Alteration of PAFAH1B1 in Human Lung Cancer and Its Roles in Tumor Progression and Poor Survival

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ABSTRACT

Rationale and Objectives: Genomic DNA copy number variation is a hallmark of cancer. In our previous array-comparative genomic hybridization (array-CGH) study, we showed that PAFAH1B1 was amplified in lung cancer patients, suggesting that PAFAH1B1 is a potential oncogene in lung cancer.

Methods: In this study, we have determined the mRNA and protein expression level of PAFAH1B1 in 91 lung cancer patients using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC). Main Results: The PAFAH1B1 mRNA and protein overexpression frequency were 61.5% (56/91) and 56% (51/91) in lung cancer patients. The results indicated that mRNA and protein overexpression level of PAFAH1B1 was significantly associated with late stage (mRNA: \( P=0.001 \), protein: \( P=0.05 \)) and poor survival in lung adenocarcinoma (\( P=0.049 \)). Conclusions: The results revealed the roles of overexpressed PAFAH1B1 in tumor progression and poor survival in lung cancer.

Key words: PAFAH1B1, lung cancer, progression, oncogene

Introduction

Genomic DNA copy number variation is a hallmark of cancer and can lead to alteration in expression and functions of genes residing within the affected chromosomal region (Davies et al., 2005; Schwab, 1999). In our previous study, we generated a non-gapped array-comparative genomic hybridization (array-CGH) microarray representing 18 human chromosome imbalance hotspot regions. Our study involved a large cohort of cancer patients including 40 Asian and 20 Caucasian lung cancer patients, and revealed one of the novel lung cancer-related genes, PAFAH1B1, which was amplified in both Asian and Caucasian lung cancer patients (GEO: GSE21276). The results suggested that PAFAH1B1 is a potential oncogene in lung cancer.

The PAFAH1B1 (also named LIS1) gene was first cloned in patients with Miller-Dieker lissencephaly syndrome (MDLS), a disorder of neural development characterized by agyria and facial abnormalities, and classic lissencephaly (type I, LIS1), a disorder of isolated agyria (Ledbetter et al., 1992). PAFAH1B1 is composed 11 exons (Fig. 1A) and encodes the 45K non-catalytic subunit of the brain isoform of platelet activating factor (PAF) acetylhydrolase, a PAF-inactivating enzyme (Hattori et al., 1994; Lo Nigro et al., 1997). PAFAH1B1 is a predicted microtubule-associated protein with N-terminal coiled-coil domain, one Lissencephaly type-1-like homology (LisH) motif

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domain and eight WD40-repeat domains (Fig. 1B). It was reported that PAFAH1B1 may enhance neuronal migration by acting as a microtubule-associated protein (Cardoso et al., 2000). PAFAH1B1 was suggested to play some roles in invasion of human neuroblastoma (Messi et al., 2008; Suzuki et al., 2007).

Here, we sought to extend our previous finding that PAFAH1B1 was amplified in lung cancer patients by evaluating the mRNA and protein expression level of PAFAH1B1 in 91 lung cancer patients. In addition, the roles of PAFAH1B1 in tumor progression and poor survival in lung cancer were examined.

Materials and methods

Clinical samples preparation and DNA/RNA extraction

Tissues were collected after obtaining appropriate institutional review board permission and informed consent from the recruited patients. Surgically resected tumor tissue and corresponding normal tissue were collected from 91 patients diagnosed with primary non-small cell lung cancer (NSCLC) admitted to Taipei Veterans General Hospital, Taiwan. Histological classification was determined according to the WHO classification system and the tumor-node-metastasis system. Information on the age, sex, tumor type, tumor stage and smoking history of the patients was obtained from hospital records. Total RNA from tumors and normal lung tissues was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was generated using SuperScript reverse transcriptase (Invitrogen).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

qRT-PCR was used to measure the mRNA expression of four candidate genes in 91 NSCLC tumor and the corresponding normal samples. qRT-PCR was conducted using the ABI 7900 Sequence Detection System (PE Applied Biosystems). Total RNA (4 μg) from tumors and the corresponding normal tissues were reverse transcribed into cDNA and amplified using the SYBR Green PCR Master Mix (Roche) and specific primers for each candidate genes. The primer sequences for PAFAH1B1 are: forward ATG GGT CGT AGC AAC AAA GG and reverse TCT TCA TGC ATC GCT TGT TC. The annealing temperature was 58°C. The relative amount of PAFAH1B1 amplification products was calculated using the standard curve-based method and then normalized to the relative amount of ß-actin as detected in the same run. The primer sequences for ß-actin are: forward GGC GGC ACC ACC ATG TAC CCT and reverse AGG GGC CGG ACT CGT CAT ACT. The annealing temperature was 58 °C. Cutoff value for overexpression was the mean of normal lung tissue expression.

Immunohistochemistry (IHC)

The protein expression level of PAFAH1B1 was evaluated by IHC in 91 NSCLC samples.
Paraffin blocks of tumors were cut into 5-μm slices and then processed using standard deparaffinization and rehydration techniques. Polyclonal antibody against PAFAH1B1 (1:800; Novus, Littleton, CO) was used as the primary antibody to detect the protein expression. The evaluation of the IHC was conducted blindly without knowledge of the clinical and pathologic characteristics of the cases. The samples were graded high expression when >50% tumor cells were stained positive using adequate staining in surrounding normal stromal and epithelial cells.

**Statistical analysis**

A two-tailed t test was used to determine the statistical significance of difference in mRNA expression level of PAFAH1B1 in clinical samples. Chi-square test was conducted to examine the association between overexpression of candidate genes and clinico-pathological parameters, including sex, age, tumor type, tumor stage, and smoking. Survival curves were calculated according to the Kaplan-Meier method, and comparison was performed using the log-rank test. A $P < 0.05$ was considered to be statistically significant.

**Results**

PAFAH1B1 mRNA is overexpressed in lung cancer, and is significantly associated with late stage lung cancer patients.

To determine the mRNA expression level of the PAFAH1B1 gene in lung cancer, qRT-PCR of PAFAH1B1 was conducted with cDNA from tumors and corresponding normal tissues of 91 lung cancer patients. The mean mRNA expression level of PAFAH1B1 analyzed in the tumor tissues was significantly higher than in the corresponding normal tissues in lung cancer patients ($P < 0.001$) (Fig. 2). The PAFAH1B1 mRNA overexpression was defined using the mean mRNA expression of normal lung tissue as a cutoff value. The PAFAH1B1 mRNA overexpression frequency was 61.5% (Table 1). To determine the statistical significance of difference in mRNA expression level of PAFAH1B1 in clinical samples. Chi-square test was conducted to examine the association between mRNA overexpression of PAFAH1B1 and clinico-pathological parameters of 91 lung cancer patients, including age, sex, smoking, tumor type, and tumor stage. The results showed that no significant correlation of PAFAH1B1 mRNA expressions was detected in these clinico-pathological parameter, except that PAFAH1B1 mRNA overexpression was significantly associated with late stage ($P=0.05$), suggesting that the
alteration of PFAH1B1 gene may be involved in tumor progression (Table 1).

PFAH1B1 protein is overexpression in lung cancer, and is significantly associated with poor survival in lung adenocarcinoma.

To determine PFAH1B1 protein expression level in lung cancer patients, IHC was performed on paraffin blocks from 91 Asian patients (Fig. 3A). The frequency of PFAH1B1 protein overexpression was 56% in lung cancer. The association between PFAH1B1 protein overexpression and clinico-pathological parameters of 91 lung cancer patients was examined using the chi-square test, including age, sex, smoking, tumor type, and tumor stage. The results showed that no significant correlation of PFAH1B1 protein expressions was detected in these clinico-pathological parameter, except that PFAH1B1 protein overexpression was significantly associated with late stage lung cancer ($P=0.001$). The mRNA expression level of PFAH1B1 showed significant correlation with PFAH1B1 protein expression level ($P=0.045$) (Table 2). Note that, Kaplan-Meier survival curves indicated that lung cancer patients with overexpression of PFAH1B1 protein showed poor survival in adenocarcinoma ($P < 0.049$) (Fig. 3B).

**Discussions**

In our previous array-CGH study, we found several candidate genes which were located in the common chromosome alteration regions in both Asian and Caucasian, such as PFAH1B1 gene on 17p13.3. In array-CGH data, PFAH1B1 showed gain of copy number in 60% of Asian and 70% of Caucasian patients. In addition, chromosome region 17p13.3 harboring PFAH1B1 gene demonstrated association with advanced tumor stage in array-CGH data (GEO: GSE21276). In previous reports, PFAH1B1 was considered to function in microtubule movement by colocalization with NudE nuclear distribution gene E homolog-like 1 (NDEL1) and cytoplasmic dynein heavy chain (CDHC) in neuron cell (Soukoulis et al., 2005; Suzuki et al., 2007; Wynshaw-Boris, 2007). In addition, decreased PFAH1B1 protein expression in human neuroblastoma cell lines led to reduced cell migration and invasiveness (Aumais et al., 2001; Messi et al., 2008). However, the role of PFAH1B1 in other cancers has never been examined.

Here, we have determined the mRNA and protein overexpression frequency of PFAH1B1 to be 61.5% and 56% in 91 Taiwanese lung cancer patients, respectively. The clinical correlation results indicated that mRNA and protein overexpression level of PFAH1B1 was significantly associated with late stage ($P=0.001$–0.05) and poor survival in lung adenocarcinoma ($P=0.049$). Chi-square test was conducted to examine the correlation between the mRNA and protein expression level of PFAH1B1. In our results, the concordant group, such as positive of expression in both protein and mRNA and negative of expression in both protein and mRNA, was detected in 61.5% [(36+20)/91] of lung cancer patients. The mRNA expression level of PFAH1B1 showed significant correlation with PFAH1B1 protein expression level ($P=0.045$). However, the discordant group, such as positive of protein expression without positive of mRNA expression and negative of protein expression.
without negative of mRNA expression, was detected in 38.5% [(15+20)/91] of lung cancer patients. The discordant group may imply that other mechanisms such as mutations of the gene, dysfunction of the regulatory proteins, or the unknown degeneration system of PAFAH1B1 could be involved in the discordant expression of mRNA and protein. Note that Tian et al. (2011) found that microRNA 302/367 regulated the mRNA and protein expression of PAFAH1B1 through the 3'UTR regions. Our previous array-CGH data suggested that PAFAH1B1 overexpression may via gene amplification in lung cancer. In addition, PAFAH1B1 may be down-regulated by microRNA 302/367 (Tian et al., 2011). In conclusion, overexpression of PAFAH1B1 correlates with late stage disease and poor survival, suggesting that PAFAH1B1 may serve as a prognostic biomarker of non-small cell lung cancer metastasis.

Acknowledgments

We thank Taipei Veterans General Hospital for providing clinical samples.

Table 2. Association between protein expression level of PAFAH1B1 and lung cancer clinicopathological parameter.*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total†</th>
<th>PAFAH1B1 protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Over-expression</td>
</tr>
<tr>
<td>Overall</td>
<td>91</td>
<td>51 (56)</td>
</tr>
<tr>
<td>Age &lt;65</td>
<td>35</td>
<td>24 (68.6)</td>
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<tr>
<td>≥65</td>
<td>56</td>
<td>27 (48.2)</td>
</tr>
<tr>
<td>Sex Male</td>
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<td>30 (51.7)</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>21 (63.6)</td>
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<tr>
<td>Smoker Yes</td>
<td>33</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>17 (58.6)</td>
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<tr>
<td>Tumor type</td>
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<td></td>
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<tr>
<td>ADC</td>
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<td>36 (62.1)</td>
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<tr>
<td>SCC</td>
<td>31</td>
<td>14 (45.2)</td>
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<tr>
<td>Tumor stage</td>
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<tr>
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<td>23 (42.6)</td>
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<td>36 (64.3)</td>
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<tr>
<td>normal expression</td>
<td>35</td>
<td>15 (42.9)</td>
</tr>
</tbody>
</table>

* The P values with significance are shown as superscripts.
† Total number of samples in some categories is less than the overall number analyzed because clinical data or molecular data was not available for these samples.


肺癌病人 PAFAH1B1 基因變異參與癌症惡化

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摘    要

目的：基因體結構變異為腫瘤形成的表徵之一。於本實驗室先前的研究，我們針對肺癌族群進行微矩陣比較基因體的雜交分析 (array-CGH)，其結果顯示 PAFAH1B1 基因具有基因體結構擴增的現象。因此，我們推測 PAFAH1B1 基因為肺癌有潛力的致癌基因。材料與方法：本研究利用即時定量反轉錄 PCR (q-RT PCR) 及免疫組織染色法 (IHC) 對 91 位肺癌族群檢測 PAFAH1B1 基因其 mRNA 及蛋白層次變異情形。結果：PAFAH1B1 基因為 mRNA 層次的過度表現頻率為 61.5%，於蛋白層次過度表現頻率為 56%，且此基因於 mRNA 及蛋白層次的過度表現皆與病人的晚期具有相關性 (mRNA：P=0.001，蛋白層：P=0.05)，且屬於腺細胞癌的病人於蛋白層次的過度表現具有較差的預後 (P=0.049)。結論：其結果顯示 PAFAH1B1 基因於肺癌族群具有過度表達的變異，且可能參與肺癌細胞轉移過程。

關鍵詞：PAFAH1B1、肺癌、癌症惡化、致癌基因

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