**KIF5B/RET** fusion gene in surgically-treated adenocarcinoma of the lung

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**Abstract.** Recently, a novel fusion gene resulting from a linkage between the kinesin family member 5B gene (KIF5B; 10p11.22) and the rearranged during transfection gene (RET; 10q11.21) was identified in non-small cell lung cancer (NSCLC). However, the correlation between the KIF5B/RET fusion gene status and the clinicopathological features of surgically-treated lung cancer has not been well characterized. In this study, we have independently investigated the KIF5B/RET fusion gene status in 371 surgically-treated NSCLCs (270 were adenocarcinomas and 101 were squamous cell carcinomas), 60 breast cancers, 11 metastatic lung cancers from colon cancers and thyroid papillary adenocarcinoma cases at the Nagoya City University Hospital. The fusion gene status was analyzed by an RT-PCR-based assay and by using direct sequencing. We detected 3 of 270 cases of KIF5B/RET fusion genes in adenocarcinomas (1.1%) consisting of female and never smokers with mixed subtype adenocarcinomas. The fusion genes were detected exclusively with other mutations, such as EGFR, Kras, Braf, erbB2 mutations, and EML4/ALK fusion. KIF5B/RET fusion was not detected in the cases with squamous cell carcinoma or other types of cancers. From the 3 cases, 2 were KIF5B (exon 15); RET (exon 12) fusions with papillary dominant and 1 case was KIF5B (exon 22); RET (exon 12) fusion with solid dominant adenocarcinoma. The matched normal lung tissues did not display translocation. We reported KIF5B/RET fusion genes as a driver somatic mutation of lung adenocarcinomas. The clinicopathological backgrounds of the KIF5B/RET fusion-positive patients were similar with those of the EML4/ALK fusion-positive patients. The chimeric oncogene may be a promising molecular target for the personalized diagnosis and treatment of NSCLC.

**Introduction**

Lung cancer is the leading cause of cancer mortality worldwide. Although smoking is known to be a major risk factor of lung cancer, 25% of lung cancer patients worldwide are never smokers (1). In Asian countries, 30-40% of non-small cell lung cancer (NSCLC) patients are never smokers. NSCLC in never smokers tends to be driven by a single somatic mutation (1). The identification of activating mutations of the epidermal growth factor receptor (EGFR) is one of the most important discoveries in the field of lung cancer. EGFR mutations which are present primarily in women, in never smokers and in Asians are sensitive to EGFR-targeted therapy, such as gefitinib (2). The EML4/ALK fusion gene, formed by chromosomal rearrangement, has been identified in NSCLC (3). The EML4/ALK fusion genes are present primarily in young patients and in patients with little or no smoking habits (4). Lung cancer identified with the ALK fusion gene is present in ~5% of NSCLC patients and is sensitive to the ALK inhibitors (5,6).

A novel fusion gene resulting from a linkage between the kinesin family member 5B (KIF5B) gene and the rearranged during transfection (RET) gene was identified in NSCLC (7-10), including a Japanese group (9,10). KIF5B and RET are located at 10p11.22 and at 10q11.21, respectively. Since there is 10.6 Mb between KIF5B and RET, a long inversion event is necessary to form the fusion gene (7,8). This fusion gene is more frequent in never smokers and in Asian patients with adenocarcinoma and exists exclusively with other mutations, such as EGFR, Kras, Braf, erbB2 or EML4/ALK fusions (8-10).

In the present study, we independently investigated the KIF5B/RET fusion gene status in surgically-treated Japanese lung cancer patients, as well as other types of cancers at a single institution using a RT-PCR based assay. The findings were analyzed in reference to the clinicopathological features of NSCLC.

**Patients and methods**

*Patients.* The study group included 371 patients with adenocarcinoma of the lung (270 were diagnosed as adenocarcinoma and 101 were squamous cell carcinoma) who had undergone surgery at the Department of Surgery, Nagoya City University Hospital.
between 1997 and 2011. The lung tumors were classified according to the 7th edition of the General Rule of Clinical and Pathological Record of Lung Cancer in Japan. All tumor samples were immediately frozen and stored at -80°C until assayed. The study was approved by the ethics committee of the hospital.

The clinical and pathological characteristics of the 270 adenocarcinoma patients were as follows: 172 cases at stage I, 40 at stage II, 51 at stage III and 7 at stage IV. The mean age was 65.8±8.8 years (range, 38-88). Among the 270 adenocarcinoma patients, 160 were male and 110 were female. One hundred and eighteen patients were non-smokers. As regards the mutation statuses of EGFR (2,8,11,12), Kras (8,13,14), Braf (8,15), erbB2 (8,12,16) and EML4/ALK fusion (8), the samples from these patients were previously analyzed. Sixteen samples overlapped with a previous study (8). Pathological findings were confirmed by an independent pathologist (S.S.). The clinical and pathological characteristics of the 101 patients with squamous cell carcinoma were as follows: 57 cases at stage I, 25 at stage II and 19 at stage III. The mean age was 67.3±8.8 years (range, 29-85). Among the patients, 88 were male and 13 were female. Four patients were non-smokers.

Moreover, 60 patients with breast cancer, 1 patient with papillary adenocarcinoma of the thyroid and 11 patients with metastatic lung tumors from colorectal adenocarcinoma were also investigated. All patients had undergone surgery at the Department of Surgery, Nagoya City University Hospital. Among the 60 patients with breast cancer, 29 were diagnosed with scirrhous carcinoma, 16 as solid tubular carcinoma, and 15 as papillotubular carcinoma.

PCR assay for KIF5B/RET. Total RNA was extracted from lung cancer tissues using an Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. RNA concentration was determined by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Rockland, DE, USA). RNA (1 µg) was reverse transcribed using the first strand cDNA synthesis kit with 0.5 µg oligo(dT)12 (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The reaction mixture was incubated at 25°C for 15 min, 42°C for 60 min, 99°C for 5 min and then at 4°C for 5 min. The cDNA concentration was determined by a NanoDrop ND-1000 Spectrophotometer. Each cDNA (1 µl) was used for the PCR analysis. The PCR reactions were performed using an Ex Taq kit (Takara Bio Inc., Shiga, Japan) in a 50-µl reaction volume. The primer sequences for screening the KIF5B/RET fusion gene were as follows: forward primer, 5'-AAATGAGCTCAACAGATGGCGTAA-3' (at exon 12 of KIF5B gene) and reverse primer, 5'-AGAACCAAGTTCTTGAGCTGTA-3' (at exon 12 of RET gene). The cycling conditions were as follows: initial denaturation at 98°C for 10 sec, followed by 40 cycles at 94°C for 10 sec, 58°C for 10 sec and 72°C for 30 sec. Amplified DNAs were separated on 2% agarose gels, and the bands were visualized using ethidium bromide and an image was captured under ultraviolet transillumination. Case 1 showed bands at 681 bp (termvariant 1) and case 3 showed a band at 1395 bp (termvariant 3).

Statistical analysis. The overall survival of patients with lung adenocarcinoma was examined using the Kaplan-Meier method, and differences were examined by the log-rank test. The other clinicopathological characteristics were examined using the Student's t-test and χ² test as appropriate. The analyses were performed using the Excel software and differences were considered significant when the P-value was <0.05.

Results

PCR assays. For the screening purpose, we performed a RT-PCR assay for the KIF5B/RET fusion gene using several primer sets. Three of 126 adenocarcinoma samples from patients lacking EGFR, Kras, Braf and erbB2 mutations had PCR products, suspected as KIF5B/RET fusion genes (Fig. 1). Matched adjacent normal lung tissues available in all 3 cases had no bands, suggesting that the translocations were somatic. As shown in Table I, all 3 patients were female and non-smokers with mixed subtype adenocarcinomas. Two cases displayed bands at 681 bp (suggested as variant 1) (8) and 1 case showed a band at 1395 bp (suggested as variant 3) (8). Lung adenocarcinomas (n=144) with either EGFR, Kras, Braf, erbB2 or EML4/ALK gene mutations and 101 patients with squamous cell carcinoma of the lung were also analyzed but there was no KIF5B/RET fusion. In summary, 3/123 (2.4%) of female and 3/184 (1.6%) of never smoker NSCLC patients possessed the KIF5B/RET fusion genes.

Of the 270 lung adenocarcinoma patients, 126 lacked EGFR, Kras, Braf and erbB2 mutations. To identify the characteristics of KIF5B/RET fusion-positive patients, these 126 patients were...
Table I. *KIF5B-RET* fusion found in patients with wild-type adenocarcinoma.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Differentiation</th>
<th>Gender</th>
<th>Agea</th>
<th>BI</th>
<th>Stage</th>
<th>Acinar</th>
<th>Solid</th>
<th>Pap</th>
<th>Micropap</th>
<th>Lepidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Well</td>
<td>Female</td>
<td>64</td>
<td>0</td>
<td>Ia</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>V1</td>
<td>Moderate</td>
<td>Female</td>
<td>58</td>
<td>0</td>
<td>IIIa</td>
<td>40</td>
<td>-</td>
<td>50</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>V3</td>
<td>Poor</td>
<td>Female</td>
<td>79</td>
<td>0</td>
<td>Ia</td>
<td>20</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

Wild-type, with no mutations of *EGFR, Kras, BRAF, HER2* and *EML4/ALK* fusion. *Age, in years. BI; Brinkman index; Pap, papillary; Micropap, micropapillary.

Table II. Clinicopathological data of 126 patients with wild-type lung adenocarcinoma.

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. of samples (%)</th>
<th>(+) n=3 (%)</th>
<th>(-) n=123 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>126</td>
<td>67.0±10.8</td>
<td>65.2±9.3</td>
<td>0.741a</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;=65</td>
<td>67 (53.2)</td>
<td>2 (66.7)</td>
<td>65 (52.8)</td>
</tr>
<tr>
<td></td>
<td>&gt;65</td>
<td>59 (46.8)</td>
<td>1 (33.3)</td>
<td>58 (47.2)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>104 (82.5)</td>
<td>0 (0)</td>
<td>104 (84.6)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22 (17.5)</td>
<td>3 (100)</td>
<td>19 (15.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>Never smoker</td>
<td>23 (18.3)</td>
<td>3 (100)</td>
<td>20 (16.3)</td>
</tr>
<tr>
<td></td>
<td>Smoker</td>
<td>103 (81.7)</td>
<td>0 (0)</td>
<td>103 (83.7)</td>
</tr>
<tr>
<td>Stage</td>
<td>I</td>
<td>74 (58.7)</td>
<td>2 (66.7)</td>
<td>72 (58.5)</td>
</tr>
<tr>
<td></td>
<td>II-IV</td>
<td>52 (41.3)</td>
<td>1 (33.3)</td>
<td>51 (41.5)</td>
</tr>
</tbody>
</table>

*aStudent's t-test; bYates' χ² test.

considered as wild-type. Table II summarizes clinicopathological features of the wild-type adenocarcinoma patients. Wild-type patients 3/126 (2.4%) had *KIF5B-RET* fusion genes. The mean age of the *KIF5B-RET* fusion-positive patients was 67.0±10.8 years, whereas that of the *KIF5B-RET* fusion-negative patients was 65.2±9.3 years. There was a significant association between *KIF5B-RET* fusion and gender (P=0.002). There was also an association between *KIF5B-RET* fusion and smoking status (P=0.003). As regards age and stage, there was no association with *KIF5B-RET* fusion (Table II). In addition, 60 patients with breast cancer, 1 patient with papillary adenocarcinoma of the thyroid, and 11 patients with metastatic lung tumors from colorectal adenocarcinoma were also analyzed. There was no *KIF5B-RET* fusion in all the patients with other types of cancer.

*Sequencing analysis for the KIF5B-RET gene.* From the direct sequencing of the PCR products (681 bp), 2 cases showed junctions between exon 15 of the *KIF5B* gene and exon 12 of the *RET* gene (Fig. 2), termed variant 1 (V1) (8). These cases were papillary dominant mixed subtype adenocarcinomas (Fig. 3). However, for the longer product (1395 bp), we were unable to detect the *KIF5B-RET* fusion point by direct sequencing. Thus, we designed a new sequencing primer at exon 17 of the *KIF5B* gene. The sequencing results from the PCR product showed the junction between exon 22 of the *KIF5B* gene and exon 12 of the *RET* gene (Fig. 2), termed variant 3 (V3) (8). The case was solid dominant mixed subtype adenocarcinoma (Fig. 3). Given the genetic sequence, all fusions contained both a dimerization unit (coiled-coil domain of *KIF5B*) and a tyrosine kinase unit (from *RET*).

*Correlation between KIF5B-RET translocation and patient outcomes in NSCLC.* The overall survival of the 126 patients with wild-type lung adenocarcinoma was analyzed in reference to the *KIF5B-RET* fusion gene statuses. The median observation period of the *KIF5B-RET* fusion-positive patients was 51.7 months (0.6-60.7 months after the primary operation) and that of the *KIF5B-RET* fusion-negative patients was 36.2 months (0.5-146.6 months after the primary operation). The overall survival of the *KIF5B-RET* fusion-positive patients was 100% at 5 years, whereas that of the *KIF5B-RET* fusion-
negative patients was 61.3% at 5 years and 46.0% at 10 years (Fig. 4). Although the number of KIF5B/RET fusion-positive cases was small, there was no significant difference between the KIF5B/RET fusion statuses (P=0.352). One KIF5B/RET fusion (variant 1) case showed recurrence of lung cancer; however, the patient had a good response to the pemetrexed treatment.

Discussion

In the present study, 371 NSCLC tissues including 270 adenocarcinoma and 101 squamous cell carcinoma were investigated to identify the clinicopathological characteristics of KIF5B/RET fusion gene statuses. The KIF5B/RET fusion genes were detected exclusively with mutations of EGFR, Kras, Braf,
erbB2 and EML4/ALK fusion. The KIF5B/RET fusion genes were detected in 3 cases of adenocarcinomas, but not in adjacent normal lung tissues or other types of cancer, suggesting that the fusions were oncogenic driver mutations, specific for lung adenocarcinomas.

RET has previously been reported as an activated oncogene in papillary thyroid carcinoma, where chromosomal translocations lead to the formation of PTC/RET fusion genes (16,17). Moreover, activated RET has been reported in pancreatic cancer (18), prostate cancer (19) and melanoma (20). Heterodimers of MET with RET have previously been reported. These heterodimers have differential roles in tumor development and they provide insight into the function of transphosphorylated RET as partners of MET in MET-amplified lung cancers (21). Thyroid cancers and cell lines harboring PTC/RET translocations are sensitive to the multikinase inhibitor, sorafenib, or sunitinib that inhibits RET (22-25), suggesting that the KIF5B/RET gene fusion may identify a drug-sensitive subset of NSCLCs. The full length KIF5B/RET gene (variant 1) introduced into Ba/F3 cells has shown IL-3-independent growth consistent with oncogenic transformation (8). As the KIF5B/RET fusion gene overexpresses the chimeric RET receptor tyrosine kinase, subsequently leading to spontaneous cellular transformation, the inhibition of RET receptor tyrosine kinase activity may suppress tumor progression of KIF5B/RET fusion-positive lung cancer. These KIF5B/RET Ba/F3 cells were sensitive to sunitinib, sorafenib and vandetanib, multi-targeted kinase inhibitors that inhibit RET, but not gefitinib, an EGFR kinase inhibitor (8). Sunitinib, but not gefitinib, inhibited RET phosphorylation in the KIF5B/RET Ba/F3 cells. As regards the chromosomal translocations, the KIF5B/ALK fusion gene has also been detected in lung cancer (26). KIF5B fusions were originally found in hypereosinophilia (27), and all these fusions contained a dimerization unit (coiled-coil domain) which induces homodimerization (26,27).

In our study, all 3 KIF5B/RET cases were female, never smokers, with mixed subtype adenocarcinomas, similar to EGFR (2,8,12) or EML4/ALK (3,4,8) or erbB2 (12) mutations. Takahashi et al reported that EML4/ALK fusion was present in 1.6% of patients with NSCLC and occurred more frequently in females and non-smokers (28). Inamura et al reported that EML4/ALK fusion was present in 3.4% of patients with NSCLC (29) and correlated with acinar-predominant (P<0.0001) and non- or light smokers (P=0.04) (30). Yoshida et al reported that solid or acinar growth patterns were more common in ALK-rearranged lung carcinomas (31). In the present study, KIF5B/RET fusions were present in patients with similar clinicopathological backgrounds compared to ALK fusions in Japanese NSCLC patients. In addition, a papillary pattern was frequently similar to the PTC/RET translocation in thyroid cancers.

As regards prognosis, the overall survival in the KIF5B/RET fusion-positive patients was 100% at 5 years, whereas that in the fusion-negative patients was 61.3% at 5 years and 46.0% at 10 years. Although the 3 cases are insufficient to discuss, the patients with KIF5B/RET fusion-positive lung cancer may have better prognosis due to the sensitivity to pemetrexed treatment for recurrence 39 months after surgery. Camidge et al reported that, in comparison with EML4/ALK-negative patients, EML4/ALK-positive patients had a significantly longer progression-free survival on pemetrexed (32). However, there are conflicting published data regarding the natural history and clinical outcomes of EML4/ALK-positive NSCLC (33,34). In our case, a less advanced stage, gender and smoking status may influence the survival data, and additional samples and data are required to conclude the correlation between the fusion status and prognosis. To identify the clinicopathological characteristics of KIF5B/RET fusion-positive lung cancer, further studies are warranted.

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