The -31 G/C promoter gene polymorphism of survivin in Turkish patients with colorectal cancer

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(Index words: colorectal cancer, polymorphism, apoptosis, survivin, promoter)

Abstract

Introduction Survivin (also known as birc5) is the first protein discovered among the apoptosis-regulating gene family referred to as inhibitor of apoptosis proteins (IAPs). It is expressed and controlled during cellular differentiation and development in human beings. Survivin expression has been shown in a number of cancers and has been associated with cancer development.

Objective In our study, we compared normal and tumoural tissue samples, which were obtained from 100 patients diagnosed with colorectal cancer, at Department of Pathology, Istanbul University.

Methods The present study employed PCR-RFLP to identify the -31 G/C polymorphism in the promoter region of the survivin gene. Distribution of the survivin polymorphism was compared between control and tumoural tissue samples using the chi-square test.

Results Comparison of all samples revealed that there was significant difference in distribution of survivin promoter -31G/C between tumour and normal tissue of the patient group ($p<0.05$). When genotypes were compared according to gender, there was no statistically significant difference in the distribution of survivin promoter -31G/C in females $p=0.420$ or males $p=0.309$.

Conclusion A significant difference was seen in distribution of C allele in tumour tissue compared to normal tissue.

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Introduction

Survivin is known as an inhibitor of apoptosis protein which is crucial for the control of cell division as well as cell survival [1]. Survivin is active in cell cycle regulation and also in tumour development [2]. Survivin is the only protein among inhibitor of apoptosis proteins (IAPs)with two functions. The first of these two functions is to inhibit apoptosis by binding to caspases. This function has been demonstrated particularly in cells which need to undergo continuous cell division and also in certain cancers. The second function of survivin is segregation of chromosomes towards the opposite poles by binding to the mitotic spindle during anaphase. Survivin expression reaches a peak and inhibits apoptosis at the cell cycle checkpoint during mitosis. The survivin gene is located in the telomeric region of the long arm (q) of chromosome 17, and spans 14.7 kb with a baculovirus repeat domain. Its expression has been shown in a number of malignant tumour types with the exception of thymus, basal clonal epithelium, endothelial cells and cells derived from neural stem cells during angiogenesis. However, it is absent in normal, differentiated tissues in adult humans [3]. A number of single nucleotide polymorphisms (SNPs) have been identified in the promoter region of the survivin gene. One of these SNPs is the -31 G/C polymorphism (rs9904341) of the CDE/CHR repressor binding domain at position -31 of the ATG start codon. The repressor protein cannot bind as this polymorphism (G/C) alters the sequence in the CDE/CHR repressor binding domain, resulting in excessive mRNA synthesis in cancer cell lines [2, 4].

Colorectal cancers are among the commonest cancers worldwide. Occurrence and development of colorectal cancers results from a series of genetic alterations leading

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to irregular epithelial cell differentiation [5-7]. Studies show that alterations such as genetic stability in colorectal epithelial cells cause prolonged survival in epithelial cells. The advanced malignant alteration in these cells is associated with apoptosis related changes. Presence of survivin expression in such patients reduce apoptosis in tumour cells and decrease life expectancy [8-12].

Furthermore, studies have suggested the -31 G/C polymorphism of the promoter CDE/CHR repressor element region as a risk factor for the development of gastric cancer and lung cancer [12-16]. In light of available data, our aim in the present study is to investigate the changes in the -31 G/C polymorphism at the CDE/CHR repressor element region in human colorectal cancers.

Methods

Characteristics of the study group

In the present study we used normal and tumoural tissue samples of 100 patients diagnosed with colorectal cancer at the Pathology Department of Istanbul University Cerrahpasa Faculty of Medicine from 2008 to 2012. We also selected a control group of 100. All patients and controls were selected from the Caucasian Turkish population. The prognostic and metastasis related information of these patients were unavailable and we used tissues only from patients with pathologically confirmed cancers of colorectal origin. The study group consisted of 41 female patients and 59 males. Mean age of the patients was 61. The study group included 44 patients with Grade 1, 9 patients with Grade 2, and 47 patients with Grade 3 tumours. Tissues initially underwent macroscopic evaluation, and normal tissues were distinguished from tumour tissues. Tissues were investigated under the microscope for any tumoural infiltration and those observed to be infiltrated were excluded from the study. Normal tissues and tumour tissues obtained from these patients were formalin-fixed and embedded in paraffin. Sections of 4-10 µm thickness were obtained from the tissue, stained with hematoxylin and eosin, dehydrated with alcohol series and were finally allowed to dry in uncovered glass in open air. The age and carcinoma related information of the patients are presented in Table 1.

*Determination of -31G/C polymorphis*m

Sections of 4-10 µm thickness were obtained from paraffin-embedded tissues, paraffin was removed with xylene, and the tissues were then dehydrated through alcohol series. Subsequently, the sections obtained from the tissues were incubated at 55°C overnight in 50 µl of extraction buffer containing 100 mM Tris-HCl, 2 mM ethylenediaminetetraacetic acid, pH: 8.0, 400 mg/mL proteinase K. The samples were then processed by boiling at 100°C for proteinase K inactivation. A sample volume of 2 µl DNA was used for each PCR reaction. For each reaction, 5'-GTTCTTTGAAAGCAGTCGAG-3' (forward) and 5'-GCCAGTTCTTGAATGTAGAG-3' (reverse) primers were used for the relevant gene region. The specified PCR conditions were 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 90 seconds, and extension at 72°C for 90 seconds, and completed with a final elongation phase at 72°C for 5 minutes. PCR products of 341 bp were obtained. The amplicon was subjected to restriction with the restriction enzyme EcoO1091 (Takara) at 37°C for 2 hours. Restriction products were stained with ethidium bromide and were observed under ultraviolet light in 2% agarose gel (Figure 1). The restriction resulted in two bands for the G allele (236 and 105 bp) while the C allele was not restricted. PCR and enzyme restriction protocols were applied as previously specified in the literature [4, 5]. At the end of the study, 10% of the sample selected randomly was analysed again the genotype findings were consistent with the previous findings.

Table 1. Demographic and clinical characteristics of cases and controls

Characteristics	*Control Group (n=100)*	*Patient group (n=100)*
	N	N
Age years (Mean ±SD),	55+-8	61 ± 11
≤57 years	62	58
> 57 years	38	42
Gender		
Female	43	41
Male	47	59
Grade		
1		44
2		9
3		47
Tumour Size		
T1		32
T2		25
T3		43
Tumour Location		
Colon		60
Rectum		31
Other parts of CRT		9

Statistical analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Company, Chicago, IL, USA). The genotype frequencies were assessed using the Hardy-Weinberg analysis while the expected frequencies were assessed using the chi-square test. Distribution of Survivin

promoter allele types among the normal and tumoural tissues of the patients was evaluated using chi-square test. Odd ratio (OR) values and 95% confidence intervals were calculated with unconditional logistic regression. Genotypes and clinico-pathological characteristics of patients with colorectal cancers were compared by using chi-square tests. The normal and tumour tissues of the patient group were separately compared with the control group by means of the Chi-square test, p-value below 0.05 was considered significant.

Results

Distribution of the -31 G/C polymorphism of survivin gene promoter (including 100 tumoural colorectal cancer tissues and 100 normal tissues) are presented in Tables 2 and 3. A significant difference was found in the -31 G/C polymorphism between the normal and tumoural tissues. The -31 G/C polymorphism demonstrated no significant difference according to the tumour localization, size, growth or differentiation characteristics. In the control group; GG, GC and CC were found to be 41, 48, 11, respectively. There was no significant difference between the control group and genotype distribution of normal and tumour tissues in the patient group (Table 3).

When genotypes of the tumour and normal tissues were compared according to gender, there was no statistically significant difference in the distribution of survivin promoter -31G/C in females p=0.906) or males (p= 0.780). However, a significant difference was seen for the C allele compared to the control group.

Figure 1 shows polymerase chain reaction-restriction fragment length polymorphism analysis to detect -31 C/G polymorphism of survivin promoter. Polymerase chain reaction products (341-bp) were digested with restriction enzyme EcoO109I, and visualized and analyzed by 2% agarose gel. In the patients group lanes 2-3 the normal genotype of tumour tissue is GG; Lanes 4-5 tumour tissue is CC; Lanes 6-7 normal and tumour tissues have same genotype. In the control group lanes 1and 3 is CC, lane 2 is GC and lane 4 is GG.

Table 2. Distribution of genotypes in normal and tumour tissues in patients according to grade, gender, and tumour location

The C-31G polymorphism	*Normal Tissue (N=100)*			*Tumour Tissue (N=100)*			*P-value*
Genotype (%)	GG	GC	CC	GG	GC	CC	
Grade 1-2	12	31	10	11	33	12	
Grade 3	13	21	13	9	24	9	0.501
Age							
≤ 57 years	9	28	10	8	27	12	0.617
> 57 years	11	30	12	7	33	13	0.723
Gender							
Female	7	23	11	6	23	12	0.420
Male	18	29	12	15	36	8	0.309
Tumour Location							
Colon	26	19	15	24	20	16	0.450
Rectum	13	10	8	11	9	11	0.930
Other parts of CRT	3	4	2	2	5	2	0.783

X^2 McNemar-Bowker Test for genotype between normal and tumour groups.

Table 3. Distribution of genotypes among case and control groups

-31G/C Genotype	*Control (n=100)*	*Patients normal tissue (n=100=%)*	*P-value*	*Patients tumour tissue (n=100=%)*	*P-value*
GG	41	25	0.015	20	0.0038
GC	48	52		57	
CC	11	23		21	

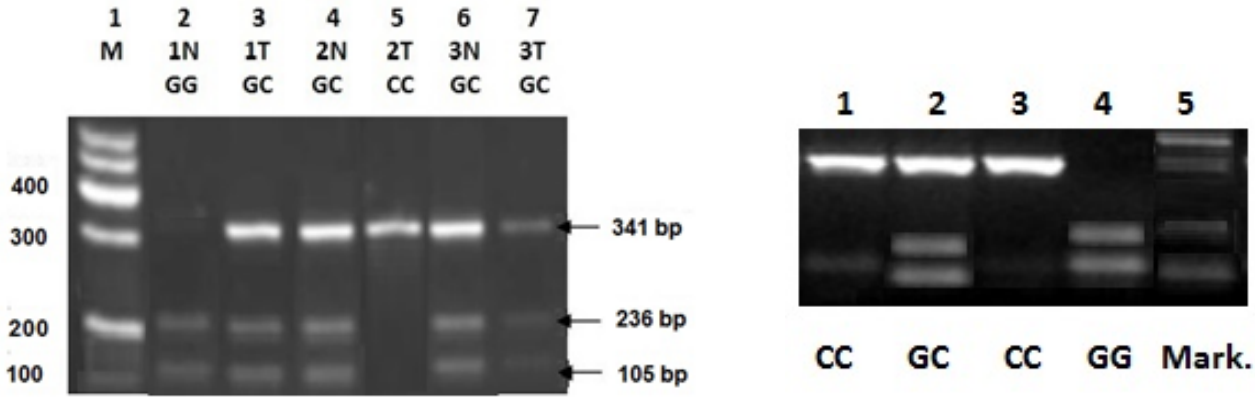

Figure 1. **PCR-RFLP Left: Patient group Lane 1; 100-bp DNA ladder (MBI Fermentas); lanes 2-3, 4-5, 6-7; pairs of normal tissue and tumour tissue from same patient. Right: Control group Lane 5 is marker for 100 pb.**

Discussion

We evaluated the -31 G/C polymorphism by comparing the genotypes of the normal tissues and tumour tissues versus genotypes in the control group. Genotypes of the tumour tissues and normal tissues in the patient group were found to be significantly different compared to the control group. This is the first study to compare survivin -31 G/C polymorphism between normal and tumour tissues in patients with colorectal cancers. We demonstrated differences between tumour tissue in the patient group and control in our study.

Survivin is an anti-apoptotic gene known to play an important role in cell cycle regulation. There is no doubt that excessive expression of the survivin gene plays a crucial clinical role, and this has been demonstrated by studies of various cancer types. In the present study, we investigated whether the -31G/C polymorphism in the survivin gene contributes to the tumour development compared to normal tissues in colorectal cancers. Consistent with the previous studies indicating an elevated frequency for the -31C allele in patients with lung and gastric cancer, our results demonstrated that the -31CC genotype and -31C allele are associated with a significantly increased risk for colorectal cancers. Despite the existing evidence regarding the survivin promoter polymorphism -31G/C and cancer susceptibility, the -31G/C polymorphism has also been investigated in human epidemiological studies to determine whether there was any possible association between this polymorphism and development of cancer [3, 8-12,14].

The polymorphism investigated in the present study may increase the risk of gastrointestinal cancer, particularly cancers of gastric and colorectal origin. The transition from normal tissue to tumour tissue may be of great importance as it has been reported that the survivin -31G/C polymorphism might increase the risk of gastrointestinal cancer, especially gastric and colorectal cancers [17]. Study findings differ regarding -31G/C polymorphism. The results obtained in studies on survivin -31G/C vary across different countries, and different findings are reported in different populations and with different tumour locations [12, 17, 18]. According to our results, a change from genotype G to C was seen in the patient group.

The results of the meta-analysis by Yan Liu *et al*. indicate that survivin -31GC polymorphism is associated with an increased risk of GIT cancer. In the analysis stratified by cancer types, significant associations were observed between survivin -31G/C polymorphism and an increased risk for colorectal and gastric cancers. However, the lack of association between survivin -31G/C polymorphism and oesophageal cancer risk may be due to the insufficient number of eligible studies and the influence of different genetic and environmental factors. Owing to the complex functional mechanism and regulatory roles of survivin in tumour genesis, the relationship between survivin -31G/C polymorphism and GIT cancer susceptibility has been widely studied; however, the findings of these studies have proven to be inconsistent [17]. A clinical genetic study suggests that the CC genotype of survivin -31G/C polymorphism may increase colorectal cancer risk among Caucasians [3].

A statistical association has been reported for CC genotype and oesophageal cancer susceptibility [2]. Finally, polymorphism of -31 G/C change from country to country. The CC allele has been reported to increase the risk of gastrointestinal cancer. To the best of our knowledge, only one study has been conducted in Turkish population, which evaluated hepato-cellular carcinoma,

and reported no significant difference [19].

In our study, we found a significantly higher distribution of the C allele group of patients with colorectal cancer compared to the control group. However this study had a small sample size. We believe that more significant and robust results may be obtained with a larger sample size.

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