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p53 codon 72 polymorphism in bladder cancer – no evidence of association with increased risk or invasiveness

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Abstract We studied the effect of the p53 gene Arg72Pro polymorphism on bladder cancer susceptibility in a case control study of 121 bladder cancer patients and 114 age-sex matched controls to determine whether this polymorphism is a biomarker for the risk and how aggressive the disease is. Genomic DNA was obtained from venous blood samples for genotype determination by PCR and restriction digestion. The genotype frequencies in the patient group were Arg/Arg: 0.3553, Arg/Pro: 0.4711, Pro/Pro: 0.1736, and in the control group Arg/Arg: 0.3684, Arg/Pro: 0.4825, Pro/Pro: 0.1491. The distribution of genotypes between the two groups was not statistically different ($\chi^2 = 0.260$, *df*: 2, $P = 0.878$). The patient group was subdivided into two groups as superficial bladder cancer ($n = 88$) and invasive bladder cancer ($n = 33$), according to the presence of muscle invasion. The distribution of genotypes in the superficial group was Arg/Arg: 0.3409, Arg/Pro: 0.5114, Pro/Pro: 0.1477 and in the invasive group Arg/Arg: 0.3940, Arg/Pro: 0.3636, Pro/Pro: 0.2424. No association was observed with the invasiveness of the tumor ($\chi^2 = 2.542$, *df*: 2, $P = 0.281$). Stratification of the data by tobacco exposure did not result in a significant difference in genotype frequencies. These data do not support an association between the p53 Arg72Pro polymorphism and bladder cancer.

Keywords Bladder cancer · *TP53* polymorphism · Genetic susceptibility

Introduction

The Arg72Pro polymorphism of the p53 gene has been known since 1987 [10]; however, its significance as a genetic susceptibility factor for cancer is still a matter of controversy. The association studies between this polymorphism and cervix [11, 13], lung [3, 9, 14], breast [12, 15], and esophagus cancers [5, 8] reveal disconcordant results. With respect to bladder cancer, no association was observed in two studies [1, 9]. However, a Taiwanese group recently reported that Pro/Pro genotype is predominant in invasive tumors [1]. This observation is quite interesting, since it is known that p53 mutations are associated with high-grade, and high-stage bladder tumors [2].

In this study, we determined the genotype frequency of the p53 gene Arg72Pro polymorphism in bladder cancer patients and age-sex matched controls. Our aim was to understand whether this polymorphism is a biomarker associated with susceptibility to bladder cancer and to study its relationship to tumor invasiveness in the Turkish population.

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Materials and methods

Peripheral blood samples were collected from 121 bladder cancer patients and 114 age-sex matched controls. Information about sex, age, lifetime tobacco exposure of the patient, and histopathology of the tumor was obtained from medical records (transitional cell carcinoma, mean age: 60.15, SD:11.10, range: 25–87, percentage of smokers: 72.0, male-female ratio: 5:1). The age-sex matched control group was comprised of non-cancer patients from the Atatürk Chest Disease Research Hospital (mean age: 59.33, SD: 13.58, range: 23–79, percentage of smokers: 63.8, male-female ratio: 5:1). Informed consent was obtained from all subjects. Genomic DNA was isolated from 700 µl blood by standard phenol-chloroform extraction. P53 Arg72Pro polymorphism was determined by polymerase chain reaction (PCR) and restriction digestion. Briefly,

amplification was carried out using primers P53+ (5′-TCCCCCCTTgCCgTCCCAA-3′) and P53- (5′-CgTgCAAgTCACAgACTT-3′) [13]; then the 279 bp amplified product was digested with *Bst*U1 enzyme [3] and electrophoresis carried out in 2% agarose gels. The presence of the restriction site resulted in two fragments of 160 bp, and 119 bp which was indicative of the Arg allele (Fig 1). Based on the pathology report of the surgical specimens the cancer group was subdivided into two groups as superficial (Ta and T1), and invasive (≥T2a) by AJCC staging. The Chi-square test was used for statistical analyses.

Results

The distribution of the p53 Arg72Pro genotypes in the patient and the control groups is shown in Table 1. The genotype frequencies in the patient group were Arg/Arg: 0.3553, Arg/Pro: 0.4711, Pro/Pro: 0.1736, and in the control group Arg/Arg: 0.3684, Arg/Pro: 0.4825, Pro/Pro: 0.1491. A significant difference between the two groups was not found ($\chi^2 = 0.260$, *df*: 2, $P = 0.878$). The patient group was subdivided into two groups as superficial bladder cancer ($n = 88$) and invasive bladder cancer ($n = 33$) according to the absence or presence of muscle invasion, respectively. The distribution of genotypes in the superficial group was Arg/Arg: 0.3409, Arg/Pro: 0.5114, Pro/Pro: 0.1477 and in the invasive group Arg/Arg: 0.3940, Arg/Pro: 0.3636, Pro/Pro: 0.2424. No association was observed with the invasiveness of the tumor ($\chi^2 = 2.542$, *df*: 2, $P = 0.281$) (Table 2). We evaluated the effect of tobacco exposure on the genotypic distribution of p53 alleles in the 111 patients for whom information on smoking status was available. Among smokers ($n = 80$), distribution of the genotypes was Arg/Arg: 0.3125, Arg/Pro: 0.4750, Pro/Pro: 0.2125 and among non-smokers ($n = 31$), Arg/Arg: 0.3871, Arg/Pro: 0.4839, Pro/Pro: 0.1290. Based on these results an association was not observed ($\chi^2 = 1.199$, *df*: 2, $P = 0.549$).

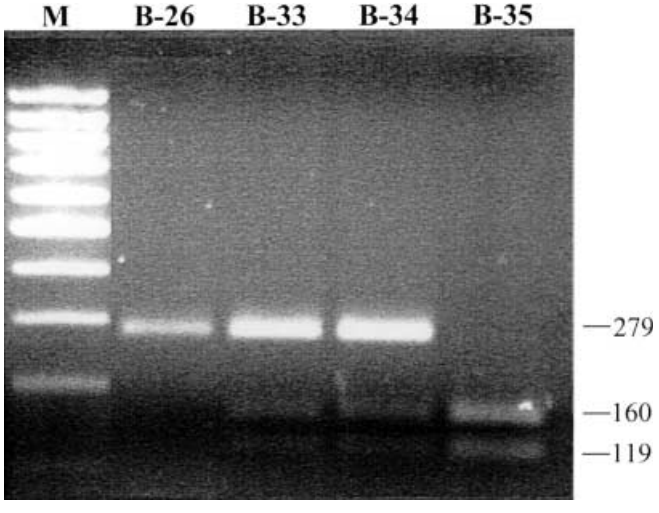

Fig. 1 P53 Arg72Pro genotyping experiments. Polymerase chain reaction products were digested with *Bst*U1 and separated on 2% agarose gel. Arg encoding allele produces two fragments of 160 bp and 119 bp, and Pro encoding allele produces an uncut fragment of 279 bp. B-26 is an example of Pro/Pro homozygote, B-33, and B-34 are Arg/Pro heterozygotes, and B-35 is an Arg/Arg homozygote. M is the GeneRuler 100 bp DNA Ladder (MBI Fermentas)

Table 1 Distribution of P53 Arg72Pro genotypes in the age and sex-matched controls and bladder cancer patients: $\chi^2 = 0.260$, $df = 2$, $P = 0.878$

	Arg/Arg (%)	Arg/Pro (%)	Pro/Pro (%)	Total (%)
Case	43 (35.53)	57 (47.11)	21 (17.36)	121 (100)
Control	42 (36.84)	55 (48.25)	17 (14.91)	114 (100)

Table 2 Distribution of P53 Arg72Pro genotypes in superficial and invasive bladder tumors: $\chi^2 = 2.542$, $df = 2$, $P = 0.281$

	Arg/Arg (%)	Arg/Pro (%)	Pro/Pro (%)	Total (%)
Superficial	30 (34.09)	45 (51.14)	13 (14.77)	88 (100)
Invasive	13 (39.40)	12 (36.36)	8 (24.24)	33 (100)

Discussion

An association between the p53 Arg72Pro polymorphism and cancer risk has been reported for breast [12], cervix, esophagus [8], and lung [3] cancers. Although the biological basis of these observations is not very well established, working models backed up by experimental data suggest that the Arg form is more prone to degradation after infection with HPV, making the Arg allele bearing individuals more susceptible to cervix cancer [13]. The Pro form hinders the protective activity of the p53 gene after exposure to cigarette smoke in adenocarcinoma of the lung [3]. Smoking is a definite [7] and HPV infection is a probable risk factor for bladder cancer [4].

We investigated the p53 Arg72Pro polymorphism in bladder cancer patients since cigarette smoke is an important etiologic factor for bladder cancer in Turkey [6]. Furthermore, we studied the relationship between this polymorphism and invasiveness of the tumor. When the genotype frequencies of the patient and the control groups were compared, none of the p53 Arg72Pro genotypes were found to be associated with a significantly increased bladder cancer risk (Table 1). Stratification of the data by tobacco exposure did not result in a significant difference in genotype frequencies. This lack of association parallels the results of two previous bladder cancer studies [1, 9]. With respect to the invasiveness of the tumor, we did not find a significant association with any one of the p53 genotypes. The only other study which addresses the association between tumor invasiveness and p53 genotype, which was carried out in a Taiwanese population, is not in agreement with our findings [1]. The risk of cancer imposed by a particular polymorphism in a specific population is a complex phenomenon. It is dependent on both environmental factors, such as the exposure to specific carcinogens, and the presence of other allelic modifiers modulating the

cancer risk in that population. This may account for the differences between the Taiwanese and the Turkish populations.

In conclusion, we did not detect an excess of any of the p53 Arg72Pro genotypes either in the bladder cancer versus control or in the superficial bladder cancer versus invasive bladder cancer groups. Therefore, p53 Arg72-Pro polymorphism does not appear to be a bladder cancer-related biomarker for the Turkish population.

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References

1. Chen WC, Tsai FJ, Wu JY, Wu HC, Lu HF, Li CW (2000) Distributions of p53 codon 72 polymorphism in bladder cancer-proline form is prominent in invasive tumor. Urol Res 28: 293
2. Cordon-Cardo C, Cote RJ, Sauter G (2000) Genetic and molecular markers of urothelial premalignancy and malignancy. Scand J Urol Nephrol Suppl 205: 82
3. Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, Wiencke JK, Christiani DC (2000) The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 9: 1037
4. Griffiths TRL, Mellon JK (2000) Human papillomavirus and urological tumours: II. Role in bladder, prostate, renal and testicular cancer. BJU Int 85: 211
5. Guimares DP, Lu SH, Snijders P, Wilmotte R, Herrero R, Lenoir G, Montesano R, Meijer CJLM, Walboomers J, Hainaut P (2001) Absence of association between HPV DNA, TP53 codon 72 polymorphism, and risk of oesophageal cancer in a high risk area of China. Cancer Lett 162: 231
6. Gumus B, Aras O, Atesci YZ, Muezzinoglu T (1999) Aetiological factors of bladder cancer in the Aegean region of Turkey between the years 1985–1996. Int Urol Nephrol 31: 197
7. Johansson SL, Cohen SM (1997) Epidemiology and etiology of bladder cancer. Semin Surg Oncol 13:291
8. Kawaguchi H, Ohno S, Araki K, Miyazaki M, Saeki H, Watanabe M, Tanaka S, Sugimachi K (2000) p53 polymorphism in human papillomavirus-associated esophageal cancer. Cancer Res 60: 2753
9. Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi SI (1993) Germ line polymorphisms of p53 and CYP1A1 genes involved in human lung cancer. Carcinogenesis 14: 1085
10. Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J, Crawford LV (1987) Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol 7: 961
11. Rosenthal AN, Ryan A, Al-Jehani RM, Storey A, Harwood CA, Jacobs IJ (1998) p53 codon 72 polymorphism and risk of cervical cancer in UK. Lancet 352: 871
12. Sjalander A, Birgander R, Hallmans G, Cajander S, Lenner P, Athlin L, Beckman G, Beckman L (1996) p53 polymorphisms and haplotypes in breast cancer. Carcinogenesis 17: 1313
13. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L (1998) Role of a p53 polymorphism in the development of human papillomavirus- associated cancer. Nature 393: 229
14. To-Figueras J, Gene M, Gomez-Catalan J, Galan C, Firvida J, Fuentes M, Rodamilans M, Huguet E, Estape J, Corbella J (1996) Glutathione-S-Transferase M1 and codon 72 p53 polymorphisms in a northwestern Mediterranean population and their relation to lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 5: 337
15. Weston A, Pan CF, Ksieski HB, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, Brower ST, Senie RT, Wolff MS (1997) p53 haplotype determination in breast cancer. Cancer Epidemiol Biomarkers Prev 6: 105