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ORIGINAL RESEARCH

The *interleukin-18* gene promoter -607 A/C polymorphism contributes to non-small-cell lung cancer risk in a Chinese population

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Abstract: The purpose of the present study was to determine the relationship between interleukin-18 (IL-18) -607 A/C polymorphism and the risk of non-small-cell lung cancer (NSCLC) and its impact on the serum IL-18 level. The genotyping of IL-18 -607 A/C polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results showed that the AA/AC genotype distribution in NSCLC patients was significantly higher than that of healthy controls (P=0.02). However, no significant differences were found between the two subgroups when stratified by clinical characteristics. Furthermore, serum IL-18 levels were found to be significantly higher in the NSCLC patients than in the controls (P=0.01) as detected by enzyme-linked immunosorbent assay analysis. There was no correlation between serum IL-18 levels and different genotypes. In conclusion, these findings suggest that IL-18 -607 A/C polymorphism increases the risk of NSCLC in the Chinese population, and this polymorphism could not functionally affect the IL-18 levels.

Keywords: IL-18, polymorphism, NSCLC

Introduction

Lung cancer is the leading cause of human cancer deaths worldwide. Most patients with lung cancer are diagnosed with an advanced, unresectable disease and have a poor prognosis. Non-small-cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer cases. The overall 5-year survival rate of patients with lung cancer is less than 16%.[1] The etiology of the lung cancer, to date, is still unknown. It is accepted that the pathogenesis of lung cancer is multifactorial, and the genetic background may be one of the critical etiologic factors.

Interleukin-18 (IL-18), an 18-kDa cytokine, belongs to the interleukin-1 superfamily.[2] It has been reported that IL-18 has both anticancer and procancer effects on lung cancer.[3–5] IL-18 has pleiotropic efficacy in activating natural killer cell cytotoxic effect and enhancing Th1 immune response, mainly by stimulating the expression of interferon-γ and tumor necrosis factor-α and consequently causing the elimination of tumor cells in vivo.[6,7] It has been proposed that IL-18 modulates the immune system for attacking cancer cells through the suppression of tumor growth and angiogenesis in ovarian cancer, inhibition of breast cancer cell proliferation, potential invasion, and enhancement of cytotoxic effects on colon carcinoma cells.[8–10] Furthermore, increased levels of IL-18 have been reported in head and neck squamous cell and hepatocellular carcinoma; esophageal, gastric, ovarian, colon, skin cancer; and myeloid leukemia cells.[11–14]

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The *IL-18* gene is located on chromosome 11q22.2-q22.3. The single nucleotide polymorphic (SNP) position of -607A/C (rs1946518) has confirmed the impact on *IL-18* activity and expression in tissues.[6,15] To date, numerous studies have explored the role of *IL-18* SNPs in diseases, such as rheumatoid arthritis,[16] type 1 diabetes,[17] immune thrombocytopenia,[18] and several cancers,[19–22] including lung cancer.[23] In this study, we investigated the role of *IL-18* gene promoter polymorphism -607A/C in NSCLC occurrence and progress in order to provide data for screening high-risk Han Chinese individuals.

Materials and methods

Subjects

A total of 500 patients and 500 healthy controls were recruited in this study for the determination of genotyping of the IL-18 -607 A/C polymorphism. Serum IL-18 levels were determined in 90 NSCLC patients and 30 controls. The diagnosis of NSCLC was established by histopathological examination of biopsy or resected tissue specimens. An inclusion criterion was that NSCLC was confirmed by histology. The exclusion criteria were that clinical staging was indistinct and cases lacked specific clinical data. The healthy controls were from the health examination center. The study was approved by the hospital-based Ethics Committee of the General Hospital of Chinese PLA, Beijing, People's Republic of China. Written informed consent was obtained from all of the subjects.

Genotyping

Peripheral blood samples were collected in vacuum tubes with 5% ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted using DNA purification kit (Tiangen Biotech, Beijing, People's Republic of China) according to the manufacturer's instruction. The genotyping of the IL-18 -607 A/C polymorphism was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The forward primer 5′-CTTTGCTATCATTCCAGGAA-3′ and the reverse primer 5′-TAACCTCATTCAGGACTTCC-3′ were used for PCR analysis. The 20 μL PCR mixture contained 50–150 ng of genomic DNA and 10 μL 2× PCR mix (Tiangen Biotech). For PCR amplification, an initial denaturation at 95°C for 5 minutes was performed followed by 36 cycles at 95°C for 30 seconds, 64°C for 50 seconds, 72°C for 20 seconds, and a final extension at 72°C for 10 minutes. MseI (New England Biolabs, Beverly, MA, USA) was used to detect this A–C transition. The IL-18 CC genotype was expected to show three DNA bands at the positions of 199 bp, 73 bp, and 29 bp, whereas the AA genotype was expected to show three bands (101/98 bp, 73 bp and 29 bp), and the heterozygote was expected to have four bands (199 bp, 101/98 bp, 73 bp, and 29 bp). To confirm the genotyping results, 20% PCR-amplified DNA samples were examined by DNA sequence, and the results were 100% concordant.

ELISA assay for serum IL-18 levels

Serum samples were collected from all the subjects. The blood samples were collected into serum tubes with an accelerating agent for serum separation and kept at a room temperature for 30 minutes before centrifugation for 20 minutes at a minimum of 1,500× *g*. Serum was then isolated and stored at −80°C until further use.

Serum IL-18 levels were measured by enzyme-linked immunosorbent assay (ELISA) kits (NeoBioscience Technology Co., Ltd, Shenzhen, People's Republic of China) according to the manufacturer's instructions.

Statistical analysis

The statistical analysis was performed using SPSS statistical software version 18.0 (SPSS Inc., Chicago, IL, USA). The genotype distribution and genotype frequencies were analyzed by chi-square test. The results are presented as mean ± standard deviation (SD). The data were analyzed by the nonparametric Mann–Whitney *U*-test. *P*-values <0.05 were considered to be statistically significant.

Results

The demographic and clinical characteristics of the subjects including NSCLC patients and healthy controls are shown in Table 1. Most of the NSCLC patients had squamous cell

Table 1 Clinical characteristics of the subjects including NSCLC patients and healthy controls

Characteristic	Patients, n (%)	Controls, n (%)
Age (years)		
≤60	189 (37.8)	168 (33.6)
>60	311 (62.2)	332 (66.4)
Sex		
Male	293 (58.6)	301 (60.2)
Female	207 (41.4)	199 (39.8)
Smoking status		
Ever	354 (70.8)	340 (68.0)
Never	146 (29.2)	160 (32.0)
Histological type		
Squamous carcinoma	261 (52.2)	
Adenocarcinoma	239 (47.8)	
Lymph node metastasis		
Negative	160 (32.0)	
Positive	340 (68.0)	
Clinical stage		
I	35 (7.0)	
II	83 (16.6)	
III	190 (38.0)	
IV	192 (38.4)	

Abbreviation: NSCLC, non-small-cell lung cancer.

Table 2 IL-18 -607 A/C polymorphism in NSCLC patients and healthy controls

	NSCLC (N=500), n (%)	Control (N=500), n (%)	χ^2	*P*-value
Genotype frequency				
AA	165 (33.0)	152 (30.4)	5.36	0.07
AC	273 (54.6)	260 (52.0)		
CC	62 (12.4)	88 (17.6)		
Allele frequency				
Allele A	603 (60.3)	564 (56.4)	3.13	0.08
Allele C	397 (39.7)	436 (43.6)		
Genotype frequency				
AA or AC	438 (87.6)	412 (82.4)	5.30	0.02
CC	62 (12.4)	88 (17.6)		
Genotype frequency				
AA	165 (33.0)	152 (30.4)	0.78	0.38
CC or GC	335 (67.0)	348 (69.6)		

Abbreviations: IL-18, interleukin-18; NSCLC, non-small-cell lung cancer.

carcinoma (52.2%) and adenocarcinoma (47.8%). A total of 23.6% patients had NSCLC stage I+II and 76.4% had stage III+V.

In this study, the genotype and allele frequency of IL-18 -607 A/C polymorphism were detected for the first time in Chinese NSCLC patients and controls. The results showed that the AA/AC genotype distribution in the NSCLC patients was significantly higher than that of the controls (P=0.02), and a significant difference (P<0.07) was observed between the homozygous AA and CC genotypes (Table 2).

To verify the association between the IL-18 -607 A/C polymorphism and clinical characteristic features, we performed stratified analyses for combined genotypes – AA genotype versus AC + CC genotype and CC genotype versus AC + AA genotype – in NSCLC patients according to age at admission, sex, smoking status, histological type, lymph node metastasis, and clinical stage. Unfortunately, no significant differences were found (Table 3).

The serum IL-18 levels were determined using ELISA. The results demonstrated that the IL-18 expression was significantly increased in NSCLC patients compared with the controls (P=0.01) (as shown in Figure 1). According to the genotype distribution, the 90 NSCLC patients were divided into three subgroups: 30 patients with AA genotype, 40 with AC, and 20 with CC. No association was found between serum IL-18 levels and different genotypes (as shown in Figure 2).

Discussion

Lung cancer is the most common malignancy worldwide, and the pathogenesis of this disease is still unclear. In recent years, the important role played by genetic predisposition in lung cancer has promoted substantial interest. IL-18 is a pleiotropic proinflammatory cytokine. This is the first report that describes a comprehensive assessment of the relationship between IL-18 -607 A/C polymorphism and NSCLC risk and whether this polymorphism influences its occurrence in the Chinese Han population.

In this study, the PCR–RFLP method was used to detect *IL-18* gene -607A/C, and an ELISA kit was used

Table 3 Association of IL-18 -607 A/C polymorphism with clinicopathological characteristics of NSCLC patients

Characteristic	Cases, n (%)	Genotype number		Genotype number		χ^2	*P*-value
		AA, n (%)	AC + CC, n (%)	CC, n (%)	AC + AA, n (%)		
Age (years)							
≤60	189 (37.8)	65 (34.4)	124 (65.6)	24 (12.7)	165 (87.3)	0.27[a]	0.61[a]
>60	311 (62.2)	100 (32.2)	211 (67.8)	38 (12.2)	273 (87.8)	0.02[b]	0.87[b]
Sex							
Male	293 (58.6)	91 (31.1)	202 (68.9)	38 (13.0)	255 (87.0)	1.21[a]	0.27[a]
Female	207 (41.4)	74 (35.7)	133 (64.3)	24 (11.6)	183 (88.4)	0.21[b]	0.65[b]
Smoking status							
Ever	354 (70.8)	120 (33.9)	234 (66.1)	47 (13.3)	307 (86.7)	0.44[a]	0.51[a]
Never	146 (29.2)	45 (30.8)	101 (69.2)	15 (10.3)	131 (89.7)	0.86[b]	0.35[b]
Histological type							
Squamous carcinoma	261 (52.2)	91 (34.9)	170 (65.1)	36 (13.8)	225 (86.2)	0.86[a]	0.35[a]
Adenocarcinoma	239 (47.8)	74 (31.0)	165 (69.0)	26 (10.9)	213 (89.1)	0.98[b]	0.32[b]
Lymph node metastasis							
Negative	160 (32.0)	53 (33.1)	107 (66.9)	18 (11.3)	142 (88.7)	0.001[a]	0.97[a]
Positive	340 (68.0)	112 (32.9)	228 (67.1)	44 (12.9)	296 (87.1)	0.29[b]	0.59[b]
Clinical stage							
I+II	118 (23.6)	37 (31.4)	81 (68.6)	14 (11.9)	104 (88.1)	0.19[a]	0.66[a]
III+IV	382 (76.4)	128 (33.5)	254 (66.5)	48 (12.6)	334 (87.4)	0.04[b]	0.84[b]

Notes: [a]AA compared with AC + CC; [b]CC compared with AC + AA.
Abbreviations: IL-18, interleukin-18; NSCLC, non-small-cell lung cancer.

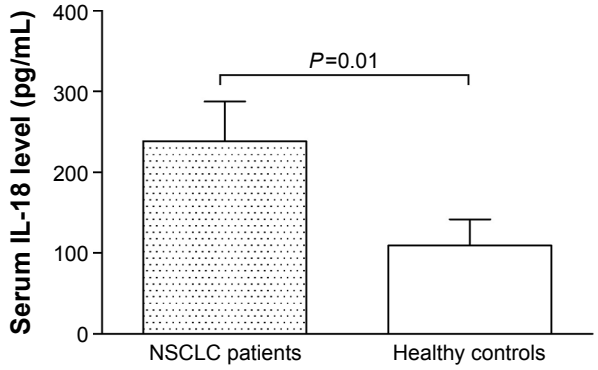

Figure 1 Serum IL-18 level in NSCLC patients and healthy controls.
Notes: The IL-18 level was detected by ELISA. The serum IL-18 levels in the NSCLC patients (238.6±48.88 pg/mL) increased more significantly than in the controls (109.6±32.38 pg/mL) (*P*=0.01). No association was found between serum IL-18 levels and different genotypes. The values are shown as mean ± standard deviation.
Abbreviations: IL-18, interleukin-18; NSCLC, non-small-cell lung cancer; ELISA, enzyme-linked immunosorbent assay.

to determine serum IL-18 levels. The results showed that the AA/AC genotype distribution in the NSCLC patients was significantly higher than that in the controls (*P*=0.02). Furthermore, stratified analyses for combined genotypes, AA genotype versus AC + CC genotype and CC genotype versus AC + AA genotype, were performed in NSCLC patients according to age at admission, sex, smoking status, histological type, lymph node metastasis, and clinical stage. However, no significant differences were found, implying that the IL-18 expression increased significantly in NSCLC patients (*P*=0.01) regardless of their clinical characteristics. According to the genotype distribution, no association was found between serum IL-18 levels and different genotypes, which is inconsistent with a previous report,[23] possibly because of the different ethnic population.

In conclusion, the current study demonstrates the important role of IL-18 -607 A/C polymorphism in increasing the risk of NSCLC in the Chinese population, but this SNP could not functionally affect the IL-18 levels. Thus, further studies are needed in a larger and ethnically different population to confirm this genetic influence on lung cancer.

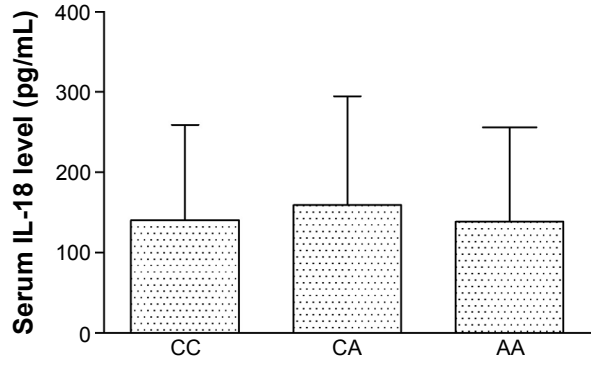

Figure 2 Serum IL-18 level in different genotypes of NSCLC patients.
Note: No association was found between serum IL-18 levels and genotypes.
Abbreviations: IL-18, interleukin-18; NSCLC, non-small-cell lung cancer.

Disclosure

The authors report no conflicts of interest in this work.

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