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

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Apoptosis plays an essential role in the elimination of mutated or transformed cells from the body. Therefore, polymorphisms of apoptosis-related genes may lead to an alteration in apoptotic capacity, thereby affecting the occurrence of *TP53* mutations in lung cancer. We investigated the relationship between potentially functional polymorphisms of apoptosis-related genes and *TP53* mutations in non-small cell lung cancer (NSCLC). Twenty-seven single nucleotide polymorphisms in 20 apoptosis-related genes were genotyped by a sequenome mass spectrometry-based genotyping assay in 173 NSCLCs and the associations with *TP53* mutations in the entire coding exons (exons 2-11), including splicing sites of the gene, were analyzed. None of the 27 polymorphisms was significantly associated with the occurrence of *TP53* mutations. This suggests that apoptosis-related genes may not play an important role in the occurrence of *TP53* mutations in lung cancer.

Key Words: Lung Neoplasms; Apoptosis; TP53 Mutation; Polymorphism, Genetic

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 chemo- and radio-therapy (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,

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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). Repeated sequencing was done using additional pieces of the same individual tumor samples 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

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Table 1. *TP53* mutations by genotypes of apoptosis-related gene polymorphisms

Genes	Polymorphism*		CR (%)	MAF	HWE *P*	Mutation by genotypes, n/N†				Adjusted OR (95% CI)¶		
Symbol (Alias)	ID no.	Base (AA) change				WW	WV	VV	*P*‡	Dominant	Recessive	Codominant
TNFSF1 (LTA)	rs1041981	C > A (T60N)	95.4	0.43	0.53	22/51	30/85	11/29	0.66	0.75 (0.35-1.60)	0.92 (0.37-2.31)	0.86 (0.52-1.43)
TNFSF6 (FASL)	rs763110	-844C > T	93.6	0.25	0.80	32/91	24/60	5/11	0.72	1.21 (0.60-2.47)	1.07 (0.28-4.16)	1.14 (0.65-2.01)
TNFSF10 (TRAIL)	rs3136586	-489A > G	95.4	0.51	0.19	12/42	29/74	18/47	0.39	1.62 (0.71-3.72)	0.96 (0.44-2.09)	1.16 (0.72-1.85)
TNFRSF1A (TNFR1)	rs4149570	-329G > T	95.4	0.39	0.80	26/62	26/77	10/26	0.61	0.75 (0.35-1.60)	0.92 (0.37-2.31)	0.86 (0.52-1.43)
TNFRSF1B (TNFR2)	rs1061622	T > G (M196R)	96.0	0.15	0.52	48/120	15/41	1/5	0.64	0.70 (0.32-1.52)	0.58 (0.05-6.94)	1.18 (0.32-4.32)
	rs1061624	*188G > A	94.8	0.39	0.44	26/58	32/83	6/23	0.29	1.03 (0.50-2.13)	0.67 (0.23-1.98)	0.92 (0.54-1.57)
TNFRSF6 (FAS)	rs10788624	-4403T > C	91.3	0.41	0.09	27/61	22/66	14/31	0.36	0.80 (0.38-1.68)	0.91 (0.36-2.30)	0.89 (0.55-1.44)
	rs2234767	-1377G > A	97.7	0.43	0.33	21/52	32/89	12/28	0.76	1.00 (0.48-2.10)	1.07 (0.42-2.72)	1.02 (0.62-1.69)
	rs1800682	-670A > G	98.3	0.47	0.50	19/46	30/89	15/35	0.53	0.98 (0.45-2.13)	1.03 (0.44-2.44)	1.00 (0.61-1.64)
TNFRSF10B (DR5)	rs1047266	C > T (A67V)	98.3	0.22	0.36	36/102	26/62	2/6	0.68	1.20 (0.59-2.41)	1.32 (0.19-9.37)	1.18 (0.64-2.19)
CASP10	rs13006529	T > A (L522I)	98.3	0.20	0.64	44/109	19/53	3/8	0.86	0.79 (0.39-1.62)	0.79 (0.16-3.96)	0.83 (0.46-1.50)
CASP9	rs4645978	-1263A > G	100.0	0.40	0.97	23/63	34/83	9/27	0.73	1.61 (0.79-3.30)	0.89 (0.34-2.35)	1.23 (0.74-2.20)
	rs4645981	-712C > T	95.4	0.22	0.89	41/99	20/58	1/8	0.22	0.95 (0.45-1.99)	0.21 (0.02-1.98)	0.81 (0.44-1.52)
	rs1052571	T > C (V28A)	97.7	0.40	0.97	22/62	33/81	8/26	0.62	0.73 (0.36-1.48)	0.82 (0.20-3.35)	0.79 (0.45-1.39)
CASP8	rs3834129	-652 6N del	99.4	0.23	0.77	42/102	20/60	4/10	0.61	0.67 (0.33-1.36)	0.74 (0.18-3.10)	0.74 (0.42-1.30)
	rs3769818	IVS12-19C > T	97.7	0.28	0.61	29/86	29/71	5/12	0.62	1.27 (0.63-2.56)	0.95 (0.27-3.39)	1.15 (0.66-1.99)
CASP7	rs11593766	T > G (D4E)	98.8	0.11	0.10	53/133	12/38	0/0	0.35	0.55 (0.24-1.30)	-	0.55 (0.24-1.30)
	rs2227310	C > G (D255E)	97.1	0.40	0.72	23/59	30/83	12/26	0.66	0.72 (0.34-1.53)	1.12 (0.44-2.81)	0.89 (0.54-1.48)
CASP3	rs2705897	IVS5-4T > G	97.1	0.20	0.88	39/108	22/53	2/7	0.71	1.20 (0.58-2.49)	1.82 (0.24-13.8)	1.22 (0.64-2.33)
APAF1	rs2289315	-529G > A	93.6	0.18	0.11	38/113	20/41	4/8	0.18	2.08 (0.96-4.50)	1.28 (0.28-5.85)	2.33 (0.65-8.29)
BCL2	rs2279115	-938C > A	96.5	0.42	0.33	20/60	32/75	12/32	0.54	1.31 (0.63-2.73)	0.84 (0.35-2.01)	1.07 (0.66-1.72)
BID	rs8190315	A > G (S56G)	96.0	0.09	0.68	52/136	12/29	0/1	0.69	0.97 (0.40-2.38)	-	0.96 (0.40-2.30)
BIRC5 (Survivin)	rs9904341	91C > G	98.8	0.49	0.32	21/47	28/79	15/45	0.47	0.57 (0.26-1.26)	0.74 (0.33-1.66)	0.72 (0.44-1.18)
BRACA1 (BRCC1)	rs799917	C > T (P817L)	95.4	0.32	0.63	24/74	31/75	6/16	0.53	1.58 (0.77-3.22)	1.61 (0.47-5.52)	1.44 (0.83-2.51)
BRACA2 (BRCC2)	rs144848	T > G (N372H)	96.0	0.24	0.37	36/97	21/57	3/12	0.71	0.84 (0.41-1.72)	0.41 (0.10-1.71)	0.77 (0.44-1.35)
PTGS2 (COX2)	rs5275	*427T > C	90.2	0.18	0.91	43/104	12/47	3/5	0.10	0.46 (0.21-1.05)	2.10 (0.31-14.02)	0.64 (0.32-1.26)
TP53	rs1042522	G > C (R72P)	96.2	0.39	0.20	20/66	31/71	12/29	0.25	1.63 (0.79-3.37)	1.28 (0.52-3.18)	1.33 (0.82-2.16)

*Information about polymorphisms and IDs were obtained from NCBI database (http://www.ncbi.nlm.gov.SNP). In the reference sequence, the transcription start site was counted as +1; †Number of cases with *TP53* mutation/number of total cases; ‡Two-sided χ^2 test for the frequency of *TP53* mutations according to genotypes; ¶ORs, 95% CIs were calculated using multivariate regression analysis, adjusted age, gender, smoking status, tumor histology, and pathologic stage. AA, amino acid; CR, call rate; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; W, wild-type allele; V, variant allele; OR, odds ratio; CI, confidence interval; TNFSF, tumor necrosis factor superfamily; LTA, lymphotoxin alpha; FASL, FAS ligand; TRAIL, TNFR apoptosis-inducing ligand; TNFRSF, TNF receptor superfamily; DR, death receptor, CASP, caspase; APAF1, Apoptotic peptidase activating factor 1; BCL2, B-cell CLL/lymphoma 2; BID, BH3 interacting domain death agonist; BIRC5, Baculoviral IAP repeat containing 5; BRACA1, breast cancer 1, early onset; PTGS2, prostaglandin-endoperoxide synthase 2; COX2, cyclooxygenase-2; TP53, tumor protein 53.

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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