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Original Article

CD40 -1C>T polymorphism and the risk of lung cancer in a Chinese population

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Abstract: Background: The co-stimulatory molecule CD40 plays an important role in anti-tumor responses by promoting cytotoxic T lymphocyte (CTL) activity and differentiation of helper T cells. Growing evidence suggests that single nucleotide polymorphisms (SNPs) in CD40 are associated with the susceptibility to cancer. This study investigated the association between the CD40 -1C/T SNP (rs1883832) and lung cancer in a Chinese population. Methods: We conducted a hospital-based case-control study including 105 lung cancer patients and 109 healthy control subjects. The -1C/T SNP in CD40 was genotyped by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), and its association with lung cancer susceptibility was evaluated. Results: The distribution of the genotypes of CD40-1C/T was significantly different between lung cancer patients and controls. The frequency of the TT genotype (adjusted *P* = 0.017; overall risk [OR] = 2.94; 95% confidence interval [CI] = 1.21-7.13) and TT/CT genotype (adjusted P = 0.020; OR = 1.95; 95% CI = 1.11-3.43) were significantly higher in lung cancer patients than that in controls. When the cases were categorized by tumor histology, the TT genotype was associated with a significantly increased risk of squamous cell carcinoma (adjusted OR = 6.53; 95% CI = 1.97-21.61; *P* = 0.002). Conclusion: Our findings suggest that the CD40 -1C/T SNP (rs1883832) is correlated with the susceptibility to lung cancer in Chinese, and the TT genotype may further increase the risk of lung cancer.

Keywords: CD40, lung cancer, single nucleotide polymorphism

Introduction

Lung cancer is the most commonly diagnosed malignancy worldwide and the leading cause of cancer-related death, with an incidence rate of 46.08/100,000 and a mortality of 37/100,000 [1]. Seventy percent of patients with lung cancer have already reached an advanced stage by the time they are diagnosed. Although significant progress has been made toward understanding interindividual predispositions associated with lung cancer, there are few useful biomarkers to identify susceptive individuals in the general population. Because of the frequent occurrence and detectability of SNP, many studies have investigated the relationship between genetic polymorphisms and the risk of lung cancers [2].

CD40 is a member of the tumor necrosis factor receptor (TNFR) family and is expressed on the surfaces of antigen presenting cells (APC) such as B cells, monocytes, dendritic cells (DC), as well as non-immune cells such as endothelial cells, epithelial cells, and malignant tumor cells. CD40 plays a key role in stimulating cytotoxic lymphocytes and interactions between CD40 and its ligand, CD154, regulate activity of APC and the immune response of T cells and B cells [3]. Moreover, CD40 signaling is critical in anti-tumor responses, acting to promote cytotoxic T lymphocyte (CTL) responses and differentiate helper T cells towards Th1 cells [5, 6]. Yet some studies reveal that CD40 can promote tumor proliferation through particular pathways [5]. For example, CD40 promotes tumor angiogenesis by inducing the expression of vascular endothelial growth factor (VEGF) [7]. Other research shows that CD40 promotes tumor growth by affecting the inflammatory microenvironment [8]. Due to the complex function of

Table 1. Characteristics of the study population

Characteristic information	Cases (n = 105)	Controls (n = 109)
Age (years)	58±11	56±10
Sex		
Male	71	70
Female	34	39
Smoking[a]		
Yes	63	60
No	42	49
Histology		
Adenocarcinoma	44	
Squamous	32	
Small Cell Carcinoma	29	

[a]The smoking history is identified by the definition of WHO of 1997: consecutive or accumulative smoking time exceeds 6 months.

CD40, many tumors and autoimmune disorders are related to CD40 expression [9-12].

In the Han Chinese population, there are approximately 45 SNPs identified in the CD40 gene. The -1C>T SNP is associated with the expression of CD40 on the surfaces of B cells and DC [13], and this polymorphism has been studied in several malignancies, including lymphomas and breast cancer [11, 14-17]. To further verify the role of the CD40-1C/T SNP in the risk of lung cancer, we conducted a hospital-based case-control study in a Han Chinese population from the ShaanXi Province, located in northwest China.

Materials and methods

Subjects

All subjects in this study were unrelated Han Chinese from northwest China. The cases were patients with histopathologically confirmed primary lung cancer in the Respiratory Department of the Xijing Hospital (the First Affiliated Hospital of the Fourth Military Medical University, Shannxi Province) from 2011 to 2013. The diagnosis of lung cancer was based on surgical and pathological reports as well as radiography results. Subjects were excluded if they had histories of malignancies, autoimmune disorders, respiratory disorders, or hereditary diseases. Patients who had infections within 4 weeks before the study were also excluded. Personal information was obtained from medical files. In the control group, 110 healthy controls were recruited randomly from volunteers at The Medical Examination Center of Xijing Hospital. All healthy controls were frequency-matched (1:1) to patients based on age, gender and smoking status. During the statistical analysis, we removed the samples failed in DNA genotyping. Ultimately, this study included 105 lung cancer patients and 109 healthy controls. This study was approved by the Institutional Review Board of the Fourth Military Medical University and informed consent was obtained from each participant.

DNA extraction and genotyping

Genomic DNA was extracted from blood samples using TIANamp Genomic DNA Kits (Beijing, China) according to manufacturer protocols. The CD40 -1C/T SNP was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. Primer sequences were designed by Primer Premier software and synthesized by Sangon Biotech (Shanghai, China). The primer sequences were:

Sense, 5'-ACACAGCAAGATGCGTCCCTAAACT-3' (Tm = 65.6°C); Anti-sense, 5'-TCCTTCTCATTCCCCACTCCCAACT-3' (Tm = 68.2°C).

The length of the PCR product was 334 bp. PCR was performed in a total volume of 50 μL containing: 200 ng genomic DNA, 25 μL Premix Taq (TaKaRa TaqTM Version 2.0 plus dye) (TaKaRa, Dalian, China), 1 μL of each primer (20 μM), ddH_2O were added to bring samples to a final volume of 50 μL. The cycling conditions were consisted of: 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 45 s at 72°C. After the 35 cycles, there is a final extension step for 7 min at 72°C. After purification with TIANamp PCR Purification Kits (Beijing, China), PCR products were digested by the restriction enzyme Ncol I at 37°C for 6 h in a volume of 20 μL containing: 1 μL of Ncol I (2 U); 2 μL of 10× K Buffer; 2 μL of 0.1% bovine serum albumin (BSA); 10 μL of PCR product; and 5 μL of ddH_2O. The restriction digest products were analyzed by electrophoresis on a 2.0% agarose gel. The -1CC homozygotes showed 2 fragments (106 bp and 228 bp), the -1TT homozygotes showed only 1 fragment (334 bp), and the -1CT hetero-

Table 2. Genotypes and allele frequencies of CD40-1C/T in lung cancer patients and controls

Analysis model	Allele or Genotype	Cases (*n* = 105) No. (%)	Controls (*n* = 109) No. (%)	Crude OR (95% CI)	Crude P	Adjusted OR (95% CI)[a]	Adjusted P[a]
	Allele						
	C	122 (58.10)	152 (69.72)	1.00 (Ref)		1.00 (Ref)	
	T	88 (41.90)	66 (30.28)	1.66 (1.12-2.47)	0.012	1.72 (1.15-2.57)	0.008
Genetic model	Genotype						
Co-Dominant	CC	36 (34.29)	53 (48.62)	1.00 (Ref)		1.00 (Ref)	
	CT	50 (47.62)	46 (42.20)	1.60 (0.89-2.87)	0.114	1.72 (0.95-3.14)	0.074
	TT	19 (18.10)	10 (9.17)	2.80 (1.17-6.71)	0.021	2.94 (1.21-7.13)	0.017
Additive				1.65 (1.11-2.47)	0.014	1.72 (1.14-2.59)	0.010
Dominant	CC	36 (34.29)	53 (48.62)	1.00 (Ref)		1.00 (Ref)	
	CT/TT	69 (65.71)	56 (51.38)	1.81 (1.05-3.15)	0.034	1.95 (1.11-3.43)	0.020
Recessive	CT/CC	86 (81.90)	99 (90.83)	1.00 (Ref)		1.00 (Ref)	
	TT	19 (18.10)	10 (9.17)	2.19 (0.96-4.96)	0.061	2.22 (0.97-5.05)	0.059

[a]Adjusted for age, smoking status and gender.

zygotes showed 3 fragments (334 bp, 106 bp, and 228 bp). To ensure the accuracy of genotyping for this PCR-RFLP analyse, 10% of the patient and control samples were randomly selected for validation by direct sequencing.

Statistical analyses

The cases and controls were compared using Student's t-tests for continuous variables and χ^2 tests for categorical variables. The Hardy-Weinberg equilibrium was tested in the control group with the expected genotype frequencies. The cancer risk associated with the genotypes was estimated as odds ratios (OR) with 95% confidence intervals (CI) using logistic regression. Crude ORs and ORs adjusted for possible confounders (gender, age and smoking status) were calculated. Multivariate logistic regression analyses were performed to analyze the association between genotypes and lung cancer risk after stratifying subjects according to age (median years), gender, smoking status. *P* values <0.05 were considered significant for all of analyses. Statistical analyses were performed using SPSS 18.0.

Results

Basic characteristics of the lung cancer patients and controls are summarized in **Table 1**. No statistically significant differences in gender, age, or smoking status were observed between the lung cancer patients and controls (*P*>0.05), which suggested adequate matching based on those variables. The genotype and allele frequencies of the CD40 -1C/T polymorphism in 105 lung cancer patients and 109 control subjects are shown in **Table 2**. The genotype distributions of the polymorphism among the controls were within the Hardy-Weinberg equilibrium (χ^2 = 0.0001; *P*>0.05).

A significant difference regarding the frequency of allele T was found between the lung cancer patients (41.9%) and controls (30.3%) (adjusted *P* = 0.008; OR = 1.72; 95% CI = 1.15-2.57). Significantly increased lung cancer risks were suggested to be associated with the TT genotype of CD40-1C/T compared with the CC genotype (adjusted P = 0.017; OR = 2.94; 95% CI = 1.21-7.13). In a dominant model, compared to the CC genotype, the TT/CT genotype was associated with a significantly increased risk of lung cancer (adjusted P = 0.020; OR = 1.95; 95% CI = 1.11-3.43). When the cases were categorized by tumor histology, we found the incidence of the CD40-1C/T polymorphisms in squamous cell carcinoma cases differed significantly from that of the controls. The CC genotype was observed in 31.3% patients versus 48.6% in controls. The CT genotype was observed in 42.2% of patients versus 37.5% of controls and the TT genotype was observed in 31.3% of patients versus 9.2% of controls (adjusted *P* in additive model = 0.004). Compared to the CC genotype, the TT genotype significantly increased the risk of squamous cell carcinoma (adjusted OR = 6.53; 95% CI = 1.97-21.61; *P* = 0.002).

The association between the CD40-1C/T polymorphism and the risk of lung cancer was further examined by grouping the subjects according to gender, age and smoking status (**Table**

Table 3. The genotype frequencies of CD40-1C/T in different histopathology type of lung cancer

Histopathology type	Genotype, No. (%)			Adjusted OR (95% CI)[a]		
	CC	CT	TT	CC	CT	TT
Controls	53 (48.60)	46 (42.20)	10 (9.20)			
Squamous	10 (31.30)	12 (37.50)	10 (31.30)	1.0	1.60 (0.61~4.16)	6.53 (1.97~21.61)[b]
Adenocarcinoma	17 (38.60)	22 (50.00)	5 (11.40)	1.0	1.55 (0.72~3.34)	1.72 (0.50~5.85)
Small Cell Carcinoma	9 (31.03)	16 (55.20)	4 (13.79)	1.0	2.04 (0.81~5.14)	2.39 (0.60~9.50)

[a]Adjusted for age, gender and smoking status. [b]P = 0.002.

3). The effect of the TT genotype on the risk of lung cancer was statistically significant in males (adjusted OR = 4.14; 95% CI = 1.32-13.00; *P* = 0.015), whereas it had no significant effect in females (adjusted OR = 1.39; 95% CI = 0.30-6.42; *P* = 0.672) (**Table 4**). When stratified according to median age, the TT genotype was associated with a significantly increased risk of lung cancer in older individuals (adjusted OR = 4.53; 95% CI = 1.12-18.28; P = 0.034), and the CT genotype had significant effect in younger individuals (adjusted OR = 2.70; 95% CI = 1.14-6.37; *P* = 0.024). However, in an additive model, the three genotypes were similar in younger individuals (adjusted OR = 1.76; 95% CI = 0.97-3.21; P = 0.064) and older individuals (adjusted OR = 1.77; 95% CI = 0.99-3.16; *P* = 0.056). With regards to smoking, the occurrence of the TT genotype was significantly different between the lung cancer patients and controls (adjusted OR = 3.62; 95% CI = 1.01-12.94; *P* = 0.047).

Discussion

Most previous studies that examined links between the CD40 -1C>T gene polymorphism and cancers suggested that the T-containing genotypes could significantly increase the risk of tumors. The association we observed between the polymorphism in CD40 -1C>T gene and the susceptibility to lung cancer has not been previously reported. In this case-control study, we found a significantly different distribution of polymorphisms between the lung cancer patients and the controls. Notably, the frequencies of the T-containing genotypes (TT and TT/CT) in lung cancer patients were much higher than that in the controls, indicating that T-containing mutations may increase the risk of lung cancer, which was consistent with previous studies.

Although the mechanism underlying this association remains to be elucidated, one possible explanation is that the CD40 -1C/T polymorphism change from a C allele to T allele could cause major alterations in the initiation of gene translation. The -1C>T SNP places at the 5'-untranslated region within the Kozak consensus sequence, which is in most eukaryotic mRNAs and facilitates the binding of mRNA to the small subunit of the ribosome and then promotes the initiation of translation [13, 18]. Accordingly, this SNP might affect the translation activity of CD40 gene, and subsequent cell reactivity to the stimulation of cytokines and T cells [19]. A previous study showed that the TT genotype was related to lower circulating levels of soluble CD40 and reduced CD40 expression levels on the surfaces of monocyte-derived activated dendritic cells and B cells [20]. Moreover, the T-containing genotypes could influence the stability of mRNA-ribosome complex, thus down-regulating CD40 expression and increasing susceptibility to breast cancer [14]. Interestingly the CC genotype enhanced CD40 translation and has been shown to induce the development of autoimmune diseases, such as Grave's disease [20].

In this study, we found that the TT genotype was associated with a significantly increased risk of squamous cell carcinoma, suggesting that different histopathological types may have different etiologies, not only in relation to environmental risk factors but genetic susceptibility. However, the small sample size in this case group did not allow examination of gene-environment interactions, and therefore larger studies are needed to explore this.

After adjusting for possible confounders (age and smoking status), we observed that men with the TT variant genotype had a higher risk of lung cancer, but surprisingly, we found no additional risk in women, even with the same level of tobacco exposure. We also found that the association between the CD40 -1C>T variant and lung cancer risk was more pronounced

Table 4. Stratification analysis of the CD40-1C/T genotype frequencies in lung cancer cases and controls

Variables	Genotype, No. (%)						Adjusted OR (95% CI)			
	Cases (*n* = 105)			Controls (*n* = 109)			Co-dominant model			Additive model
	CC	CT	TT	CC	CT	TT	CC	CT	TT	
Gender										
Female	10 (29.41)	19 (55.88)	5 (14.71)	14 (35.90)	20 (51.28)	5 (12.82)	1.0	1.38 (0.48~3.93)[a]	1.39 (0.30~6.42)[a]	1.22 (0.59~2.54)[a]
Male	26 (36.62)	31 (43.66)	14 (19.72)	39 (55.71)	26 (37.14)	5 (7.14)	1.0	1.85 (0.89~3.84)[a]	4.14 (1.32~13.00)[a,e]	1.97 (1.19~3.27)[a,e]
Age (year)[d]										
≤57	14 (26.92)	31 (59.62)	7 (13.46)	28 (46.67)	25 (41.67)	7 (11.67)	1.0	2.70 (1.14~6.37)[b,e]	2.28 (0.64~8.11)[b]	1.76 (0.97~3.21)[b]
>57	22 (41.51)	19 (35.85)	12 (22.64)	25 (51.02)	21 (42.86)	3 (6.12)	1.0	1.15 (0.48~2.78)[b]	4.53 (1.12~18.28)[b,e]	1.77 (0.99~3.16)[b]
Smoking status										
Non-smoking	13 (30.95)	21 (50.00)	8 (19.05)	21 (42.86)	22 (44.90)	6 (12.24)	1.0	1.56 (0.60~4.10)[c]	2.20 (0.61~7.94)[c]	1.50 (0.80~2.79)[c]
Smoking	23 (36.51)	29 (46.03)	11 (17.46)	32 (53.33)	24 (40.00)	4 (6.67)	1.0	1.77 (0.82~3.83)[c]	3.62 (1.01~12.94)[c,e]	1.85 (1.07~3.22)[c,e]

[a]Adjusted for age and smoking status. [b]Adjusted for gender and smoking status. [c]Adjusted for gender and age. [d]The median of age is 57. [e]P<0.05.

in smokers. This finding is consistent with previous results that the effect of two other CD40 SNPs (rs3765459 and rs1535045) on non-small cell lung cancer (NSCLC) was only significant for smokers [21], indicating that genetic susceptibility of CD40 variants is often associated with smoking status.

In conclusion, our study provides evidence that links CD40-1C/T polymorphisms and lung cancer susceptibility in a Chinese population. Additional studies are needed to better understand the different pathways and factors that contribute to these associations. With advances in clinical molecular biology, genetic information can be readily utilized in clinical decision making.

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Disclosure of conflict of interest

None.

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