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P53 intronic variant G13964C analyses in cases with colon cancer

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ABSTRACT

Nucleotide alterations in p53 intron 6 have been reported to be associated with the dysregulation of p53 function and tumor development. G13964C base change functioned as dominant mutation similar to the more common missense, nonsense and splice-site mutations. To detect the G13964C variant PCR-RFLP assay was used. In this study, DNA was isolated from colon cancer tissue samples of 35 cases (19 female and 16 male) diagnosed to be colon carcinoma. In this study, we found that mutations were present in 30 (85.7%) of 35 cases enrolled into study. In 7 (23.3%) cases G/G, 21 (70.0%) cases G/C and 2 (6.7%) C/C genotypes were found. In 5 (14.3%) cases DNA isolation could not be obtained. Our results indicate that heterozygotes for the GC allele have higher frequency than other alleles and one of the reasons of colon cancer may be related to GC allele frequency. [Turk J Cancer 2009;39(2):51-55]

KEY WORDS: Colon cancer, G13964C, PCR-RFLP, polymorphism, TP53

INTRODUCTION

When Lane and Crawford (1) and Linzer et al. (2) first described p53 protein as a tumor antigen in 1979, they probably never imagined that its gene would become the most important tumor suppressor gene ever studied. It took some time to realize that the p53 gene was a tumor suppressor, not an oncogene, as was thought at the beginning (3-8). Frequent deletion of the 17p13.1 region (p53 locus) was associated with mutation of the remaining allele in a variety of tumors, including sporadic colon cancer (6,9,10).

Intronic variants may affect gene regulation through aberrant splicing or through disruption of critical DNA-protein interactions. The p53 G13964C variant is not within the consensus splice site and there is thus far no direct evidence that it affects the expression of p53. It is not associated with over-expression of p53, which is a hallmark of missense mutations in p53. Nonetheless, there is some indirect evidence that this variant has a functional role (11-13). Functional analysis using an *in vitro* cell survival assay demonstrated that lymphoblastoid cell lines derived from patients with the G13964C variant exhibited a reduced level of apoptosis after chemotherapy and prolonged cell survival following DNA damage. The constitutional p53 intronic G to C base change at nucleotide 13964 (GenBank accession number X54156/U94788), was found by Buller et al. (14) in patients with ovarian and breast cancer.

There is now substantial evidence that p53 gene abnormalities are frequently associated with the pathogenesis of neoplasias, particularly solid tumors, like breast, lung and colon cancer (15). The role of the p53 gene in a variety of cellular processes, including transcription, DNA repair, cell cycle control and apoptosis, makes it a potential marker for the detection of patients at higher risk for developing cancer (16). Most of the reported mutations in the p53 gene cluster within the region coding the DNA-binding domain (17) impairing the DNA binding or transactivation functions of the protein, thereby inhibiting its key role in cell cycle control (18). There is accumulating evidence that novel mechanisms of gene regulation, including mutations in the splice, donor and acceptor sites or enhancer, intron and promoter elements, may be important in regulating gene expression (19). In addition to the numerous coding region mutations, the p53 gene also contains several polymorphisms and non-coding region mutations. The regulatory role of intronic sequences has been recognized only recently. Some germline polymorphisms include a variable number of tandem repeat regions in intron 1, various different RFLPs in introns 1, 6 and 7, a 16 bp insertion in intron 3 as well as transversions in introns 2 and 10 (20-25). Among these, the intron 3 and intron 6 polymorphisms have been widely analyzed as possible cancer susceptibility modifiers.

The G to C base change at intron 6 nucleotide 13964 of the p53 gene destroys a natural restriction site. The p53 G13964C variant is not within the consensus splice site and there is thus far no direct evidence that it affects expression of the p53 gene. Nonetheless, recent data suggests a functional role for this variant leading to stabilization, possibly inactivating the p53 protein, thereby contributing to tumorigenesis (26).

In order to determine the importance of the G13964C p53 variant in multiple-case Australian breast cancer families, it has been genotyped this variant in the youngest affected member of 71 breast cancer families and 143 control individuals. Researchers results suggest that the rare 13964C allele is no more common in breast cancer families than in control individuals. This suggests that the variant is not a high-risk mutation but, as suggested previously, is more likely to be a benign polymorphism (14).

MATERIALS AND METHODS

Patients

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 ages of female 40-90 (mean age 65.95±2.76) and ages of male 40-79 (mean age 61.67±3.07).

DNA extraction

Genomic DNA was extracted from paraffin-embedded tissues using E.Z.N.A Tissue DNA Kit (Omega Biotek, USA) following the protocol. In order to determine p53 G13964C variant genetic polymorphisms were performed in DNA extracted from paraffin-embedded sections, using the Polymerase Chain Reaction (PCR) technique. In brief, 4 μm sections of the tumor were treated with xylene (Merck, Germany), ethanol (Merck, Germany), proteinase K, phenol (Merck, Germany) and chloroform (Merck, Germany). Pellets were resuspended in milliQ water and stored at -20 °C. Then, 5 μL aliquots of DNA were subjected to PCR, using Master Mix (Eppendorf, Düsseldorf, Germany) together with the following primers.

Genotyping of p53 G13964C polymorphism

The genotypes of p53 G13964C polymorphism was determined using PCR based restriction fragment length polymorphism (RFLP) method (27). PCR condition was 5 min at 95, followed by 36 cycles of 30 s at 95, 30 s at 59, and 35 s at 72, and with a final extension at 72 for 10 min. A 10 μL aliquot of PCR product was digested overnight at 37 °C in a 20 μL reaction volume containing 10 units of BstHHI (SibEnzyme, USA). After overnight digestion, the amplified products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer at 100 V for 45 min and viewed on a transilluminator (Syngene, UK) using ethidium bromide. The genotypes of G13964C variant homozygotes for CC were represented by a DNA band with the size of 207 bp, whereas GG homozygotes were represented by DNA bands with sizes of 175 bp and 32 bp. Heterozygotes GC displayed a combination of both alleles (207, 175 and 32 bp) (Figure 1).

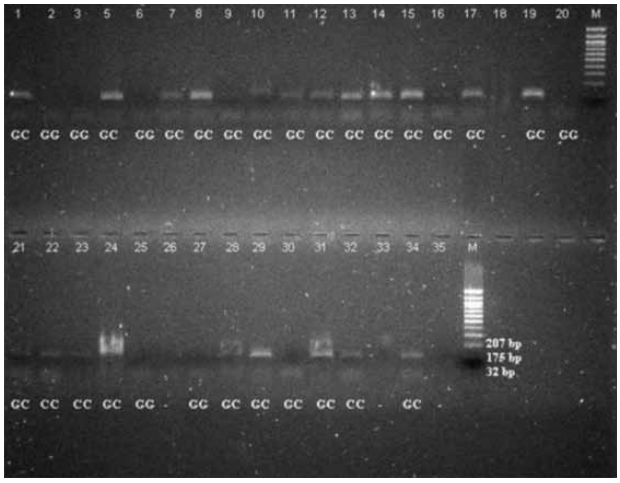

Fig 1. PCR-RFLP genotyping for the p53 G13964C variant. PCR products (207 bp) were digested with BstHHI restrictase (G > C substitution destroys the BstHHI site) and analysed on 2% agarose gels. Products amplified from the homozygous GG genotype were digested to 175 and 32 bp fragments (the 32 bp fragment is not visible on the gel); the heterozygous GC genotype corresponded to three bands of 207, 175 and 32 bp. Molecular Weight Marker DNA 100 bp Ladder (1200-1100-1000-900-800-700-600-500-400-300-200-100)

Statistical analysis

Statistical analysis was performed with the use of statistical package SPSS 9.0. Differences between the samples were determined to be statistically significant at P<0.05. Computerised data base included the following variables: age, sex, tumor status, TP53 gene polymorphism status and genotypes. Chi-square test was used for comparison of proportions.

RESULTS

Tissues were collected from 35 cases with colon cancer; 19 females (mean age 65.95±12.34) and 16 males (mean age 61.67±11.88) (p=0.31).

The frequencies of the G/G, G/C and C/C genotypes for p53 G13964C were 3 (20.0%), 11 (73.3%) and 1 (6.7%), respectively in the age 60 and down group and were 4 (26.7%), 10 (66.7%) and 1 (6.7%), respectively in the age 61 and up group. No significant difference in the genotype distribution was found between these two age groups (p=0.91).

The frequencies of the G/G, G/C and C/C genotypes for p53 G13964C were 4 (23.5%), 12 (70.6%) and 1 (5.9%), respectively in the females and were 3 (23.1%), 9 (69.2%) and 1 (7.7%), respectively in the males. No significant difference in the genotype distribution was found between these two sex groups (p=0.98).

The frequencies of the G/G, G/C and C/C genotypes for p53 G13964C were 1 (12.5%), 6 (75.0%) and 1 (12.5%), respectively in the well-, were 6 (30.0%), 13 (65.0%) and 1 (5.0%), respectively in the moderate-, and were 0 (0%), 2 (100.0%) and 0 (0%), respectively in the

Table 1
Distribution of p53 intron 6 G13964C genotype and allele frequency by clinicopathological parameter of colon cancer cases

Factor	p	n	Status at P53 intron 6 G13964C		
			GG n (%)	GC n (%)	CC n (%)
Age					
60 and down	0.91	15	3 (20.0)	11 (73.3)	1 (6.7)
61 and up		15	4 (26.7)	10 (66.7	1 (6.7)
Sex					
Female	0.98	17	4 (23.5)	12 (70.6)	1 (5.9)
Male		13	3 (23.1)	9 (69.2)	1 (7.7)
Tumor Grade					
Well	0.69	8	1 (12.5)	6 (75.0)	1 (12.5)
Moderate		20	6 (30.0)	13 (65.0)	1 (5.0)
Poor		2	-	2 (100.0)	-
Total Frequency		30	7 (23.3)	21 (70.0)	2 (6.7)

poor-tumor grade. No significant difference in the genotype distribution was found between these three tumor groups (p=0.69).

In this study, our results indicate that individuals heterozygous for (G/C) were the most prevalent genotypes in colorectal cancer patients when compared for age, sex and tumor grades. These alleles have higher frequencies than the other alleles and they may have an increased colon carcinoma risk and it may be an important biomarker in colon cancer prognosis. The results are shown in table 1.

DISCUSSION

The G13964C intronic variant was detected in eight individuals in total, none of whom had a family history of Li Fraumeni syndrome. Because the G13964C intronic variant was identified in individuals aged from 21 to 62 years with no evidence of cancer at the time of recruitment and with no reported family history of breast cancer, the present results suggest that this variant is not associated with an increased risk for breast cancer. These findings contradict the results found by Lehman et al. *(*26), who did not observe the variant in 171 sporadic breast cancer control individuals and reported a significantly elevated frequency in familial breast cancer. The present study would have had 80% power to detect an odds ratio of 4.4, and therefore we can conclude that the G13964C is not a high-risk mutation for breast cancer. Instead, our results support the proposal that the G13964C site is a benign polymorphism, as originally suggested by Buller et al *(*14). The variant lies within the noncoding region of the gene and is not associated with over-expression of p53 *(*14).

In conclusion, our data indicate that the p53 G13964C variant is a germline mutation associated with a high risk of cancer. Our results indicate that individuals heterozygous for the GC allele have higher frequency than other alleles and that colon cancer may be related to GC allele frequency.

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