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Original Article

Correlation between CD226 polymorphism (rs763361) and the susceptibility of lung cancer in nonsmoking Chinese population

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Abstract: This study aimed to research the relationship between *CD226* rs763361 and the susceptibility of lung cancer. A small case-control study of 83 healthy controls and 120 patients, was carried out in the Han population of northwest China. Whole genome DNAs were extracted and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used to detect the *CD226* rs763361 polymorphism. Logistic regression model was used to evaluate the statistical association between the *CD226* rs763361 polymorphism and the susceptibility of lung cancer. The final statistical results demonstrated that in nonsmoking group, TT genotype is more susceptible to lung cancer than CT/CC genotype in *CD226* gene rs763361 (OR=3.4846, 95% CI: 1.1932-10.1762, *P*=0.0224,). In the female group, the TT genotype ratio of patients is higher than that of the controls (OR=3.4090, 95% CI: 0.9449-12.2987, *P*=0.0610), which might hint a suggestive association between *CD226* rs763361 polymorphism and the susceptibility of lung cancer. Meanwhile, our results found that *CD226* rs763361 polymorphism has little effect on pathology in patients group with the susceptibility of lung cancer. Our findings suggested that *CD226* rs763361 polymorphism may correlate with the susceptibility of lung cancer in Chinese northwest population.

Keywords: Lung cancer, CD226 rs763361, single nucleotide polymorphism

Introduction

Lung cancer, over the past 30 years, has substituted for liver cancer and become the number one cause of mortality among patients who developed malignant tumors in China [1]. In China, according to the research data, between 1990 and 2011, the mortality has increased 464.84%, which significantly affected the health of the population [1]. As we all know, so far, the causes of the occurrence of the lung cancer are still unknown, and the pathological types and clinical classifications of lung cancer were varied, which indicated that its pathogenesis is multiplex and multifactorial. It is now generally acknowledged that the risk factors of the lung cancer were smoking and exposure to secondhand smoke, air pollution, many chromosomal changes and chronic pulmonary disease [2-5]. In those papers, we can see that the pathogenesis of lung cancer involves a multilevel and complex process including gene of private individuals and environmental pollution.

CD226 (DNAX accessory molecule 1, DNAM-1), a type 1 transmembrane protein belongs to the Ig-supergene family, is expressed a wide spectrum on the immune cells, such as activated T lymphocytes, natural killer (NK) cells, platelets, endothelial cells and NK T cells, and participated in the adhesion and co-stimulation of these cells through the ligands CD112 and CD155 [6, 7]. In recent years, some researchers focus on the relationship between CD226 genetic polymorphism and autoimmune diseases or cancer, for example, juvenile idiopathic arthritis, gastric cancer, type 1 diabetes, cervical squamous cell carcinoma, celiac disease, multiple sclerosis, Grave's disease and so on [8-12]. But until

Table 1. Characteristics of lung cancer patients and healthy controls

Characteristic	Patients (n=120)	Controls (n=83)	*P* value
Gender			
Female	37 (30.83%)	28 (33.73%)	0.6631
Male	83 (69.17%)	55 (66.27%)	
Age			
Mean ± SD	56.94±10.63	54.82±9.64	0.1479
≤56 years	59 (49.17%)	45 (54.22%)	0.4791
>56 years	61 (50.83%)	38 (45.78%)	
Smoking history			
Non smoker	50 (41.67%)	38 (45.78%)	0.5607
Ever smoker	70 (58.33%)	45 (54.22%)	

now, there are little researches about the susceptibility of lung cancer and CD226 rs763361 SNP.

In this article, we found that in nonsmoking group, TT genotype is more susceptible to lung cancer than CT/CC genotype in *CD226* gene rs763361 (OR=3.4846, 95% CI: 1.1932-10.1762, *P*=0.0224). Meanwhile, in the female group, the TT genotype ratio of patients is higher than that of the controls (OR= 3.4090, 95% CI: 0.9449-12.2987, *P*=0.0610), hinted a suggestive association between *CD226* rs763361 polymorphism and the susceptibility of lung cancer. The results will help us to further understand the susceptibility and the pathogenic factors of lung cancer, improving diagnosis and ultimately making a better prognosis for therapy.

Materials and methods

Ethics statement

The study design was approved by Ethics Committee Subjects of Xijing Hospital, Xi'an, China. Before recruited from hospital, patients and healthy controls signed the informed consent.

Study subjects

A total of 203 Chinese individuals who were not relatives from northwest China, were recruited from Xijing Hospital from 2011 to 2013. Among them, 120 patients were diagnosed with lung cancer according to the clinical diagnostic criteria with an average age at 56.94 years. All the information of the age, the gender, the smoking history of recruited patients was obtained from case history. At the same time, 83 healthy controls were randomly recruited as the volunteers with an average age of 54 years, who had done the physical examination in Xijing hospital with no history of respiratory disorders, malignancy, and autoimmunity disorders.

DNA extraction and PCR

he 2 ml peripheral blood samples from each patients and healthy controls were gathered and frozen at -20°C. Genomic DNA was extracted from peripheral blood using the TIANamp Blood DNA kit (TIANGEN, Beijing, China), and then stored at -20°C. The *CD226* gene rs763361 polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The *CD226 gene* containing the locus rs763361 was amplified. The forward primer and the reverse primer of the ampliconic sequence are 5'-CTGCGAGAGAAGGTTGGATAGTTGAC-3', 5'-CTTGTCCCATATCATGCCTGCATT-3', respectively. The amplification reaction system: 1 μl of genomic DNA (50 ng/μl), 1 μl of each primer (20 μM), 25 μl of Premix Taq (TaKaRa Taq™ Version 2.0 plus dye) (TaKaRa, Dalian, China), and 22 μl of double distilled water, a total of 50 μl. The PCR condition were initially 94°C denatured for 5 min, then amplified by 35 cycles of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C, and the final extended for 10 min at 72°C. The specificity of amplified products was authenticated by agarose gel electrophoresis. And then dealt the rest of 40 μl amplified products with 10 U of *Msp* I each reaction, overnight at 37°C. Using the 2% agarose gel identified the digested products of *CD226* gene rs763361. The rs763361 T allele was not cut by the incision enzyme *Msp* I and produced the 213 bp fragment, the rs763361 C allele was cut and produced two parts of 73 and 140 bp. The abandoned samples which without the inconsistent results or without the enzyme-digested product were not reckoned in the study.

Statistical analysis

Statistical analyses were performed using STATA 13.0 software (StataCorp, College Station, Texas, USA). We set the significant difference *P*<0.05, the homogeneity of variance test level to 10%, the confidence interval (CI) treated as

Table 2. Genotype frequencies of the SNPs in CD226 gene in controls and patients

Genotype	Controls		Patients		Adjusted OR (95% CI)[a]	*P* value
	N (83)	(%)	N (120)	(%)		
CC	26	(31.33)	41	(34.17)		
CT	41	(49.40)	44	(36.67)	0.7028 (0.3647, 1.3544)[b]	0.2921[b]
TT	16	(19.28)	35	(29.17)	1.4883 (0.6819, 3.2487)[c]	0.3180[c]
CT+TT	57	(68.67)	79	(65.83)	0.9179 (0.5017, 1.6793)[d]	0.7810[d]

[a]: The gender, age, smoking history was adjusted in the multivariate logistic regression model. [b]: CT VS CC. [c]: TT VS CC. [d]: CC VS CT+TT.

95%. The quantitative data of the cases and healthy controls were descripted by mean ± standard deviation. Using t test analyzed data of the groups, when the variance is homogeneous. Using t' test analyzed data of the groups, when the variance is inhomogeneous. Ratings data between groups were analyzed using Wilcoxon rank sum test. Qualitative data between groups were analyzed using chi-squared (X^2) test. To explore the relationship between disease and SNP genotype, we established the univariate logistic regression model. Adjusted the effects of the age, gender, smoking history in the multivariate logistic regression model, then evaluated the statistical association between the SNP and the susceptibility of lung cancer, which through doing the stratified analysis, according to the subsections of the age, the gender, the smoking history, and the pathological types. In this statistics, four kinds of biological models of coding SNP were considered: additive model, co-dominant model, dominant model and recessive model (the partial results were exhibited in this paper).

Results

Characteristics of lung cancer patients and healthy controls

The statistical description of the characteristics (gender, age or whether smoking) of the 120 patients and the 83 healthy controls in our study are presented in **Table 1**. Divided into several groups. In the gender groups, the percentage of female in patients and controls are 30.83% and 33.73%, the percentages of male are 69.17% and 66.27% respectively. The percentage of >56 years in patients and the controls is similar, as well as the percentage of <56 years. In the nonsmoking group, the frequencies of the patients and the controls are 41.67% and 45.78%. The frequencies of the patients and controls in the smoking group are also approximate equivalent (58.33% and 54.22%). In terms of the gender, the age, the smoking history, there are no significant statistically differences (P>0.05), which indicated that the two groups came from the same population.

Genotype frequencies of the rs763361 SNPs in CD226 gene in controls and patients

The genotype frequencies of the SNPs in CD226 gene in controls and patients was shown in **Table 2**. The results shown that in codominant model, there are no significant difference between patients and healthy controls in the genotype frequencies of TT vs CC, CT vs CC, CC vs CT+TT.

Recessive model analysis of association between CD226 polymorphisms and susceptibility of lung cancer

The frequency of the CD226 rs763361 polymorphism in recessive model is shown in **Table 3**. The frequency of TT genotype within the nonsmoking history of lung cancer patients group is increased compared with that of the healthy controls, and the P value was 0.0224 (OR=3.4846, 95% CI: 1.1932-10.1762). We also observed that the P value of smoker group is 0.9578, which is great higher than the nonsmoking group. In the stratified analysis of the age and the gender, there is no significant differences between patients and healthy controls. However, in the female group of the stratified analysis, the P value is 0.0610. Depending on this data, we guessed that TT genotype is more susceptible than the CC+CT genotype to develop the lung cancer in female people. When the patients and the controls were compared, the frequency of TT genotype is higher in the former, which suggested that the frequency of TT genotype are different in the two groups. However, a clear association between the female groups needs to confirm using more data.

Table 3. Recessive model analysis of association between CD226 polymorphisms and susceptibility of lung cancer

Variables	Control		Patients		Adjusted[b] OR (95% CI)[c]	*P* value
	CC+CT (%)	TT (%)	CC+CT (%)	TT (%)	CC+CT vs. TT	
All samples	67 (80.72)	16 (19.28)	85 (70.83)	35 (29.17)	1.8196 (0.9188, 3.6034)	0.0860
Gender						
Female	24 (85.71)	4 (14.29)	24 (64.86)	13 (35.14)	3.4090 (0.9449, 12.2987)	0.0610
Male	43 (78.18)	12 (21.82)	61 (73.49)	22 (26.51)	1.4039 (0.6169, 3.1950)	0.4187
Age[a]						
≤56	37 (82.22)	8 (17.78)	41 (69.49)	18 (30.51)	2.1061 (0.8086, 5.4854)	0.1272
>56	30 (78.95)	8 (21.05)	44 (72.13)	17 (27.87)	1.4488 (0.5539, 3.7896)	0.4498
Smoking history						
Never smoker	32 (84.21)	6 (15.79)	31 (62.00)	19 (38.00)	3.4846 (1.1932, 10.1762)	0.0224
Ever smoker	35 (77.78)	10 (22.22)	54 (77.14)	16 (22.86)	1.0251 (0.4093, 2.5670)	0.9578

[a]: The median of the age is 56. [b]: The gender, age, smoking history was adjusted in the multivariate logistic regression model. [c]: OR odds ratio. 95% CI: 95% confidence interval.

Table 4. The genotype frequencies of CD226 rs763361 in different histopathology types of lung cancer

Histopathology type	Genotype		Adjusted[a] OR (95% CI)[b]	*P* value
	CC+CT	TT		
Controls	67 (80.72)	16 (19.28)		
Small cell lung cancer	28 (70.00)	12 (30.00)	1.8860 (0.7805, 4.5569)	0.1587
Adenocarcinoma	32 (68.09)	15 (31.91)	2.0305 (0.8772, 4.7003)	0.0981
Squamous cell carcinoma	25 (80.65)	6 (19.35)	0.9741 (0.3291, 2.8834)	0.9622
Adenosquamous carcinoma	0 (0.00)	2 (100.00)		

[a]: The gender, age, smoking history was adjusted in the multivariate logistic regression model. [b]: OR odds ratio. 95% CI: 95% confidence interval.

The genotype frequencies of CD226 rs763361 in different histopathological types of lung cancer

The percentages of pathological types of *CD226* rs763361 were shown in **Table 4**. The four different histopathological frequencies were counted and the recessive model was used to evaluate the difference between CC+CT genotype and TT genotype in small cell lung cancer group, adenocarcinoma group, squamous cell carcinoma group and adenosquamous carcinoma group respectively. The *P* value in small cell lung cancer and the adenocarcinoma are 0.1587 and 0.0981 respectively, but the *P* value in squamous carcinoma is 0.9622.

Discussion

So far, three SNP loci of *CD226* gene located in exon sequence were found, including rs763361, rs727088 and rs34794968 [13]. A series of epidemiologic studies showed that *CD226* gene rs763361 SNP is correlated with multiple autoimmune diseases such as type 1 diabetes (T1D), systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis (JIA) diseases [8, 13, 14]. Meanwhile, *CD226* rs727088 polymorphic variants mutated at a functional site lead to the decreased expression level of CD226 at the transcriptional level and protein level [13]. And now two published papers reported that *CD226* rs727088 SNP was associated with the susceptibility of gastric cancer and cervical squamous cell carcinoma (CSCC) respectively [9, 11]. The *CD226* rs34794968 was not solely associated with diseases, but exerted synergistic effects combined with the other two polymorphic sites of *CD226* [13, 15]. Bossini-Castillo L et al. reported that the TCG haplotype (containing the three SNP locus of CD226 polymorphism: rs763361, rs727088, rs34794968)

was associated with systemic sclerosis-related pulmonary fibrosis [15].

The present case-control study is focused on the association between CD226 rs763361 and the susceptibility of lung cancer. The TT genotype is more susceptible to lung cancer than CT/CC genotype in nonsmoking group (*P*=0.0224, OR=3.4846, 95% CI: 1.1932-10.1762), when the gender, and the age were adjusted in the multivariate logistic regression model. Interestingly, in the results of stratified analysis, the OR value of the female group is 3.4090, *P* value is 0.0610. Then, we speculated that the TT genotype is susceptible to lung cancer in comparison with CC/CT genotypes in female people. However, this possibility requires more data to prove.

Then, we would like to further explore the potential reasons why the TT genotype is more susceptible to lung cancer than CT/CC genotype in nonsmoking Chinese population. In 2011, Dieudé et al. demonstrated that the *CD226* rs763361 T allele, particularly the TT genotype is correlated with systemic sclerosis (SSc) and SSc-related fibrosing alveolitis in Europeans [16]. We suspected that the CD226 rs763361 mutation in the TT genotyping, may change the tail phosphorylation condition of CD226 protein and then affecte the physical structures of LFA-1, which combined with ICAM-1 [17, 18]. On one hand, these changes perhaps obstructed the coordinated expression of CD226 and LFA-1, which impeded the education and cytotoxic function of NK cells [19]. On the other hand, these changes may weaken the activation of T cells and break the balance between CTLA-4 and CD28 in T cells, as the abnormal function of CTLA-4 is correlated with CD226 rs763361 mutation locus in mouse model of type 1 diabetes [20].

According to the previous studies, many factors were associated with the risk of lung cancer in nonsmoking groups, such as the gene epidermal growth factor receptor (*EGFR*), *PIA3CA*, semaphorin *(SEMA5A)*, the surroundings cooking oil fume exposure and so on [21-25]. And now, our research suggested that TT genotype in *CD226* gene rs763361 is associated with nonsmoking lung cancer and female groups. In short, the susceptible factors of lung cancer were complicated, and our studies may contribute to further explore its susceptible factors.

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Disclosure of conflict of interest

None.

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