Identification of KIF5B-RET and GOPC-ROS1 Fusions in Lung Adenocarcinomas through a Comprehensive mRNA-Based Screen for Tyrosine Kinase Fusions

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Abstract

**Background:** The mutually exclusive pattern of the major driver oncogenes in lung cancer suggests that other mutually exclusive oncogenes exist. We conducted a systematic search for tyrosine kinase fusions by screening all tyrosine kinases for aberrantly high RNA expression levels of the 3′ kinase domain (KD) exons relative to more 5′ exons.

**Methods:** We studied 69 patients (including five never smokers and 64 current or former smokers) with lung adenocarcinoma negative for all major mutations in KRAS, EGFR, BRAF, MEK1, HER2, and for ALK fusions (termed “pan-negative”). A NanoString-based assay was designed to query the transcripts of 90 tyrosine kinases at two points: 5′ to the KD and within the KD or 3′ to it. Tumor RNAs were hybridized to the NanoString probes and analyzed for outlier 3′ to 5′ expression ratios. Presumed novel fusion events were studied by rapid amplification of cDNA ends (RACE) and confirmatory reverse transcriptase PCR (RT-PCR) and FISH.

**Results:** We identified one case each of aberrant 3′ to 5′ ratios in ROS1 and RET. RACE isolated a GOPC-ROS1 (FIG-ROS1) fusion in the former and a KIF5B-RET fusion in the latter, both confirmed by RT-PCR. The RET rearrangement was also confirmed by FISH. The KIF5B-RET patient was one of only five never smokers in this cohort.

**Conclusion:** The KIF5B-RET fusion defines an additional subset of lung cancer with a potentially targetable driver oncogene enriched in never smokers with “pan-negative” lung adenocarcinomas. We also report in lung cancer the GOPC-ROS1 fusion originally discovered and characterized in a glioma cell line.

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Introduction

The management of lung adenocarcinoma has been transformed by the discovery of targetable driver oncogenes such as EGFR mutations and ALK fusions (1, 2). It has also become apparent that these major driver oncogenes, along with KRAS, HER2, and others, are mutually exclusive. Indeed, this remarkable, mutually exclusive pattern of mutations suggests that other mutually exclusive driver oncogenes remain to be discovered. On the basis of extensive genotyping data generated by our group and others (3–5), it is estimated that approximately 60% of lung adenocarcinomas in the United States contain one of the following known driver oncogenes, all mutually exclusive with rare exceptions: KRAS, EGFR, ALK fusion, BRAF, HER2, NRAS, and MEK1. This pattern of mutations in more than 60% of cases suggests a general dependence of lung adenocarcinoma on mutational activation of certain downstream signaling pathways [mitogen-activated protein kinase (MAPK), AKT] and provides a rationale for a systematic screen of the remaining, “pan-negative” cases for other driver oncogenes. As part of such a screening effort, we have used an RNA-based approach to systematically search for evidence of novel tyrosine kinase fusions in “pan-negative” lung adenocarcinomas.

The overall rationale for our exon-level mRNA-based screen for the detection of fusion genes is based on the observation that most gene fusions that lead to the formation of a chimeric fusion protein cause an intragenic discontinuity in the RNA expression level of the exons that are 5′ or 3′ to the fusion point in one or both of the fusion partners. This is attributable to the differences in the strength or activity of the promoters of the two translocation partner genes. In addition, in some cases,
**Translational Relevance**

The identification of druggable driver oncogenes such as mutated *EGFR* and *HER2* and *ALK* fusions represents a major advance in the therapy of lung adenocarcinoma. However, the majority of patients with lung adenocarcinoma still do not have a targeted treatment option. Here, we focused on patients with lung adenocarcinoma without any identifiable driver oncogenes to search for new potential targets. In this enriched patient population, we used a NanoString-based strategy to search for new tyrosine kinase fusion genes. With this approach, we identified and confirmed one case each of a novel *KIF5B-RET* fusion and a *GOPC-ROS1* (*FIG-ROS1*). Although RET and ROS1 fusions represent small percentages of lung adenocarcinoma, they are of immediate clinical interest. ROS1 fusions have been shown to respond clinically to targeted treatment with crizotinib, and preclinical data suggest that RET fusions should respond to RET inhibitors.

### Discovery samples

We identified 69 "pan-negative" lung adenocarcinomas with frozen tumor available for RNA extraction based on data from ongoing reflex clinical genotyping of resected lung adenocarcinomas at MSKCC (4). These samples were not selected for demographic features or smoking history, and these data are listed in Supplementary Table S1. The summary of the data for these parameters are as follows: 64 smokers (50 former smokers including 5 with 5 pack-years or less, 14 current) and 5 never smokers (all female); 66 white non-Hispanic; and 3 Hispanic or Black. There were no Asian patients in this study population. The genotyping process used to identify these samples as "pan-negative" for all known lung adenocarcinoma driver oncogenes is outlined in Fig. 1 and briefly summarized here: tumor samples were subjected to extended molecular testing under MSKCC IRB protocols, consisting of a prospective screen for all recurrent mutations in 8 key genes (*EGFR*, *HER2*, *KRAS*, *NRAS*, *BRAF*, *MEK1*, *PIK3CA*, *AKT1*) in all lung adenocarcinomas using the Sequenom platform. The panel variability for each gene in the NanoString assay, we included 17 *KRAS*-mutated lung adenocarcinomas, 11 *EGFR*-mutated lung adenocarcinomas, and 37 samples of non-neoplastic lung tissue procured at time of lung cancer surgery.

### Materials and Methods

#### Assay validation samples and negative control samples

To validate the performance of the NanoString assay design, we used cell lines and patient tumor samples with known fusions. The cell lines included H2228 and H3122 (both *EML4-ALK*+ lung adenocarcinomas), K299 (*NPM1-ALK*+ anaplastic large cell lymphoma), HCC78 (*CD74-ROS1*+ lung adenocarcinoma), and U118 (*FIG-ROS1*+ glioma). The patient tumor samples, tested under Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) Institutional Review Board (IRB) protocols as described in more detail below, included 75 lung adenocarcinoma samples of which 24 were positive for, and 51 were negative for, evidence of ALK fusion by ALK FISH and at least one other method, either *EML4-ALK* real-time PCR (RT-PCR) or immunohistochemistry (IHC) using the D5F3 ALK monoclonal antibody (Cell Signaling). As negative control samples to establish the range of normal 5'–3' expression ratio...
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interrogates these 8 genes for the presence of 91 point mutations (5). *EGFR* exon 19 deletions, exon 20 insertions, and *HER2* exon 20 insertions were screened by PCR product sizing assays (7, 8). Cases containing *PIK3CA* mutations, even as the sole detectable mutation, were not excluded because *PIK3CA* mutations are known to frequently co-occur with other driver mutations (9). Cases negative with the preceding assays (except *PIK3CA*) were then screened by FISH for evidence of ALK fusions using the Abbott/Vysis ALK breakapart FISH assay.

**NanoString assay for kinase fusions**

The NanoString nCounter system is a fluorescence-based platform to detect individual mRNA molecules without PCR amplification in a quantitative and highly multiplexed fashion (10, 11). In this system, each capture probe and reporter probe together query a contiguous 100-bp region and only 100 ng RNA is needed per reaction. Our NanoString assay design (Fig. 2) was based upon the known genomic properties of existing tyrosine kinase fusions, namely, that these fusions typically occur upstream of the exons encoding the kinase domain. The exons encoding the kinase domain GXGXXG motif for all 90 tyrosine kinases and 3 serine/threonine kinases (*BRAF, ARAF, CRAF*) were identified as described elsewhere (12). All exons were labeled according to ENSEMBL numbering. On the basis of this mapping, 2 100-bp regions were selected for each gene transcript, a 5' probe pair located far upstream of the kinase domain exons, and the second located within those exons or further 3'. The 100-bp regions were selected to straddle exon boundaries to reduce the risk of interfering signal from gDNA. Detailed sequence information for all the kinase gene target regions is provided in Supplementary Table S2. Each sample was analyzed using 100 to 200 ng of total RNA per assay. All kinase genes and control genes were assayed simultaneously in multiplexed reactions. The raw data were normalized to the nCounter system spike-in positive and negative controls in each sample. The normalized results are expressed as the relative mRNA level. NanoString count data were converted to the log2 scale (1 was added to all data to avoid problems with zeros). The data were then normalized with the quantile method using the normalizeBetweenArrays procedure from the Bioconductor R package. The 3'/5' ratios were calculated for each kinase gene in the assay, and samples with outlier ratios were visualized on log scale plots. Samples with specific tyrosine kinase genes with 3'/5' ratios below –4 on the log2 scale were considered outliers if only seen in rare pan-negative samples and none of the other control samples.

**5'RACE**

Rapid amplification of cDNA ends (RACE) was conducted with the use of 5' RACE system (5' system for Rapid Amplification of cDNA Ends, version 2.0, Invitrogen). The primers used to identify aberrant ROS1 transcripts in RNA in 5' RACE reaction were ROS1-GSP1 primer (5'-GAGGAG-ACCTTCATACATATTT-3') for cDNA synthesis and ROS1-GSP2 (5'-AAGACAAAGAGTGCTGAGCCTCGGC-3') and ROS1-GSP3 (5'-CTGGCATAGAAGATTAAAGAATC-3') for the nested PCR reaction. The primers used to identify

![Figure 2. Principle of NanoString assay for evidence of tyrosine kinase fusions. As functional tyrosine kinase fusions typically occur upstream of the exons encoding the kinase domain, probes were designed to measure the expression at 2 regions for each gene transcript, a 5' probe pair located far upstream of the kinase domain exons, and the second located within those exons or further 3'. Kinase fusions often cause an imbalance in the RNA expression level of these 2 regions attributable to stronger activation of the promoter of the fusion partner gene and/or, in some cases, loss of the nononcogenic, reciprocal fusion gene due to an unbalanced translocation event. The 3'/5' expression ratios are calculated for each kinase gene in the assay, and samples with outlier ratios were visualized on log scale plots.](image-url)
aberrant RET transcripts in RNA in 5’ RACE reaction were RET-GSP1 primer (5'-GATGAAGCAGCTTCATCTCAGG-3') for cDNA synthesis and RET-GSP2 (5'-GAGCAGTGAAGTTT-CCAGGAGTCTC-3') and RET-GSP3 (5'-CTAGAGTTTTTGCACTTTTCCAAAATTC-3') for the nested PCR reaction. For sequencing, the RACE PCR product was cloned using TOPO TA Cloning Kit (Invitrogen), purified with Qiaprep Spin Miniprep Kit (QIAGEN) and sequenced by Sanger sequencing.

**RT-PCR for KIF5B-RET**

To detect the presence of the KIF5B-RET fusion transcript, we conducted RT-PCR using several independent sets of forward and reverse primers, to ensure that the RT-PCR products were short (<350 bp). Specifically, primer pairs for RT-PCR were 5'-CTGAGATGATGCCATTTTACAAATTTCCAAAATTC-3' (RET_Ex12) and 5'-TGGATGATTTGTGATGACACCC-3' (KIF5B_Ex23) with 5'-AGATTTTCTCCAAAACGAGTCC3'-3' (RET_Ex12). Total RNA (500 ng) was reverse-transcribed to cDNA using Superscript II Reverse Transcriptase (Invitrogen). For cDNA synthesis, we used the gene specific RET exon 14 reverse primer, 5'-CAGGAGCCTAATTTGGCG-3'. cDNA (corresponding to 25 ng total RNA) was subjected to PCR amplification using HotStarTaq Master Mix Kit (QIagen). The reactions were carried out in a thermal cycler under the following conditions: 35 cycles of 94°C for 1 minute, 57°C for 2 minutes, and 72°C for 2 minutes, with a final extension for 7 minutes at 72°C. For sequencing, the RT-PCR product was cloned using TOPO TA Cloning Kit (Invitrogen), purified with Qiaprep Spin Miniprep Kit (QIAGEN) and sequenced by Sanger sequencing.

**RET breakapart FISH assay**

A RET breakapart FISH assay was developed using bacterial artificial chromosome (BAC) clones based on the UCSC Genome Browser database (http://genome.ucsc.edu/). BAC clones were ordered from the Children’s Hospital Oakland Research Institute (Oakland, CA). BAC DNAs were extracted using BACMAX DNA purification kit (Epicentre Biotechnologies) and labeled with either SpectrumOrange-dUTP (red) or SpectrumGreen-dUTP (green) using the nick-translation kit (Vysis/Abbott Molecular). RET 5’-probe, a combination of BAC clones RP11-633E1 and RP11-124011, was labeled in green; RET 3’-probe, a combination of BAC clones RP11-718J13 and RP11-54P13 was labeled in red. Four-micrometer formalin-fixed, paraffin-embedded (FFPE) sections generated from FFPE blocks of tumor specimens were pretreated by deparaffinizing in xylene and dehydrating in ethanol. Dual-color FISH was conducted according to the protocol for FFPE sections from Vysis/Abbott Molecular with a few modifications. FISH analysis and signal capture were conducted on a fluorescence microscope (Zeiss) coupled with ISIS FISH Imaging System (MetaSystems). We analyzed 100 interphase nuclei from each tumor specimen.

**Results**

**NanoString assay validation**

To evaluate the performance of the assay design for a known fusion, we studied 75 lung adenocarcinoma RNA samples (6 extracted from frozen tissue; 69 from FFPE blocks) of which 24 were positive for, and 51 were negative for, evidence of ALK fusion by ALK breakapart FISH and at least one other method, either EML4-ALK RT-PCR or IHC using the D5F3 ALK monoclonal antibody (Cell Signaling). The ALK 5’ to 3’ expression ratios were highly nonoverlapping between the 2 groups, with 74 of 75 cases being correctly separated (Fig. 3A). To gauge the sensitivity of the assay, we examined serial dilutions of RNAs from cell lines H2228, H3122 (both EML4-ALK+ lung adenocarcinomas) and K299 (NPM-ALK+ anaplastic large cell lymphoma), into RNA from the HL60 leukemia cell line (Fig. 3B). We conservatively interpreted these results as suggesting that samples with at least 25% ALK fusion–positive tumor cell content should be readily detectable, an acceptable sensitivity range for a discovery assay. Other control samples that were appropriately positive included 2 ROS1 fusion–positive cell lines (HCC78, U118; shown as negative controls...
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**GOPC-ROS1 fusion**

RACE analysis of the sample with the aberrant ROS1 5’ to 3’ ratio isolated a fusion of GOPC to the Golgi-associated PDZ and coiled-coil motif containing (GOPC) gene, previously known as Fused in Glioblastoma (FIG; Fig. 5). This in-frame fusion of GOPC exon 7 to ROS1 exon 35 was also confirmed by an independent RT-PCR (Fig. 5), and the RT-PCR product was also sequence-verified. This tumor was from a 68-year-old white female former smoker with an 82 pack-year smoking history. Histologically, the tumor was a combined adenocarcinoma and small cell carcinoma (Fig. 5). Routine diagnostic IHC studies showed that the small cell carcinoma component was positive for TTF-1 and CD56 and had an MIB1 proliferation rate of nearly 100%. The adenocarcinoma was weakly positive for CD56 and negative for TTF-1, Napsin-A, synaptophysin, and chromogranin. Both components were negative for 4A4, 34BE12, and CK5/6, supporting the diagnosis. This patient’s stage IIIA lung cancer was resected in August 2010, followed by adjuvant cytotoxic chemotherapy completed by December 2010, without subsequent recurrence.

**KIF5B-RET fusion**

RACE analysis of the sample with the most aberrant RET 5’ to 3’ ratio isolated a fusion of RET to the kinesin family 5B gene (KIF5B; Fig. 6). This in-frame fusion of KIF5B exon 15 to RET exon 12 was likewise also confirmed by sequencing of independent RT-PCR products. This patient was a 60-year-old female never smoker, and the tumor was a 2.8 cm adenocarcinoma with predominantly papillary and acinar growth patterns with some solid and micropapillary components (Fig. 6). The tumor nuclei displayed frequent and prominent intranuclear inclusions. In routine diagnostic IHC studies, the tumor cells were positive for CK7, TTF-1, and PE10 and negative for CK20 and thyroglobulin, consistent with pulmonary origin. Using BAC clones, we developed a FISH assay for RET rearrangement (see Materials and Methods). This showed narrow but consistent split signals in the tumor nuclei from this case (Fig. 6), consistent with the signal pattern of an intrachromosomal rearrangement such as the inversion between KIF5B and RET that would generate this gene fusion. This patient’s small, incidentally detected adenocarcinoma was resected in March 2009 without subsequent recurrence or treatment.

Further screening by RT-PCR for KIF5B-RET using multiple primer combinations (see Materials and Methods) did not identify any additional positive samples in 48 of the remaining 68 samples NanoString study set with sufficient material for RT-PCR.

**Discussion**

The discovery of ALK fusions in lung adenocarcinoma in 2007 resulted in renewed interest in kinase fusions as oncogenic drivers in common solid cancers (13, 14). Soon thereafter, on the basis of marked tumor shrinkages observed in patients with ALK rearrangements in a phase I trial of crizotinib, a cohort of patients with ALK rearrangements was added and showed an overall response rate of...
61% with a median response duration of 12 months, and a subsequent phase II trial of patients with ALK fusion–positive lung adenocarcinomas found a radiographic response rate higher than 50% (15). Together, these studies led to the accelerated approval of crizotinib by the U.S. Food and Drug Administration in August 2011 for this molecular subset defined by a novel kinase fusion (16, 17). These recent developments prompted us to design and conduct a more systematic screen for kinase fusions in lung adenocarcinomas without a currently known oncogenic driver, that is, “pan-negative” lung adenocarcinomas.

Using the present NanoString-based assay to screen 69 “pan-negative” lung adenocarcinomas for evidence of fusions involving 90 tyrosine kinase genes, we identified novel ROS1 and RET fusions in 2 samples. This may suggest that undiscovered tyrosine kinase fusions are unlikely to account for many or most “pan-negative” lung adenocarcinomas. However, we acknowledge that there are limitations to the present assay design: the sensitivities for detecting outlier 5' to 3' ratios may be variable based on the expression level of the fusion gene and the expression level of the native gene in non-neoplastic cells. Furthermore, it is likely that additional 5' and 3' probes for each gene would allow more robust scoring of outlier 5' to 3' ratios. As with any high-throughput genomic technology, it is not possible to experimentally validate the performance of the assay for each of the genes tested. There are also advantages to this NanoString-based assay, notably it extends the analyzable sample types beyond the high-quality samples usually required for array-based platforms or next-generation sequencing–based chimeric transcript detection and is, therefore, well suited to limited clinical sample RNAs (100–200 ng) of variable quality extracted from either frozen tissues or FFPE tumor blocks. It is also relatively cost-efficient (approximately $200/sample). Our validation studies and successful identification of 2 novel fusions support this type of assay as a discovery platform, but considerable additional validation studies would be needed to establish this as a clinical diagnostic platform for one or more tyrosine kinase fusions. Nonetheless, the labor-intensive, inherently low-throughput nature of FISH makes screening for multiple possible fusion genes (ALK, ROS1, RET) cumbersome and will drive the search for more multiplexed diagnostic approaches such as this one or others.

We have identified for the first time the fusion of ROS1 with GOPC, previously known as FIG, in a lung adenocarcinoma. Although karyotype data were not available in our case, these 2 genes are only 134 KB apart in the same orientation at 6q22 so this fusion is consistent with an intrachromosomal deletion, namely, del(6)(q22q22). The GOPC-ROS1 fusion has previously only been found in the U118MG human glioblastoma multiforme cell line (18). The GOPC-ROS1 fusion protein has been shown to have constitutively active kinase activity, and its transforming
potential has been shown in a mouse transgenic model where it resulted in glioblastomas in an Ink4a;Arf-null background (19).

**ROS1** fusions in lung cancer, specifically **SLC34A2-ROS1** and **CD74-ROS1**, were first reported by Rikova and colleagues in 2007 (13). Interestingly, additional **ROS1** fusion partners reported since then in lung cancer have included **TPM3**, **SDC4**, **EZR**, and **LRIG3**, but not **GOPC** (20). All 6 lung cancer **ROS1** fusions tested by Takeuchi and colleagues were found to be transforming in 3T3 cells (20). Together, these data strongly support **ROS1** fusions as driver events, even as possible subtle functional differences among the many related **ROS1** fusion proteins described in lung cancer remain to be explored in lung epithelial cells or transgenic mouse models in future studies.

This molecular subset of lung adenocarcinoma is of immediate clinical interest given the activity of crizotinib for this target, recently shown in cell line experiments and clinically in a single **ROS1** FISH–positive patient who experienced a complete response to this agent (21). The expansion of the possible **ROS1** fusion partners in lung cancer to **GOPC** further underscores the heterogeneity of **ROS1** fusions and the difficulty in using RT-PCR assays for comprehensive detection of these patients. The size of this molecular subset among lung adenocarcinomas was 1.2% (13 of 1,116) in one study (20), 2.6% (18 of 644) in a second study (21), and 1.5% (2 of 152) in a third study limited to Asian never smokers (22). In the present study, we found it in 1 of 69 “pan-negative” lung adenocarcinomas, which in our testing experience make up about 40% of lung adenocarcinomas, and therefore our overall prevalence of **ROS1** fusions would be approximately 0.6%. It should be noted that certain breakapart FISH assay designs...

![Figure 6. Identification of KIF5B-RET fusion. A, sequence of product of 5’RACE shows an in-frame fusion of KIF5B exon 15 to RET exon 12 in this cancer from a 60-year-old never smoker white female. B, histology shows lung adenocarcinoma with predominantly papillary and acinar growth patterns (left). Nuclei are pleomorphic and display prominent intranuclear pseudoinclusions (right). C, FISH analysis shows splitting of green (5’ probe) and red (3’ probe) signals of RET breakapart FISH assay. Inset, normal bone marrow cells showing fused or overlapping red and green signals only.](https://www.aacrjournals.org/doi/10.1158/1078-0432.CCR-12-0838)
for ROS1 fusions would fail to detect the GOPC-ROS1 fusion because their 5' probe overlaps or includes GOPC, which is only 134 KB upstream, and therefore no significant change in FISH signal patterns would be apparent. This is the case with a commercially available assay (Abnova) as well as the assay used by Bergethon and colleagues (21). Therefore, studies based on such FISH assays may underestimate the prevalence of ROS1 fusions by missing GOPC-ROS1 cases. In contrast, the 5' ROS1 probe used by Takeuchi and colleagues (20) includes the 5' end of ROS1 and the region between ROS1 and GOPC, and therefore a GOPC-ROS1 fusion case may show just a loss of the 5' ROS1 probe in such an assay design due to the genomic interstitial deletion. If the GOPC-ROS1 fusion occurs by a more complex mechanism such as an insertion/duplication, it may not be detectable by any of these ROS1 FISH assays. Finally, we should note that, while the present article was under review, another group has reported the detection of a GOPC-ROS1 fusion in lung cancer using IHC for ROS1 as a screening approach (23).

More recently, as the present report was in preparation, several independent groups (20, 24–26) also reported the discovery of KIF5B-RET fusions in lung adenocarcinomas, at an overall prevalence of approximately 1.3% (27). On the basis of the same estimates provided above for ROS1 fusions, our overall prevalence of RET fusions would be approximately 0.6%. Notably, our KIF5B-RET–positive tumor was detected in 1 of only 5 never smokers in our discovery set, suggesting that screening of “pan-negative” never smokers may be a more efficient strategy to identify this rather small subset of patients. It is possible that more cases with kinase fusions might have been detected if our study group had been intentionally enriched for never smokers. Our KIF5B-RET fusion involved exon 15 of KIF5B and exon 12 of RET, thus retaining a portion of the dimerization domain of KIF5B and the entire kinase domain of RET, analogous to RET fusion proteins in papillary thyroid carcinoma. This fusion structure is also the most commonly reported of the seven isoforms described so far (21). Cytogenetically, as KIF5B and RET are approximately 10.6 Mb apart on chromosome 10 in opposite orientations, the KIF5B-RET fusion may occur most simply by a pericentric inversion of 10p11.22-q11.21. RET is a well-established oncogene in thyroid cancer, both in the context of point mutations in medullary thyroid cancer and other fusions in papillary thyroid cancer (28). The oncogenic potential of KIF5B-RET itself has been established by transfection of NIH 3T3 cells in which it leads to anchorage-independent growth in vitro and tumor formation in xenografted mice (20, 25) and by transfection of Ba/F3 cells in which it results in interleukin-3–independent growth (20, 24). Although no human lung cancer cell line with an endogenous KIF5B-RET fusion has been identified, the phenotypes elicited in heterologous cells by transfection with KIF5B-RET cDNA are sensitive to multi-kinase inhibitors whose targets include RET, such as vandetanib, sunitinib, and sorafenib (20, 24, 25), supporting the concept of prospectively genotyping lung cancers for RET fusions to identify patients for clinical trials of such agents in this new molecular subset of lung adenocarcinoma. It is interesting to note that agents such as sunitinib (29, 30), sorafenib (31), and cabozantinib (or XL184) in unselected patients with non–small cell lung cancer have been associated with partial response rates in the 2% to 10% range. Given the multi-kinase targeting of these agents, it has been difficult to elucidate why some patients benefit but it is now tempting to speculate that some of these partial responses may have been in tumors containing a RET fusion.

Finally, it is notable that the 2 most common lung fusions so far, EML4-ALK and KIF5B-RET, are both intrachromosomal events, as is the only other RET fusion described so far in lung cancer, CCDC6-RET, reported in one case by Takeuchi and colleagues (20). Although most ROS1 fusion partners are on other chromosomes, the GOPC-ROS1 fusion reported here and another recently described ROS1 fusion, EZR-ROS1 (20), are also intrachromosomal rearrangements. These observations are intriguing given the link between radiation and intrachromosomal rearrangements established in papillary thyroid cancer based on epidemiologic and mechanistic data. Specifically, papillary thyroid cancers that develop following nuclear disasters are more likely to harbor RET rearrangements (instead of BRAF or RAS mutations; refs. 32, 33) and the fusion partners are more often CCDC6 ("RET-PTC1" fusion) and NCOA4 ("RET-PTC3" fusion), both on chromosome 10 (32). RET fusions can be induced in vitro by irradiating human thyroid epithelial cells and these 3-chromosome 10 loci (RET, CCDC6, NCOA4) are often juxtaposed in the interphase nuclei of thyroid epithelial cells, which may facilitate coincident DNA strand breakage by a single radiation track and/or illegitimate recombination (34–36). These considerations, along with the increased risk of lung cancer in atomic bomb survivors and uranium miners (37, 38), suggest that a possible etiologic role for environmental radiation should be explored in never smokers whose tumors harbor KIF5B-RET or possibly EML4-ALK.

Disclosure of Potential Conflicts of Interest
M.G. Kris has a commercial research grant from Pfizer, Inc. and Boehringer Ingelheim and is a consultant/advisory board member for Pfizer, Inc. M. Ladanyi has been a consultant/advisory board member for NanoString Technologies, Inc. No potential conflicts of interest were disclosed by the other authors.

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References


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