Short Communication

FoxP3 genetic variants and risk of non-small cell lung cancer in the Chinese Han population

Yan-Qi He, Qiao Bo, Wei Yong, Zhi-Xin Qiu, Ya-Lun Li, Wei-Min Li

Abstract

CD4+CD25+ regulatory T cell-mediated immunosuppression is one of the crucial mechanisms that tumor cells use to evade the immune system. The forkhead box P3 (FoxP3) gene regulates regulatory T-cell development and function and may modulate the susceptibility to non-small cell lung cancer (NSCLC). Because a single nucleotide polymorphism (SNP) within the FoxP3 gene (rs3761548 in the promoter region) is associated with susceptibility to Graves’ disease, this study detected rs3761548 in a hospital-based case–control study. A total of 192 NSCLC patients and 259 healthy subjects were recruited for the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis of FoxP3 SNP. The data showed that the A allele of rs3761548 significantly increased NSCLC risk (P = 0.000, OR = 2.32, 95%CI = 1.736–3.102). The AC genotype, AA genotype, and the combined A variant genotype (AA + AC) were also associated with a higher risk of NSCLC (OR [95%CI] = 2.147 [1.419–3.247], 4.413 [2.359–8.255], and 2.563 [1.746–3.761], respectively). Moreover, a significantly higher frequency of AA + AC genotype was observed in patients with stage II NSCLC (OR, 2.053; 95%CI, 1.033–4.078). In conclusion, the data from the current study demonstrated for the first time the association of the FoxP3 SNP with a risk of developing NSCLC in the Chinese Han population.

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death in the world. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer cases, and includes two predominant subtypes, adenocarcinoma and squamous cell carcinoma (SCC) (Dutt et al., 2011; Minna et al., 2002; Wistuba, 2007). The prognosis of NSCLC remains poor, with a five-year overall survival rate of about 15% (Ferlay et al., 2010). However, there are significant prognostic differences between early and late stages of NSCLC (Naruke et al., 2001), indicating that early detection of NSCLC is key survival of NSCLC patients. A major risk factor associated with NSCLC is tobacco smoking, followed by asbestos and other mineral exposures, air pollution, and personal and family histories of lung cancer. Nevertheless, it is critical to understand the contribution of genetic and environmental interactions in the development of NSCLC (Kriek et al., 1993; Li et al., 2004; Spitz et al., 2007; Vineis and Husgafvel-Pursiainen, 2005). For example, it has been shown that when exposed to similar environmental and occupational elements, only some individuals develop NSCLC, whereas other NSCLC patients have not been exposed to any risk factors. This discrepancy suggests that there is individual variation in cancer susceptibility in the general population and there are other unknown factors (such as genetics or host predisposition or immune defense system) that contribute to NSCLC pathogenesis (Amos et al., 1992). A previous meta-analysis using 41 published studies showed that the lung cancer family history was a risk factor of lung cancer (Lissowska et al., 2010).

The host immune defense has been shown to play a role in modulating human carcinogenesis. In the host immune defense system, regulatory T cells (Tregs) play a key role in sustaining self-tolerance and immune homeostasis via suppressing a wide variety of physiological and pathological immune responses against self and nonself (Nishikawa and Sakaguchi, 2010; Sakaguchi, 2004; Shevach, 2002; Takahashi and Sakaguchi, 2003). For example, the deficiency or dysfunction of CD4+CD25+ Treg cells, which are the most physiologically relevant Treg population, causes a breach in self-tolerance and produces autoimmune diseases in normal animals and in humans (such as severe allergy and inflammatory bowel disease) (Fontenot et al., 2003; Hori et al., 2003). Recently published data demonstrated that CD4+CD25+ Tregs dominantly infiltrate into tumors and hinder immune response against tumor cells (Nishikawa and Sakaguchi, 2010). This data suggests that Treg cell-mediated immunosuppression is one of the crucial tumor immune evasion mechanisms, and may contribute to the failure of tumor immunotherapy (Khattri et al., 2003; Nishikawa and Sakaguchi, 2010).
The forkhead box P3 (FoxP3) gene is primarily expressed in regulatory T cells under normal physiological conditions. The FoxP3 gene is located on the X chromosome at Xp11.23 (Bennett et al., 2001; Wang et al., 2009) to encode FoxP3 protein, which regulates T cell activation and functions as a transcriptional repressor to downregulate cytokine production in T cells (Gao et al., 2010; Horis et al., 2003; McHugh et al., 2002; Schubert et al., 2001; Takahashi et al., 2000). Normally, FoxP3 is expressed in epithelial cells from various organs (e.g., thymus and lung) (Gupta et al., 2007; Karonkas et al., 2008; Katoh et al., 2010). However, during tumorigenesis, FoxP3 is also expressed in a variety of cancers, such as lymphoma, lung, ovary, and prostate cancers (Kono et al., 2006; Wei et al., 2004). Mutations of FoxP3 cause immune dysregulation, polyendocrinopathy, and X-linked syndrome (IPEX) in human body or breast and prostate cancers (Spitz et al., 2004). In addition, FoxP3 gene polymorphisms result in the lack of functional CD4+CD25+ Tregs, and therefore induce some autoimmune diseases, including type 1 diabetes and inflammatory primary biliary cirrhosis (Bassuny et al., 2003; Park et al., 2005; Wildin et al., 2002). A single nucleotide polymorphism (SNP) of the FoxP3 gene (rs3761548) is associated with susceptibility of development and intractability of Graves’ disease (Owen et al., 2006).

Thus, in this study, we analyzed FoxP3 gene SNP (rs3761548) for association with the risk of NSCLC in the Chinese Han population using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique.

2. Materials and methods

2.1. Study subjects

In this study, we carried out a hospital-based case–control study by recruiting 192 NSCLC patients (141 males and 51 females, mean age of 57.42 ± 10.26 years) from The West China Hospital of Sichuan University between April 2008 and December 2011. The diagnosis of NSCLC was confirmed by histopathological examination of the resected or biopsy tissue specimens in all cases. The control population consisted of 259 healthy subjects (181 males and 78 females, mean age: 58.90 ± 12.91 years) from a routine healthy examination in the same hospital. All subjects were from the Chinese Han population living in Sichuan province of southwest China. Those with secondary lung tumors or other serious diseases were intentionally excluded. The demographic and clinical information were collected, including age at admission, tobacco smoking, histological diagnosis, tumor size, and tumor–nude metastasis clinical stage according to the American joint committee on cancer 2010 guidelines (de Mello et al., 2012; Sculier et al., 2008). The study was approved by our hospital ethics committee and all the subjects provided informed consent.

2.2. PCR and restriction enzyme digestion

Genomic DNA was extracted from 200 μL of EDTA-anticoagulated peripheral blood samples using a commercial DNA isolation kit from Biotek (Beijing, China) according to the manufacturer's instructions. Primers were designed by using Primer 3 online software (MIT, US). The polymerase chain reaction (PCR)-polyacrylamide gel electrophoresis (PAGE) method was used to assess FoxP3 polymorphism. Specifically, genomic DNA from the case and control was amplified by PCR with specific primer sets. The primers used for amplification of the rs3761548 were 5′-GGCGAGGTGAGAAGGCAGAA-3′ and 5′-CAAGTGTCGAAGGCGAAA-3′. PCR reaction was performed in a total volume of 25 μL, including 2.5 μL of PCR buffer, 1.5 mmol/L of MgCl2, 0.15 mmol/L of dNTPs, 0.5 μmol/L of each primer, 100 ng of genomic DNA and 1 U of Taq DNA polymerase. The PCR conditions were 94 °C for 4 min, followed by 32 cycles of 30 s at 94 °C, 30 s at 63.2 °C and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. PCR products were then digested overnight with a specific restriction enzyme and the digested PCR products were separated in a 6% polyacrylamide gel and stained with 1.5 mg/ml argent nitrate. The restriction enzyme used for A/C was PstII; allele A was uncuttable and the fragment was 155 bp; and allele C was cuttable, yielding two fragments of 75 and 80 bp. Approximately 10% of the samples were randomly selected for repeating the assay, and the results were 100% concordant.

2.3. Statistical analysis

The genotype frequencies were obtained by direct counting and Hardy–Weinberg equilibrium was tested by a chi-square test. The comparison of genotype and allele frequency between NSCLC and control groups were analyzed by the Pearson chi-square test; Odds ratio (OR) and respective 95% confidence intervals (CI) were reported to evaluate the effects of any difference between alleles and genotypes. Differences were considered significant when P < 0.05. The statistical analysis was performed by using SPSS medical statistical software version 16.0 (SPSS Inc., Chicago, IL).

3. Results

The cohort of 192 NSCLC patients with a mean age of 57.4 year old contained slightly more men (73.4%) than women (26.6%), whereas the 259 control individuals with a mean age of 58.9 year old consisted of 69.5% men and 30.1% women. Between case and control, the age and gender were well balanced and the distribution of rs3761548 A/C allele frequencies was also in Hardy–Weinberg equilibrium (P = 0.066 and P = 0.073), indicating that the frequencies fell into the expected equilibrium and were thus randomly distributed. In NSCLC cases, adenocarcinoma represented 54.69%, and squamous cell carcinoma 45.31% (stage III 33.3%, stage II 43.75%, and stage III + IV 22.9%).

The genotype and allele frequencies of FoxP3 SNP in 192 NSCLC patients and 259 control subjects were summarized in Table 1. Briefly, the A allele of rs3761548 is significantly higher in the NSCLC cases compared to the controls (40.1% versus 22.4%, P = 0.000). The frequency of combined A variant genotype (AA + AC) was significantly higher in the NSCLC cases compared to the controls (60.9% versus 37.8%, P = 0.000), which was consistent with the A allele distribution. When we used the CC genotype as a reference, we found that both AC and AA genotypes were associated with a higher risk of developing NSCLC (OR, 2.147; 95%CI, 1.419–2.347 and OR, 4.413; 95%CI, 2.359–8.255). It is also shown that the combined A variant genotype (AA + AC) was associated with a higher risk in developing NSCLC (OR, 2.563; 95%CI, 1.746–3.761).

In order to determine the association between the polymorphism of FoxP3/rs3761548 and certain clinicopathological features, we conducted stratified analyses for combined genotypes with the CC genotype versus the AA + AC genotype in NSCLC patients according to age at admission, smoking status, histological types, tumor size, clinical stage, tumor differentiation, and lymph node metastasis (Table 2). The frequency of the combined genotype at the rs3761548 locus was significantly distinct from the clinical stages of NSCLC patients, such that there was a

<table>
<thead>
<tr>
<th>SNP genotype/allele</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>OR (95% CI)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3761548</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>75 (39.1%)</td>
<td>161 (62.2%)</td>
<td>1.00 (reference)</td>
<td>0.000</td>
</tr>
<tr>
<td>AC</td>
<td>80 (42.7%)</td>
<td>80 (30.9%)</td>
<td>2.147 (1.419–2.347)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>37 (19.3%)</td>
<td>18 (6.9%)</td>
<td>4.413 (2.359–8.255)</td>
<td></td>
</tr>
<tr>
<td>AA + AC</td>
<td>117 (60.9%)</td>
<td>98 (37.8%)</td>
<td>2.563 (1.746–3.761)</td>
<td>0.000</td>
</tr>
<tr>
<td>C</td>
<td>230 (59.9%)</td>
<td>402 (77.6%)</td>
<td>1.00 (reference)</td>
<td>0.000</td>
</tr>
<tr>
<td>A</td>
<td>154 (40.1%)</td>
<td>116 (22.4%)</td>
<td>2.320 (1.736–3.102)</td>
<td></td>
</tr>
</tbody>
</table>

a The Pearson chi-squared test for either genotype distributions or allele frequencies between the cases and controls.

b OR odds ratio, CI confidence intervals.
significantly higher frequency of AA + AC genotype observed in patients with stage II, compared to stage I (OR, 2.053; 95% CI, 1.033–4.078). However, there was no statistically significant association of rs3761548 polymorphism with age, smoking status, histological types, tumor size, tumor differentiation or lymph node metastasis (as shown in Table 2, all P > 0.05).

4. Discussion

To the best of our knowledge, the current study is the first to assess the association of genetic variants of FoxP3 gene with the risk of NSCLC. In this hospital-based, case–control study, we analyzed FoxP3 gene SNP for NSCLC susceptibility in a Chinese Han population. Our data demonstrated that FoxP3 SNP/rs3761548 was signifi-
cantly associated with risk of NSCLC, suggesting that FoxP3 polymorphism might be involved in pathogenesis of NSCLC in the Chinese Han population. We demonstrated that AA, AC, and the combined A variant genotype (AA + AC) within the FoxP3 gene 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.

Indeed, it has been shown that alteration of the human immune system contributes to the development of human cancer, and that there is functional immune surveillance against tumorigenesis. Previous studies have shown that cancer patients harbor T cells reactivity with their own cancer cells (Dunn et al., 2004; Van Der Bruggen et al., 2002; Yamaguchi and Sakaguchi, 2006). In contrast, CD4+CD25+ FoxP3+ regulatory T (Treg) cells expressing high levels of Foxp3 play an important role in the tumor escape mechanism (Hinz et al., 2007). Karagöz et al. showed that Treg cells and the percentages of these cells among total lymphocytes were higher in the advanced stage lung cancer patients compared with the control healthy volunteers (Karagöz et al., 2010). Petersen et al. found that patients with stage I NSCLC have a higher proportion of tumor Treg cells relative to T-cell lymphocytes, and have a significantly higher risk of tumor recurrence (Petersen et al., 2006). These correlations support the hypothesis that Foxp3 plays an important role in the development of NSCLC. Therefore, an investigation of the association between Foxp3 SNPs and NSCLC may further increase our understanding of NSCLC pathogenesis, and may help to develop new diagnostic and gene therapeu-

Moreover, many T-cell-mediated autoimmune diseases share predisposing genes, of which the Fox3 gene has received much attention (Bassuny et al., 2003; Park et al., 2005). For example, in the investigation of association of Fox3 polymorphisms with allergic rhinitis, genotypes of allergic rhinitis disease patients revealed increased heterogeneity (Zhang et al., 2009). It has also reported that genotypes of Graves’ disease and Crohn’s disease patients revealed increased homo-

There are some limitations to the current study that merit attention. For example, the current study had a relatively small population size, which may limit the statistical power of our analyses. Larger population-based studies are expected to confirm our current data. Moreover, our study only involved Chinese Han patients. Research on other ethnic population will be needed to determine the role of poly-
morphisms in NSCLC.

5. Conclusions

In conclusion, the current study showed that Foxp3 AA and AC genotypes had an effect on the risk of NSCLC in a Chinese Han population. Additionally, the combined AA + AC genotype was significantly associated with stage II patients. The precise mechanism by which Foxp3 gene polymorphism influences the pathogenesis of NSCLC remains to be determined. Future studies are warranted to investigate

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Association of FoxP3 polymorphism/rs3761548 with clinicopathological characteristics from NSCLC patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td># of cases (%)</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>111 (57.8%)</td>
</tr>
<tr>
<td>Never</td>
<td>81 (42.2%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≥ 60</td>
<td>108 (56.3%)</td>
</tr>
<tr>
<td>&lt; 60</td>
<td>84 (43.7%)</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>105 (54.7%)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>87 (45.3%)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>≤ 5 cm</td>
<td>130 (67.7%)</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>62 (32.3%)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>64 (33.1%)</td>
</tr>
<tr>
<td>II</td>
<td>84 (43.8%)</td>
</tr>
<tr>
<td>III + IV</td>
<td>44 (22.9%)</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>120 (67.2%)</td>
</tr>
<tr>
<td>Moderate or well</td>
<td>63 (32.8%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>93 (48.4%)</td>
</tr>
<tr>
<td>Positive</td>
<td>99 (51.6%)</td>
</tr>
</tbody>
</table>

\(^a\) The Pearson chi-squared test for either genotype distributions or allele frequencies between the cases and controls.

\(^b\) OR odds ratio, CI confidence intervals.
whether the SNP rs3761548 affects expression levels of Foxp3 protein and determine the role of Foxp3 protein in the regulation of Treg cell activity. These studies will be important to further our understanding of the genetic basis for NSCLC development.

Conflict of interest

The authors declare no conflict of interest.

References


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