RICTOR Amplification Defines a Novel Subset of Patients with Lung Cancer Who May Benefit from Treatment with mTORC1/2 Inhibitors

Haiying Cheng¹, Yiyu Zou¹, Jeffrey S. Ross², Kai Wang³, Xuewen Liu³, Balazs Halmos¹,³, Siraj M. Ali², Huijie Liu¹, Amit Verma¹, Cristina Montagna⁵, Abraham Chachoua¹, Sanjay Goel¹, Edward L. Schwartz¹, Changcheng Zhu², Jidong Shan⁵, Yiting Yu¹, Kira Gritsman¹, Roman Yelensky², Doron Lipson³, Geoff Otto², Matthew Hawryluk², Philip J. Stephens², Vincent A. Miller², Bilal Piperdi¹, and Roman Perez-Soler¹

ABSTRACT

We identified amplification of RICTOR, a key component of the mTOR complex 2 (mTORC2), as the sole actionable genomic alteration in an 18-year-old never-smoker with lung adenocarcinoma. Amplification of RICTOR occurs in 13% of lung cancers (1,016 cases) in The Cancer Genome Atlas and at a similar frequency in an independent cohort of 1,070 patients identified by genomic profiling. In the latter series, 11% of cases harbored RICTOR amplification as the only relevant genomic alteration. Its oncogenic roles were suggested by decreased lung cancer cell growth both in vitro and in vivo with RICTOR ablation, and the transforming capacity of RICTOR in a Ba/F3-cell system. The mTORC1/2 inhibitors were significantly more active against RICTOR-amplified lung cancer cells as compared with other agents targeting the PI3K–AKT–mTOR pathway. Moreover, an association between RICTOR amplification and sensitivities to mTORC1/2 inhibitors was observed. The index patient has been treated with mTORC1/2 inhibitors that led to tumor stabilization for more than 18 months.

SIGNIFICANCE: RICTOR amplification may define a novel and unique molecular subset of patients with lung cancer who may benefit from treatment with mTORC1/2 inhibitors.

Cancer Discov; 5(12); 1262–70.
© 2015 AACR.

INTRODUCTION

The advent of targeted therapeutics against oncogenic genomic alterations has revolutionized the treatment paradigm in subgroups of patients with lung cancer. However, targetable genomic alterations have not been identified in nearly half of lung adenocarcinomas (1). We evaluated an 18-year-old male never-smoker with lung adenocarcinoma for possible targeted therapy. The only genomic alteration identified by a hybridization capture–based next-generation sequencing assay (FoundationOne) was amplification of RICTOR.

RICTOR, the defining component of the mTOR complex 2 (mTORC2; ref. 2), is the upstream kinase of several AGC kinase family members, including AKT, SGK, S6K mutants, and several PKC isoforms (3, 4). Activation of RICTOR–mTORC2 modifies actin organization and promotes cell proliferation and survival. The less well-characterized mTOR-independent functions of RICTOR regulate cell morphology, migration, and protein degradation (3, 5). In the context of...
these diverse functions, mTORC2 has critical oncogenic roles in regulating cancer cell migration, invasion, and metastasis in gliomas and in breast, ovarian, prostate, and colorectal cancers (6–8). Specifically, PTEN-induced prostatic oncogenesis is dependent on RICTOR dosage (8). Recently, the overexpression of RICTOR was demonstrated to induce malignant glioma formation in a transgenic mouse model (9).

In this study, we determined the prevalence of RICTOR amplification in two independent series of human lung carcinoma cases, tested the effects of blockade of RICTOR signaling in lung cancer cells both in vitro and in vivo, and demonstrated clinical benefit in a patient with RICTOR-amplified lung carcinoma when treated with dual mTORC1/2 inhibitors. We also determined the association between RICTOR amplification and sensitivities to mTORC1/2 inhibitors.

RESULTS
Identification of RICTOR Amplification as the Sole Actionable Genomic Alteration in a Young Patient with Lung Cancer

A young male never-smoker was diagnosed with lung adenocarcinoma in May 2010 at the age of 18 (Fig. 1A). Lung cancer in patients this young is exceedingly uncommon. He was considered to have either locally advanced or metastatic disease, given that his PET/CT revealed possible bilateral disease, given that his PET/CT revealed possible bilateral lesions. Initial focused genomic testing was negative for actionable target in the tested gene panel in 11% of 85 cases. Interestingly, 14.6% in small cell lung cancer (7/48), 8.7% in large cell neuroendocrine carcinoma (2/23), 8.4% in adenocarcinoma (61/724), and 7.4% in squamous cell carcinoma (8/108). Interestingly, RICTOR amplification was the sole potentially actionable target in the tested gene panel in 11% of 85 cases. The median age for these patients with RICTOR amplification as the only actionable alteration is 64 years old, including 58% female (49/85) and 42% male (36/85). One third of the cases with RICTOR amplification (29/85) had alterations in other genes within the PI3K–AKT–mTOR pathway. In addition, 26% (22/85) and 14% (12/85) of these patients had alterations in EGFR and KRAS as an additional alteration in lung adenocarcinomas (Fig. 1B, Supplementary Fig. S3A and S3B).

Nonfocal RICTOR amplifications were detected in 21 cases, and they were excluded from the analysis.

The Effects of RICTOR Knockdown on RICTOR-Amplified and RICTOR-Nonamplified Lung Cancer Cells

We screened a tissue microarray of lung cancer cell lines for RICTOR amplification using the FISH assay. The majority of cell lines had normal copy numbers of RICTOR with a ratio of RICTOR/control around 1, but 3 of 27 non–small cell lung cancer (NSCLC) cell lines, including H23, H1734, and H1703, were found to have a ratio of RICTOR/control of 2, indicating amplification of RICTOR (Fig. 2A). Of note, in addition to RICTOR amplification, H23 also harbors a KRAS mutation, whereas H1703 has a PDGFR amplification.

The effects of siRNA-mediated RICTOR knockdown in RICTOR-amplified H23 and H1703 cells were next examined. Both siRNAs diminished RICTOR expression, and the decrease in both AKT$^{S473}$ phosphorylation and colony formation was consistent with a functional effect of this intervention (Fig. 2A). Similarly, inducible shRNA RICTOR knockdown was also associated with significantly reduced proliferation of both H23 and H1703 cells (Fig. 2B and C). We next examined the role of RICTOR in lung tumor growth.

Published OnlineFirst September 14, 2015; DOI: 10.1158/2159-8290.CD-14-0971
Figure 1. A, timeline of diagnosis, treatment, and responses of the index patient with RICTOR amplification. Inset, Western blot: CC-223 therapy was associated with modest inhibition of pAKT S473 and p4EBP1 in patient’s blood cells (PBMC). RICTOR amplification, mTORC1/2 inhibitors, and their responses shown in red. Carbo, carboplatin; Dx, diagnosis; dz, disease; ImmunoRx, immunotherapy; NGS, next-generation sequencing; pem, pemetrexed; POD, progression of disease; PR, partial response.

B, tile plot showing the gene alterations in each individual with RICTOR-amplified tumors, focusing on the commonly assessed genomic alterations in lung cancer (RTK/RAS/RAF) and PI3K/AKT/mTOR alterations. For the full list, please refer to Supplementary Fig. S3A and S3B. Note: The case of the index patient was sequenced in 2012 using the FoundationOne 182 gene panel, and all 85 RICTOR-amplified cases presented in B used the FoundationOne 236 gene panel. Thus, the index patient is not included in the 85-case amplified series.
Figure 2. The effects of genetic knockdown of RICTOR and pharmacologic inhibition on RICTOR-amplified lung cancer cells. A, FISH in lung cancer cell lines: both H23 and H1703 cells have a ratio of RICTOR/control of 2, and thus are RICTOR amplified. Western blotting and colony formation assays: the effects of transient siRNA-mediated RICTOR knockdown on RICTOR-amplified H23 cells and H1703 cells. *, \( P < 0.05 \). B, Western blot: the effects of inducible shRNA-mediated RICTOR knockdown on RICTOR-amplified H23 cells and H1703 cells. The addition of doxycycline led to RICTOR knockdown in cells. C, colony formation assay: the addition of doxycycline led to RICTOR knockdown and was associated with decreased colony formation. *, \( P < 0.05 \). D, xenograft mouse models: the effects of inducible shRNA-mediated RICTOR knockdown on H23 cells and H1703 xenografts. *, \( P < 0.05 \). E, the effects of PI3K/AKT/mTOR inhibitors on RICTOR-amplified H23 cells. Among all the examined agents targeting the PI3K-AKT-mTOR pathways, BEZ235 (dual PI3K/mTOR inhibitor) and AZD2014 (dual mTORC1/2 inhibitor) were the most potent and showed similar inhibition of pmTOR, pAKT\(^{S473}\), and pS6.
in vivo using murine xenografts. As shown in Fig. 2D, inducible RICTOR knockdown significantly reduced H1703 xenograft tumor growth. Taken together, genetic ablation of RICTOR is associated with growth inhibition of RICTOR-amplified lung cancer, consistent with RICTOR’s role as an oncogenic driver.

The in vitro potency of inhibitors targeting different components of the P13K–AKT–mTOR pathways, including BEZ235 (dual PI3K/mTOR inhibitor), AZD2014 (dual mTORC1/2 inhibitor), RAD001 (everolimus, a rapamycin analogue and mTORC1 inhibitor), MK2206 (AKT inhibitor), and LY294002 (PI3K inhibitor), as well as erlotinib (EGFR tyrosine kinase inhibitor), was determined. Among all the examined agents, BEZ235 and AZD2014 were the most potent in RICTOR-amplified H23 cells in a concentration-dependent manner. As expected for dual mTORC1/2 inhibitors, both agents decreased phosphorylation of S6RP, AKT, and mTOR (Fig. 2E). In contrast, RAD001 inhibited only pS6 and slightly increased pAKT(S473), suggestive of a feedback effect between mTORC1 and mTORC2. Analogous results were observed in H1703, another RICTOR-amplified lung cancer cell line (data not shown).

We also examined the role of RICTOR in lung cancer cells that lack RICTOR amplification but contain other molecular abnormalities. We found that RICTOR knockdown also reduced colony formation in NSCLC cell lines that lack RICTOR amplification, such as A549 (KRAS mutated) and HCC827 (EGFR mutated; Fig. 3A and B). Furthermore, when RICTOR expression was continuously decreased with doxycycline treatment of tumor-bearing mice for around 4 weeks, the growth of A549 and HCC827 lung tumor xenografts was markedly reduced by 75% and 66% (P < 0.05; Fig. 3C). Taken together, these results indicate that RICTOR plays important roles in regulating cell survival and proliferation in RICTOR-expressing lung cancer cells. A potential negative implication of growth inhibition in nonamplified tumors is the possible toxicity to normal cells. However, clinical studies have shown that mTORC1/2 inhibitors, such as CC-223 and MLN0128, which block RICTOR–mTORC2 signaling, are generally well tolerated in unselected patients with cancer (14). Thus, RICTOR-targeted therapy may potentially be a novel therapeutic strategy for both RICTOR-amplified lung cancers and RICTOR-expressing but nonamplified tumors.

**Association between RICTOR Amplification and Sensitivities to mTORC1/2 Inhibitors in a Panel of Lung Cancer Cell Lines**

Ideally, an association between the sensitivity of a pure RICTOR inhibitor and RICTOR levels in cells with the same genetic background would best support our hypothesis. However, a pure RICTOR or mTORC2 inhibitor has not yet been reported. Thus, we tested dual mTORC1/2 inhibitors that block both mTORC1 and mTORC2 signaling. A trend associating RICTOR amplification with sensitivity to mTORC1/2 inhibitors, such as MLN0128 and AZD2014, was notable (Table 1; data for AZD2014 not shown). The IC50 values for RICTOR-amplified H23 and H1703 cells were quite low, around 10 nmol/L. Furthermore, silencing of RICTOR rendered RICTOR-amplified H23 or H1703 cells markedly more resistant to mTORC1/2 inhibitors, likely by eliminating the relevant target, RICTOR amplification, in those cells. In contrast, the IC50 of MLN0128 did not change as much in RICTOR-nonamplified cells, such as A549, HCC827, and PC9 after RICTOR ablation, indicating that RICTOR may not be as relevant a target for mTORC1/2 inhibitors in those cells. Of note, the potential sensitivity of the cells to mTORC1 inhibition may complicate or mask the association between RICTOR amplification and the effects of the dual mTORC1/2 inhibitors because RICTOR is a key component of mTORC2 but not mTORC1. Taken together, there is an association between RICTOR amplification and sensitivities to mTORC1/2 inhibitors in NSCLC cells.

**The Ability of RICTOR Induction to Transform Ba/F3 Cells to Growth Factor Independence and Its Association with Sensitivity to mTORC1/2 Inhibitors**

The previously tested lung cancer cell lines have multiple different genetic alterations. To examine the effects of RICTOR amplification in isolation, we examined Ba/F3 cells that were transfected with a construct to inducibly overexpress RICTOR in the presence of doxycycline (Fig. 3D). These cells, which are normally dependent on IL3 for proliferation, were transformed to IL3 independence by the upregulation of RICTOR (Fig. 3E), supporting the role of RICTOR as an oncogene.

Furthermore, in isogenic Ba/F3–RICTOR cells in which the RICTOR level can be regulated by the addition of doxycycline, the IC50 of two structurally different mTORC1/2 inhibitors (MLN0128 and AZD2014) decreased with higher RICTOR levels (Fig. 3F, P < 0.05). This indicates that RICTOR amplification is associated with improved sensitivity to mTORC1/2 inhibitors. Thus, the results are consistent with RICTOR amplification as a potential oncogenic driver that can also sensitize Ba/F3 cells to mTORC1/2 inhibitors.

**DISCUSSION**

In the present study, we identified RICTOR amplification as the sole actionable genomic alteration in an 18-year-old patient with lung cancer by a highly sensitive hybrid capture-based next-generation sequencing strategy (15). Reviews of the TCGA database and an independent series revealed that RICTOR amplification occurs in 8% to 13% of patients with lung cancer. In the second dataset, we found that it occurs either as the sole potentially actionable gene target (11%) or with other genomic alterations. The RICTOR ablation–associated decrease in cancer cell growth in RICTOR-amplified NSCLC cells and the transforming capacity of RICTOR-dependent Ba/F3 cells suggest that RICTOR regulates cancer cell survival and proliferation, consistent with a role as an oncogenic driver in some settings.

Dual mTORC1/2 blockers were found to be the most active agents against RICTOR-amplified lung cancer cells among the inhibitors of PI3K–AKT–mTOR tested. Consistent with this in vitro finding, our index patient had tumor stabilization for more than 18 months from treatment with dual mTORC1/2 inhibitors, such as previously CC-223 and currently MLN0128. Both agents are orally available, ATP-competitive inhibitors of mTOR kinase that block both mTORC1 and mTORC2 signaling and are in clinical development. This was a striking result when compared with the typical outcome in a heavily pretreated patient with NSCLC that has progressed on multiple regimens previously (16).
Figure 3. A, FISH in lung cancer cell lines revealed that neither A549 nor HCC827 cells were RICTOR amplified. B, Western blotting and clonogenic assays: the effects of siRNA-mediated RICTOR knockdown on RICTOR-expressing but nonamplified A549 and HCC827 cells. *, P < 0.05. C, the in vivo effects of RICTOR knockdown in RICTOR-expressing but nonamplified A549 and HCC827 xenograft mouse models. *, P < 0.05. D, Western blot: RICTOR expression in parental Ba/F3 cells and inducible Ba/F3–RICTOR cells. Ba/F3–RICTOR were inducible Ba/F3 cells with RICTOR overexpression in the presence of doxycycline. IL3 was withdrawn for 2 days for the parental Ba/F3 cells and was not added for 1 month for the rest. Of note, although the inducible Ba/F3–RICTOR system had some “leakage” of RICTOR expression in the absence of doxycycline, the addition of doxycycline caused higher IL3-independent RICTOR expression and cell survival. E, relative viable cells after withdrawal of IL3. F, modulation of IC_{50} of two structurally different mTORC1/2 inhibitors, MLN0128 and AZD2014, when RICTOR was upregulated by the addition of doxycycline in Ba/F3–RICTOR cells. *, P < 0.05 when comparing IC_{50} of Ba/F3–RICTOR cells in the absence of doxycycline versus IC_{50} in the presence of doxycycline. It appears that higher levels of RICTOR rendered Ba/F3–RICTOR cells more sensitive to mTORC1/2 inhibitors.
In contrast, previous studies have shown limited clinical benefit from mTORC1/2 inhibitors in unselected patients with lung cancer (17). Our findings suggest that stratifying patients by genomic profiles, specifically RICTOR amplification, for future incarnations of these trials may lead to better patient selection. Results were just published of a phase I trial on CC-223 in patients with advanced solid tumors (18). Among 28 patients (2 with NSCLC) who were enrolled and who received CC-223, the best responses were 1 partial response in a patient with breast cancer (response duration, 220 days) and 8 subjects with stable disease with response duration ranging from 36 to 168 days. Our index patient had stable disease for 12 months (±365 days) on CC-223, which represents a 66% increase over the best results observed with the patient who had a partial response of 220 days. Therefore, he seems to be an outlier in the context of the available clinical data with CC-223. Furthermore, his disease rapidly progressed after 3 months of combined immunotherapy, but was stabilized again with another dual mTORC1/2 inhibitor, MLN0128. Taken together, our patient had much longer disease control with mTORC1/2 inhibitors in comparison with available published data.

There are several potential reasons for the index patient’s not having had a more robust response. First, the dose of mTORC1/2 inhibitors that he received may not have been sufficient to adequately inhibit the targets in the tumor. Second, because CC-223 and MLN0128 are inhibitors of mTORC1/2 but not specific RICTOR inhibitors, they will not block mTORC2-independent RICTOR signaling. It is also possible that the type of tumor response to targeted therapies against amplification of oncogenes may differ from targeted therapies against driver gene mutations and translocations. ERBB2/HER2 amplification-targeted therapy, including trastuzumab, is so far the only FDA-approved treatment targeting gene amplification. Unlike the impressive responses associated with specific kinase inhibitors against EGFR and other driving mutations, single-agent trastuzumab therapy was associated with an overall response rate of only 15% and duration of response of 9.1 months in the second-/third-line monotherapy trial conducted in patients with HER2-positive metastatic breast cancer (19). On the other hand, targeted therapy against ERBB2 amplification has transformed the field of breast cancer management, providing further impetus to investigate a potential paradigm for the use of RICTOR-targeted therapy for RICTOR-amplified lung cancer.

In addition to its role in NSCLC, a relatively high degree of RICTOR amplification was also observed in small cell lung cancer (14.6%), which is similar to the previously reported frequency (20, 21). The mTORC1 inhibitors, such as everolimus (RAD001; ref. 22), and inhibitors targeting both mTORC1 and mTORC2 have been tested in small cell lung cancer with very limited responses in unselected patients (22). Thus, future studies will be needed to investigate whether patients with small cell lung cancer can be stratified based on RICTOR amplification to derive clinical benefits from mTORC1/2 inhibitors.

In summary, the initial clinical data presented here suggest that patients with RICTOR-amplified NSCLC may benefit from treatment with dual mTORC1/2 inhibitors, especially in cases where RICTOR is the sole oncogenic driver. Our clinical and laboratory observations provide the rationale for reevaluating dual mTORC1/2 inhibitors as a potential therapy for patients with lung cancer, as stratified by genomic profiling to assess both RICTOR amplification and the remainder of the genomic background, as our index patient may have benefited from the absence of other known oncogenic drivers in responding to mTORC2-targeted therapy. A first key step in that direction will be to identify specific RICTOR inhibitors, and for that purpose, a high-throughput drug screen is currently under way.

### Table 1. IC₅₀ values of MLN0128 (mTORC1/2 inhibitor) in a panel of NSCLC cells

<table>
<thead>
<tr>
<th>Cell line (R4 cells: inducible RICTOR knockdown cells)</th>
<th>Control without doxycycline</th>
<th>RICTOR knockdown with doxycycline</th>
<th>Resistance ratio</th>
<th>RICTOR amplification (yes = 4 copies; no = 2 copies)</th>
<th>Other genomic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>H23-R4</td>
<td>12</td>
<td>169</td>
<td>14</td>
<td>Yes</td>
<td>KRAS mutation</td>
</tr>
<tr>
<td>H1703-R4</td>
<td>9.4</td>
<td>48.4</td>
<td>5.1</td>
<td>Yes</td>
<td>PDGFR amplification</td>
</tr>
<tr>
<td>A549-R4</td>
<td>5.7</td>
<td>13.0</td>
<td>2.3</td>
<td>No</td>
<td>KRAS mutation</td>
</tr>
<tr>
<td>HCC827-R4</td>
<td>9.6</td>
<td>13.4</td>
<td>1.4</td>
<td>No</td>
<td>EGFR mutation</td>
</tr>
<tr>
<td>PC9-R4</td>
<td>21.1</td>
<td>31.7</td>
<td>1.5</td>
<td>No</td>
<td>EGFR mutation</td>
</tr>
<tr>
<td>H157</td>
<td>20.1</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td>KRAS mutation, TP53-E298</td>
</tr>
<tr>
<td>H460</td>
<td>23.1</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td>KRAS mutation, PIK3CA-ES45K</td>
</tr>
<tr>
<td>H2228</td>
<td>22.6</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td>ALK translocation</td>
</tr>
<tr>
<td>H322</td>
<td>43.7</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>H3122</td>
<td>32.1</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td>ALK translocation</td>
</tr>
</tbody>
</table>

NOTE: The IC₅₀ for RICTOR-amplified H23 and H1703 were quite low, around 10 nmol/L. RICTOR knockdown rendered RICTOR-amplified H23 or H1703 cells markedly more resistant to mTORC1/2 inhibitors, likely by eliminating the relevant target, RICTOR amplification, in those cells. In contrast, the IC₅₀ of MLN0128 did not change as much in RICTOR-nonamplified cells, such as A549, HCC827, and PC9 after RICTOR ablation, indicating that RICTOR may not be such a relevant target for mTORC1/2 inhibitor in those cells. Taken together, there is an association between RICTOR amplification and sensitivities to mTORC1/2 inhibitors in NSCLC cells. R4 cells: inducible RICTOR knockdown cells. IC₅₀: 50% inhibitory concentration. Drug resistance ratio: obtained from IC₅₀ values, by comparing the IC₅₀ in the presence of doxycycline and the IC₅₀ in the absence of doxycycline.
METHODS

Cell Lines and Material

The H23, H1703, A549, HCC827, H157, H460, H2228, H3122, and H322 NSCLC cell lines were obtained from the American Type Tissue Collection and were grown in RPMI 1640 supplemented with 10% FBS and 1x antibiotic/antimycotic (Invitrogen). Among them, cell lines H23, HCC827, H1703, and A549 were tested and authenticated by short tandem repeat profiling in August 2014 and 2015, and the rest were not authenticated. PC9 cells were a gift from Dr. Susumu Kobayashi (Harvard Medical School, Boston, MA). Ba/F3 cells were obtained from G. Gilliland (Brigham and Women’s Hospital, Boston, MA). RICTOR-dependent Ba/F3 cells were developed by transfecting Ba/F3 cells with a doxycycline-inducible plasmid for the overexpression of wild-type RICTOR in the presence of doxycycline (RICTOR–pTetOne, modified from Tet-One Systems, Clontech Laboratories, Inc., and generated by GENEWIZ, Inc.). BEZ235, AZD2014, RAD001 (everolimus), erlotinib, MK2206, and LJ294002 were obtained from Selleck Chemicals; cisplatin was obtained from Sigma-Aldrich.

Immunoblotting and Antibodies

Western blot analysis was performed as described (23). Antibodies against RICTOR, phospho-mTOR, phospho-EGFR, phospho-AKT S473, phospho-S6RP (S235/236), phospho-4EBP1 (T37/46), phospho-ERK1/2, tubulin, and GAPDH were from Cell Signaling Technology. The RICTOR antibody used in IHC was from Bethyl Laboratories.

PBMC Isolation and Analysis of Patient’s Specimen

The patient’s PBMCs were isolated by Ficoll density gradient centrifugation (24). The collection and analysis of this patient’s sample were carried out in accordance with the Declaration of Helsinki, and the protocols were approved by the Institutional Review Board of Albert Einstein College of Medicine. Informed consent for genomic analysis was obtained from the patient.

IHC

IHC was performed as previously described (23) with formalin-fixed primary lung tumor tissue slides. RICTOR antibody was used at a dilution of 1:5,000 (optimal dilution for overnight incubation) at room temperature.

Tissue Microarray of Lung Cancer Cell Lines

The formalin-fixed, paraffin-embedded tissue microarray of lung cancer cell lines includes 27 NSCLC cell lines.

Clonogenic Survival Assay

As previously described (25), logarithmically growing cells were plated in triplicate. All drugs were administered in 4 weeks. Tumors were measured with calipers twice weekly, and size was calculated as length × width²/2. The protocol was approved by the Institutional Animal Care and Use Committee.

Inducible shRNA Knockdown

The Thermo Scientific Open Biosystems Expression Arrest TRIPZ Lentiviral shRNAmir was used per the manufacturer’s manual (TRIPZ Human RICTOR shRNA, Thermo Fisher Scientific). Pooled NSCLC cells with inducible shRNA–RICTOR were utilized within a month following transduction.

FISH

FISH for the detection of copy-number variations mapping to the RICTOR locus (chr5:38,938,023-39,074,510) was performed using the following locus-specific probes: BAC clones RP11-315A2 (chr5:38,840,944-38,995,845) and RP11-44D11 (chr5:39,184,412-39,868,485) both mapping to chr5p13.1 and spanning 545,902 bp across the RICTOR locus, and a control reference clone RP11-204D12 (chr5:95,677,452-95,844,184) mapping distal to the RICTOR region of interest (chr5q13 and 56Mb distal the RICTOR-specific clones). FISH was performed as previously described (27). Similar to HER2 amplification, RICTOR amplification is defined as a ratio of RICTOR/control ≥ 2.

Xenograft Mouse Studies

SCID mice (The Jackson Laboratory; female, 9–10 weeks old) were inoculated subcutaneously with 5 × 10⁶ NSCLC cells. After 2 days, mice were divided into a vehicle control (water) group and a doxycycline group, with 5 mice in each group. Doxycycline was administered by oral gavage: 100 mg/kg/day (28). The same volume of water was given to the control group. A total of 20 daily doses were administered in 4 weeks. Tumors were measured with calipers twice weekly, and size was calculated as length × width²/2. The protocol was approved by the Institutional Animal Care and Use Committee.

Genomic Profiling of Clinical Lung Cancer Samples

The hybridization capture-based next-generation sequencing was performed using the FoundationOne test (Foundation Medicine). The detailed laboratory and computational methods used in the FoundationOne assay have been reported (15). Data were generated from two consecutive versions of the test, targeting increasing numbers of genes. Hybridization capture baits for the RICTOR gene were identical for both versions of the test. Actionable genomic alterations are defined as those linked to FDA-approved drugs or agents under study in targeted therapy registered clinical trials. Copy-number amplifications are defined as copy number ≥ 6. Local site permissions were obtained to use clinical samples for this study.

Statistical Analysis

All data are expressed as mean ± SD from at least triplicate experiments. Statistical analysis was performed by one-way or two-way ANOVA as appropriate, using Statistica 6.0 (StatSoft). Differences were considered significant at P < 0.05.

Disclosure of Potential Conflicts of Interest

J.S. Ross reports receiving a commercial research grant from Foundation Medicine and has ownership interest (including patents) in the same. S.M. Ali has ownership interest (including patents) in Foundation Medicine. R. Yelensky has ownership interest (including patents) in Foundation Medicine. D. Lipson has ownership interest (including patents) in Foundation Medicine. D.J. Stephans has ownership interest (including patents) in Foundation Medicine. V.A. Miller has ownership interest (including patents) in Foundation Medicine. B. Piperdi is Senior Principal Scientist, Clinical Research, at V.A. Miller has ownership interest (including patents) in Foundation Medicine. M. Hawryluk has ownership interest (including patents) in Foundation Medicine. D. Lipson has ownership interest (including patents) in Foundation Medicine. R. Yelensky has ownership interest (including patents) in Foundation Medicine.
Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Cheng, J.S. Ross, X. Liu, S.M. Ali, C. Montagna, A. Chachoua, S. Goel, C. Zhu, J. Shan, K. Gritsman, V.A. Miller, B. Piperdi, R. Perez-Soler
Administrative, technical, or material support (i.e., reporting and organizing data, constructing databases): H. Cheng, J.S. Ross, K. Wang, H. Liu, R. Perez-Soler
Study supervision: H. Cheng, J.S. Ross, A. Chachoua, R. Perez-Soler

Acknowledgments
The authors thank Yinhui Song for her technical support in performing the FISH assays.

Grant Support
The work was supported by a Paul Calabresi Career Development Award for Clinical Oncology from NIH (5K12CA132783-04 to R. Perez-Soler, B. Piperdi, and H. Cheng), the American Cancer Society (RSG-08-303-01 to B. Halmos), and the LUNGevity Foundation Targeted Therapeutics Award (to H. Cheng and B. Halmos).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertised in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 26, 2014; revised September 8, 2015; accepted September 10, 2015; published OnlineFirst September 14, 2015.

REFERENCES

1270 | CANCER DISCOVERY | DECEMBER 2015 www.aacrjournals.org

Downloaded from cancerdiscovery.aacrjournals.org on April 12, 2017. © 2015 American Association for Cancer Research.
CANCER DISCOVERY

RICTOR Amplification Defines a Novel Subset of Patients with Lung Cancer Who May Benefit from Treatment with mTORC1/2 Inhibitors

Haiying Cheng, Yiyu Zou, Jeffrey S. Ross, et al.

Cancer Discov 2015;5:1262-1270. Published OnlineFirst September 14, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/2159-8290.CD-14-0971

Supplementary Material
Access the most recent supplemental material at:
http://cancerdiscovery.aacrjournals.org/content/suppl/2015/09/12/2159-8290.CD-14-0971.DC1

Cited articles
This article cites 28 articles, 10 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/5/12/1262.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/5/12/1262.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.