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Original Article

IL-21 polymorphisms rs907715 and rs2221903 are associated with decreased non-small cell lung cancer susceptibility

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Abstract: The etiology of lung cancer is still incompletely understood. Previous studies have suggested the association between IL-21 polymorphisms and autoimmune diseases, however, little is known about its role in lung cancer susceptibility. Here, we investigated the role of two SNPs of IL-21 gene in a cohort of non-small cell lung cancer (NSCLC) patients. A total of 128 NSCLC patients and 156 healthy controls were genotyped. Multivariate logistic regression was used to analyze the association between IL-21 polymorphisms and NSCLC risk. Our data showed that both rs907715 and rs2221903 were significantly associated with lung cancer susceptibility, and patients carrying rs907715A (P = 0.007, adjusted OR = 0.60, 95% CI = 0.42-0.87) or rs2221903G (P = 0.020, adjusted OR = 0.52, 95% CI = 0.30-0.90) allele had a decreased risk of NSCLC. Further study identified that the association between IL-21 polymorphisms and NSCLC risk was limited to lung adenocarcinoma. Haplotype analysis revealed that the AG (P = 0.006, OR = 0.072 95% CI = 0.011-0.451) and AA (P = 0.022, OR = 0.657, 95% CI = 0.458-0.941) haplotypes of rs907715/rs2221903 were associated with a decreased risk of NSCLC, whereas the GA (P = 0.0001, OR = 1.932, 95% CI = 1.378-2.710) haplotype was associated with an increased risk. In conclusion, our study demonstrates the association between IL-21 polymorphisms (rs907715 and rs2221903) and NSCLC risk in a Chinese Han population, indicating their potential role in lung cancer detection and treatment.

Keywords: SNP, IL-21, NSCLC, susceptibility

Introduction

Lung cancer is still the leading cause of cancer associated mortality [1], and its incidence is increasing yearly with the major environmental problem in China [2]. As the most common type that accounts for most of the lung cancer cases, NSCLC is mainly composed of adenocarcinoma, squamous carcinoma and large cell carcinoma, and is characterized by relatively low sensitivity to chemotherapy. The recent novel recognitions of cancer immunotherapy derived from the extensive studies have greatly benefited the clinical management of NSCLC patients [3], however, NSCLC is still considered as an aggressive malignancy with high risk of metastasis and dismal prognosis. Therefore, elucidating its mechanisms of pathogenesis is of considerable urgency.

Cell mediated immunity is believed to play a crucial roles in the immune surveillance of tumor cells. Previous studies have demonstrated the increased CD4+ T regulatory cells (Tregs) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) in NSCLC patients [4], indicating the suppressed immune response. Cytokines constitute critical nodal points for the appropriate switches of signaling cascades in immune cells. Importantly, the aberrant production of a series cytokines such as TGF-β, IL-17 and IL-10 has been proved to be significantly involved in the development and metastasis of lung cancer by suppression of cell immunity and maintenance of tumor microenvironment [5-8]. IL-21 is a

Table 1. Baseline characteristics

	Case	Control
Age (years)	53.3±9.3	53.0±10.6
Sex		
Male	98	119
Female	30	37
Smoking status		
Yes	82	81
No	46	75
Family history		
Yes	20	16
No	108	140
Histological type		
Adenocarcinoma	68	
Squamous cell carcinoma	55	
Other	5	

newly identified modulator of immune cells such as natural killer cells and cytotoxic T cells. Current understandings of its role are largely limited to the autoimmune diseases, only a few experimental researches have showed that IL-21 exerted great beneficial effects on melanomas and the adenocarcinoma of breast [9]. In addition to the certain environmental risk factors such as cigarette smoking and benzopyrene, the genetic factors that linked to lung cancer susceptibility including single nucleotide polymorphisms (SNPs) have attracted increasing attentions. Additionally, several SNPs in the encoding gene of IL-21 have been shown to be related to the susceptibility of autoimmune diseases and cancers [10-14]. However, no evidence on its role in lung cancer has been found in the published literature. Rs907715 and rs2221903, two polymorphism sites that locate in the intron region, have been delineated to be associated with systemic lupus erythematosus and Grave's disease [15, 16]. Whether the two polymorphism sites within IL-21 gene are associated with the cancer susceptibility has been seldom tested. In light of the previous reports demonstrating the critical association of the polymorphisms with IL-21 production, we suspected that the two previously reported functional SNPs might represent susceptible genetic factors for NSCLC.

In this study, we included a cohort of NSCLC patients; the IL-21 polymorphisms were tested. We show that rs907715 and rs2221903 are associated with the susceptibility of NSCLC, which reveals the previously unknown role of these two polymorphisms in the development lung cancer.

Study population, materials and methods

Study population

A total of 128 patients who were newly diagnosed as NSCLC in Shandong Cancer Hospital from July 2013 to February 2015 were included in this study. All the diagnoses were confirmed by pathohistological examination. The histological type of each patient was also determined. The number of patients with adenocarcinoma, squamous carcinoma or other types of NSCLC was 68, 55 or 5, respectively. The healthy control people were randomly recruited from the individuals who underwent routine physical examination in the outpatient department. Individuals with severe complications such as cardiovascular, hepatic and renal disease were excluded from this study. We interviewed each individual face-to-face to obtain the information of smoking status and family history. Informed consents were obtained from all the study subjects. The study protocol was approved by the Ethics Committee of Shandong Cancer Hospital.

Genotyping

The blood sample of each individual was collected, followed by genomic DNA isolation by aTIANamp Blood DNA Kit purchased from Tiangen biotechnology Co., Ltd (Beijing, China) according to the protocol provided by the manufacturer. The blood DNA samples were stored into a -70°C freezer. The Taqman assay was used to discriminate different genotypes. We obtained assay on-demand genotyping kits for rs907715 (C_8949748_10) and rs2221903 (C_16167441_10) from Applied Biosystems (Foster City, USA). The PCR reaction was conducted with a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Haplotype analysis was performed with the online software SHEsis [17] (http://analysis.bio-x.cn/myAnalysis.php).

Statistical analysis

The comparison of age between NSCLC patients and healthy controls were performed by independent t-test. All the other categorical variables in the baseline characteristics and Hardy-Weinberg equilibrium (HWE) were tested using χ^2 test. The association of polymorphis-

Table 2. Genotype distributions of rs907715 and rs2221903

	NSCLC (n = 128) N (%)	Control (n = 156) N (%)	*p*-value	OR (95% CI)
rs907715				
Genotype frequency				
GG	67 (52.4)	58 (37.2)	-	reference
GA	52 (40.6)	71 (45.5)	0.077	0.63 (0.38-1.05)
AA	9(7.0)	27 (17.3)	0.001	0.25 (0.11-0.59)
Recessive model				
GA + GG	119 (93.0)	129 (82.7)	-	reference
AA	9 (7.0)	27 (17.3)	0.035	0.40 (0.17-0.94)
Dominant model				
GG	67 (52.3)	58 (37.2)	-	reference
GA + AA	61 (47.7)	98 (62.8)	0.077	0.63 (0.38-1.05)
Allele frequency				
G	186 (72.7)	187 (59.9)	-	reference
A	70 (27.3)	125 (40.1)	0.007	0.60 (0.42-0.87)
HWE (p)	0.800	0.513		
rs2221903				
Genotype frequency				
AA	106 (82.8)	109 (69.9)	-	reference
AG	21 (16.4)	44 (28.2)	0.007	0.44 (0.24-0.80)
GG	1 (0.8)	3 (1.9)	0.327	0.31 (0.03-3.18)
Recessive model				
AA + AG	127 (99.2)	153 (98.1)	-	reference
GG	1 (0.8)	3 (1.9)	0.783	0.72 (0.07-7.57)
Dominant model				
AA	106 (82.8)	109 (69.9)	-	reference
AG + GG	22 (17.2)	47 (30.1)	0.007	0.44 (0.24-0.80)
Allele frequency				
A	234 (91.4)	261 (83.7)	-	reference
G	22 (8.6)	51 (16.3)	0.020	0.52 (0.30-0.90)
HWE (P)	0.971	0.549		

ORs were adjusted for age, sex, smoking status, family history and histological type.

ms with lung cancer susceptibility was performed by multivariate logistic regressions. The odds ratios (ORs) were adjusted for age, sex, smoking status and family history. Fisher's exact test was used to compute the *p* value in the haplotype analysis. All the statistical analyses were two-sided, $P<0.05$ was deemed as statistical significance. The statistical analyses were conducted with spss 19.0 software package.

Results

Baseline characteristics

We included 128 NSCLC patients and 156 healthy controls in the present study. The demographic characteristics were shown in **Table 1**, no significant difference regarding age (53.3±9.3 vs. 53.0±10.6, P>0.05), sex (χ^2 = 0.003, P>0.05) and family history (χ^2 = 1.831, P>0.05) was observed. The numbers of patients were 68, 55 and 5 for adenocarcinoma, squamous cell carcinoma and other types of NSCLC, respectively.

Association between IL-21SNPs (rs907715 and rs2221903) and NSCLC susceptibility

The genotype and allele distribution of the rs907715 and rs2221903 polymorphisms were shown in **Table 2**. Both the rs907715 and rs2221903 genotype distribution were in consistent with HWE (P>0.05). We observed that the frequency of AA genotype of rs907715 was significantly lower in NSCLC patients, multivariate logistic regression analysis suggested patients with rs907715 AA genotype had a decreased susceptibility to NSCLC (7% vs. 17.3%, adjusted OR = 0.25,95% CI = 0.11-0.59, P = 0.001). Similar result was obtained when we combined GG and AA genotype to construct a recessive model (adjusted OR = 0.40, 95% CI = 0.17-0.94, P = 0.035). Lower susceptibility of NSCLC was also found in patients carrying A allele (27.3% vs. 40.1%, adjusted OR = 0.60, 95% CI = 0.42-0.87, P = 0.007). For rs2221903 polymorphism, the frequency of heterozygous AG genotype was significantly lower in case group (16.4% vs. 28.2%, P = 0.007), and individuals with AG genotype have 0.44-fold risk of NSCLC compared with those with AA genotype (adjusted OR = 0.44, 95% CI = 0.24-0.80). Same result was observed when we combined AG and GG to construct a dominant model (17.2% vs. 30.1%, adjusted OR = 0.44, 95% CI =0.24-

Table 3. Association between IL-21 allele frequency and histological type in patients

	Adenocarcinoma	Control	*p*-value	OR (95% CI)
rs907715				
G	103 (75.7)	187 (59.9)	-	reference
A	33 (24.3)	125 (40.1)	0.005	0.51 (0.32-0.82)
rs2221903				
A	126 (92.6)	261 (83.7)	-	reference
G	10 (7.4)	51 (16.3)	0.039	0.46 (0.22-0.96)
	SCC	Control	*p*-value	OR (95% CI)
rs907715				
G	75 (68.2)	187 (59.9)	-	reference
A	35 (31.8)	125 (40.1)	0.247	0.76 (0.47-1.21)
rs2221903				
A	100 (90.9)	261 (83.7)	-	reference
G	10 (9.1)	51 (16.3)	0.085	0.52 (0.25-1.09)

ORs were adjusted for age, sex, smoking status and family history.

Table 4. Haplotype analysis of rs907715 and rs2221903 in NSCLC patients and healthy controls

rs907715	rs2221903	NSCLC	Control	*P* value	OR (95% CI)
A	A	27.0%	36.1%	0.022	0.657 [0.458-0.941]
A	G	0.3%	4.0%	0.006	0.072 [0.011-0.451]
G	A	64%	47.9%	0.0001	1.932 [1.378-2.710]
G	G	8.7%	12.0%	0.194	0.694 [0.400-1.207]

0.80, P = 0.007). The G allele distribution of rs2221903 was also lower in case group (8.6% vs. 16.3%, adjusted OR = 0.52, 95% CI = 0.30-0.90, P = 0.020).These data suggested that the two SNPs of IL-21 were associated with decreased susceptibility of NSCLC.

IL-21 SNPs are associated with the susceptibility of adenocarcinoma, but not squamous cell carcinoma

We next performed stratified analysis to evaluate the association of IL-21 SNPs and cancer risk in different histological types of NSCLCs. As displayed in **Table 3**, we found that both the frequency of A allele for rs907715 (24.3% vs. 40.1%, adjusted OR = 0.51, 95% CI = 0.32-0.82, P = 0.005) and G allele for rs2221903 (7.4% vs. 16.3%, adjusted OR = 0.46, 95% CI = 0.22-0.96, P = 0.039) were significantly lower in patients with adenocarcinoma compared with healthy controls, whereas the distribution of both alleles were not significantly decreased in patients with SCC (P = 0.76 for rs907715 A allele and 0.52 for rs2221903 G allele).

Haplotype analysis of rs907715 and rs2221903

The result of haplotype analysis was shown in **Table 4**, we identified that both the frequencies of AA (OR = 0.657 95% CI = 0.458-0.941, P = 0.022) and AG (OR = 0.072 95% CI = 0.011-0.451, P = 0.006) haplotype were significantly lower in case group, whereas the frequency of GA haplotype was significantly higher (OR = 1.932 95% CI = 1.378-2.710, P = 0.0001). These results suggested that the AA and AG were associated with decreased NSCLC susceptibility, whereas the GA haplotype did the opposite.

Discussion

In the present case-control study, we identified a novel association between two SNPs within IL-21 gene and NSCLC susceptibility. We found that the frequencies of rs907715G/A and rs2221903A/G were significantly decreased in NSCLC patients. Our further evidence showed that individuals carrying IL-21 SNPs had a decreased susceptibility to adenocarcinoma subtype rather than squamous cell carcinoma, which indicated that the association might be cell type specific. Our study therefore demonstrates that IL-21 SNPs rs907715 and rs2221903 might be favorable in the tumor genesis of adenocarcinoma of lung. And to the best of our knowledge, it is the first evidence showing the association of IL-21 SNPs with lung cancer susceptibility.

IL-21 is a newly discovered cytokine and is thought to play critical roles in innate immunity and adaptive immunity. Mainly secreted by CD4+ T cells, IL-21 shows pleotropic effects on immune systems by affecting the functions of B cell, CD8+ T cell, Tregs and etc [18]. Previous studies revealed its diverse roles in lymphoid cell development, inflammation response and autoimmune diseases [18]. Particularly, recent

evidences in mice have showed that IL-21 administration might represent a promising therapeutic method for melanoma and renal tumor [19], suggesting the possible broad implications of IL-21 in cancers. Additionally, concerning the previous studies reporting the impaired functions of CD4+ and CD8+ T cell in lung cancer patients [3, 20], IL-21 presumably plays a critical role in lung cancer development by enhancing immune-surveillance. Current consensus that the interplay between genetic factors and environmental stimuli contributes to the oncogenic potential represents a good model to illustrate the underlying etiology of lung cancer. In recent years, a great effort has been done to elucidate the genetic basis of lung cancer susceptibility, and the identification of a large set of SNPs has opened up a new avenue for cancer detection and prognosis. Several cytokine related SNPs have already been established as susceptible factors for lung cancer. For example, polymorphisms in encoding gene of IL-6, IL-10 and IL-17A have been proved to be associated with lung cancer susceptibility or prognosis [21-23]. However, little attention has been drawn with respect to the role of IL-21 in lung cancer. Intriguingly, recent evidences by Xiao et al. and You et al. addressed the critical association of rs907715 and rs12508721 SNPs in IL-21 with thyroid cancer and breast cancer [10, 11], respectively. Our results, which elucidated that rs907715 and rs2221903 is associated with decreased susceptibility of NSCLC, are in consistent with their findings. Previous evidence showed that rs907715 is associated with increased IL-21 production [12], our results therefore also indicate the crucial role of IL-21 in cell mediated immunity to lung cancer cells. In our study, we report that rs2221903 synergistically acted as a protective SNP in lung cancer development with rs907715, giventhat previous study reported a lack of association between rs2221903 and IL-21 production [12], the association between rs2221903 and lung cancer susceptibility might be explained by currently unknown mechanisms. An interesting finding in our stratification analysis is that the association of the two SNPs showed preference in lung adenocarcinoma rather than lung SCC. Although the mechanism is unknown, the genetic heterogeneity of lung cancer would provide some possible insights into this result.

Despite the several studies demonstrating the association between IL-21 SNPs and cancer susceptibility, the actual functions of these gene polymorphisms are still unknown. Both the two studied SNPs in our study are located in the intron of IL-21 gene, and thus may not represent functional SNPs. However, rs907715 and rs2221903 have been shown in linkage disequilibrium with several SNPs and selected as tag SNPs in several studies [10, 11, 16]. The variant status of the two studied SNPs may surrogate the functional mutation. In our study, we detected no linkage disequilibrium between rs907715 and rs2221903 (data not shown), our further haplotype analysis delineated that AA and AG haplotype (rs907715/rs2221903) is a protective predictor, whereas GA haplotype might be detrimental.

It should be mentioned that several limitations exist in our study. Firstly, as a chart-based and single-centered study, the data only reflect a small ethnic group of Chinese Han population, whether the rs907715 and rs2221903 polymorphisms of IL-21 are associated with lung cancer susceptibility in other ethnic groups still needs to be identified. Secondly, as lung cancer is significantly associated with the smoking exposure, the exposure time and level of patients upon diagnosis was unknown, which limited the strength of our data. Finally, the sample size is still relatively small, and the unique association between IL-21 SNPs and lung adenocarcinoma requires to be confirmed in a larger sample size.

In conclusion, the current study deciphered the association between the IL-21 SNPs (rs907715 and rs2221903) and NSCLC susceptibility in a Chinese Han population. More importantly, the association was specifically limited to lung adenocarcinoma. Although no causal link has been established, our study undoubtedly provided insights into the role of polymorphisms of IL-21 in lung cancer, and it merits future investigation.

Disclosure of conflict of interest

None.

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