Original Article

LKB1 Gene Alterations in Surgically Resectable Adenocarcinoma of the Lung

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Abstract

Purpose. Germline mutations of LKB1 (also known as SKT11; locus 19p13.3) cause the occurrence of autosomal dominant Peutz–Jeghers syndrome (PJS). Nearly half of the non-small cell lung cancer (NSCLC) cell lines and one-third of lung adenocarcinoma in Caucasian patients have an LKB1 mutation.

Methods. This study examined the mutational hot spots of the LKB1 gene in surgical resectable lung adenocarcinoma. Exons 1, 6, and 7 of the LKB1 gene were sequenced in 174 Japanese patients with lung adenocarcinoma (including 157 men and 17 women).

Results. Only five patients had LKB1 gene alterations (2.9%). All of them were male smokers, and no LKB1 mutation was observed in any of the females. The details of LKB1 alterations were: one 5 bp deletion in intron 5, one Gly to Phe substitution at codon 279 of exon 6, and three Pro to Leu substitutions at codon 281 of exon 6. The P281L alteration and 5 bp deletion in the intron 5 were found to be germline polymorphisms. The G279F was confirmed to be a novel somatic mutation. None of the five patients with an LKB1 alteration showed either an EGFR or K-ras mutation.

Conclusion. The LKB1 gene alteration is rare in Japanese patients with lung adenocarcinoma, and is generally limited to male smokers.

Key words LKB1 · Mutation · Alteration · Adenocarcinoma · Smoking · Polymorphism

Introduction

Germline mutations of the LKB1 gene (also known as STK11) cause Peutz–Jeghers syndrome (PJS), which is autosomal dominant.1–3 Individuals with PJS typically exhibit mucocutaneous melanin pigmentation and develop hamartomatous polyps in the gastrointestinal tract.4,5 However, the most important problem with PJS is the increased risk of cancer development,6 including breast, ovarian, pancreatic, and lung cancers. The types and pattern of the mutations with PJS patients have been extensively studied.7–10 The majority of the germline LKB1 mutations result in truncation of the protein, leading to its dysfunction. The LKB1 gene is thought to act as a tumor suppressor in PJS. The LKB1 gene is implicated in the regulation of multiple biological processes, including G1 cell-cycle arrest,11 p53-mediated apoptosis,12 Wnt signaling,13,14 transforming growth factor-β signaling,15 and cell polarity.17 LKB1 is predicted to have a potential to be a tumor suppressor in sporadic tumors. However, a somatic LKB1 gene mutation has been identified only in a small fraction of various tumors, such as malignant melanomas, pancreatic cancers, and breast cancers.18–21 Half of the lung cancer cell lines and one-third of the primary lung adenocarcinoma in Caucasians harbor somatic LKB1 gene alterations.22,23 However, the frequency of somatic LKB1 mutation in the lung cancer patients in Japanese is less than 10%.24,25 The LKB1 gene mutation is found, almost exclusively, in adenocarcinoma, males, and smokers. In this study we evaluated the LKB1 gene alterations in Japanese surgically resectable lung adenocarcinoma patients.

Materials and Methods

Lung adenocarcinoma tissue specimens were obtained at Nagoya City University Hospital by surgical excision from 174 patients between 1997 and 2006. The research was approved by the Institutional Review Board of the hospital. All patients consented to the use of their tissues for the present analysis. None of the patients...
showed any clinical symptoms of PJS. Genomic DNA was extracted using the Wizard SV Genomic DNA purification system (Promega) according to the manufacturer's instructions. There were 157 (90%) male patients including 130 smokers, and 22 were light smokers (Brinkman Index [BI] ≤500). The 17 female patients included were all smokers. The age at diagnosis ranged from 35 to 88 years (median 66 years). One hundred and five patients had stage I, 24 had stage II, 38 had stage III, and 7 had stage IV. The epidermal growth factor receptor (EGFR) and v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras) mutations in this cohort had all been determined previously.

One hundred and seventy-four lung adenocarcinoma genomic DNA samples were amplified by polymerase chain reaction (PCR) reaction for LKB1 exons 1, 6, and 7. The primers and the PCR conditions have been previously reported.25 The PCR products were purified using a MiniElute PCR purification kit (Qiagen, Hilden, Germany) and then directly sequenced using an ABI PRISM 3100 Genetic Analyzer and analyzed by the ABI PRISM SeqScape Software package, Version 2.1.1 (Applied Biosystems, Carlsbad, CA, USA). Sequencing was performed from the forward and reverse sides for mutation-positive samples.

Results

One hundred and seventy-four adenocarcinoma tumor samples were subjected to conventional genomic DNA sequencing of the exons 1, 6, and 7, which include the kinase domain of LKB1 and the mutational hot spots in PJS. No alterations were detected in exon 1 and exon 7. Five alterations were detected in exon 6 (Table 1). Among them, one was 5 bp deletion in intron 5 (moderately differentiated adenocarcinoma), and others were base substitutions resulting in amino acid changes, Gly to Phe substitution at codon 279 (moderately differentiated adenocarcinoma; Fig. 1A) and Pro to Leu substitutions at codon 281 (Fig. 1B). The P281L included one well-differentiated adenocarcinoma and two poorly differentiated adenocarcinomas. The LKB1 somatic alteration has been previously reported to be limited to male smokers with adenocarcinoma. This study investigated for the first time the LKB1 hot spot in the males with adenocarcinoma, and found five cases with alterations. All five were smokers. No patients had double cancers in the organs other than the lung. One patient with the P281L polymorphism had double cancer in the same lobe of the lung. In contrast, no mutation was detected in 17 Japanese female patients with adenocarcinoma, all of whom were smokers. We could detect no mutation among the female smokers.

DNA from matched normal lung tissues of these five patients was investigated to see whether the detected alteration was a somatic or germline change. The Gly to Phe change at codon 279 was not detected in the DNA of normal tissue (Fig. 1A). The 5 bp deletions in intron 5 and P281L (Fig. 1B) were detected in the DNA of normal tissues, and were thought to be germline polymorphisms. The incidence of the somatic LKB1 gene mutation in males with adenocarcinoma was 0.6%.

Table 1. LKB1 alterations in lung adenocarcinoma patients

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Brinkman Index</th>
<th>Histology</th>
<th>pStage</th>
<th>Alteration</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>M</td>
<td>660</td>
<td>AD (Mod)</td>
<td>IIIA</td>
<td>835 G→T</td>
<td>279 G→F</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>M</td>
<td>1800</td>
<td>AD (Well)</td>
<td>IA</td>
<td>836 G→T</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>M</td>
<td>1600</td>
<td>AD (Well)</td>
<td>IIB</td>
<td>842 C→T</td>
<td>281 P→L</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>M</td>
<td>950</td>
<td>AD (Por)</td>
<td>Intron 15406-15410 deletion</td>
<td>842 C→T</td>
<td>281 P→L</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>1600</td>
<td>AD (Por)</td>
<td>IA</td>
<td>842 C→T</td>
<td>281 P→L</td>
</tr>
</tbody>
</table>

AD, adenocarcinoma; Well, well differentiated; Mod, moderately differentiated; Por, poorly differentiated