Tohoku J. Exp. Med., 2016, **238**, 279-286

Association of Single Nucleotide Polymorphisms in the Apoptosis-Related Genes *TP63* and *CD40* with Risk for Lung Cancer in a Chinese Han Population

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Apoptosis plays a critical role in tumorigenesis. TP63 inhibits the pro-apoptosis function of TP53, and CD40 increases expression of anti-apoptotic proteins. Two single nucleotide polymorphisms (SNPs), rs6790167 (g243059A>G) in intron 9 of *TP63* and rs1535045 (g6194C>T) in intron 1 of *CD40* respectively, may affect the susceptibility of lung cancer. To evaluate the association of these SNPs with lung cancer, we performed a case-control study with 258 patients, including 149 adenocarcinoma and 47 small cell lung cancer, and 270 controls. Genotyping was conducted using allele-specific polymerase chain reaction and pyrosequencing. We found that rs6790167 and rs1535045 are associated with the risk of lung adenocarcinoma (P = 0.048) and small cell lung cancer (P = 0.019), respectively. Non-smoking males carrying the GG genotype of rs6790167 had higher risk for lung adenocarcinoma than individuals carrying the AA genotype (OR = 7.58, 95% CI: 2.43-23.65). Compared to the TT genotype of rs1535045, non-smoking women with the CC genotype had higher risk for lung adenocarcinoma (OR = 4.20, 95% CI: 1.34-13.12). After stratified analysis based on clinical characteristics, the frequency of the CC genotype of rs1535045 was higher in patients at I-II stages (P = 0.013) or patients whose tumor markers were negative (P = 0.003). Individuals carrying both the GG genotype of rs6790167 and the CC genotype of rs1535045 were associated with significantly higher risk for lung adenocarcinoma. Thus, the polymorphisms in the *TP63* and *CD40* genes are associated with lung cancer in a Chinese Han population.

Keywords: apoptosis; CD40; lung cancer; single nucleotide polymorphism; TP63
Tohoku J. Exp. Med., 2016 April, **238** (4), 279-286.

Introduction

Lung cancer is the most common malignant tumor that occurs around the world and is currently one of cancers with the highest mortality. In 2012, around 1.8 million new cases of lung cancer were documented, accounting for 13% of the total number of cancers occurring worldwide. During the same period, 1.6 million deaths due to lung cancer were reported, which accounted for 19.4% of the total cancer-related deaths (Torre et al. 2015). In China, the incidence and mortality of lung cancer have grown rapidly in the past decades. In 2012, 652,842 new diagnostic cases and 587,182 deaths were documented (IARC, International Agency for Research on Cancer 2012). Lung cancer is caused by both environmental factors and genetic factors. Although smoking is recognized as one of the main risk factors for lung cancer (Ruano-Ravina et al. 2003), only less than 20% of smokers develop lung cancer, suggesting that genetic susceptibility to lung cancer in different individuals is highly variable (Lam et al. 2004). Development of lung cancer is a complex multi-stage process that involves multiple genes. It is not only related to the activation of oncogenes and inactivation of tumor suppressor genes, but also closely associated with apoptosis (Ramezanpour et al. 2014).

Received October 27, 2015; revised and accepted March 1, 2016. Published online April 8, 2016; doi: 10.1620/tjem.238.279.
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The inactivation of pro-apoptotic genes and the inhibition of apoptosis gene overexpression are two important causes of lung cancer.

TP63 is a member of the TP53 family. The human *TP63* gene is located on chromosome 3q28 and has a high degree of sequence and structural homology to TP53. TAp63, a TP63 isoform, activates promoters of TP53 target genes, thereby inducing cell cycle repression and triggering apoptosis (Helton et al. 2008).

CD40 belongs to the tumor necrosis factor receptor (TNF-R) superfamily. The human *CD40* gene is located on chromosome 20q13, and encodes an important costimulatory molecule that regulates the cellular and humoral immunity (Elgueta et al. 2009). CD40 activates the transcription factor NF-κB, increases synthesis of anti-apoptotic proteins, induces VEGF secretion by tumor cells or endothelial cells, and promotes endothelial cell proliferation and tumor angiogenesis (Korniluk et al. 2014).

Single nucleotide polymorphisms (SNPs) are tumor molecular markers that have been extensively investigated in the past few decades and have also been utilized an important tool in screening individuals at high-risk for cancer. In recent years, genetic variants in apoptosis-related genes associated with susceptibility to lung cancer have been identified (Hu et al. 2011; Pathak et al. 2014), including *CD40* and *TP63* (Wang et al. 2008; Miki et al. 2010; Hu et al. 2014; Yin et al. 2014). Rs6790167 (g243059A>G) is located in intron 9 of *TP63* gene and may be involved in the transcription regulation. Previous studies indicated that genetic variants in *TP63* were associated with the response to DNA damage (McDade et al. 2012). Rs1535045 (g6194C>T) is located in intron 1 of *CD40* gene, and the SNP region has potential to affect the chromatin structure and transcription of *CD40* (Pathak et al. 2014).

In the present case-control study, we evaluated the association of *TP63* and *CD40* polymorphism with lung cancer susceptibility by comparing the genotype distributions of rs6790167 and rs1535045 between patients with lung cancer and healthy controls in a Chinese Han population.

Materials and Methods

Study subjects

Patients in the case group were selected from new cases that were pathologically diagnosed as lung cancer at the General Hospital of Hainan Land Reclamation between June 2014 and July 2015. All cases were not treated with chemotherapy and radiation therapy, and had no primary tumors in other body parts. The control group was composed of healthy individuals who underwent physical examination at the same hospital and had no previous history of cancer. The gender ratio and the age (± 5 years) of the controls were matched with those of the lung cancer patients. All study subjects were unrelated Han Chinese.

Epidemiological investigations

General information such as gender, age, smoking history, family history of cancer, occupational exposure, and other habits were collected from the study participants by use of a questionnaire. Clinical information for the case group included pathological type, clinical stage, distant metastasis, and tumor markers. Individuals who had smoked ≥ 1 cigarettes/day and continued smoking for more than six months were defined as smokers; otherwise, designated as non-smokers. All patients were screened for serum carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCCA), cancer antigen 199 (CA 199), cancer antigen 125 (CA 125), and cancer antigen 153 (CA 153); those with values higher than the reference was defined as abnormal, or otherwise, normal. The Ethics Committee of the General Hospital of Hainan Land Reclamation reviewed and approved the present study. All study participants provided their informed consent.

Genotyping

Three microliters of fasting and EDTA anti-coagulated peripheral blood samples were collected from each subject. Genomic DNA was extracted using a blood genome extraction kit (Tiangen, Beijing, China). The concentration and purity of the extracted DNA were determined using a UV spectrophotometer. The DNA samples were stored at −20°C until analysis.

The polymorphisms rs6790167 in the *TP63* gene and rs1535045 in the *CD40* gene were genotyped using allele-specific polymerase chain reaction (AS-PCR). Primers were designed using the Gene Quest software (primer sequences are presented in Table 1). Each AS-PCR system comprised a total volume of 20 μL, which included 10 μL of 2 × TaqMan Master Mix, 1 μL of the forward primer (F), 1 μL of the reverse primer (R), 1 μL of the allele-specific primer (RA or FG or RC or RT), 2 μL of genomic DNA (equivalent to 50 ng), and 5 μL of ddH_2O. The reaction conditions included a pre-denaturation step at 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 59°C for 30 s. and 72°C for 60 s, and a final extension at 72°C for 10 min. The PCR products were analyzed via electrophoresis in a 2% agarose gel.

All samples underwent duplicate pyrosequencing using the PyroMark ID system (Qiagen, Hilden, Germany) with the SNP sequencing program mode, following the manufacturer's manual. The SNP analysis software was used for sequencing and interpretation.

Statistical analysis

The SPSS 18.0 software was used for statistical analysis. The samples, which were employed as a representative study population of the Han Chinese, were tested for Hardy-Weinberg (HW) equilibrium. The chi-square test was used to compare genotypes and risk factors between the case group and the control group. Odds ratio (OR) and its 95% confidence interval (CI) were used to represent relative risk. Unconditional logistic regression analysis was used to analyze the correlation between genotypes and lung cancer. All statistical tests were two-sided. The significance level α was set as 0.05.

Results

Characteristics of study participants

A total of 258 patients with lung cancer and 270 healthy controls were enrolled in the present study (Table 2). There was no significant difference in age and sex between the case and control groups. General information on the study participants is presented in Table 2. There

Table 1. Primers and probes used for *CD40* rs1535045 and *TP63* rs6790167 genotyping.

Primers	Sequences (5′ → 3′)	Product Size, bp
CD40 rs1535045		
AS-PCR		
F	CTACTTTAGAGGGCTGTAGATTC	
R	ACAAGAAGCCCTCAATAGATA	
RC	TTACCTCTTTCCAGCTCCG	
RT	TTACCTCTTTCCAGCTCCA	
pyrosequencing		65
F	TGAAGCAATGGCTCTTAGGG	
R	CCCCTTTACCTCTTTCCAGCT	
Sequencing primer	TTACCTCTTTCCAGCTC	
TP63 rs6790167		
AS-PCR		
F	CCAGCGTTTCGTCAGAAC	
R	TCTCTAGCCCTCTCCACTATATG	
RA	CCTTTCCCATTGTCACAGATGAT	
FG	TTTTACAGTATGATCATCATCTCG	
pyrosequencing		83
F	TTTGAATGGGCTTTTAC AGTATGA	
R	AGCAGCTTCAACTGACTAAGACAC	
Sequencing primer	TTCCCATTGTCACAGA	

F, forward primer; R, reverse primer; FG, forward allele-specific primer; RC / RT / RA, reverse allele-specific primer.

were 136 (54.0%) smokers in the case group, which was significantly higher than the 89 (33.0%) smokers in the control group (P = 0.000). Approximately 10.5% of patients in the case group had a family history of cancer, which was higher than that of the control group (7.9%), but the difference was not statistically significant (P = 0.118).

HW equilibrium test

The genotype distribution of rs6790167 in the *TP63* gene (P = 0.091) and rs1535045 in the *CD40* gene (P = 0.347) did not significantly deviate from HW equilibrium in both patient and control groups, indicating that the genotype observations were consistent with the genotype expectations in these two loci and thus serve as a good representative sample of the study population.

Association of CD40 and TP63 polymorphisms with lung cancer

The genotype distribution of rs1535045 and rs6790167 is presented in Table 3. Compared to the AA genotype, individuals who harbored the GG genotype had a significantly higher risk for lung cancer (OR = 1.88, 95% CI: 1.10-3.20) and lung adenocarcinoma (OR = 2.02, 95% CI: 1.10-3.70), respectively. The allele frequency of rs1535045 in the *CD40* gene significantly differed between the case and control groups (P = 0.013). Individuals who harbored the CC genotype had a higher risk for lung cancer than those with the TT genotype (OR = 1.84, 95% CI: 1.01-3.35).

Stratified analysis by smoking status

Stratified analysis by smoking status revealed that frequencies of the CC genotype of rs1535045 and the GG genotype of rs6790167 in non-smoking patients with lung cancer were significantly higher than those of the control subjects. Individuals who harbored the GG genotype had significantly higher risk than the AA genotype for lung cancer (OR = 3.68, 95% CI: 1.70-7.99). In the smoking group, the allele frequency of these SNPs did not significantly differ between patients with lung cancer and the control subjects (Table 4).

Stratified analysis by gender in non-smokers

Because the smoking rate of females was very low in the present study, we further stratified the non-smokers by gender, which showed that the frequency of the CC genotype of rs1535045 was significantly higher in non-smoking female patients with lung cancer (OR = 4.67, 95% CI: 1.49-14.59) and lung adenocarcinoma (OR = 4.20, 95% CI: 1.34-13.12), whereas the frequency of the GG genotype of rs6790167 was significantly higher in non-smoking male patients with lung cancer (OR = 6.00, 95% CI: 2.20-16.36) and lung adenocarcinoma (OR = 7.58, 95% CI: 2.43-23.65). Moreover, the similar correlation can also be found in lung adenocarcinoma (Table 5).

Table 2. General information of lung cancer patients and healthy controls.

Variables	Case (%)	Control (%)	χ^2	P
Gender				
Male	184 (71.3)	193 (71.5)	0.002	0.967
Female	74 (28.7)	77 (28.5)		
Age				
⩽ 61years	124 (48.1)	116 (43.0)	1.384	0.239
> 61years	134 (51.9)	154 (57.0)		
Smoking status				
Yes	136 (52.7)	89 (33.0)	23.406	0.000*
No	122 (47.3)	181 (67.0)		
Family history of cancer				
Yes	27 (10.5)	18 (6.7)	2.442	0.118
No	231 (89.5)	252 (93.3)		
Histology				
Adenocarcinoma	149 (57.8)			
Squamous cell carcinoma	52 (19.2)			
Small cell lung cancer	47 (18.2)			
Others	10 (3.9)			
Stage				
Ⅰ + Ⅱ	89 (34.5)			
Ⅲ + Ⅳ	169 (65.5)			
Tumor marker				
Abnormal	154 (59.7)			
Normal	104 (40.3)			
Distant metastasis				
Yes	85 (32.9)			
No	173 (67.1)			

χ^2: the chi-square value; P-value between patients and controls using χ^2 test.
*Statistically significant.

Table 3. Genotype frequencies of *TP63*/*CD40* SNPs in patients and controls.

Genotype	Control (%) (n = 270)	Case (%) (n = 258)	Adenocarcinoma (%) (n = 149)	OR (95% CI)[a]	P[a]	OR (95% CI)[b]	P[b]
TP63 rs6790167							
AA	98 (36.3)	80 (31)	47 (31.5)	1.00 (reference)		1.00 (reference)	
GA	140 (51.9)	129 (50)	71 (47.7)	1.13 (0.77 - 1.65)	0.532	1.06 (0.67 - 1.66)	0.808
GG	32 (11.9)	49 (19.0)	31 (20.8)	1.88 (1.10 - 3.20)	0.020*	2.02 (1.10 - 3.70)	0.022*
CD40 rs1535045							
TT	32 (11.9)	22 (8.5)	15 (10.1)	1.00 (reference)		1.00 (reference)	
TC	132 (48.9)	102 (39.5)	58 (38.9)	1.12 (0.62 - 2.05)	0.703	0.94 (0.47 - 1.86)	0.854
CC	106 (39.3)	134 (51.9)	76 (51.0)	1.84 (1.01 - 3.35)	0.045*	1.53 (0.78 - 3.02)	0.219

[a]relative risk between case and control.
[b]relative risk between adenocarcinoma and control.
*Statistically significant.
CI, confidence interval; OR, odds ratio.

Stratified analysis according to clinical characteristics

To further understand the relationship between genetic variants and clinical characteristics in the case group, we stratified the case group according to various clinical characteristics (Table 6). The results showed that the frequency of CC genotype of rs1535045 was higher in patients at I-II stages (P = 0.013) or patients whose tumor markers were negative (P = 0.003). Compared to the control group, rs6790167 and rs1535045 are associated with high risk of lung adenocarcinoma (P = 0.019) and small cell lung cancer

Table 4. Genotype frequencies of *CD40/TP63* SNPs and smoking status in patients and controls.

Genotype	Non-smoking				Smoking			
	Case (%) (n = 122)	Control (%) (n = 181)	OR (95% CI)	P	Case (%) (n = 136)	Control (%) (n = 89)	OR (95% CI)	P
CD40 rs1535045								
TT	9 (7.4)	20 (11.0)	1.00 (reference)		13 (9.6)	12 (13.5)	1.00 (reference)	
TC	45 (36.9)	92 (50.8)	1.09 (0.46 - 2.58)	0.850	57 (41.9)	40 (44.9)	1.32 (0.54 - 3.18)	0.542
CC	68 (55.7)	69 (38.1)	2.19 (0.93 - 5.15)	0.068	66 (48.5)	37 (41.6)	1.65 (0.68 - 3.98)	0.265
TP63 rs6790167								
AA	38 (31.1)	70 (38.7)	1.00 (reference)		42 (30.9)	28 (31.5)	1.00 (reference)	
GA	58 (47.5)	98 (54.1)	1.09 (0.65 - 1.82)	0.741	71 (52.2)	42 (47.2)	1.13 (0.61 - 2.08)	0.702
GG	26 (21.3)	13 (7.2)	3.68 (1.70 - 7.99)	0.001*	23 (16.9)	19 (21.3)	0.81 (0.37 - 1.75)	0.587

*Statistically significant.
CI, confidence interval; OR, odds ratio.

Table 5. Genotype frequencies of *CD40/TP63* SNPs in non-smoking patients and controls.

Genotype	Control (%) (n = 181)	Case (%) (n = 122)	Adenocarcinoma (%) (n = 87)	OR (95% CI)[a]	P[a]	OR (95% CI)[b]	P[b]
CD40 rs1535045							
Female							
TT	14 (18.9)	5 (7.2)	5 (8.8)	1.00 (reference)		1.00 (reference)	
TC	36 (48.6)	24 (34.8)	16 (28.1)	1.87 (0.59 - 5.86)	0.281	1.24 (0.38 - 4.05)	0.716
CC	24 (32.4)	40 (58.0)	36 (63.2)	4.67 (1.49 - 14.59)	0.005*	4.20 (1.34 - 13.12)	0.010*
Male							
TT	6 (5.6)	4 (7.5)	2 (6.7)	1.00 (reference)		1.00 (reference)	
TC	56 (52.3)	21 (39.6)	12 (40.0)	0.56 (0.14 - 2.19)	0.403	0.64 (0.12 - 3.58)	0.612
CC	45 (42.1)	28 (52.8)	16 (53.3)	0.93 (0.24 - 3.60)	0.920	1.07 (0.20 - 5.83)	0.941
TP63 rs6790167							
Female							
AA	28 (37.8)	24 (34.8)	20 (35.1)	1.00 (reference)		1.00 (reference)	
GA	42 (56.8)	37 (53.6)	31 (54.4)	1.03 (0.51 - 2.07)	0.939	1.03 (0.49 - 2.16)	0.931
GG	4 (5.4)	8 (11.6)	6 (10.5)	2.33 (0.62 - 8.72)	0.200	2.10 (0.52 - 8.42)	0.289
Male							
AA	42 (39.3)	14 (26.4)	8 (26.7)	1.00 (reference)		1.00 (reference)	
GA	56 (52.3)	21 (39.6)	9 (30.0)	1.13 (0.51 - 2.47)	0.769	0.84 (0.30 - 2.37)	0.747
GG	9 (8.4)	18 (34.0)	13 (43.3)	6.00 (2.20 - 16.36)	0.000*	7.58 (2.43 - 23.65)	0.000*

[a]relative risk between case and control.
[b]relative risk between adenocarcinoma and control.
*Statistically significant.
CI, confidence interval; OR, odds ratio.

(P = 0.048), respectively (Table 7).

Association of the interactions between the CD40 and TP63 genes with lung cancer

Logistic regression analysis showed that there were gene-gene interactions between *CD40* and *TP63*. Individuals who carried both the CC of rs1535045 and the

Table 6. Association of clinic pathological criteria with *CD40/TP63* SNPs in lung cancer patients.

Variables	CD40 rs1535045			P	TP63 rs6790167			P
	CC	TC	TT		GG	GA	AA	
Case (%)	134 (51.9)	102 (39.5)	22 (8.5)		49 (19.0)	129 (50)	80 (31)	
Histology								
Adenocarcinoma (%)	76 (51.0)	58 (38.9)	15 (10.1)	0.585	31 (20.8)	71 (47.7)	47 (31.5)	0.597
Others (%)	58 (53.2)	44 (40.4)	7 (6.4)		18 (16.5)	58 (53.2)	33 (30.3)	
Stage								
Ⅰ + Ⅱ (%)	57 (64.0)	28 (31.5)	4 (4.5)	0.013*	10 (11.2)	49 (55.1)	30 (33.7)	0.070
Ⅲ + Ⅳ (%)	77 (45.6)	74 (43.8)	18 (10.7)		39 (23.1)	80 (47.3)	50 (29.6)	
Tumor marker								
Normal (%)	66 (63.5)	28 (26.9)	10 (9.6)	0.003*	16 (15.4)	54 (51.9)	34 (32.7)	0.477
Abnormal (%)	68 (44.2)	74 (48.1)	12 (7.8)		33 (21.4)	75 (48.7)	46 (29.9)	
Metastasis								
No (%)	98 (56.6)	67 (38.7)	8 (4.6)	0.003*	34 (19.7)	84 (48.6)	55 (31.8)	0.801
Yes (%)	36 (42.4)	35 (41.2)	14 (16.5)		15 (17.6)	45 (52.9)	25 (29.4)	

*Statistically significant.

Table 7. Genotype frequencies of *CD40/TP63* SNPs in 3 major histological types of lung cancer.

Variables	CD40 rs1535045			P	TP63 rs6790167			P
	CC	TC	TT		GG	GA	AA	
Conrol (%)	106 (39.3)	132 (48.9)	32 (11.9)		32 (11.9)	140 (51.9)	98 (36.3)	
Small cell lung cancer (%)	28 (59.6)	13 (27.7)	6 (12.8)	0.019*	8 (17.0)	23 (48.9)	16 (34.0)	0.616
Adenocarcinoma (%)	76 (51.0)	58 (38.9)	15 (10.1)	0.066	31 (20.8)	71 (47.7)	47 (31.5)	0.048*
Squamous cell carcinoma (%)	26 (50.0)	25 (48.1)	1 (1.9)	0.066	10 (19.2)	31 (59.6)	11 (21.2)	0.072

P-value between patients and controls using χ^2 test.
*Statistically significant.

GG of rs6790167 had a significantly higher risk than those with the TT of rs1535045 and the AA of rs6790167 for lung cancer (OR = 3.67, 95% CI: 1.0-13.23) and lung adenocarcinoma (OR = 5.83, 95% CI: 1.06-32.02), respectively (Table 8).

Discussion

In recent years, a number of genome-wide association studies (GWAS) and candidate gene case-control studies have confirmed that apoptosis-related gene polymorphisms are associated with lung cancer risk (Wang et al. 2008; Broderick et al. 2009; Hu et al. 2011; Zhang et al. 2014). In the present study, we evaluated the associations of SNPs in the apoptosis-related genes *CD40* (rs1535045) and *TP63* (rs6790167) with lung cancer in a Chinese Han population. We found that the CC genotype of rs1535045 and the GG genotype of rs6790167 were associated with a higher risk for lung cancer. Stratified analysis showed that rs6790167 was associated with lung cancer in non-smoking males, whereas rs1535045 was associated with lung cancer in non-smoking females with early lung cancer or negative tumor markers. Individuals who harbored both the CC genotype of rs1535045 and the GG genotype of rs6790167 had a higher risk for lung cancer.

The association of the *TP63* gene polymorphisms and lung cancer susceptibility has been confirmed in several studies. In a study of European populations, Wang et al. (2011) observed that rs10937405 (g38968C>T), rs17429138 (g189245593A>G) and rs4396880 (g12006G>A) in *TP63* were associated with susceptibility to lung cancer. In an investigation involving East Asian populations (i.e., Japan and South Korea), Miki et al. (2010) showed that rs4488809 (g12046T>C) and rs10937405 in *TP63* were associated with susceptibility to lung adenocarcinoma. Another study reported that only SNP rs10937405 in the *TP63* gene was closely associated with risk for lung adenocarcinoma in

Table 8. Correlation of *TP63*/*CD40* SNPs with risk for lung cancer.

Genotype		Control (n = 270)	Case (n = 258)	Adenocarcinoma (n = 149)	OR (95% CI)[a]	P[a]	OR (95% CI)[b]	P[b]
AA	TT	10	5	2	1.00 (reference)		1.00 (reference)	
	TC	50	39	21	1.56 (0.49 - 4.94)	0.447	2.10 (0.42 - 10.42)	0.355
	CC	38	36	24	1.90 (0.59 - 6.08)	0.278	3.16 (0.64 - 15.67)	0.143
GA	TT	18	15	11	1.67 (0.47 - 5.96)	0.430	3.06 (0.56 - 16.62)	0.183
	TC	66	38	22	1.15 (0.37 - 3.62)	0.809	1.67 (0.34 - 8.20)	0.526
	CC	56	76	38	2.71 (0.88 - 8.38)	0.074	3.40 (0.70 - 16.36)	0.110
GG	TT	4	2	2	1.00 (0.13 - 7.45)	1.000	2.50 (0.26 - 24.34)	0.423
	TC	16	25	15	3.13 (0.90 - 10.84)	0.066	4.69 (0.88 - 25.00)	0.056
	CC	12	22	14	3.67 (1.02 - 13.23)	0.042*	5.83 (1.06 - 32.02)	0.031*

[a]relative risk between case and control.
[b]relative risk between adenocarcinoma and control.
*Statistically significant.
CI, confidence interval; OR, odds ratio.

non-smoking Asian women (Hosgood et al. 2012). A GWAS study of 2,331 Chinese patients with lung cancer and 3,077 healthy controls revealed that only rs4488809 was associated with susceptibility to lung cancer in Chinese Han populations (Hu et al. 2011). The results of the present study showed that in the non-smoking male population, individuals with the GG genotype of rs6790167 in the *TP63* gene had a higher risk for lung cancer than those with the GA or AA genotypes. On the other hand, in the non-smoking female or smoking male population, rs6790167 in the *TP63* gene was not associated with susceptibility to lung cancer. Besides, the variation in *TP63* (rs6790167) is associated with the risk of lung adenocarcinoma, which is in agreement with the findings of Wang et al. (2011). In contrast, Pathak et al. (2014) reported that rs6790167 in the *TP63* gene is an independent risk factor for African-American women with non-small cell lung cancer. These results suggest that differences in associations may be attributable to the ethnicity of the study population.

The *CD40* gene is closely related to the development of cancer, chronic inflammatory diseases, and autoimmune diseases such as breast cancer, lymphoma, Graves' disease, chronic obstructive pulmonary disease (COPD), and systemic lupus erythematosus (SLE). The current association studies of *CD40* gene polymorphisms have been focused on these diseases (Hsiao et al. 2008; Liu et al. 2009; Shuang et al. 2011; Piotrowski et al. 2013). However, studies on *CD40* and lung cancer susceptibility are limited. Our results confirmed that the CC genotype of rs1535045 is associated with a higher risk for lung adenocarcinoma in non-smoking women, which is in agreement with the findings of Pathak et al. (2014). We also determined that in the early stages of lung cancer (I + II), the frequency of the CC genotype was significantly higher than that of the TC and TT genotypes, thus suggesting a higher risk for early lung cancer or a lower positive rate of serum tumor markers in individuals with this genotype. Therefore, we believe that detection of the rs1535045 polymorphism in the *CD40* gene may contribute to early diagnosis of lung cancer.

In addition, we determined that there were gene-gene interactions between rs1535045 in the *CD40* gene and rs6790167 in the *TP63* gene. Individuals who harbored both the CC genotype of rs1535045 and the GG genotype of rs6790167 had higher risk for lung adenocarcinoma. The binding of CD40 to its receptors activates transcription factor NF-κB and increases the synthesis of anti-apoptotic proteins, as well as activates TGF-β signaling pathways (Kim et al. 2015). Since TP63 is downstream target of NF-κB (Wu et al. 2010), the expression of an isoform of TP63 (ΔNp63) is increased when CD40 activated NF-κB (Fukunishi et al. 2010). ΔNp63 may inhibit the pro-apoptosis function of TP53 and promote tumorigenesis by competitively binding to the TAp63-binding site (Westfall et al. 2003). These mechanisms may explain the synergistic effect between rs1535045 in the *CD40* gene and rs6790167 in the *TP63* gene.

Because this research was a hospital-based case-control study, selection bias could not be completely ruled out. Future population-based case-control studies of larger samples confirming the associations of rs1535045 in the *CD40* gene and rs6790167 in the *TP63* gene with lung cancer susceptibility are thus warranted. In addition, the sample size of the present study was relatively small, thereby limiting statistical power of the analysis. To this end, we are prepared to conduct further validation studies using a large, multi-center study population.

Conflict of Interest

The authors declare no conflict of interest.

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