

RESEARCH ARTICLE

RTN4 3'-UTR Insertion/Deletion Polymorphism and Susceptibility to Non-Small Cell Lung Cancer in Chinese Han Population

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Abstract

Nogo protein, encoded by gene reticulon-4 (RTN4), includes three major isoforms by different splicing, named Nogo-A, Nogo-B and Nogo-C. Nogo proteins play an important role in the apoptosis of cells, especially in tumor cells. RTN4 single nucleotide polymorphisms (SNPs) can influence the efficiency of transcription and translation thus being related with an individual's predisposition to cancer. The CAA insertion/deletion polymorphism (rs34917480) within RTN4 3'-UTR has been reported to be associated with many cancer types. In order to investigate the relationship between this polymorphism and susceptibility to non-small cell lung cancer (NSCLC) in the Chinese population, we conducted the present case-control study including 411 NSCLC patients and 471 unrelated healthy controls. The genotype distributions were significantly different between cases and controls ($p=0.014$). We found that the del allele could significantly increase NSCLC risk (ins/ins vs ins/del: $p=0.007$, OR 1.46, 95% CI=1.11-1.93; dominant model: $p=0.004$, OR 1.47, 95% CI=1.13-1.92 and allele model: $p=0.008$, OR 1.35, 95% CI=1.08-1.67). This association was stronger in participants over 60 years old, males and smokers. We therefore conclude that the CAA insertion/deletion polymorphism (rs34917480) contributes to non-small cell lung cancer risk in Chinese population. Age, sex and environmental exposure are also related to carcinogenic effects of rs34917480

Keywords: RTN4 - polymorphism - rs34917480 - non-small cell lung cancer - susceptibility.

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Introduction

Lung cancer is one of the most common cancers with an increasing incidence in the human population. More than one million patients died of lung cancer around the world every year (Hecht, 1999). Lung cancer ranks first in male cancer mortality rate and ranks second in females just following breast cancer. Lung cancer is also known as bronchus lung cancer, because nearly all the lung cancer originated in the bronchial epithelium. Lung cancer includes two subtypes, non-small cell lung cancer (NSCLC) and small lung cancer (SCLC). 80-85% of lung cancer cases are NSCLC (adeno-, squamous cell- and large-cell carcinoma) (Liam et al., 2014). Surgical resection should be the first choice to the treatment of NSCLC. The 5-year survival rate of NSCLC after surgery can be up to 50% before occurring lymph node

metastasis. However, most patients were diagnosed with advanced NSCLC in their first surgical treatment losing the opportunity for radical resection. Therefore, early diagnosis and early treatment still play a pivotal role in the treatment of lung cancer. Genetic variations are thought to lead to different susceptibilities to NSCLC for individuals (Piao et al., 2013). So, finding available molecular genetic markers are important for early diagnosis and treatment.

RTN4 (reticulon-4) gene, which mapped to chromosome 2p12-14 (Yang et al., 2000), plays an important role in the inhibition of axonal regeneration, vascular remodeling, apoptosis and inhibition of tumor. RTN4 gene produced three Nogo isoforms A, B and C through differential splicing and varied promoter usage (Oertle et al., 2003). Nogo-A is mainly expressed in the central nervous system; Nogo-B is expressed in various tissues, such as endothelial cells and smooth muscle cells

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(Acevedo et al., 2004); Nogo-C is highly expressed in the central nervous system, as well as in skeletal muscle (GrandPre et al., 2000). Recently, many researchers have paid much attention to the peripheral role of Nogo protein in the nervous tissue, but less in other tissues. Some evidences have shown that Nogo proteins play an important role in apoptosis (Tagami et al., 2000; Li et al., 2001; Chen et al., 2006; Kuang et al., 2006; Tashiro et al., 2013).

Nowadays, more and more researchers have started to focus on the polymorphisms of RTN4 3'-UTR. 3'-UTR of eukaryotic mRNA has been proved to regulate the expression of gene, and also involve in the regulation of translation initiation, mRNA stability and subcellular localization (Gray et al., 1998; Jansen, 2001; Mitchell et al., 2001). rs34917480 is located on RTN4 3'-UTR and the CAA insertion/deletion polymorphism is associated with the occurrence of schizophrenia (Novak et al., 2002; Novak et al., 2006). Thus, the polymorphism may affect the expression RTN4. However, the association between the polymorphism and the susceptibility to NSCLC in Chinese Han population has remained unknown. Therefore, by conducting this case-control study, we want to investigate the association between rs34917480 and NSCLC risk in Chinese population.

Materials and Methods

Study populations

411 unrelated non-small cell lung cancer patients (292 males and 119 females) were recruited from Soochow municipal hospital between July, 2011 and September, 2012. Control subjects were 471 unrelated healthy individuals (338 males and 133 females) from a routine health survey at Soochow municipal hospital during the same period. Control subjects were matched to cases for sex and age at the ratio of 1:1.15. This project has been approved by Soochow University Ethics Committee. All participants have signed a written informed consent for donating their blood samples.

Determination of genotypes

All blood samples were collected and stored in EDTA-anticoagulant tubes. A Chelex method was used to extract genomic DNA of blood samples (Walsh et al., 1991). The primers of PCR were according to Shaoqing Shi's method (Shi et al., 2012). PCR was performed in a total volume of 20 μ L, including 2 μ L 10 \times PCR buffer, 1.5 mM MgCl₂, 0.15 mM dNTPs, 0.5 mM of each primer, 50 ng of genomic DNA, and 1.0 U of Taq DNA polymerase. The PCR conditions were 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 61°C, and 30 s at 72°C, with a final elongation at 72°C for 10 min. The PCR products were analyzed by 6% polyarylamide gel electrophoresis and visualized by silver nitrate staining. For CAA polymorphism, the CAA deletion yields a 124-bp band, and the CAA insertion yields a 127-bp band.

About 10% of the samples were randomly selected to perform the repeated assay and the reproducibility was 100%.

Statistical analysis

The Hardy-Weinberg equilibrium in control subjects was tested using goodness-of-fit χ^2 test. Differences in frequency distributions of the genotypes, alleles and the selected demographic variables between cases and controls were evaluated by χ^2 test. The mean ages were compared using t-test. Logistic regression analyses were conducted to calculate odds ratio (OR) and 95% confidence interval (95%CI) to evaluate the risk of NSCLC. Multivariate adjustments were made for age, sex and smoking status. We further performed the stratification analyses according to age (≤ 60 , > 60), sex (male, female) and smoking status (non-smoker, smoker). The genetic models were used as follows: codominant model (AA vs AB & AA vs BB); dominant model (AA vs AB BB); recessive model (AA AB vs BB) and overdominant model (AA BB vs AB), assuming B is the risk allele. $p < 0.05$ was regarded as statistical significance. All data analyses were carried out by using SPSS 18.0 statistical software.

Results

Subject characteristics

The distributions of characteristics in cases and controls were summarized in Table 1. The final analysis included 411 NSCLC cases and 471 healthy controls. By the frequency-matched study design, there were no statistical differences in the distributions of age ($p = 0.105$), sex ($p = 0.823$) and smoking status ($p = 0.418$) between cases and controls.

Genotype distributions and NSCLC risk

The genotype frequencies of rs34917480 in this analysis were summarized in Table 2. The observed genotype frequencies in controls were agreed with Hardy-Weinberg equilibrium ($p = 0.413$). The genotype frequencies were significantly different between the cases and controls ($p = 0.014$). The del allele was more frequent among cases than among controls, and the difference was statistically significant ($p = 0.008$).

Multivariate logistic regression analysis was conducted after adjustment by age, sex and smoking in genetic models, the results showing in Table 3. In the dominant model, the del allele of rs34917480 was associated with a significantly increased risk of NSCLC compared with ins/ins genotype (OR=1.47, 95%CI=1.13-1.92, $p = 0.004$). The ins/del genotype significantly increased lung cancer susceptibility in the codominant model (OR=1.46, 95%CI=1.11-1.93, $p = 0.007$) and in the overdominant model (OR=1.41, 95%CI=1.07-1.85, $p = 0.014$).

Stratification analysis of NSCLC risk

We further calculated the association between rs34917480 and NSCLC risk stratified by variables including age, sex and smoking status. The results are shown in Table 4. The potential association of rs34917480 del allele with the risk of NSCLC is more evident in older subjects and smokers. For males, the significant results were observed in the codominant model (OR=1.43, 95%CI=1.03-1.98, $p = 0.032$) and the dominant model (OR=1.47, 95%CI=1.07-2.01, $p = 0.018$).

Discussion

The occurrence of lung cancer is the comprehensive result of genetic-environment interactions, and increasing studies have certified that genetic variants of important genes play major roles in the susceptibility to lung cancer. In this study, we investigated the association between genetic variant within RTN4 (rs34917480) and NSCLC risk, which was the first time conducted on NSCLC, as far as we know. The result showed that rs34917480 increased NSCLC susceptibility in Chinese population.

RTN4 gene, containing eight introns and nine exons and locating on chromosome 2p12-14, can encode three proteins by different splicing, named Nogo-A, Nogo-B and Nogo-C (Oertle et al., 2003). In recent years, more and more attentions have been drawn to the functions of RTN4 gene. One study has reported that the absence of Nogo-B enhances apoptosis of hepatic stellate cells and the overexpression of Nogo-B inhibits apoptosis (Tashiro et al., 2013). But there is an inconsistent result that Nogo-B interacted with Bcl-XL and Bcl-2, thus promoting Bcl-XL and Bcl-2 to locate on endoplasmic reticulum (ER), and decreasing the anti-apoptosis activity of them (Tagami et al., 2000). Some researchers believed that the overexpression of Nogo-B induces cell apoptosis through ER stress and ER-specific signal pathways (Kuang et al., 2006). Strikingly, Nogo-B was claimed to be potent pro-apoptosis protein in certain tumor cells

when it ectopically overexpressed. Transient transfecting Nogo-B into carcinoma cell lines (CGL4, SaOS-2) can induce the cell apoptosis (Li et al., 2001). Nogo-B induces vascular smooth muscle cells apoptosis by activation of the JNK/p38 MAPK signaling pathway (Zheng et al., 2011). The overexpression of Nogo-B protein can induce apoptosis in cancer cells, but not in normal cell lines (Watari et al., 2003). Nogo-C expressed in HEK 293 cell confers apoptosis by inducing caspase-3 and p53 activation through the JNK-c-Jun-dependent pathway (Chen et al., 2006). Moreover, by transferring mutant p53 protein from nucleus to cytoplasm and decreasing the expression of c-Fos, Hsp70 protein, Nogo-c inhibited SMMC7721 cell growth and promoted its apoptosis. Nogo-C is expressed differently in hepatocellular carcinoma and its paracancerous tissues (Chen et al., 2005). Some researchers have shown that knockdown of Nogo-A in cardiomyocytes markedly attenuated hypoxia/reoxygenation-induced apoptosis (Sarkey et al., 2011). Increasing evidences show that Nogo proteins play an important role in the apoptosis of cells, especially in tumor cells.

The 3'-UTR of eukaryotic mRNAs takes part in the translation initiation, mRNA stability and localization. The CAA insertion/deletion polymorphism (rs34917480) locates at 4548-4554 of RTN4 (AY102279) 3'-UTR and we found the absence of CAA allele will increase NSCLC risk. This result suggested that RTN4 3'-UTR was associated with NSCLC risk and rs34917480 altered the function of 3'-UTR. Recently, two studies have shown that this polymorphism of RTN4 3'-UTR was significantly associated with increased cervical squamous cell carcinoma risk (Shi et al., 2012) and uterine leiomyomas (UL) risk (Zhang et al., 2013). The polymorphism with

Table 1. Characteristics of the Cases and Controls

| Variables | Case No. (%) | Control No. (%) | <i>P</i> ^a |
|---------------|--------------|-----------------|-----------------------|
| All subjects | 411 (100) | 471 (100) | |
| Age (Mean±SD) | 59.61±11.35 | 60.41±12.87 | 0.105 |
| ≤60 | 206 (50.1) | 210 (44.6) | |
| >60 | 205 (49.9) | 261 (55.4) | |
| Sex | | | 0.823 |
| Male | 292 (71.0) | 338 (71.8) | |
| Female | 119 (29.0) | 133 (28.2) | |
| Smoking | | | 0.418 |
| Yes | 218 (53.0) | 236 (50.1) | |
| No | 193 (47.0) | 235 (49.9) | |

*SD: Standard error; a Two-sided χ^2 test for distributions between cases and controls

Table 2. The Genotype and Allele Distributions Among Cases and Controls

| Genotypes/Alleles | Cases No. (%) | Controls No. (%) | <i>P</i> ^a |
|-------------------|---------------|------------------|-----------------------|
| ins/ins | 205 (49.9) | 281 (59.7) | 0.014 |
| ins/del | 185 (45.0) | 172 (36.5) | |
| del/del | 21 (5.1) | 18 (3.8) | |
| ins | 595 (72.4) | 734 (77.9) | 0.008 |
| del | 227 (27.6) | 208 (22.1) | |

** χ^2 test for genotype and allele distributions between cases and controls

Table 3. Logistic Regression Analysis of the Association between rs34917480 and Lung Cancer Risk

| Genetic model | Case No. (%) | Control No. (%) | OR* (95%CI) | <i>P</i> [*] |
|-----------------|--------------|-----------------|------------------|-----------------------|
| Genotypes | | | | |
| Codominant | | | | |
| ins/ins | 205 (49.9) | 281 (59.7) | 1.00 (reference) | |
| ins/del | 185 (45.0) | 172 (36.5) | 1.46 (1.11-1.93) | 0.007 |
| del/del | 21 (5.1) | 18 (3.8) | 1.26 (0.91-1.75) | 0.166 |
| Dominant | | | | |
| ins/ins | 205 (49.9) | 281 (59.7) | 1.00 (reference) | |
| ins/del+del/del | 206 (50.1) | 190 (40.3) | 1.47 (1.13-1.92) | 0.004 |
| Recessive | | | | |
| ins/ins+ins/del | 390 (94.9) | 453 (96.2) | 1.00 (reference) | |
| del/del | 21 (5.1) | 18 (3.8) | 1.18 (0.85-1.63) | 0.326 |
| Overdominant | | | | |
| ins/ins+del/del | 226 (55.0) | 299 (63.5) | 1.00 (reference) | |
| ins/del | 185 (45.0) | 172 (36.5) | 1.41 (1.07-1.85) | 0.014 |

*Adjusted by sex, age and smoking

Table 4. Stratification Analysis for Associations Between rs34917480 and Lung Cancer Risk in Genetic Models

| Character | ins/ins vs ins/del | | OR* (95%CI) | <i>P</i> [*] | Dominant | | OR* (95%CI) | <i>P</i> [*] | Overdominant | | OR* (95%CI) | <i>P</i> [*] | |
|-----------|--------------------|---------|-------------|-----------------------|----------|---------|-------------|-----------------------|--------------|---------|-------------|-----------------------|-------|
| | Case | Control | | | Case | Control | | | Case | Control | | | |
| Age | ≤60 | 105/92 | 116/85 | 1.23 (0.83, 1.84) | 0.306 | 105/101 | 116/94 | 1.21 (0.82, 1.78) | 0.338 | 114/92 | 125/85 | 1.21 (0.82, 1.79) | 0.342 |
| | >60 | 100/93 | 165/87 | 1.79 (1.22, 2.64) | 0.003 | 100/105 | 165/96 | 1.85 (1.27, 2.70) | 0.001 | 112/93 | 174/87 | 1.67 (1.15, 2.45) | 0.008 |
| Sex | Male | 143/132 | 198/127 | 1.43 (1.03, 1.98) | 0.032 | 143/149 | 198/140 | 1.47 (1.07, 2.01) | 0.018 | 160/132 | 211/127 | 1.36 (0.99, 1.87) | 0.059 |
| | Female | 62/53 | 83/45 | 1.35 (0.79, 2.30) | 0.277 | 62/57 | 83/50 | 1.28 (0.76, 2.16) | 0.360 | 66/53 | 88/45 | 1.38 (0.81, 2.34) | 0.238 |
| Smoking | Yes | 99/106 | 143/88 | 1.65 (1.12, 2.43) | 0.012 | 99/119 | 143/93 | 1.74 (1.19, 2.55) | 0.004 | 112/106 | 148/88 | 1.52 (1.04, 2.23) | 0.031 |
| | No | 106/79 | 138/84 | 1.22 (0.81, 1.82) | 0.337 | 106/87 | 138/97 | 1.15 (0.78, 1.70) | 0.470 | 114/79 | 151/84 | 1.24 (0.84, 1.85) | 0.280 |

*Adjusted by age, sex and smoking

RNT4 3'-UTR could be molecular marker for detecting malignancy.

In the subgroup analysis, we also found the association between rs34917480 and NSCLC risk was more apparent among elders, males and smokers. These results indicated that these factors can impact the effect of this SNP site. As the functional role of rs34917480 remained unknown, this investigation provided experimental basis for further research.

There were some limitations for this study. The relatively small sample size may cause instability to the result. And the information of environmental exposure was not detailed, such as the explicit cigarette smoking history and drinking consumption.

In summary, we have provided the initial evidence that the CAA polymorphism in RTN4 3'-UTR associated with non-small cell lung cancer in Chinese Han population. However, further study will be required to investigate the mechanism of this polymorphism in the development of NSCLC.

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