Original Article

The programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with non-small cell lung cancer risk in a Chinese Han population

Liu Yin1, Huishu Guo2, Lei Zhao3, Jinguang Wang3

1Department of Oncology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, Liaoning, China; 2Central Laboratory, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, Liaoning, China; 3Department of Thoracic Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, Liaoning, China. *Joint first authors.

Received August 26, 2014; Accepted October 21, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: It has been proposed that genetic factors contribute to the susceptibility of non-small cell lung cancer (NSCLC). The programmed death-1 (PD1) is an immunoinhibitory receptor belonging to the CD28/B7 family. The aim of this study is to investigate the relationship between PD-1.5 C/T and NSCLC risk in a Chinese population. A population-based case-control study was conducted in 324 NSCLC patients and 330 cancer-free controls. The genotype of the PD-1.5 C/T was determined by using a polymerase chain reaction assay. Statistically significant difference was observed when the patients and controls were compared according to CC+CT versus TT (OR=2.34, 95% CI 1.35-4.06, P=0.003). The C allele was significantly associated with NSCLC risk (OR=1.421, 95% CI 1.10-1.82, P=0.006). Compared to TNM stage I+II, PD-1.5 C/T significantly increased advanced NSCLC risk (OR=2.66, 95% CI 1.07-6.63, P=0.03). The results from this study suggested that PD-1.5 C/T was potentially related to NSCLC susceptibility in Chinese Han population.

Keywords: Non-small cell lung cancer, programmed death-1, polymorphism, genetics

Introduction

Lung cancer is one of the most common human cancers and the leading cause of cancer-related deaths with non-small cell lung cancer (NSCLC) accounting for 80% of all primary lung cancers. Despite early detection screening protocols, improved surgical techniques, and advanced radio and chemotherapeutic regimens, little progress has been made in altering the natural progression of the disease, the 5-year survival rate of patient with NSCLC is only 15% [1]. Current knowledge regarding NSCLC is not a single disease but a collection of diseases with distinct pathogeneses by molecular mechanisms. Genetic play an integral role in the transformation, promotion and progression of cancer [2].

Programmed death-1 (PD1) is an immunoinhibitory receptor belonging to the CD28/B7 family. PD1 expressed by activated T cells, B cells and myeloid cells, downregulates B- and T-cell responses [3]. PD-L/PD1 interaction between PD1 and its ligand (PD-L) can activate the specific cytoplasmic tail such as immune receptor tyrosine based inhibitory motif (ITIM) that begins intracellular signal transduction pathways which mediate exhausted T cell and reduce activation and proliferation of T cell [4]. Zhang et al. found that positive PD-L1 expression was significantly associated with more advanced T status, N status, and pathologic stage in NSCLC [5]. Thus, PD1 might play an important role in the NSCLC. However, little is known about the relationship between genetic polymorphism in PD-1 and susceptibility to NSCLC. In this study, we aimed to analyze PD-1.5 C/T for association with the risk of NSCLC in a Chinese Han population.

Methods

Study subjects

A population-based case-control study was conducted in 324 NSCLC patients and 330 cancer-free controls between 2010 and 2013 from...
The First Affiliated Hospital of Dalian Medical University, China. All subjects were ethnically homogeneous Chinese Han population and from the city of Dalian 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 5': GGAGCATGTGATCAACG 3' and reverse 5': AAGAGCTGTCATCCATC 3'. Polymerase chain reaction (PCR) was performed in a total volume of 20 μL, including 2.0 μL 10× PCR buffer, 1.5 mM MgCl2, 0.25 mM dNTPs, 0.5 mM 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 58°C, and 30 s at 72°C, with a final elongation at 72°C for 5 min. The PCR products were analyzed by 3% agarose gel electrophoresis and visualized by ethidium bromide staining. The allelic discrimination of PD-1.5 C/T was determined by the numbers and the positions of the band on the gels.

**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 con-
controls in demographic characteristics and frequencies of genotypes were evaluated by using chi-square ($\chi^2$) test. Hardy-Weinberg equilibrium (HWE) was also tested by a chi-square ($\chi^2$) test. Differences were considered significant when $P < 0.05$.

**Results**

The cohort of 324 NSCLC patients contained slightly more men (50.3%) than women (49.7%). Between case and control, the age, gender, and smoking habits were well balanced. The distribution of PD-1.5 C/T frequencies was also in HWE ($P=0.26$ and $P=0.63$), indicating that the frequencies fell into the expected equilibrium and were thus randomly distributed. In NSCLC cases, adenocarcinoma represented 37.7%, and squamous cell carcinoma represented 62.3% (stage I+II 28.7% and stage III+IV 71.3%). The main characteristics of NSCLC cases and controls were shown in Table 1.

The genotype and allele frequencies of PD-1.5 C/T were shown in Table 2. The frequencies of CC, CT and TT genotypes in the patients were 61.1%, 32.7%, and 6.2% and were 54.8%, 31.8%, and 13.4% in the controls, respectively. Heterozygous (CT) genotype disclosed a statistically significantly increased risk of developing NSCLC (OR=2.22, 95% CI 1.23-4.02, $P=0.008$). Homozygous (CC) genotype also showed an increased risk of NSCLC (OR=2.40, 95% CI 1.37-4.24, $P=0.002$). Statistically significant difference was observed when the patients and controls were compared according to CC+CT versus TT (OR=2.34, 95% CI 1.35-4.06, $P=0.003$). The C allele was significantly higher in the NSCLC cases compared to the controls (77.5% versus 70.8%). The C allele was significantly associated with NSCLC risk (OR=1.421, 95% CI 1.10-1.82, $P=0.006$).

In order to determine the association between the polymorphism of PD-1.5 C/T and certain clinicopathological features, we conducted stratified analyses for combined genotypes with the TT genotype versus the CC+CT genotypes in NSCLC patients according to gender, age at admission, smoking status, histology, and TNM stage. There was a significantly higher frequency of CC+CT genotypes observed in patients with stage III+IV, compared to stage I+II (OR =2.66, 95% CI 1.07-6.63, $P=0.03$). There was no statistically significant associations of PD-1.5 C/T with gender, age, smoking status, and histology (Table 3).

**Discussion**

To the best of our knowledge, this is the first study to assess the association of PD-1.5 C/T with the risk of NSCLC. In this case-control study, we analyzed PD-1.5 C/T for NSCLC susceptibility in a Chinese Han population. Our results suggested that PD-1.5 C/T was signifi-

### Table 3. Association of PD-1.5 C/T with clinicopathological characteristics in NSCLC patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Genotype numbers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (%)</td>
<td>CC+CT (%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>163 (50.3%)</td>
<td>153 (50.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>161 (49.7%)</td>
<td>151 (49.7%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60</td>
<td>170 (50.1%)</td>
<td>159 (52.3%)</td>
</tr>
<tr>
<td>&gt; 66</td>
<td>154 (49.9%)</td>
<td>145 (47.7%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>168 (51.9%)</td>
<td>160 (52.6%)</td>
</tr>
<tr>
<td>Yes</td>
<td>156 (48.1%)</td>
<td>144 (47.4%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>202 (62.3%)</td>
<td>195 (65.1%)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>122 (37.7%)</td>
<td>109 (35.9%)</td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>93 (28.7%)</td>
<td>83 (27.3%)</td>
</tr>
<tr>
<td>III+IV</td>
<td>231 (71.3%)</td>
<td>221 (72.7%)</td>
</tr>
</tbody>
</table>

TNM, tumour-node-metastasis staging system.
PD-1 Polymorphism and NSCLC

cantly associated with the risk of NSCLC, suggesting that PD-1.5 C/T might be involved in pathogenesis of NSCLC in the Chinese Han population. We demonstrated that CC, CT, and the combined C variant genotype (CC+CT) within the PD-1.5 C/T were associated with an increased risk of NSCLC. Patients carrying those genotypes had a higher risk for NSCLC than those carrying the other genotypes. Furthermore, we also found that this polymorphism was significantly associated with advanced NSCLC risk.

Our results show a significant association between PD-1.5 C/T and NSCLC. Hua et al. reported that the C allele frequency was more in breast cancer patients than those in control individuals in Chinese population [6]. In addition, Mojtahedi et al. showed a significant association between PD-1.5 polymorphism and colon cancer [7]. Furthermore, Savabkar and colleagues found that PD-1.5 C/T polymorphism may affect the gastric cancer risk and prognosis in an Iranian population [8].

PD-1.5 C/T is a synonymous variation that does not change final amino acid sequence of the protein, thus, this significant association may be PD-1.5 C/T variation linkage disequilibrium with other PD-1 gene polymorphisms that may lead to alter the PD-1 expression level [9]. Lin et al. investigated PD-1.5 C/T polymorphism in rheumatoid arthritis (RA) and SLE, and indicated the association of the CT genotype and T allele with susceptibility to RA, but not SLE [9]. It was suggested that the T allele might be associated with the increased activity of T cells.

Currently a number of studies are ongoing to test the efficacy of investigational PD-1 inhibitor, Lambrolizumab and Nivolumab. Data on MK-3475 (Lambrolizumab) from phase I study of 38 patients with advanced NSCLC who had received at least 2 prior therapies was presented at the 15th World conference on Lung cancer by Garon et al. [10]. It is exciting to see that preclinical success of some of the immunotherapeutic agents is being reflected onto actual clinical success as seen with PD-1 inhibitors.

This present study had limitations that should be taken into account when interpreting the findings. 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 PD-1.5 C/T. 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.

In conclusion, the current study showed that PD-1.5 C/T had an effect on the risk of NSCLC in a Chinese Han population. Additionally, the combined CC and CT genotypes were significantly associated with advanced NSCLC risk.

Acknowledgements

This work was supported by The National Natural Science Foundation of China (No. 81273919) and The National Basic Research Program of China (973 Program, No. 2013CB531703).

Disclosure of conflict of interest

None.

Address correspondence to: Jinguang Wang, Department of Thoracic Surgery, The First Affiliated Hospital of Dalian Medical University, No. 193 Li-anhe Road, Dalian 116011, Liaoning, China. Tel: 86-0411-83635963-2062; E-mail: yinliu111222@126.com

References


