Int J Clin Exp Med 2015;8(5):8153-8157
www.ijcem.com /ISSN:1940-5901/IJCEM0004054

Original Article

The association between NFKB1-94ins/del ATTG polymorphism and non-small cell lung cancer risk in a Chinese Han population

Yue Wang[1], Libo Chen[2], Liming Pan[3], Jinru Xue[1], Haixiang Yu[1]

[1]Department of Thoracic Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, P.R. China; [2]Department of Ultrasound, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, P.R. China; [3]Department of Pediatrics, First Affiliated Hospital of Jilin University, Changchun 130033, Jilin, P.R. China

Received November 22, 2014; Accepted February 2, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: This study aimed to clarify the influence of a common insertion/deletion polymorphism (-94ins/del ATTG, rs28362491) in the Nuclear factor-κB1 (NFKB1) promoter on non-small cell lung cancer (NSCLC) susceptibility. We genotyped the NFKB1 -94ins/del ATTG polymorphism by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and assessed the association with NSCLC risk, clinicopathological parameters in a case-control study of 421 cases and 425 controls. Heterozygous (ID) genotype disclosed a statistically significantly increased risk of developing NSCLC (OR = 1.57, 95% CI 1.13-2.19, *P* = 0.007). Homozygous (II) genotype also showed an increased risk of NSCLC (OR = 1.87, 95% CI 1.27-2.75, *P* = 0.001). Statistically significant difference was observed when the patients and controls were compared according to ID + II versus DD (OR = 2.01, 95% CI 1.47-2.76, *P*<0.001). The I allele was significantly higher in the NSCLC cases compared to the controls (52.9% versus 45.1%). The I allele was significantly associated with NSCLC risk (OR = 1.37, 95% CI 1.12-1.65, *P* = 0.001). There was a significantly higher frequency of ID + II genotypes observed in smokers, compared to non-smokers (OR = 1.99, 95% CI 1.22-3.24, *P* = 0.005) and in patients with stage III + IV, compared to stage I + II (OR = 2.16, 95% CI 1.34-3.49, *P* = 0.002). This study suggested that NFKB1 -94ins/del ATTG polymorphism was significantly associated with NSCLC risk in Chinese Han population.

Keywords: Non-small cell lung cancer, nuclear factor-κB, polymorphism; genetics

Introduction

Lung cancer is the most common cause of cancer death, with more than 226,000 new cases in the United States in 2012, and the majority of lung cancer cases are non-small cell lung cancer (NSCLC), which accounts for approximately 80% of lung cancer [1]. Despite the enormous improvements made in chemotherapy and radiotherapy over the past few decades, the outlook for patients with NSCLC was dismal, with only slightly more than 15% of patients alive 5 years after diagnosis [2]. Thus, it is urgent to find new prognostic markers and therapeutic strategies to improve treatment of NSCLC.

Nuclear factor-κB (NF-κB) was initially identified in 1986 as a transcription factor which binds to a 10 bp DNA element in kappa immunoglobulin light-chain enhancer in B cells [3]. The NF-κB family consists of p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel (Rel), and RelB. The major form of NF-κB is a heterodimer of the p50 and p65/RelA subunits which are encoded by the NFKB1 and NFKB2 genes, respectively [4].

The human NFKB1 gene is mapped to chromosome 4q24 and encodes a 50 kDa DNA-binding protein (p50) that can act as a master regulator of inflammation and cancer development [5]. A common insertion (ins)/deletion (del) (-94ins/del ATTG, rs28362491) polymorphism of NFKB1 gene promoter exerts functional effects on the transcription of the NFKB1 [6]. Since the 4 bp ins/del polymorphism produces a relatively large sequence change and its location is proximal to binding sites that are important to pro-

Table 1. Characteristics of the cases and controls

Characteristics	Case (%)	Control (%)	*P* value
Gender			
Male	216 (51.3%)	207 (48.7%)	0.45
Female	205 (48.7%)	218 (51.3%)	
Age (years)			
≤ 60	210 (49.9%)	230 (54.1%)	0.22
> 60	211 (50.1%)	195 (45.9%)	
Smoking status			
No	224 (53.2%)	231 (54.3%)	0.74
Yes	197 (46.8%)	194 (45.7%)	
Histology			
Squamous cell carcinoma	267 (63.4%)		
Adenocarcinoma	154 (36.6%)		
TNM stage			
I + II	139 (33.0%)		
III + IV	282 (67.0%)		
HWE	0.369	0.595	

TNM, tumour-node-metastasis staging system; HWE, Hardy-Weinberg equilibrium.

Table 2. Genotype and allele frequencies of NFKB1 -94ins/del ATTG polymorphism in cases and controls

Genotype/allele	Case (%)	Control (%)	OR (95% CI)	*P* value
DD	89 (21.2%)	131 (30.8%)	1 (Reference)	
ID	219 (52.0%)	205 (48.2%)	1.57 (1.13-2.19)	0.007
II	113 (26.8%)	89 (20.9%)	1.87 (1.27-2.75)	0.001
II + ID	332 (78.8%)	243 (69.1%)	2.01 (1.47-2.76)	< 0.001
D	397 (47.1%)	467 (54.9%)	1 (Reference)	
I	445 (52.9%)	383 (45.1%)	1.37 (1.12-1.65)	0.001

DD, del/del; DI, ins/del; II, ins/ins.

moter regulation, the ATTG deletion (D) allele displays significantly reduced promoter activity and it is also involved in lower levels of p50 protein. Thus, this polymorphism seems to be relative to several diseases such as colorectal cancer, and rheumatoid arthritis [7, 8]. However, the role of NFKB1 -94ins/del ATTG polymorphism in NSCLC has not been determined. Thus, in this study, we aimed to analyze NFKB1 -94ins/del ATTG polymorphism for association with the risk of NSCLC in a Chinese Han population.

Methods

Study subjects

A hospital-based case-control study was conducted in 421 NSCLC patients and 425 cancer-free controls between 2011 and 2014 from China-Japan Union Hospital of Jilin University, China. All subjects were ethnically homogeneous Chinese Han population and from the city of Jilin and its surrounding region. All patients underwent a series of examinations of pathologic stages by board-certified pathologists. Tumor types and stages were determined according to the World Health Organization classification. The controls were randomly selected from healthy individuals who underwent routine physical examination in the same area during the same time period as the case study. Controls had no individual history of cancer. Information on individuals was gathered from both cases and controls. The study protocol was approved by the Institutional Review Boards of the hospital.

Genotyping and quality control

The blood samples were collected from each enrolled subjects. The genomic DNA was extracted from peripheral venous blood using the Axygen DNA isolation kit (Axygen, USA). DNA fragments containing the polymorphism were amplified with the forward primer 5'-TGG GCA CAA GTC GTT TAT GA-3' and 5'-CTG GAG CCG GTA GGG AAG-3'. The PCR reaction was carried out in a 20 ml reaction mixture containing 1 × Phusion High-Fidelity PCR Master Mix (Thermo Scientific, Finland) and 0.25 mM of each primer. The PCR cycle consisted of an initial denaturation step at 98°C for 30 s, followed by 35 cycles of denaturation (98°C for 5 s), annealing (65°C for 5 s) and extension (72°C for 5 s), and a final extension at 72°C for 5 min. The 281 bp (deletion allele) or 285 bp (insertion allele) products generated were then digested using PflMI (Van91I) restriction enzyme (Thermo Scientific, Finland). The wild type (deletion) genotype did not contain PflMI (Van91I) restriction site, hence the PCR product of 281 bp remained undigested. The insertion variants were cleaved by PflMI (Van91I)

Table 3. Association of *NFKB1* -94ins/del ATTG polymorphism with clinicopathological characteristics in NSCLC patients

Characteristics	Case (%)	II + ID (%)	DD (%)	OR (95% CI)	*P* value
Gender					
Male	216 (51.3%)	168 (50.6%)	48 (52.8%)	1 (Reference)	
Female	205 (48.7%)	164 (49.4%)	41 (47.2%)	1.09 (0.68-1.74)	0.71
Age (years)					
≤ 60	210 (49.9%)	159 (48.0%)	41 (47.2%)	1 (Reference)	
> 66	211 (50.1%)	173 (52.0%)	48 (52.8%)	0.97 (0.61-1.55)	0.90
Smoking status					
No	224 (53.2%)	165 (49.7%)	59 (66.3%)	1 (Reference)	
Yes	197 (46.8%)	167 (50.3%)	30 (33.7%)	1.99 (1.22-3.24)	0.005
Histology					
Squamous cell carcinoma	267 (63.4%)	216 (65.1%)	51 (57.3%)	1 (Reference)	
Adenocarcinoma	154 (36.6%)	116 (34.9%)	38 (42.7%)	0.72 (0.45-1.16)	0.18
TNM stage					
I + II	139 (33.0%)	97 (29.2%)	42 (47.2%)	1 (Reference)	
III + IV	282 (67.0%)	235 (70.8%)	47 (52.8%)	2.16 (1.34-3.49)	0.002

TNM, tumour-node-metastasis staging system.

restriction enzyme into two fragments of 240 bp and 45 bp. Heterozygotes showed all three bands. Ten percent of the samples were subjected to randomly repeat blind assays and all results were consistent.

Statistical analysis

All statistical analyses were performed by the Statistical Package for Social Sciences for Windows software (Windows version release 18.0; SPSS, Inc., Chicago, IL, USA). The frequencies of allele and genotype in cases and controls were calculated by gene counting method. Differences between cases and controls in demographic characteristics and frequencies of genotypes were evaluated by using chi-square (χ^2) test. Hardy-Weinberg equilibrium (HWE) was also tested by a chi-square (χ^2) test. Differences were considered significant when $P < 0.05$.

Results

The cohort of 421 NSCLC patients contained slightly more men (51.3%) than women (48.7%), whereas the 425 control individuals consisted of 48.7% men and 51.3% women. Between case and control, the age, gender, and smoking habits were well balanced (**Table 1**). The distribution of NFKB1 -94ins/del ATTG polymorphism frequencies was also in HWE ($P = 0.369$ and $P = 0.595$), indicating that the frequencies fell into the expected equilibrium and were thus randomly distributed. In NSCLC cases, adenocarcinoma represented 36.6%, and squamous cell carcinoma represented 63.4% (stage I + II 33.0% and stage III + IV 67.0%).

The genotype and allele frequencies of NFKB1 -94ins/del ATTG polymorphism were shown in **Table 2**. The frequencies of DD, ID and II genotypes in the patients were 21.2%, 52.0%, and 26.8% and were 30.8%, 48.2%, and 20.9% in the controls, respectively. Heterozygous (ID) genotype disclosed a statistically significantly increased risk of developing NSCLC (OR = 1.57, 95% CI 1.13-2.19, $P = 0.007$). Homozygous (II) genotype also showed an increased risk of NSCLC (OR = 1.87, 95% CI 1.27-2.75, $P = 0.001$). Statistically significant difference was observed when the patients and controls were compared according to ID + II versus DD (OR = 2.01, 95% CI 1.47-2.76, $P < 0.001$). The I allele was significantly higher in the NSCLC cases compared to the controls (52.9% versus 45.1%). The I allele was significantly associated with NSCLC risk (OR = 1.37, 95% CI 1.12-1.65, $P = 0.001$).

In order to determine the association between the polymorphism of NFKB1 -94ins/del ATTG polymorphism and certain clinicopathological features, we conducted stratified analyses for

combined genotypes with the DD genotype versus the ID + II genotypes in NSCLC patients according to gender, age at admission, smoking status, histology, and TNM stage. There was a significantly higher frequency of ID + II genotypes observed in smokers, compared to non-smokers (OR = 1.99, 95% CI 1.22-3.24, *P* = 0.005) and in patients with stage III + IV, compared to stage I + II (OR = 2.16, 95% CI 1.34-3.49, *P* = 0.002) (**Table 3**). There was no statistically significant associations of NFKB1-94ins/del ATTG polymorphism with gender, age, and histology (**Table 3**).

Discussion

In this case-control study, we analyzed NFKB1 -94ins/del ATTG polymorphism for NSCLC susceptibility in a Chinese Han population. Our results suggested that NFKB1 -94ins/del ATTG polymorphism was significantly associated with the risk of NSCLC, suggesting that NFKB1 -94ins/del ATTG polymorphism might be involved in pathogenesis of NSCLC in the Chinese Han population. We demonstrated that II, ID, and the combined I variant genotype (II + ID) within the NFKB1 were associated with an increased risk of NSCLC. Patients carrying those genotypes had a higher risk for NSCLC than those carrying the other genotypes. However, further studies are warranted to elucidate how these genotypes contribute to NSCLC. Furthermore, we also found that this polymorphism was significantly associated with advanced NSCLC risk and smokers with NSCLC.

As a subunit of the NF-κB complex, p50 is encoded by the NFKB1 gene [4]. The over-expression of p50 (NF-κB1) was observed in various malignancies [9, 10], including non-small cell lung carcinoma, colon cancer, prostate cancer, breast cancer, bone cancer, and brain cancer. Considering that p50 over-expression is frequently observed in various tumor tissues, p50 is potentially involved in tumorigenesis. The probable mechanism behind the observed association may be linked to the expression and activity of p50 (NF-κB1), which regulates important cellular events such as apoptosis and cell death independent of the NF-κB complex [11]. Using a reporter assay, a previous study found that the -94ins/del ATTG polymorphism has regulatory influence on NFKB1 gene expression and that the activity of the ins allele is twice as high as that of the del allele [6].

This present study had some limitations. First, this was a hospital-based case-control study, thus selection bias cannot be excluded and the participants may not be representative of the general population. Second, this present study only analyzed -94ins/del ATTG polymorphism in NFKB1. Finally, caution should be taken when interpreting these data since the population was exclusively from China, which reduces the possibility of confounding from ethnicity, but it does not permit extrapolation of the results to other ethnic groups. Thus, more studies are still required to validate the results of this study [12, 13].

In conclusion, the current study showed that NFKB1 -94ins/del ATTG polymorphism could impact the risk of NSCLC in a Chinese Han population.

Disclosure of conflict of interest

None.

Address correspondence to: Haixiang Yu, Department of Thoracic Surgery, China-Japan Union Hospital of Jilin University, No. 126 Xiantai Road, Changchun 130033, Jilin, P.R. China. Tel: 86-0431-84641032; E-mail: yuhaixiangjilin@126.com

References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[2] Pastorino U. Lung cancer screening. Br J Cancer 2010; 102: 1681-6.

[3] Sen R, Baltimore D. Inducibility of K immunoglobulin enhancer-binding protein NF-kB by a posttranslational mechanism. Cell 1986; 47: 921-8.

[4] Chen F, Castranova V, Shi X, Demers LM. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem 1999; 45: 7-17.

[5] Sun XF, Zhang H. NFKB and NFKBI polymorphisms in relation to susceptibility of tumour and other diseases. Histol Histopathol 2007; 22: 1387-98.

[6] Karban AS, Okazaki T, Panhuysen CI, Gallegos T, Potter JJ, Bailey-Wilson JE, Silverberg MS, Duerr RH, Cho JH, Gregersen PK, Wu Y, Achkar JP, Dassopoulos T, Mezey E, Bayless TM, Nouvet FJ, Brant SR. Functional annotation of

a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. Hum Mol Genet 2004; 13: 35-45.

[7] Song S, Chen D, Lu J, Liao J, Luo Y, Yang Z, Fu X, Fan X, Wei Y, Yang L, Wang L, Wang J. NFκB1 and NFκBIA polymorphisms are associated with increased risk for sporadic colorectal cancer in a southern Chinese population. PLoS One 2011; 6: e21726.

[8] López-Mejías R, García-Bermúdez M, González-Juanatey C, Castañeda S, Miranda-Filloy JA, Gómez-Vaquero C, Fernández-Gutiérrez B, Balsa A, Pascual-Salcedo D, Blanco R, González-Álvaro I, Llorca J, Martín J, González-Gay MA. NFKB1-94ATTG ins/del polymorphism (rs28-362491) is associated with cardiovascular disease in patients with rheumatoid arthritis. Atherosclerosis 2012; 224: 426-9.

[9] Mukhopadhyay T, Roth JA, Maxwell SA. Altered expression of the p50 subunit of the NF-kappa B transcription factor complex in non-small cell lung carcinoma. Oncogene 1995; 11: 999-1003.

[10] Bours V, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-kappa B transcription factor and cancer: high expression of NF-kappa B- and I kappa B-related proteins in tumor cell lines. Biochem Pharmacol 1994; 47: 145-9.

[11] Yu Y, Wan Y, Huang C. The biological functions of NF-kappaB1 (p50) and its potential as an anti-cancer target. Curr Cancer Drug Targets 2009; 9: 566-71.

[12] Maria TG, Vasileios KE, Panagiotis PS, Kostas SN. Changes of acute-phase protein levels in the serum of lung cancer patients following radiotherapy. Int J Clin Exp Med 2013; 6: 50-6.

[13] Nikolaos T, Maria T, Ioannis KD, Georgios L, Nikolaos P, Stamatina D, Christos P, Georgios K, Vasileios B, Anna E. Dermatomyositis as an early manifestation and a significant clinical precursor of lung cancer: report of a rare case and review of the current literature. Int J Clin Exp Med 2013; 6: 105-9.