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Kinome sequencing reveals RET G691S polymorphism in human neuroendocrine lung cancer cell lines

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Abstract

Neuroendocrine (NE) lung tumors comprise 20–25% of all invasive lung malignancies. Currently, no effective treatments are available to cure these tumors, and it is necessary to identify a molecular alteration(s) that characterizes NE lung tumor cells. We aimed to identify a kinase mutation(s) associated with NE lung tumor by screening 517 kinase-encoding genes in human lung cancer cell lines. Our next-generation sequencing analysis of six NE lung tumor cell lines (four small cell lung cancer lines and two non-small cell lung cancer lines) and three non-NE lung tumor lines revealed various kinase mutations, including a nonsynonymous mutation in the proto-oncogene *RET* (c.2071G>A; p.G691S). Further evaluation of the *RET* polymorphism in total 15 lung cancer cell lines by capillary sequencing suggested that the frequency of the minor allele (A-allele) in NE lung tumor lines was significantly higher than its frequency in a reference population ($p = 0.0001$). However, no significant difference between non-NE lung tumor lines and a reference group was detected ($p = 1.0$). Nevertheless, neither *RET* expression levels were correlated with the levels of neuron-specific enolase (NSE), a key NE marker, nor vandetanib and cabozantinib, small molecule compounds that inhibit *RET*, affected NSE levels in lung cancer cells. Our data suggest a potential association of G691S *RET* polymorphism with NE lung tumor, proposing the necessity of more thorough evaluation of this possibility. The dataset of kinase mutation profiles in this report may help choosing cell line models for study of lung cancer.

Keywords

lung cancer; neuroendocrine; *RET*; G691S polymorphism

Introduction

Lung cancer is currently the most common cancer worldwide, comprising nearly 13% of new cancer cases each year and being the leading cause of cancer mortality (Jemal et al. 2011; Siegel et al. 2012). Lung cancers are mainly classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which account for approximately 87% and 13% of all lung cancer cases, respectively (Govindan et al. 2006). Lung cancers are also divided into various subtypes according to their different characteristics (Govindan et al.

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Conflict of Interest Statement

All authors declare that they have no conflict of interest

2006; Herbst et al. 2008). For example, neuroendocrine (NE) phenotypes characterize a spectrum of lung tumors from the low grade typical carcinoid and intermediate grade atypical carcinoid to the high grade large cell NE carcinoma and SCLC (Rekhtman 2010; Travis 2009; van Meerbeeck et al. 2011). NE lung tumors comprise 20–25% of all invasive lung malignancies, among which SCLC is the major whereas carcinoid tumors and large cell NE carcinomas are the minor (Swarts et al. 2012; Travis 2009). Multiple studies have revealed various genetic alterations in non-NE lung tumors, including adenocarcinoma and squamous cell lung carcinoma (Bergethon et al. 2012; Dutt et al. 2011; Hammerman et al. 2011; Kohno et al. 2012; Lipson et al. 2012; Pao et al. 2004; Soda et al. 2007; Takeuchi et al. 2012). However, such information on NE lung tumors is relatively limited, demanding identification of genetic alterations associated with NE lung tumors.

The proto-oncogene *RET* (*RE*arranged during *Trans*fection) encodes a receptor tyrosine kinase, which is activated by glial cell line-derived neurotrophic factor (Asai et al. 2006). While *RET* is essential for early development of the enteric nervous system and the kidney, and for spermatogenesis, different mutations in *RET* are associated with several human tumors with NE characteristics (de Groot et al. 2006). For example, multiple endocrine neoplasia type 2A (MEN2A), MEN2B and familial medullary thyroid carcinomas are attributed to the germline mutations that constitutively activate RET (Plaza Menacho et al. 2005; Santoro et al. 1995). Certain *RET* polymorphisms have also been characterized for their additive effects. For example, it was reported that RET L769L and S836S single nucleotide polymorphisms (SNP) are associated with increased risks of sporadic medullary thyroid carcinoma (Ceolin et al. 2012). It was also reported that the G691S SNP in *RET* exon 11 (rs1799939; c.2071G>A) may have a functional role as a genetic modifier in MEN2A (Robledo et al. 2003), medullary thyroid carcinoma (Cardot-Bauters et al. 2008; Elisei et al. 2004; Lantieri et al. 2012), desmoplastic melanoma (Barr et al. 2012; Narita et al. 2009), and pancreatic cancer (Sawai et al. 2005). *RET* mutations have also been reported in lung cancer, albeit not frequently. For example, a single somatic point mutation in *RET* (A664D in the juxta-membrane domain) was detected in two of seven SCLC specimens, although its functional significance is yet unclear (Futami et al. 1995). Allelic loss of the *RET* locus was also detected in SCLC patients (Futami et al. 2003). In addition, recent studies detected the *RET* gene fusions, i.e., *KIF5B-RET* and *CCDC6-RET*, in about 2% of lung adenocarcinoma patients (Kohno et al. 2012; Lipson et al. 2012; Takeuchi et al. 2012), and several somatic *RET* mutations, including R820L in the kinase domain, and A1051T in the cytoplasmic domain in SCLC patients (Peifer et al. 2012; Rudin et al. 2012). Therefore, *RET* may be involved in lung tumorigenesis. Nevertheless, *RET* mutations that characterize NE lung tumor have not been reported.

Human genome encodes 518 protein kinases, and deregulation of many kinases has been established as a major mechanism for cancer development (Taylor and Kornev 2011). In this study, we conducted kinome sequencing of nine human lung cancer lines to identify genetic alterations that may characterize NE lung tumor. Our study reveals various kinase mutations, including those that were previously reported. Further, we detected relatively high occurrence of G691S *RET* variant in lung cancer lines, specifically in lines with NE

phenotype, suggesting a potential association of the G691S *RET* polymorphism with NE lung tumor.

Material and Methods

Cell culture and reagents

Human SCLC cell lines (DMS53, NCI-H209, NCI-H69, SHP77, NCI-H889, NCI-H345, and NCI-H82) and human NSCLC cell lines (A549, NCI-H23, NCI-H460, NCI-H1155, NCI-H358, NCI-H727, NCI-H1770, and NCI-H125) were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, and 1% penicillin streptomycin, as previously described (Hong et al. 2013). All SCLC and the two NSCLC lines, NCI-H1155 and NCI-H727, were previously characterized as NE tumors (Carney et al. 1985; Gazdar and Minna 1996; Linnoila 1996). Human primary diploid fibroblasts IMR90 were maintained in MEM (Invitrogen, Carlsbad, CA) supplemented with 10% bovine growth serum, 1% sodium pyruvate, 1% MEM non-essential amino acids, and 1% penicillin streptomycin. Vandetanib was purchased from Invitrogen and LC Laboratories (Woburn, MA). Cabozantinib was purchased from Selleckchem (Huston, TX).

Genomic DNA extraction

Genomic DNA was extracted using PureLink Genomic DNA Kit (Invitrogen) according to the manufacture's protocol. The concentration of DNA and its high quality ($A_{260/280}$ of 1.8–2) were determined using the NanoVue Plus spectrophotometer (GE Healthcare Biosciences, Pittsburgh, PA).

Kinome sequencing

6.5 µg genomic DNA per cell line was used for kinome sequencing. Cell lines used were 4 SCLC lines (DMS53, NCI-H69, SHP77, and NCI-H889), five NSCLC lines (NCI-H23, NCI-H460, NCI-H1155, NCI-H358, and NCI-H727), and one normal control (IMR90). Briefly, genomic DNA specimens were enriched for targeted sequences using SureSelect Human Kinome Kit (Agilent Technologies, Santa Clara, CA). The sequencing targets were 612 genes, encoding 517 protein kinases, 12 phosphatidylinositol 3-kinase (PI3K) domain-containing genes, 12 diglyceride kinases, 6 PI3K regulatory components, 9 inositol polyphosphate kinases, 9 phosphatidylinositol phosphate (PIP)4/PIP5 kinases, 46 cancer related genes, and associated untranslated regions (Supplemental Table 1). Exon-enriched libraries were then sequenced using the Illumina high-throughput Genome Analyzer IIx sequencing system (Illumina, San Diego, CA) at the facility of Expression Analysis (Durham, NC). Analysis of sequence data was performed by Expression Analysis.

Immunoblot analysis

Cells were lysed in 62.5mM Tris (pH 6.8)/2% sodium dodecyl sulfate mixed with the protease inhibitor cocktail (Sigma, St. Louis, MO) and briefly sonicated before determining the protein concentration using the bicinchoninic acid reagent (Pierce, Rockford, IL). 40 µg of protein was resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane filter (Bio-Rad, Hercules, CA). Membrane filters were blocked in 0.1M Tris (pH 7.5)/0.9% NaCl/0.05% Tween 20 with 5% nonfat dry milk and incubated with the

appropriate antibodies. Antibodies were diluted as follows: neuron specific enolase (NSE), 1:2000 (Thermo Scientific, Rockford, IL); RET, 1:1000 (Santa Cruz Biotechnology, Santa Cruz, CA); phospho-RET (Tyr1062), 1:1000 (R&D Systems, Minneapolis, MN); poly(ADP-ribose) polymerase (PARP), 1:1000; B-cell lymphoma 2 (Bcl-2), 1:2000; cleaved lamin A, 1:2000 (Cell Signaling Biotechnology, Danvers, MA); β -actin, 1:15000 (Sigma). The Supersignal West Femto and Pico chemiluminescence kits (Pierce) were used for visualization of the signal. Images of immunoblots were taken and processed using ChemiDoc XRS+ and Image Lab version 3.0 (Bio-Rad).

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed by reverse transcription of 1 μ g total RNA isolated using Trizol (Invitrogen) and the subsequent 30 cycles of PCR using Taq polymerase (Invitrogen), as described elsewhere (Wu et al. 2013). To detect RET splicing variant 2 (RET51), AGGGGCGGAAGATGAAGA and ACCTTTCACAAAGAAATGTAACTATC were used. To detect RET splicing variant 4 (RET9), AGGGGCGGAAGATGAAGA and TGCAGAGGGGACAGCGGTGCTAGAA were used. As a control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified using CGGAGTCAACGGATTTGGTCGTAT and AGCCTTCTCCATGGTGGTGAAGAC.

Genotyping the RET polymorphism rs1799939

To determine the genotype at the locus of G691S *RET* (SNP rs1799939), a 302 base pair region in exon 11 of *RET* was amplified by PCR using TGGCGGTGCCAAGCCTCACACCAC and GACCCTCACCAGGATCTTGAAGGC. The PCR products were run on 2% agarose gel electrophoresis and were extracted for purification using GeneJET Gel Extraction Kit (Fisher Scientific – USA, Pittsburgh, PA). Approximately 50 ng of purified PCR products were sequenced using ATCCACTGTGCGACGAGC at Retrogen (San Diego, CA).

Statistical analysis

Genotype frequencies in the tested cell lines and the reference population were compared using multivariate logistic regression analysis for the co-dominant, dominant and recessive models. These analyses were performed using SNPStat (Sole et al. 2006). Allelic frequencies were compared using the Fisher's exact test (Preacher 2001). The odds ratios (OR) and 95% confidence intervals (CI) were calculated using the online statistical calculator, MedCalc. $P < 0.05$ was determined as being significant. As a reference general population, a multinational population (P1) from the National Center for Biotechnology Information (NCBI) SNP database was utilized. The P1 population consisted of 102 people of multi-racial origin and the genotype distribution at the G691S polymorphism (rs1799939) in this population is accessible at NCBI website. Based on Fisher's exact test, our sample size of all cell lines was adequate to detect 25% difference in the frequency of A-allele between the study and the reference groups with 76% power; and sample size of NE lines was adequate to detect 42% difference with 95% power.

Accession codes

Kinome sequencing raw data in this study are deposited at the Sequence Read Archive at NCBI (Accession: PRJNA248384 ID: 248384) and can be downloaded from <http://www.ncbi.nlm.nih.gov/bioproject/248384>.

Results

Kinome sequencing of human lung cancer cell lines

To identify kinase mutations associated with NE lung cancer, we performed exome sequencing of 517 protein kinases, 12 PI3K domain-containing genes, 12 diglyceride kinases, 6 PI3K regulatory components, 9 inositol polyphosphate kinases, 9 PIP4/PIP5 kinases, and 46 cancer related genes (Supplemental Table 1) in four SCLC and five NSCLC cell lines. Among these cell lines, all SCLC and two NSCLC (NCI-H1155 and NCI-H727) cell lines were previously characterized as NE tumors (Carney et al. 1985; Gazdar and Minna 1996; Linnoila 1996). Our kinome sequencing revealed a number of mutations, and those genes that exhibit nonsynonymous or frameshift mutations in at least one cell line are listed in the Supplemental Table 2. This sequencing also detected many mutations previously reported in these cell lines, which validates cell line identity (Supplemental table 3).

To determine the extent of overlap between our data and the data of other studies, we compared our results with the studies of genome-wide mutation profiles in SCLC patients conducted by Rudin et al. (2012) and Peifer et al. (2012). Although harboring different mutations, 160 kinase genes were commonly identified by us and Rudin et al. (2012), while 172 kinase genes were commonly identified by us and Peifer et al. (2012). Among these, 82 genes were common to all three studies (Fig. 1), of which 31 genes harbored different mutations in the same or adjacent exons in at least two studies (Table 1). Seven of these 31 genes exhibit identical mutations between the studies of Peifer et al. (2012) and Rudin et al. (2012). These mutations are *CDKN2A*^{A57T}, *LMTK2*^{P850L}, *MYLK*^{S287I}, *RBI*^{R251X}, *ROS1*^{A1043S}, *USP28*^{N48Y}, and *VRK2*^{Y187F} (Table 1). However, these mutations were not present in the lung cancer lines examined in this study. Of note, we found that NCI-H460 harbors *PIK3CA*^{E545K}, which was also detected in SCLC patients by Rudin et al. (2012) but not by Peifer et al. (2012) (Table 1). These data demonstrate that different human lung cancer lines harbor various mutations in the kinase-encoding genes.

In addition to the mutations above, we also found two non-synonymous mutations in the *RET* proto-oncogene. Intriguingly, the NE lung tumor lines, DMS53, NCI-H69, SHP77, NCI-H1155, and NCI-H727, exhibited a common polymorphism (rs1799939), which substitutes GGT (Gly) to AGT (Ser) at codon 691 in the protein juxta-membrane domain (Table 1). However, this mutation was not detected in any of the tested non-NE tumor lines, although NCI-H23 exhibited CGC (Arg) to CTC (Leu) substitution at codon 77 in the extracellular domain of RET, as previously reported (Abaan et al. 2013) (Table 1).

G691S *RET* variant is relatively frequent in NE lung cancer lines

Detection of G691S *RET* variant led us to further evaluate the genetic variation in in NE lung cancer cell lines. First, we verified the *RET* kinome sequencing results from the 9 lung cancer lines by sequencing their *RET* exon 11 region using PCR and the Sanger method. Besides, we also analyzed three additional SCLC and three additional NSCLC cell lines. This 2nd sequencing analysis confirmed the initial sequencing results and, furthermore, identified additional cell lines that harbor G691S *RET* variant (Table 2; Supplemental Fig. 1). In these 15 cell lines, overall frequency of the G691S allele (the “A allele” of the *RET* SNP rs1799939) was 43.3%, while NE and non-NE lines exhibited 60% and 10% of G691S allele frequency, respectively (Table 3). Importantly, the A allele frequency in 10 NE lines was significantly different from the allele frequency in the reference population ($p = 0.0001$), which is 17.6% according to the NCBI SNP database (see Materials and Methods for details). However, the A allele frequency in the 5 non-NE lung tumor lines was not significantly different from the allele frequency in the reference population ($p = 1.0$).

We next analyzed the genotypes distribution at rs1799939 (i.e., AA, GA, and GG) in lung cell lines in comparison with the reference population (Table 4). We found that the frequency of AA genotype is significantly higher in lung cell lines than in the reference population ($p = 0.0011$) whereas the frequency of GA genotype was not significantly different (Table 4). These data suggest that the G691S allele may be present more frequently in lung cancer, especially lung NE tumors, than in general population.

RET expression is not correlated with NSE expression in lung tumor lines

The correlation between the G691S *RET* variant and NE lung tumor lines led us to investigate whether expression of RET (wild type and RET^{G691S}), at mRNA and protein levels, is correlated with the levels of NSE in lung cancer cell lines. NSE is a key NE marker (Kasprzak et al. 2007), which is strongly expressed in NE lung tumor lines (Fig. 2A). As determined by RT-PCR of the *RET* splicing variants 2 (RET51) and 4 (RET9), and by Western blotting of RET (total and phosphorylated at Tyr1062), our analyses did not reveal any correlation between the levels of RET and NSE (Fig. 2). Therefore, although G691S *RET* mutation is correlated with NE lung tumor lines, expression levels of RET or RET^{G691S} are heterogeneous in the tumor lines.

We also investigated whether the receptor tyrosine kinase inhibitors, vandetanib (trade name Caprelsa, AstraZeneca) and cabozantinib (trade name Cometriq, Exelixis) could affect NSE expression in NCI-H209, NCI-H1155, and NCI-H125 cells, because these NSE-positive cell lines exhibited relatively high levels of RET autophosphorylation at Tyr1062, an indication of RET activation (Asai et al. 2006), and because vandetanib and cabozantinib can inhibit RET along with other receptor tyrosine kinases activated by vascular endothelial growth factor, epidermal growth factor, or hepatocyte growth factor (Carlomagno et al. 2002; Wedge et al. 2002; Yakes et al. 2011). In our analysis, neither of these inhibitors affected NSE levels in cells, although these inhibitors induced expected effects in these cell lines, i.e., downregulated RET activity, as indicated by RET autophosphorylation at Tyr1062, and suppressed cell survival, as indicated by altered expression levels of lamin A, Bcl-2, or

PARP (Supplemental Fig. 2). These data suggest that RET may not be involved in the maintenance of NE phenotype in lung cancer.

Discussion

Currently, no effective therapeutic modalities are available for the treatment of NE lung tumors. Particularly, SCLC is rarely treated by surgery due to its early metastasis and extensiveness at first diagnosis (van Meerbeeck et al. 2011). While this demands better understanding of the molecular alterations underlying NE lung tumors, this also limits the procurement of suitable fresh-frozen tumor specimens. Therefore, cell lines are an indispensable model for the study of these diseases. In this regard, genomic profiles of human lung tumor cell lines are important information for the design of future research, especially now that the genomic profiles of human lung tumors from patients are becoming available. We expect that the catalog of kinase genes whose alterations were analyzed in comparison with the results of two other integrative analyses of SCLC (Peifer et al. 2012; Rudin et al. 2012) may facilitate the usage of the cell lines examined in our study as a model for the identification and characterization of putative oncogenic mutations. Of note, although their mutations were not identical, 31 kinase-encoding and other cancer-related genes were commonly detected by this study as well as by the two previous studies (Peifer et al. 2012; Rudin et al. 2012).

In the human lung tumor cell lines, we detected two *RET* mutations, *RET*^{G691S} and *RET*^{R77L}. Our data suggest that the G691S *RET* SNP (c.2071G>A) may be associated with NE lung tumors. Intriguingly, G691 *RET* variant was reported only in one SCLC patient (Futami et al. 2003), but it was not reported in the studies of recent genome wide mutational analyses (Peifer et al. 2012; Pleasance et al. 2010; Rudin et al. 2012; Sos et al. 2012). It is possible that this polymorphism was overlooked in those studies because common polymorphisms are regarded as less significant than a novel mutation. Because allelic variation can be associated with the susceptibility to a certain disease, further analysis of G691S *RET* SNP in a larger scale is necessary to determine the significance of the *RET* polymorphism in NE lung tumor.

In addition to its association with a few tumor types featuring the phenotypes relevant to NE characteristics (Barr et al. 2012; Cardot-Bauters et al. 2008; Elisei et al. 2004; Narita et al. 2009; Sawai et al. 2005), G691S *RET* variant has been suspected for its role in tumorigenesis as a genetic modifier, particularly in MEN2A (Robledo et al. 2003). It is therefore conceivable that the G691S *RET* variant may also have a role in NE lung tumorigenesis. Nevertheless, our data did not reveal any correlation between the expression levels of RET, both mRNA and protein, and NSE. Moreover, the multi-receptor tyrosine kinase inhibitors, vandetanib and cabozantinib, did not affect the levels of NSE in the NE lung tumor lines expressing active RET, although the inhibitors suppressed RET activity. Given these data, RET does not appear to be necessary for the maintenance of NE phenotype in lung cancers. Nevertheless, it is possible that G691S *RET* mutation has a specific role at an early stage of NE lung carcinogenesis but, afterward, its role becomes unnecessary.

Conclusion

Our study provides the kinome sequencing results of human lung tumor cell lines, which may help facilitate future research using these cell lines. Our data suggest a potential association of G691S *RET* polymorphism with NE lung tumor, proposing the necessity of more thorough evaluation of this possibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
MEN2	multiple endocrine neoplasia type 2
NCBI	National Center for Biotechnology Information
NE	neuroendocrine
NSE	neuron-specific enolase
NSCLC	non-small cell lung carcinoma
OR	odds ratio
<i>RET</i>	<i>RE</i> arranged during <i>Tr</i> ansfection
SCLC	small cell lung carcinoma
SNP	single nucleotide polymorphism

References

- Abaan OD, Polley EC, Davis SR, Zhu YJ, Bilke S, Walker RL, Pineda M, Gindin Y, Jiang Y, Reinhold WC, et al. The exomes of the nci-60 panel: A genomic resource for cancer biology and systems pharmacology. *Cancer Res.* 2013; 73:4372–4382. [PubMed: 23856246]
- Asai N, Jijiwa M, Enomoto A, Kawai K, Maeda K, Ichihara M, Murakumo Y, Takahashi M. Ret receptor signaling: Dysfunction in thyroid cancer and hirschsprung's disease. *Pathol Int.* 2006; 56:164–172. [PubMed: 16634961]
- Barr J, Amato CM, Robinson SE, Kounalakis N, Robinson WA. The ret g691s polymorphism is a germline variant in desmoplastic malignant melanoma. *Melanoma Res.* 2012; 22:92–95. [PubMed: 22189301]
- Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, Massion PP, Siwak-Tapp C, Gonzalez A, Fang R, et al. Ros1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol.* 2012; 30:863–870. [PubMed: 22215748]

- Cardot-Bauters C, Leteurtre E, Leclerc L, Vantghem MC, Do Cao C, Wemeau JL, d'Herbomez M, Carnaille B, Barbu V, Pinson S, et al. Does the ret variant g691s influence the features of sporadic medullary thyroid carcinoma? *Clin Endocrinol (Oxf)*. 2008; 69:506–510. [PubMed: 18331611]
- Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, Ryan AJ, Fontanini G, Fusco A, Santoro M. Zd6474, an orally available inhibitor of kdr tyrosine kinase activity, efficiently blocks oncogenic ret kinases. *Cancer Res*. 2002; 62:7284–7290. [PubMed: 12499271]
- Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD. Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res*. 1985; 45:2913–2923. [PubMed: 2985257]
- Ceolin L, Siqueira DR, Ferreira CV, Romitti M, Maia SC, Leiria L, Crispim D, Ashton-Prolla P, Maia AL. Additive effect of ret polymorphisms on sporadic medullary thyroid carcinoma susceptibility and tumor aggressiveness. *Eur J Endocrinol*. 2012; 166:847–854. [PubMed: 22345297]
- de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM. Ret as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. *Endocr Rev*. 2006; 27:535–560. [PubMed: 16849421]
- Dutt A, Ramos AH, Hammerman PS, Mermel C, Cho J, Sharifnia T, Chande A, Tanaka KE, Stransky N, Greulich H, et al. Inhibitor-sensitive fgfr1 amplification in human non-small cell lung cancer. *PLoS One*. 2011; 6:e20351. [PubMed: 21666749]
- Elisei R, Cosci B, Romei C, Bottici V, Sculli M, Lari R, Barale R, Pacini F, Pinchera A. Ret exon 11 (g691s) polymorphism is significantly more frequent in sporadic medullary thyroid carcinoma than in the general population. *J Clin Endocrinol Metab*. 2004; 89:3579–3584. [PubMed: 15240649]
- Futami H, Egawa S, Takasaki K, Tsukada T, Shiraishi M, Yamaguchi K. Allelic loss of DNA locus of the ret proto-oncogene in small cell lung cancer. *Cancer Lett*. 2003; 195:59–65. [PubMed: 12767512]
- Futami H, Egawa S, Tsukada T, Maruyama K, Bandoh S, Noguchi M, Yamaguchi K. A novel somatic point mutation of the ret proto-oncogene in tumor tissues of small cell lung cancer patients. *Jpn J Cancer Res*. 1995; 86:1127–1130. [PubMed: 8635999]
- Gazdar AF, Minna JD. Nci series of cell lines: An historical perspective. *J Cell Biochem Suppl*. 1996; 24:1–11. [PubMed: 8806089]
- Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, Spitznagel EL, Piccirillo J. Changing epidemiology of small-cell lung cancer in the united states over the last 30 years: Analysis of the surveillance, epidemiologic, and end results database. *J Clin Oncol*. 2006; 24:4539–4544. [PubMed: 17008692]
- Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W, Brace LE, Woods BA, Lin W, Zhang J, et al. Mutations in the ddr2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer Discov*. 2011; 1:78–89. [PubMed: 22328973]
- Herbst RS, Heymach JV, Lippman SM. Lung cancer. *N Engl J Med*. 2008; 359:1367–1380. [PubMed: 18815398]
- Hong SK, Kim JH, Starenki D, Park JI. Autophagy sensitivity of neuroendocrine lung tumor cells. *Int J Oncol*. 2013; 43:2031–2038. [PubMed: 24126619]
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011; 61:69–90. [PubMed: 21296855]
- Kasprzak A, Zabel M, Biczysko W. Selected markers (chromogranin a, neuron-specific enolase, synaptophysin, protein gene product 9.5) in diagnosis and prognosis of neuroendocrine pulmonary tumours. *Pol J Pathol*. 2007; 58:23–33. [PubMed: 17585539]
- Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, Sakamoto H, Tsuta K, Furuta K, Shimada Y, et al. Kif5b-ret fusions in lung adenocarcinoma. *Nat Med*. 2012; 18:375–377. [PubMed: 22327624]
- Lantieri F, Caroli F, Ceccherini I, Griseri P. The involvement of the ret variant g691s in medullary thyroid carcinoma enlightened by a meta-analysis study. *Int J Cancer*. 2012; 132:2808–2819. [PubMed: 23180660]
- Linnoila RI. Spectrum of neuroendocrine differentiation in lung cancer cell lines featured by cytomorphology, markers, and their corresponding tumors. *J Cell Biochem Suppl*. 1996; 24:92–106. [PubMed: 8806093]

- Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, Curran JA, Balasubramanian S, Bloom T, Brennan KW, et al. Identification of new alk and ret gene fusions from colorectal and lung cancer biopsies. *Nat Med.* 2012; 18:382–384. [PubMed: 22327622]
- Narita N, Tanemura A, Murali R, Scolyer RA, Huang S, Arigami T, Yanagita S, Chong KK, Thompson JF, Morton DL, et al. Functional ret g691s polymorphism in cutaneous malignant melanoma. *Oncogene.* 2009; 28:3058–3068. [PubMed: 19561646]
- Oie HK, Russell EK, Carney DN, Gazdar AF. Cell culture methods for the establishment of the nci series of lung cancer cell lines. *J Cell Biochem Suppl.* 1996; 24:24–31. [PubMed: 8806091]
- Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, et al. Egf receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A.* 2004; 101:13306–13311. [PubMed: 15329413]
- Peifer M, Fernandez-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, Plenker D, Leenders F, Sun R, Zander T, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nat Genet.* 2012; 44:1104–1110. [PubMed: 22941188]
- Pettengill OS, Sorenson GD, Wurster-Hill DH, Curphey TJ, Noll WW, Cate CC, Maurer LH. Isolation and growth characteristics of continuous cell lines from small-cell carcinoma of the lung. *Cancer.* 1980; 45:906–918. [PubMed: 6266631]
- Phelps RM, Johnson BE, Ihde DC, Gazdar AF, Carbone DP, McClintock PR, Linnoila RI, Matthews MJ, Bunn PA Jr, Carney D, et al. Nci-navy medical oncology branch cell line data base. *J Cell Biochem Suppl.* 1996; 24:32–91. [PubMed: 8806092]
- Plaza Menacho I, Koster R, van der Sloot AM, Quax WJ, Osinga J, van der Sluis T, Hollema H, Burzynski GM, Gimm O, Buys CH, et al. Ret-familial medullary thyroid carcinoma mutants y791f and s891a activate a src/jak/stat3 pathway, independent of glial cell line-derived neurotrophic factor. *Cancer Res.* 2005; 65:1729–1737. [PubMed: 15753368]
- Pleasant ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, Lin ML, Beare D, Lau KW, Greenman C, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature.* 2010; 463:184–190. [PubMed: 20016488]
- Preacher, KJ.; Briggs, NE. [Accessed 24 October 2013] Calculation for fisher's exact test: An interactive calculation tool for fisher's exact probability test for 2×2 tables. 2001. <http://quantpsy.org>
- Rekhtman N. Neuroendocrine tumors of the lung: An update. *Arch Pathol Lab Med.* 2010; 134:1628–1638. [PubMed: 21043816]
- Robledo M, Gil L, Pollan M, Cebrian A, Ruiz S, Azanedo M, Benitez J, Menarguez J, Rojas JM. Polymorphisms g691s/s904s of ret as genetic modifiers of men 2a. *Cancer Res.* 2003; 63:1814–1817. [PubMed: 12702567]
- Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS, Bergbower EA, Guan Y, Shin J, Guillory J, et al. Comprehensive genomic analysis identifies sox2 as a frequently amplified gene in small-cell lung cancer. *Nat Genet.* 2012; 44:1111–1116. [PubMed: 22941189]
- Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, et al. Activation of ret as a dominant transforming gene by germline mutations of men2a and men2b. *Science.* 1995; 267:381–383. [PubMed: 7824936]
- Sawai H, Okada Y, Kazanjian K, Kim J, Hasan S, Hines OJ, Reber HA, Hoon DS, Eibl G. The g691s ret polymorphism increases glial cell line-derived neurotrophic factor-induced pancreatic cancer cell invasion by amplifying mitogen-activated protein kinase signaling. *Cancer Res.* 2005; 65:11536–11544. [PubMed: 16357163]
- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin.* 2012; 62:10–29. [PubMed: 22237781]
- Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, et al. Identification of the transforming eml4-alk fusion gene in non-small-cell lung cancer. *Nature.* 2007; 448:561–566. [PubMed: 17625570]
- Sole X, Guino E, Valls J, Iniesta R, Moreno V. Snpstats: A web tool for the analysis of association studies. *Bioinformatics.* 2006; 22:1928–1929. [PubMed: 16720584]

- Sos ML, Dietlein F, Peifer M, Schottle J, Balke-Want H, Muller C, Koker M, Richters A, Heynck S, Malchers F, et al. A framework for identification of actionable cancer genome dependencies in small cell lung cancer. *Proc Natl Acad Sci U S A*. 2012; 109:17034–17039. [PubMed: 23035247]
- Swarts DR, Ramaekers FC, Speel EJ. Molecular and cellular biology of neuroendocrine lung tumors: Evidence for separate biological entities. *Biochim Biophys Acta*. 2012; 1826:255–271. [PubMed: 22579738]
- Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, Asaka R, Hamanaka W, Ninomiya H, Uehara H, et al. Ret, ros1 and alk fusions in lung cancer. *Nat Med*. 2012; 18:378–381. [PubMed: 22327623]
- Taylor SS, Kornev AP. Protein kinases: Evolution of dynamic regulatory proteins. *Trends Biochem Sci*. 2011; 36:65–77. [PubMed: 20971646]
- Travis WD. Lung tumours with neuroendocrine differentiation. *Eur J Cancer* 45 Suppl. 2009; 1:251–266.
- van Meerbeeck JP, Fennell DA, De Ruysscher DK. Small-cell lung cancer. *Lancet*. 2011; 378:1741–1755. [PubMed: 21565397]
- Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, Boffey SJ, Valentine PJ, Curwen JO, Musgrove HL, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res*. 2002; 62:4645–4655. [PubMed: 12183421]
- Wu PK, Hong SK, Veeranki S, Karkhanis M, Starenki D, Plaza JA, Park JJ. A mortalin/hspa9-mediated switch in tumor-suppressive signaling of raf/mek/extracellular signal-regulated kinase. *Mol Cell Biol*. 2013; 33:4051–4067. [PubMed: 23959801]
- Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, Qian F, Chu F, Bentzien F, Cancilla B, et al. Cabozantinib (XL184), a novel met and vegfr2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther*. 2011; 10:2298–2308. [PubMed: 21926191]

Fig. 1. Comparison of genes identified by this study (Sosonkina et al.) and two other studies (Peifer et al. 2012, and Rudin et al. 2012).

Genes mutated in lung cancer cell lines were compared with the genes mutated in SCLC patients. 82 common genes that harbor identical or different mutations are listed. These mutations are described in Supplemental Table 2.

Fig. 2. Expression of RET in lung cancer cell lines

(A) Total cell lysates of lung cancer lines maintained in RPMI medium supplemented with 10% fetal bovine serum were analyzed by Western blotting for phosphorylated RET (p-RET), RET, and NSE expression. GAPDH is the loading control. (B) Total RNA extracted from the lung cancer cell cultures in (A) were examined by RT-PCR for expression of RET splicing variants 2 and 4. GAPDH is the control for use of equal amount of RNA for PCR.

The 31 genes harboring identical or closely located mutations detected in this study and in one or two reference studies Peifer et al. (2012) and Rudin et al. (2012).

Table 1

Gene	Location	Exon	Mutation; (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
ACVR1C isoform 1	2q22.3	exon 9	c.1169A>G; p.D390G		NCL-H23, NCL-H727 (hetero*)
		exon 3		c.4521G>T; p.V151L	NCL-H358 (hetero)
ATM	11q22.3	exon 37	c.5600A>C; p.Q1867P	c.G5557A; p.D1853N (rs1801516)	NCL-H727, NCL-H345 (hetero)
		exon 10		c.G1541A; p.G514D (rs2235000)	NCL-H23 (homo)
		exon 22		c.G3192T; p.M1064I	NCL-H69 (hetero)
		exon 28		c.C4138T; p.H1380Y	NCL-H23 (homo)
		exon 38		c.A5756C; p.Q1919P	NCL-H23 (homo)
		exon 43		c.C6205T; p.Q2069X	NCL-H69 (hetero)
		exon 48		c.C7004T; p.T2335I (rs3092831)	NCL-H1155 (hetero)
BMPRIA	10q23.2	exon 11		c.1175A>G; p.N392S	
		exon 10		c.A911G; p.Q304R	NCL-H23 (hetero)
				c.G1051A; p.G351S	NCL-H1155 (hetero)
CCNE1	19q12	exon 9		c.T779G; p.V260G	NCL-H69 (hetero)
		exon 12	c.1111G>T; p.D371Y	c.A1183C; p.T395P	NCL-H23, NCL-H1155, NCL-H358, NCL-H727, IMR90 (hetero)
		exon 3		c.G202A; p.D68N	NCL-H23 (hetero)
CDKN2A	9p21.3	exon 2	c.169G>A; p.A57T	c.169G>A; p.A57T	NCL-H69 (hetero)
ERBB4	2q34	exon 27	c.3320G>C; p.C1107S	c.A3328C; p.T1110P	SHP77 (hetero)
		exon 3		c.G320T; p.G107V	SHP77 (hetero)
		exon 21		c.G2635T; p.G879X	NCL-H727 (hetero)
		exon 25		c.2965_2965del; p.989_989del	NCL-H727 (hetero)
FLT4	5q35.3	exon 9		c.G1213T; p.A405S	NCL-H69 (hetero)
		exon 10	c.1267_1268insC; p.Q423fs	c.1335C>G; p.C445W	
		exon 13		c.A1952G; p.Y651C	DMS53 (hetero)

Gene	Location	Exon	Mutation; (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
IGF1R	15q26.3	exon 19		c.C1921T; p.P641S (rs55667289) c.C2670G; p.H890Q (rs448012)	NCL-H727 (hetero) NCL-H460 (hetero)
		exon 6	c.1411C>T; p.R471C	c.1454G>T; p.R485I	NCL-H727 (hetero)
KALRN	3q21.2	exon 42		c.T6082G; p.S2028A	NCL-H69 (hetero)
		exon 43		c.6199C>T; p.Q2067X	
		exon 5		c.G875A; p.R292Q	NCL-H1155 (hetero)
		exon 13		c.T2179A; p.S727T	NCL-H1155 (homo)
		exon 14		c.A2480G; p.E827G	NCL-H1155 (hetero)
		exon 41		c.G5998T; p.E2000X	NCL-H69 (hetero)
		exon 48		c.G6916A; p.G2306R	NCL-H69 (hetero)
		exon 51		c.C7367G; p.T2456S	NCL-H23 (hetero)
LMTK2	7q21.3	exon 11	c.2549C>T; p.P850L	c.2549C>T; p.P850L	
		exon 12		c.G4202A; p.S1401N (rs45488394)	NCL-H23 (hetero)
LRRK2	12q12	exon 35		c.5044C>A; p.L1682I	
		exon 34		c.T4939A; p.S1647T (rs11564148)	DMS53, NCL-H889, NCL-H23 (hetero)
	exon 14			c.C1653G; p.N551K (rs7308720)	NCL-H1155 (homo); NCL-H460, NCL-H358 (hetero)
				c.G4193A; p.R1398H (rs7133914)	NCL-H1155 (homo); NCL-H460, NCL-H358 (hetero)
	exon 30			c.C4355T; p.T1452I	NCL-H358 (hetero)
				c.A6241G; p.N2081D (rs33995883)	NCL-H727 (homo)
	6q23.3	exon 15	C2089G>A; p.G697S	c.G2042T; p.G681V	NCL-H358 (homo)
				c.A2021T; p.D674V	SHIP77 (hetero)
MAST1	19p13.2	exon 7		c.C1220T; p.T407M	NCL-H23 (homo)
		exon 14	c.1537C>T; p.L513F	c.C1517A; p.T506N c.G1630A; p.D544N	NCL-H69 (hetero) NCL-H1155 (hetero)

Gene	Location	Exon	Mutation; (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
MAST2	1p34.1	exon 29		c.4039G>C; p.A1347P	DMS53 (homo); NCI-H358 (hetero)
		exon 8		c.G893A; p.R298Q	NCI-H1155 (hetero)
	exon 10			c.T1164G; p.D388E (rs11211247)	SHP77, NCI-H889, NCI-H358 (hetero)
		exon 27		c.A3590G; p.K1197R (rs1052607)	DMS53 (homo); NCI-H358, NCI-H345 (hetero)
	exon 29			c.A5018G; p.K1673R (rs34070850)	SHP77 (hetero)
MET	7q31.2	exon 12		c.2711_2713del; p.904_905del	DMS53 (hetero)
		exon 13	c.2823A>G; p.I941M		
	exon 14			c.C2908T; p.R970C (rs34589476)	NCI-H69 (hetero)
MYLK	3q21.1	exon 10	c.860G>T; p.S287I	c.860G>T; p.S287I	NCI-H23 (hetero)
				c.T810A; p.D270E	DMS53 (hetero)
				c.T782C; p.V261A (rs3796164)	NCI-H23 (hetero)
	exon 17		c.2360G>T; p.W787L		
	exon 18			c.3199_3201del; p.1067_1067del	NCI-H69, NCI-H23 (hetero)
				c.T2582C; p.L861P	NCI-H69, NCI-H23 (hetero)
				c.C2742A; p.D914E	NCI-H69, NCI-H23 (hetero)
				c.C62A; p.P21H (rs28497577)	NCI-H69, NCI-H23, NCI-H460 (hetero)
	exon 4				
MYO3A	10p12.1	exon 22		c.G2497T; p.A833S (rs33947968)	NCI-H1155 (homo)
		exon 23		c.2534C>A; p.A845D	
	exon 27			c.G3028A; p.E1010K	NCI-H23 (homo)
NEK2	1q32.3	exon 8		c.T1307C; p.L436P	NCI-H727 (homo)
		exon 3		c.G401A; p.G134D	NCI-H460 (hetero)
NTRK3	15q25.3	exon 7	c.729G>C; p.W243C	c.G669T; p.E223D	NCI-H727 (hetero)
				c.731T>C; p.I244T c.625C>A; p.L209I	
PAK7	20p12.2	exon 5		c.956c>T; p.P319L	
		exon 6	c.1056G>T; p.R352S	c.G1004C; p.R335P (rs11700112) c.G1322T; p.S441I	NCI-H460 (hetero) DMS53 (hetero)

Gene	Location	Exon	Mutation ¹ (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
		exon 7		c.G1532A; p.S511N (rs2297345)	DMS53, NCI-H460, NCI-H727, NCI-H345 (hetero); NCI-H23 (homo)
PIK3CA	3q26.32	exon 10	none	c.1633G>A; p.E545K	NCI-H460 (hetero)
				c.1624G>C; p.E542Q	
	exon 2	exon 7		c.307_315del; p.103_105del	NCI-H69 (hetero)
		exon 13		c.A1173G; p.I391M (rs3729680)	SHP77 (homo)
	exon 14	exon 14		c.T1930C; p.Y644H	NCI-H889, NCI-H23 (hetero)
		exon 18		c.G2119A; p.E707K	NCI-H889 (hetero)
PSKH2	8q21.3	exon 3	c.1038C>G; p.P346P	c.C2529G; p.D843E	NCI-H1155 (hetero)
		exon 5	c.388C>G; p.R130Q	c.A1006G; p.I336V (rs16876805)	NCI-H23 (homo)
PTEN	10q23.31	exon 5	c.388C>G; p.R130G	c.G1097T; p.R366M	NCI-H460 (homo)
		exon 6	c.495A>T; p.G165G		
	exon 7		c.494G>A; p.G165E		
RBI	13q14.2	exon 8	c.751C>T; p.R251X	c.G697T; p.R233X	NCI-H1155 (homo)
		exon 22	c.2214A>G; p.T738T		
RET	10q11.21	exon 11		c.G2071A; p.G691S (rs1799939)	DMS53, NCI-H69, SHP77, NCI-H1155 (homo); NCI-H727 (hetero)
		exon 14	c.2459G>T; p.R820L		
	exon 19			c.3151G>A; p.A1051T	
	exon 2			c.G230T; p.R77L	NCI-H23 (homo)
ROR2	9q22.31	exon 7	c.1082G>C; p.G361A	c.T1165C; p.C389R	NCI-H69 (hetero)
			c.1069G>T; p.E357X		
	exon 9	exon 9	c.2155G>A; p.A719TX	c.C2134T; p.P712S	NCI-H23 (hetero)
		exon 6		c.G1720T; p.V574L	NCI-H69 (hetero)
				c.A733G; p.T245A (rs10820900)	DMS53, NCI-H345 (homo)

Gene	Location	Exon	Mutation; (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
ROS1	6q22.1	exon 42	c.6710G>T; p.G2237V	c.6731A>G; p.E2244G	DMS53, NCI-H889, NCI-H345 (hetero); NCI-H23, NCI-H1155 (homo)
		exon 21	c.3127G>T; p.A1043S	c.3127G>T; p.A1043S	DMS53, NCI-H889, NCI-H345 (hetero); NCI-H23, NCI-H1155 (homo)
		exon 6		c.G500A; p.R167Q (rs2243380)	NCI-H1155 (homo); NCI-H345 (hetero)
		exon 26		c.G4177T; p.D1393Y	NCI-H69 (hetero)
TP53 isoform 1	17p13.1	exon 8	c.817C>T; p.R273C	c.817C>T; p.R273C	NCI-H1155 (homo)
		exon 4	c.818G>T; p.R273L	c.112C>T; p.Q38X	DMS53 (hetero)
		exon 7	c.736A>G; p.M246V	c.725G>A; p.C242Y	NCI-H23 (homo)
ULK4	3p22.1	exon 22	c.2194_splice; e22-3	c.C722T; p.S241F (rs28934573)	DMS53 (hetero)
		exon 24	c.496_497insACAAGCAGT; p.Q165_S166insYKQ	c.496_497insACAAGCAGT; p.Q165_S166insYKQ	NCI-H727 (hetero)
		exon 11	c.G511T; p.E171X	c.G511T; p.E171X	NCI-H69 (homo)
USP28	11q23.2	exon 25	c.142A>T; p.N48Y	c.142A>T; p.N48Y	NCI-H358 (hetero)
		exon 18	c.3082G>T; p.E1028X	c.G3133A; p.E1045K	NCI-H358 (hetero)
		exon 21	c.3082G>T; p.E1028X	c.G3133A; p.E1045K	NCI-H358 (hetero)

Gene	Location	Exon	Mutation; (refSNP number)		Cell line (mutation type)
			Peifer et al. (2012)	Rudin et al. (2012)	
		exon 7		c.C757T; p.Q253X	NCL-H1155 (hetero)
		exon 16		c.G1829A; p.R610Q	NCL-H727 (hetero)
		exon 24		c.G2893A; p.E965K	NCL-H23 (homo)
VRK2	2p16.1	exon 8	c.560A>T; p.Y187F	c.560A>T; p.Y187F	NCL-H69, NCL-H460 (homo); SHP77, NCL-H889, NCL-H23, NCL-H345, NCL-H358, NCL-H727 (hetero)
		exon 7		c.A499G; p.I167V (rs1051061)	

* Hetero, heterozygous; homo, homozygous.

Table 2

Characterization of cell lines panel.

Number	Type	Phenotype	NSE expression	RET status	Sequencing method
DMS53	SCLC	NE	low	exon11:c.G2071A; p.G691S, homo	NGS, capillary
NCI-H209	SCLC	NE	high	exon11:c.G2071A; p.G691S, homo	capillary
NCI-H82	SCLC	NE	high	exon11:c.G2071A; p.G691S, hetero	capillary
NCI-H69	SCLC	NE	high	exon11:c.G2071A; p.G691S, homo	NGS, capillary
NCI-H889	SCLC	NE	high	wild type	NGS, capillary
NCI-H345	SCLC	NE	high	wild type	NGS, capillary
SHP77	SCLC	NE	medium	exon11:c.G2071A; p.G691S, homo	NGS, capillary
A549	NSCLC	non-NE	low	exon11:c.G2071A; p.G691S, hetero	capillary
NCI-H23	NSCLC	non-NE	none	exon2:c.G230T; p.R77L, homo	NGS, capillary
NCI-H460	NSCLC	non-NE	none	wild type	NGS, capillary
NCI-H1155	NSCLC	NE	high	exon11:c.G2071A; p.G691S, homo	NGS, capillary
NCI-H358	NSCLC	non-NE	none	wild type	NGS, capillary
NCI-H727	NSCLC	NE	none	exon11:c.G2071A; p.G691S, hetero	NGS, capillary
NCI-H125	NSCLC	Mixed*	low	wild type	capillary
NCI-H1770	NSCLC	NE	high	wild type	capillary

Hetero, heterozygous; homo, homozygous; NE, neuroendocrine; NGS, next-generation sequencing; NSE, neuron-specific enolase; NSCLC, non-small cell lung cancer; SCLC, small-cell lung cancer.

* This cell line displayed mixed phenotype according to Phelps et al. (1996).

Table 3
Genotypes distribution at *RET* rs1799939 and frequencies of the A allele in cell lines and reference population ^a.

	Genotypes				A-allele frequency	p-value ^b	OR (95% CI) ^c
	AA	GA	GG	GA			
Reference population	4	28	70		0.176		
Total lines (n=15)	5	3	7		0.433	0.003	3.57 (1.59 – 7.99)
NE lines (n=10)	5	2	3		0.6	0.0001	7 (2.67 – 18.36)
non-NE lines (n=5)	0	1	4		0.1	1.0	0.52 (0.06 – 4.22)

^a Described in Materials and Methods.
^b Fisher's exact test.

^c OR = odds ratio of A-allele occurring in the study group vs the reference population; IC = 95% confidential interval.

Table 4

Logistic regression analysis of genotypes distribution at *RET* rs1799939 in 15 cell lines and in the reference population[‡] (n=102)

Genotype	OR (95% CI) [*]	P
GG	1.00 ^a	0.0049
GA	1.07 (0.26 – 4.44)	
AA	12.50 (2.71 – 57.57)	
GA + AA vs. GG	2.50 (0.83 – 7.49) ^b	0.1
GA vs. GG + AA	0.66 (0.17 – 2.52)	0.53
AA vs. GG + GA	12.25 (2.83 – 53.12) ^c	0.0011

^aCo-dominant model of inheritance.

^bDominant model of inheritance.

^cRecessive model of inheritance.

[‡]as described in Material and Methods.

^{*}odd ratio (OR) and 95% confidential interval (CI).