Int J Clin Exp Med 2015;8(7):11519-11523
www.ijcem.com /ISSN:1940-5901/IJCEM0007343

Original Article

Cytotoxic T lymphocyte-associated antigen-4 +49A>G polymorphism and the risk of non-small cell lung cancer in a Chinese population

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Received February 25, 2015; Accepted May 26, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: Background: CTLA-4 is a potent immunoregulatory molecule and plays a pivotal role in the negative regulation of T-cell proliferation and activation. Previously, the association between CTLA-4 +49A>G polymorphism and the risk of NSCLC has been investigated in several studies, however, their results were inconsistent. Therefore, we aimed to investigated the association between CTLA-4 +49A>G polymorphism and the risk of NSCLC in a Chinese population. Methods: We recruited 231 NSCLC patients and 250 healthy controls in the present case-control study. PCR-RFLP was used to analyze the polymorphism of CTLA-4. The chi-squared test was used to examine differences between NSCLC patients and controls. The odds ratio (OR) and its 95% confidence interval (95% CI) were obtained by logistic regression methodology to determine correlations between the CTLA-4 polymorphism and the incidence of NSCLC. Results: When the AA genotype was used as the reference group, the GG genotype was significantly associated with increased risk for NSCLC (OR=2.181, 95% CI: 1.244-5.198; P=0.007), however, the AG genotype was not significantly associated with increased risk for NSCLC (OR=2.018, 95% CI: 0.826-3.881; P=0.099). Under the dominant model of inheritance, the AG+GG genotype was significantly associated with increased risk for NSCLC (OR=3.271, 95% CI: 1.827-4.559; P=0.015). In addition, the G allele had a 2.754-fold higher risk of NSCLC in comparison with the A allele (OR=2.754, 95% CI: 1.365-6.891, P=0.005). Conclusions: Our data provided evidence that the CTLA-4 +49A>G polymorphism is associated with increased risk of NSLCL in Chinese population.

Keywords: CTLA-4, NSCLC, polymorphism, risk

Introduction

Lung cancer is the most commonly diagnosed cancer and is the leading cause of cancer death in males, as well as being the fourth most commonly diagnosed cancer and the second leading cause of cancer death in females [1]. Approximately 80-85% of lung cancers are classified as non-small cell lung cancer (NSCLC), the majority of patients presenting with unresectable advanced disease. Current knowledge regarding NSCLC is not a single disease but a collection of diseases with distinct pathogeneses by molecular mechanisms. Genetic play an integral role in the transformation, promotion and progression of NSCLC [2].

The cytotoxic T-lymphocyte antigen 4(CTLA4, CD152) gene, located on chromosome 2q33, is composed of four exons that encode separate functional domains: leader sequence, extracellular domain, transmembrane domain, and cytoplasmic domain [3]. CTLA4 is a potent immunoregulatory molecule and plays a pivotal role in the negative regulation of T-cell proliferation and activation [4]. CTLA-4 can induce FAS-independent apoptosis of activated T cells [5], which may inhibit immune function of T lymphocytes. In addition, antitumor T cells play a pivotal role in immune surveillance of cancer cells [6].

CTLA-4 gene possesses several vital SNPs, such as the +49A/G (rs231775), -318C/T (rs5742909), CT60G/A (rs3087243), -1661A/G (rs4553808), and -1722T/C (rs733618) SNPs, etc [7, 8]. Previously, the association between CTLA-4 +49A>G polymorphism and the risk of

Table 1. Clinicopathological characteristics of 231 NSCLC patients and 250 controls

		NSCLC (n=231)		Controls (n=250)		
Character	Subgroup	Number	%	Number	%	*P*-value
Gender	Male	145	62.77	142	56.80	0.194
	Female	86	37.23	108	43.20	
Age	<65	109	47.19	121	48.40	0.855
	≥65	122	52.81	129	51.60	
Smoking status	Ever	187	80.95	142	56.80	<0.001
	Never	44	19.05	108	43.20	
Alcohol drinking	Ever	142	61.47	121	48.40	0.004
	Never	89	38.53	129	51.60	

Table 2. Genotype and allele frequencies of CTLA-4 +49A>G polymorphism among NSCLC cases and controls

CTLA-4 +49A>G polymorphism	NSCLC (n=231)		Controls (n=250)		*P*-value
	n	%	n	%	
Genotype					
AA	53	22.94	108	43.20	<0.001
AG	101	43.72	91	36.40	
GG	77	33.33	51	20.40	
Allele					
A	207	44.81	307	61.40	<0.001
G	255	55.19	193	38.60	

NSCLC has been investigated in several studies, however, their results were inconsistent [9-11]. Therefore, we aimed to investigated the association between CTLA-4 +49A>G polymorphism and the risk of NSCLC in a Chinese population.

Material and methods

Study subjects

In the present case-control study, we recruited 231 NSCLC patients with histologically confirmed diagnoses between August 2009 and March 2014. Patients should meet the following criteria: (1) all cases should meet lung cancer diagnostic criteria announced by World Health Organization (WHO); (2) they should be primary NSCLC; (3) they have not received chemotherapy or radiation therapy. During the same period, 250 healthy controls with no evidence of lung or other cancers were randomly recruited from a medical examination center in the same hospital. Participants were unrelated ethnic Han Chinese. Face-to-face interviews of patients and healthy control subjects were conducted by two trained interviewers, collected information including demographic data (name, gender, age, etc.) and drinking, smoking status. At recruitment, written informed consents about the study were obtained from all subjects. The research was approved by the institutional Review Board of Hebei General Hospital.

DNA extraction and genotyping

DNA was extracted from 2 ml peripheral blood obtained from each participant using a Genomic DNA Extraction Kit according to the manufacturer's protocol. Aliquot DNA was stored at 20°C until used. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the polymorphism of CTLA-4. The PCR was carried out in an AmpGene DNA thermal cycler 4800 and reaction mixtures of the total volume of 25 μL included 10 μg genomic DNA, 5 pmol of each primer, and 1X PCR mix containing 200 μmol/L of each dNTP, 5 μL of 10X reaction buffer, and 1.25 U Taq Gold Polymerase and 4 mmol/L $MgCl_2$. Primers 5'-AAGGGCTCAGCTGAACCTGGT and 5'-CTGCTGAAACAAATGAAACCC were used to amplify the 152-bp DNA fragment of the CTLA-4 +49A/G polymorphism. The PCR is followed by an overnight digestion with the restriction enzyme BstEII. Ten percent of the samples were confirmed by direct sequencing of PCR products to verify the accuracy of genotyping.

Statistical analysis

The compatibility with the Hardy-Weinberg equilibrium was calculated with HWE program (http://linkage.rockefeller.edu/ott/linkutil.htm). The chi-squared test was used to examine differences between NSCLC patients and con-

Table 3. The association of CTLA-4 +49A>G polymorphism with the risk of NSCLC

	Patients	Controls	OR (95% CI)	*P* value
General genotype				
AA	53	108	1.00 (Reference)	
AG	101	91	2.018 (0.826-3.881)	0.099
GG	77	51	2.181 (1.244-5.198)	0.007
Dominant genotype				
AA	53	108	1.00 (Reference)	
AG+GG	178	142	3.271 (1.827-4.559)	0.015
Recessive genotype				
AA+AG	154	199	1.00 (Reference)	
GG	77	51	1.972 (0.781-3.883)	0.127
Allele frequency				
A	207	307	1.00 (Reference)	
G	255	193	2.754 (1.365-6.891)	0.005

trols. The odds ratio (OR) and its 95% confidence interval (95% CI) were obtained by logistic regression methodology to determine correlations between the CTLA-4 polymorphism, and the incidence of NSCLC. All analyses were performed using SPSS 18.0 software (SPSS, Inc. Chicago, IL, USA), and a P<0.05 was considered to be statistically significant.

Results

Clinicopathological characteristics of 231 NSCLC patients and 250 controls

Data on 231 lung cancer cases (145 males, 86 females) and 250 healthy controls (142 males, 108 females) were available for analyses. The distribution of the main clinicopathological characteristics was shown in **Table 1**. No significant difference in age or sex distribution was observed between NSCLC cases and controls (P=0.855 and P=0.194, respectively). However, there were more smokers (P<0.001) and drinkers (P=0.004) in NSCLC cases group than that in control group.

Genotype and allele frequencies of CTLA-4 +49A>G polymorphism among NSCLC cases and controls

The genotype distribution of CTLA-4 +49A>G polymorphism deviated from Hardy-Weinberg equilibrium in both NSCLC patients and controls (P>0.05), indicating that it was plausible that selective forces are operating in the population. The allele and genotype frequencies of CTLA-4 +49A>G polymorphism for the NSCLC cases and controls are presented in **Table 2**. The G allele revealed significantly increased frequency in NSCLC patients compared to healthy controls (55.19% vs. 38.60%, P<0.001). Among 231 NSCLC patients, 53 (22.94%) displayed a AA genotype, 101 (43.71%) with a AG genotype and 77 (33.33%) with a GG genotype. Among 250 healthy controls, 108 (43.20%) displayed a AA genotype, 91 (36.40%) with a AG genotype and 51 (20.40%) with a GG genotype. Therefore, there was a significant difference of CTLA-4 +49A>G polymorphism genotype distribution between NSCLC group and control group (P<0.001).

The association of CTLA-4 +49A>G polymorphism with the risk of NSCLC

When the AA genotype was used as the reference group, the GG genotype was significantly associated with increased risk for NSCLC (OR=2.181, 95% CI: 1.244-5.198; P=0.007), however, the AG genotype was not significantly associated with increased risk for NSCLC (OR=2.018, 95% CI: 0.826-3.881; P=0.099, shown in **Table 3**). Under the dominant model of inheritance, the AG+GG genotype was significantly associated with increased risk for NSCLC (OR=3.271, 95% CI: 1.827-4.559; P=0.015). However, under the recessive model of inheritance, the GG genotype was not significantly associated with increased risk for NSCLC (OR=1.972, 95% CI: 0.781-3.883; P=0.127). In addition, the G allele had a 2.754-fold higher risk of NSCLC in comparison with the A allele (OR=2.754, 95% CI: 1.365-6.891, P=0.005).

Discussion

Lung cancer is a biological complex disease highly relevant to factors such as environment, occupation, smoking, and the genetic factor also plays an important role, the difference between individual cell cycle, DNA repair and apoptosis control may decide to different individual genetic susceptibility to tumor [12]. The

research of the correlation between gene polymorphism and lung cancer will help to clarify the pathogenesis of lung cancer, including its formation and development, and play an important part of the diagnosis and prognosis of patients with lung cancer.

CTLA-4 is a immunoregulatory molecule and plays a critical role in limiting the potency of tumor immunity through antagonizing T cell activation [13, 14]. CTLA-4 binds to B7-1 and B7-2 and disrupts IL-2 production, IL-2 receptor expression, and cell cycle progression of activated T cells [15]. In addition to a significant involvement in regulation of tolerance to human "self" antigens, CTLA-4 also attenuates antitumor response by elevating T-cell activation threshold, thus inducing occurrence of cancer [16]. The aforementioned evidence suggests that CTLA-4 blockade may be an effective way to suppress cancer progression.

CTLA-4 is a highly polymorphic gene with more than 100 single-nucleotide polymorphisms [8]. These SNPs encode leader sequence, and regulate several independent function domains, including extracellular domain, transmembrane domain, and cytoplasmic domain [17]. Previous investigations revealed significant associations of polymorphisms in CTLA4 gene and the susceptibility to various types of cancers such as cervical cancer, breast cancer, bladder cancer, non-Hodgkin's lymphoma and multiple myeloma [3, 18-21]. Polymorphism at CTLA4 +49A/G was associated with reduction in inhibitory function of CTLA4. A49G dimorphism (Thr/Ala exchange in a peptide) leads to the expression of defective receptor, as a result, the inhibitory effect of CTLA4 on lymphocyte T cell activation is impaired [22]. Previously, the association between CTLA-4 +49A>G polymorphism and the risk of NSCLC has been investigated in several studies, however, their results were inconsistent [9-11]. Therefore, we aimed to investigated the association between CTLA-4 +49A>G polymorphism and the risk of NSCLC in a Chinese population.

In the present study, the genotype distribution of CTLA-4 +49A>G polymorphism deviated from Hardy-Weinberg equilibrium in both NSCLC patients and controls, indicating that it was plausible that selective forces are operating in the population. The G allele revealed significantly increased frequency in NSCLC patients compared to healthy controls. Moreover, there was a significant difference of CTLA-4 +49A>G polymorphism genotype distribution between NSCLC group and control group. When the AA genotype was used as the reference group, the GG genotype was significantly associated with increased risk for NSCLC. Under the dominant model of inheritance, the AG+GG genotype was significantly associated with increased risk for NSCLC. In addition, the G allele had a 2.754-fold higher risk of NSCLC in comparison with the A allele. In conclusion, our data provided evidence that the CTLA-4 +49A>G polymorphism is associated with increased risk of NSLCL in Chinese population.

Disclosure of conflict of interest

None.

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