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Original Article

The relationship between *XPG* rs17655 polymorphism and the risk of lung cancer

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Abstract: Purpose: This study aimed at to investigate the relationship between DNA repair gene xeroderma pigmentatosum group G (*XPG*) polymorphisms and the onset risk of lung cancer. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was utilized to detect the genotypes of *XPG* rs17655 polymorphism in 70 lung cancer patients and 79 healthy controls. The χ^2 test was used to analyze the frequencies differences of genotypes and allele frequencies in case and control groups. The association of *XPG* rs17655 polymorphism with lung cancer susceptibility was showed by odds ratio (OR) and 95% confidence interval (95% CI). Results: In *XPG* rs17655 polymorphism, the frequency distributions of CG/GG genotypes in case and control groups had statistically significant differences ($P<0.05$). Compared with CC genotype, people with CG/GG genotype had a significantly increased risk of developing lung cancer (OR=2.38, 95% CI=1.23-4.62). Stratification analysis on smoking status discovered that G allele in CG/GG genotype could significantly increase the risk which smoking population suffered from lung cancer (OR=3.40, 95% CI=1.37-8.40). Meanwhile, the stratification analysis on pathological types found that the frequencies of genotype CG/GG had obvious difference between the two groups. Compared with CC genotype, CG/GG genotype apparently aggrandized the onset risk of lung squamous carcinoma (OR=2.89, 95% CI=1.25-6.70). Conclusions: *XPG* rs17655 polymorphism can remarkably increase the onset risk of lung cancer, especially in smokers and it is correlated with the pathological types of lung cancer.

Keywords: *XPG*, lung cancer, polymorphism, stratification analysis

Introduction

As a malignant tumor, lung cancer has become one of the global health problems because of its high morbidity and mortality [1]. In recent years, it has been the second leading cause of death in Chinese urban area [2-4]. Lung cancer has the highest cumulative risk in our county due to the aging of the population, the industrialization of the towns, the environmental pollution and destruction, and especially the increasing smoking population. Smoking is a environmental factor that has the closest relationship with the onset of lung cancer and about 85%-90% of lung cancer patients have smoking history [5-7]. Molecular epidemiological study indicates that lung cancer is a result of the complex interactions between internal genetic factors and external environmental factors. However, exposing the same environment, only a part suffer from lung cancer, which suggests that the genetic factors of the patients play a vital role in lung cancer development. Therefore, gene polymorphism is paid attention to exploring the ethology and pathology of lung cancer.

Research has shown that DNA repair mechanisms are of great importance to maintain the integrity of DNA and prevent the generation of tumors. Nucleotide excision repair (NER) is the most flexible repair pathway in DNA repair system [8-11]. Xeroderma pigmentatosum group G (XPG) gene involved in the NER pathway is an important member of the human DNA repair gene family [12]. The change of *XPG* in the NER pathway may affect the DNA repair capacity and lead to the dysfunction of the NER signaling pathway so as to cause the occurrence of tumors [13, 14]. Single nucleotide polymorphism (SNP) rs17655 in the coding area of *XPG*

Table 1. Research objects and basic characteristics

Group	Sex		Age		Smoking		Drinking	
	Male	Female	≤55	>55	Yes	No	Yes	No
Case n=70	41	29	49	21	46	24	50	20
Control n=79	43	36	54	25	37	42	53	26
P	0.62		0.86		0.02		0.59	

was proved by studies to be related to the susceptibility to non-small cell lung cancer (NSCLC) [15].

Therefore, in this case-control study, we extracted the blood DNA samples of 70 lung cancer patients and 79 healthy controls to conduct the genotyping of the *XPG* rs17655 polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and further investigate the association between *XPG* gene polymorphism and the susceptibility to lung cancer.

Materials and methods

Study objects

In the case group of this study, 70 lung cancer patients diagnosed by histopathology from The Fourth Affiliated Hospital of China Medical University between May, 2012 and June, 2014 were collected, including 41 males and 29 females. The patients with the mean age of 46.23±6.56 years old were all Chinese Han population. According to pathological types, the cases were assigned to 3 groups: lung adenocarcinoma group (35 cases), lung squamous carcinoma group (26 cases) and other pathological types group (9 cases). 79 healthy controls (43 males and 36 females) with the mean age of 45±8.52 years old were from the physical examination center of the same hospital in the same period with the cases. The persons were excluded which have the family history of autoimmune diseases, infection and tumors in the control group. The controls were frequency-matched with the cases in sex and age. All subjects were fully informed the study procedures and they had no objection to sign the informed consents. This study was carried out on the premise of abiding the norms of medical Ethics Committee of The Fourth Affiliated Hospital of China Medical University, and the subjects had no relationship by blood.

Sample collection

We collected 2 mL fasting venous blood from every participant and put in the tube with EDTA anticoagulant. Genome DNA was extracted with the whole blood DNA extraction kit according to the manufacturer instruction. Then the samples were preserved at -20°C for standby application.

Genotype determination

The genotypes of *XPG* rs17655 polymorphism were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR amplification primers of *XPG* gene were designed by Primer premier 5.0 software and synthesized by Shanghai Sangon Biotech, Co., Ltd. The forward primer sequence was: 5'-GACCTGCCTCTCAGAATCAT-3', and the reverse primer sequence was 5'-CCTCGCACGTCTTAGTTT-3'. The total PCR reaction mixture consisted of 25 μL, with 2.0 μL template DNA, 0.5 μL forward primer, 0.5 μL reverse primer, 12.5 μL 2× Master Mix (including pfu DNA Polymerase 0.05 U/μL, $MgCl_2$ 4 mmol/L, dNTPs 0.4 mmol/L; Aidlab Biotechnologies Co., Ltd), and finally ddH_2O made up the rest volume. PCR amplification started with a initial denaturation step at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s and at the end extension at 72°C for 7 min. Amplification products were detected with 2% agarose gel electrophoresis (AGE).

The mixture of 10 μL PCR products were incubated in a water bath at 37°C for overnight with 1 μL restriction enzyme *Hsp92* II (from Fermentas company). This digestion fragments were separated by 3% AGE and stained with ethidium bromide.

Statistical analysis

The differences comparison between two groups was conducted by χ^2 test. The Hardy-Weinberg equilibrium (HWE) about the frequency distributions of genotypes was inspected to ascertain the randomness of the study subjects. Odds ratio (OR) and 95% confidence interval (95% CI) were used to represent the association strength between gene polymorphism and lung cancer susceptibility. All of the statistical interpretation was performed by SPSS 18.0 software, with $P<0.05$ implying statistically significant difference.

Table 2. Relationship between the distributions of *XPG* rs17655 SNP and the onset of lung cancer

Group	Genotype			P	OR (95% CI)
	CC	CG	GG		
Total					
Case n=70 (%)	25 (35.7)	27 (38.6)	18 (25.7)	-	-
Control n=79 (%)	45 (57.0)	26 (32.9)	8 (10.1)	0.01	2.38 (1.23-4.62)
Nonsmoking					
Case n=24 (%)	14 (58.3)	7 (29.2)	3 (12.5)	-	-
Control n=42 (%)	22 (52.4)	15 (35.7)	5 (11.9)	0.80	0.79 (0.29-2.16)
Smoking					
Case n=46 (%)	16 (23.9)	19 (43.5)	11 (32.6)	-	-
Control n=37 (%)	23 (62.2)	11 (29.7)	3 (8.1)	0.01	3.40 (1.37-8.40)

Results

Research objects and basic characteristics

As was shown in **Table 1**, the sex, age and drinking status indicated no statistical significance of comparable difference between the case and control groups (P>0.05). Smokers were more frequent in case group than in control group, and the difference was statistically significant (P<0.05). The genotypes distribution of *XPG* rs17655 polymorphism conformed to HWE, suggesting our study population possessed a representativeness.

The result of the genotyping in XPG rs17655 polymorphism

The genotyping result of *XPG* rs17655 polymorphism indicated there existing three genotypes through the digesting of restriction enzyme, namely, CC GC, GG. Among them, the common homozygote CC was 271 bp fragment; and the mutant homozygote GG included two fragments 227 bp and 44 bp; and the heterozygous genotype CG was three fragments 271 bp, 227 bp and 44 bp.

Correlation between XPG rs17655 SNP distribution and the onset risk of lung cancer

This study contained 70 cases and 79 controls. The genotype distributions of the control group satisfied with HWE (P>0.05). The genotype frequencies of CC and CG/GG were 35.7%, 64.3% respectively in the case group. The frequencies of genotype CC, CG and GG in control group were 57.0%, 32.93% and 10.1% respectively. It could be concluded that the genotype distribution differences between the case and control groups had statistical significance (χ^2= 8.384, *P*=0.01). Compared with CC genotype, G allele existed in genotype CG and GG increased the risk of developing lung cancer (OR=2.38, 95% CI=1.23~4.62). Stratification analysis on smoking status proved that the distribution of genotypes was significantly different between smoking and nonsmoking groups (P<0.05). Smokers with genotype CG/GG had significantly higher risk of developing lung cancer than nonsmoking carriers (OR=3.40, 95% CI= 1.37~8.40) (**Table 2**). Smoking might increase the onset risk of lung cancer, and it was a risk factor of this disease. In addition, smokers with CG/GG genotype were more tendency to suffering from lung cancer than persons with CG/GG genotype. Therefore, the interaction might exist between *XPG* rs17655 polymorphism and smoking.

Association between XPG polymorphisms and the pathological types of lung cancer

Stratification analysis on the pathological types demonstrated that *XPG* rs17655 polymorphism might be relevant with the onset risk of lung squamous carcinoma (P<0.05). In lung squamous carcinoma group, patients with the mutant genotype CG/GG had higher onset risk than patients in the other two groups (OR=2.89, 95% CI=1.25~6.70). CG/GG genotype was a risk factor of lung squamous carcinoma (**Table 3**).

Discussion

The main effect of *XPG* protein as endonuclease is to remove the damage DNA fragment from 3' side and maintain the stability of DNA structure. *XPG* is the core gene of DNA repair system and its high expression can increase the DNA repair activity of tumor cells, which tumor cells will have stronger invasion and proliferation abilities [16, 17]. Besides, the 1104th codon C→G mutation of *XPG* in No.15 exon can lead to the amino acid replacement of Asp→His. *XPG* Asp1104His polymorphism is located in the C terminal of *XPG* protein. This latter has

Table 3. Correlation between *XPG* rs17655 SNP and different lung cancer pathological types

Group	Genotype			P	OR (95% CI)
	CC	CG	GG		
Control n=79 (%)	45 (57.0)	26 (32.9)	8 (10.1)	-	-
Adenocarcinoma n=35 (%)	11 (31.4)	14 (40.0)	10 (28.6)	0.02	2.89 (1.25-6.70)
Squamous carcinoma n=26 (%)	10 (38.5)	9 (34.6)	7 (26.9)	0.12	2.12 (0.86-5.25)
Others n=9 (%)	4 (44.4)	4 (44.4)	1 (11.2)	0.50	1.65 (0.41-6.63)

functional interactions with others DNA repair gene, such as *XPB*, *XPD*, *p62* and *p44*. However, *XPG* His1104Asp polymorphism can cause defects of such interactions and finally affect the repair function of NER pathway [13]. Wei et al. have reported that poor DNA repair ability can increase the onset risk of lung cancer [18]. Therefore, studies on the relationship between *XPG* rs17655 SNP and the genetic susceptibility to lung cancer have become the focal point.

It is generally known that lung cancer has become one of the serious diseases that affect the health of Chinese residents. Lung cancer is a complex process with the involvement of multiple factors and genes. It is not caused by a single gene, but by the cooperation of environment factors and genetic factors. As the initial factors of tumor development, environmental carcinogens can directly and/or indirectly attack host cells after entering the body. If DNA damages caused by carcinogens were too severe, or DNA repair capacity decreased due to the roles of genetic factors, which may result in somatic mutation, and finally cells cancerization will develop [19-22]. In recent years, many studies on the association between *XPG* His1104Asp SNP and tumors susceptibility presented different results. Chang et al. reported that in Americans, the result showed that genotype GG increased the risk of developing lung cancer based in African Americans, compared with CC genotype [23]. The study was conducted by Cui et al. among Caucasians, Mexicans, African Americans and Asian Americans to find that GG genotype in recessive model could decrease the onset risk of lung cancer [24]. However, other study discovered that *XPG* His1104Asp polymorphism had no effects on Chinese population [25].

The results of this study on the correlation between *XPG* rs17655 polymorphism and the onset risk of lung cancer showed that the distribution differences of mutant genotype CG/GG between case group and control group had statistical significance. CG and GG genotypes carriers had higher risk of developing lung cancer than CC genotype carriers, which indicated that genotype CG/GG significantly increased the risk of lung cancer development. Further stratification analyses on smoking status and the pathological types of lung cancer suggested that the risk of developing lung cancer increased in smokers with G allele. Both smoking and CG/GG genotype play an independent role in the onset of lung cancer and meanwhile, these two risk factors also existed the interaction. In addition, *XPG* rs17655 polymorphism CG/GG genotype was found to be related to the onset of lung squamous carcinoma, and it was a high risk factor of the disease.

However, the present study just took one SNP into consideration and the interaction between this SNP and others relative SNPs from the NER pathway related genes are also not be researched. The specific function and biochemical mechanism of *XPG* defects leading to tumor still remain unclear at present. Studies on genetic susceptibility to lung cancer at home and abroad are in early stages. Small sample size, ethnic and regional differences and different genetic backgrounds of different ethnicgroups may be the reasons causing different study results. So, in order to further investigate the relationship between the NER pathway gene (*XPG*) SNPs and lung cancer risk with the larger sample size. Besides, researches on different polymorphisms from the different genes of the NER pathway are conducive to the prevention and treatment of lung cancer.

Disclosure of conflict of interest

None.

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