

Frequency of TP53 codon72 polymorphism in cases with colon cancer

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ABSTRACT

Recent studies indicated that the Arg allele is preferentially mutated and retained in various human cancers arising in Pro/Arg heterozygotes and it may be an important biomarker in colon cancer prognosis. In this study, DNA was isolated from paraffine-embedded colon tumor tissue samples of 35 cases diagnosed as colon carcinoma. We have observed PCR-RFLP genotyping for the codon 72 exon 4 polymorphism (Arg72Pro) of the p53 gene. In this study, we detected that TP53 codon 72 polymorphism was present in 27 (77.1%) of 35 cases enrolled into the study. In 14 (51.9%) cases Arg/Arg, 11 (40.7%) cases Arg/Pro and 2 (7.4%) cases Pro/Pro genotype frequencies were found. In 8 (22.9%) cases DNA isolation were not obtained. Our results point out that individuals homozygous for the Arg allele have higher frequency than other alleles and that colon cancer may be related to Arg allele frequency. [Turk J Cancer 2009;39(1):5-10]

KEY WORDS: Colon cancer, Arg72Pro, PCR-RFLP, polymorphism, TP53

INTRODUCTION

Colorectal cancer development is a complex, gradual, multistep process, in which many factors are known to be implicated. The molecular and histological changes involved are also well described, though not well understood. Most of these cancers are adenocarcinomas (95%), showing a high frequency of p53 mutations, mainly at advanced stages of colorectal cancer progression (1). The p53 tumor suppressor gene is of critical importance for the regulation of cell cycle and maintenance of genomic integrity (2,3). The p53 tumor-suppressor gene, located on chromosome 17p13, is one of the most commonly mutated genes in all types of cancer (4,5). Frequent deletion of the 17p13.1 region (p53 locus) is associated with mutation of the remaining allele in a variety of tumors, including sporadic colon cancer (6). To date, several polymorphisms in the wild-type p53 gene locus have been described (7). The most important p53 polymorphism is the restriction fragment length polymorphism in codon 72 of exon 4 coding for proline (72Pro: CCC) or arginine (72Arg: CGC). This polymorphism has been reported to be associated with some tumor types [Pro: bladder cancer, lung cancer; Arg: hepatocellular carcinoma, cervical cancer, human papilloma virus (HPV)-associated cervical cancer] (8-14).

Alterations of the p53 tumor suppressor gene is also a mutational event involved in colorectal mucosa carcinogenesis. The normal p53 protein inhibits proliferation of cells with DNA damage; alterations in both, or sometimes even only one of its alleles may interfere with this function (15,16). P53 point mutations are found in about 50%

of colorectal cancers. An acquired defect of p53 or loss of the wild-type allele generally tends to result in an alteration of cell cycle regulation. This molecular event plays an important role in colorectal carcinogenesis (17,18).

The wild-type p53 gene exhibits a polymorphism at codon 72 in exon 4, with a single nucleotide change that causes a substitution of proline for arginine (Arg72Pro) (19). The Arg72Pro polymorphism is located in a proline-rich region (residues 64-92) of the p53 protein, where the 72Pro amino acid constitutes one of five PXXP motifs resembling a SH3 binding domain. The region is required for the growth suppression and apoptosis mediated by p53 but not for cell cycle arrest. The two polymorphic variants of wildtype p53 have been shown to have some different biochemical and biological properties (20). Since Storey et al. (21) established an association of p53 Arg72Pro with cervical cancer, the p53 polymorphism has been studied as a risk factor in various cancers with inconsistent results.

In addition to gene mutations, several reports have focused on p53 polymorphisms as risk factors for malignant disease. The alleles of the polymorphism in codon 72, exon 4, encode an arginine amino acid (CGC, Arg72) with a positive charged basic side chain and a proline residue (CCC, Pro72) with a nonpolar aliphatic side chain (14, 22-25).

This study was undertaken to investigate the association of p53 codon 72 polymorphism with colon cancer cases. The purpose of this study was to investigate whether p53 Arg at position 72 would represent a risk factor for colorectal cancer cases. Our results indicate that individuals carrying the arginine allele have an increased colon carcinoma risk compared to proline homozygotes and it may be an important biomarker in colon cancer prognosis.

MATERIALS AND METHODS

To investigate the codon 72 polymorphism paraffin-embedded tissue samples from 35 patients with colon cancer were analysed by PCR-RFLP. Tissues were collected from 35 cases with colon cancer; females aged 40-90 (mean age 65.95±2.76) and males aged 40-79 (mean age 61.67±3.07). Genomic DNA was extracted from paraffin-embedded tissues using EZNA Tissue DNA Kit

(Omega) following the protocol. According to the exon 4 sequence primers P1(5'TTGCCGTCCCAAGCAATGG ATGA3') and P2(5'TCTGGGAAGGGACAGAAGATG AC3') (MWG-BIOTECH) were designed. 5 µl DNA was amplified in a reaction, that contained 0.2 µmol dNTP mix (Dr. Zeydanlı-TURKEY), 0.5 unit Taq DNA polymerase (BIORON) and primers, with a total volume of 50 µl. The PCR was performed with 10 pmol 0.5µl (P1+P2) for 36 cycles (predenaturing for 5 minutes at 95°C, denaturing for 30 seconds at 95°C, annealing for 30 seconds at 59°C and extension for 35 seconds at 72°C) in thermal cycler (Primus 96 Advanced, Peqlab) for amplification product of 199 bp. 15 µl of PCR products was diluted with 4 µl of 6X loading dye (Dr. Zeydanlı-TURKEY) and separated in 2% agarose gel (Serva) stained with ethidium bromide (Dr. Zeydanlı-TURKEY) and observed under UV-Light transilluminator (CSL). After then 4th exon of p53 were amplified by PCR method and p53 mutation analyses were performed using BstFNI restriction enzyme determined for 4th exon. Agarose gel electrophoresis of p53 Arg72Pro region PCR products were digested with BstFNI (Figure 1). Thus, the proline (Pro/Pro) allele is identified by the presence of a single fragment of 199 bp and the arginine (Arg/Arg) allele by two fragments of 113 and 86 bp, respectively. Heterozygous (Arg/Pro) samples display all three fragments of 199 bp, 113 bp and 86 bp.

RESULTS

Tissues were collected from 35 cases with colon cancer; females aged 40-90 (mean age 65.95±2.76) and males aged 40-79 (mean age 61.67±3.07); p=0.03. The relationship among p53 codon 72 genotype and various clinical and pathologic variables previously shown to influence p53 mutational status is shown in table 1.

p53 codon 72 genotype was assessed on DNA extracted from paraffin-embedded tissue of 35 colon cancer patients. The analysis of the p53 codon 72 genotype on the DNA from the colon cancer tissue revealed 2 (5.7%) proline homozygotes (Pro/Pro), 11 (31.4%) arginine/proline heterozygotes (Arg/Pro), and 14 (40.0%) arginine homozygotes (Arg/Arg). In 8 (22.9%) cases DNA isolation were not obtained. Our results indicate that individuals homozygous for the Arg allele have higher frequency than other alleles and that colon cancer may be related to Arg allele frequency (Table1).

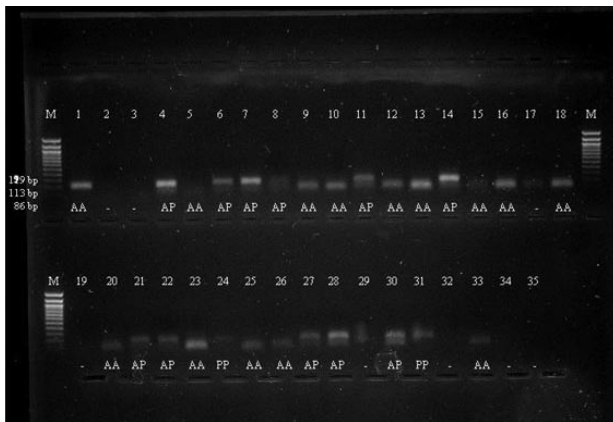


Fig 1. Agarose gel electrophoresis of p53 Arg72Pro region PCR products were digested with BstFNI. Molecular Weight Marker DNA 100 bp Ladder, (AA: Arg/Arg homozygous genotype (113 bp and 86 bp); AP: Arg/Pro heterozygous genotype (199 bp, 113 bp and 86 bp); PP: Pro/Pro homozygous genotype (199 bp). Molecular Weight Marker DNA 100 bp Ladder (1200-1100-1000-900-800-700-600-500-400-300-200-100)

Codon 72 arginine and proline alleles were investigated by PCR-based digestion analysis. The recognition site (CGCG) of the restriction enzyme was present only in the arginine encoding allele. Thus, the proline (Pro/Pro) allele was identified by the presence of a single fragment of 199 bp and the arginine (Arg/Arg) allele by two fragments of 113 and 86 bp, respectively. Heterozygous (Arg/Pro) samples display all three fragments of 199 bp, 113 bp and 86 bp.

The distribution of the Arg/Arg, Arg/Pro and Pro/Pro genotype frequencies were 51.9%, 40.7% and 7.4%, re-

spectively in both sexes. The frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 43.8%, 50.0% and 6.3%, respectively among women and were 63.6%, 27.3% and 9.1%, respectively among men ($p=0.498$). The frequencies of the well, moderate, poor for tumor grade were 22.9%, 71.4% and 5.7%, respectively in both sexes. The frequencies of the well, moderate, poor for tumor grade was 30.0%, 60.0% and 10.0%, respectively among women and were 13.3% and 86.7%, respectively among men ($p=0.183$).

The frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 51.9%, 40.7% and 7.4%, respectively in both age groups. The frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 53.8%, 38.5% and 7.7%, respectively in the 60 and down age group and the frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 50.0%, 42.9% and 7.4%, respectively in the 61 and up age group ($p=0.973$).

The corresponding frequencies were 51.9 for the arginine allele and 7.4 for the proline allele. The corresponding frequencies of the Arg/Arg, Arg/Pro and Pro/Pro genotypes in the patients were 40.0%, 31.4% and 5.7%, respectively. A significant difference between alleles were found for the Arg/Arg genotype frequencies compared with (grouped) Arg/Pro and Pro/Pro genotype frequencies. Table 1 shows the obtained risk estimation for colorectal cancer with its corresponding confidence intervals.

Table 1
Correlation between genetic and clinopathologic data of patients with colorectal cancer

Factor	p	n	Status at codon 72		
			Arg/Arg [n(%)]	Arg/Pro[n(%)]	Pro/Pro[n(%)]
Age	0,983				
60 and down		13	7 (53.8)	5 (38.5)	1 (7.7)
61 and up		14	7 (50.0)	6 (42.9)	1 (7.4)
Sex	0,498				
Female		16	7 (43.8)	8 (50.0)	1 (6.3)
Male		11	7 (63.6)	3 (27.3)	1 (9.1)
Tumor Grade	0,753				
Well		7	3 (42.9)	4 (57.1)	-
Moderate		18	10 (55.6)	6 (33.3)	2 (11.1)
Poor		2	1 (50.0)	1 (50,0)	-
Frequency		27	14 (51.9)	11 (40.7)	2 (7.4)

In 11 of 35 (40.7%) Arg/Pro patients, the loss of heterozygosity (LOH) at the p53 locus was confirmed by PCR-RFLP. Statistical analysis revealed a significant increase in the presence of the Arg allele in the colon cancer cases indicating that the Arg allele represents a high-risk polymorphism in colon cancer in our colorectal cancer group.

In this study, homozygosity for arginine was the most prevalent genotype in colorectal cancer patients. The frequency of codon 72 proline/arginine p53 gene polymorphism was correlated with a higher risk of colorectal cancer. Arginine/Arginine genotype frequencies was more prevalent in colorectal cancer cases.

DISCUSSION

Recently, several studies have provided evidence that p53 polymorphism at codon 72 may be associated with certain tumors, such as cervical cancer, breast cancer, lung cancer, and hepatocellular carcinoma. In particular, both Arg and Pro alleles have been shown to be associated with a high risk of malignancy. Indeed, an early involvement of p53 in the progression from colonic dysplasia to invasive colon cancer is suggested by the finding of mutated alleles in the inflamed bowel mucosa of patients with ulcerative colitis (UC) and cancer (26).

Cancer is a multi-step mechanism occurring as a result of series of progressive genetic alterations. So far, molecular genetic studies revealed that a group of tumor suppressor gene and protooncogen alterations were effective in cancer formation. p53 is the most important tumor suppressor gene that is effective in cancer formation and it is very effective in progression of colon cancer. Due to this, detection of p53 mutations are thought to be an important factor in prognosis and early diagnosis. In this study, we aimed to detect the frequency of mutations of p53, exon 4 Arg72Pro genotypes and their relations with progression of the disease. Relevant to this, by PCR, amplification of 4th exon were performed from isolated DNA samples of tumor tissues of colon cancer diagnosed in 35 cases and mutation existence was tested by enzyme restriction. As a result, the analysis of the p53 codon 72 genotype on the DNA from the colon cancer tissues revealed 2 (5.7%) proline homozygotes (Pro/Pro), 11 (31.4%) arginine/proline heterozygotes (Arg/Pro), and 14 (40.0%) arginine homozygotes (Arg/Arg).

In this study, we investigated p53 codon 72 polymorphism in a different age, sex and tumor grade of colorectal carcinoma in colon cancer cases. Considering tumor tissue DNA, we found a significantly higher frequency of 72Arg in colorectal tumors. There was an apparent association of the Arg genotype with tumor progression. Schneider-Stock et al. (14) found preferential mutation of the Arg allele in a group of colorectal adenocarcinomas. They also reported selective loss of the Pro allele in tumors with loss of heterozygosity (LOH), resulting in a positive association between Arg prevalence and Dukes progression. The authors discarded the possibility of HPV as a potential mechanism for the higher frequency of Arg alleles in colorectal tumors and hypothesized that carcinogenic exposure may selectively affect the p53 Pro allele in the development of colon cancer.

The p53 gene is one of the most extensively studied human genes because of its role as a tumor suppressor gene. Its diverse functions include DNA binding, cell cycle control, DNA repair, differentiation, genomic plasticity, and apoptosis. Thus, the overall function of p53 is to maintain genomic integrity as a whole, providing a protective effect against tumorigenesis. Recent studies indicated that the Arg allele is preferentially mutated and retained in various human cancers arising in Pro/Arg heterozygotes. These findings suggest that this polymorphism acts as an intragenic modifier of mutant p53 behavior and has an effect on the biological activity of p53 (27). Also, experimental evidence suggests that the p53 protein is related to cell aggressiveness and tumor metastasis (28). Recent studies have shown that a common polymorphism at codon 72 of the p53 gene results in two alleles, the arginine (Arg) and proline (Pro) isoforms, which differ biologically and biochemically (29). In this sense, it appears that this polymorphism may be associated with differential susceptibility to cancer. Several studies conducted in different countries reported significant associations between p53 polymorphism and human cancer. However, the data available for most cancers remain inconclusive, including colorectal cancer (30).

In this study we evaluated the association between the risk of developing colon cancer and the genotype at codon 72 by PCR and restriction enzyme digestion. The length of fragments in our cancer group were similar to those reported by other authors in Chinese population (27).

Buyru et al. (31) reported that the purpose of their study was to investigate whether p53 Arg at position 72 would represent a risk factor for breast cancer in the Turkish population. Their results indicate that individuals carrying the arginine allele have a 3-fold increased breast carcinoma risk compared to proline homozygotes. Statistical analysis revealed a significant increase in the presence of the Arg allele in Turkish colon cancer patients when compared with controls indicating that the Arg allele represents a high-risk polymorphism in breast cancer in their study group. This finding is discordant with some reports which have not found a difference in the frequency of the Arg allele between colon cancer patients. Similar inconsistency for the codon 72 alleles exists also for other tumors (31). In this study, the relationship among tumor grade and Arg-Pro genotypes in our cancer group were similar to those reported by other authors (14,24).

Perez et al. (32) reported that p53 codon 72 arginine homozygous genotype may represent a genetic predisposing factor for colon cancer development. However, further studies are needed in order to elucidate the role of p53 codon 72 polymorphism in colorectal cancer. Frequencies in our study are very close to the frequencies from La Plata, Argentina and Spanish people (33). Pérez et al. (32) reported that 58.5% of the cases were Arg/Arg, 37.7% were Arg/Pro and 3.8% were Pro/Pro. The corresponding frequencies were 0.77 for the arginine allele and 0.23 for the proline allele, which is consistent with our results.

Our study is in agreement with Schneider-Stock et al.'s (14) study, in which considering tumor tissue DNA, they found a significantly higher frequency of the 72Arg in colorectal tumors. There was an apparent association of the Arg genotype with tumor progression, with metastases showing a bias toward the Arg genotype. There was only one metastasis with Pro/Pro homozygous con-

stitution. They explained that all allelic losses at codon 72 of p53 exon 4 were directed toward the loss of the proline allele. They suggest that the preferential loss of the 72Pro variant and/or mutations of 72Arg, also seen in their study for colorectal cancer and colorectal liver metastases, is of biologic significance. Thus, they tested all tumors for p53 mutations. Indeed, tumors that had retained the 72Arg allele often carried p53 mutations, with the "gain-of-function" type occurring with remarkably high frequency. 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. In addition, the TP53 codon 72 polymorphism has been reported to be associated with colorectal tumor malignant potential, age of onset and survival (14, 32-34). Further studies, therefore, should be performed to analyze this potential role of the TP53 polymorphism for colorectal tumor characteristics.

In summary, we found a preferential loss of the 72Pro allele and mutations in the retained 72Arg in colorectal cancer. Our results indicate that individuals carrying the arginine allele may have increased colorectal carcinoma risk compared to proline homozygotes. Further studies should be performed to analyze the potential role of the TP53 polymorphism for colorectal tumor characteristics.

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