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Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies

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Abstract

Applying a next-generation sequencing assay targeting 145 cancer-relevant genes in 40 colorectal cancer and 24 non–small cell lung cancer formalin-fixed paraffin-embedded tissue specimens identified at least one clinically relevant genomic alteration in 59% of the samples and revealed

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AUTHOR CONTRIBUTIONS

D.L. and R.Y. designed experiments and algorithms, and performed analyses. J.W. and G.M.F. performed data analyses. A.P. and G.O. performed RNA preparations and prepared the cDNA libraries and sequencing. M.J. designed the experiments and performed analyses. J.A.C. and K.C.M. performed laboratory and project management. S.R.D. and T.B. performed tissue pathology preparations and extractions. J.S.R. provided tissue specimens and the pathology review. S.B., K.W.B., A.D., L.G., F.J., E.W. and Z.Z. performed DNA library preparation and sequencing. P.J.S., M.T.C. and P.A.J. designed experiments, analyzed data and wrote the manuscript. T.P., H.N., L.-S.G., C.E.S., J.K., H.S., H.R.K. and S.P. provided subject specimens. M.C. performed genotyping of subjects with lung cancer, conducted *in vitro* studies and analyzed data. D.E. performed *in vitro* studies.

COMPETING FINANCIAL INTERESTS

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two gene fusions, *C2orf44-ALK* in a colorectal cancer sample and *KIF5B-RET* in a lung adenocarcinoma. Further screening of 561 lung adenocarcinomas identified 11 additional tumors with *KIF5B-RET* gene fusions (2.0%; 95% CI 0.8–3.1%). Cells expressing oncogenic *KIF5B-RET* are sensitive to multi-kinase inhibitors that inhibit RET.

We analyzed genomic DNA from 40 colorectal cancer (CRC) and 24 non–small cell lung cancer (NSCLC) formalin-fixed paraffin-embedded (FFPE) specimens using an assay that captures and sequences 2,574 coding exons representing 145 cancer-relevant genes (genes that are associated with cancer-related pathways, targeted therapy or prognosis), plus 37 introns from 14 genes that are frequently rearranged in cancer (Supplementary Table 1). We sequenced this 606,676-bp content, selected using solution phase hybridization, to an average coverage of 229 ×, with 84% of exons being sequenced at ≥ 100 × coverage (Supplementary Table 2 and Supplementary Methods). To maximize mutation-detection sensitivity in heterogeneous cancer biopsies, we validated the test to detect base substitutions at a ≥ 10% mutant allele frequency with ≥ 99% sensitivity and to detect indels at a ≥ 20% mutant allele frequency with ≥ 95% sensitivity, with a false discovery rate of < 1% (data not shown).

Among 40 CRCs, we identified 125 alterations in 21 genes. We found at least one alteration in 39 out of 40 tumors (with a range of 1–9 alterations), and 62.5% (25 out of 40) of the tumors harbored at least two classes of DNA alteration (Fig. 1 and Supplementary Table 3). *TP53* and *APC* were altered in 80% (32 out of 40) and 67.5% (27 out of 40) of CRCs, respectively, with both being mutated at higher frequencies than are reported in the Catalogue of Somatic Mutations in Cancer[1]. Additionally, 11 genes were mutated, amplified or rearranged in multiple CRCs: *KRAS* (10), *BRAF* (6), *FBXW7* (5), *ATM* (2), *BCL2L1* (2), *BRCA2* (2), *CDH1* (2), *ERBB3* (2), *GNAS* (2), *PIK3CA* (2) and *SMAD4* (2). *ALK*, *CDK8*, *LRP1B*, *MYC*, *MSH6*, *RICTOR*, *SMAD2* and *STK11* were each altered in one tumor (Fig. 1a). Notably, 52.5% of CRCs (21 out of 40) harbored at least one alteration that has been linked to a clinical treatment option or is currently being investigated in clinical trials of new targeted therapies. Examples of this include mutations in *KRAS* and *BRAF* (cetuximab[2,3] or panitumumab resistance[3]), *FBXW7* (anti-tubulin resistance[4]), *BRCA2* (poly-(ADP-ribose) polymerase (PARP) inhibitor trials[5]), *GNAS* (MEK or ERK inhibitor trials[6]) and *PIK3CA* (phosphoinositide-3-kinase, catalytic, alpha polypeptide (PI3K) and mechanistic target of rapamycin (mTOR) inhibitor trials[7]) (Supplementary Table 4).

We detected a fusion gene between *C2orf44* and *ALK* in sequencing data from one subject with CRC (Supplementary Fig. 1). This in-frame fusion starts at the canonical exon 20 recombination site that was previously reported for *ALK* gene fusions[8,9]. *C2orf44*, on chromosome 2, contains a coiled-coil domain; the *C2orf44-ALK* fusion results from a 5,194,955-bp tandem duplication (Fig. 1b,c). Complementary DNA (cDNA) sequencing identified 75 read pairs spanning the fusion junction (data not shown) and an 89.8-fold increase in 3′ *ALK* expression beginning at exon 20 relative to exons 1–19, suggesting that the *C2orf44-ALK* fusion transcript results in ALK kinase overexpression (Fig. 1d). Immunohistochemistry (IHC) was negative for ALK staining (data not shown). Clinical detection of *ALK* rearrangements is currently performed using fluorescence *in situ* hybridization with *ALK* break-apart probes[9] or by RT-PCR using *ALK*-rearrangement–specific primers. Given the structure of this gene fusion, probably neither method would have detected it. These findings suggest that a previously unrecognized subset of individuals with CRC may harbor genetic alterations that may respond to treatment with crizotinib or other ALK inhibitors.

Among 24 NSCLCs, we identified 50 alterations in 21 genes, with at least one alteration being present in 83% (20 out of 24) of the tumors (with a range of 1–7 alterations). Twelve

genes were altered in multiple tumors: *KRAS* (10), *TP53* (7), *STK11* (4), *LRP1B* (3), *JAK2* (3), *EGFR* (2), *BRAF* (2), *CDKN2A* (2), *CTNNB1* (2), *MDM2* (2), *PIK3CA* (2) and *ATM* (2). *APC*, *CCNE1*, *CDK4*, *MLH1*, *MSH6*, *NF1*, *RB1*, *RET*, and *TSC1* were each altered in one tumor (Fig. 2a and Supplementary Table 3). In 72% (36 out of 50) of the NSCLCs, at least one alteration was associated with a current clinical treatment or targeted therapy trial, including mutations in *KRAS* (epidermal growth factor receptor (EGFR) kinase inhibitor resistance[10] and PI3K and MEK inhibitor trials[7]), *BRAF* (v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitor trials, including those with vemurafenib[11] and GSK 2118436 (ref. 11)), *EGFR* (gefitinib or erlotinib sensitivity[12]), *MDM2* (nutlin trials[13]), *CDKN2A*, *CCNE1* and *CDK4* (cyclin-dependent kinase 4 (CDK4) inhibitor trials[14,15]) and *PIK3CA* (PI3K and mTOR inhibitor trials[7,16]) (Supplementary Table 4).

In addition to the known NSCLC gene alterations, we made two notable discoveries. The first was a G1849T (V617F) *JAK2* mutation present in three subjects at a low allele frequency (4–10%). Although commonly observed in myelodysplastic syndromes, this *JAK2* mutation has not been described in solid tumors[1]. Sequencing additional NSCLCs will be required to characterize the role of *JAK2* mutations in NSCLC, and clinical trials will be necessary to assess whether they predict sensitivity to JAK2 inhibitors[17].

We also detected an 11,294,741-bp pericentric inversion on chromosome 10 generating a new gene fusion joining exons 1–15 of *KIF5B* to exons 12–20 of *RET* (Fig. 2b and Supplementary Fig. 2) in a lung adenocarcinoma from a 44-year-old, male ‘never smoker’ of European ancestry; this fusion is denoted K15;R12, based on the last *KIF5B* and first *RET* exons in the fusion. *KIF5B* exons 1–15 comprise the kinesin motor and coiled-coil domains that mediate homodimerization, whereas exon 15 is a known *KIF5B-ALK* fusion site in individuals with NSCLC[18]. *RET* exons 12–20 encode the tyrosine kinase portion of the *PTC-RET* fusions observed in ~35% of papillary thyroid carcinomas[19] (Fig. 2c). cDNA sequencing identified 490 unique read pairs spanning the fusion junction (data not shown) and detected a 7.3-fold *RET* expression increase beginning at exon 12 relative to exons 1–11 (Fig. 2d). IHC showed focal moderate cytoplasmic immunoreactivity for RET protein expression (Fig. 2e).

An additional 117 NSCLCs (from 92 individuals of European ancestry, 5 African-American individuals and 20 individuals of unknown ancestry) screened by IHC showed moderate to intense RET staining in 22 samples. RT-PCR and cDNA sequencing of RNA from 15 tumors that had RET expression using IHC identified one additional *KIF5B-RET* fusion in a male former smoker of European ancestry (Supplementary Table 5).

We evaluated tumors from 121 individuals of European ancestry and 405 Asians, comprising only never or limited former smokers, by RT-PCR for *KIF5B-RET* fusions, with 1 out of 121 (0.8%) European-ancestry and 9 out of 405 (2%) Asian subjects testing positive for the fusion. None of the fusion-positive tumors contained mutations in *EGFR*, *ERBB2*, *BRAF* or *KRAS* or rearrangements of *EML4-ALK* or *ROS1*, resulting in a total of 10 individuals with *RET* rearrangements out of 159 (6.3%) subjects without previously known driver mutations (Supplementary Table 5). The lack of known oncogenic mutations in individuals positive for the *KIF5B-RET* fusion raises the possibility that the *RET* fusion is a driving oncogenic event. Of note, all additional 12 *KIF5B-RET* fusions (from the 11 individuals described above) were identified in lung adenocarcinomas, which comprised 561 of the 643 cases screened, in addition to the original 24 individuals, for an overall occurrence rate of 2.0% (95% CI 0.8–3.1%).

The 13 total *RET* fusions (from 12 individuals) comprise four unique transcripts: eight from K15;R12 (variant 1), three from K16;R12 (variant 2), one from K22;R12 (variant 3) and one

from K15;R11 (variant 4) (the subject with variant 4 also had the K15;R12 variant) (Supplementary Figs. 2 and 3). The *KIF5B* portions of the variants differ, but all retain the coiled-coil domain necessary for homodimerization. No identified *RET* fusion was amplified.

Thyroid cancers and cell lines harboring *PTC-RET* translocations are sensitive to sorafenib, which inhibits RET[20], suggesting the *KIF5B-RET* gene fusion in NSCLC may be druggable. *KIF5B-RET* expression in Ba/F3 cells led to oncogenic transformation, as determined by interleukin-3 (IL-3)-independent growth. These cells were sensitive to sunitinib, sorafenib and vandetanib, which are all multi-targeted kinase inhibitors that inhibit RET but not gefitinib, which is an EGFR kinase inhibitor (Fig. 2f). Sunitinib, but not gefitinib, inhibited RET phosphorylation in Ba/F3 cells with the KIF5B-RET fusion protein (Fig. 2g). These findings suggest that RET kinase inhibitors should be tested in prospective clinical trials for therapeutic benefit in individuals with NSCLC that carry *KIF5B-RET* rearrangements.

Overall in this study, 52.5% of subjects with CRC and 71% of subjects with NSCLC had genomic alterations that were directly linked to a clinical therapeutic option, including two new gene fusions. Identifying even a small subpopulation of affected individuals with gene fusions who may be potentially responsive to targeted therapy, as exemplified by the discovery of *C2orf44-ALK* and *KIF5B-RET*, may have major therapeutic relevance, as highlighted by the recent US Food and Drug Administration's approval of crizotinib for the treatment of NSCLC that harbors rearrangements in *ALK*[9]. These findings in aggregate show the potentially large clinical impact of a single multiplex test that requires minimal DNA from FFPE tumor biopsies.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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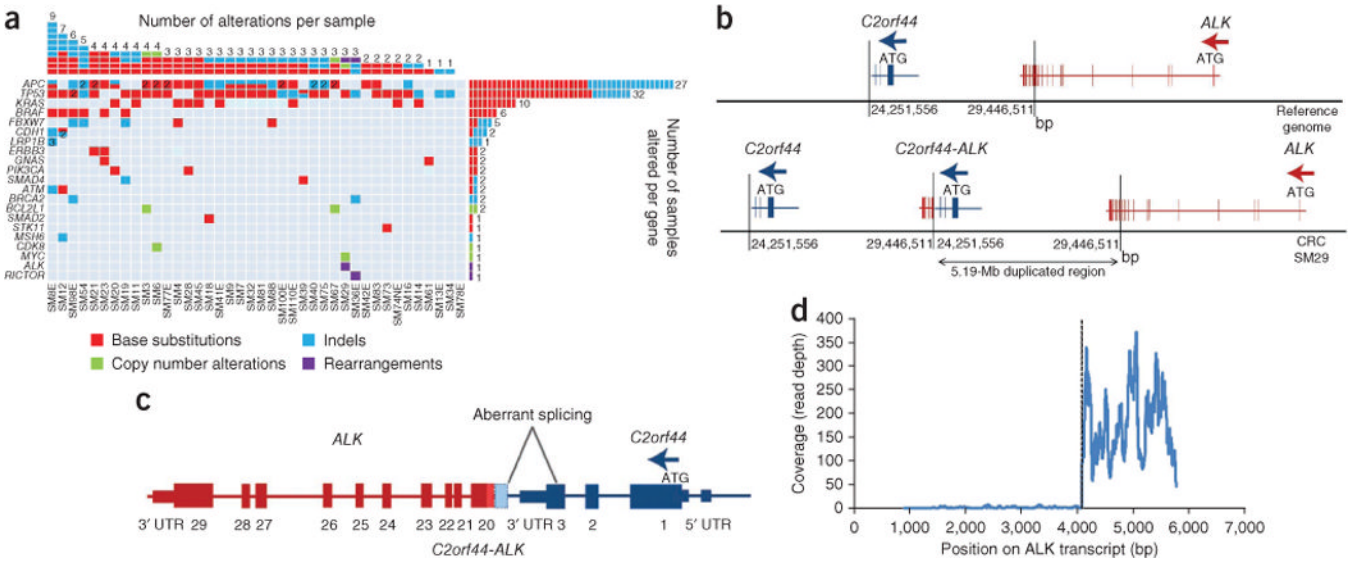

Figure 1.

DNA alterations identified in 40 CRC FFPE specimens. (**a**) The columns in the table denote samples, and the rows denote genes. A number inside the cell indicates the number of alterations of a specific type identified here. (**b**) A 5,194,955-bp tandem duplication generates an in-frame *C2orf44-ALK* gene fusion. (**c**) The RNA sequence of the *C2orf44-ALK* gene fusion shows aberrant splicing. UTR, untranslated region. (**d**) RNA sequencing shows an 89.8-fold increase in expression of *ALK* beginning at exon 20 relative to exons 1–19.

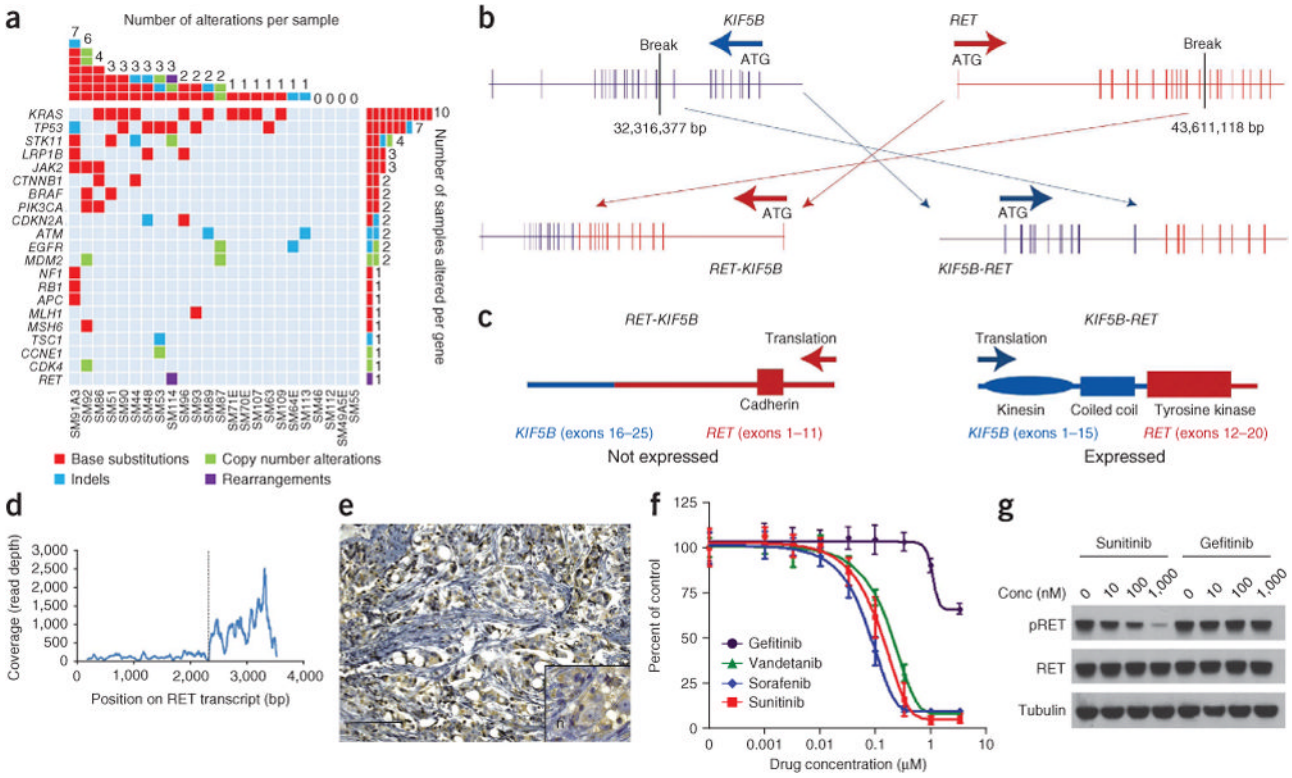

Figure 2.

DNA alterations identified in 24 NSCLC FFPE specimens. (**a**) The columns in the table denote samples, and the rows denote genes. (**b**) An 11,294,741-bp inversion generates an in-frame *KIF5B-RET* gene fusion and the reciprocal *RET-KIF5B* fusion. (**c**) Protein domain structure of the RET-KIF5B and KIF5B-RET fusions. (**d**) RNA sequencing shows a 7.3-fold increase in expression of RET beginning at exon 12 relative to exons 1–11. (**e**) Focal moderate cytoplasmic immunoreactivity for RET protein expression (using avidin-biotin peroxidase). Scale bar, 100 μm; inset, 10 μm. (**f**) Ba/F3 cells with the *KIF5B-REF* fusion were treated with different drugs at the indicated concentrations, and viable cells were measured after 72 h of treatment and plotted relative to untreated controls. (**g**) Cells from **e** were treated with increasing concentrations of sunitinib or gefitinib for 6 h, and immunoblotting was used to detect the indicated proteins. Conc, concentration; pRET, phosphorylated RET.