RESEARCH ARTICLE

Association Between Survivin Gene Polymorphisms and the Susceptibility to Colon Cancer Development in the Turkish Population

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Abstract

Background: Colon cancer is one of the most common cancers worldwide. Apoptosis is a necessary physiological process for cell elimination which is very important both cellular homeostasis and cell proliferation and differantiation. Dysregulation can lead to uncontrolled cell growth and tumor development. Survivin, a member of the IAP family, plays a key role in promotion of cell proliferation as well as inhibition of apoptosis in cancer cells. The aim of this study was to investigate whether specific genetic polymorphisms of survivin could be associated with colon cancer development and progression in a Turkish population. Our study is the first to our knowledge to investigate the relationship between colon cancer risk and survivin gene polymorphisms. Materials and Methods: The relation between colon cancer and survivin -31 G/C (rs9904341), -241 C/T (rs17878467) and -625 C/G (rs8073069) polymorphism in promotor site of survivin gene associated with apoptosis was investigated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: Individuals with -31C allele and CC genotype were found to have a higher risk of developing colon cancer (OR=13.4, p=0.01). The -241 CT genotype considerably increased the risk of colon cancer (OR=12.0, p=0.0001). However, there was no significant varaition of the survivin -625 C/G polymorphism among colon cancer patients and controls in our study. Conclusions: This study provides the first evidence that survivin -31 G/C and -241 C/T SNP significantly contribute to the risk of colon cancer in the Turkish population.

Keywords: Colon cancer - survivin - polymorphism - PCR-RFLP - susceptibility - Turkey

Asian Pac J Cancer Prev, **15 (20)**, 8963-8967

Introduction

Cancer is the abnormal cell growth that causes cell proliferation and morbidity due to multiple gene expressions, and if not treated, it may result in tissue invasion and metastasis leading to death. Gastrointestinal cancers (GICs) are the most common malignant tumors along with lung and breast cancer in the world (Liu et al., 2013; Turgut et al., 2014). Among GICs, the colon cancer is the most common and malignant cancer in human. More affecting older age groups, the incidence of this disease increases after 50 age and is most often seen in 60-70 years (Jemal et al., 2004). Both dietary and lifestyle factors place patients at an increased risk of developing colon cancer. Excessive fatty diet, excess consumption of red meat, less ingestion of dietary fiber, obesity, smoking and alcohol use are very important risk factors in the development of colon cancer (Fenoglio- Preiser et al., 1999; Anne et al., 2007; Shike et al., 1990).

Colon cancer is thought to occur as a result of mutations in oncogenes and tumor suppressor genes or DNA mismatch repair gene change (Uchida et al., 1998). There are two different pathways in the development of colon cancer that have been seen the gradual accumulation of multiple mutations. At first of them is APC/β- catenin way that has been known as adenoma-carcinoma process. This mechanism is seen in approximately 80% of sporadic cancers. The second is associated with inactivation of the DNA repair gene. This was observed in 10-15 % of sporadic cancers (Al-Sohaily et al., 2012). Apoptosis, also known as programmed cell death, has evolutionarily conserved, and it is a necessary process for organ development, tissue remodeling and the suppression of the immune response (Qiao et al., 2009). Inhibitor of Apoptosis (IAP) proteins is a family of proteins with anti-apoptotic functions that contribute to the evasion of apoptosis. IAP proteins are expressed at high levels in a variety of human cancers including colon cancer (Srinivasula et al., 2008; Altieri et al., 2010; Mace et al., 2010). At 16.5 kDa, survivin is the smallest mammalian member of the inhibitor-of-apoptosis

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(IAP) gene family (Riedl et al., 2001). Alternative splicing of survivin pre-mRNA produces five different mRNAs, which potentially encode five distinct proteins, survivin, survivin 2B, survivin DEx3, survivin 3B13 and survivin 2α (Ryan et al., 2009). Survivin regulates two essential cellular processes; it inhibits apoptosis and promotes cell proliferation (Riedl et al., 2001). Survivin play a main role in cancer progression because it prevents apoptosis by inhibition of caspase 3 and caspase 7, and by regulating the G2 and M phases of the cell cycle (Verdecia et al., 2000). Overexpression of survivin is associated with neoplasia and metastasis. As a result, survivin might be used as a biomarker and a primary chemotherapeutic target for the detection and treatment of gastrointestinal tract cancer, especially esophageal, gastric, and colon cancers (Altieri, 2003; Montorsi et al., 2007; Li et al., 2008). Survivin -31 G/C, -241 C/T and -625 C/G polymorphisms may be changing the function of cells expressing survivin gene. Also these polymorphisms may be affecting cancer progression, thus we hypothesized that the Survivin -31 G/C, -241 C/T and -625 C/G polymorphisms could influence colon cancer risk.

Materials and Methods

Subjects

This study was conducted 59 (25 female, 34 male), 67 (27 female, 40 male) and 66 (26 female, 40 male) patients with colon cancer for survivin -31 G/C, -241 C/T and -625 C/G gene polymorphisms respectively and 45 (25 female, 20 male) healthy control matched subjects who were in the follow-up Duzce Faculty of Medicine in Duzce University. The mean ages of colon cancer patients for survivin -31 G/C, -241 C/T and -625 C/G polymorphisms were 65.94±13.08, 66.07±12.96, 65.82±12.99 years, respectively. The mean age of control groups were 52.54±11.65. The specimens were taken after obtaining informed consent and the study was conducted prospectively. Medical Ethics Committee of Duzce Medical Faculty approval was obtained for the study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human S ubjects).

DNA extraction and genotyping survivin gene polymorphisms

DNA extraction procedure was explained before (Arslan et al., 2012; Yaykasli et al., 2014). DNA isolation from paraffin embedded tissue was performed using Analytik Jena BlackPREP FFPE DNA KitTM (Germany). Blood specimens for control group were collected in tubes containing EDTA and DNA samples were extracted from whole blood by a Blood DNA Extraction Kit (Invitrogen, PureLinkTM, Carlsbad, California, USA). The - 31 G/C, - 241 C/T, and - 625 C/G genotypes of survivin were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers for PCR (Invitrogen, USA), the restriction enzymes (Fermantas, Lithuania), and the fragments length after digestion are shown in Table 1 (Han et al., 2009). Amplification was carried out on Bioneer My GenieTM 96 Gradient Thermal Block (Daejeon, Korea). The annealing temperature was 66 0C, 61 0C, and 61 0C for - 31G/C, - 241C/T, and - 625 C/G, respectively. After digested by restriction enzymes, the digested products were analyzed on 3% agarose gels and stained with ethidium bromide.

Statistical analysis

SPSS 15.0 was used for statistical analysis of this study. Data are expressed as means±SD. Values of $p<0.05$ were considered statistically significant. Comparison of numerical variables between groups was performed by Student's t-test and comparison of categorical variables between groups was performed by chi-square test. Relationship between all variables were controlled by logistic regression analysis.

Results

Subject characteristics

The analysis included different number of colon cancer patients for each survivin polymorphism and 45 healthy (without any cancer diagnosis) controls. In the age range 25-89, 59 patients diagnosed with colon cancer were included in this study to examine survivin -31 G/C polymorphism. For survivin -241C / T polymorphism, 68 colon cancer patients (between the ages of 25-89) were investigated in the study. 66 colon cancer patients (aged 25-89) were examined for survivin -625 C/G polymorphism in this study. Colon cancer patients were compared with a control group of 45 subjects (aged 31-82). There was no significant difference statistically between colon cancer cases and controls in terms of gender, smoking and alcohol parameters for survivin -31 G/C, -241 C/T and -625 C/G polymorphisms (Table 2).

Association between survivin polymorphisms and risk of colon cancer

According to the results of the statistical analysis of survivin -31 G/C polymorphism, genotype distribution

Table 1. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism-Based Assay of Survivin SNPs

SNP	Position	Sequence of primer	Enzyme	Interpretation(bp)
rs9904341	-31	F: 5'-CGT TCT TTG AAA GCA GTC GAG-3' R: 5'-TGT AGA GAT GCG GTG GTC CT-3'	Eco0109I (Fermentas FastDigest FD0264)	CC 329 CG 329,234,92 GG 234.92
rs17878467	-241	F: 5'-GAT TAC AGG CGT GAG CCA CT-3' R: 3'-GTG TGC CGG GAG TTG TAG TC-3'	HaeII (Fermentas FastDigest FD2184)	TT 159 CT 159,95,64 CC 95,64
rs8073069	-625	F: 5'-TGT TCA TTT GTC CTT CAT GCGC-3' R: 5'-CCA GCC TAG GCA ACA AGA GCAA-3'	BstUI (Fermentas Fast Digest FD0924)	CC 125 CG 125,104,21 GG 104,21

Table 2. Characteristics of Study Groups

SNP	Parameters		Control	Patients	P value
Survivin -31 G/C	Gender	Female	24 (53.5%)	25 (42.4%)	0.267
		Male	21 (46.7%)	34 (57.6%)	
	Smoking	Yes	7 (18.9%)	10 (18.9%)	0.995
		No	30 (81.1%)	43 (81.1%)	
	Alcohol	Yes	1 (2.7%)	6 (11.3%)	0.133
		No	36 (97.3%)	47 (88.7%)	
Survivin -241 C/T	Gender	Female	25 (55.6%)	27 (40.3%)	0.112
		Male	20 (44.4%)	40 (59.7%)	
	Smoking	Yes	7 (18.9%)	12 (20.3%)	0.865
		No	30 (81.1%)	47 (79.7%)	
	Alcohol	Yes	1 (2.7%)	6 (10.2%)	0.171
		No	36 (97.3%)	53 (89.8%)	
Survivin -625 C/G	Gender	Female	25 (55.6%)	26 (39.4%)	0.093
		Male	20 (44.4%)	40 (60.6%)	
	Smoking	Yes	7 (18.9%)	11 (18.3%)	0.943
		No	30 (81.1%)	49 (81.7%)	
	Alcohol	Yes	1 (2.7%)	6 (10%)	0.177
		No	36 (97.3%)	54 (90%)	

*p values less than 0.05 denoted statistical significance; **n=number of individuals, Chi-square test was used to compare gender , smoking and alcohol in the study group

Table 3. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism-Based Assay of Survivin SNPs

SNP	Genotype	Control (n=45)		Patient (n=59)		Univariate analysis	Logistic regression	
		Number	%	Number	%	P	OR	P
-31 G/C	GG (reference)	25	55.6	16	26.7	0.011	1.00	---
	GC	16	35.6	35	58.3		5.39	0.006
	CC	4	8.9	9	15		13.40	0.01
-241 T/C	TT	3	6.7	8	11.8	0.0001	2.04	0.44
	CT	7	15.6	39	57.4		12.00	0.0001
	CC (reference)	35	77.8	21	30.9		1.00	---
-625 C/G	GG (reference)	13	28.9	38	56.7	0.0001	1.00	---
	CG	18	40	29	43.3		0.000	0.998
	CC	14	31.1	0	0		0.80	0.68

*p values less than 0.05 denoted statistical significance; **n=number of individuals, Chi-square test and logistic regression were used to compare genotype in the study group

was determined statistically significant difference between colon cancer patients and controls ($p<0.05$). Risk of developing colon cancer was found to be significantly higher in individuals carrying homozygous for the C allele (OR=13.4). Also, colon cancer risk in individuals carrying heterozygous GC genotype was found to be higher than individuals carrying homozygous G allele (OR=5.39). Results was shown in Table 3. Genotype distribution of survivin -241 C/T polymorphism statistically significant difference was found between colon cancer patients and control ($p<0.05$). The heterozygous CT genotype was determined to increase the risk of developing colon cancer (OR=12.0, $p=0.0001$) (Table 3). Genotype distribution of Survivin -625 C/G polymorphism was examined, a statistically significant difference was not found between patients with colon cancer and the control group ($p<0.05$) (Table 3).

Discussion

Colon cancer is seen a common form of cancer in populations that are economically advanced. 5 to 10% of colon cancer cases is inherited, remainder occurs sporadically (Macaron et al., 2014). Causes and mechanisms of colon cancer are associated with genetic and environmental factors, especially diet. (Azcarate-Peril et al., 2011). Apoptosis, necessary for the proper development of an organism, is an essential genetic session (Thompson et al., 1995). The inhibitor of apoptosis proteins (IAPs) is a family of antiapoptotic proteins, including inhibit initiator and effector caspases to prevent apoptosis (Mace et al., 2010). Survivin, which is member an inhibitor of apoptosis protein (IAP), is involved in the regulation of apoptosis and in cell cycle control. In vivo and in vitro studies have shown that increased expression of survivin plays an important role in the development and progression of malignant neoplasms by reducing tumor cell apoptosis (Riedl et al., 2001; Johnson et al., 2004). Therefore, survivin might be used as one of the most important diagnostic and prognosis markers for many types of cancers (Li et al., 2013). As a result of our study, survivin -31 G/C and -241 C/T polymorphisms statistically significant difference between colon cancer and control. Survivin -31 CC genotype and genotype -31 GC were found to be significantly higher risk of developing colon cancer. C allele was observed to be significantly increased risk of developing colon cancer. Survivin -241 CT genotype was found to be significantly higher risk of developing colon cancer. However, no significant difference was found for survivin -625 C/G polymorphism between colon cancer patients and controls. Similarly, Han et al (2009) have shown carrying of homozygous

C allele for -31 G / C polymorphism may be associated with ovarian cancer and they have not found a statistically significant importance for -625 C/G polymorphism between patients with ovarian cancers and controls. But, they have not found a significant relationship between 241 C/T, and ovarian cancer. Jaiswal et al (2011) have determined carrying homozygous C allele for -31 G/C polymorphism increases 2.6 fold risk of bladder cancer. Also, homozygous C allele for survivin -31 C/G has been shown that increased cancer risk in urothelial cancer (Wang et al., 2009), bladder cancer (Kawata et al., 2011), nasopharyngeal carcinoma (Ma et al., 2011), esophagus cancer (Upadhyay et al., 2011), prostate cancer (Chen et al., 2013) and colorectal cancer (Gazouli et al., 2009; Li et al., 2013) studies. Yazdani et al. (2012) also have demonstrated that GC and CC genotype distributions of survivin -31 G/C polymorphism were found statistically significant differences between thyroid cancer patients and controls. Gazouli et al. have investigated survivin -31 GC polymorphism in sporadic colorectal cancer. At the end of their study, they have found that survivin -31 C allele and CC genotype was higher in patients with colorectal cancer than control group. Li et al. (2013) have determined statistically significant of CC genotype frequency compared with GG and GC genotypes for survivin -31 G/C in colorectal cancer patients. However, there were no significant difference of survivin -241 C/T polymorphism distributions among cases and controls in their study. In contrast to our results, carrying G allele for survivin -31 C/G has reported that increased cancer risk in renal cell carcinoma (Qin et al., 2012), stomach cancer (Borges et al., 2011) and lung cancer (Jang et al., 2008) studies. Additionally, GG genotype for survivin -31 C/G polymorphism has been shown a risk factor in keratocystic odontogenic tumor development (Andric et al., 2012). Aynaci et al. (2013) has reported that individuals carrying survivin 31 GC genotype had a significantly decreased risk of having non-small cell lung cancer. Yang et al (2009) have reported that -625 CC genotype increased risk of squamous cell esophagus carcinoma. Some previous studies have shown that survivin -31 G/C, -625 C/G polymorphisms were not associated with hepatocellular carcinoma (Li et al., 2012), non-small cell lung cancer (Dai et al., 2010) and cervical cancer (Borbely et al., 2007). Kostic et al (2013) have demonstrated that survivin -31 G/C polymorphism was not a risk factor for oral squamous cell carcinoma and skin basal cell carcinoma. In conclusion, our findings provide new evidence for the association between survivin -31 G/C and -241 C/T SNP and the risk of colon cancer in a Turkish population. As a result of this study showing compliance with the previous studies, survivin gene may be a potential marker for colon cancer but further functional studies are required to clarify this issue in detail. Our results need to be confirmed in larger cohorts in order to better understand their role in development of colon cancer.

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