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ARTICLE

p53 Codon 72 Polymorphism in Basal Cell Carcinoma of the Skin

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Basal cell carcinoma (BCC) is the most prevalent cancer in Iran. A common polymorphism at codon 72 of exon 4 of p53 tumor suppressor gene has been reported to be associated with increased inheritable susceptibility to several cancers. In the present study the frequency of p53 codon 72 polymorphism in 91 patients with BCC of skin, compared to 465 healthy normal individuals, was investigated. In total, there was no significant difference in the p53 genotypes between patients and controls. However, there was an apparent increase in the *Arg*/*Arg* genotype among those BCC patients who had a history of occupational sun exposure, compared to non-exposed patients (46.3% vs. 23.1%, P=0.11). A trend of increase in the frequency of *Arg* allele among sun-exposed patients was also observed (69.4% vs. 53.8%, P=0.07). Comparison of the genotype frequencies between sun-exposed patients and normal controls confirmed the accumulation of *Arg*/*Arg* genotype in these patients (46.3% vs. 34.8%, P = 0.07). In addition, the frequency of *Arg* allele was significantly higher in sun-exposed patients compared to controls (69.4% vs. 58.2%, P=0.03). Our results suggest that *Arg* allele at codon 72 of p53 gene might affect the risk of ultraviolet-induced basal cell carcinoma. (Pathology Oncology Research Vol 12, No 1, 29–33)

Key words: basal cell carcinoma, p53, polymorphism, sun exposure

Introduction

Skin cancer is the most common cancer among different populations worldwide. Among the three main types of skin cancer, basal cell carcinoma (BCC) is the most prevalent cancer in Iran, a country populated by a non-European Caucasoid population. The most important risk factor known for skin carcinogenesis is solar UV radiation.[1] However, there are cumulative data pointing to the importance of genetic factors in susceptibility to the disease. Thus far, mutations and polymorphisms in PTCH and p53 genes have been suggested to play a role in the development of BCC.[2] Recent reports have shown a significant association between *Arg72* homozygosity in p53 tumor suppressor gene and susceptibility to non-melanoma skin cancers in renal transplant patients, mostly in white Caucasians.[3] However, there is no report on this polymorphism in the darker-skinned populations which are significantly resistant to the effects of sunlight.

The polymorphism is a G/C substitution in codon 72 of p53 gene, which results in an *Arg*/*Pro* change in the sequence of encoded amino acids.[4] The *Arg*/*Pro* polymorphism occurs in a proline-rich domain and results in alteration of electrophoretic mobility of the protein.[5,6] The codon 72 polymorphism has also been shown to affect the behavior of certain p53 mutants and their potential of transforming cells.[7,8] In the presence of *Arg* allele, conformational p53 mutants have been more potent in binding to p73 and neutralizing p73-induced apoptosis,[7] which enhances tumorigenesis and provides a selective growth advantage to tumor cells.[8] Increased susceptibility of *Arg72* containing p53 protein to degradation by human papillomavirus (HPV) has also been reported.[9] In this regard, a strong association of *Arg72* homozygosity with HPV-induced cervical cancer has been suggested.[10-13] In addition, a selective retention of *Arg72* alleles and a higher aggressiveness of *Arg72*-containing ovarian tumors have been shown.[14] However, the issue is still

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controversial in some other types of cancers and in some ethnic groups.[15-20]

This study was undertaken to investigate the association of p53 codon 72 polymorphism with BCC in southern Iranian patients.

Patients and Methods

In this study we investigated the frequency of p53 codon 72 polymorphism in 91 patients with BCC of skin, compared to 465 ethnically-matched healthy blood donors. Peripheral blood samples were collected in 10 ml volume by venous puncture method, and genomic DNA was extracted from peripheral blood lymphocytes by salting out method. The extracted DNA was examined by an allele-specific polymerase chain reaction described by Soulitzis et al.[21] To detect the p53 codon 72 polymorphism, two primer sets in separate tubes were used, one to amplify the *Arg* allele and the other to amplify the *Pro* allele as follows: *Arg*F: TCC CCC TTg CCg TCC CAA, *Arg*R: CTg gTg Cag ggg CCA CgC, *Pro*F: gCC AgA ggC TgC TCC CCC, *Pro*R: CgT gCA AgT CAC AgA CCT.

Each set of primers were used in a different tube in a total volume of 25 µl containing 0.3 mM dNTPs, 1.5 mM $MgCl_2$, 2U Taq DNA polymerase (Sinagen, Iran) and 1x buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl). The amplification was performed for 35 cycles under a touchdown program; by denaturation at 94° for 30 s, annealing at 68°C to 62°C for ten cycles and 62°C to 58°C for 25 cycles, and extension at 72°C for 30 s in each cycle. The PCR product of the *Arg* allele was 141 bp, while the product of the *Pro* allele was 177 bp (*Figure 1*).

At the time of blood sampling, demographic and clinical data were recorded in a questionnaire. The location of lesions, number of lesions, history of occupational sun exposure and history of malignancies were also recorded.

The calculated frequencies were analyzed by Chi-square test using EPI-info 2000 and SPSS 10.0 for Windows software.

Results

In total 58 male and 33 female BCC patients were studied of which 54 individuals had a history of occupational sun exposure. Mean age of patients was found to be 59.16 ± 1.37 years and median age of patients was 59 years. The mean age at diagnosis was 57.70 ± 1.87 years and median age at diagnosis was 59 years. Twenty-nine (31.87%) out of 91 patients were farmers. The number of lesions varied from 1 to 11 with different sizes. *Table 1* shows the patients' information.

In total, 34 (37.4%) BCC patients and 162 (34.8%) normal individuals had *Arg*/*Arg* genotype, while 10 (11%) BCC patients and 86 (18.5%) normal individuals had

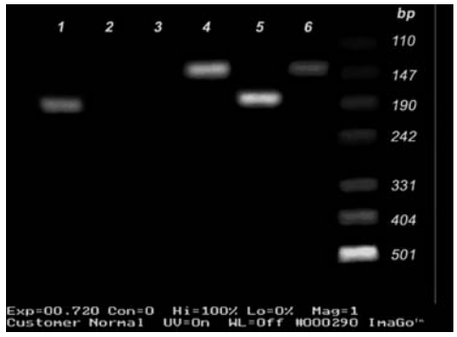

Figure 1. *PCR amplification of the p53 codon 72 Arg allele (141 bp) and Pro allele (177 bp). The result of amplification for Pro homozygote (lanes 1 and 2), Arg homozygote (lanes 3 and 4) and Pro/Arg heterozygote (lanes 5 and 6) genotypes are indicated. The reaction in lane 1 (Pro) and lack of reaction in lane 2 (Arg) indicate that the first individual is Pro homozygote, while lack of reaction in lane 3 (Pro) and the existence of PCR-amplified Arg band in lane 4 indicate that individual 2 is Arg homozygote. The reaction in both lanes (5 and 6) indicates that individual 3 is heterozygote.*

Pro/*Pro* genotype. The frequencies of heterozygous BCC and healthy individuals were 47 (51.6%) and 217 (46.7%), respectively. There was no significant difference in the frequencies of p53 alleles and genotypes between patients and controls (*Table 2*, P=0.24 and P=0.22). However, there was an apparent increase in *Arg*/*Arg* genotype among those BCC patients who had a history of occupational sun exposure compared to non-exposed patients (46.3% vs. 23.1%, P=0.11). A trend of increase in the frequency of *Arg* allele among sun-exposed patients was also observed (69.4% vs. 53.8%, P=0.07). Among the control group, 64 had a history of occupational sun exposure, for which the frequencies of *Arg*/*Arg* and *Pro*/*Pro* genotypes were found to be 21 (32.8%) and 10 (15.6%), respectively. The frequencies of these genotypes in sun-exposed patients were found to be 25 (46.3%) and 4 (7.4%), respectively. In this regard, an increase in the *Arg*/*Arg* genotype and a decrease in *Pro*/*Pro* genotype among patients was observed, but did not reach statistical significance (P=0.2). Comparison of the allele frequencies revealed an increase in the *Arg* allele and a decrease in *Pro* allele among sun-exposed patients compared to sun-exposed healthy individuals. However, the difference was not statistically significant (P=0.11).

Comparison of the genotype frequencies between sun-exposed patients and normal controls confirmed the accumulation of *Arg*/*Arg* genotype in these patients (46.3% vs. 34.8%, P=0.07). In addition, the frequency of *Arg* allele was significantly higher in sun-exposed patients compared to controls (69.4% vs. 58.2%, P=0.03).

The genotype and allele frequencies were also compared between 63 control individuals aged more than 45 years

and the 91 patients. There was no significant difference in the frequencies between patients and age-matched controls (P=0.73). *Table 3* shows the genotype frequencies of this comparison. There was also no significant difference in the frequency of codon 72 polymorphism between patients and controls in regard to age, gender, number of lesions, location of lesion, exposure of lesion, history of recurrence and history of skin malignancy. There was also no significant difference in the studied frequencies between patients who lived in rural and urban areas.

Discussion

Our results are in accordance with previous reports suggesting that there is no direct association between p53 codon 72 polymorphism and BCC in immunocompetent patients.[3] However, the comparison of the sun-exposed patients with control healthy individuals revealed a significant difference in the frequency of *Arg* allele and a marginal difference in the frequency of *Arg*/*Arg* genotype. A non-significant increase both in the *Arg*/*Arg* genotype

Table 1. **Characteristics of patients**

	Number	*%*
Gender		
Male	58	63.7
Female	33	36.3
Occupation		
Farmer	29	31.9
Housewife	13	14.3
Teacher/Clerk	8	8.8
Worker	8	8.8
Driver	7	7.7
Army officer	6	6.6
Other	13	14.2
ND	7	7.7
Lesion exposure		
Sun-exposed	72	79.1
Non-exposed	9	9.9
Both	2	2.2
ND	8	8.8
Location of lesions		
Nose	25	27.4
Head	14	15.4
Cheeks	11	12.1
Eye	5	5.5
Temple	5	5.5
Forehead	4	4.4
Ear	4	4.4
Arm	3	3.3
Chin	1	1.1
Underarm	1	1.1
Breast	1	1.1
Other	11	12.1
ND	6	6.6
Number of lesions		
1	67	73.6
2	7	7.7
3	7	7.7
5	3	3.3
11	1	1.1
ND	6	6.6
Approximate diameter of lesion (mm)		
4	1	1.1
5	20	21.9
8	5	5.5
9	6	6.6
10	19	20.9
15	12	13.2
20	8	8.8
25	2	2.2
30	4	4.4
40	2	2.2
50	2	2.2
75	1	1.1
100	1	1.1
ND	8	8.8
Recurrence		
Yes	14	15.4
No	44	48.3
ND	33	36.3
Skin malignancy		
Yes	3	3.3
No	47	51.6
ND	41	45.1
Sunscreen use		
Yes	4	4.4
No	59	64.8
ND	28	30.8
Living area		
Rural	37	40.7
Urban	42	46.1
ND	12	13.2
Total	91	100

ND: Not determined

Table 2. **Genotype and allele frequencies in patients and controls**

	Arg/Arg	*Pro/Pro*	*Arg/Pro*	*Arg*	*Pro*
Patients	34	10	47	115	67
Controls	162	86	217	541	389
		P=0.22		P=0.24	

Table 3. **Genotype frequencies in patients and age-matched controls**

	Arg/Arg	*Pro/Pro*	*Arg/Pro*
Patients	34	10	47
Controls	25	9	29
		P=0.73	

and in the *Arg* allele frequency among sun-exposed patients compared to the non-exposed patients was also obvious. The most important risk factor of BCC is solar UV radiation as for other skin malignancies.[22] Ethnicity, color of unexposed skin, latitude, pattern and amount of sun exposure, other radiations, Arsenic exposure, Xeroderma pigmentosum, Bazex syndrome, and Gorlin's syndrome are other minor risk factors in the common population.[23] As can be seen among the sunlight non-exposed patients of this study, the effect of sunlight and *Arg* allele have simultaneously decreased. Therefore, the significance of this allele is highlighted when interacting environmental factors are taken into consideration.

Environmental UV exposure is an early event in skin carcinogenesis, which can induce harmful mutations in the p53 gene.[24] This is in part related to the abundance of high UV absorbent conjugated bonds in the structure of DNA.[25] Among BCC patients, UVB signature point mutations occur frequently in the p53 gene.[26] A dysfunctional p53 protein will lose its tumor suppressive and cell cycle arrest-inducing effects. In addition, the mutated protein will be non-functional or less functional in apoptosis induction. An inherent *Arg* allele which acquires mutations after epigenetic interference is more likely to inhibit downstream apoptotic pathways.[7] Therefore, the risk of p53 codon 72 polymorphism comes into existence when epigenetic factors such as UV radiation and HPV virus infection are taken into consideration.[11,13] This effect might be more important when recessive less functional p53 mutants are present in the tumor cells.[8] As it has been reported, presence of *Arg72* in the mutant allele or preferential retention of *Arg72* allele in the tumoral tissue (*Arg* bias) provides a selective growth advantage to tumor cells during the stage of tumorigenesis.[8]

BCC of the skin is the most common cancer in humans worldwide, and is mainly considered a disease of fair-skinned people.[27] The estimated prevalence of skin cancers in Fars province has been reported to be 9.7 in 100,000, which comprises 18.3% of the total registered cancers in this area.[28] BCC accounts for 68% of all registered skin cancers in this area,[28] in which the most important type of high and long-term solar UV exposure is occupational sun exposure. p53 codon 72 polymorphism shows a latitude-related distribution; i.e., higher prevalence of *Pro* allele in high latitude areas. It is usually concluded that dark-skinned populations have a higher frequency of the *Pro* allele. In our study the frequencies of the *Pro* allele (41.8%) and *Pro*/*Pro* genotype (18.5%) in normal individuals were lower than that of *Arg* allele and *Arg*/*Arg* genotype. These frequencies are very close to the reported frequencies from Greece,[29] and are consistent with the north-south pattern of *Pro* allele distribution.[30] However, this is most likely to be associated with the genes and not with skin color itself, as none of our patients had a fair-skinned phenotype, although this phenotype is not rare among Iranians. These results are consistent with the study of Armstrong et al. in which a weak evidence of increase in risk of BCC with increasing fairness of the skin was reported.[30]

In conclusion, our results suggest that *Arg72* allele might affect the risk of occupational UV-induced BCC in Iranian population.

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