NSCLC Driven by DDR2 Mutation Is Sensitive to Dasatinib and JQ1 Combination Therapy

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Abstract

Genetically engineered mouse models of lung cancer have demonstrated an important role in understanding the function of novel lung cancer oncogenes and tumor-suppressor genes identified in genomic studies of human lung cancer. Furthermore, these models are important platforms for preclinical therapeutic studies. Here, we generated a mouse model of lung adenocarcinoma driven by mutation of the discoidin domain receptor 2 (DDR2) gene combined with loss of TP53. DDR2L63V/TP531/1 mice developed poorly differentiated lung adenocarcinomas in all transgenic animals analyzed with a latency of 40 to 50 weeks and a median survival of 67.5 weeks. Mice expressing wild-type DDR2 with combined TP53 loss did not form lung cancers. DDR2L63V/TP531/1 tumors displayed robust expression of DDR2 and immunohistochemical markers of lung adenocarcinoma comparable with previously generated models, though also displayed concomitant expression of the squamous cell markers p63 and SOX2. Tumor-derived cell lines were not solely DDR2 dependent and displayed upregulation of and partial dependence on MYCN. Combined treatment with the multitargeted DDR2 inhibitor dasatinib and BET inhibitor JQ1 inhibited tumor growth in vitro and in vivo. Together, these results suggest that DDR2 mutation can drive lung cancer initiation in vivo and provide a novel mouse model for lung cancer therapeutics studies. Mol Cancer Ther; 14(10). 2382–9. ©2015 AACR.

Introduction

Lung cancer is the leading cause of cancer-related death in the United States and worldwide (1). More than 85% of lung cancer patients are diagnosed with non–small cell lung cancer (NSCLC), including adenocarcinoma (50% of lung cancers), squamous cell carcinoma (SQC; 30%), and large-cell carcinoma (10%; ref. 2). Technologic advances in recent years, including the application of next-generation sequencing (NGS), have allowed researchers to construct large databases describing the molecular features of human lung tumors. These efforts have been accompanied by the generation of a number of new models of lung cancer, notably genetically engineered mouse models (GEMM), which have facilitated the study of candidate human lung cancer oncogenes and tumor-suppressor genes in vivo. These models have been used for a number of purposes, including studies of tumor formation using one or more genetic alterations found in human cancers, studies of the impact of specific genetic changes on the tumor and its microenvironment, and for studies of anticancer therapies. EGFR-, Kras-, BRAF-, and ERBB2-mutated and -translocated EML4-ALK lung adenocarcinoma models have been generated and used for studies providing important insights into the mechanisms of response and resistance to clinically relevant–targeted lung cancer therapies (3–7). Lung cancer genome studies continue to nominate an increasing number of candidate lung cancer oncogenes and tumor suppressors in lung adenocarcinoma, and more recently, in lung SCC. Some candidate genomic alterations from studies of squamous cell lung cancers include amplification of SOX2, PDGFRα, and FGFR1 and mutations of KEAP1, STK11, ERBB4, and DDR2 (Discoidin domain receptor 2; refs. 8, 9).

DDR2 is a type I transmembrane receptor tyrosine kinase (RTK). DDR kinases are widely expressed in human tissues, are activated by collagens, and have roles in cell adhesion, migration, proliferation, and survival when activated by ligand binding and phosphorylation (10). DDR kinases play a role in cancer progression by regulating the interactions of tumor cells with their surrounding collagen matrix. DDR2 mutations have been reported in multiple tumor types, including lung cancer, breast cancer, brain cancer, gynecologic cancer, and prostate cancer (10). We previously reported the identification of novel somatic mutations in the DDR2 gene at a frequency of 3.8% in a sample set of 290 squamous cell lung cancer samples (11). Overall, 11 mutations were found throughout the entire gene and located in various DDR2 domains, including L63V, I120M, and D125Y, within the collagen-binding discoidin 1 domain; L239R and G253C within the discoidin 2 domain; G505S in the cytosolic domain; and G290R within the discoidin 3 domain.
Materials and Methods

Generation of the DDR2wt- and L63V-mutant mouse cohort

The full-length DDR2wt cDNA was obtained from Origene and cloned into expression vector pBS31 (21). L63V and I638F mutations were generated by site-directed mutagenesis using the Quickchange Site Directed Mutagenesis Kit (Strategene) and cloned into expression vector pBS31 (21). L63V and I638F mutations were shown to confer oncogenicity in NIH-3T3 fibroblasts in a colony forming assay in soft agar (11).

DDR2 can interact with multiple proteins resulting in complex signaling processes. Src has been shown to phosphorylate DDR2 resulting in subsequent DDR2 autophosphorylation (12). Thus, DDR2–Src interactions may play a key role in DDR2-initiated signaling. The signaling networks downstream of DDR2 include MAPK (13) and phosphoproteomic studies have nominated SHP-2 as a critical mediator of DDR2 signaling (14). Acquisition of an EMT phenotype in MDCK and human breast epithelial cells has also been shown to induce DDR2 expression (15). Activation of DDR2 regulates the EMT driver SNAIL1 stability by stimulating ERK2 activity, in a Src-dependent manner. DDR2-mediated stabilization of SNAIL1 promotes breast cancer cell invasion and metastasis in vivo (16). However, the transcription factors and mechanisms involved in upregulation of DDR2 during EMT have not been elucidated.

Four kinase inhibitors, dasatinib, imatinib, ponatinib, and nilotinib were identified initially as inhibitors of DDR1 and DDR2 by chemical proteomic profiling studies (17, 18), suggesting that tumors with activated DDR2 signaling may be targeted by agents that are already FDA approved for other indications. In preclinical studies, dasatinib was shown to inhibit proliferation in two lung cancer cell lines with DDR2 mutations both in vitro and in vivo and in cases of response to dasatinib in lung cancer patients with DDR2 mutations have been published (11, 19). However, studies have shown that DDR2 inhibition with dasatinib leads to both adaptive (20) and acquired resistance with resistance mechanisms, including DDR2 gatekeeper mutation, NFI loss and activation of parallel RTK pathways, including EGFR, IGF1R, and MET (13).

Although several studies performed in cellular models have suggested that DDR2 mutations may be clinically relevant, data are lacking to prove that DDR2 mutations can drive lung cancer in vivo. Here, we report that the conditional overexpression of the DDR2 L63V-mutant downstream of a murine CCSP promoter can promote tumorigenesis in genetically engineered mice with a phenotype of lung adenocarcinoma. In addition, MYCN was elevated in the tumors driven by this DDR2 mutation and cell lines derived from the murine tumors were partially dependent on NMYC. We showed that the bromodomain inhibitor IQ1, an inhibitor of MYC-driven malignancies, plus the nonselective DDR2 inhibitor dasatinib as combination therapy could suppress growth of these tumors. Together, these data suggest that DDR2 mutations can contribute to lung cancer formation in vivo.

Histology and immunohistochemistry

Mice were sacrificed with CO2, half of the dissected tumors were snap-frozen in liquid nitrogen for preparation of protein lysates and the left lung tissue was fixed in 10% neutral-buffered formalin for 24 hours at room temperature, and then transferred to 70% ethanol, embedded in paraffin, and sectioned at 5 μm for IHC staining. Hematoxylin and eosin (H&E) stains were performed in the Department of Pathology in Brigham and Women’s Hospital (Boston, MA). Immunohistochemistry was performed using previously described methods (23). Anti-DDR2 antibody was from Bethyl Laboratories. All the other antibodies used for dynamic markers are listed in Table 1.

Gene expression profiling analysis

RNA was extracted from snap-frozen Kras/p53 and DDR2L63V/p53 tumor tissue using TRIzol (Invitrogen) and further purified by the RNeasy MinElute Cleanup Kit (Qiagen). Arrays were performed at Dana-Farber Cancer Institute facility on Affymetrix mouse Gene1.0ST arrays. Data were preprocessed and normalized using the GenePattern Preprocess module with default parameters followed by GenePattern Comparative Marker Selection.

Table 1. Antibodies used for immunohistochemistry

<table>
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<tr>
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<td>M3575</td>
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<tr>
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RNAi and cell culture

MYCN expression was knocked down using MYCN siRNA (m) obtained from Santa Cruz Biotechnology, Inc. Cell lines 3941 DDR2 and 858 were seeded at 2 × 10^5 cells per well in 6-well tissue culture plates. Control- and N-Myc siRNA–transfected cells were used for immunoblot analysis and counted at 24 and 48 hours requiring a total of 12 seeded wells. Single drugs and combinations were added the following day at concentrations of 10 nmol/L, 50 nmol/L, 100 nmol/L, 500 nmol/L, 1 µmol/L, and 10 µmol/L and after 5 days a standard 96-well plate luminometer was used to measure cell proliferation. Comparison of untreated cells with those treated at a given concentration was used to determine the percentage of survival. For proliferation analysis, mean values were calculated from samples in triplicate and SEs were calculated by Microsoft Excel. GraphPad Prism software was used to determine IC_{50} values.

Western blotting

Snap-frozen tissues or cells cultured in 6-well plates were homogenized with RIPA buffer containing phosphatase and protease inhibitors (Thermo Fisher Scientific), lysates were cleared by centrifugation, protein concentration was determined using the Bradford reagent (Bio-Rad), and 100 µg of lysate was loaded per sample. Immunoblots were then performed using the NuPAGE System (Invitrogen) per the manufacturer’s instructions. Primary antibodies used were NMYC (Santa Cruz Biotechnology), DDR2 (Bethyl Laboratories), β-actin (Sigma), and Vinculin (Sigma). Secondary antibodies used were horseradish peroxidase mouse and rabbit (Pierce) and ECL prime (Thermo Fisher Scientific) was used for protein detection.

Gene expression changes were confirmed using real-time PCR. Total cellular RNA was prepared from the cells by using the Trizol Reagent System (Invitrogen) per the manufacturer’s instructions. Cells were seeded in triplicate at 1,500 cells per well in 96-well clear-bottomed plates. Single drugs and combinations were added the following day at concentrations of 10 nmol/L, 50 nmol/L, 100 nmol/L, 500 nmol/L, 1 µmol/L, and 10 µmol/L and after 5 days a standard 96-well plate luminometer was used to measure cell proliferation. Comparison of untreated cells with those treated at a given concentration was used to determine the percentage of survival. For proliferation analysis, mean values were calculated from samples in triplicate and SEs were calculated by Microsoft Excel. GraphPad Prism software was used to determine IC_{50} values.

**Results**

**DDR2I638F expression is oncogenic in the lung**

Given that somatic DDR2 mutants have been observed in human NSCLC and shown to be potential therapeutic targets in cellular studies, we sought to generate a mouse model of lung cancer driven by mutated DDR2 to examine whether expression of DDR2 mutants could initiate lung tumorigenesis in vivo. To address this question, we selected the L63V and L638F mutations for characterization, given that they appeared to be the most oncogenic in NIH-3T3 assays reported previously (11). These DDR2 mutations were introduced in the human DDR2 gene by site-directed mutagenesis and cloned into pB831 Ipln vector followed by a tetrO minimal CMV promoter and an ATG and an FRT site (21). DDR2^{wt}, DDR2^{L63V}, and DDR2^{L638F} transgenic mice were then generated by injection of the construct into FVB/N fertilized eggs. Progeny were genotyped by PCR. Founders were crossed with CCSP-rTA as well as conditional Tp53-deficient allele (p53^{fli3L}) mice to accelerate tumor formation as no tumors were observed in the DDR2 transgenics alone with 2 years of observation. The complex model tet-op-DDR2^{wt};CCSP-rTA;p53^{fli3L} (DDR2^{wt};p53 hereafter), tet-op-DDR2^{L63V};CCSP-rTA;p53^{fli3L} (DDR2^{L63V};p53 hereafter), and tet-op-DDR2^{L638F};CCSP-rTA; p53^{fli3L} (DDR2^{L638F};p53 hereafter) were confirmed by genotyping and expanded for subsequent analyses and experiments (Fig. 1A). DDR2^{wt};p53, DDR2^{L63V};p53, and DDR2^{L638F};p53 mice were placed on a continuous doxycycline diet at 6 weeks of age to induce DDR2 expression, and Adenovirus-Cre intranasally instilled to delete Tp53 on the same day. Lungs of induced mice were visualized by serial MRI every 8 weeks and mice were also sacrificed every 8 weeks for histologic examination.

Expression of DDR2 was targeted to lung type II alveolar cells by crossing mice carrying club (originally known as Clara) cell...
secretory protein (CCSP)–regulated reverse tetracycline transactivator (rtTA) transgene, regulated by tetracycline (tet)-responsive elements, which allowed for DDR2 expression in lung pneumocytes after doxycycline administration. The median survival of DDR2L63V;p53 mice was 67.5 weeks (Fig. 1B) and there was no mortality observed in the DDR2wt;p53 or DDR2L63F;p53 models at 2 years of observation. After 20 to 30 weeks of doxycycline treatment, histologic examination of DDR2L63V; p53 mice demonstrated the development of focal or diffuse bronchioalveolar carcinoma (BAC), in some cases preceded by precancerous adenomatous lesions in the airway epithelia (Fig. 1C). After 30 to 40 weeks on doxycycline, the DDR2L63V; p53 mice developed small intrabronchial carcinomas in the distal (Fig. 1C) and proximal (Fig. 1C) bronchioalveolar locations. After 50 to 60 weeks, all of the tested DDR2L63V; p53 mice developed lung adenocarcinoma (Fig. 1C). In agreement with the pathologic observations, MRI images also showed gradual tumor development of DDR2L63V; p53 mice after 40 weeks of doxycycline induction (Supplementary Fig. S1A). The DDR2wt;p53 and DDR2L63F;p53 models only showed epithelial hyperplasia (Supplementary Fig. S1B); none of tested mice developed malignant tumors within 2 years. Both immunohistochemical (IHC) stains and Western blotting confirmed DDR2 expression in tumor nodules (Fig. 1D and Supplementary Fig. S1C and S1D) and lack of p53 (Supplementary Fig. S2A). The majority of DDR2L63V tumors showed marked SPC staining, implying a type II pneumocyte origin, as expected, given the use of the CCSP promoter. Interestingly, CCSP staining was nearly negative (Supplementary Fig. S2B). One possibility is that these tumors are of club cell origin, which is then followed by altered differentiation leading to loss of the CCSP expression marker. The same phenomenon was observed previously for the tet-opt-EGFR-T790M-L858R;CCSP-rtTA mouse model (22).

**NSCLC driven by DDR2L63V mutant is morphologically adenocarcinoma but with p63 and SOX2 expression**

Because somatic DDR2 mutations are more frequently found in human SCC, we explored whether the DDR2L63V model we generated displayed any markers of lung SCC. p63 is a well-known marker of squamous differentiation and overexpression of this gene has been consistently identified in lung SCCs by global gene-expression profiling or by IHC (26, 27). Several other markers are widely used in the subclassification of lung carcinomas, including CK5/6 and TTF1 (26–28). Most human SCC cases display a p63+/TTF1- immunophenotype, whereas most adenocarcinoma cases show the opposite expression pattern: TTF1+, p63−. SOX2 is a transcription factor reported as an important gene in SCC and is also used as histologic marker of human SCC (29, 30). As controls for this analysis, we used LSL-KrasG12D/C0;p53−/− (Kras;p53 hereafter) genetically engineered mice that develop SCC and is also used as histologic marker of human SCC (29, 30). Interestingly, DDR2L63V tumors displayed typical adenocarcinoma morphology with strong TTF1 expression but also displayed some p63 and SOX2 staining (Fig. 2). This mixed phenotype is not unique to the tumors induced by DDR2L63V and has been previously reported with indelic deletion of the tumor-suppressor Lkb1 along with KrasG12D mutant (5).
MYCN is elevated in DDR2L63V tumors

Given the long latency of the DDR2L63V model, we generated two tumor-derived cell lines (3941 and 3942) to study signaling and therapies in more detail. Both cell lines demonstrated expression of DDR2 that was augmented by culture in doxycycline, as would be expected (Fig. 3A). The cell line 855 displayed in this figure is one of several previously described cell lines generated from murine Krasp53 tumors (24). Interestingly, both the 3941 and 3942 cell lines proliferated well in culture in the presence of or absence of doxycycline with no discernible difference in phenotype, suggesting that they were not dependent on ongoing DDR2 expression and raising the possibility that additional genomic alterations may be contributing to transformation in the DDR2-mutated mouse model. To support this observation, we performed siRNA-mediated knockdown of DDR2 in the 3941 and 3942 cell lines. Although the degree of knockdown in these lines was only approximately 50% as measured by real-time PCR (Supplementary Fig. S3A), there was no effect seen on the proliferation of these cell lines or of the Krasp53 cell line 858, which was selected on the basis of transfectibility (Supplementary Fig. S3B).

To address the possibility that other genetic alterations may be playing an important role in the tumors from the DDR2L63V; p53−/− mice, we performed microarray analyses to compare gene-expression profiles of these two tumor-derived cell lines from the DDR2L63V; p53−/− mouse lines to those from three mouse Krasp53 tumors (24). This was performed after comparative immunoblot analyses of these cell lines did not show any differential phosphorylation of RAS/MAPK or PI3K effectors as compared with the Kras−/− cell lines (data not shown). Of note, the cell lines studied were derived from tumors that developed typical adenocarcinoma on pathology, and p53 was conditionally deleted at the same ages. Differential gene-expression analysis using the GenePattern module identified 541 genes differentially upregulated in the DDR2L63V tumors as compared with the Kras tumors using a fold-change cutoff of 2.0 and a corrected P value of less than 0.001 (Supplementary Data File). A representative heatmap of the top differentially expressed genes is shown in Fig. 3B. Manual inspection of these top differentially expressed genes focused our attention on MYCN, a member of the myc family of proto-oncogenes. The MYC gene is expressed in a wide variety of tissues, whereas MYCN expression is restricted to early stages of embryonic development, amplification of MYCN is frequently found in a number of advanced-stage tumors, including lung cancer, contributing to a myriad of phenotypes associated with growth, invasion, and drug resistance (31).

In the microarray data, MYCN expression was 37-fold higher in the DDR2L63V tumor-derived cell lines, and immunoblotting demonstrated elevated MYCN protein levels in 3941 and 3942 cell lines as compared with Kras lines 634, 855, and 858 (Fig. 3C). Analysis of gene-expression data from The Cancer Genome Atlas (TCGA) demonstrated a trend toward higher expression of MYCN in DDR2-mutated lung SCCs (mean RSEM 176 vs. 127), but this difference was not statistically significant. DDR2 and MYCN expression levels were weakly correlated across the TCGA cohort (Pearson correlation 0.22). We did not note any upregulation of MYCN in two previously described NSCLC cell lines with DDR2 mutations (NCI-H2286 and HCC-366) as compared with other NSCLC cell lines described in the Cancer Cell Line Encyclopedia. Given increased MYCN expression in the DDR2-mutated lines (Fig. 3C), we assessed whether MYCN loss would have an effect on their proliferation. siRNA was used to knockdown MYCN in two previously described NSCLC cell lines with DDR2 mutations (634, 855, and 858) (Fig. S4). These two cell lines were also probed for sensitivity to a previously reported nonselective DDR2 inhibitor, sarcatinib, given studies linking SRC and DDR2 activity and no differences in sensitivity were observed.

NSCLC driven by DDR2L63V is sensitive to dasatinib and JQ1 combination therapy

We next probed the 3941 and 3942 cell lines for sensitivity to two previously reported nonselective DDR2 inhibitors, dasatinib and ponatinib, and compared the sensitivity with three previously generated Krasp53 cell lines (634, 855, and 858). We did not observe any differences in the sensitivity of these cell lines to these two chemical inhibitors (dasatinib: 3941 and 3942 DDR2 IC50 1.96 and 0.682 μmol/L, respectively, as compared with Kras 634, 855, 858 with IC50 0.484, 1.76, and 0.557 μmol/L, respectively. Ponatinib: 3941 and 3942 DDR2 IC50 0.579μmol/L and 0.978μmol/L, respectively, as compared with Kras 634, 855, 858 IC50 2.26, 2.31, and 2.57 μmol/L, respectively; Supplementary Fig. S4). These two cell lines were also probed for sensitivity to a previously reported SRC inhibitor sarcatinib, given studies linking SRC and DDR2 activity and no differences in sensitivity were observed (sarcatinib: 3941 and 3942 DDR2 IC50 2.69 and 5.49 μmol/L, respectively, as compared with Kras 634, 855, 858 with...
IC50 4.69, 11.67, and 8.86 μmol/L, respectively; Supplementary Fig S4). To control for any intrinsic drug resistance of the 3941 or 3942 cell lines as compared with the Kras-mutated lines, we compared their sensitivity with etoposide and noted no inherent chemoresistance in the DDR2-mutated lines (3941 and 3942 DDR2 IC50 0.840 and 0.439 μmol/L, respectively, as compared with Kras 634, 855, and 858 with IC50 2.49, 1.53, and 1.00 μmol/L, respectively; Supplementary Fig S4).

Given evidence that MYCN knockdown in DDR2-mutated cell lines decreased cellular proliferation, we reasoned that combined inhibition of DDR2 and NYMC might further suppress proliferation of these models. To this end, we assessed the impact of the combination IQ1, a prototype bromodomain and extraterminal bromodomain inhibitor that has been previously shown to suppress MYC activity (32, 33), in the 3941 and 858 cell lines. We observed enhanced sensitivity of 3941 to IQ1 alone (3941 DDR2 IC50 of 160 nmol/L as compared with Kras 658 IC50 of 761 nmol/L) and to the combination of IQ1 and dasatinib as compared with 858 (3941 DDR2 IC50 of 1.0 nmol/L as compared with Kras 858 IC50 of 10.8 nmol/L; Fig 4A). To assess this combination treatment in a more physiologic setting, we performed a xenograft study where Nu/Nu mice were injected with 3941 DDR2 cells and treated with vehicle, dasatinib, IQ1, or dasatinib plus IQ1 combination. Tumor formation was monitored twice weekly for 3 continuous weeks. Consistent with our previous observation (11), dasatinib showed antitumor efficacy on the DDR2L63V mouse model as compared with vehicle control. IQ1 alone showed antitumor efficacy; however, the combination treatment of IQ1 and dasatinib displayed the most potent effect with growth inhibition of all tested tumors (Fig 4B and Cand Supplementary Fig S5, P < 0.001; individual tumor responses are shown in Supplementary Fig S5). Although they were not as sensitive as the murine lines to IQ1 alone, IQ1 potentiated the effects of dasatinib in the DDR2-mutated NSCLC cell lines NCI-H2286 and HCC366 with IC50 values of 10 and 14 nmol/L respectively, for dasatinib with 1 μmol/L IQ1, a one-log reduction from their typical dasatinib IC50 (Supplementary Fig S6).

**Discussion**

Here, we have presented a novel genetically engineered mouse model of lung cancer driven by L63V mutation in DDR2. These studies were motivated by prior work suggesting that DDR2 mutations may be oncogenic and confer sensitivity to FDA-approved tyrosine kinase inhibitors, including dasatinib, imatinib, nilotinib, and ponatinib. Because all of the prior work was completed in lung cancer cell lines or other cellular models, we sought to explore whether mutated DDR2 could drive lung cancer formation in an organism and observed a phenotype of lung adenocarcinoma with a relatively long latency. The adenocarcinoma phenotype was not surprising, given our use of the CCSP promoter to drive expression of mutated DDR2 in cell types known to be precursors for lung adenocarcinoma as well as prior reports of DDR2 mutations in lung adenocarcinoma (34). The recent publication of several models of mouse lung SCCs also suggests that an inflammatory insult and/or a genetic lesion–driving squamous metaplasia is required for SCC formation (23, 35), neither of which was present in this model.

One of the challenges in advancing DDR2 mutations as a potential therapeutic target in lung cancer has been the lack of recurrent point mutations in the gene as well as the infrequency of DDR2 mutations overall. Studies in cell lines have suggested that only a subset of patient-derived mutations confer gain-of-function phenotypes, and the ability to discern "driver" from "passenger" events in DDR2 has not been well established in an experimental system with conflicting reports in some cases, such as DDR2 L638F, which was not oncogenic in our mouse model nor in prior proteomic studies, but was transforming in some cellular systems (11, 14). Our results, therefore, call into question whether L638F is really a gain-of-function mutation or is operating via a different mechanism as compared with L63V. Interestingly, two cases of response to dasatinib in patients with DDR2 S768R mutations have been reported, suggesting that at least some patient-derived mutations are likely to be therapeutic targets, though dasatinib therapy has been associated with toxicity in lung cancer patients (36). In this work, we established a single point...
mutation (L63V) of DDR2 as oncogenic in vivo, and the generation of additional cellular and animal models will be necessary to discern which DDR2 mutations are truly oncogenic, given that it is likely that many DDR2 mutations will be passenger events given the high background mutation rate of lung cancers. This work does, however, serve as a proof-of-concept of the oncogenicity of mutated and not wild-type DDR2 in a mouse model.

Although we observed lung cancer formation at a high penetrance in the DDR2 L63V model, the latency was long and the fact that we were able to grow tumor-derived cell lines in the absence of doxycycline induction suggests that, although DDR2 mutants can play a role in lung cancer initiation, there are likely to be other important cooperating oncogenic alterations that facilitate ongoing proliferation of DDR2-mutated tumors. This idea is in agreement with human cancer-derived cell line studies that suggest that a number of oncogenic pathways are activated in DDR2-mutated cell lines and can substitute for DDR2 in the context of DDR2 inhibition by small-molecule inhibitors. Although these studies have largely focused on compensatory RTK pathways, our observation that MYCN can play a role in DDR2-mutated tumors also indicated that other mutations are present along with DDR2 and include mutations in Kras (codon 61), Fgfr2, Irs2, Csf1r, and Daxx, suggesting that DDR2 mutations are not sufficient alone to drive tumorigenesis and require cooperating events.

In conclusion, our work extends prior cellular studies that have demonstrated that DDR2 mutations are found in NSCLCs, and that a subset of these mutations are oncogenic both in culture systems and in the context of a transgenic mouse model. Our model can facilitate additional studies of the role of mutated DDR2 in lung cancer as well as serve as a platform for therapeutic studies aimed at targeting DDR2.

Disclosure of Potential Conflicts of Interest
M. Meyerson reports receiving a commercial research grant from Bayer, has ownership interest (including patents) in Foundation Medicine and LabCorp, and is a consultant/advisory board member for Foundation Medicine. P.S. Hammerman is a consultant/advisory board member for Molecular MD. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: C. Xu, M. Meyerson, K.-k. Wong, P.S. Hammerman
Development of methodology: C. Xu, K.-k. Wong, P.S. Hammerman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Xu, Y. Zhang, H. Asahina, E.M. Beauchamp, K.-k. Wong, P.S. Hammerman
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Xu, K.A. Buczkowski, E.M. Beauchamp, Y.Y. Li, K.-k. Wong, P.S. Hammerman

Figure 4. NSCLC driven by DDR2L63V is sensitive to dasatinib and JQ1 combination therapy. A, proliferation of Krasp53 cell lines 858 and DDR2L63V/p53 cell line 3941 grown for 5 days in the presence of varying concentrations of dasatinib, JQ1, and a combination of 1 μmol/L JQ1 and varying dasatinib concentrations. B and C, tumor volume measurement in a xenograft study of the DDR2L63V/p53 (3941) lung cancer cell line. Nu/Nu mice were treated with dasatinib, JQ1, and dasatinib plus JQ1 combination for 3 weeks following tumor formation. B, average tumor volumes. C, harvested tumor nodule sizes.
Writing, review, and/or revision of the manuscript: C. Xu, K.A. Buczokowski, H. Ashina, K.-k. Wong, P.S. Hammerman
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