SPANDIDOS PUBLICATIONS

ONCOLOGY REPORTS 28: 1187-1192, 2012

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

KEISUKE YOKOTA[1], HIDEFUMI SASAKI[1], KATSUHIRO OKUDA[1], SHIGEKI SHIMIZU[2], MASAYUKI SHITARA[1], YU HIKOSAKA[1], SATORU MORIYAMA[1], MOTOKI YANO[1] and YOSHITAKA FUJII[1]

[1]Department of Oncology, Immunology and Surgery, Nagoya City University Graduate School of Medical Sciences, Mizuho-cho, Mizuho-ku, Nagoya 467-8601; [2]Department of Pathology, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Osaka 591-8555, Japan

Received May 9, 2012; Accepted June 28, 2012

DOI: 10.3892/or.2012.1908

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 cinicopathological 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.

Correspondence to: Dr Hidefumi Sasaki, Department of Oncology, Immunology and Surgery, Nagoya City University Graduate School of Medical Sciences, Kawasumi 1, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan
E-mail: hisasaki@med.nagoya-cu.ac. jp

Key words: KIF5B/RET, chromosomal rearrangement, non-small cell lung cancer, adenocarcinoma

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 pathologic 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$(dT)_{16}$ (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'-AGAACCAAGTTCTTCC GAGGAAT-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 98˚C for 10 sec and 68˚C for 1 min. The products were purified using a Qiagen PCR purification kit (Qiagen, Valencia, CA). To confirm the variant, further primer sets for the *KIF5B/RET* fusion gene were used: forward primers, 5'-TAAGGAAATGACCAACCAACCACCAG-3' (for variant 1) or 5'-GTGAAACGTTGCAAGCAAGCAGTTAG-3' (for variant 3), and reverse primer, 5'-CCTTGACCACTTTTCC AAATTC-3'. The cycling conditions were as follows: initial denaturation at 94˚C for 10 sec, followed by 40 cycles at 94˚C for 30 sec, 62˚C for 30 sec (variant 1) or 40 sec (variant 3) 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. Amplified fragments were then subjected to direct sequence analysis. To confirm the fusion point, a designed sequencing primer, 5'-TAGTCCAGCTTCGAGCACAA-3' was also used.

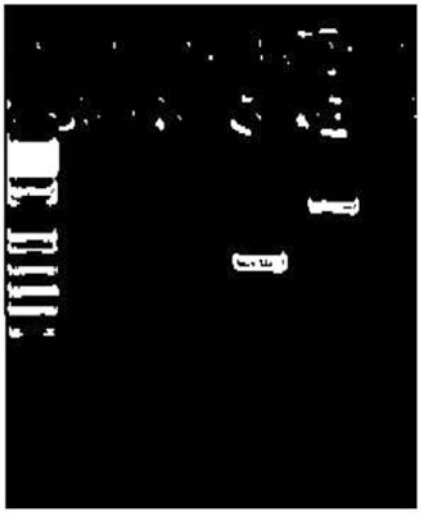

Figure 1. Amplified DNAs were separated on 2% agarose gels, and the bands were visualized by ethidium bromide and photographed under ultraviolet transillumination. Case 1 showed bands at 681 bp (termed variant 1) and case 3 showed a band at 1395 bp (termed variant 3).

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 χ^2 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.

Variant	Differentiation	Gender	Age[a]	BI	Stage	Acinar	Solid	Pap	Micropap	Lepidic
V1	Well	Female	64	0	Ia	-	-	70	-	30
V1	Moderate	Female	58	0	IIIa	40	-	50	10	-
V3	Poor	Female	79	0	Ia	20	40	30	30	-

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

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

		KIF5B/RET fusion		
Factors	No. of samples (%)	(+) n=3 (%)	(-) n=123 (%)	P-value
Mean age (years)	126	67.0±10.8	65.2±9.3	0.741[a]
Age				
≤65	67 (53.2)	2 (66.7)	65 (52.8)	0.911[b]
>65	59 (46.8)	1 (33.3)	58 (47.2)	
Gender				
Male	104 (82.5)	0 (0)	104 (84.6)	0.002[b]
Female	22 (17.5)	3 (100)	19 (15.4)	
Smoking				
Never smoker	23 (18.3)	3 (100)	20 (16.3)	0.003[b]
Smoker	103 (81.7)	0 (0)	103 (83.7)	
Stage				
I	74 (58.7)	2 (66.7)	72 (58.5)	0.756[b]
II-IV	52 (41.3)	1 (33.3)	51 (41.5)	

[a]Student's t-test; [b]Yates' χ^2 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*) (Fig. 2).

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-

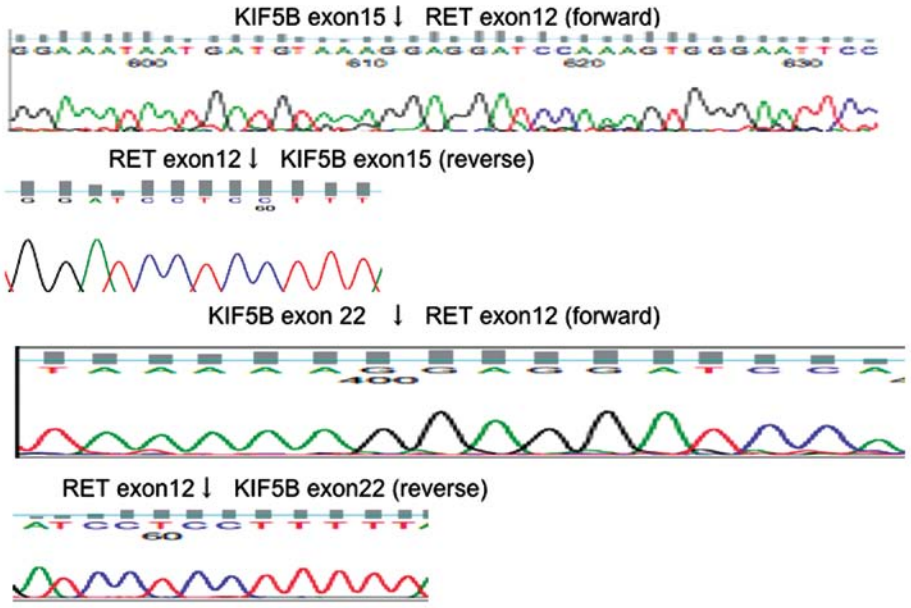

Figure 2. Case 1 showed junctions between exon 15 of the *KIF5B* gene and exon 12 of the *RET* gene (upper panel), and case 3 showed a junction between exon 22 of the *KIF5B* gene and exon 12 of the *RET* gene (lower panel). Both fusions contained both a dimerization unit (coiled-coil domain of *KIF5B*) and a tyrosine kinase unit (from *RET*).

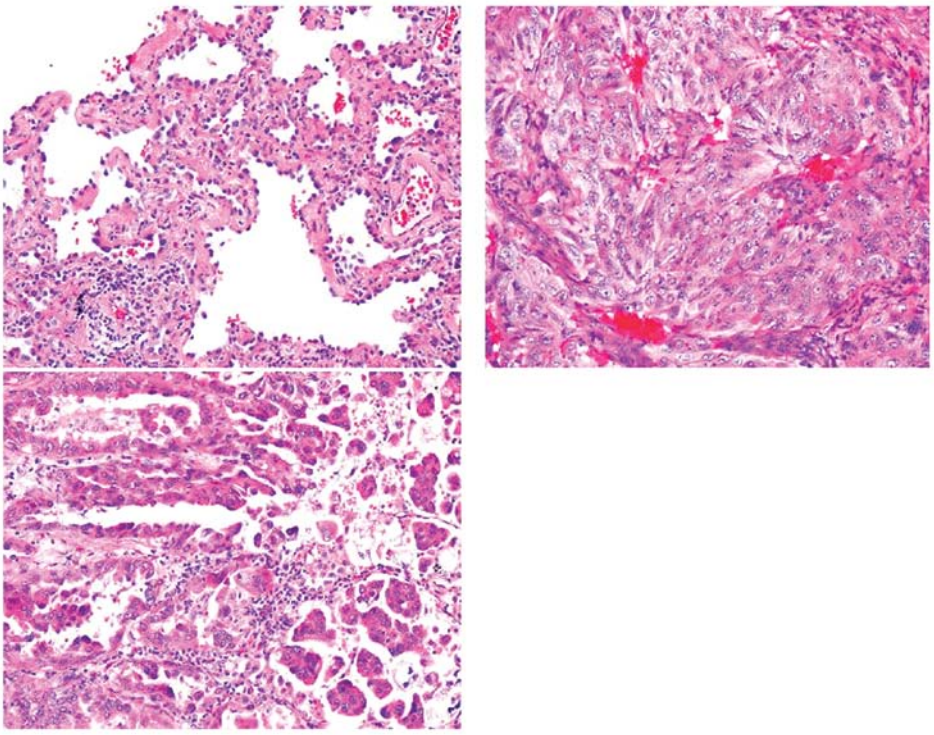

Figure 3. The variant 1 type (*KIF5B* exon 15; *RET* exon 12 fusion) cases were papillary dominant mixed subtype adenocarcinomas (left panel); the variant 3 type (*KIF5B* exon 22; *RET* exon 12 fusion) case was solid dominant mixed subtype adenocarcinoma (right panel).

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 clinicopthological characteristics of *KIF5B*/*RET* fusion gene statuses. The *KIF5B*/*RET* fusion genes were detected exclusively with mutations of *EGFR*, *Kras*, *Braf*,

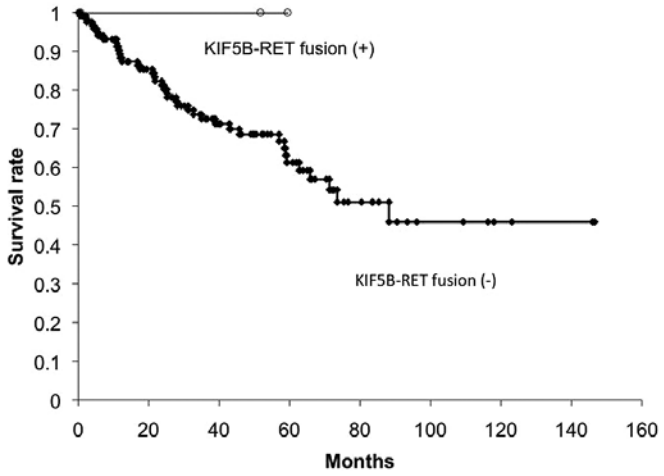

Figure 4. Kaplan-Meier curve shows overall survival of 126 patients with lung adenocarcinoma according to *KIF5B*/*RET* fusion. The overall survival of the fusion-positive patients was 100% at 5 years, whereas that of the fusion-negative patients was 61.3% at 5 years and 46.0% at 10 years. There was no significant difference in prognosis according to *KIF5B*/*RET* fusion (log-rank test, P=0.352).

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, multitargeted 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.

Acknowledgements

The authors would like to thank Mrs. Miki Mochizuki and Yuka Toda for their excellent technical assistance. This study was supported by Grants-in-Aid for Scientific Research, Japan Society for the Promotion of Science (JSPS) (nos. 23659674, 24592097 and 21591820).

References

1. Lee YJ, Kim JH, Kim SK, *et al*: Lung cancer in never smokers: change of a mindset in the molecular era. Lung Cancer 72: 9-15, 2011.
2. Paez JG, Jänne PA, Lee JC, *et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
3. Soda M, Choi YL, Enomoto M, *et al*: Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 448: 561-566, 2007.
4. Wong DW, Leung EL, So KK, *et al*: The EML4-ALK fusion gene is involved in various histologic types of lung cancers from non smokers with wild-type EGFR and KRAS. Cancer 115: 1723-1733, 2009.
5. Pao W and Girard N: New driver mutations in non-small-cell lung cancer. Lancet Oncol 12: 175-180, 2011.
6. Shaw AT, Yeap BY, Solomon BJ, *et al*: Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 12: 1004-1012, 2011.
7. Ju YS, Lee WC, Shin JY, *et al*: Fusion of KIF5B and RET transforming gene in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 22: 436-445, 2012.

8. Lipson D, Capelletti M, Yelensky R, *et al*: Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 18: 382-384, 2012.
9. Kohno T, Ichikawa H, Totoki Y, *et al*: KIF5B-RET fusions in lung adenocarcinoma. Nat Med 18: 375-377, 2012.
10. Takeuchi K, Soda M, Togashi Y, *et al*: RET, ROS1 and ALK fusions in lung cancer. Nat Med 18: 378-381, 2012.
11. Sasaki H, Endo H, Konishi A, *et al*: EGFR mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler. Clin Cancer Res 11: 2924-2929, 2005.
12. Sasaki H, Shimizu S, Endo K, *et al*: EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer 118: 180-184, 2006.
13. Sasaki H, Hikosaka Y, Kawano O, Moriyama S, Yano M and Fujii Y: Evaluation of Kras mutation and copy number gain in non-small cell lung cancer. J Thorac Oncol 6: 15-20, 2011.
14. Sasaki H, Okuda K, Kawano O, *et al*: Nras and Kras mutation in Japanese lung cancer patients: genotyping analysis using LightCycler. Oncol Rep 18: 623-628, 2007.
15. Sasaki H, Kawano O, Endo K, *et al*: Uncommon V599E Braf mutations in Japanese patients with lung cancer. J Surg Res 133: 203-206, 2006.
16. Grieco M, Santoro M, Berlingieri MT, *et al*: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60: 557-563, 1990.
17. Alberti L, Carniti C, Miranda C, Roccato E and Pierotti MA: RET and NTRK1 proto-oncogenes in human diseases. J Cell Physiol 195: 168-186, 2003.
18. Zeng Q, Cheng Y, Zhu Q, *et al*: The relationship between overexpression of glial cell-derived neurotrophic factor and its RET receptor with progression and prognosis of human pancreatic cancer. J Int Med Res 36: 656-664, 2008.
19. Dawson DM, Lawrence EG, MacLennan GT, *et al*: Altered expression of RET proto-oncogene product in prostatic intraepithelial neoplasia and prostate cancer. J Natl Cancer Inst 90: 519-523, 1998.
20. Ohshima Y, Yajima I, Takeda K, Kumasaka M, Matsumoto M and Kato M: c-RET molecule in malignant melanoma from oncogenic RET-carrying transgenic mice and human cell lines. PLoS One 5: e10279, 2010.
21. Tanizaki J, Okamoto I, Sakai K and Nakagawa K: Differential roles of trans-phosphorylated EGFR, HER2, HER3, and RET as heterodimerisation partners of MET in lung cancer with MET amplification. Br J Cancer 105: 807-813, 2011.
22. Henderson YC, Ahn SH, Kang Y and Clayman GL: Sorafenib potently inhibits papillary thyroid carcinomas harboring RET/PTC1 rearrangement. Clin Cancer Res 14: 4908-4914, 2008.
23. Carlomagno F, Anaganti S, Guida T, *et al*: BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst 98: 326-334, 2006.
24. Kim DW, Jo YS, Jung HS, *et al*: An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. J Clin Endocrinol Metab 91: 4070-4076, 2006.
25. Dawson S, Conus NM, Toner GC, Raleigh JM, Hicks RJ, McArthur G and Rischin D: Sustained clinical responses to tyrosine kinase inhibitor sunitinib in thyroid carcinoma. Anticancer Drugs 19: 547-552, 2008.
26. Takeuchi K, Choi YL, Togashi Y, *et al*: KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 15: 3143-3149, 2009.
27. Score J, Curtis C, Waghorn K, *et al*: Identification of a novel imatinib responsive KIF5B-PDGFRA fusion gene following screening for PDGFRA overexpression in patients with hypereosinophilia. Leukemia 20: 827-832, 2006.
28. Takahashi T, Sonobe M, Kobayashi M, *et al*: Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. Ann Surg Oncol 17: 889-897, 2010.
29. Inamura K, Takeuchi K, Togashi Y, *et al*: EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. J Thorac Oncol 3: 13-17, 2008.
30. Inamura K, Takeuchi K, Togashi Y, *et al*: EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol 22: 508-515, 2009.
31. Yoshida A, Tsuta K, Nakamura H, *et al*: Comprehensive histologic analysis of ALK-rearranged lung carcinomas. Am J Surg Pathol 35: 1226-1234, 2011.
32. Camidge DR, Kono SA, Lu X, *et al*: Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. J Thorac Oncol 6: 774-780, 2011.
33. Yang P, Kulig K, Boland JM, *et al*: Worse disease-free survival in never-smokers with ALK$^+$ lung adenocarcinoma. J Thorac Oncol 7: 90-97, 2012.
34. Wu SG, Kuo YW, Chang YL, *et al*: EML4-ALK translocation predicts better outcome in lung adenocarcinoma patients with wild-type EGFR. J Thorac Oncol 7: 98-104, 2012.