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Original Article

Association between *EGFR* polymorphisms and the risk of lung cancer

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Abstract: Target: The study aimed to investigate the role of epidermal growth factor receptor (*EGFR*) rs6965469 and rs763317 polymorphisms in the occurrence and development of lung cancer. Methods: We used polymerase chain reaction-ligation detection reaction (PCR-LDR) method to detect the genotypes of *EGFR* rs6965469 and rs763317 polymorphisms and the data were analyzed by GeneMapper software. Odds ratios (ORs) with 95% confidence intervals (CIs) was calculated by χ^2 test to estimate the significance difference of genotype and allele frequencies in case and control groups. ORs and 95% CIs were adjusted by logistic regression analysis with age, gender, drinking and smoking. The genotypes distributions of control group were tested by Hardy-Weinberg equilibrium (HWE). Results: The genotypes frequencies of controls for rs6965469 and rs763317 polymorphims were consistent with HWE. The distribution of rs6965469 TT genotype in two groups was significantly different ($P<0.05$) and TT genotype was associated with an increased risk of lung cancer (OR=6.92, 95% CI=1.33-36.00). AA genotype and A allele of rs763317 were also the susceptible factors of lung cancer. Individuals with AA genotype or A allele were more likely to suffer lung cancer (AA vs. GG: OR=7.20, 95% CI=1.33-39.07; A vs. G: OR=2.61, 95% CI=1.04-6.59). Conclusions: The *EGFR* rs6965469 and rs763317 polymorphisms may be risk factors for lung cancer.

Keywords: *EGFR,* lung cancer, polymorphisms

Introduction

Lung cancer is one of malignancies that has the largest number of patients all over the world. Its global incidence is rising year by year and it has become one of the cancers with high morbidity and mortality [1]. Over the past twenty years, the morbidity and mortality of lung cancer in China has been on the rise, too [2]. With the sustained growth of tobacco consumption, severe environment contamination and changes in life style, lung cancer seriously affects people's health and lives in our country [3-5]. However, the pathogenesis of lung cancer is still not fully clear. Smoking, occupation, environmental exposure, chronic lung infection, familial inheritance and the decrease of immune function are all risk factors of lung cancer. The occurrence of lung cancer is a complex process with polygenes, multiple factors and multiple stages.

Among the genes, epidermal growth factor receptor (*EGFR*) has become a focus in the research field of lung cancer. *EGFR* is a peptide with a strong ability to promote cells proliferation and differentiation. Overexpression of *EGFR* can abnormally activate its downstream signal pathway, which leads to cells transformation and proliferation. Numerous studies have showed that high expression and mutation of *EGFR* are closely related to tumors [6-10]. Additionally, Han et al. found that the level of *EGFR* protein was higher in the lung cancer tissues compared with normal tissues [11]. Furthermore, *EGFR* polymorphisms were also correlated with susceptibility to lung cancer [12-14]. However, there are few correlative studies about *EGFR* rs6965469 and rs763317 polymorphisms and lung cancer.

Our study based on a case-control experiment chose 50 lung cancer patients and 52 healthy persons to explore the relevance of *EGFR* polymorphisms (rs6965469 and rs763317) with lung cancer susceptibility. The polymerase chain reaction-ligation detection reaction (PCR-LDR) method was used to perform the genotyping for *EGFR* polymorphisms.

Table 1. Primer sequences of rs6965469 and rs763317 polymorphisms

SNP	Primers	Primer sequence
rs6965469	Forward	5'-TCTGTTCCCTGGAATCCATC-3'
	Reverse	5'-TTTGAGTGCCCAAAAAGACA-3'
rs763317	Forward	5'-TCCTTCAGCAAAACCCTCAG-3'
	Reverse	5'-GGACTCCAGTCCAATTTTTCA-3'

Materials and methods

Selection of subjects

50 patients diagnosed as lung cancer by clinical data and histopathology were enrolled from Laiwu People's Hospital. All the patients, aged 29~80 years (mean age of 50.6±10.9), received no surgery, radiotherapy or chemotherapy. 52 synchronous healthy persons, aged 18~76 (mean age of 56.8±11.6), were matched by sex and age with cases as the control group. The individuals who have family history of tumors or other serious lung diseases were excluded. Written consents were obtained from the unrelated subjects. Our study was conducted under the permission of the ethics committee of Laiwu People's Hospital.

DNA extraction

2 ml fasting peripheral venous blood of every subject was collected in the morning and put in the EDTA anticoagulation tube. Genome DNA was extracted using AxyPrep-96 whole blood genomic DNA kit (Axygen company, America) according to the instructions. DNA concentration and purity were evaluated by NanoDrop 2000 and preserved at -20°C for use.

Inspection of EGFR rs6965469 and rs763317 polymorphisms

The genotypes of rs6965469 and rs763317 polymorphisms were inspected at Shanghai Biowing Applied Biotechnology Co., Ltd by polymerase chainreaction-ligation detection reaction (PCR-LDR) method. The primer sequences were shown in **Table 1**. PCR conditions were as follows: denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, annealing at 72°C for 1 minute and finally extension at 72°C for 7 minutes. Then LDR with the above PCR products as a template was performed and the probe sequences were shown in **Table 2**. The LDR parameters were: denaturation at 95°C for 2 minutes, 35 cycles of denaturation at 94°C for 30 seconds, denaturation at 60°C for 2 minutes. Finally, the LDR products were tested on the an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA). Data analyses were performed using GeneMapper software.

Sequencing result

EGFR rs6965469 polymorphism had three genotypes: CC homozygotic type, TT homozygotic type and CT heterozygous type. Rs763317 polymorphism was presented as GG homozygotic type, AA homozygotic type and GA heterozygous type.

Statistical analyses

The genotype and allele distributions of *EGFR* polymorphisms in the two groups were compared by χ^2 test and the subject representativeness of control group was tested by Hardy-Weinberg Equilibrium (HWE). The clinical data between groups were analyzed by non-conditional logistic regression. All the statistical analyses were performed by using SPSS 18.0 software, with significant level at $P<0.05$.

Results

Characteristics of the subjects

Our study contained 50 cases and 52 controls. There were no significant differences of sex, age and drinking status between two groups ($P>0.05$). However, significant differences in smoking status existed in two groups ($P<0.05$) (**Table 3**).

Analysis of EGFR rs6965469 and rs763317 polymorphisms and lung cancer risk

The genotype and allele distributions of *EGFR* rs6965469 and rs763317 polymorphisms were shown in **Table 4**. The genotypes distributions of control group satisfied with HWE ($P>0.05$). The adjusted results by age, sex, environmental conditions and drinking and smoking status indicated that rs6965469 TT genotype could increase the onset risk of lung cancer (OR=6.92, 95% CI=1.33-36.00), while frequencies of T allele in two groups showed no statistically significant differences ($P>0.05$).

Table 2. Probe sequences in LDR analysis

SNP	Probe	Probe sequence (5'→3')	Length of LDR products
rs6965469	Modify	P-TATATTCTTCAATGTGATCTGATAGTTTTTTTTTTTTTTT-FAM	
	C	TTTTTTTTTTTTTTTACAACAGCCTTCATAGTACGGCTTG	82 bp
	T	TTTTTTTTTTTTTTTTTACAACAGCCTTCATAGTACGGCTTA	84 bp
rs763317	Modify	P-CTTTGACATTCCAGGTTTCCTCATGTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
	A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAATGCAGAATGTGTTGCACTTTAT	114 bp
	G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAATGCAGAATGTGTTGCACTTTAC	116 bp

Table 3. Basic characteristics of the subjects

Information	Case (n=50)	Control (n=52)	*P*
Sex			
Male	32	29	0.43
Female	18	23	
Age			
≤50	12	17	0.38
>50	38	35	
Smoking status			
Smoking	35	25	0.03
Non-smoking	15	27	
Drinking status			
Drinking	36	32	0.30
Non-drinking	14	20	

The frequency of rs763317 AA genotype in case group was higher than that of control group with significant level at $P<0.05$ and AA genotype carriers showed higher risk of developing lung cancer than GG genotype (OR=7.20, 95% CI=1.33-39.07). A allele also could increase the risk of lung cancer compared with G allele (OR=2.61, 95% CI=1.04-6.59).

Discussion

Lung cancer is a malignant disease under the control of polygenes and multifactors with complex biological characteristics. A mass of studies have verified genetic factors play vital role in the development of tumors, especially genetic variants. It is also applied to the pathogenesis of lung cancer. Yin et al. made a conclusion that PD-1.5C/T polymorphism was associated with the increased susceptibility of non-small cell lung cancer in Chinese Han Population [15]. In addition, *CHRNA3* polymorphisms can significantly increase the occurrence risk of lung cancer in Chinese Han population who are smokers according to the study of Zhou et al. [16].

Studies in recent years have found that *EGFR* plays an important role in the development of malignant tumors [17]. Kim et al. reported *EGFR* was overexpressed in patients with gastric cancer compared with healthy person, suggesting that *EGFR* may be a risk factor of gastric cancer [18]. Genetic mutations and excessive expression of *EGFR* are correlated with breast cancer, head and neck squamous cell carcinomas, colorectal cancer, pancreatic cancer and lung cancer [19-23]. All these results prove that *EGFR* is closely related to the development of tumors.

EGFR is a powerful promoter of the cell division that mainly works in the way of autocrine or paracrine. After the combination of *EGFR* and *EGF* on the target cell membranes, the receptor is activated when part of intracellular tyrosine residues are phosphorylated, then the receptor identifies and phosphorylates the target protein containing tyrosine residues in cell, thus activates dormant nuclear transcription factors to regulate the expression of some genes and cells proliferation and division [24, 25].

In present study, the frequencies of rs6965469 TT genotype and T allele in case group exhibited significant differences with those of controls. After the adjustment of age, sex, smoking and drinking status, the distribution of T allele showed no statistically significant differences in two groups. TT genotype could still increase the risk of lung cancer, so did the rs763317 AA genotype. A allele was also one of genetic-susceptibility factor of lung cancer. Our result was consistent with a previous research that found that *EGFR* rs763317 polymorphism was related to the susceptibility to lung cancer [26].

Our study found that the mutant genotypes of rs6965469 and rs763317 polymorphisms were related with an increased risk of lung can-

Table 4. Comparison of the genotype and allele distribution of *EGFR* rs6965469 and rs763317in two groups

Groups	rs6965469				
	CC	CT	TT	C	T
Case (n, %)	24 (48.0)	15 (30.0)	11 (22.0)	63 (63.0)	37 (37.0)
Control (n, %)	34 (65.4)	16 (30.8)	2 (3.8)	84 (80.8)	20 (19.2)
OR (95% CI)	Reference	1.33 (0.55-3.19)	7.79 (1.58-38.4)	Reference	2.47 (1.31-4.65)
OR[a] (95% CI)	Reference	1.10 (0.44-2.78)	6.92 (1.33-36.00)	Reference	2.51 (0.96-6.58)
Groups	rs763317				
	GG	GA	AA	G	A
Case (n, %)	19 (38.0)	22 (44.0)	9 (18.0)	60 (60.0)	40 (40.0)
Control (n, %)	32 (61.5)	18 (34.6)	2 (3.9)	82 (78.8)	22 (21.2)
OR (95% CI)	Reference	2.06 (0.89-4.78)	7.58 (1.48-38.8)	Reference	2.49 (1.34-4.61)
OR[a] (95% CI)	Reference	2.08 (0.85-5.11)	7.20 (1.33-39.07)	Reference	2.61 (1.04-6.59)

Note: [a]means the results were adjusted by sex, age, drinking and smoking status.

cer. However, in the study we overlooked the influence from the interaction of gene-gene and gene-environment. Additionally, the role of *EGFR* polymorphisms are different in different races and districts. Therefore, further well-designed researches with more races and large-sample size are needed to verify the relevance between *EGFR* polymorphisms with lung cancer susceptibility.

Disclosure of conflict of interest

None.

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