ONLINE METHODS

Study participants. Participants were drawn from 14 studies (**Supplementary Table 1**). Cases had histologically confirmed lung cancer. Each participating study obtained informed consent from study participants and approval from its respective institutional review board for this study. Studies obtained institutional certification permitting data sharing in accordance with the US National Institutes of Health (NIH) Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies (GWAS), with the exception of the component of the GELAC study that was not scanned at the NCI.

Genotyping and quality control. Genome-wide scanning data came from two sources. Internal sets (HKS, SNU, CNULCS, SWHS, YLCS and components of samples from Japan and GELAC) were genotyped at the NCI CGR Laboratory using the Illumina 660W SNP microarray. External sets were genotyped as follows: (i) samples from CAMSCH, FLCS, GDS, SLCS, TLCS and WLCS were genotyped on contract at Gene-Square Biotech in Beijing using the Illumina 660W SNP microarray; (ii) samples from GELAC were genotyped on contract at GeneTech Biotech in Taiwan on the Illumina 370K SNP microarray in a pilot project, and remaining samples were genotyped on contract at deCODE Genetics in Iceland using the Illumina 610Q SNP microarray and initially reported elsewhere[11]; (iii) a subset of samples from Japan were genotyped at MSKCC using the Illumina 610Q SNP microarray; and (iv) samples from Singapore were genotyped at the Genome Institute of Singapore on the Illumina 660W SNP microarray. The scanned intensity data from external sources were collected, and genotypes were clustered and called at the CGR using Illumina Genome Studio v2011.1 on the basis of the GenTrain2 calling algorithm. Genotype clusters were estimated from samples with preliminary completion rates of greater than 98% per cluster group.

Genotyping was attempted for a total of 5,568 samples on the Illumina 660W SNP microarray at the CGR. Six samples could not be loaded into Illumina Genome Studio because of their low intensities, and 16 samples failed to scan because of broken chips. In addition, a total of 5,946 samples were genotyped at Gene-Square Biotech (3,828), deCODE Genetics and GeneTech Biotech (1,232), MSKCC (374) and the Genome Institute of Singapore (512); the distribution of samples genotyped per SNP microarray chip was as follows: Illumina 660W (4,340), Illumina 610Q (1,494) and Illumina 370K (112). Seven samples (all from Gene-Square Biotech) could not be loaded into Illumina Genome Studio because of their low intensities. In addition, 111 samples from 4 studies (FLCS, GDS, SLCS and TLCS) were excluded due to laboratory processing errors. The combined 11,374 samples with genotypes mapped to 11,025 unique individuals drawn from 14 studies.

We subsequently performed quality control filtering at the sample level in 19 quality control groups (**Supplementary Table 6**). Samples were exclude that had low completion rates ($n = 725$ samples) and extreme mean heterozygosity rates ($n = 116$). Thresholds were chosen on the basis of the sample completion rate or sample mean heterozygosity distribution for each quality control group (**Supplementary Table 6**) and on the basis of discordant expected duplicate samples ($n = 6$). There were samples that were excluded for multiple reasons, and the total number of unique samples excluded was 761 (**Supplementary Table 6b**). Genotype data for the remaining 10,613 samples were merged, resulting in data from 10,312 unique individuals. The genotype concordance rate for expected duplicates ($n = 311$) was greater than 99.9%. Further quality control analysis at the individual level led to the exclusion of samples with (i) gender discordance ($n = 94$); (ii) less than 86% Asian ancestry ($n = 3$); (iii) first-degree relatives who were also genotyped in the study ($n = 136$ subjects); and (iv) incomplete phenotype or unknown histology, as well as those who had ever smoked or were deemed ineligible ($n = 15$). Thus, the total number of scanned subjects after both quality control and analytic exclusions was 10,054 (5,510 cases and 4,544 controls). A summary of the number of excluded loci by study is shown in **Supplementary Table 6c**.

TaqMan custom genotyping assays (Applied Biosystems) were designed and optimized for 13 SNPs, including 9 in the NCI scan data and 4 surrogates not in this scan. In an analysis of 385 samples from 7 studies, comparison of the Illumina calls with the results from TaqMan assays conducted at the NCI CGR showed an average concordance rate of 99.97% (with a range of 99.7–100%) for the overlapping 9 SNPs. The Cancer Institute and Hospital at the Chinese Academy of Medical Sciences also conducted TaqMan genotyping for 7 SNPs on 201 previously scanned samples from 5 studies. Comparison of the Illumina calls with the results of TaqMan assays showed an average concordance rate of 99.93% (with a range of 99.5–100%). In examining the concordance between rs2395185 (scan) and its perfect surrogate rs28366298 (TaqMan), we applied genotype mapping GG→AA, GT→AC and TT→CC to confirm reproducibility of genotyping between platforms.

For the replication phase, we analyzed an additional 3,933 individuals (1,023 cases and 2,910 controls) with TaqMan data, and an additional 79 individuals (76 cases and 3 controls) genotyped using the Illumina 660W array at Gene-Square Biotech were available for analysis. Thus, the final number of subjects included in the analyses was 14,066 (6,609 cases and 7,457 controls; **Supplementary Table 1**). SNP assays with locus call rates lower than 90% or Hardy-Weinberg equilibrium P values less than 1.0×10^{-7} in each quality control group were excluded. In total, 596,032 SNPs remained in the analytic data set. After setting the minimum minor allele frequency (MAF) to 0.01, we excluded 83,806 loci from the association analysis. Thus, 512,226 SNPs were analyzed in the association studies reported here.

Statistical analyses. Data analysis and management were performed with GLU (Genotyping Library and Utilities version 1.0), a suite of tools available as an open-source application for the management, storage and analysis of GWAS data. Assessment of the population structure of study participants was performed with the GLU struct.admix module using the Japanese in Tokyo, Japan (JPT) and Han Chinese in Beijing, China (CHB), Utah residents of Northern and Western European ancestry (CEU) and Yoruba from Ibadan, Nigeria (YRI) samples as the reference populations (HapMap Build 28). A set of 33,165 SNPs with low pairwise correlation ($r^2 < 0.01$) was selected for this analysis. Three individuals were estimated to have less than 86% Asian ancestry (**Supplementary Fig. 4**).

The genotypes for all subject pairs were computed for cryptic relatedness using the GLU qc.ibds module with the same set of selected SNPs. In addition to 68 pairs of unexpected duplicates, we detected 33 parent-offspring and 41 full-sibling pairs. For the 142 unexpected duplicates and first-degree relative pairs, 1 subject from each simple pair was excluded. For each family with multiple relative pairs detected, only one randomly chosen subject was included in the principal-components analysis (PCA). To address the underlying population substructure, PCA was conducted using the GLU struct.pca module, a program similar to EIGENSTRAT[49,50], with the same set of SNPs (**Supplementary Fig. 5a,b**). Three samples with less than 86% Asian ancestry were excluded on the basis of PCA.

Association analysis. Association analyses were conducted using logistic regression, adjusted for age (in 10-year categories), study group and eigenvectors, if they were significant when analyzed in the base models. For analysis of all cases versus controls, we adjusted for EV1, EV2 and EV4. For analysis of adenocarcinoma cases versus controls, we adjusted for EV2 and EV4. For analysis of squamous cell cases versus controls, we adjusted for EV8. Each SNP genotype was coded as a count of minor alleles (trend effect). A score test with 1 degree of freedom was performed on all genetic parameters in each model to determine statistical significance. The unscaled λ value for all cases versus controls in the main effect model was 1.014, and λ_{1000} was 1.003, with corrected λ calculated as $\lambda_{corrected} = 1 + (\lambda - 1) \times (n_{case}^{-1} + n_{control}^{-1})/(2 \times 10^{0-3})$.

We assessed heterogeneity in genetic effects across studies using the Cochran's Q statistic, which conforms to a χ-squared distribution with $k - 1$ degree of freedom, where k is the number of studies.

For the inclusion of TaqMan data for the SNPs that failed assay design (rs2395185 and rs10197940), we conducted a fixed-effects meta-analysis by combining the aggregate results from their perfect surrogates (rs28366298 and rs2290368, respectively) scanned in the GWAS with their own results based only on the additional TaqMan samples not used in the GWAS association analyses.

Genotype-environment interactions with environmental tobacco smoke were assessed using logistic regression for studies with such information available and adjusted by age, study group, the main effect of the SNP and environmental tobacco smoke, and the interaction term.

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Estimate of recombination hotspots. To identify recombination hotspots in the region, we used SequenceLDhot[51], a program that uses the approximate marginal likelihood method[52] and calculates likelihood ratio statistics at a set of possible hotspots. Drawn from scanned controls, 100 individuals were randomly sampled from Han Chinese, Japanese and Korean samples. Three independent recombination hotspot inferences were analyzed and are represented as three different colored lines in **Figure 1**. Specifically, for the *VTI1A* regional plot, genotypes of 70 SNPs spanning chromosome 10 114,362,000–114,593,000 (UCSC Genome Build hg18) were phased using PHASE v2.1 (ref. 53) to calculate background recombination rates. The PHASE outcome was used as direct input for the SequenceLDhot program, and LD was estimated as r^2 for 70 SNPs within a ~230-kb region, and a heatmap was drawn using the snp.plotter program[54]. Similarly, we started with the genotypes of 63 SNPs for the *ROS1-DCBLD1* regional plot and the genotypes of 59 SNPs for the HLA class II locus.

Imputation analysis. To begin to fine map newly identified regions, we imputed all the SNPs catalogued in 1000 Genomes Project data, March 2012 release, and the DCEG Imputation Reference Set version 1 (ref. 27). The IMPUTE2 program[28] was used to impute a 1-Mb region centered on the index SNP for each of the three regions, using recommended default settings. Imputed SNPs with INFO of <0.3 were excluded from association analysis using the SNPTEST program v2.3 (see URLs), which considered probabilistic genotypes out of imputation. Because 1000 Genomes Project data was based on the NCBI Build 37 reference genome, we conducted liftOver (see URLs) on our scan data from Build 36 to 37 before imputation.

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KIF5B-RET fusions in lung adenocarcinoma

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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

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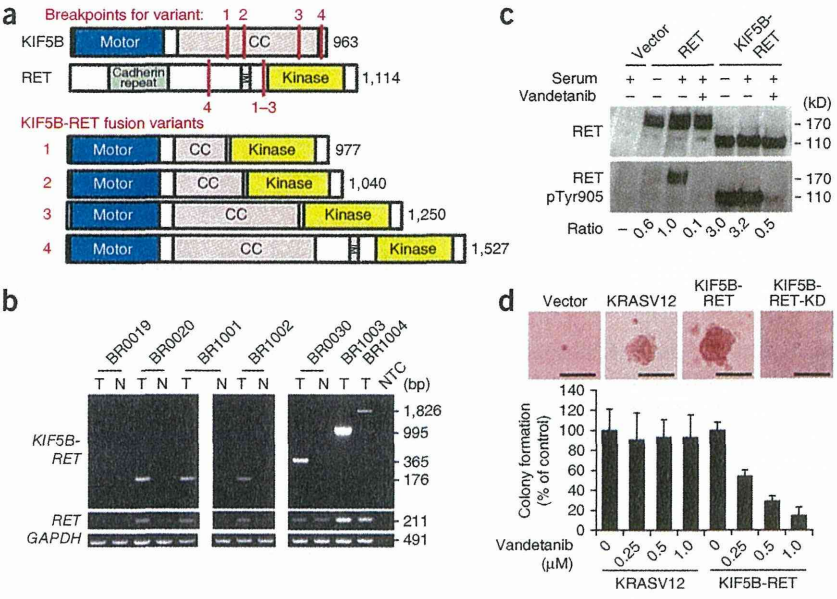

Figure 1 *KIF5B-RET* fusions in LADC. (**a**) Schematic representations of the wild-type KIF5B and RET proteins as well as the four fusion variants identified in this study. The breakpoints for each variant are indicated with red lines. CC, coiled coil; TM, transmembrane. (**b**) Detection of *KIF5B-RET* 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.

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

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	Thyroglobin 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.

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**.

Note: Supplementary information is available on the Nature Medicine website.

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RNA sequencing: H.I., K.Y., M.H., T.N. and H.S. Sequence data processing: Y.T., S.C. and I.Y. Molecular biological analyses: T.K., Y.S., R.I., H. Ogiwara, T.O., M.E., A.J.S., H. Okayama, A.H., Y.A. and S.O. Clinical and pathological analyses: K.T., K.F., V.S., S.W., I.S. and H.T. Manuscript writing: T.K., H.I. and T.S. Study design: T.K., H.I., C.C.H., T.Y., J.Y. and T.S.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Review Article

RET fusion gene: Translation to personalized lung cancer therapy

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Development of lung adenocarcinoma (LADC), the most frequent histological type of lung cancer, depends in many cases on the activation of "driver" oncogenes such as *KRAS*, epidermal growth factor receptor (*EGFR*), and anaplastic lymphoma kinase (*ALK*). Inhibitors that target the EGFR and ALK tyrosine kinases show therapeutic effects against LADCs containing *EGFR* gene mutations and *ALK* gene fusions, respectively. Recently, we and others identified the *RET* fusion gene as a new targetable driver gene in LADC. The RET fusions occur in 1–2% of LADCs. Existing US Food and Drug Administration-approved inhibitors of RET tyrosine kinase show promising therapeutic effects both *in vitro* and *in vivo*, as well as in a few patients. Clinical trials are underway to investigate the therapeutic effects of RET tyrosine kinase inhibitors, such as vandetanib (ZD6474) and cabozantinib (XL184), in patients with *RET* fusion-positive non-small-cell lung cancer. (*Cancer Sci* 2013; 104: 1396–1400)

Personalized Therapy of LADC

Lung cancer is the leading cause of cancer-related mortality worldwide. Lung adenocarcinoma (LADC) is the most frequent type of lung cancer. LADC occurs both in smokers and non-smokers, and its incidence is increasing.[1] Genome analyses of LADC show that these tumors contain distinct genetic alterations that activate oncogenes.[2,3] Genetic alterations that result in the activation of several oncogenes are detected in a mutually exclusive manner (Fig. 1); of the hundreds of genes mutated in each case of LADC, these oncogenes are considered to be "driver genes".[4] Remarkably, molecular targeted therapy using inhibitory drugs against activated oncogene products has begun to replace conventional chemotherapy using cytotoxic drugs, even for first-line use.[2]

The epidermal growth factor receptor (*EGFR*) gene is activated by single amino acid substitution mutations or in-frame amino acid deletion mutations in 10–20% of LADC cases in the USA and in 30–40% of cases in East Asia.[2] Tumors harboring these *EGFR* mutations respond to EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib and gefitinib, thereby improving progression-free survival and quality of life.[5,6] In addition, 3–5% of LADC harbor fusions that result in the activation of the anaplastic lymphoma kinase (*ALK*) gene; such mutations are mutually exclusive with *EGFR* mutations. Inhibitors, such as crizotinib, that target ALK tyrosine kinase show marked therapeutic effects against ALK fusion-positive LADCs.[7–9] These results indicate that personalized therapy for LADC using TKIs selected on the basis of somatic genetic alterations has been realized already; indeed, 20% of USA/European and 40% of Asian LADC patients benefit from such therapies.

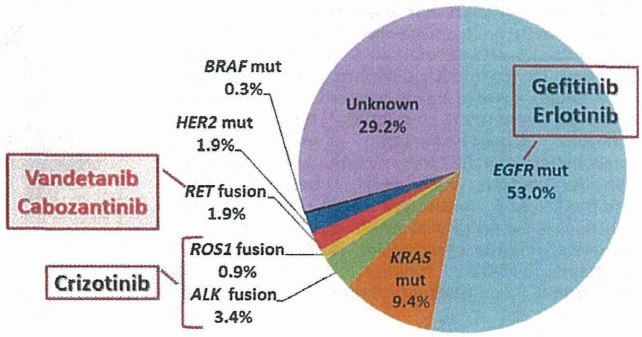

Fig. 1. Pie chart showing the fraction of Japanese lung adenocarcinoma patients that harbor "driver" gene mutations. Surgical specimens from 319 stage I–II lung adenocarcinomas deposited in the National Cancer Center Biobank (Japan) were subjected to analysis. The *EGFR*, *KRAS*, *BRAF*, and *HER2* mutations (mut) were examined using the high resolution melting method, whereas *ALK*, *ROS1* and *RET* fusions were examined by RT-PCR.[12,31] The protocol for this research project has been approved by the institutional review board of the National Cancer Center.

Discovery of the *RET* Fusion Gene as a New Targetable Driver Gene

In 2012, four studies, including one by our group, identified fusions of the *RET* (rearranged during transfection) oncogene[10–13] (Fig. 2). *RET* is a well-known driver oncogene kinase for thyroid cancer, and both activating mutations and fusions of this gene have been observed.[14,15] Germline gain-of-function mutations in *RET* predispose carriers to multiple endocrine neoplasia type 2, which is characterized by medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism, and also to familial medullary thyroid carcinoma syndrome. Somatic gain-of-function *RET* mutations have been observed in 30–50% of sporadic medullary thyroid cancer, and somatic *RET* gene fusions have been observed in 30–50% of sporadic papillary thyroid cancer. The US Food and Drug Administration (FDA) have approved two inhibitory drugs, vandetanib (ZD6474) and cabozantinib (XL184), for the treatment of advanced medullary thyroid cancer. The molecular process for generating a *RET* fusion is similar to the mechanism underlying *ALK* fusion: the most frequent *RET*

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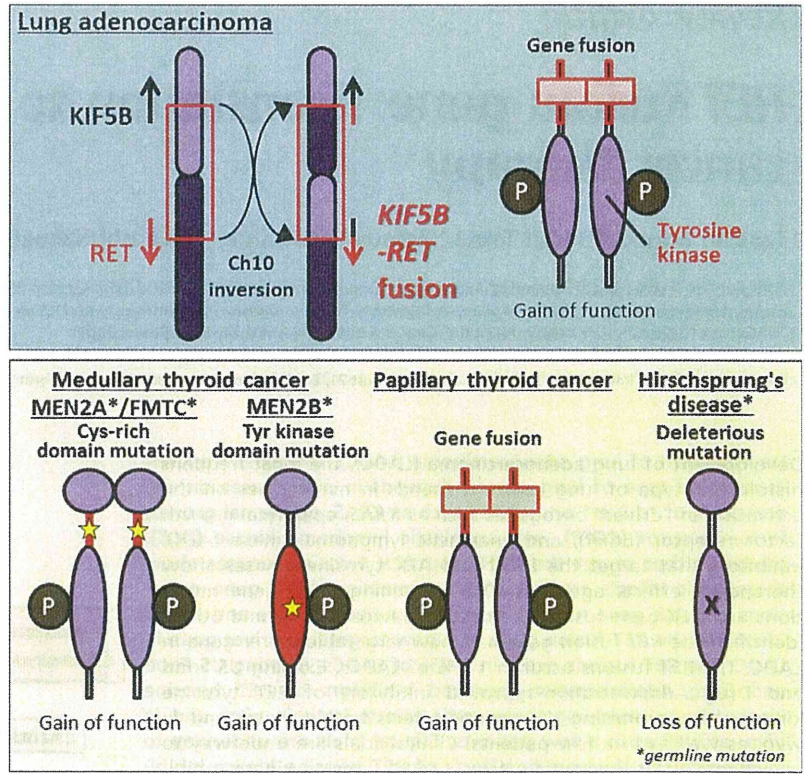

Fig. 2. Involvement of the *RET* gene in lung and thyroid carcinogenesis and in a developmental disorder. Upper panel, somatic inversion in chromosome 10 results in *KIF5B–RET* fusions. The RET fusion protein has constitutive tyrosine (Tyr) kinase activity, representing a gain-of-function alteration. Lower panel, *RET* alterations in other diseases. A germline gain-of-function mutation of *RET* drives thyroid carcinogenesis in patients with multiple endocrine neoplasia type 2 (MEN2). Somatic gain-of-function mutation and translocation of *RET* cause medullary and papillary thyroid cancers, respectively. Germline loss-of-function *RET* mutations cause Hirschsprung's disease, a hereditary disorder characterized by the absence of enteric ganglia in variable segments of intestine. FMTC, familial medullary thyroid carcinoma; P, phosphorylation; X, inactivating mutation.

fusion, *KIF5B–RET*, is generated by a pericentric inversion in chromosome 10, whereas the most frequent *ALK* fusion, *EML4–ALK*, is generated by a paracentric inversion in chromosome 2 (Fig. 2).

Four different strategies resulted in the discovery of the same *RET* fusion gene (Table 1, Fig. 3). We carried out whole-transcriptome sequencing using RNA from 30 snap-frozen surgical LDAC specimens to identify novel fusion-gene transcripts.[12] Ju *et al.*[13] analyzed the whole genome and transcriptome of a single young (33-year-old) LADC patient. Lipson *et al.*[11] carried out targeted-capture sequencing of 145 cancer-relevant genes from genomic DNA obtained from 24 formalin-fixed paraffin-embedded tumor samples to identify genes mutated or fused in LADC. Takeuchi *et al.*[10] carried out a FISH-based screen against known fusion kinase and partner genes to detect rearrangement of oncogenes in >1500 LADC cases.

To date, *RET* fusions have been identified that involve four fusion partners comprising nine subtypes of fusion variants: *KIF5B*, *CCDC6/PTC/H4*, *NCO4/PTC3/ELE1*, and *TRIM33 /PTC7*.[16] The latter three partners are also fused to *RET* in thyroid cancer, whereas *KIF5B* is not. The deduced features of the proteins encoded by all types of *RET* fusion gene are similar to those of ALK: coiled-coil domains in the N-terminal fusion partners cause the RET domains to dimerize, resulting in activation of RET tyrosine kinase in the absence of ligands (Fig. 2). The ligand-independent dimerization and constitutive activation of RET protein are also caused by gain-of-function mutations and translocations of *RET*, which have been detected in sporadic and hereditary thyroid cancers.[15] In fact, autophosphorylation of the KIF5B–RET fusion protein, representing RET protein activation, was observed in LADC tissues harboring the corresponding *RET* fusion gene,[12] as well as in cells cultured in the absence of serum. The transforming and signal-addictive activities of KIF5B–RET fusion proteins are suppressed by FDA-approved drugs (e.g., vandetanib, sorafenib, and sunitinib), which themselves suppress RET kinase.[10–12] In addition, the LADC cell line, LC-2/ad, which harbors a *CCDC6–RET* fusion, is sensitive to these drugs both *in vitro* and *in vivo*.[17,18] Unfortunately, these drugs are not approved for use as treatments for lung cancer; however, the existing data led us to investigate their therapeutic effects in clinical trials, as described below.

Prevalence and Characteristics of *RET* Fusion-Positive LADC

Several studies have validated the presence of *RET* fusion in a small subset of non-small-cell lung cancers (NSCLCs).[16,19–24] The total number of examined cases has reached approximately 5000 (Table 1). Most of the positive cases are LADC, but several cases involve other histological types of NSCLC, such as adenosquamous carcinoma.[19,20] The *RET* fusions are present in 1–2% of NSCLC/ADC of patients of both Asian and European descent. Several studies indicate that *RET* fusion occurs preferentially in young, never-smoker, and light-smoker patients.[10,12,20]

The LADCs harboring *KIF5B–RET* fusions are well or moderately differentiated, similar to LADCs harboring *EGFR* mutations. This is in contrast to *EML4–ALK* fusion-positive LADCs, which tend to show signet-ring and mucinous cribriform patterns.[10] Those LADCs harboring *CCDC6–RET* fusions show such histological features.[10,18]

In our previous study, we did not detect *RET* fusions in a screen of 234 squamous cell, 17 large cell, and 20 small-cell lung cancers.[12] Adenocarcinomas of other organs, such as colon (n = 200) and ovary (n = 100), were also negative for *RET* fusion. To date, whole-transcriptome analysis of other organs has not identified *RET* fusions in cancers outside the lung. Therefore, *RET* fusion may occur mainly in LADC and papillary thyroid cancer.

Table 1. Prevalence of *RET* gene fusion in non-small-cell lung cancer (NSCLC)

Institution	No. of cases examined	No. of *RET* fusion (+) cases	*RET* fusion%	Fusion type	Ref.
	NSCLC/lung adenocarcinoma				
National Cancer Center, Japan	704/433	7/7	1.0/1.6	*KIF5B–RET*: 7	12
Japan Foundation for Cancer Research, Japan	1482/1119	13/13	0.9/1.2	*KIF5B–RET*: 12 *CCDC6–RET*: 1	10
Foundation Med, USA	643/561	12/12	1.8/2.1	*KIF5B–RET*: 12	11
Seoul National University, Korea	21/21 (Driver mutation −)	3/3	14/14	*KIF5B–RET*: 3	13
Chinese Academy of Sciences, China	202/202 (Driver mutation −)	2/2	1.0/1.0	*CCDC6–RET*: 2	24
Nagoya City University, Japan	371/270	3/3	0.8/1.1	*KIF5B–RET*: 3	23
Memorial Sloan-Kettering Cancer Center, USA	69/69 (Driver mutation −)	1/1	1.4/1.4	*KIF5B–RET*: 1	21
Fudan University Shanghai Cancer Center, China	936/633	13/11	1.4/1.7	*KIF5B–RET*: 9 *CCDC6–RET*: 3 *NCOA4–RET*: 1	20
Tongji University School of Medicine, China	392/231	6/4	1.5/1.7	*KIF5B–RET*: 6	19
Korea Research Institute of Bioscience and Biotechnology, Korea	6/6 (Female non-smoker)	1/1	17/17	*CCDC6–RET*: 1	22
Memorial Sloan-Kettering Cancer Center, USA	31/31 (Driver mutation −)	5/5	16/16	*KIF5B–RET*: 2 *TRIM33–RET*: 1 (Unknown: 2)	16
Total	4857/3576	66/62	1.4/1.8	*KIF5B–RET*: 55 *CCDC6–RET*: 7 *NCOA4–RET*: 1 *TRIM33–RET*: 1	

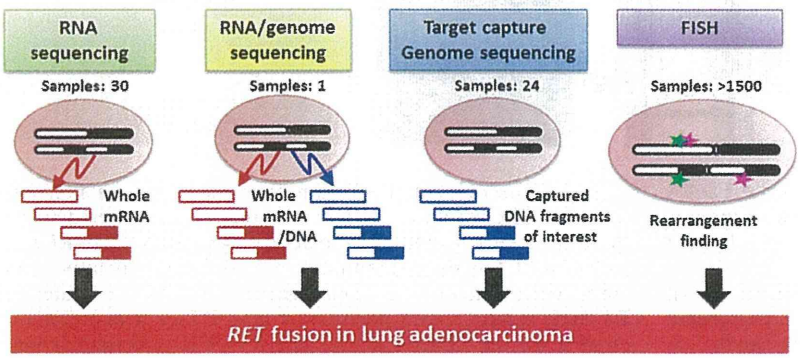

Fig. 3. Strategies used to identify *RET* fusion in lung adenocarcinoma. Four different methods were used to identify novel oncogenic fusions in lung adenocarcinomas.[10–13]

Therapeutic Effects of RET TKIs in Patients with *RET* Fusion-Positive NSCLC

In clinical trials, the ALK TKI, crizotinib, showed a dramatic therapeutic effect against NSCLCs harboring *ALK* gene fusions. Crizotinib was approved for use in the USA in August 2011 and for use in Japan in March 2012.[8] Considering that the *ALK* gene fusion was first identified in NSCLC in 2007, approval has been achieved extremely rapidly. Consequently, the discovery of the *RET* fusion has raised expectations that patients with NSCLCs harboring *RET* fusions will soon benefit from targeted therapy using existing RET TKIs.

Several commercially available multikinase inhibitors, such as vandetanib (ZD6474), cabozantinib (XL184), sorafenib, sunitinib, lenvatinib (E7080), and ponatinib (AP24534), have activity against the RET kinase; however, no selective RET inhibitors have yet been developed for clinical use. Several phase II clinical trials have been initiated to investigate the therapeutic effects of such multikinase inhibitors in patients with advanced *RET* fusion-positive NSCLC (Table 2). As for previous clinical trials of ALK TKIs, all of these trials have open-label and single-arm designs, with response rate as the primary endpoint. One study, carried out by Drilon *et al.* at the Memorial Sloan-Kettering Cancer Center (NCT01639508),

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Table 2. Ongoing phase II clinical trials of RET tyrosine kinase inhibitors in patients with *RET* fusion-positive non-small-cell lung carcinoma

Trial number†	Drug (pharmaceutical company)	Study design	Primary end-point	Enrolment no.	Study start
NCT01639508	Cabozantinib/XL184 (Exelixis)			25	July 2012
UMIN000010095	Vandetanib/ZD6474 (AstraZeneca)			17	Feb 2013
NCT01823068	Vandetanib/ZD6474 (AstraZeneca)	Open-label, single arm	Response rate	17	April 2013
NCT01877083	Lenvatinib/E7080 (Eisai)			20	April 2013
NCT01813734	Ponatinib/AP24534 (ARIAD)			20	June 2013

†Detailed information is available at http://clinicaltrials.gov/ or https://upload.umin.ac.jp.

Table 3. Response of lung adenocarcinoma patients to RET tyrosine kinase inhibitors

Patient	*RET* fusion gene	Inhibitor	Ethnicity	Sex	Age, years	Pathological diagnosis	Smoking history (pack-year)	Response (% decrease)	Reference
1	*TRIM33–RET*	Cabozantinib	Caucasian	Female	41	Papillary adenocarcinoma	Never-smoker	Partial response (66)	16
2	*KIF5B–RET*	Cabozantinib	African-American	Female	75	Poorly differentiated adenocarcinoma	Never-smoker	Partial response (32)	16
3	*KIF5B–RET*	Cabozantinib	Caucasian	Female	68	Mixed subtype adenocarcinoma	Never-smoker	Stable disease	16
4	*KIF5B–RET*	Vandetanib	Caucasian	Male	58	Poorly differentiated adenocarcinoma	Former smoker (5)	Decrease in size	26

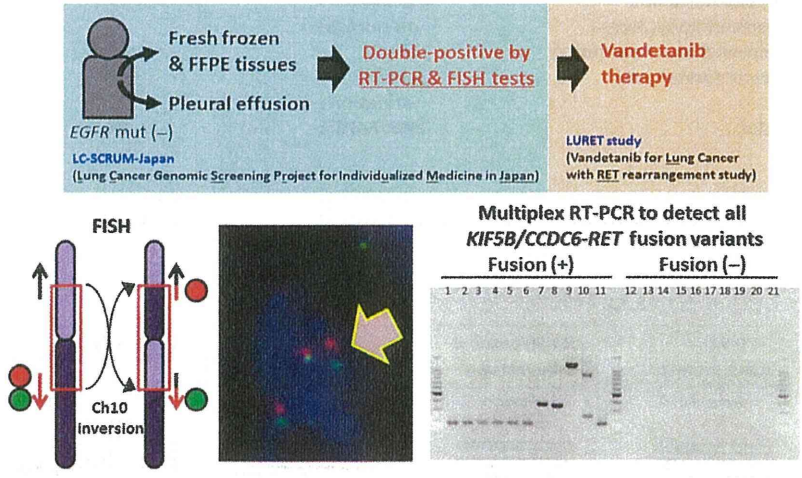

Fig. 4. Consolidated Standards of Reporting Trials diagram of the Lung Cancer Genomic Screening Project for Individualized Medicine in Japan (LC-SCRUM) and the Lung Cancer with *RET* rearrangement (LURET) study in Japan. The LC-SCRUM screen identified 17 *RET* fusion-positive cases from non-squamous non-small-cell lung carcinoma cases without epidermal growth factor receptor (*EGFR*) mutations (mut). The *RET* fusion-positive cases are defined as being positive in both RT-PCR and subsequent FISH tests. Representative pictures of these tests are shown. Fusion-positive cases were treated with vandetanib in the LURET study. Ch10, chromosome 10; FFPE, formalin-fixed paraffin-embedded.

is testing cabozantinib, a drug recently approved by the FDA for the treatment of thyroid cancer. The therapeutic responses of the first three patients to be treated with cabozantinib were reported to be promising (Table 3).[16]

The other phase II clinical trial was initiated by our own group in Japan (UMIN00001009). This trial, designated LURET (Lung Cancer with *RET* rearrangement study), is investigating the therapeutic effects of vandetanib in 17 patients with *RET* fusion-positive NSCLC (Table 2). Because vandetanib is a multikinase inhibitor that is effective against EGFR and vascular endothelial growth factor, this drug was previously examined for its therapeutic efficacy in advanced NSCLC patients in several "all-comer" clinical trials.[25] Those trials were carried out without considering gene alterations in determining eligibility, and the trials did not show significantly greater therapeutic effects than pre-existing therapeutic regimens. Therefore, only *RET* fusion-positive cases, which represent 1–2% of all NSCLCs, are eligible for the LURET study.

To evaluate eligibility for this study, we established a diagnostic method for detecting *RET* fusions using a combination of RT-PCR and FISH (Fig. 4). In this study, RNAs from frozen biopsy tissue or pleural effusion from patients with non-squamous NSCLCs without *EGFR* mutations are subjected to RT-PCR; this method enables us to detect all seven *KIF5B–RET* and *CCDC6–RET* variants identified to date.[16] The positive cases are then subjected to break-apart and fusion FISH to validate the RT-PCR results. Cases positive by both RT-PCR and FISH are eligible for the LURET study. The RT-PCR screening is being carried out in >100 hospitals throughout Japan by a consortium designated LC-SCRUM (Lung Cancer Genomic Screening Project for Individualized Medicine in Japan). The therapeutic results will be obtained within 2 years.

Notably, a recent study reported that one patient with LADC harboring a *KIF5B–RET* fusion responded to vandetanib (Table 3). The patient was Caucasian male and a former smoker. Tumor shrinkage was observed starting in the first week, and continued for 4 weeks.[26]

Perspective

The *RET* gene is predicted to be an additional therapeutic target for therapy against LADC. Three other oncogene kinases,

HER2 (activated by inflame insertion mutations), BRAF (activated by point mutation), and ROS1 (activated by gene fusion) are also promising targets for personalized therapy in addition to EGFR and ALK (Fig. 1). In fact, inhibition of these kinases has yielded therapeutic effects in several lung cancer patients. The LADCs harboring *HER2* mutations responded to therapy with anti-HER2 antibodies and HER2 TKIs.[27] One LADC case harboring a *BRAF* mutation responded to therapy with vemurafenib, an FDA-approved drug for the treatment of melanoma.[28] The ALK TKI, crizotinib, suppresses the activity of the ROS1 tyrosine kinase due to the high structural similarity between the ALK and ROS1 tyrosine kinase domains. Consistent with this, a significant portion of the LADC patients with *ROS1* fusions that were enrolled in a clinical trial responded to crizotinib.[29] Therefore, developing therapies that target RET and other kinases means that increasing numbers of LADC patients will benefit from personalized therapy (Fig. 1). Thus, LADC represents a type of cancer in which "precision cancer medicine"[30] based on somatic gene alterations will be realized.

Acquisition of drug resistance is a serious problem for therapies based on TKIs. The LADCs harboring ALK fusions become resistant to crizotinib by acquiring second-site mutations in the gatekeeper region of ALK tyrosine kinase.[7] Those LADCs harboring *ROS1* fusions also become resistant to crizotinib, in this case through second-site mutations in the gatekeeper region of ROS1.[29] Therefore, *RET* fusion-positive LADCs might also acquire resistance to RET TKIs through the same mechanism. Clinical trials of RET TKIs as a treatment for fusion-positive NSCLCs should be carried out carefully, and focus both on efficacy and the acquisition of resistance.

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Disclosure Statement

The authors have no conflict of interest.

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