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KIF5B-RET fusions in lung adenocarcinoma

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Abstract

We identified in-frame fusion transcripts of *KIF5B* (the kinesin family 5B gene) and the *RET* oncogene, which are present in 1–2% of lung adenocarcinomas (LADCs) from people from Japan and the United States, using whole-transcriptome sequencing. The *KIF5B-RET* fusion leads to aberrant activation of RET kinase and is considered to be a new driver mutation of LADC because it segregates from mutations or fusions in *EGFR*, *KRAS*, *HER2* and *ALK*, and a RET tyrosine kinase inhibitor, vandetanib, suppresses the fusion-induced anchorage-independent growth activity of NIH3T3 cells.

A considerable proportion of LADCs, the most common histological type of lung cancer that comprises ~40% of the total cases, develops through activation of oncogenes, for example, somatic mutations in *EGFR* (10–50% of cases) or *KRAS* (10–30% of cases) or fusion of *ALK* (5% of cases), in a mutually exclusive manner[1–4]. Tyrosine kinase inhibitors (TKIs) targeting the EGFR and ALK proteins are effective in the treatment of LADCs that carry *EGFR* mutations and *ALK* fusions[1–3], respectively.

We performed whole-transcriptome sequencing (RNA sequencing)[5] of 30 LADC specimens from Japanese individuals to identify new chimeric fusion transcripts that could be targets for therapy[3,5,6]. These LADCs were 2 carcinomas with *EML4-ALK* fusions, 4 with *EGFR* or *KRAS* mutations and 24 without these fusions or mutations (Supplementary Table 1). Identifying candidate fusions represented by >20 paired-end reads and validation by Sanger sequencing of the RT-PCR products (Supplementary Methods) led to the identification of seven fusion transcripts, including *EML4-ALK* (Supplementary Table 1). We detected one of these fusions between *KIF5B* on chromosome 10p11.2 and *RET* on chromosome 10q11.2 in subject BR0020 (Fig. 1 and Supplementary Fig. 1a). We then further investigated this fusion, as fusions between *RET* and genes other than *KIF5B* have previously been shown to drive papillary thyroid tumor formation[6,7].

RT-PCR and a Sanger sequencing analysis of 319 LADC specimens from Japanese individuals (Supplementary Table 2), including 30 that had been subjected to whole-transcriptome sequencing, revealed that 1.9% (6 out of 319) expressed *KIF5B-RET* fusion transcripts (Fig. 1b and Supplementary Fig. 1b). We identified four variants in these six tumors, and all of these variants were in frame (Fig. 1a).

A genomic PCR analysis of the six tumors that were positive for RET fusions revealed somatic fusions of the *KIF5B* introns 15, 16, 23 or 24 at chromosome 10p11.2 with the *RET* introns 7 or 11 at 10q11.2 (Supplementary Fig. 1c,d), indicating that a chromosomal inversion had occurred between the long and short arms in the centromeric region of chromosome 10 (Supplementary Figs. 1e and 2). We verified this chromosomal inversion using fluorescence *in situ* hybridization, which revealed a split in the signals for the probes

that flank the *RET* translocation sites in tumors positive for the *KIF5B-RET* fusion (Supplementary Fig. 2).

The tumors positive for the *KIF5B-RET* fusion were all well or moderately differentiated (Table 1 and Supplementary Fig. 3). None of the subjects with these tumors had a history of thyroid cancer, and none showed abnormal findings in their thyroid tissues as determined by computed tomography or positron emission tomography before surgery for LADC. All five examined tumors with the *KIF5B-RET* fusion were positive for thyroid transcription factor 1 (TTF-1) and napsin A aspartic proteinase (Napsin A)[8] but were negative for thyroglobulin[9], indicating that they were of pulmonary origin (Table 1 and Supplementary Fig. 3). The LADCs that were positive for the *KIF5B-RET* fusion showed twofold to 30-fold higher *RET* expression than non-cancerous lung tissues (Fig. 1b and Supplementary Figs. 4 and 5). An immunohistochemical analysis using an antibody against the C-terminal region of the RET protein detected positive cytoplasmic staining in the tumor cells of the fusion-positive LADCs (Table 1 and Supplementary Fig. 3b) but did not detect this staining in any of the non-cancerous lung cells. A western blot analysis confirmed the expression of the fusion proteins in the LADCs (Supplementary Fig. 6).

To address the prevalence of *KIF5B-RET* fusions in LADCs from individuals of non-Asian ancestry, we examined LADCs in cohorts from the United States and Norway (Supplementary Table 2). We detected a fusion transcript in 1 of the 80 (1.3%) subjects from the United States (an individual of European ancestry) (Supplementary Fig. 7), but we detected no fusion transcripts in the 34 subjects from Norway (Supplementary Table 3); *KIF5B-RET* fusions occurred in 1–2% of LADCs in both Asians and non-Asians. The individual from the United States with the *RET* fusion was classified as an ‘ever smoker’, whereas the six individuals from Japan with the *RET* fusion were ‘never smokers’ (Table 1). Therefore, prevalence of LADC with regard to smoking status is unclear. We did not detect the *KIF5B-RET* fusion in other major subtypes of lung cancer, including 234 squamous-cell, 17 large-cell and 20 small-cell lung carcinomas (Supplementary Table 3). The fusion was also not present in other types of adenocarcinomas, including those of the ovary ($n = 100$) and colon ($n = 200$) (data not shown), suggesting that it is specific to LADC.

All seven subjects with LADC harboring the *KIF5B-RET* fusion were negative for *EGFR, KRAS* and *ALK* mutations or fusions and were negative for mutations in *HER2*, which is an additional driver mutation in LADC[10] (Table 1 and Supplementary Table 4). The mutually exclusive nature of the *RET* fusions and other oncogenic alterations[1,2,11] suggests that the *KIF5B-RET* fusion is a driver mutation. All proteins encoded by the four *KIF5B-RET* fusion variants contained the KIF5B coiled-coil domain, which functions in protein dimerization[12], and retained the full RET kinase domain, similar to other types of oncogenic *RET* fusions observed in thyroid tumors (Fig. 1a)[13]. The KIF5B-RET proteins are likely to form a homodimer through the coiled-coil domain of KIF5B, causing an aberrant activation of the kinase function of RET in a manner similar to the *PTC-RET* and *KIF5B-ALK* fusions[7,14]. In fact, the N-terminal portion of the KIF5B coiled-coil region, which is retained in all variants, has been predicted to have the ability to dimerize through two coiled-coil structures[15]. Consistently, when the *KIF5B-RET* variant 1 was exogenously expressed in H1299 human lung cancer cells without wild-type or fusion *RET* expression, Tyr905, which

is located in the activation loop of the RET kinase site[15,16], was phosphorylated in the absence of serum stimulation, indicating an aberrant activation of *RET* kinase[16,17] by fusion with *KIF5B* (Fig. 1c). This phosphorylation was suppressed by vandetanib, a TKI against RET (as well as other tyrosine kinases, including EGFR and VEGFR)[18] (Fig. 1c and Supplementary Fig. 8).

Expression of exogenous *KIF5B-RET*, but not *KIF5B-RET*-KD (a kinase-dead mutant corresponding to S765P in wild-type RET[17]), induced morphological transformation (Supplementary Fig. 9) and anchorage-independent growth of NIH3T3 fibroblasts in a way that was analogous to the induction caused by mutant *KRAS* (KRASV12) (Fig. 1d). Consistently, phosphorylation of Tyr905 was higher in the KIF5B-RET protein than in the KIF5B-RET-KD protein. The anchorage-independent growth induced by *KIF5B-RET* was suppressed by treatment with vandetanib (<1 μM), whereas the growth induced by mutant *KRAS* was not (Fig. 1d). These results are similar to those observed for *RET* fusions in thyroid cancer[19]. We also detected phosphorylation of the KIF5B-RET protein at Tyr905 in fusion-positive LADC specimens (Supplementary Fig. 6). These results suggest that the *RET* fusions are a previously unidentified LADC driver mutation and a potential target for existing TKIs, including vandetanib, which has been recently approved by the US Food and Drug Administration for the treatment of thyroid cancer[18]. Further studies are warranted to promote molecular subtype diagnoses and personalized therapy options for LADC. For this purpose, both the clinical and biological features of this fusion are being investigated. For further information, please see the Supplementary Note and Supplementary Tables 5 and 6.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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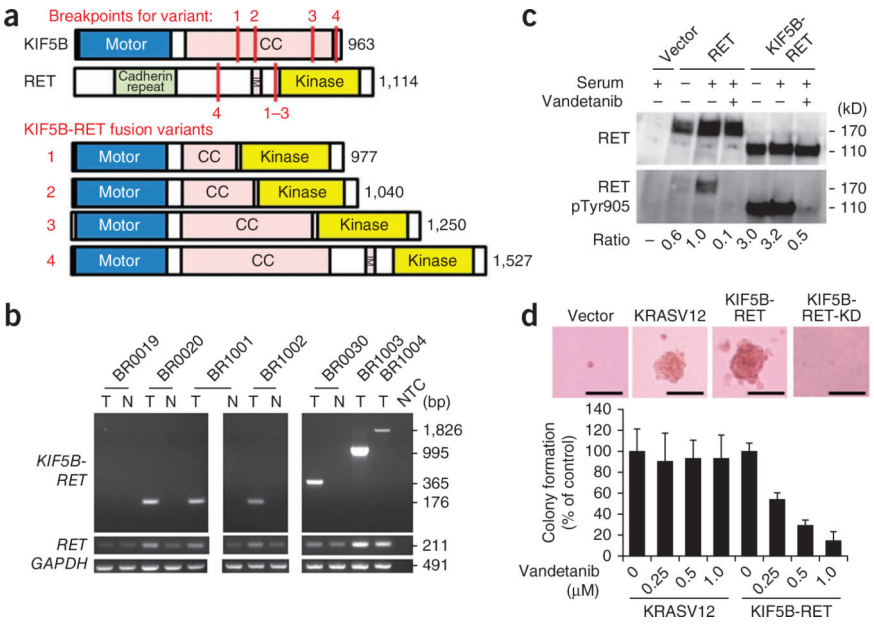

Figure 1.

KIF5B-RET fusions in LADC. **(a)** Schematic representations of the wild-type KIF5B and RET proteins as well as the four fusion variants identified in this study. The breakpoints for each variant are indicated with red lines. CC, coiled coil; TM, transmembrane. **(c)** Detection of *KIF5B-RET* fusions by RT-PCR. RT-PCR products for the *RET* kinase domain (exons 12 and 13) and *GAPDH* are shown below. Six LADCs positive for *KIF5B-RET* fusions (T) are shown, with four corresponding non-cancerous lung tissues (N), a no-template control (NTC) and one LADC that was negative for the fusion (BR0019). **(c)** Activation of RET kinase activity in the KIF5B-RET protein and the suppression of this activity by vandetanib. H1299 lung cancer cells were transfected with an empty vector, wild-type RET (RET) or KIF5B-RET expression plasmids and treated either with DMSO (serum) or vandetanib, as indicated. The ratios of phosphorylated Tyr905 (pTyr905) RET to total RET signals with respect to wild-type RET after the serum treatment are listed below the gels. (**d**) Anchorage-independent growth of NIH3T3 cells expressing KIF5B-RET protein and the suppression of this growth by vandetanib. Representative pictures of colonies without vandetanib treatment (top). Scale bars, 50 μm. Bar graph showing the percentage (± s.d.) of colonies formed after treatment with the indicated amounts of vandetanib (average results of three independent experiments) with respect to those formed by DMSO-treated cells. The study was approved by the institutional review boards of institutions participating in this study.

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Table 1

Characteristics of lung adenocarcinomas with the *KIF5B-RET* fusion

Sample	Country	Sex	Age[a]	Smoking	*KIF5B-RET* fusion[b]	Pathological stage	Pathological findings	RET staining	TTF-1 staining	Napsin A staining	Thyroglobulin staining
BR0020	Japan	Male	57	Never	K15; R12 (variant 1)	IIB	Moderately differentiated ADC	+	+	+	−
BR1001	Japan	Female	65	Never	K15; R12 (variant 1)	IB	Well differentiated ADC	+	+	+	−
BR1002	Japan	Female	64	Never	K15; R12 (variant 1)	IB	Well differentiated ADC	+	+	+	−
BR0030	Japan	Male	57	Never	K16; R12 (variant 2)	IA	Well differentiated ADC	+	+	+	−
BR1003	Japan	Male	28	Never	K23; R12 (variant 3)	IA	Well differentiated ADC	+	+	+	−
BR1004	Japan	Female	71	Never	K24; R8 (variant 4)	IA	Moderately differentiated ADC	NT	NT	NT	NT
NCI1580	USA	Male	63	Ever[c]	K15; R12 (variant 1)	II	Moderately differentiated ADC	NT	NT	NT	NT

[a] Age in years.

[b] Fused exon numbers of *KIF5B* (K) and *RET* (R); and variant types (in parentheses) are shown. None of the subjects had oncogenic *EGFR, KRAS, HER2* or *ALK* mutations or fusions.

[c] The number of pack years smoked for this subject is not known. NT, not tested.