Abstract. Background/Aim: Heat-shock proteins (HSPs) are molecular chaperones which modify the structures and interactions of other proteins. The aim of our study was to investigate HSP90AA1, HSP90AB1 and HSP90B1 gene polymorphisms in patients with non-small cell lung cancer (NSCLC).

Materials and Methods: Ninety-seven patients with NSCLC and 97 healthy controls were included in the study. Real-time polymerase chain reaction was used for genotyping. Results: The frequency of mutant CC genotype for HSP90AA1 (rs4947C/T), mutant AA genotype for HSP90AB1 (rs13296A/G) and mutant CC genotype for HSP90B1 (rs2070908 C/G) was significantly higher in the patient group than in controls (p=0.019, p=0.004 and p=0.036, respectively). The frequency of patients with homozygote mutant allele was also significantly higher than that of controls and possessing of the mutant genotype increased the risk for disease by approximately 2.9, 4.8, 1.9 for HSP90AA1, HSP90AB1 and HSP90B1, respectively. The present study appears to be the first of its kind to report data on these gene polymorphisms in patients with NSCLC in the Turkish population.

The Significance of HSP90AA1, HSP90AB1 and HSP90B1 Gene Polymorphisms in a Turkish Population with Non-small Cell Lung Cancer

ENDER COSKUNPINAR1, NERGIZ AKKAYA1, PINAR YILDIZ2, YASEMIN MUSTERI OLTULU1, ENGIN AYNACI3, TURGAY ISBIR4 and ILHAN YAYLIM1

1Department of Molecular Medicine, Institute for Experimental Medicine (DETAE), Faculty of Pharmacy, Istanbul University, Capa, Istanbul, Turkey;
2Third Clinic, Yedikule Teaching Hospital for Chest Diseases and Thoracic Surgery, Istanbul, Turkey;
3Department of Chest Diseases, Faculty of Medicine, Medipol University, Istanbul, Turkey;
4Department of Medical Biology, School of Medicine, Yeditepe University, Istanbul, Turkey

Correspondence to: Associate Professor B. Pinar Yildiz, Yedikule Chest Disease and Surgery Training and Research Hospital, Third clinic, Zeytinburnu, Istanbul, Turkey. Tel: +90 05333585708, e-mail: pinary70@yahoo.com

Key Words: NSCLC, HSP90AA1, HSP90B1, HSP90AB1, SNPs, Turkish population.
**Materials and Methods**

**Study groups.** Ninety-seven patients diagnosed as having NSCLC (92 men; 5 women) and 97 healthy individuals (68 men; 29 women) were admitted to the Istanbul Yedikule Chest Diseases and Thoracic Surgery Training Hospital and consecutively included in the case–control study. Patients who were referred to our clinic and diagnosed with NSCLC were informed of the study. Ninety-seven healthy persons without any malignancy were selected for the control group that comprised only individuals with a negative family history of cancer. The patient and control groups were matched for age. All participants signed an informed consent form before enrollment and Institutional Ethical committee (Istanbul University, School of Medicine Ethical Committee, November 14, 2011; no. 1825) approval was obtained for the study. Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood according to the kit protocol (Roche Diagnostics GmbH, Mannheim, Germany).

**Genotyping by real-time-PCR.** Three single nucleotide polymorphisms (rs4947C/T, rs13296 A/G and rs2070908 C/G) were analyzed in three different genes: HSP90AA1 [TTTGAAACAG AAGAAGAAAACA(A/C)TATCAAATGTATGACGAGGT TT], HSP90AB1 [TCTGAAACCCCCATCTCGA TCTTGGG (A/G) TTTTGCTCATCTCTATCTCTCTCT], HSP90B1 [GGGTGAAA GCGGCCCGACCTGCTTG(C/G)GGTGACGCACGCG CGCGT] by using quantitative real-time PCR (RT-PCR LightCycler; Roche Diagnostics). Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was isolated from blood with a spin column kit (Roche Diagnostics) according to the manufacturer’s instructions. Quantitative RT-PCR for genotyping was performed on the LightCycler 1.5 system using 1 μl of hybridization probe pair (Light Cycler Fast Start DNA Master HybProbe) labeled with 3'-fluorescein and 5'-LightCycler Red. The following protocol was used for amplification; initial denaturation at 95°C for 10 min, followed by 40 cycles with denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 10 s. An additional melting curve analysis was performed at 95°C for 30 s, 40°C for 2 min and 75°C for 0 s in order to detect non-specific amplifications.

**Statistical analysis.** All statistical analyses were carried out using the SPSS version 17.0 (IBM, Armonk, NY, USA) statistical package for Windows. The Chi-square test was used to assess both the prevalence of the genotypic distribution, and allelic differences between groups. The associations between expression status and clinicopathological parameters were analyzed using Chi-square and Fisher’s exact test. The relative associations between patients and controls were assessed by calculating crude Garth’s odds ratios (ORs) and 95% confidence intervals (95% CIs). The threshold for significance was p<0.05. Linkage disequilibrium between different polymorphisms was assessed using D0 and r2 values obtained with the Haploview program (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). A multivariate logistic regression model was used to investigate the effects of genotypes and alleles after adjustment for age. Values of p<0.05 were considered to be statistically significant.

**Results**

The frequency of males was significantly higher for patients (94.8%) in comparison to the control group (70.1%), with a 7.8-fold increased risk for lung cancer (p<0.0001, Chi-square=20.541, OR=7.847, 95% CI=2.888-21.320). From a total of 97 patients with NSCLC included in our study, the histological tumor type was defined as squamous cell in 32 (33%) cases, adenocarcinoma in 20 (21%), malignant carcinoid tumor in 1 (1%), large cell carcinoma in 6 (6%), while 38 (39%) were described as unclassified NSCLC.

The distribution of the HSP90AA1 (rs4947C/T) genotypes in the patient group was significantly different when compared to that of the controls (p=0.019) (Table I). The prevalence of TC heterozygosity was 25.8% (25/97) in patients and 17.5% (17/97) in the control group. The frequency of homozygous CC genotype (14.4%) was significantly higher in patients than in controls (5.02%) (p=0.019). Moreover, the frequency of the C allele was higher in controls when compared with NSCLC (27.3% and 13.9% respectively; p=0.001, Chi-square=10.64) (Table I). The distribution of genotype and alleles and comparisons between study groups is shown in Table I.

Genotypic and allelic frequencies for HSP90AB1 (rs13296A/G) in patients with NSCLC and controls are listed in Table II. Statistically significant differences in both distribution of genotypes and alleles of HSP90AB1 (rs13296A/G) were found. The frequency of the homozygous AA genotype was almost five-fold higher in NSCLC compared to controls (p=0.004), and that of individuals with A allele, similarly, 1.7-fold higher (Chi-square=7.2, p=0.007) (Table II).

The distribution of HSP90B1 (rs2070908 C/G) genotype and allelic frequency are shown in Table III. The frequency of the homozygous CC genotype was almost two-fold higher in patients when compared to controls (p=0.036). In addition, the frequency of C allele carriers was higher in NSCLC than controls (Chi-square=6.5, p=0.01).

The risk of metastasis was related to GA heterozygocity for the HSP90B1 genotype (p=0.026). When we compared patients with advanced-stage (stage 3 and 4) and early-stage (stage 1 and 2) disease, individuals carrying A alleles of the HSP90AB1 gene had a 1.79-fold increased odds for advanced-stage disease (p=0.018, OR=1.786, 95% CI=1.022-3.120).

No linkage disequilibrium was found between the three genes. Haplotypes were evaluated for association with NSCLC (Table IV). The frequencies of individuals with haplotype-1 (CGA), haplotype-3 (TCA), and haplotype-5 (TCG) were significantly higher in NSCLC when compared to controls (p-values 0.0286, 0.0357 and 0.001, respectively) (Table IV).
Discussion

In the current study, real-time quantitative PCR was used to investigate the possible association between polymorphisms in \textit{HSP90AA1} (rs4947C/T), \textit{HSP90AB1} (rs13296A/G), \textit{HSP90B1} (rs2070908C/G) and NSCLC. To our knowledge, this is the first work to evaluate the collective impact of these single-nucleotide polymorphisms on NSCLC in our ethnic population. According to our results, the frequency of CC genotype for \textit{HSP90AA1} (rs4947C/T), AA genotype for \textit{HSP90AB1} (rs13296A/G) and CC genotype for \textit{HSP90B1} (rs2070908) was significantly higher in NSCLC when compared to controls (\(p\)-value of 0.019, 0.004 and 0.036, respectively).

HSP molecular chaperones guide the normal folding, intracellular disposition and proteolytic turnover of many of the key regulators of cell growth, differentiation and survival. Recent studies have reported that HSP90 and HSP70, in particular, might have an important role in the process of carcinogenesis (17-19). HSP90 was localized not only in cancer cells, but also in cells adjacent to cancer cells. It was also shown that both \textit{HSP90\alpha} and \textit{-\beta} mRNAs were overexpressed in the cytoplasm around the nucleus of cancer cells (20). Overexpression of HSP90\alpha has been reported in various types of cancer, such as human leukemia cells (18) and pancreatic carcinomas (19). In addition, HSP90\beta has been reported to inhibit apoptosis and cell differentiation (21). Currently, HSP90 inhibitors are being developed as anticancer agents. Early studies have shown promising results in a subgroup of solid tumors such as ALK-rearranged NSCLC (22).

Although lung cancer has complex and various genetic abnormalities, the molecular nature of carcinogenesis is not fully-understood. Understanding the complex networks that lead to cancer development could lead to development of molecular target-based anticancer treatment modalities which might allow for better prognosis of this deadly disease. Increased expression of HSP90 proteins has been shown in different types of tumors (23). Up-regulation of both cytosolic HSP\alpha and HSP90\beta have been demonstrated in various tumors, including lung cancer, with data for HSP90\beta being considerably limited (24, 25). Prognostic implication and relationship with metastatic potential has been implicated by the same authors (25). Inhibition of HSP90 can lead to degradation of multiple oncogenic signaling proteins which are involved in tumor progression (26). HSP90\alpha has been shown to be associated with poor prognosis in human breast cancer (27). In our study, we showed that the risk of metastasis was related to the GA heterozygocity for \textit{HSP90AB1} genotype (\(p=0.026\)). Then we compared patients with advanced-stage (stage 3 and 4) and early-stage (stage 1 and 2) disease, are found individuals carrying A alleles of \textit{HSP90AB1} gene had a 1.78-fold increased risk for advanced-stage disease (\(p=0.018\)). Our results suggest that \textit{HSP90AB1} (rs13296A/G) polymorphism might have a prognostic impact in NSCLC.

In a study investigating the role of \textit{HSP90} Gln488His (C>G) polymorphism as a potential risk factor for breast cancer, a significant association between HSP90 G allele and breast cancer risk was detected (28). Urban \textit{et al.} (29) aimed
to characterize single-nucleotide polymorphism of human cytosolic HSP90 genes (HSP90AA1 and HSP90AB1). It was concluded that all HSP90 single-nucleotide polymorphisms would have a limited effect on predicting functional sequence in key domains of the human HSP90 proteins. Their DNA samples originated from 26 Caucasians, 27 African Americans, 18 Chinese, 13 Japanese, 10 Mexicans and seven Southeast Asians, and a total of 15 exonic single-nucleotide polymorphisms were identified in their study. To date, there have been limited data examining the presence of cytosolic HSP90 SNPs in the human population (29-31). This study is the first, to our knowledge, which analyzed HSP90 gene variants in a Turkish population. We showed that genotype distribution for each gene and allelic predominance is significantly different in NSCLC compared to healthy controls. We clearly showed that the frequency of the patients homozygous for the mutant allele of all genes (HSP90AA1, HSP90AB1, HSP90B1) was also significantly higher than in controls. Possessing a mutant genotype increases the odds of disease by approximately 2.9-, 4.8- and 1.9-fold, respectively. It can be concluded that all HSP90 single-nucleotide polymorphisms might be related to an increased risk of lung cancer. Such a hypothesis needs to be analyzed with large population-based multicenter studies.

To our knowledge, these are the first data to evaluate the presence of HSP90 gene variants in a Turkish population. We showed that genotype distribution for each gene and allelic predominance is significantly different in NSCLC compared to healthy controls. We clearly showed that the frequency of the patients homozygous for the mutant allele of all genes (HSP90AA1, HSP90AB1, HSP90B1) was also significantly higher than in controls. Possessing a mutant genotype increases the odds of disease by approximately 2.9-, 4.8- and 1.9-fold, respectively. It can be concluded that all HSP90 single-nucleotide polymorphisms might be related to an increased risk of lung cancer. Such a hypothesis needs to be analyzed with large population-based multicenter studies.

Acknowledgements

The present work was supported by a grant from the Scientific Research Projects Coordination Unit of Istanbul University (Project No: 21163). The Authors would like to thank Mrs. Aylin Çayirli for her help in the data collection.

References


Table IV. Results of the haplotype analysis in patients and controls (HSP90AA1, HSP90AB1 and HSP90B1 genes). The frequencies of haplotype-1 (CGA), haplotype-3 (TCA), haplotype-5 (TCG) were significantly higher in patients with NSCLC when compared with controls.

<table>
<thead>
<tr>
<th>Haplotype no.</th>
<th>Haplotype</th>
<th>Frequency</th>
<th>Total</th>
<th>NSCLC</th>
<th>Controls</th>
<th>( \chi^2 )</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CGA</td>
<td>0.385</td>
<td>0.439</td>
<td>0.330</td>
<td>4.793</td>
<td>0.0286</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CCA</td>
<td>0.162</td>
<td>0.135</td>
<td>0.190</td>
<td>2.179</td>
<td>0.1399</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TCA</td>
<td>0.150</td>
<td>0.188</td>
<td>0.112</td>
<td>4.41</td>
<td>0.0357</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>TGA</td>
<td>0.097</td>
<td>0.074</td>
<td>0.120</td>
<td>2.416</td>
<td>0.1201</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TCG</td>
<td>0.090</td>
<td>0.137</td>
<td>0.042</td>
<td>10.78</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CGG</td>
<td>0.066</td>
<td>0.077</td>
<td>0.055</td>
<td>0.74</td>
<td>0.3895</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CCG</td>
<td>0.044</td>
<td>0.051</td>
<td>0.038</td>
<td>0.377</td>
<td>0.5391</td>
<td></td>
</tr>
</tbody>
</table>


