RESEARCH ARTICLE

TP53 Gene *Pro72Arg* (*rs1042522*) Single Nucleotide Polymorphism as Not a Risk Factor for Colorectal Cancer in the Iranian Azari Population

Milad Asadi[1], Dariush Shanehbandi[1], Armin Zarintan[1], Negar Pedram[1], Behzad Baradaran[1], Venus Zafari[2], Masoud Shirmohamadi[3], Shahriyar Hashemzadeh[2]*

Abstract

Background: The p53 protein participates critically in several cellular functions such as cell growth and DNA repair. Polymorphisms in the *TP53* locus have repeatedly been implicated in the pathogenesis of cancers all over the world. Over 200 single nucleotide polymorphisms (SNPs) have been characterized, but one well-known example at at codon 72, *Pro72Arg* (*rs1042522*), has the displayed inconsistent results with regard to cancer risk. Herein, we aimed to evaluate whether *Pro72Arg* (*rs1042522*) single nucleotide polymorphism (SNP) in *TP53* gene might be associated with risk of colorectal cancer in the Iranian Azari population. **Methods:** Blood samples were taken from 100 healthy controls and 100 colorectal cancer patients with Iranian-Azeri ethnicity. Genotyping was performed with Tetra-ARMS PCR. **Results:** The alleles of the *TP53* gene *Pro72Arg* SNP did not significantly differ in prevalence between patients and controls (P>0.05). Additionally, genotypes of *Pro72Arg* SNP were not significantly associated with colorectal cancer risk in the studied population. **Conclusions:** *Pro72Arg* SNP of *TP53* gene may not be involved in the disease pathogenesis in Iranian Azari patients with colorectal cancer.

Keywords: *TP53- Pro72Arg* polymorphism- colorectal cancer

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Introduction

Colorectal cancer (CRC) is one of the most prevalent and lethal disorders worldwide, and significant raising occurrence of CRC has been indicated throughout the world in the past two decades (Pisani et al., 1999; Li et al., 2005). Like other cancers, genetic variations have shown to have crucial role in CRC development (Calvert and Frucht, 2002; Rupnarain et al., 2004). Epidemiological studies have established that mutations in the tumor-suppressor gene *TP53* occur in approximately half of all colorectal cancers, eventuates in promoting the malignant transformation of adenomas (Vogelstein et al., 1988). The p53 protein is a key tumor suppressor that has been widely studied in colorectal cancer, but no predictive or prognostic role in the clinical practice has been proposed to date (Walther et al., 2009).

P53 is an important apoptotic protein that is involved in most of critical cellular processes (Gomez-Lazaro et al., 2004). In G1 phase of cell cycle, activation of p53 in response to DNA damage leads to DNA repair or starts cell death machinery, resulting in apoptosis (Robles and Harris, 2001). Given its function, p53 gene mutations and single nucleotide polymorphisms (SNP) are important in all types of human cancers (Børresen-Dale, 2003; Khan et al., 2005). Additionally, aberrant expression and mutations of p53 have been reported frequently in CRC (Ahnen et al., 1998; Soong et al., 2000; Elsaleh et al., 2001; Liang et al., 2002). Besides, about 14 SNPs have been identified in wild type *TP53* gene, which could change the function of p53 protein (Soussi and Béroud, 2001; Olivier et al., 2002).

One of most common SNPs of the TP53 gene is *Pro72Arg* (*rs1042522*), located in proline rich domain of p53, that is important in normal p53 function (Dumont et al., 2003a). Studies show that the arginine (Arg) variant is able to induce apoptosis faster and more efficient than proline (Pro), while the Pro variant is better for inducing cycle arrest (Thomas et al., 1999). It has been reported that *Pro72Arg* SNP in *TP53* gene can increase risk of cancers. However, there is some conflicts about the role of this SNP in colorectal cancer. In some populations, CRC has been observed to have association with *Pro72Arg* SNP in *TP53* gene, while this association has not been established in some other populations (Zhu et al., 2007; Dastjerdi et al., 2008).

Therefore, for the first time, to our best knowledge, we aimed to investigate the effect of *TP53* gene *Pro72Arg* SNP in risk of CRC in Iranian Azari population in a

[1]Immunology Research Center, [2]Tuberculosis and Lung Diseases Research Center, [3]Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. For Correspondence: shahriar_90@yahoo.com

case-control study.

Material and Methods

Sampling

This study has been designed and performed according to the institutional bioethical guidelines by the Ethical Committee of Tabriz University of Medical Sciences. Blood samples of 100 patients with histologically confirmed for colorectal cancer were taken from patients referred to Imam Reza Hospital of Tabriz University of Medical Sciences. Additionally, 100 matched healthy individuals with no history of any cancers as control group were recruited. Written informed consent was obtained from all the patients. Clinical and pathological characteristics of patients are summarized in Table 1. About 5 ml of venous blood was taken from each patient and control through EDTA-anticoagulated venoject tubes. Afterwards, genomic DNA was extracted from peripheral blood using salting-out approach as described previously (Miller et al., 1988)

Tetra-ARMS PCR

Tetra-amplification refractory mutation system–polymerase chain reaction (T-ARMS-PCR), which is a rapid and simple technique for studying of SNPs (Ye et al., 2001), was conducted for allele and genotype detection. The primers used for the position was designed using oligo7 and were blasted in NCBI website: http://www.ncbi.nlm.nih.gov/tools/primer-blast/ (Table 2). Two allele-specific (inner) primers have been designed in opposed directions and, in combination with the common primers, can simultaneously amplify both the mutant allele and wild type alleles in a single-tube PCR. PCR-amplified DNA samples were electrophoresed in agarose gel (2%). After that, 3 amplified samples were examined by DNA sequencing in order to validate the results determined by T-ARMS-PCR.

Genotyping was performed by T-ARMS PCR using the Taq-PCR Master Mix (Cat. No: 5200350-0050, Lot. No: 13C18, Amplicon, Denmark) and thermocycler PCR System (Applied Biosystems, Foster City, CA, USA). Each reaction mixture contained a total volume of 20µl (master mix 10µl, forward and reverse inner primer 1µl each, forward and reverse outer primer 0.2µl each, and H2O 7.6µl). The thermocycler conditions were: 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds 56°C for 30 seconds, and 72°C 40 seconds and final extension of 72°C for 10 minutes.

Statistical analysis

Statistical analysis was performed using the SPSS software version 21 (SPSS, Chicago, IL, USA). Genotype and allelic distribution between case and control groups were implemented by Chi-Square test. Pearson's χ^2-tests were applied to test for significance differences of both genotype and allele frequencies between two groups. All probability values were calculated from two-tailed test. Moreover, the odds ratio (OR) and 95% confidence interval (CI) were calculated. The genotype distributions of chosen SNPs were tested for deviation from Hardy-Weinberg equilibrium in case and control. The Bonferroni correction approach was exerted in multiple statistical testing (i.e. P-value was set to <0.01) to recognize a statistically significant results, adjusting the multiple comparison, and controlling the false discovery rate (FDR) (Benjamini and Hochberg, 1995). Additionally, the SHEsis online software was exerted for analyzing the haplotype and genotype, and also Hardy-Weinberg equilibrium (Yong and Lin, 2005).

Results

The demographic and laboratory data of the patients are summarized in Table 1. The distribution of the alleles and genotypes for all variations in case and healthy control groups did not show any significant deviation from the Hardy–Weinberg equilibrium (Table 3).

The C allele of the *TP3* gene *Pro72Arg* SNP was found in 36% of patients and 36.5% of controls. This allele did not have significantly different distribution between the studied groups (OR=0.97, 95% CI: 0.73-1.30; P=0.98). Moreover, there was not a significant (OR=1.02, 95% CI: 0.76-1.36; P=0.98) difference of G allele distribution between patients (64%) and healthy controls (63.5%). The CC (Pro/Pro) genotype was observed in 15% of CRC patients and 12% of the healthy subjects, and had an insignificantly much occurrence in patients compared with controls (OR=1.23, 95% CI: 0.69-2.17; P=0.46). On the other side, both GC (Arg/Pro) and GG (Arg/Arg) genotypes of *Pro72Arg* SNP in *TP3* gene were not represented significantly different between two study

Table 1. Demographic and Clinicopathological Characteristics of The Patients with Colorectal Cancer From Iranian Azari Population

Characteristics (n=100)	Value
Age	47 ± 2.8 (years)
Sex	59 males, 41 females
Histology	36 (Good), 45 (Moderate), 19 (Poor)
Lymph node metastasis	74 (Yes), 26 (No)
Venous invasion	64 (Yes), 36 (No)
AJCC stage classification	7.2 ± 1.8
Liver metastasis	43 (Yes), 57 (No)

Table 2. Primers Used in T-ARMS PCR, the Amplicon S size, and Melting Temperature (°C) of Each Primer

SNP	Primer Type	Sequence	Amplicon Size	Tm (°C)
TP53 G/C	Forward inner primer (G allele)	5′ GCTGCTGGTGCAGGGGCCAGGG 3′	200	60
	Reverse inner primer (C allele)	5′CCAGAATGCCAGAGGCTGCTCCGCG 3′	247	60
	Forward outer primer	5′TGCAGGGGGATACGGCCAGGCATTGAAGTC3′	493	60
	Reverse outer primer	5′TGGGGGGCTGAGGACCTGGTCCTCT3′		60

Table 3. Allele and Genotype Distribution of *TP53* Pro72Arg SNP in Patients with Colorectal Cancer and Healthy Controls

dbSNP	Alleles/ Genotypes	Case (n=100) N (%)	Control (n=100) N (%)	*P* Value	Adj. *P*	OR (95% CI)
TP53 Gene Pro72Arg (rs1042522)	C	71 (36%)	73 (36.5%)	0.98	0.98	0.97 (0.73-1.30)
	G	129 (64%)	127 (63.5%)	0.98	0.98	1.02 (0.76-1.36)
	CC (Pro/Pro)	15 (15%)	12 (12%)	0.46	0.76	1.23 (0.69-2.17)
	GC (Arg/Pro)	41 (41%)	48 (48%)	0.52	0.24	0.78 (0.52-1.16)
	GG (Arg/Arg)	44 (43%)	40 (40%)	0.87	0.47	1.15 (0.77-1.71)
HWE		0.56	0.66			

HWE, Hardy–Weinberg Equilibrium

groups (OR=0.78, 95% CI: 0.52-1.16; P=0.52 and OR=1.15, 95% CI: 0.77-1.71; P=0.87, respectively).

None of the clinical outcomes of the CRC patients had associations with the genotypes of the *Pro72Arg* SNP in TP3 gene.

Discussion

In this study, our purpose was to evaluate the role of the *TP53* gene *Pro72Arg* SNP in Iranian Azeri population with CRC. This genetic association study revealed that *TP53* gene *Pro72Arg* SNP did not impress the susceptibility of CRC in Iranian Azari population. This polymorphism may not be a risk factor for CRC in Iranian Azeri population.

In *TP53* gene, *Pro72Arg* (*rs1042522*) is a G/C variation at the second position of codon 72 in exon 4, which results in Arg72 or Pro72 protein change. Remarkable ethnic variations have been identified in this SNP. It has been reported that the Arg72 variant was the more prevalent in Caucasians, while the Pro72 variant was more frequent in Chinese and African-Americans (Beckman et al., 1994; Langerod et al., 2006). the Residue 72 is found within a proline-rich region and has been suggested to impress the structure of the SH3-binding domain in p53 protein. A number of functional differences have been identified between the Arg72 and Pro72 variants. Studies show that Arg72 variant is more effective in stimulating apoptosis, a function that associates with Mouse double minute 2 homolog (MDM2) protein, which mediates the nuclear export and mitochondrial localization of p53 (Dumont et al., 2003b). On the other side, the Pro72 variant has been observed to be more efficient in inducing cell-cycle arrest (Pim and Banks, 2004) as well as DNA repair (Siddique and Sabapathy, 2006). Other different functions between these two variants are the capacity to bind to the transcriptional machinery components, which may function as transcription activation or repression of the transformation of cells in primary phases of development (Thomas et al., 1999).

Development of CRC have been associated with nucleotide alternations in DNA such as mutation in *TP53* gene (Sotamaa et al., 2005; Whibley et al., 2009). One of the important gene variations in *TP53* gene is *Pro72Arg*, which results in replacement of proline in codon 72 instead of arginine (from CGC to CCC in exon 4, rs1042522). This SNP has been reported as a risk factor for several cancers (Langerød et al., 2002; Shen et al., 2002; Soulitzis et al., 2002; Mahasneh and Abdel-Hafiz, 2004; Noma et al., 2004; Kalemi et al., 2005).

Studies have already evaluated the effect of *Pro72Arg* SNP in proneness to CRC in various ethnic groups (Pérez et al., 2006; Zhu et al., 2007; Dastjerdi et al., 2008). In this study, we examined the association of *Pro72Arg* SNP in 100 colorectal adenocarcinoma cases and 100 healthy individuals in Iranian Azari population and found that this variant did not change significantly the CRC risk. Moreover, no significant association was observed between three genotypes of this SNP and clinical features of the CRC patients. Our results was in accord with some previous studies, while in discord with some others (Pérez et al., 2006; Zhu et al., 2007; Dastjerdi et al., 2008).

The observed contradiction in results might magnified ethic roles in cancer especially in CRC. The employed technique for genotyping may be another reason for discrepancy in the results. Epidemiologic studies have indicated that distribution of *Pro72Arg* SNP varies in different populations. The mutant variant (Arg) of this SNP has frequency been seen in Asian and African populations in comparison to the wild type (Pro). However, in Europeans and United States, wild type has higher frequency (Beckman et al., 1994; Lu et al., 2004; Zhu et al., 2005). Our population did not have the same frequency pattern as seen in Asians.

There are a number of limitation and caveats in this investigation that need to be addressed. We tried to genotype maximum possible patients and healthy individuals. However, the more the sample size, the higher the power of the observations and obtained results. Moreover, the method we applied here was ARMS-PCR; although if the Real-time allelic discrimination method was used, the results would be more reliable.

In conclusion, all in all, this was the first study designed to assess the role of the *TP53* gene *Pro72Arg* SNP in a replicated case-control of Iranian Azeri population with colorectal cancer. The current study indicated no association between the mentioned gene variant and CRC in Iranian Azari population. This polymorphism may not be a risk factor for CRC in Iranian Azeri population. However, further studies are still needed to validate these data in different ethnic populations of Iran.

Disclosure of conflict of interests

None.

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