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Original Article

Evidence for an association of TP53 codon 72 polymorphism with sporadic colorectal cancer risk in Isfahan

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Abstract

BACKGROUND: A common polymorphism at codon 72 of TP53 gene has been associated with increased risk for many human cancers. We studied this TP53 polymorphism in colorectal adenocarcinomas in small population selected from Isfahan city.

METHODS: Samples: We undertook a case-control study on 180 controls and 180 paraffin block specimens of sporadic colorectal adenocarcinomas. PCR amplification of TP53 codon 72 polymorphism: TP53 codon 72 genotypes were detected by PCR using specific primer pairs for amplifying the Proline or the Arginine alleles.

STATISTICAL ANALYSES: The $\chi 2$-test was used to assess the significance of any difference in the prevalence of TP53 codon 72 polymorphism between colorectal cancer patients and controls.

RESULTS: In control samples, the genotype distribution for TP53 polymorphism showed 28.3%, 48.9% and 22.8% for the Arginine/Arginine, Arginine/Proline and Proline/Proline genotypes, respectively. In the cancer group 40% of the cases were Arginine/Arginine, 42.2% were Arginine/Proline and 17.8% were Proline/Proline. A significant difference between cases and controls was found for the Arginine/Arginine genotype compared with (grouped) Arginine/Proline and Proline/Proline genotypes (Odds Ratio = 1.686 (1.085-2.620), P = 0.02).

CONCLUSIONS: TP53 codon 72 polymorphism may be a genetic predisposing factor for colorectal adenocarcinomas in Isfahan city.

KEYWORDS: Colorectal adenocarcinoma, TP53, Arginine, Proline, Polymorphism.

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TP53 is the most important tumor suppressor gene that is involved in many pathways such as apoptosis, cellular transcriptional regulation, and cell cycle control.[1,2] The p53 protein has important role in cell cycle control, being involved in G1-phase arrest for DNA repairs or activation of the cell death machinery.[3] The protein accumulates in the cytoplasm following DNA damage, and then translocates to the nucleus and activates gene transcription machinery for cell cycle arrest to allow repair of damaged DNA.[4] Also p53 protein, in response to an excessive DNA damage, would activate programmed cell death pathway through transcriptional control of several genes.[5,6] TP53, located on chromo-

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some 17p13, is one of the most mutated genes affecting many types of human cancers.[7,8] In addition to mutations, several polymorphisms in the wild-type TP53 gene locus have been detected, which could alter its function.[9,10] Among the 14 polymorphisms identified in the TP53 gene, the most common in the general population associated with cancer development is the codon 72 Arg (Arginine) to Pro (Proline) substitution.[11] The TP53 Arg72Pro, located in exon 4 at codon 72 and involved in guanine to cytosine nucleotide exchange, leads to nonconservative change of an Arg to Pro. This polymorphism is located in a proline-rich region (residues 64-92) of TP53, homologous to the SH3 binding which is necessary for the protein to completely induce apoptosis.[12] These two polymorphic forms of TP53 gene have different primary structure and electrophoretic migration properties[11] with some different biochemical and biological potentials including different binding to compartments of the transcriptional machinery and different ability to activation of transcription, but they do not differ in their property to bind DNA.[13,14] Also the two polymorphic variants of TP53 have different potential to degradation by the HPV E6 protein.[15] It is observed that the Pro variant involves in activation of transcription to a higher level compared to the Arg variant and it is better for inducing cell cycle arrest, while the Arg variant is able to induce apoptosis faster and more efficiently than the Pro variant.[13]

Colorectal cancer is one of the most common causes of cancer-related death in the world and it is the fourth commonest malignancy after lung, breast and prostate cancers.[16] Many factors such as sex, age, diet and a variety of genetic factors influence the risk of developing colorectal cancer.[17,18] There are a number of syndromes with Mendelian dominant inheritance in which there is a primary predisposition to benign or malignant tumors of colon. These syndromes are causes of only 2-6% of colorectal cancer cases. Most of the patients do not have Mendelian dominant inheritance, and many researchers suggest that other genetic factors might predispose the patients to this kind of cancer.[19-21] Colorectal carcinogenesis is a complex multistage process that shows a high frequency of TP53 alterations and the large majority of these cancers are adenocarcinomas.[18,22]

Because of functional differences between the two polymorphic variants of TP53, genotype at codon 72 may affect susceptibility to colorectal cancer development. TP53 codon 72 polymorphism has been associated with the risk of developing various human cancers such as lung,[23] esophageal,[24] cervical,[25] bladder,[26] breast,[27-30] head and neck,[31] pancreas,[32] nasopharynx[33] and liver[34] cancers, although the results are still controversial. The role of codon 72 polymorphism of TP53 gene had been noted in the colorectal cancer patients in many populations including Argentina,[35] Taiwan,[36] Spain,[37] China,[38] Japan,[39-41] Turkey,[42] Germany[43] France,[44] Sweden[45] and the USA.[46] It had been shown that the codon 72 polymorphism varies greatly in different ethnic populations[47] and this ethnic difference might have a significant effect on cancer risk in different ethnic populations. However, the role of the polymorphism in relation to colorectal cancer risk in the Iranian population has not been reported. This study explores a possible association between colorectal cancer and this polymorphism in a small population selected from Isfahan city.

Materials and methods

Study population and samples:

We performed a case-control study on 180 paraffin blocks of sporadic colorectal adenocarcinomas and 180 healthy controls, in order to examine possible associations between the Arg72Pro alleles and the risk of cancer. Incidental colorectal cancer cases (histologically confirmed) attending the Alzahra Hospital (Isfahan) over the period 2002-2006 made up the case group. Proximal tumors were defined as occurring in the cecum through to the transverse colon; tumors in the splenic flexure, descending and sigmoid colon, were defined as being distal. Other disorders of colorectal re-

gion such as HNPCC, familial adenomatous polyposis, Inflammatory Bowel Disease (IBD), hamartoma, simultaneous occurrence of adenomas, previous or synchronous adenocarcinomas were excluded from this study. As control group, we used peripheral blood from 180 healthy age and sex matched persons. Controls were noncancerous persons who had already undergone colonoscopy.

DNA isolation from colorectal tissue and blood samples:

Genomic DNA from the tumors and blood samples was prepared using High pure PCR Template preparation DNA isolation kit (Roche, Germany) for tissue and whole blood, according to manufacturer's instructions.

PCR amplification of TP53 codon 72 polymorphism:

The TP53 codon 72 Pro allele was detected by PCR using the primer pair p53Pro+/ p53Pro- (p53Pro+: 5′-GCCAGAGGCTGCTCCCCC; and P53Pro-: 5′-CGTGCAAGTCACAGACTT) and the p53 codon 72 Arg allele by the primer pair p53Arg+/p53Arg- (p53Arg+: 5′-TCCCCCTTGCCGTCCCAA and p53Arg-: 5′-CTGGTGCAGGGGCCACGC).[15] Between 100 to 300 nanograms DNA was used as template in a 25 µl PCR reaction mixture containing 1.5 µmol MgCl2, 1 U Taq polymerase (Sinagen) and 2 µmol either of the primer pairs.

PCR cycling conditions were carried out with an initial denaturation step for 3 min at 94 ^{o}C, followed by 35 cycles of 30 s at 94 ^{o}C, 30 s at 60^{o}C (for Arg) or 54 ^{o}C (for Pro) and 30 s at 72 ^{o}C. A final extension step was performed at 72 ^{o}C for 5 min. The PCR reaction was done separately for each of the two polymorphic variants. The amplified products were subjected to electrophoresis on 1% agarose gel in 1× TBE buffer and visualized on a transilluminator using ethidium bromide.

Statistical analyses:

The χ2-test was used to assess the significance of any difference in the prevalence of TP53 codon 72 polymorphism between colorectal cancer patients and controls. The odds ratio and 95% CI (Confidence Intervals) were used as a measure of the strength of the association. Statistical significance level was set to $P \leq 0.05$.

Results

This analysis included 180 adenocarcinomas and 180 cancer-free control subjects. The general and clinicopathological characteristics of the cases are shown in Table 1. The age of 180 patients (77 women and 103 men) ranged from 35 to 91 years (mean age of men 67.15 ± 12.24 years, mean age of women 64.13 ± 15.76 years).

Table 1. General and clinicopathological data of patients with colorectal Cancer.

Factor	N (%)
Sex	
Male	57.2 (% 103)
Female	42.8 (%77)
Age	
≤ 59	67 (37.2 %)
≥ 60	62.8 (%113)
Localization	
Proximal	68.3 (% 123)
Distal	31.7(% 57)
Dukes stage	
A-B	23.9(% 43)
C-D	137 (76.1 %)
TNM staging	
I	25(% 45)
II	31.7(% 57)
III	36.1(% 65)
IV	13 (7.2 %)

To analyze the codon 72 polymorphism, we used a PCR-based assay that specifically amplifies either TP53 Pro or TP53 Arg allele and gives a PCR product by using specific primers for Pro allele (fig.1) and/or Arg allele (fig.2), respectively. Detection of TP53 codon 72 polymorphism by allele specific PCR was successfully conducted in all cases and controls. The distribution of the three different genotypes of codon 72 in exon 4 of TP53 in our cases and controls is shown in table 2. In control samples, the genotype distribution for p53 polymorphism showed 28.3%, 48.9% and 22.8% for the Arg/Arg, Arg/Pro and Pro/Pro genotypes, respectively. Allelic frequencies corresponded to 0.528 for the Arg allele and 0.472 for the Pro allele (table 3). In the cancer

group, 40% of the cases were Arg/Arg, 42.2% were Arg/Pro and 17.8% were Pro/Pro (table 2). The corresponding frequencies in this group were 0.611 for the Arg allele and 0.389 for the Pro allele (table 3). A significant difference between cases and controls was found for the Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes (Odds Ratio = 1.686 (1.085-2.620), P = 0.02). The Arg allele was found more often in patients than controls (Odds Ratio = 1.406 (1.064-1.891), P = 0.024).

Table 2. Distribution of TP53 codon72 polymorphism genotypes among colorectal cancer cases and controls in Isfahan

Genotype	Cases(N=180)		Controls(N=180)		Odds Ratio (95% CI)
	N	%	N	%	
A/A	72	40%	51	28.3%	1.686* (1.085-2.620)
A/P	76	42.2%	88	48.9%	
P/P	32	17.8%	41	22.8%	

A/A: Arg/Arg genotype;
A/P: Arg/Pro genotype;
P/P: Pro/Pro genotype;
N: number
CI: Confidence Intervals
*: $\chi 2$ test, P = 0.02 (Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes)

Table 3. Allelic frequencies of TP53 codon 72 among colorectal cancer cases and controls in Isfahan

Allele	Patients	Controls	Odds Ratio (95% CI)
Arg	0.611	0.528	1.406* (1.064-1.891)
Pro	0.389	0.472	

CI: Confidence Intervals
*: $\chi 2$ test, P = 0.024

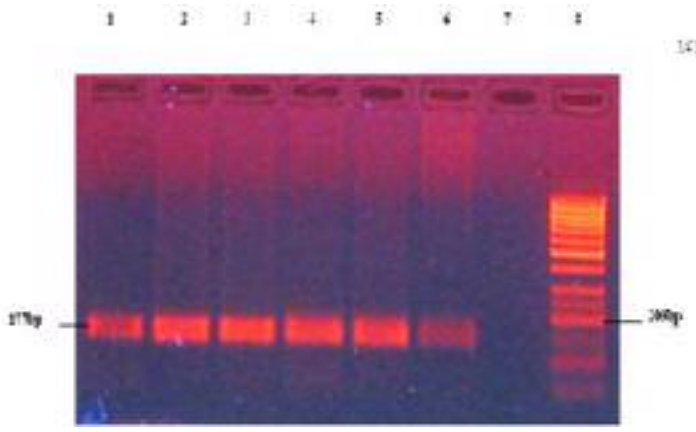

Figure 1. PCR amplification of the TP53 codon 72(electrophoresis in 1% agarose gel) in 6 colorectal adenocarcinoma specimens. lane 1-6: positive for Pro allele (177bp). lane7: negative control. lane8: DNA marker.

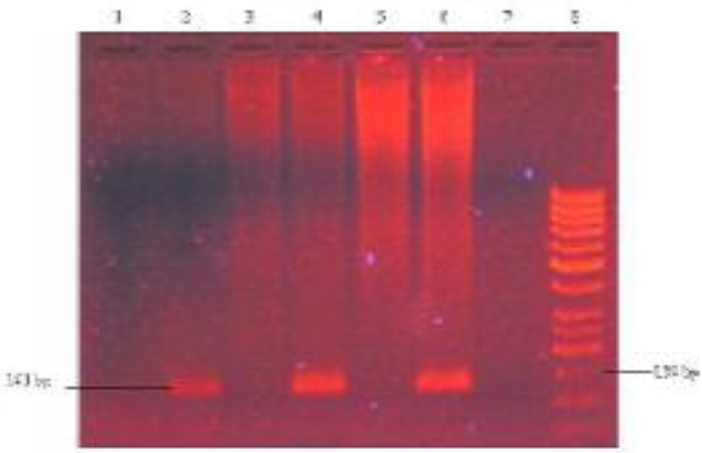

Figure 2. PCR amplification of the TP53 codon 72(electrophoresis in 1% agarose gel) in 6 colorectal adenocarcinoma specimens. lane 2, 4, 6 : positive for Arg allele (141bp). lane 1, 3, 5 : negative for Arg allele. lane7: negative control. lane 8: DNA marker.

Discussion

Molecular alterations have been associated with the development of colorectal cancer, including mutations at the TP53 tumor suppressor gene.[23] TP53 is polymorphic at amino acid 72 of the protein that it encodes, thus p53

protein may contain either an Arg or a Pro residue at this position. An association of the TP53 codon 72 polymorphism with several cancers susceptibilities has been reported.[23-34] In particular, both Arg and Pro alleles have been shown to be associated with a high risk of malignancy. The role of the Arg/Pro polymorphism in colorectal cancer susceptibility has been examined in several studies,[35-46] which have reported controversial results. We investigated the genotype frequencies of TP53 codon 72 in 180 sporadic colorectal adenocarcinomas and 180 healthy individuals from Isfahan and found a significant difference between cases and controls for the Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes (Odds Ratio = 1.686 (1.085-2.620), P = 0.02). The Arg allele was found more often in patients than in controls (Odds Ratio = 1.406 (1.064-1.891), P = 0.024). These findings are in agreement with the original study of Storey et al on cervical cancer.[15] They showed that p53Arg72 protein is more susceptible to degradation by the HPV E6 proteins, and degradation of p53 protein by HPV E6 is correlated with increased risk for HPV-associated cancers. In this study we did not consider HPV infections in accordance to detection of p53 genotypes and it is an important issue for future studies. Our finding also seems to be consistent with the results reported by Perez et al[35] which support an appreciable association between the Arg allele and colorectal cancer. However, there are contradictory findings about the mechanisms which lead to the increase of the Arg allele in human cancers[48-50] implicating that the involvement of TP53 polymorphism in human cancers demands further studies.

In contrast to our findings, other studies reported that the Pro allele had a significant effect on colorectal cancer risk,[36-39] and some studies did not show a significant association of the polymorphism with colorectal cancer risk.[40-46] The contradictory results about association of this polymorphism with colorectal cancer risk in different studies may be due to differences of allele frequencies between ethnic groups. It is well known that the distribution of TP53 codon 72 polymorphism varies in different geographic regions and ethnicities. According to the literature, general populations from Africa and Asia exhibit high frequencies of the Pro allele compared to the Arg one, while lower prevalences of Pro are found in populations of Latin America, the United States and Europe.[47,51-53] In this study, the frequency for the Arg allele was higher than Pro one in the both cancer and control groups and therefore it is not consistent with the results of other studies on Asian populations.

It is already determined that this polymorphism acts as an intragenic modifier of mutant p53 behavior[54] and has an effect on the biological activity of p53, so the different genotypes of p53 my have different biochemical and biological potentials and may affect susceptibility to colorectal cancer development.

Other factors may interfere in colorectal cancer risk such as genetic heterogeneity in the pathogenesis of colorectal cancer, different environmental factors, and sample size limitations. It is also possible that TP53 codon 72 polymorphism could be in linkage disequilibrium with other putative etiological variants[45] which would likely differ across different ethnic populations.

The present study was not controlled for other potential predisposing factors, such as smoking or life-style habits. This is an important issue to be addressed in further studies in order to assess the role of TP53 polymorphism in this tissue.

In conclusion, the findings of the present study indicate that TP53 codon 72 polymorphism may be a genetic predisposing factor for colorectal adenocarcinomas and p53Arg72 protein may be correlated with possible increased risk of this kind of cancers in Isfahan.

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