RESEARCH ARTICLE

Genetic Variation in PDCD6 and Susceptibility to Lung Cancer

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Abstract

Lung cancer is the most common type of cancer and one of the leading causes of death in the world. Genetic factors play an important role in its development. PDCD6, the encoding gene for programmed cell death protein 6, may function as a tumor suppressor gene. Non-small cell lung cancer (NSCLC) contributes about 80% to newly histologically diagnosed lung cancer patients. To explore the relationship between PDCD6 and NSCLC, we examined two single nucleotide polymorphisms(rs3756712 G/T andrs4957014 G/T, both in the intron region) of the PDCD6gene.A hospital-based case-control study was carried out including 302 unrelated NSCLC patients and 306 healthy unrelated subjects. Significantly increased NSCLC risk was found to be associated with the T allele of rs4957014 (P=0.027, OR=0.760, 95%CI=0.596-0.970). The genotype and allele frequencies of rs3756712 did not shown any significant difference between NSCLC group and controls (P=0.327, OR=0.879, 95%CI=0.679-1.137). In conclusion, we firstly demonstrated the association between the PDCD6 gene and risk of NSCLC in a Chinese Han population.

Keywords: PDCD6 - lung cancer - single nucleotide polymorphism

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Introduction

Lung cancer is the most common type of cancer and one of the leading causes of death in the world (Jemal et al., 2011). In China, data from the national death survey and cancer registration of China mainland showed that there would be 2.6 million new cancer incidences and 1.8 million cancer deaths in 2005. It was estimated that lung cancer was also leading cause of death in most cities in China (Chen et al., 2009). In Sichuan, the death rate of lung cancer was 49.66/105 in males, while 20.18/105 in females (Dai et al., 2012). In general, non-small cell lung cancer (NSCLC) contributes about 80% to newly histologically diagnosed lung cancer patients. Despite considerable therapeutic progress, the prognosis of NSCLC patients remains poor, with a 5-year overall survival (OS) of less than 15% (Sculier et al., 2008). Surgery is the best curative approach in the early stages (I and II). However, even in these patients, the 5-year mean OS is less than 70% (Felip et al., 2005). It is indicated that the situation of lung cancer are becoming serious all over the world.

Many epidemiologic studies show that environment, including cigarette smoking, occupational exposure to asbestos, metals, and welding fumes, air pollution, and malnutrition or diets are risk factors for lung cancer (Spitz et al., 2007). However, in some individuals, lung cancer is not associated with any of those factors, suggesting that other factors such as the genetic factors or host contribute to a predisposition to develop lung cancer (Amos et al., 1992). In 2010, Lissowska et al. (2010). performed a meta-analysis involving 41 studies, and found that the lung cancer family history was a risk factor of lung cancer. Therefore, it is reasonable to suspect that genetic factors might contribute to the development of lung cancer.

PDCD6, the coding gene of programmed cell death protein 6, also known as apoptosis-linked gene 2 (ALG-2), is a novel member of the penta-EF-hand (PEF) family. PDCD6 is the only so far known calcium-binding protein (Tarabykina et al., 2004).PDCD6, not only directly participate in FAS- induced cell death (Jung et al., 2001), and interact with SH3-binding domain containing proteins such as AIP1/Alix promoting cell death (Missotten et al., 1999; Vito et al., 1999), it also activates TNF-induced ASK1-JNK cell apoptotic signaling (Ichijo et al., 1997). Moreover, study also revealed a proliferative role of PDCD6. Subramanian et al. came to the conclusion that PDCD6 is generally down-regulated in melanoma cells compared to normal melanocytes (Subramanian et al., 2004). la Cour et al. (2003). found that the PDCD6 expression levels in 4 different types of lung metastasis are similar or lower than in the corresponding primary tumors. All the data suggests that PDCD6 may function as a tumor suppressor gene.

It has been studied that apoptosis, a conserved and regulated cell suicide process, is closely linked with carcinogenesis and plays an important role in pathogenesis of lung cancer (McGrath 2011; Shih et al., 2011). Current researches show that genetic polymorphisms can explain and contribute to inter-individual differences in oncogenesis and differentiation (Kutikhin 2011; Gu et al.,

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2012; Nakada et al., 2012; Xu et al., 2012). In 2011, Wei et al. (2011). performed a meta-analysis of polymorphisms in ERCC1 and XPD genes and NSCLC, indicated that NSCLC was significantly associated with polymorphism of ERCC1 C1118T. Dogu et al (2012). found that the gene polymorphism of MDR1 associated with the genetic susceptibility to NSCLC. To our knowledge, there is no report provided relationship between PDCD6 genetic polymorphisms and lung cancer up to now.

In our study, we genotyped two single nucleotide polymorphism(SNP) in PDCD6 gene (rs3756712 G/T andrs4957014 G/T, both in the intron region) and investigated their association with the risk of lung cancer.

Materials and Methods

Study subjects

A hospital-based case-control study was carried out including 302 unrelated lung cancer patients (216 males and 86 females, mean age: 58.56±10.20 years) enrolled from the West China Hospital of Sichuan University between April 2008 and December 2011. The clinical diagnosis of lung cancer was confirmed by histological examination of resected or biopsy specimens in all cases. The control group consisted of 306healthy unrelated subjects (219 males and 87 females, mean age: 57.90±11.91 years) from a routine healthy survey in the same hospital. All subjects were Han population living in Sichuan province of southwest China. Those with second lung tumors or other serious disease were intentionally excluded. A simple questionnaire was used to collect information on demographic characteristics, including sex, age at diagnosis, clinical stage, tumor differentiation and tumor type. The study was approved by the hospital ethics committee and all subjects gave informed consent.

PCR amplification and restriction enzyme digestion

Genomic DNA was extracted from 200 μl EDTA-anticoagulated peripheral blood samples by using a commercial DNA isolation kit from Bioteke (Peking, China), according to the manufacturer's instructions. Primers were established with the On-line software (http://frodo.wi.mit.edu/primer3/). The polymerase chain reaction (PCR)-polyacrylamide gel electrophoresis (PAGE) method was used to genotype the two polymorphisms of PDCD6. DNA fragments containing the polymorphisms were amplified by PCR using primer pairs respectively. The primers used for amplification of the rs4957014 were 5'-tggtgtttcataccattgacacttgc-3', and 5'-CTCAGAACCAAGCAGGTTCCTTCA-3', the primers used for amplification of the rs3756712 were 5'-TACAGTGGCAAAGGACCACA-3', and 5'-CACATTCCAGCACTCACCAC-3'. PCR reaction was performed in a total volume of 25 μl, including 2.5 μl 10× PCR buffer, 1.5mmol/L of $MgCl_2$, 0.15 mmol/L of dNTPs, 0.5 μmol/L of each primer, 100 ng of genomic DNA and 1U of Taq DNA polymerase. The PCR conditions were 94 °C for 4min, followed by 32 cycles of 30 s at 94 °C, 30 s at 62 °C and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. PCR products were digested overnight with specific restriction enzyme and the digested PCR products were separated by a 6% polyacrylamide gel and stained with 1.0 mg/ml argent nitrate: HphI for rs4957014G/T, allele G is cuttable, yielding two fragments of 13 and 100 bp, allele T is uncuttable and the fragment is still 113bp. RsaI for rs3756712, allele G is cuttable, yielding two fragments of 66 and 99 bp, allele T is uncuttable and the fragment is still 165bp. The genotypes were confirmed by the DNA sequencing analysis (BigDye®Terminator v3.1 Cycle Sequencing Kits; Applied Biosystems, Foster City, CA). About 10% of the samples were randomly selected to perform the repeated assays and the results were 100% concordant.

Statistical analysis

The genotype frequencies were obtained by direct counting and Hardy-Weinberg equilibrium was tested by a chi-square test. The comparison of genotype and allele frequency between NSCLC and control group were analyzed by the Pearson chi-squared test; Odds ratio (OR) and respective 95% confidence intervals (CI) were calculated using logistic regression to evaluate the effects of any difference between alleles, genotypes. Differences were considered significant when $P<0.05$. The analysis was performed with SPSS medical statistical software (version 16.0; SPSS Inc.).

Results

These two SNPs were successfully analyzed in 302 NSCLC and 306 control subjects. Genotype distributions of these two SNPs in our cases and control subjects were in accordance with that expected under the Hardy-Weinberg equilibrium ($P>0.05$), indicating that the frequencies fell into the expected equilibrium and were thus randomly distributed. The genotyped and allele frequencies of these two SNPs for 302 NSCLC patients and 306 control subjects were calculated and are summarized in Table 1. As shown in Table 1, significantly increased NSCLC risk was found to be associated with T allele of rs4957014 (P=0.027, OR=0.760, 95%CI=0.596-0.970). The genotype and allele frequencies of rs3756712 was not shown any significant difference between NSCLC group and controls (P=0.327, OR=0.879, 95%CI=0.679-1.137).

Table 1. Genotype and Allele Frequencies of Two SNPs in PDCD6 Gene in Lung Cancer and Normal Controls

SNP genotype/allele	Patient	control	χ^2	P-Value	OR (95% CI)
rs3756712					
TT	168(0.556)	168(0.549)	4.446	0.108	
TG	120(0.397)	111(0.363)			
GG	14(0.046)	27(0.088)			
T	456(0.755)	447(0.730)	0.96	0.327	0.879(0.679-1.137)
G	148(0.245)	165(0.270)			
rs4957014					
TT	155 (0.513)	134 (0.438)	4.918	0.085	
TG	124(0.411)	136(0.444)			
GG	23(0.076)	36(0.118)			
T	434(0.719)	404(0.660)	4.841	0.027	0.760(0.596-0.970)
G	170(0.281)	208(0.340)			

Significant P values (<0.05) are in boldface; OR, odds ratio; CI, confidence intervals; Frequencies are displayed in parenthesis

Table 2. Analysis of Patient Characteristics and Polymorphism of rs4957014

Characteristics	No of cases	Genotype No.			P-Value	Allele No.		P-Value	OR
		GG	GT	TT		G	T		(95%CI)
Sex									
Male	216	18(0.083)	87(0.403)	111(0.514)	0.731	123(0.285)	309(0.715)	0.777	1.058
Female	86	5(0.058)	37(0.430)	44(0.512)		47(0.273)	125(0.727)		(0.713-1.571)
Age									
≤60	170	14(0.082)	66(0.388)	90(0.529)	0.648	94(0.276)	246(0.724)	0.757	1.057
>60	132	9(0.068)	58(0.439)	65(0.492)		76(0.288)	188(0.712)		(0.740-1.511)
Histological types									
Adenocarcinoma	164	13(0.079)	71(0.433)	80(0.488)	0.627	97(0.296)	231(0.704)	0.395	0.856
Squamous cell carcinoma	138	10(0.072)	53(0.384)	75(0.543)		73(0.264)	203(0.736)		(0.598-1.224)
Clinical stage									
I	102	10(0.098)	44(0.431)	48(0.471)		64(0.314)	140(0.686)		
II	121	5(0.041)	49(0.405)	67(0.554)	0.175	59(0.244)	183(0.756)	0.099	0.705 (0.464-1.069)
III	62	7(0.113)	25(0.403)	30(0.484)	0.919	39(0.314)	85(0.685)	0.988	1 (0.620-1.623)
IV	17	1(0.059)	6(0.353)	10(0.588)	0.647	8(0.235)	26(0.765)	0.356	0.673 (0.288-1.568)
Tumor differentiation									
Poor	204	16(0.078)	83(0.407)	105(0.515)		115(0.282)	293(0.718)	0.974	0.993
Moderate or Well	98	7(0.071)	41(0.418)	50(0.510)		55(0.281)	141(0.719)		(0.680-1.451)
Lymph node metastasis									
Negative	156	8 (0.051)	65(0.417)	83(0.532)		81(0.260)	231(0.740)	0.217	1.25
Positive	146	15(0.103)	59(0.404)	72(0.493)		89(0.305)	203(0.695)		(0.876-1.783)

Significant P values (<0.05) are in boldface; OR, odds ratio; CI, confidence intervals; Frequencies are displayed in parenthesis

In order to determine the association between the polymorphism of rs4957014 and certain clinical pathologic features, we conducted stratified analyses for allelic frequency and genotype distribution in NSCLC patients with sex, different age, histological types, clinical stage, tumor differentiation and lymph node metastasis. Results of stratified analyses are presented in Table 2. As shown in Table 2, no statistically significant association was observed with rs4957014 polymorphism and characters of sex, age, histological types, clinical stage, tumor differentiation and lymph node metastasis respectively (as shown in Table 2, all P>0.05).

Discussion

To our best knowledge, this study is the first to analyze the association between genetic variants of PDCD6 gene and NSCLC, as well as the association between these SNPs and NSCLC patients' characteristics. As the most common histological type of lung cancer, NSCLC is regarded as a diverse and heterogeneous disease. Molecular biological studies have shown that cancers carried multiple genetic and epigenetic alterations, indicating inactivation of tumor suppressor genes and activation of dominant oncogenes during the processes of carcinogenesis and subsequent progression of NSCLC (Osada et al., 2002). Many of the tumor suppressor genes and oncogenes altered in lung cancer are known to play a role in the regulation of cell cycle progression, and a considerable proportion of lung cancer-related genes are a component of the checkpoint mechanisms (Dhar et al., 2000). Such as p53 gene, regarded as a tumor suppressor gene, has performed in cell cycle arrest, apoptosis and DNA repair. Previous studies have demonstrated that unrepairable DNA damage may lead to apoptotic cell death via p53-dependent induction of the expression of various downstream genes including the pro-apoptotic Bcl-2 family, PIG3 and p53AIP (Miyashita et al., 1995; Venot et al., 1998; Oda et al., 2000a; Oda et al., 2000b; Yu et al., 2001). All these evidence have shown that genetic factor-related apoptosis may be involved in the pathogenesis of NSCLC. Apoptosis is the orchestrated collapse of a cell including membrane blebbing, condensation of chromatin, cell shrinkage, and fragmentation of DNA and occurs from embryogenesis to aging, from normal tissue homoeostasis to many human diseases (Renehan et al., 2001). It plays an essential role in the elimination of mutated or transformed cells from the body, and is crucial in normal lung cell turnover and lung development (Fine et al., 2000; Shivapurkar et al., 2003).

PDCD6 is located on chromosome 5p15.33 and encodes a 191-aa protein. A number of PDCD6 targets have been identified including AIP1/Alix, ASK1, HEED, annexin7 and more recently Raf-1, Tsg101,copines, HEBP2, c14orf32,Sec 31A (Tomsig et al., 2003; Tarabykina et al., 2004; Rual et al., 2005; Chen et al., 2005; Katoh et al., 2005; Draeby et al., 2007; la Cour et al., 2007). AIP1/Alix stands out as it has been shown in several systems to play a role in apoptosis together with PDCD6. The work of Mahul-Mellier et al. supports for PDCD6 as a pro-apoptosis protein (Mahul-Mellier et al., 2006) as previous reports (Vito et al., 1999; Chatellard-Causse et al., 2002). Tsg101has been described as an inhibitor of p53 independent cell cycle arrest and cell death in mouse embryonic fibroblasts (Krempler et al., 2002). It is therefore well possible that PDCD6, depending on the cellular context, may act as a well death promoting protein or as cell viability factor acting even through the same effector protein. It is also found that in normal tissue,

detectable PDCD6 levels are found in cells of epithelioid origin, and compared to non-endocrine cells, PDCD6 expression is higher in cells with endocrine functions (la Cour et al., 2008). All of these results together supported that PDCD6 play a crucial role in NSCLC. Therefore, investigation of the PDCD6 will help our understanding of the pathogenesis of NSCLC.

However, we do not know whether the polymorphisms of PDCD6 gene affect the susceptibility of NSCLC. In our study, we compared two SNPs in NSCLC patients and normal controls to assess whether the polymorphism of PDCD6 gene is a factor affecting the susceptibility of NSCLC. We selected two tag SNPs (rs3756712 andrs4957014) as our candidate SNP locus. Tag SNP is the representative SNP in a region of the genome with high linkage disequilibrium. Among the present study, we have found that the significant association between SNP rs4957014 and the susceptibility of NSCLC, but not the pathological features of NSCLC. It appeared that the T allele was significantly higher than that in normal control (Table 1), which indicated that allele T maybe a risk factor for NSCLC in Chinese. However, our data show that the polymorphism of rs3756712 was not associated with NSCLC. The results suggest that PDCD6 gene polymorphisms appear to play an important role in the susceptibility of NSCLC in Chinese Han population. Although SNP rs4957014 is located in intronic region of PDCD6, we speculate that it may influence protein expression by affecting conformation of the three-dimensional structure of DNA, and transcription, stability of mRNA.

However, in the current study, there may have some limitations that may affect the accuracy of the results. The sample size of the study was relatively small. In the present study, only 302 NSCLC patients and 306 control subjects have genotypes, which may cause a small effect of rs3756712 in PDCD6 gene cannot be measured. In further studies, we require a larger sample size to justify that the association between rs4957014 and NSCLC is not by chance alone. Because of genetic polymorphisms vary greatly among ethic populations, further studies in different populations are also needed to exclude a population-oriented association. The mechanism underlying the associations between these SNPs and the risk of NSCLC is not immediately evident.

In conclusion, we firstly demonstrated the association between PDCD6 gene and the risk of NSCLC patients in Chinese Han population. The results of this study show that rs4957014G/T is associated with an increased risk of NSCLC, which suggest that PDCD6 gene SNP is a risk factor for susceptibility of NSCLC. Our findings will need to be validated in future studies.

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