Polymorphisms in Apoptosis-Related Genes and TP53 Mutations in Non-Small Cell Lung Cancer

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Environmental exposure, primarily cigarette smoke, is the most important risk factor for lung cancer. The genetic and epigenetic damage caused by environmental carcinogens is considered to be the major mechanism underlying the development of lung cancer (1). Somatic mutations of the TP53 gene are among the most frequent genetic alterations detected in lung cancer and occur in ~50% of patients with non-small cell lung cancer (NSCLC) (2). TP53 mutations often occur in pre-neoplastic lesions and in histologically normal lung tissue surrounding the tumor (3). In addition, TP53 mutations have been reported to be associated with a poor prognosis, as well as with a poor response to chemotherapy and radiotherapy (4). Thus, TP53 mutations play an important role not only in the development and progression of NSCLC, but also in determining the therapeutic response. Therefore, identification of genetic factors related to the occurrence of TP53 mutations would be help to find novel ways to prevent and manage lung cancer. In view of this point, several studies have shown that polymorphisms of the genes involved in xenobiotic metabolism and DNA repair are associated with the occurrence of TP53 mutations (5-7).

Apoptosis is a highly regulated process of cell death which controls the cell number in multicellular organisms and eliminates unnecessary or damaged cells. It is assumed that a decreased ability to eliminate cells with DNA damage may facilitate the accumulation of somatic mutations, and thereby contribute to tumor initiation, progression, and metastasis (8). There is considerable interindividual variation in apoptotic capacity, and such variation is largely attributed to an individual’s genetic constitution (9). In addition, it has been reported that several polymorphisms in apoptosis-related genes affect the expression or activities of enzymes and therefore the polymorphisms are associated with the risk and prognosis of various human cancers,
including lung cancer (10, 11). Therefore, it has been hypothesized that an alteration in apoptotic capacity related to polymorphisms of apoptosis-related genes could affect the risk of TP53 mutations in lung cancer. To test this hypothesis we investigated the relationship between polymorphisms of apoptosis-related genes and TP53 mutations in NSCLCs.

Tumor and corresponding non-malignant lung tissue specimens were provided by the National Biobank of Korea-Kyungpook National University Hospital, Daegu, Korea, which is supported by the Ministry of Health, Welfare and Family Affairs. The population used in the present study was evaluated previously for TP53 mutations (12). The patients underwent curative resection at Kyungpook National University Hospital from January 2003 to July 2007 and registered collectively to the National Biobank in April 2011. All materials derived from the National Biobank were obtained under Institutional Review Board approved protocols (Approval No., KNUHBIO_10_1017). All of the patients included in this study were ethnic Koreans. Patients who underwent chemotherapy or radiotherapy prior to surgery were excluded to avoid the effects on DNA. Of 176 patients in our previous study (12), three squamous cell carcinoma patients including one harboring a TP53 mutation were excluded in the present study because of lack of available DNA. As a result, this study included 56 patients with squamous cell carcinoma and 117 patients with adenocarcinoma. There were 113 males and 60 females in the study cohort. The patients consisted of 56 never-smokers and 117 smokers. Of the 117 patients with adenocarcinoma, 54 were never-smokers. All of the tumor and macroscopically-normal lung tissue samples were obtained at the time of surgery, and rapidly frozen in liquid nitrogen, and stored at -80°C. Only tumors with greater than 80% of the tumor component were sent for analysis. This study was approved by the Institutional Review Board of Kyungpook National University Hospital. The methods and results of TP53 mutation analysis have been described in our previous study (12). Briefly, TP53 mutations of the entire coding exons (exons 2-11), including the splicing sites of the gene, were examined using PCR-based sequencing. All sequence variants were confirmed by sequencing the products of independent PCR amplifications in both directions. Among 173 tumors, there were two adenocarcinoma cases which had borderline height of minor peak for mutation positivity and were designated as wild-type TP53 in the previous study (12). Repeat sequencing was done using additional pieces of the same tumor sample to confirm the presence of TP53 mutation. As a result, compared to our previous study (12), two more TP53 mutations were additionally confirmed in two adenocarcinoma patients. Somatic TP53 mutations were detected in 66 of 173 tumors (38.2%). TP53 mutations were more frequent in males, ever-smokers, and patients with squamous cell carcinoma (50.4%, 51.3%, and 60.7%, respectively) than in females, never-smokers, and patients with adenocarcinoma (15.0%, 10.7%, and 27.4%, respectively; all comparisons, \( P < 0.001 \)). However, the TP53 mutation status was not associated with age and pathologic stage of the disease.

Due to the high number of single nucleotide polymorphisms (SNPs) in the human genome, the initial challenge was the efficient selection of potentially functional SNPs. Thus, a prioritizing strategy was created using public databases that provide diverse information on the potential phenotypic risks of SNPs. First, candidate genes involved in apoptosis and related information were collected from web-based databases that included information on the biologic pathway and potential biologic effects of SNPs. Next, SNPs with minor allele frequencies < 0.10 were excluded based on the allele frequencies recorded for East Asian populations obtained from FASTSNP. The selected SNPs were then scored according to certain phenotypic risks, and ordered according to the sum of the risk scores based on the algorithm suggested by Yuan et al. (13). Finally, 28 polymorphisms in 20 genes with high-risk scores were selected for study. Caspase8 rs383412 was genotyped by PCR-RFLP assay and the remaining 27 SNPs were genotyped using a sequenome mass spectrometry-based genotype assay. For quality control, the genotyping analysis was performed blind with respect to the patients. Approximately 10% of the samples were randomly selected to be genotyped again by PCR-restriction fragment length polymorphism or DNA sequencing. The Hardy-Weinberg equilibrium (HWE) was tested by comparing the observed and expected genotype frequencies using a goodness-of-fit chi-squared test. The association between genotypes and TP53 mutations was analyzed using the chi-squared test or Fisher’s exact test. The possible associations were evaluated by unconditional multivariate logistic regression models controlling for age, gender, smoking status, tumor histology and pathologic stage (odds ratio and 95% confidence interval) where appropriate.

Among the 28 polymorphisms genotyped, the BIRC5 rs2071214A > G SNP was shown to deviate from the HWE (\( P < 0.05 \)), and was thus excluded from further analysis. The identification numbers, genotype and minor allele frequencies, genotyping call rates, and \( P \) values for the HWE of the remaining 27 polymorphisms are shown in Table 1. None of the 27 polymorphisms were significantly associated with the occurrence of TP53 mutations under dominant, recessive, and codominant models (Table 1).

Apoptosis plays a critical role in the maintenance of genomic integrity by eliminating cells with un-repairable DNA damage (8). Thus, it is assumed that variation in apoptosis-related genes may influence the apoptotic capacity related to the elimination of DNA alterations, thereby modulating the occurrence of TP53 mutations. In the present study, there was no significant association between potentially functional polymorphisms in the apoptosis-related genes and the frequency of TP53 mutations. This is the first comprehensive study to use a multigenic analysis of
apoptosis-related gene polymorphisms in relation to TP53 mutations. However, a number of limitations in the present study need to be addressed. Our study was limited by the modest sample size, which did not have sufficient statistical power for the detection of variants that had a small effect on the occurrence of TP53 mutations; therefore, there might be type II errors. In addition, the sample size did not have sufficient statistical power to examine the associations between genotypes and specific types of TP53 mutations, such as G:C > T:A transversions and G:C > A:T transitions at CpG sites. Therefore, additional studies with larger sample sizes are required.

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