Null Results in Brief

No Association between Haplotypes of Three Variants (Codon 81, 284, and 762) in Poly(ADP-ribose) Polymerase Gene and Risk of Primary Lung Cancer[1]

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Introduction

DNA repair plays a critical role in protecting the genome from the insults of cancer-causing agents, such as those found in tobacco smoke. Reduced DNA repair capacity can increase the susceptibility to lung cancer (1). Polymorphisms in the DNA repair genes may alter the efficiency of DNA repair, thereby contributing to lung cancer susceptibility.

Base excision repair is a major DNA repair pathway for small base adducts produced by oxidation, methylation, and environmental carcinogens. PARP[3] acts as a "sensor of DNA strand breaks," intermediates of enzymatic repair of base damage during BER. In addition to its role as a nick sensor, it also interacts with the scaffold protein XRCC1 and may in this way accelerate the recruitment of DNA repair proteins to stand interruptions (2). The PARP gene is located in chromosome lq42, and two pseudogenes have been identified in 13q33-qter and 14 (3). The association between the PARP pseudogene in chromosome 13 and the risk of lung cancer has been evaluated in several racial groups. The 193-bp deletion polymorphism in the PARP pseudogene has been associated with an increased risk for adenocarcinoma of the lung in Mexican-Americans and African-Americans (4).

Recently, several candidate polymorphisms in the PARP gene have been deposited in public databases.[4] Although the functional effects of these polymorphisms have not yet been elucidated, we hypothesized that some of these variants, particularly their haplotypes, may have an effect on the DNA repair capacity, thereby modulating the susceptibility to lung cancer. To test this hypothesis, a case control study was conducted to evaluate the association between PARP genotypes/haplotypes and the risk of lung cancer. Among the candidate polymorphisms, we have focused on single nucleotide polymorphisms in coding regions [Asp81Asp, C801T in exon 2; Alal88Thr, G721A in exon 4; Ala284Ala, C1011T in exon 7; His613Glu, C1998G in exon 13; Val762Ala, T2444C in exon 17; and Cys908Thr, G2882A in exon 20; GenBank accession no. NM_001618], because these are most likely to affect the gene function. In the present study, we evaluated the association of the codon 81, 284, and 762 polymorphisms and their haplotypes with lung cancer because the codon 188, 613, and 908 polymorphisms were not detected in a preliminary study consisting of 50 lung cancer cases and 50 healthy controls.

Materials and Methods

The current study was a hospital-based case control study that included 352 lung cancer patients and 352 healthy controls. Detailed descriptions of the methods used for subject enrollment and study population have been published previously (5). Eligible cases included all patients newly diagnosed with primary lung cancer between January 2000 and December 2000 at Kyungpook National University Hospital (Daegu, Korea). There was no age, sex, or histological or stage restrictions, but patients with a previous history of cancer were excluded. The cases were 168 (47.7%) squamous cell carcinomas, 119 (33.8%) adenocarcinomas, 56 (15.9%) small cell carcinomas, and 9 (2.6%) large cell carcinomas. Controls were randomly selected from a pool of healthy volunteers who visited the general health check-up center at Kyungpook National University Hospital during the same period. The controls were frequency (1:1) matched to the cases based on sex and age (±5 years).

The codon 81, 284, and 762 genotypes were determined using a PCR-RFLP assay. The primers for the codon 81 polymorphism were 5′-TCCTTCTAGTCGCCCATGTT-3′ and 5′-ATGGGCACCATACGCTTGAT-3′, which generate a 311-bp fragment. The primers for the codon 284 polymorphism were 5′-TGCAGATCTTGGACCGAGTAGC(mutated T→C)-3′ and5′-TGCAATCTCAGGGACCTGAAGT-3′, which generate a 274-bp fragment. The primers for the codon 762 polymorphism were 5′-TTTGCTCCTCCAGGCCAAC(mutated G→C)G-3′ and 5′-TGGAAGTTTGGGACCGCTGC-3′, which generate a 210-bp fragment. The PCR reactions were performed in a 20-μl reaction volume containing 200 ng of genomic DNA, 25 pmol of each primer, 0.2 mM each deoxynucleoside triphosphate, 1 × PCR buffer [10 mM Tris-HCI (pH 8.3) and 50 mM KCl], 1.5 mM $MgCl_2$, and 1 unit of Taq polymerase (TAKARA SHUZO Co., Otus, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step of 94°C for 5 min followed by 35

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[3] The abbreviations used are: PARP, poly(ADP-ribose)polymerase; OR, odds ratio; CI, confidence interval.
[4] Internet address: http://www.ncbi.nlm.nih.gov/SNP.

Table 1 Analysis of PARP haplotype frequencies, ORs, and 95% CIs for lung cancer by selected variables

	No.[a] (%)								
	Cases			Controls			Adjusted OR (95% CI)		
	1-1-1[b]	1-2-1	2-2-2	1-1-1	1-2-1	2-2-2	1-1-1	1-2-1	2-2-2
All subjects	150 (21.3)	242 (34.4)	312 (44.3)	137 (19.5)	247 (35.1)	320 (45.4)	1.0	0.94 (0.70–1.27)	0.92 (0.69–1.22)[c]
Age									
≤61	68 (21.0)	115 (35.5)	141 (43.5)	72 (20.5)	125 (35.5)	155 (44.0)	1.0	1.04 (0.68–1.59)	0.99 (0.66–1.49)[d]
>61	82 (21.6)	127 (33.4)	171 (45.0)	65 (18.5)	122 (34.7)	165 (46.9)	1.0	0.82 (0.54–1.26)	0.82 (0.48–1.23)[d]
Sex									
Male	119 (20.7)	203 (35.2)	254 (44.1)	117 (20.3)	196 (34.0)	263 (45.7)	1.0	1.07 (0.77–1.50)	0.98 (0.72–1.35)[e]
Female	31 (24.2)	39 (30.5)	58 (45.3)	20 (15.6)	51 (39.9)	57 (44.5)	1.0	0.56 (0.27–1.12)	0.71 (0.35–1.41)[e]
Smoking status									
Never-smoker[g]	25 (20.5)	45 (36.9)	52 (42.6)	27 (16.1)	64 (38.1)	77 (45.8)	1.0	0.70 (0.35–1.39)	0.70 (0.36–1.37)[f]
<40 pys[h]	49 (21.3)	83 (36.1)	98 (42.6)	67 (20.7)	112 (34.6)	145 (44.7)	1.0	1.05 (0.66–1.69)	0.96 (0.61–1.52)[f]
≥40 pys	76 (21.6)	114 (32.4)	162 (46.0)	43 (20.3)	71 (33.5)	98 (46.2)	1.0	0.91 (0.57–1.47)	0.94 (0.60–1.47)[f]
Histologic type[i]									
Squamous	76 (22.6)	111 (33.0)	149 (44.3)				1.0	0.85 (0.59–1.24)	0.85 (0.59–1.21)[c]
Adeno	47 (19.7)	87 (36.6)	104 (43.7)	137 (19.5)	247 (35.1)	320 (45.4)	1.0	1.03 (0.67–1.58)	0.96 (0.64–1.44)[c]
Small	23 (20.5)	34 (30.4)	55 (49.1)				1.0	0.86 (0.49–1.54)	1.07 (0.63–1.83)[c]

[a] Each subject contributes two haplotypes to the analysis, thus, the total number of haplotypes in each column is twice the number of subjects.
[b] Wild allele is denoted by 1 and the polymorphic allele by 2. The order of the polymorphisms is as follows: codon 81, 284, and 762.
[c] Adjusted for age, sex, and pack-years.
[d] Adjusted for sex and pack-years.
[e] Adjusted for age and pack-years.
[f] Adjusted for age and sex.
[g] Current and former smokers.
[h] Pack-years.
[i] Nine large cell carcinomas were excluded.

cycles of 30 s at 94°C for codon 81 and 284 and 20 s at 94°C for codon 762, 20 s at 56°C for codon 81 and 762 and 20 s at 59°C for codon 284, 30 s at 72°C for codon 81 and 284 and 20 s at 72°C for codon 762, and a final elongation at 72°C for 10 min. Five microliters of the PCR products were digested overnight with 5 units of *Bcl*I (New England Biolabs, Beverly, MA) at 50°C for codon 81, 5 units of TaqI (New England Biolabs) at 65°C for codon 284, and 5 units of *Bst*UI (New England Biolabs) at 60°C for codon 762. The digestion products were separated on 6% acrylamide gel. Three possible genotypes were defined for each codon based on distinct band patterns: (*a*) codon 81: CC (311 bp), CT (311, 182, and 129 bp), and TT (182 and 129 bp); (*b*) codon 284: CC (274 bp), CT (274, 252, and 22 bp), and TT (252 and 22 bp); and (*c*) codon 762: TT (210 bp), TC (210, 190, and 20 bp), and CC (190 and 20 bp). As a quality control, the genotyping analysis was repeated twice for all subjects, and selected PCR-amplified DNA samples ($n = 2$, respectively, for each genotype) were examined by DNA sequencing to confirm the genotyping results.

The ORs and 95% CIs were calculated using an unconditional logistic regression analysis. To analyze the association between the genotypes/haplotypes and risk of lung cancer after stratification into age (median age, ≤61 years/>61 years), sex, smoking status, and cigarette consumption (median pack-years, <40 pack-years/≥40 pack-years), multiple logistic regression analyses were performed. All analyses were performed using Statistical Analysis Software for Windows, version 6.12 (SAS Institute, Gary, NC).

Results

The polymorphic allele frequencies for codon 81, 284, and 762 among the controls were 0.443, 0.787, and 0.443, respectively, and in Hardy-Weinberg equilibrium. The polymorphic allele frequencies for codon 81, 284, and 762 among the cases were 0.455, 0.805, and 0.455, respectively, which were not significantly different from those among the controls.

The three polymorphisms were in linkage disequilibrium. Theoretically, although 8 haplotypes are possible, only 3 haplotypes (C-C-T, haplotype A; C-T-T, haplotype B; and T-T-C, haplotype C) were identified in the current study population. No significant deviation was observed in the distribution of the haplotypes between the cases and controls (Table 1). When the analyses were stratified by age, sex, smoking status, and pack-years of smoking, no significant association was found between the haplotype and risk of lung cancer. Moreover, the haplotypes exhibited no apparent relationship with any of the histological types of lung cancer.

Statistical Power

For all lung cancers, this study had 80% power (two-sided test of significance, $\alpha = 0.05$) to detect an OR > 1.58 (assuming a risk effect) or <0.67 (assuming a protective effect) for carriers of haplotype B and an OR > 1.57 or <0.68 for carriers of haplotype C relative to carriers of haplotype A. For squamous cell carcinomas, this study had 80% power to detect an OR > 1.80 or <0.61 for haplotype B and an OR > 1.78 or <0.63 for haplotype C compared with haplotype A. For adenocarcinomas, this study had 80% power to detect an OR > 1.94 or <0.57 for haplotype B and an OR > 1.96 or <0.59 for haplotype C relative to haplotype A. For small cell carcinomas, this study had 80% power to detect an OR > 2.79 or <0.46 for haplotype B and an OR > 2.57 or <0.51 for haplotype C compared with haplotype A.

Study Limitations

The limitations of the current study are the same as in other case control studies, primarily, a selection and information bias, as described in a previous study by the current authors (5).

This is the first case control study of PARP polymorphisms in relation to lung cancer. Our results suggest that PARP polymorphisms (codon 81, 284, and 762) or their haplotypes do not significantly affect susceptibility to lung cancer. However, because genetic polymorphisms often vary between ethnic groups (4), additional studies are needed to clarify the association of PARP polymorphisms with lung cancer in diverse ethnic populations.

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