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Original Article

Relation between TNF-α genetic polymorphism and primary lung cancer

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Abstract: Objective: Single nucleotide polymorphism (SNP) in the promoter region of tumor necrosis factor-α (TNF-α) is found to be related to some inflammations and tumors. In this study, we aimed to confirm the correlation between TNF-α-308G/A polymorphism and susceptibility to primary lung cancer among Chinese population. Method: Genotyping was performed for TNF-α-308G/A polymorphism (rs1800629 locus) using high-throughput TaqMan-MGB probe. The genotype distributions in 500 healthy subjects and 500 patients with primary lung cancer were compared. Results: The frequency of GG genotype of TNF-α-308G/A polymorphism was 70.8% in case group and 88.2% in control group. The frequency of AG genotype was 21.0% in case group and 8.6% in control group. The frequency of AA genotype was 8.2% in case group and 3.2% in control group. The carriers of GA (OR=3.042, 95%: 2.077-4.454, P<0.001) and AA genotype (OR=3.192, 95% CI: 1.761-5.784, *P*<0.001) have higher risk of lung cancer. Logistic regression analysis remains the significance (T allele vs. G allele, OR=2.665, 95% CI: 1.342~4.142, P=0.008). Conclusion: TNF-α-308G/A polymorphism was significantly associated with susceptibility to primary lung cancer in a Chinese population.

Keywords: Tumor necrosis factor-α (TNF-α), single nucleotide polymorphism (SNP), small cell lung cancer, non-small cell lung cancer

Introduction

Single nucleotide polymorphism (SNP) is a single base-pair difference in the DNA sequence of individual members of a species. It represents one of the most common forms of inheritable variations. TNF-α gene is localized to chromosome 6p21.4 with a full length of 3.6 kbp. TNF-α gene is of 4 exons and 3 introns, and is a closely linked locus within the major histocompatibility complex and an important member of TNF superfamily [1-4]. TNF-α gene can initiate and regulate the generation of inflammatory cytokines. 8 SNPs were found in the TNF-α promoter region including 238, 308, 857, 863 and 1031 loci. It is believed that TNF-α promoter polymorphism is related to the transcriptional regulation of TNF-α. Report indicates that TNF-α G-308A SNP is associated with susceptibility to chronic obstructive pulmonary disease [5], lymphoma [6-8], rheumatoid arthritis [9], sarcoidosis [10], and type II diabetes [11]. We applied Taqman technique to detect TNF-α-308 polymorphism in 400 healthy subjects and 400 patients with primary lung cancer, all being Chinese. The genotype distribution was compared between the two groups, based on which the correlation between TNF-α-308G/A polymorphism and susceptibility to primary lung cancer in Chinese population was investigated.

Materials and method

Subjects

From January 2007 to January 2015, 500 healthy subjects receiving physical examination at physical examination center in our hospital were randomly included (357 males, 143 females, aged 28-70 years old, media age 54). In the meantime, 500 patients with primary lung cancer were also randomly included at this department (355 males, 145 females, aged 30-70 years old, median age 53). There was no kinship either among the healthy subjects or among the patients. The protocol was approved by Ethics Committee in our hospital. The basic information of the subjects is shown in **Table 1**.

Table 1. General characteristics of the case group and the control group n (%)

Characteristics	Controls (n=500)	Cases (n=500)	X^2/t	P
Age (years)	54.5±12.3	53.4±12.6	1.423	0.323
Gender (F/M)	143/357	145/355	1.225	0.898
Smoking	132 (26.4)	323 (64.6)	9.247	<0.001
Histologic type				
Adenocarcinoma	-	154 (30.8)		-
Squamous cell carcinoma	-	236 (45.2)		-
Arge cell lung cancer	-	78 (15.6)		-
Others	-	32 (6.4)		-

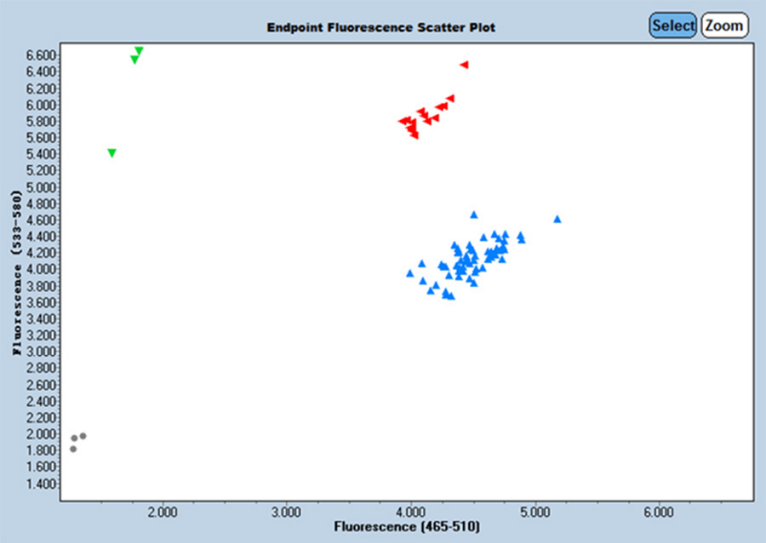

Figure 1. Genotyping results by TaqMan methods (Green dot: TT genotype; Red dot: GT genotype; Blue dot: GG genotype).

Table 2. Hardy-Weinberg equilibrium test results

Group		SNP			*P*
		GG	GA	AA	
Control (*n*=500)	Actual	441	43	16	0.081
	Expected	427	70	3	
Case (*n*=500)	Actual	354	105	41	0.074
	Expected	331	151	18	

Methods

Genomic DNA extraction from peripheral blood leucocytes: Fasting venous blood (5 ml) was drawn in the morning and placed into the tube containing EDTA. DNA extraction was performed using DNA extraction kit (Qiagen, Germany). The concentration of the extracted DNA was detected by using a UV spectrophometer, normalized to 50 ng/μL and preserved at -80°C.

DNA polymorphism analysis: As shown in **Figure 1**, genotyping was performed using Taqman technique. The genotyping results were presented as 3 kind of color dots (Red, blue, and green). The pre-designed Taqman probe and PCR primers and Master Mix for PCR amplification were manufactured by Applied Biosystems (AB. Inc. USA). For PCR, 2.5 μL of DNA template was fully mixed with 2× TaqMan Genotyping Master Mix 2.5 μL and 40×TaqMan SNP Genotyping Assay 0.125 μL. For each experiment, NTC (no-template controls) and positive control were set up. Real Time-PCR (RT-PCR) conditions: 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, 60°C for 1 min, 50 cycles. Plate read was performed on a RT-PCR, and the raw data were analyzed with SDS 2.2 software. Genotyping was performed using 7900HT Fast Real-Time PCR System (Applied Biosystems, USA).

Statistical process: Statistical analysis was performed using SPSS 18.0 software (Chicago, IL, USA). The distribution of age, gender and smoking history in case group and control group was compared by χ^2 test and t test. The correlation between gene polymorphism and susceptibility to lung cancer was analyzed by multiple logistic regressions. All tests were two-sided with α=0.05. P<0.05 was considered significantly different.

Results

Comparison of general information between case group and control group

The age and gender distribution of the two groups showed no significant differences (P> 0.05). The smoking status showed significant

Table 3. Association between the SNP of the TNF-α gene and the risk for lung cancer n (%)

Genotype allele	Controls (n=500)	Cases (n=500)	OR (95% CI)	P
GG	441 (88.2)	354 (70.8)	1 (reference)	
GA	43 (8.6)	105 (21.0)	3.042 (2.077-4.454)	<0.001
AA	16 (3.2)	41 (8.2)	3.192 (1.761-5.784)	<0.001
G allele	925 (92.5)	813 (81.3)	1 (reference)	
A allele	75 (7.5)	187 (18.7)	2.836 (2.135-3.768)	<0.001

Table 4. Logistic regression of the relation between gene polymorphism and lung cancer risk

Parameters	Beta	OR	95% CI	*P* value
TNF-α polymorphism	0.274	2.665	1.342~4.142	0.008
Smoking	0.765	2.449	1.323~4.201	0.015
Age	0.465	1.432	0.887~3.871	0.221
Histologic type	0.445	1.401	0.884~4.014	0.324

differences between the two groups (P<0.05, **Table 1**).

Hardy-Weinberg equilibrium test

The genotype distribution of the control and case group did not show significant difference from the Hardy-Weinberg equilibrium values (**Table 2**).

Correlation between genotype frequency and risk of lung cancer

The frequency of GG genotype of TNF-α-308G/A polymorphism was 70.8% in case group and 88.2% in control group. The frequency of AG genotype was 21.0% in case group and 8.6% in control group. The frequency of AA genotype was 8.2% in case group and 3.2% in control group. The carriers of GA (OR=3.042, 95%: 2.077-4.454, P<0.001) and AA genotype (OR= 3.192, 95% CI: 1.761-5.784, P<0.001) have higher risk of lung cancer (**Table 3**).

Logistic regression results

After adjustment of the confounders such as smoking, age, gender, and histologic type, the TNF-α-308G/A polymorphism was independent associated with lung cancer (OR=2.665, 95% CI: 1.342~4.142, P=0.008, **Table 4**).

Discussion

Lung cancer is among the malignancies that pose the greatest global threat to human life. The etiology, prevention, diagnosis and treatment of lung cancer are global concerns. The epidemiological studies indicate lung cancer is the result of combined action of environmental and genetic factors. Although over 80% of lung cancers can be attributed to tobacco exposure, only less than 20% of smokers finally get lung cancer under the same intensity of tobacco exposure [12]. This implies the interindividual variation of genetic susceptibility to lung cancer. SNP is the genetic polymorphism that has the widest distribution and the highest prevalence. The features such as high density, representativeness, genetic stability and easy detectability make SNP an outstanding genetic marker in relevant studies. Several SNPs are found to be associated with the onset, development, treatment and prognostic prediction of lung cancer [13, 14]. We aimed to reveal the potential connections between TNF-α-308G/A polymorphism and primary lung cancer by using Taqman technique in a large-sample analysis. Because TNF-α gene is a closely linked locus within the major histocompatibility complex, MHC also plays a certain role in some diseases. This may serve as the molecular basis for the occurrence of some tumors.

We found that the frequency of genotypes of TNF-α-308G/A polymorphism was statistically significant between lung cancer patients and healthy controls (P<0.05), which agreed with the findings of other studies. Both AG genotype and AA genotype are the risk factors of primary lung cancer as compared with GG genotype [15]. After adjustment of confounders, the TNF-α-308G/A polymorphism was independent associated with lung cancer (OR=2.665, 95% CI: 1.342~4.142, P=0.008.

In conclusion, TNF-α-308G/A polymorphism is correlated with genetic susceptibility to primary lung cancer. The occurrence of tumors involves multiple factors, and our findings shed some new light on the understanding of etiology of lung cancer.

Disclosure of conflict of interest

None.

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