (67.5%).

Mean age at diagnosis of bladder cancer was 64.7 with extreme ages at 35 and 86 years old. The majority of the samples were obtained from males (42 of 46); there was a significant gender difference ($P < 0.0001$). In addition, DNA from 38 healthy age-matched (mean age 61.2) males volunteers was included as controls.

Figure 1 shows the PCR products obtained after amplification with arginine- or proline-specific primers. Table 1 depicts the genotype frequency distribution of the three different biallelic DNA polymorphisms in the p53gene for the 41 bladder cancer patients and 38 healthy controls subjects. Genotype frequencies observed in cases and controls were in Hardy-Weinberg equilibrium. Frequencies of Arg/Arg, Arg/Pro and Pro/Pro genotypes among cases were 17% (7/41), 66% (27/41), and 17% (7/41), while in controls the frequencies were 15.8% (6/38), 63.2% (24/38), and 21% (8/38), respectively. There was no significant difference in overall genotype frequency distribution between cases and controls groups. Among patients with cystitis, the frequency of Arg/Arg genotype was null, whereas the frequencies of Arg/Pro and Pro/Pro were 80% (4/5) and 20% (1/5), respectively.

The relative frequency for Arg allele is 0.5 in cases and 0.474 in controls, whereas for Pro allele the frequencies were 0.5 in cases and 0.526 in controls. No difference in frequency distribution of the two alleles was observed between bladder cancer patients and healthy individuals.

Further analysis revealed that there was almost no difference between frequencies of the rare allele (Arg/Pro + Pro/Pro) in cases and controls, and the frequencies of homozygous and heterozygous genotypes were roughly similar in cases and controls.

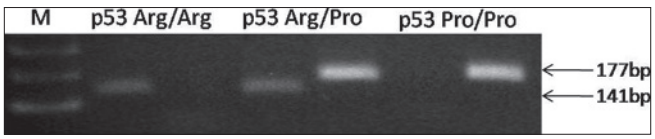

Figure 1: Analysis of p53 codon 72 polymorphism by polymerase chain reaction using allele-specific primers. Arg allele-specific primer: 141 bp and Pro allele-specific primers: 177 bp. M: 100 bp ladder

The distribution of TP53 Arg72Pro according to stage and grade of tumors was also tested and reported in Table 2. The analysis shows that there was no significant association between stage/grade and homozygous genotypes (Arg/Arg or Pro/Pro) or heterozygous genotype (Arg/Pro).

Our study found higher frequency of rare allele in low stage (pTa/pT1 = 90.9%) than in high stages (pT2/higher = 57.1%), but this difference was not statistically significant ($P = 0.27$). Also, no difference in frequency of rare allele was observed between high (85.2%) and low (84.6%) grade of bladder tumors ($P = 0.64$). The frequency of the Arg allele was higher (71.45%) than the Pro allele (28.55%) in high stage of bladder tumors ($P = 0.85$), this is probably due to the weak number of cases.

DISCUSSION

The*TP53* gene has a central role in tumor suppression by initiating apoptosis or inducing cell arrest at the G1/S phase in response to DNA damage and seems to play a prominent role in bladder cancer. Since the identification of the *TP53* Arg72Pro polymorphism,[49] a number of studies[35-39,41,50,51] have investigated the genetic effect of the *TP53* Arg72Pro polymorphism on bladder cancer susceptibility. However, the results were controversial.

Some investigations revealed high risks of bladder cancer development, due to either arginine or proline for codon 72

Table 1: Genotype frequencies of TP53 Arg72Pro in patients

Genotype	Bladder cancer *n* (%)	Controls *n* (%)	*P* value
Arg/Arg	7 (17)	6 (15.8)	0.488
Arg/Pro	27 (66)	24 (63.2)	0.932
Pro/Pro	7 (17)	8 (21)	0.644

Arg: Arginine, Pro: Proline, TP: Tumor protein

Table 2: Association between TP53 codon 72 phenotypes and clinicopathologic characteristics

	Total	p53 polymorphism		
		Arg/Arg (%)	Arg/Pro (%)	Pro/Pro (%)
Tumor stage				
pTa-T1 (%)	33	3 (9.1)	23 (69.7)	7 (21.2)
≥pT2 (%)	7	3 (42.9)	4 (57.1)	0 (0)
		P=0.09	0.84	0.42
Tumor grade				
Low (%)	13	2 (15.4)	10 (76.9)	1 (7.7)
High (%)	27	4 (14.8)	17 (63)	6 (22.2)
		P=0.67	0.6	0.49
Adenocarcinoma	1	1	0	0
Cystitis	5	0	4	1

Arg: Arginine, Pro: Proline, TP: Tumor protein

polymorphism; while others failed to show any association between the p53 polymorphism and cancer. This variety of results is likely due to ethnical factors. Indeed, a meta-analysis study has shown[45] an evidence for association between Arg72 carriers and reduced bladder cancer risk among Asians but not among whites, Africans, or Turk population. Therefore, additional studies are warranted to evaluate the effect of this functional polymorphism on bladder cancer risk in different ethnicities, especially in Africans. This study, conducted for the first time in Moroccan population, aims to evaluate whether codon 72 polymorphism of p53 is implicated or not in bladder cancer. Frequencies obtained of this polymorphism in cancer cases were 17%, 66%, and 17% for Arg/Arg, Arg/Pro and Pro/Pro, respectively. The heterozygote variant is predominantly distributed compared with homozygous forms and such distribution was observed in Greek population with 64.7% as Arg/Pro frequency.[38] No association between different forms of polymorphism and bladder cancer was found and our finding is supported by a study performed in a neighbor country[39] and by another study realized in a different ethnical group.[41] In controls, frequencies of Arg/Arg, Arg/Pro and Pro/Pro were 15.8%, 63.2%, and 21%, respectively. The polymorphic variant distribution between cases and controls was slightly different but statistically not significant.

Also, when the patient group was stratified according to clinicopathological data, we found no significant correlation between any polymorphic variant and tumor stage and/or grade. Indeed, a higher frequency of rare allele (Arg/Pro + Pro/Pro) was detected in low stage (pTa/pT1 = 90.9%) than in (pT2/higher = 57.1%), but this difference was not statistically significant (P = 0.27). This is in tune with previous studies showing the predominance of rare allele in advanced bladder tumors.[36,40]

In addition, no difference in frequencies distribution of the two alleles was observed between bladder cancer patients and healthy individuals. However, a significant decrease in the frequency of the Pro allele with increased latitude was reported with a frequency of 63% in Africans and 50% in African-Americans.[41] Moreover, the frequency of the Arg allele was higher (71.45%) than the Pro allele (28.55%) in high stage of bladder tumors, suggesting that Arg allele could be more involved in developing bladder tumor in Moroccan population. This is supported by Soulitzis,[38] who reported a significant correlation between the frequency of the Arg allele and an increased risk of developing bladder cancer in the Greek population.

CONCLUSION

Our overall results are not concordant with an association between codon 72 polymorphism and risk of developing bladder cancer. Moreover, the frequency of the Arg allele was higher than the Pro allele in high stage of bladder tumors and enlarging the sampling will be necessary to confirm this association in Moroccan population.

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