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

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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 codon 72, Pro72Arg (rs1042522), has 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-Azari 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, which could change the function of p53 protein (Soussi and Béroud, 2001; Olivier et al., 2002).

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

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Material and Methods

Sampling
This study has been designed and performed according to the institutional ethical 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 10 minutes. 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 χ²-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                  | 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 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’ GCTGCTGGTGCAAGGCTCCAGGG 3’ | 200 | 60 |
|       | Reverse inner primer (C allele) | 5’ CCCAGAATGCCAGAGGGCTCCAGGG 3’ | 247 | 60 |
|       | Forward outer primer           | 5’ TGCAGGAGGGATACGGCCAGGCATTTGAGTGC 3’ | 493 | 60 |
|       | Reverse outer primer           | 5’ TGGGGGGCTGAGGACCTGGTCTCTC 3’ | 493 | 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) | Control (n=100) | 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, Hardy–Weinberg Equilibrium

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 Abdell-Hafiz, 2004; Noma et al., 2004; Kalemì 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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References

Ahnen DJ, Feigl P, Quan G, et al (1998). Ki-ras mutation and p53 overexpression predict the clinical behavior of colorectal cancer: a southwest oncology group study. Cancer Res, 58, 1149-58.

Beckman G, Birgander R, Sjilander A, et al (1994). Is p53 polymorphism maintained by natural selection?. Hum Hered, 44, 266-70.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol, 57, 289-300.

Barresen-Dale AL (2003). TP53 and breast cancer. Hum Mutat, 21, 292-300.

Calvert PM, Frucht H (2002). The genetics of colorectal cancer. Ann Intern Med, 137, 603-12.

Dastjerdi MN, Salehi M, Mohajeri MR, et al (2008). Evidence for an association of TP53 codon 72 polymorphism with sporadic colorectal cancer risk in Isfahan. J Res Med Sci, 13, 317-23.

Dumont P, Leu J-J, Della Pietra AC, et al (2003a). The codon 72 polymorphism of p53 may affect the function of the protein. Nat Genet, 33, 357-65.

Dumont P, Leu J-J, Della Pietra AC, et al (2003b). The codon 72 polymorphism and breast cancer. Hum Mutat, 23, 57-65.

Dumont P, Leu J-J, Dell’a Pietra AC, et al (2003a). The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet, 33, 357-65.

Dumont P, Leu J-J, Della Pietra AC, et al (2003b). The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet, 33, 357.

Elsaleh H, Powell B, McCaul K, et al (2001). P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin Cancer Res, 7, 1343-9.

Gemmigni F, Moreno V, Landi S, et al (2004). A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. Oncogene, 23, 1954-6.

Gomez-Lazaro M, Fernandez-Gomez F, Jordan J (2004). p53: twenty five years understanding the mechanism of genome protection. J Physiol Biochem, 60, 287-307.

Robles AI, Harris CC (2001). p53-mediated apoptosis and genomic instability diseases. Acta Oncol, 40, 696-701.

Kalemli TG, Lambropoulos AF, Guzorguev M, et al (2005). The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. Cancer Lett, 222, 57-65.

Khan SA, Thomas HC, Toledano MB, et al (2005). p53 Mutations in human cholangiocarcinoma: a review. Liver Int, 25, 704-16.

Langerod A, Bokholm IR, Bregård A, et al (2002). The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epidemiol Biomarkers Prev, 11, 1684-8.

Langerod A, Burdette L, Yeager M, et al (2006). Pattern of genetic variation in the tp53 locus indicates linkage disequilibrium extends across the flanking genes, ATP1B2 and WDR79. Hum Mutat, 26, 2157-65.

Li M, Yuan Y-H, Han Y, et al (2005). Expression profile of cancer-testis genes in 121 human colorectal cancer tissue and adjacent normal tissue. Clin Cancer Res, 11, 1809-14.

Liang JT, Huang KC, Cheng YM, et al (2002). P53 overexpression predicts poor chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV colorectal cancers after palliative bowel resection. Int J Cancer, 97, 451-7.

Lu X-M, Zhang Y-M, Lin R-Y, et al (2004). p53 polymorphism in human papillomavirus-associated Kazakh’s esophageal cancer in Xinjiang, China. World J Gastroenterol, 10, 2775-8.

Mahasneh AA, Abdel-Hafiz SS (2004). Polymorphism of p53 gene in Jordanian population and possible associations with breast cancer and lung adenocarcinoma. Saudi Med J, 25, 1568-73.

Miller S, Dykes D, Polesky H (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res, 16, 1215.

Noma C, Miyoshi Y, Taguchi T, et al (2004). Association of p53 genetic polymorphism (Arg72Pro) with estrogen receptor positive breast cancer risk in Japanese women. Cancer Lett, 210, 197-203.

Olivier M, Eeles R, Hollstein M, et al (2002). The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat, 19, 607-14.

Pérez LO, Abba MC, Dulout FN, Golijov CD (2006). Evaluation of p53 codon 72 polymorphism in adenocarcinomas of the colon and rectum in La Plata, Argentina. World J Gastroenterol, 12, 1426-9.

Pim D, Banks L (2004). p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer, 105, 196-23.

Pisani P, Parkin DM, Bray F, et al (1999). Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer, 83, 18-29.

Rupnarain C, Dlaminzi Z, Naicker S, et al (2004). Colon cancer: genomics and apoptotic events. Biol Chem, 385, 449-64.

Shen H, Zheng Y, Sturgis EM, et al (2002). P53 codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Lett, 183, 123-30.

Siddique M, Sabapathy K (2006). Trp53-dependent DNA-repair is affected by the codon 72 polymorphism. Oncogene, 25, 3489.

Soong R, Powell B, Elsahel H, et al (2000). Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma: influence of tumour site, stage, adjuvant chemotherapy and type of mutation. Eur J Cancer, 36, 2053-60.

Sotamiaa K, Liyanarachchi S, Mecklin JP, et al (2005). p53 codon 72 and MDM2 SNP309 polymorphisms and age of colorectal cancer onset in Lynch syndrome. Clin Cancer Res, 11, 6840-4.

Soulitzis N, Sourvinos G, Dokianakis DN, et al (2002). p53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett, 179, 175-83.

Soussy T, Béroud C (2001). Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer, 1, 233-9.

Thomas M, Kalita A, Labrecque S, et al (1999). Two polymorphic variants of wild-type p53 differ biochemically and biologically. MMol Cell Biol, 19, 1092-100.

Vogelstein B, Fearon ER, Hamilton SR, et al (1988). Genetic alterations during colorectal-tumor development. N Engl J Med, 319, 525-32.

Walther A, Johnstone E, Swanton C, et al (2009). Genetic and epigenetic signatures of colorectal cancer progression: Patterns and consequences. Nat Rev Cancer, 9, 301-13.

Whibley C, Pharoah PD, Hollstein M (2009). p53 polymorphisms: cancer implications. Nat Rev Cancer, 9, 95.
genotyping single nucleotide polymorphisms. *Nucleic Acids Res*, 29, e88-e.

Yong Y, Lin H (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*, 15, 97-8.

Zhu Z-Z, Cong W-M, Liu S-F, et al (2005). Homozygosity for Pro of p53 Arg72Pro as a potential risk factor for hepatocellular carcinoma in Chinese population. *World J Gastroenterol*, 11, 289-92.

Zhu Z-Z, Wang A-Z, Jia H-R, et al (2007). Association of the *TP53* codon 72 polymorphism with colorectal cancer in a Chinese population. *Jpn J Clin Oncol*, 37, 385-90.