



# Relationship between genetic polymorphisms of DNA ligase 1 and non-small cell lung cancer susceptibility and radiosensitivity

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**ABSTRACT.** The aim of this study was to examine the relationship between genetic polymorphisms in DNA ligase 1 (LIG1) and non-small cell lung cancer (NSCLC) susceptibility and radiosensitivity in a Chinese population. This was a case-control study that included 352 NSCLC patients and 448 healthy controls. Polymerase chain reaction-restriction fragment length polymorphism analysis was conducted to detect *Hae*III polymorphisms in exon 6 of the LIG1 gene in this population. This information was used to observe the effects of radiation in patients with different genotypes in order to determine the genotypes associated with radiosensitivity. The CC genotype and C allele frequency were significantly higher in the NSCLC group than in the control group ( $P = 0.012$  and  $P = 0.023$ , respectively). The relative risk of experienc-

ing NSCLC was 2.55 [95% confidence interval (CI), 1.12-3.98] for CC homozygous patients and 0.87 (95%CI, 0.46-1.88) for AA homozygous patients. Analysis of LIG1 genetic polymorphisms and radiosensitivity of NSCLC patients showed that AA homozygous patients were significantly more radiosensitive than the control group (AA vs AC,  $P = 0.014$ ; AA vs CC,  $P < 0.001$ ; AC vs CC,  $P = 0.023$ ). Therefore, the LIG1 CC genotype was associated with susceptibility to NSCLC, and the AA genotype demonstrated increased radiosensitivity compared to the AC and CC genotypes.

**Key words:** Gene; Genetics; Non-small cell lung cancer; Radiation effects; Radiation tolerance

## INTRODUCTION

Lung cancer is the most common cancer worldwide, with the highest incidence and mortality among all malignancies. According to the global cancer statistics report, more than 135 million people were diagnosed with lung cancer in 2002 alone, which accounted for 12.4% of all cancer patients, and around 1.18 million lung cancer patients died during that time (Johannesdottir et al., 2012). Currently, the role of familial genetic predisposition in lung cancer is of interest (Zhang et al., 2014). Studies of non-small cell lung cancer (NSCLC) risk factors and the effects of radiotherapy in patients of the same race or from the same geographical region are important for ascertaining whether a common genetic mechanism influences cancer susceptibility and tumor radiosensitivity. As part of the catalytic polymerization process carried out by DNA polymerase I, DNA ligase (ligase I, LIG1) fills gaps between single-stranded DNA before closing gaps in double-stranded DNA, making it important for DNA replication, repair, and recombination (Chen et al., 2014; Huan et al., 2014; Mitra et al., 2014; Soni et al., 2014). The current study identified *Hae*III polymorphisms in exon 6 of the LIG1 gene. LIG1 is involved in various DNA repair pathways, and low LIG1 expression could cause dysfunction of the immune system, which may be important for tumor incidence and development (Bazrgar et al., 2014; Han et al., 2014). In this study, we identified correlations between LIG1 genetic polymorphisms and NSCLC susceptibility and radiosensitivity in order to provide a theoretical basis for early cancer diagnosis and individualization of radiotherapy for NSCLC.

## MATERIAL AND METHODS

### Subjects

For this study, we selected 352 patients who were hospitalized with NSCLC at Nanjing Medical University Affiliated Jiangsu Cancer Hospital from 2008 to 2013. We also included 448 healthy control patients of Han ethnicity from the same region.

### Genomic DNA extraction

Peripheral blood (5 mL) was drawn from patients and controls and anticoagulated with sodium citrate. Genomic DNA was extracted using a TaKaRa Genomic DNA Extraction

KIT (code number D9081; Ningbo XingPu Company, Ningbo, China).

### Main variable definition

“Smoking” indicates that from the first cigarette to the present date, the patient smoked at least 100 cigarettes annually, or at least 2 per week, for more than 1 year. Body mass index (BMI) is an index for the degree of obesity, which is calculated as follows: BMI (kg/m<sup>2</sup>) = weight (kg)/height (m)<sup>2</sup>. Pack-year is an index of the accumulated amount of smoking, which is calculated as follows: [cigarettes per day (branch)/20 (branch/pack)] x years of smoking.

### Efficacy observation

All NSCLC patients were living with tumors and received 60 to 65 Gy radiation therapy at our hospital without pre-treatment. For clinical criteria, pre-radiotherapy and post-radiotherapy computed tomography images were used to measure tumor size before and after radiotherapy, and to calculate the percentage of tumor shrinkage. According to the evaluation criteria established by the World Health Organization, objective responses were classified as complete response (CR), partial response (PR), no change, and progressive disease. The effective response rate was equal to the CR plus PR. The remaining cases were disregarded.

### Polymorphism detection

The single nucleotide polymorphism (SNP) in the *LIG1* gene was positioned using the SNP database at the National Center for Biotechnology Information website, and specific primers were synthesized according to its nucleotide sequence: forward: 5'-ATGCCCTGTAGGTTCAATGG-3'; and reverse: 5'-TGGAGGTCTTTAGGGGCTTG-3' (Sangon Biotech Co., Ltd., Shanghai, China).

### Polymerase chain reaction (PCR) amplification

Reactions were carried out in a 20 µL system that contained DNA template (50 ng), primers (0.4 µL of 10 mM for both forward and reverse), and 2X Taq PCR MasterMix (10 µL) (Beijing Tianwei Time Technology Co., Beijing, China). The reaction conditions were as follows: pre-denaturation at 95°C for 5 min, followed by denaturation at 95°C for 30 s, 58°C for 35 s, and 72°C for 40 s for a total of 35 cycles, followed by a final extension at 72°C for 10 min. PCR products (5 µL) were separated by electrophoresis on 1.5% agarose gels with DL2000 DNA markers (Biotek, Beijing, China) as molecular standards. After electrophoresis, gels were photographed and the images were saved using a Bio-Rad gel imaging and analysis system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

### Restriction fragment length polymorphism digestion typing

Reactions were carried out in 20 µL systems that included 10X PCR buffer, *HaeIII* (*BsuRI*) enzyme (3U; Thermo Fisher Scientific, Waltham, MA, USA), PCR products (8 µL), and ddH<sub>2</sub>O. The reactions were incubated in a 37°C water bath for 16 h. Digestion products

were tested by electrophoresis on 3.0% agarose gels (5 V/cm) containing ethidium bromide. The PCR products were 165-bp fragments. Bands for wild-type AA samples appeared only at 165 bp; bands for heterozygous mutant AC samples appeared at 165, 100, and 65 bp; bands for homozygous mutant CC samples appeared at 100 and 65 bp.

## Statistics

The SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis.  $\chi^2$  tests were used to compare the genotype distributions of LIG1 in the case and control groups. A logistic regression model was established to evaluate the correlation between LIG1 genotypes and lung cancer incidence.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Subject characteristics

There were no significant differences in age or gender ratio between the case and control groups (all  $P > 0.05$ ). There were statistically significant differences in smoking, BMI, and other indicators between the 2 groups (Table 1).

**Table 1.** Clinical characteristics of study participants.

	Lung cancer (N = 352)	Control (N = 448)	P
Age, years $\pm$ SD	61.7 $\pm$ 12.8	61.9 $\pm$ 12.6	0.667
Male gender, N (%)	244 (69.3)	311 (69.4)	0.879
Smoking, N (%)	201 (57.1)	126 (28.10)	<0.001
Alcohol drinker, N (%)	143 (40.6)	129 (28.8)	<0.001
Family history of cancer, N (%)	101 (28.7)	-	-
BMI, kg/m <sup>2</sup>	21.5 $\pm$ 6.8	24.2 $\pm$ 5.9	0.026

BMI, body mass index; SD, standard deviation.

### Hardy-Weinberg genetic equilibrium test

The LIG1 genotype frequencies in the control group coincided well with expectations and were at Hardy-Weinberg genetic equilibrium, indicating a good representation.

### Relationship between LIG1 genes and lung cancer

The mutant allele C occurred at a frequency of 25.0% in the case group and 19.2% in the control group, which was a statistically significant difference ( $P = 0.002$ ; Table 2). The LIG1 wild-type AA, heterozygous mutant AC, and homozygous mutant CC genotypes occurred in the case group at frequencies of 50.0, 43.18, and 6.82%, respectively. This was compared to frequencies of 64.73, 32.14, and 3.13%, respectively, in the control group. Using the wild-type AA as a reference, the risk of lung cancer for the heterozygous AC and homozygous mutant CC genotypes after adjustment for age, gender, education, BMI, and smoking was 2.523 [95% confidence interval (CI), 1.421-5.123; Table 3].

**Table 2.** Genotype distribution of LIG1 polymorphisms.

Groups	N	Allele (%)		Genotype, N (%)		
		C	A	CC	AC	AA
Case	352	25.0	75.0	24 (6.82)	152 (43.18)	176 (50.0)
Control	448	19.2	80.8	14 (3.13)	144 (32.14)	290 (64.73)

**Table 3.** Logistic regression analysis results.

Risk factors	$\beta$	SE	Wald $\chi^2$	P	OR	95%CI
LIG1 C allele	0.446	0.132	5.011	0.003	2.523	1.421-5.123
Smoking	1.098	0.421	5.022	0.004	2.311	1.415-5.146
LIG1 C allele x smoking**	0.614	0.455	7.014	0.001	4.324	2.121-11.1222

CI, confidence interval; LIG1, DNA ligase I; OR, odds ratio; SE, standard error. \*\*Interaction between smoking and LIG1 C allele.

### Relationship between LIG1 genetic polymorphisms and NSCLC radiosensitivity

The frequency of radiation responders was higher in the AA group than in the groups with inactivating mutations in LIG1. With reference to the AA group, there was a statistically significant difference ( $P < 0.05$ ) in effective rate (CR+PR) between AC and CC genotype. The results are shown in Table 4.

**Table 4.** Effective rate between genotypes.

Genotype	Effective group (CR + PR)	Ineffective group (NC + PD)	P value
AA (N = 172)	109 (63.37%)	63 (36.63%)	P = 0.014 (AA vs AC)
AC (N = 156)	88 (56.41%)	68 (43.59%)	P < 0.001 (AA vs CC)
CC (N = 24)	7 (29.17%)	17 (70.83%)	P = 0.023 (AC vs CC)

CR, complete response; NC, no change; PD, progressive disease; PR, partial response.

## DISCUSSION

Surgery is not suitable for most NSCLC patients; therefore, radiotherapy has become the main method for treatment. However, some patients have NSCLC that is not sensitive to radiotherapy, possibly because of different genotypes. A relationship between LIG1 genetic polymorphisms and lung cancer radiosensitivity has not yet been reported. This study demonstrates that radiotherapy was more effective in patients with AA genotype NSCLC compared to other genotypes.

LIG1, which is 1 of the 4 DNA ligases in mammalian cells, is located on chromosome 19q13.2-13.3 (Chen et al., 2014; Huan et al., 2014; Mitra et al., 2014). A variety of tumor cells often lack functional LIG1. LIG1 is a DNA repair gene in a broad sense; it connects the lagging strand Okazaki fragments that occur during synthesis of double-stranded DNA. In 1998, Livak et al. (1998) reported that LIG1 had 2 variants: the exon 6 AC polymorphism and another variant at the 5' end of the complex GT repeat sequence in intron 6. Early studies demonstrated that LIG1 deficiency can cause Bloom's syndrome, which is characterized by more

frequent chromosome breaks and rearrangements, more frequent sister chromatid exchanges, and a slowdown in DNA replication. This may lead to a high frequency of cancer breaks in the immune system. Current data suggests that the relationship between LIG1 and cancer susceptibility varies in different populations. Shen et al. (2002) examined case and control groups of American Caucasians and reported that the frequency of the C allele was 49.4 and 49.0%, respectively. In a population of mixed ethnicities from the United States, Lee et al. (2008) reported that the frequency of the C allele was 43.8 and 51.5%, respectively; this C allele occurred at a frequency of 55.3 and 53.0% in an Indian population. The distribution of LIG1 alleles may differ by race. Shen et al. (2002) Lee et al. (2008) and other studies in the United States, India, and other countries have reported a risk degree of 0.8 to 1.2 for carriers of any of the wild-type alleles compared to the homozygous mutant CC allele. This study enrolled a Chinese Han population for a case-control analysis. The frequency of the C allele at this site was significantly higher in patients with NSCLC compared to the control group, indicating that the CC genotype at this site may be associated with susceptibility to NSCLC.

Our study also identified a relationship between the efficacy of radiotherapy for NSCLC patients and LIG1 genetic polymorphisms. Patients with the AA genotype were significantly more sensitive to radiotherapy. Of course, our findings require confirmation by studies with larger sample sizes. Case-control studies in multi-ethnic populations are necessary to confirm our results.

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