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RET Fusion Genes in Korean Non-Small Cell Lung Cancer

Seung Soo Yoo,[1]* Guang Jin,[2]*
Hye Jin Jung,[3] Mi Jeong Hong,[3]
Jin Eun Choi,[3] Hyo-Sung Jeon,[3]
Shin Yup Lee,[1] Jeong Ok Lim,[4]
and Jae Yong Park[1,3]

[1]Department of Internal Medicine, Kyungpook
National University School of Medicine, Daegu,
Korea; [2]Cancer Research Center, Yanbian University
School of Basic Medicine, Yanji, China;
[3]Departments of Biochemistry and Cell Biology and
[4]Bio-Medical Research Institute, Kyungpook
National University School of Medicine, Daegu,
Korea

*Seung Soo Yoo and Guang Jin contributed equally
to this work.

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Address for Correspondence:
Jae Yong Park, MD
Departments of Internal Medicine and Biochemistry and Cell
Biology, Kyungpook National University School of Medicine,
807 Hoguk-ro, Buk-gu, Daegu 702-210, Korea
Tel: +82.53-200-2631, Fax: +82.53-200-2027
E-mail: jaeyong@knu.ac.kr

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Recently, rearranged during transfection (*RET*) fusions have been identified in
approximately 1% of non-small cell lung cancer (NSCLC). To know the prevalence of *RET*
fusion genes in Korean NSCLCs, we examined the *RET* fusion genes in 156 surgically
resected NSCLCs using a reverse transcriptase polymerase chain reaction. Two *KIF5B-RET*
fusions and one *CCDC6-RET* fusion were identified. All three patients were females and
never smokers with adenocarcinomas. *RET* fusion genes were mutually exclusive from
EGFR, *KRAS* mutations and *EML4-ALK* fusion. *RET* fusion genes occur 1.9% (3 of 156) of
surgically treated NSCLC patients in Koreans.

Key Words: *RET* Fusion; *KIF5B*; *CCDC6*; Carcinoma, Non-Small-Cell Lung; Korean

Lung cancer is the most common cause of cancer-related deaths worldwide. Surgical resection is the best treatment modality for early-stage non-small cell lung cancer (NSCLC). About two thirds of NSCLC is diagnosed with locally advanced or metastatic disease and treated with chemotherapy (1). Cytotoxic chemotherapy with platinum-based doublets has been the mainstay of treatment for advanced NSCLC (2). However, recent development of molecular technology has enabled targeted therapies for NSCLC. For example, epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor, such as gefitinib, is used as 1st line chemotherapy for *EGFR* mutated adenocarcinoma (AC) (3). Crizotinib, an anaplastic lymphoma receptor tyrosine kinase (*ALK*) inhibitor, has been available after discovery of *ALK* fusion gene in NSCLC (4).

Most recently, novel fusions of rearranged during transfection (*RET*) gene have been identified in 1%-2% of NSCLC (5-9). *RET* proto-oncogene, located on chromosome 10q11.2, encodes a receptor tyrosine kinase expressed in tissues derived from neural crest (10). RET plays a crucial role in neural crest devel-

opment. Oncogenic activation of *RET* is related with inherited cancer syndromes, such as multiple endocrine neoplasia type 2 (MEN 2), which includes medullary thyroid cancer (10). *RET* rearrangements is also associated with papillary thyroid cancer after exposure to external radiation (11). In lung cancer, genomic rearrangement of *RET* partnered with kinesin family member 5B (*KIF5B*) or coiled-coil domain containing 6 (*CCDC6*) have been reported (5-9). Similarly to echinoderm microtubule-associated protein-like 4 (*EML4*), which is common fusion partner to *ALK* gene, *KIF5B* and *CCDC6* contain a coiled-coil domain (CCD). In *RET* fusion genes, a CCD of *KIF5B* or *CCDC6* functions as a dimerization unit, which induces homodimerization and activates the oncogenic protein tyrosine kinase domain by autophophorylation (12). Ligand-independent activation of *RET* fusion gene may serve as the driving force for carcinogenesis.

To date, seven variants in *KIF5B-RET* fusion and only one type in *CCDC6-RET* fusion have been reported (13). Among 7 *KIF5B-RET* fusion variants, the fusion of *KIF5B* exon 15 and

RET exon 12 (K15;R12) is the most common, and accounts for 60%-70% of *KIF5B-RET* fusion (5-7). The other variants, such as K16;R12, K23;R12, K22;R12 etc. were found infrequently.

Takeuchi et al. (5) found 13 (0.9%) *RET* fusion genes, which consist of 12 *KIF5B-RET* and 1 *CCDC6-RET*, in 1,482 Japanese NSCLCs. Wang et al. (7) discovered 13 patients (1.4%) harboring *RET* fusion genes among 936 Chinese NSCLCs. *KIF5B-RET* fusion gene was identified in 0.8% (1 of 121) NSCLCs in Caucasians (6). *KIF5B-RET* fusion gene was not found in 34 Norwegian and 5 African American (6, 9). In the present study, to know the prevalence of *RET* fusion genes in Korean NSCLCs, we investigated the *RET* fusion status of 156 surgically resected NSCLCs.

Tumor samples were provided by the National Biobank of Korea, Kyungpook National University Hospital, Daegu, Korea, which is supported by the Ministry of Health, Welfare, and Family Affairs. All materials derived from the National Biobank were obtained in accordance with the institutional review board approved protocol, Kyungpook National University Medical Center (Approval No., KNUHBIO_10_1016). This study included 156 NSCLC patients with available RNA who underwent curative surgical resection at the Kyungpook National University Hospital (Daegu, Korea) between January 2005 and July 2011. All the tissues were rapidly frozen in liquid nitrogen and stored at -80°C until assayed. Written informed consent was obtained from all patients. All patients included were ethnic Koreans. The histologic types of lung cancers were as follows: 104 patients (66.7%) with AC and 52 patients (33.3%) with squamous cell carcinoma (Supplement Table 1). There were 105 males and 51 females, with the mean age of 63.8 ± 8.7 yr. Patients consisted of 102 ever smokers and 54 never smokers. Of the 104 AC patients, 51 were never-smokers.

Total RNA was extracted from fresh frozen tissues using the RNeasy Mini kit (Qiagen, Valencia, CA, USA), and the RNA extract was incubated with RNase-free DNase I to remove contaminating DNA. Reverse transcription of total RNA was carried out using a Qiagen kit to generate complementary DNA (cDNA). Among 7 *KIF5B-RET* variants, we targeted the most common 5 *KIF5B-RET* fusion variants, K15;R12, K16;R12, K23;R12, K22;R12, and K24;R11. We conducted reverse transcriptase-polymerase chain reaction (RT-PCR) assays using sense primers 5´-ATTAG-GTGGCAACTGTAGAACC-3´ on exon 15 of KIF5B, to detect K15;R12 and K16;R12; 5´-AGCCACAGATCAGGAAAAGA-3´ on exon 22 of *KIF5B*, to detect K22;R12, K23;R12 and K24;R11; and

5´-TGCAGCAAGAGAACAAGGTG-3´ on exon 1 of *CCDC6*. Antisense primer was 5´-CAGGCCCCATACAATTTGAT-3´ on exon 12 of *RET*. PCR reactions were performed in a total volume of 20 μL containing 200 ng of cDNA, 10 pM of each primer, 0.2 mM dNTPs, 1 unit of *Taq* polymerase (Genet Bio, Daejeon, Korea), 25 mM MgCl$_2$, and 10 × reaction buffer (10 mM Tris-HCl [pH8.3], 50 mM KCl, and 1.5 mM MgCl2). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 15 min, followed by 34 cycles of 30 sec at 94°C; 30 sec at 56°C; 1 min at 72°C; and a final elongation at 72°C for 10 min. The PCR products were resolved on 1.2% agarose gels and stained with ethidium bromide for visualization under UV light. To identify the specific PCR product, the PCR products were digested 2 hr at 37°C with the *EcoR*I restriction enzymes (New England BioLabs, Beverly, MA, USA). To confirm the RFLP results, selected PCR-amplified DNA samples were examined by DNA sequencing, and the results were also 100% concordant. We also analyzed mutations in the *EGFR* (exons 18-21), *KRAS* (exon 2) and *EML4-ALK* fusion using PCR and direct sequencing, as described in our previous study (14).

Using RT-PCR, *RET* fusion transcripts were detected in 3 (1.9%) of the 156 NSCLCs. *RET* fusion genes were found only in ACs. All the three patients were females and never smokers (Table 1). None of them had previous history of thyroid cancer or radiation therapy. Among three patients, two harbored *KIF5B-RET* fusion (*KIF5B* exon 15 fused with *RET* exon 12) and one harbored *CCDC6-RET* fusion (*CCDC6* exon 1 fused with *RET* exon 12) (Fig. 1). Among 104 ACs, 26 cases (25.0%) had *EGFR* mutations, 7 cases (6.7%) had *KRAS* mutations, and 3 cases (2.9%) had *EML4-ALK* fusion. *RET* fusions were mutually exclusive with *EGFR, KRAS* mutations and *EML4-ALK* fusion.

In this study, the frequency of *RET* fusion of NSCLCs was 1.9% (2.9% in ACs), which was comparable to the other studies, which reported that the frequency of *RET* fusion was 1%-2% in East Asian patients with NSCLCs (5-7, 9). However, this was different with the frequency reported in a previous Korean study, in which two cases (10%) of the *KIF5B-RET* fusion were identified in 20 primary lung ACs (15). This is mostly because the samples in the previous study were selected among triple-negative (*EGFR, KRAS* and *EML4-ALK*) or double-negative (*EGFR* and *EML4-ALK*) ACs.

There are some limitations in the present study. Among 7 variants of *KIF5B-RET* fusion gene, we examined only five variants.

Table 1. Clinicopathological features of 3 patients with non-small cell lung cancer with *RET* fusions

Patient number	Partner genes	Sex	Age (yr)	Smoking status	Histology	Differentiation	Tumor size	Lymph node status	Stage	*EGFR*	*KRAS*	*BRAF*	*EML4-ALK*
1	*KIF5B**	F	53	Never	AC	Poor	4 cm	N2	IIIA	-	-	-	-
2	*KIF5B**	F	62	Never	AC	Well	2.4 cm	N0	IA	-	-	-	-
3	*CCDC6*†	F	59	Never	AC	Poor	4 cm	N2	IIIA	-	-	-	-

*Fusion between *KIF5B* exon 15 and *RET* exon 12; †Fusion between *CCDC6* exon 1 and *RET* exon 12. F, female; AC, adenocarcinoma.
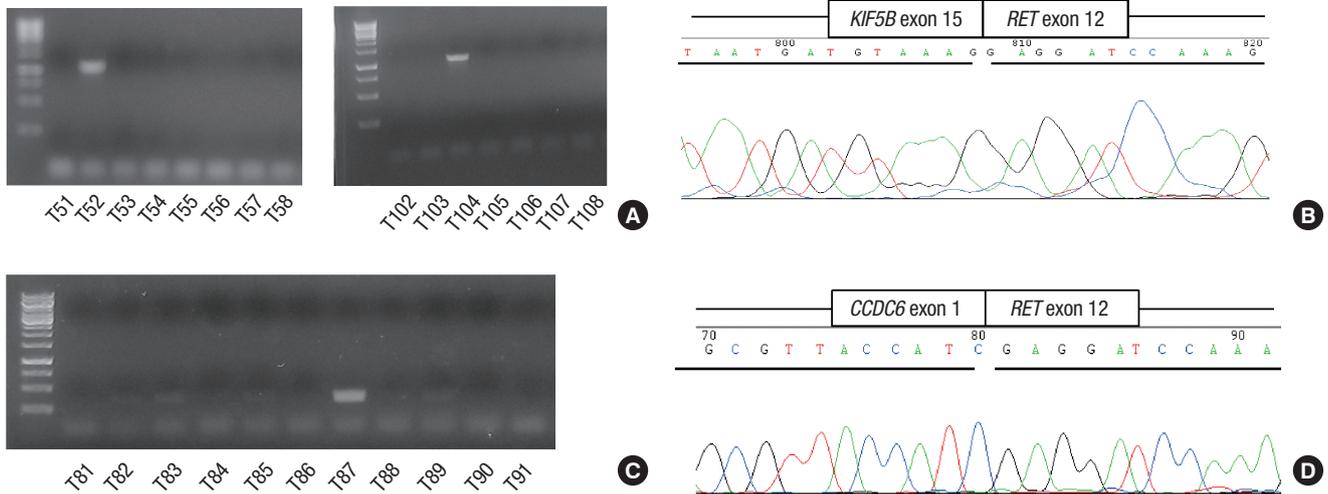
Fig. 1. Detection of *RET* fusion genes by RT-PCR and sequencing. RT-PCR results of *KIF5B-RET* fusion genes (**A**). Nucleotide sequencing of the RCR product of *KIF5B-RET* (K15;R12) (**B**). RT-PCR result of *CCDC6-RET* fusion gene (**C**). Nucleotide sequencing of the RCR product of *CCDC6-RET* (**D**).

However, the other two variants, K24;R8 and K15;R11, are extremely rare and only one case for each fusion has been reported until now (6, 9). In addition, the results of RT-PCR analysis were not further examined by fluorescence in situ hybridization (FISH). Although FISH is more effective method for detection chromosome rearrangement, the high cost and need for technical expertise limits its wide usage. Furthermore, unlike PCR, FISH cannot distinguish between different *RET* fusion variants. Therefore, we employed RT-PCR and direct sequencing to evaluate the profile of *RET* fusion genes in Korean NSCLCs.

DISCLOSURE

The authors have no conflicts of interest to disclose.

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Supplement Table 1. Characteristics of study population and 3 patients with *RET* fusion gene

Variables	No. of patients, n = 156 (%)	*RET* fusion gene		
		Positive, n = 3 (%)	Negative, n = 153 (%)	*P* value*
Age				
Mean	63.8 ± 8.7	58.0 ± 4.6	63.9 ± 8.7	0.243[†]
≤ 64	80 (51.3)	3 (100.0)	77 (50.3)	0.246
> 64	76 (48.7)	0 (0.0)	76 (49.7)	
Sex				
Male	105 (67.3)	0 (0.0)	105 (68.6)	0.034
Female	51 (32.7)	3 (100.0)	48 (31.4)	
Residential area				
Urban	100 (64.1)	3 (100.0)	97 (63.4)	0.553
Rural	56 (35.9)	0 (0.0)	56 (36.6)	
Smoking status				
Ever	102 (65.4)	0 (0.0)	102 (66.7)	0.040
Never	54 (34.6)	3 (100.0)	51 (33.3)	
FEV1/FVC (%)				
< 70	60 (40.8)	1 (33.3)	59 (41.0)	1.000
≥ 70	87 (59.2)	2 (66.7)	85 (59.0)	
Stage				
I	88 (56.4)	1 (33.3)	87 (56.9)	0.581
II or IIIA	68 (43.6)	2 (66.7)	66 (43.1)	
Histology				
SCC	52 (33.3)	0 (0.0)	52 (34.0)	0.551
AC	104 (66.7)	3 (100.0)	101 (66.0)	
Histologic grade				
Well	47 (30.1)	1 (33.3)	46 (30.1)	0.365
Moderate	89 (57.1)	1 (33.3)	88 (57.5)	
Poor	20 (12.8)	1 (33.3)	19 (12.4)	

P value was obtained by chi-squared test; [†]*P* value was obtained by Student t-test. FEV1, forced expiratory volume at one second; FVC, forced vital capacity; SCC, squamous cell carcinoma; AC, adenocarcinoma.