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Original Research

A candidate single nucleotide polymorphism in the 3' untranslated region (rs17878624) of survivin gene for NSCLC

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Abstract: Survivin is a gene that locates on human chromosome 17q25 and contains 142 amino acid. Survivin (BIRC5) is the first one of the found inhibitors of apoptosis proteins (IAPs) that is an important protein family and regulates apoptosis. It is expressed particularly in cancer cells. 3'UTR region of gene has components that is necessary for gene function and this region plays a critical role in the regulation of posttranscriptional regulation of the gene expression. Therefore, polymorphisms in this region may affect the function of the gene. The purpose of the study is to investigate possible relationship, that is associated with development and prognosis of the disease, between the 3 'UTR region (rs17878624) polymorphism and NSCLC in a Turkish society.

Key words: NSCLC; Survivin; Gene polymorphism; 3'UTR.

Introduction

Lung cancer is the most widely-spread cancer in the world since 1985. It constitutes about 12-13% of all new cancer cases and 29% of all cancer death worldwide (1). Lung cancer has been classified into Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC) by the World Health Organization. Although, conventional cancer treatment options, surgery and chemotherapy, are applied to this cancer, overall outcome is poor due to recurrence of disease in most of cases and resistance of tumor to great number of anti-cancer agents (2). New, marker is needed to identify high- risk individuals and predict disease outcomes. Survivin seems to be candidate gene for these purposes in lung cancer (especially for NSCLC). it is the gene that encodes for survivin protein which belongs to the human Inhibitors of Apoptosis Protein (IAP) family. IAPs family regulates the cell division preventing the programmed cell death – apoptosis - through inhibiting certain apoptotic proteins called caspases (3,4). Hence, survivin plays an essential role in the development and progression of neoplastic processes suppressing the cancer cell death, thus facilitating the growth of these cells (2). To date, there are eight members of IAPs family. These proteins usually consist of one to three baculovirus IAP repeat (BIR) domains, which are responsible for anti-apoptotic activity, and either a C-terminal RING or caspase activation recruitment (CARD) domain. However, survivin has unique structure containing only a single BIR domain and lacking RING and (CARD) domains (5). Survivin protein is significantly expressed in embryonic and fatal tissues and homozygous survivin deletion leads to early embryonic death, showing its essential role in cell development, differentiation and homeostasis. Whereas its amount is undetectable or very low in terminally differentiated adult tissues. Studies indicate that in contrast to normal cases in variety human cancers including lung cancer surviving gene is dramatically up-regulated (6,7). These findings prove the importance of above mentioned gene in carcinogenesis and thus makes it potential marker for early diagnosis and prognostic point of view. Genetic variation is one of the main factors that can affect the gene expression and thus can mediate the individual's predisposition to cancer. Single-nucleotide polymorphism (SNP) is most common DNA sequence variation type. Identifying the distribution frequency of certain SNP in a population is broadly used strategy in order to predict the risk and prognosis of a cancer (8). There are several SNPs identified to be related with the different region of survivin gene (9,10). The aim of this study to identify whether there is any correlation between a certain SNP (rs17878624) that is located on 3'UTR of survivin gene and NSCLC in Turkish population.

Materials and Methods

Study population and collection of specimens

In the study 163 diagnosed NSCLC patients and 151 healthy controls were examined. NSCLC patients were recruited from the Yedikule Chest Diseases and Thoracic Surgery Training Research Hospital, Istanbul. Samples were collected from Istanbul Yedikule Chest Diseases and Thoracic Surgery Training Hospital Department of Thoracic Surgery clinic. The mean ages of the patients and controls were 60.7 ± 9.57 years and 57.9 ± 9.39 years respectively. The percentage of females was 5.5%

Table 1. Characteristics and laboratory parameters of non-small cell lung cancer patients and controls.

Characteristics	NSCLC patients (N=163)	Control group (N=151)	P value
Male N (%)	154 (94.5)	114 (75.5)	
Female N (%)	9 (5.5)	37 (24.5)	
Age (years, means ±SD)	60,7 ±9,57	57,9 ±9,39	0,814
Smoking history (pack/years)	48,78± 30,850	38,98 ±27,874	0,817
WBC (10^3/mm^3)	9,11± 3,171	6,62±1,679	**0,001**
FEV1	65,64± 18,213	96,59 ±19,509	0,808
FVC	68,8934± 17,18806	98,0595± 16,54910	0,537
FEV1/FVC	78,8142 ±16,07904	96,2329 ±13,86570	0,129

*P obtained by the Student *t*-test. Data are reported as number (percentage in parentheses) or as means ± standard deviation.

for patients and 24.5% for controls, and percentage of males was 94.5% for patients and 75.5% for controls (Table 1.). In NSCLC group, all subjects were diagnosed and confirmed with histopathological examination. One hundred fifty-one healthy persons without any malignancy were selected for the control group that comprised only individuals with a negative family history of cancer. All participants signed an informed consent before enrollment and Institutional Ethical committee approval was obtained for the study. Pulmonary function test and routine biochemical examinations were done after test for voluntaries. The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine (July 08, 2010 No:376).

DNA extraction

DNA was isolated from the blood leukocytes in 10 ml EDTA by the method of Miller et al. based on sodium dodecyl sulphate lysis, ammonium acetate extraction, and ethanol precipitation (11,12). The concentration and purity of DNA were determined using an ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) at A260 and A280. Template DNA (0.5–1.0 ug) was used in a PCR under sterile conditions.

Genotyping

For the genotyping studies, the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods were used. We investigated the polymorphism in the 3' untranslated region of the Survivin gene. 0.25μmol/L of each primer was used for the reaction. The primers shown in Table 2. In a volume of 25 μl containing 1,5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris- HCl (pH:8.4), 0.16 mM each of dNTP (MBI Fermentas), and 1 unit of Taq polymerase (MBI Fermentas). Amplification was performed by initial denaturation at 94ºC for 5 minutes, followed by 35 cycles with denaturation steps at 94ºC for 30 seconds, annealing at 56ºC for 30 seconds, and extension at 72ºC for 30 seconds. The PCR programme was completed by a final extension cycle at 72ºC for 5 minutes (Miller et al., 1988). The appropriate primers (Table 2.) were used to amplify the corresponding gene of the subjects by PCR and the reaction products were digested by using the HphI restriction enzyme at 37°C. The PCR product exhibited a 741 base pair fragment for 3' untranslated region (rs17878624) polymorphism. The PCR product was digested with HphI (MBI Fermentas). Then visualized by electrophoresis on 3% agarose containing 0.5 mg/ml ethidium bromide and examined under transillumination. If there is any conflict, samples were repeated (Figure 1).

Statistical analysis

All statistical analyses were carried out using the SPSS version 17.0 for Windows. Numeric values were analyzed by student's t-test. Chi-square test used that Survivin 3' untranslated region of the prevalence of the genotype with alleles differences between groups with for assessing together. The relative associations between NSCLC patients and controls were assessed by calculating crude Gart's odds ratios (ODs) and 95% confidence intervals (95%CI). Threshold for significance was $p<0.05$.

Results

Genotype and allele frequencies for Survivin 3'UTR region (rs17878624) in NSCLC patients and controls are listed in Table 3. The distribution of the survivin 3'UTR

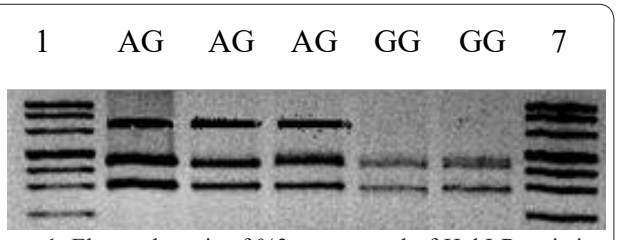

Figure 1. Electrophoresis of %3 agarose gel of HphI Restriction Enzyme digestion. PCR-RFLP to detect 3'UTR polymorphism of Survivin. Polymerase chain reaction products (741 bp) digested with restriction enzyme HphI and analyzed by 3% agarose gel. Lanes 1 and 7 = pUC Mix Marker, 8, ready- to-use DNA ladder (MBI Fermentas); lanes 2, 3, 4 = heterozygotic for G Allele; lanes 4 and 5 = homozygotic wild type genotypes.

Table 2. PCR-RFLP products of Survivin 3' UTR region (rs17878624).

	Primers	PCR product	Restriction enzyme	Restriction products
3'UTR region (rs17878624)	R:5'-TCATCTTACGCCAGACTTCAG-3' F:5-GAGAAGTGAGGGAGGAGG-3'	741 bp	HphI	AG: 741/401/340 bp GG: 401/340 bp AA: 741 bp

bp = base pair; F = Forward; R = Reverse.

Table 3. Survivin 3'UTR polymorphism allele and genotype frequencies in patients with NSCLC and controls.

	NSCLC patients (N=163)	Control group (N=151)	P value
Survivin 3'UTR Genotype			
GG	92 (56.4%)	105 (69.5%)	**0.016**
AG	71 (43.6%)	46 (30.5%)	
AA	0 (0%)	0 (0%)	
Survivin 3'UTR allele			
G	255 (78.23%)	256 (84.76%)	**0.035**
A	71 (21.77%)	46 (15.24%)	

genotypes in control and patients was found to be significantly different (p=0,016). The prevalence of the survivin 3'UTR GG homozigosity was 56.4% (92/163) in patients and 69.5% (105/151) in the control group. Individuals carrying survivin 3'UTR GG genotype had a 2-fold decreased risk for NSCLC cancer (*p=0.016, χ2:5.75; OR: 0.568 %95CI:0.357-0.903). There was no association between 3'UTR region (rs17878624) frequencies and tumor stage, lymph node and metastasis status in NSCLC patients. Survivin 3'UTR region (rs17878624) genotype and smoking (pack/years) did not reach significance.

Discussion

During cancer formation in healthy differentiated tissues, unlike Bcl-2 and other IAPs, abnormalities in apoptosis mechanisms play a critical role. In cancer the apoptosis mechanism is highly suppressed by anti-apoptotic proteins. One of the most important of these proteins is survivin which is responsible for cell cycle regulation. Apart from survivin there have been various proteins identified which are found to be upregulated in cancer cells. One other mechanism how the apoptotic signal is bypassed is the regions in the protein structure called BIR (Baculovirus IAP repeat) which binds to the caspase proteins who are the main effectors of apoptosis in a cell. One research on survivin (13) suggests that polymorphisms in the promotor sequence of the survivin gene can cause genetic modification in many other cancer cells other than small cell lung cancer cell lines. Another research (14) demonstrates the correlation between the formation of hepatocellular carcinomas and T9809C polymorphisms located at survivin gene's 3' UTR. Studies show that nasopharangeal cancer development is is dependant on the genetic make-up mir-218 and survivin genes, 3' UTR (15). Another case demonstrated Mityaev et al, suggested for the treatment of lung cancer by gene therapy up regulation of survivin promoter sequences can be paramount (16). A 2012 study by Chang et al. proved the relationship between development of esopharangeal cancer and the 3' UTR sequences of survivin and CUG-BP1 (17). From a clinical perspective, prediction of indivual patterns in treatment is mainly reliant on the patients genetic make-up of survivin gene regulation and genetic variations. It is safe to say these factors influence and affect the responses against treatment and acts as a precursor. For example, the high survivin upregulation in plevral efusions are proved to be a precursor of a bad prognosis by Lan et al. in their publication in 2010 (18-20). These studies show that survivin inhibits apoptosis and it regulates cell cycle which develops angiogenesis. These studies also show that survivin has significant expression in many kinds of human tumors. So it can be therapeutic target.

To conclude, our case study of Turkish population genetics on the survivin gene 3' UTR polymorphisms (rs17878624) show that GG genotype provides about twice as much protection from forementioned diseases. In addition to this; we also proved that survivin is association with development and prognosis of disesases, between 3'UTR region (rs17878624) polymorphism and NSCLC in Turkish population.

Interest conflict

There is no conflict of interest among the author.

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