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MET exon 14 mutation encodes an actionable therapeutic target in lung adenocarcinoma

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Abstract

Targeting somatically activated oncogenes has revolutionized the treatment of non-small cell lung cancer (NSCLC). Mutations in the gene mesenchymal-epithelial transition (*MET*) near the exon 14 splice sites are recurrent in lung adenocarcinoma and cause exon skipping (*MET* Δ 14). Here we analyzed 4,422 samples from 12 different malignancies to estimate the rate of said exon skipping. *MET* Δ 14 mutation and transcript were most common in lung adenocarcinoma. Endogenously expressed levels of *MET* Δ 14 transformed human epithelial lung cells in a Hepatocyte growth factor (HGF)-dependent manner. Additionally, overexpression of the orthologous mouse allele induced lung adenocarcinoma in a novel, immunocompetent mouse model. Met inhibition showed clinical benefit in this model. Additionally, we observed a clinical response to crizotinib in a patient with *MET* Δ 14-driven NSCLC, only to observe new missense mutations in the *MET* activation loop, critical for binding to crizotinib, upon clinical progression. These findings support genomically selected clinical trials directed towards *MET* Δ 14 in a fraction of NSCLC patients, confirm second-site mutations for further therapeutic targeting prior to and beyond acquired resistance, and provide an *in vivo* system for the study of *MET* Δ 14 in an immunocompetent host.

Keywords

Targeted therapy; Non-small cell lung cancer; acquired resistance

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Introduction

The mesenchymal-epithelial transition (*MET*) proto-oncogene has been extensively studied in human cancer. DNA amplification of wild-type *MET*, fusion of *MET* from chromosomal rearrangement and activating kinase-domain point mutations have all been identified as independent mechanisms of MET/HGF axis activation in cancer (1). Somatic mutations at or around the splice junctions of *MET* exon 14 (*METΔ14*) are a recurrent mechanism of MET activation and lead to a METΔ14 protein that lacks the intracellular juxtamembrane domain (2). The *METΔ14* mutation and transcript occurs in around 3–4% of lung adenocarcinoma (LUAD) (3,4). Exon 14 of *MET* encodes an ubiquitin ligase site (Y1003), which promotes MET degradation (5). As such, extended protein half-life has been proposed as a selective force for *METΔ14* mutations in cancer (2). Case-reports have suggested varying degrees of responsiveness to experimental- and FDA-approved agents that inhibit MET in patients with tumors harboring *METΔ14* alleles (6) but preclinical models and clinical trials are lacking to date. Finally, acquired mutations in the *MET* gene have been observed recently in NSCLCs; one (*MET*^{Y1230C}) following treatment with chemotherapy, radiation and crizotinib (7), and another (*MET*^{D1228V}) with an experimental MET inhibitor in an *EGFR*-mutant lung adenocarcinoma without pretreatment *MET* mutations (8). These reports suggest that second-site, acquired resistance might be combated by next-generation MET inhibitors. Several unanswered questions remain. Malignancies other than lung cancer have not been screened for the *METΔ14* mutation and transcript. Additionally, the role ligand (HGF) plays in signaling from the mutant receptor and the biochemical activity of this mutant protein have not been extensively characterized.

We describe the prevalence of *METΔ14* in solid tumors and define the role of *METΔ14* using both *in vitro* and *in vivo* models. We identify acquired resistance to crizotinib in a patient harboring a *METΔ14* mutation. These findings qualify *METΔ14* mutations as drivers of lung adenocarcinoma and identify a subpopulation of patients whom may benefit from further development of targeted MET/HGF therapies and suggest that combinations of type I and II MET inhibitors might be best deployed together, to prevent activation loop mutations that drive single agent resistance.

Materials and Methods

mRNA expression analysis and Genomic analysis

TCGA PanCancer exon level RNAseq data was obtained (9) and mRNA data for seven types of cancers (READ, COAD, LUSC, KIRC, HNSC, BLCA, LUAD) were downloaded from UCSC. Genomic coordinates for MET exon 13 (chr7: 116411552 -116411708), exon 14 (Chr7: 116411903 -116412043) and exon 15 (Chr7: 116414935 -116415165) were used to retrieve the expression of these three exons. We used a ratio of exon 14 to the average of neighboring exons 13 & 15 [$r = \text{exon 14} * 2 / (\text{exon 13} + \text{exon 15})$] (3). Samples with rate of skipping $R = (1-r) > 0.6$ were considered as exon 14 skipping (Δ14), including partial skipping $0.6 < R < 0.9$ and near complete skipping $R > 0.9$. In TCGA_LUAD, the samples were stratified into *MET wt* and *METΔ14* based on above predicted METΔ14 mRNA status, HGF and MET mRNA expression in two groups was plotted. Samples with METΔ14 skipping identified in latest available TCGA LUAD RNAseq exon level data (n=571) were manually

searched with corresponding MET genotyping in TCGA LUAD samples with known whole exome sequences (10). For specimens that had MET exon 14 skipping at the RNA-level, but lacked somatic mutation calls, exome sequencing BAMs were downloaded from the NCI Genomic Data Commons (GDC) (11). Soft-clipped reads near the genomic region of *MET* exon 14 were identified as potentially misaligned due to a larger deletion. The full sequence of the soft-clipped reads were realigned using BLAT (12). BLAT alignments supported larger deletions in these samples, with multiple read support. Normalized read counts were log2 transformed, scaled and centered in R program. Two-tailed student's t-test was used for two group samples statistical analysis in R (Version 2.15).

Cell lines and cell culture

The AALE parental cell line was established and maintained as described (13) (14). The AALE parental cell line and the following stable cell lines were received as gifts in 2015 from Dr. Choi and Dr. Berger in Dr. Meyerson's lab at Dana-Farber Cancer Institute. No mycoplasma testing was performed. AALE *MET* sg pool and AALE GFP sg pool were infected with sgRNA encoded by pXPR001 and selected in puromycin (1.5 µg/mL). AALE *MET* sg 1F10 single clone and 2E9 single clone were derived from transient transfection with pXPR001-*MET*sg, followed by isolation by limiting dilution. *MET* exon 14 deletion was confirmed by PCR and immunoblotting. AALE cell lines expressing ectopic *MET*Δ14, *MET*wt, RFP, *TPR-MET*, *TPR-MET*ex14 wt or *TPR-MET*ex14 Y1003F were infected with corresponding pLX302 lentivirus and selected in 1.5 µg/ml puromycin.

Protein stability

The experiment was performed as described (2). AALE cells were treated with 75ug/ml cycloheximide (CHX) after 5 min. exposure to HGF (50ng/ml). Protein half-life was calculated from exponential regression of MET protein band intensity as assessed on a LiCor® machine.

Trypsin assay

The experiment is a modified version of that previously described (15), intracellular (internalized) MET is protected from exogenous trypsin in cell culture while cell surface-displayed MET is trypsin sensitive (15). As schematized in Supplementary Fig. 2B, AALE cells were grown to 50% confluence and stimulated with HGF for various time periods. Subsequently, cells were shifted to 4 °C, washed twice with PBS and for 10 minutes in ice-cold pH 3.7 medium (pH adjusted with HCl). After two washes with ice-cold PBS the cells were treated with 0.1% Trypsin in PBS on ice for 30 minutes followed by the addition of TNS (CC-5002, Lonza) and PBS washes. The cells were lysed and handled as described in the immunoblotting section in supplementary method.

Ras Binding Domain pull-down Assays

The experiment was performed as described (16) according to Ras activation assay kit instructions (BK008, Cytoskeleton).

Mouse model

All of the animal experiments were done under protocols reviewed and approved by UCSF animal care committee. *Trp53^{fl/f}* mouse has been described (17). We used 1×10^6 active viral particles/mL for pHAGE-*MET* Δ 14 and pHAGE-GFP and 1×10^7 for pHAGE-*KRAS*^{G12D} (n=3 mice each). Mice were euthanized at protocol-defined endpoint. See supplementary methods for Preclinical Therapeutic Studies with Micro X-ray Computed Tomography (μ CT).

Clinical tumor and cell-free DNA sequencing

Pretreatment tumor sequencing at diagnosis was performed by Foundation Medicine (Cambridge, MA) (4). Cell-free DNA sequencing at the time of progression was performed by Guardant Health (Redwood City, CA) (18). GCP practice and institutional review board (IRB) regulation were followed in the study of patient material.

Results

MET Exon 14 Skipping Occurs in Lung Adenocarcinoma, but Rarely in Other Malignancies

To determine the frequency with which *MET*-encoded mRNA transcripts specifically exclude exon 14 (i.e. *MET* Δ 14) in various cancer types, we examined RNAseq from a total of 4,422 samples from the TCGA Pan-Cancer-12 set composed of cases from 12 different malignancies (9). First we examined total mRNA expression of *MET* and its ligand *HGF* and found only lung adenocarcinoma (LUAD) and clear cell kidney cancer (KIRC) had high levels of both transcripts (Supplementary Fig. S1A, S1B). Next we used exon-level analysis to calculate a ratio of *MET* exon 14 to the average of neighboring exons 13&15 [$r = \text{exon } 14^2 / (\text{exon } 13 + \text{exon } 15)$] (3) to identify 16 LUAD samples from 571 (2.8%) (10) with *MET* Δ 14 skipping (Fig. 1A, and Fig. S1C) while the other 6 cancer types have no or low (<1.2%) rates of *MET* exon 14 skipping. Fourteen of 16 *MET* Δ 14 samples (87%) showed somatically encoded DNA *MET* alterations around splice sites (4) (Fig. 1B and table S1). Fifteen out of 2,582 samples from cancers other than lung showed RNA evidence of exon 14 skipping by the above metric. No splice site mutations supporting a somatic origin for this skipping were seen in other cancer types. Total *MET* and *HGF* mRNA expression did not differ between *MET* Δ 14 and *MET*wt samples (Fig. S1D). In summary, the relatively high *MET* Δ 14 skipping rate and high total *MET* and *HGF* expression level suggest LUAD as the appropriate setting for a deeper, functional investigation of *MET* Δ 14 function.

MET Exon 14 Skipping Transforms Human Lung Epithelial Cells in an HGF-Dependent Manner

The majority of *MET* Δ 14 skipping occurs without *MET* amplification, at least in early-stage LUAD (table S1) (19). To model the effects of *MET* Δ 14 expression from the endogenous promoter in a lung cancer-relevant cell type, we used CRISPR to target the *MET* intron/exon 14 junctions in AALE cells, an immortalized, non-transformed, human tracheobronchial epithelial cell line (14). sgRNA/CAS9-editing led to *MET* exon14 skipping and *MET* Δ 14mRNA expression confirmed by RT-PCR (Fig. S2A) and immunoblotting (Fig. 2A). Importantly, this endogenously-transcribed system allowed us to decouple *MET* Δ 14's

biochemical function from overexpression, adding new knowledge to the existing ectopic expression cell line models which inevitably overexpress MET protein when introducing the mutation(4). Expression of MET Δ 14 from the endogenous locus (*MET*sg) renders AALE cells anchorage independent in an HGF dose-dependent manner (Fig. 2A), despite equivalent or even lower total protein levels of the exon-skipped receptor compared to wild type MET in *GFP*sg control AALE cells. Similarly, ectopically expressed MET Δ 14 cDNA transformed AALE cells in an HGF-dependent manner more efficiently than equivalently expressed wt MET under the same HGF concentration (Fig. 2B). TPR-MET, a cytosolic, hyperactive, HGF-independent MET fusion mutant with strong transforming ability(20) was used as a control for both colony formation and HGF independence (Fig. 2B). These results suggest that MET Δ 14 is more active than wild type MET, but depends on HGF for full receptor activation and cellular transformation.

MET Δ 14 Increases and Prolongs RAS/AKT & RAS/ERK pathway signaling

To study the mechanism(s) underlying the transforming ability of MET Δ 14, we measured signaling through the MET/HGF axis to downstream effector pathways. We observed that cells with expression of MET Δ 14 from the endogenous locus in AALE (*MET*sg) resulted in higher and more durable levels of phospho-Y1349 MET after HGF-stimulation as compared to MET wt-expressing AALE cells (Fig. 2C and 2D). MET/HGF signaling is known to activate Ras and its downstream PI3'K-AKT and MEK-ERK pathways. AKT and MEK1/2 phosphorylation likewise increase more rapidly and persist longer after HGF stimulation (Fig. 2C), suggesting that the strength and/or duration of HGF-MET signaling is increased in cells expressing MET Δ 14. MET internalization/endocytosis after HGF binding plays a direct role in tumorigenesis in part because activated MET continues to signal to effectors after internalization (15). To study MET internalization, we deployed a sgRNA/CAS9-edited AALE single clone: 1F10, harboring complete, (presumably bi-allelic) editing of *MET* (Fig. 2E). The addition of trypsin removes extracellular proteins and spares internalized MET, rendering it detectable by immunoblotting after cell lysis (Fig. S2B diagram). After HGF stimulation, MET Δ 14 is rapidly internalized and persists in an intracellular, active state longer than does wt MET (Fig. 2F), possibly explaining the prolonged MET Δ 14 signaling observed in this system.

Loss of MET exon 14 Moderately Increases MET protein stability

Deletion of Exon 14 of MET prevents ubiquitination and therefore fosters stabilization of MET protein (2). We measured the half-lives of endogenously-expressed wt MET and MET Δ 14 protein after HGF stimulation in two individual informative sgRNA/CAS9-edited AALE clones. 1F10 has complete *MET* editing (Fig. 2D), while clone 2E9 has hemizygous editing of *MET* (Fig. S3A, right lane). Immunoblotting confirmed that 1F10 expresses exclusively MET Δ 14 while the 2E9 clone expresses equivalent amounts of both wt MET (upper band) and MET Δ 14 (lower band) (Fig. S3B). Immunoblotting of cells treated with cycloheximide and serially collected at different time points (Fig. 3A) shows that MET Δ 14 has a 15% longer protein half-life ($t_{1/2}$) (Fig. S3C) in both homozygous clone 1F10 (7.4 hr) compared to wt parental (6.5 hr) and in hemizygous clone 2E9 (8.5hr vs. 7.6hr Fig. S3C). Pooled *MET*sg cells also showed similar trends, with a MET Δ 14 half-life of 7.9 hr compared to the 6.2 hr for MET wt in the *GFP*sg pool (control) (Fig. S3D and S3E). This

modest increase in $t_{1/2}$ confirms the role of exon 14-encoded Cbl-binding-site in MET stability, but suggests additional, negative regulatory functions possibly attributable to the exon 14-encoded domains.

MET exon 14-encoded Domains Regulate MET Kinase Activity beyond CBL Engagement

To dissect the effects of MET Δ 14 on protein activity from those related to protein turnover, we generated multiple AALE cell lines with stable ectopic expression of mutant *TRP-MET* alleles. Since *TRP-MET* lacks the *MET* exon 14 sequence (20), it has been used as a template upon which to study the independent effect(s) of exon 14 vs. those of the Y1003 CBL binding site on MET activity (21). We inserted either the wt or Y1003F exon 14 sequence into the *TPR-MET* fusion junction in frame with the c-terminus of *MET* (Fig. 3B diagram). We generated AALE cells with stable expression of these constructs respectively confirmed by immunoblotting (Fig. S3F). Insertion of wt exon 14 inhibits 80% of the transforming activity of TPR-MET (Fig. 3C and Fig. S3G). Introducing Y1003F, known to prevent Cbl binding (2) in exon 14 failed to reverse the inhibition of the transforming activity of TPR-MET by wt exon 14 sequence (Fig. 3C), consistent with previous reports in mouse fibroblasts (21). Similar effects are seen on RAS-GTP loading, a marker of RAS activation (Fig. 3D). Co-immunoprecipitation of alternatively-tagged proteins demonstrated that exon 14 insertion does not disrupt TPR-MET dimerization (Fig. S3H). These results confirm the previously described inhibitory role of exon 14(2) and suggest additional negative regulatory function(s) of the exon 14-encoded juxtamembrane domain of MET beyond prevention of CBL engagement.

Met Δ 15 Induces Lung Adenocarcinoma in a Novel *in vivo* Mouse Model

To explore the role of MET Δ 14 *in vivo* we utilized a mouse lung cancer model (Fig. 4A) in which lentivirus encoding a cDNA in *cis* with Cre recombinase is delivered intranasal, leading to expression of the cDNA and ablation of floxed germ line elements in the same lung cancer precursor cell. Since amplification of *MDM2* or *TP53* mutation co-occur with the vast majority of patients with *MET* exon 14 mutations (4,6), we infected immunocompetent *Trp53*^{flox} mice (22) with a lentivirus encoding mouse *Met* lacking exon15 (*Met* Δ 15, the mouse equivalent of human *MET* Δ 14) in *cis* with Cre recombinase. Lentivirus encoding *Kras*^{G12D} or GFP in *cis* with Cre served as controls. Mice receiving *Kras*^{G12D} or *Met* Δ 15 lentivirus became tachypneic and were euthanized at 3 months post infection. These animals all harbored high-grade lung adenocarcinoma while those receiving GFP + Cre virus did not (Fig. 4B and Fig. S4A). Immunohistochemistry staining showed that all tumors expressed the lung marker Ttf-1 and proliferation marker Ki67 (Fig. 4C) while the *Met* Δ 15-driven tumors specifically showed phospho-Met. Delivery of a distinct lentivirus encoding sgRNA targeting the endogenous mouse *Met* exon15 exon/intron junction in *cis* with Cre to *Trp53*^{flox} mice resulted in adenoma but not adenocarcinoma formation at three months, indicating likely additive effects of *Met* Δ 15 activity and Met overexpression or inherent differences in cDNA expression vs. sgRNA editing, or both (Fig. 4C right panel). In summary, the lentivirus delivered *Met* Δ 15 expression in combination with genetic mouse recapitulates some of the lung adenoma cases seen in human patients and is a model to study *Met* Δ 15 inhibition *in vivo*.

Therapeutic Benefit of Met inhibition *in vivo*

Crizotinib is FDA-approved for the treatment of ALK-fused NSCLC but also inhibits MET (23). Several groups have reported off-label use of crizotinib in NSCLC harboring *MET* exon 14 mutations (4),(19),(24). We deployed the *MetΔ15/Trp53^{fllox}* model to study crizotinib response *in vivo* on *MetΔ15*-driven tumors. Tumor-bearing mice were randomized by tumor volume (as assessed by micro-computed tomography performed at 8 weeks after lentiviral induction, Fig. 5A, D0 panel) to receive either crizotinib (50mg/kg/d) or vehicle. Crizotinib-treated mice had, on average, stable disease while control animals continued to progress (Fig. 5B and Fig. S4B). Immunostaining for phospho-MET showed that crizotinib treatment inhibited Met auto-phosphorylation and activation (Fig. 5C). Ki-67 reactivity (Fig. 5C and 5D) was also significantly lower in the crizotinib-treated group. These results suggest that crizotinib has a favorable, but suboptimal, therapeutic effect in this novel Met exon skipping driven mouse model of this important NSCLC subtype.

Response to crizotinib and acquired resistance in a patient with METΔ14-Mutated NSCLC

We found coincident *METΔ14* mutation with ~15× amplification, among other aberrations (Fig. 5A inserted table) in a 63-year-old, otherwise healthy, never-smoking female diagnosed with lung adenocarcinoma and pain. Systemic therapy with crizotinib was initiated after detection of *METΔ14* mutation with ~15× amplification in tumor sequencing results. Restaging PET/CT demonstrated a dramatic improvement of disseminated disease involving the liver, adrenals, multiple osseous lesions and subcutaneous disease (Fig. 6A and Fig. S5A) and a near-complete radiographic response at three months (Fig.6B). Subsequent imaging at nine months showed progression in the liver (Fig. 6C). Cell-free DNA sequencing(18) was performed in the setting of clinical progression to explore mechanisms of acquired resistance without rebiopsy. Pretreatment *MET* exon 14 skipping mutation and *MET* amplification were identified along with four new, subclonal, missense mutations in *MET*: D1228N, Y1230H, Y1230S and G1163R (amino acid numbering according to UniProtKB P08581, Fig. 6C inserted table), all of which are located in the ATP docking pocket (25) (26) and have been previously and independently reported to activate the kinase, impair crizotinib binding (23) and to lead to resistance to other MET inhibitors *in vitro* (26). These mutations occur *in trans* from one another and in quantitatively lower allele fractions than the initial MET exon 14 mutations (Fig. S5B), indicating convergent, acquired resistance through activation loop interactions with crizotinib. This is the first clinical documentation of multiple secondary mutations in a crizotinib-treated case of *METΔ14* driven lung adenocarcinoma, definitively proving that *METΔ14* is the target of crizotinib and that *METΔ14* is a therapeutic target in lung adenocarcinoma.

Discussion

The need to extend targeted approaches to larger fractions of patients with cancer is clear and compelling. We find 2.8% of lung adenocarcinomas show evidence of *MET* mRNA skipping, the vast majority of which result from acquired, somatic mutations and/or deletions in or around the exon 14-coding region of the gene, detectable with genomic profiling of *MET*. The two of 16 samples in which we observed evidence of mRNA skipping but could not detect DNA aberrations in MET could be either false positive mRNA

predictions or harbor yet unexplained mechanisms for exon 14 exclusion in the mRNA transcript of *MET*. The clinical relevance of this population of NSCLC is large, as *MET*Δ14 was at least as frequent as *ALK* and *ROS1* fusions combined in one large dataset where both fusions and skipping were evaluable (10). Our integrative genomic, biochemical, animal and clinical analyses firmly establish *MET*Δ14 as a driver event in NSCLC. We show that *MET*Δ14 expression from the endogenous, non-amplified allele is transforming in an HGF-dependent manner, suggesting HGF levels as a possible independent determinant of activity, response and resistance to therapy. Our experiments here were designed to decouple *MET*Δ14 catalytic activity from the effects of overexpression known to be transforming (27), a distinction our prior ectopic expression studies could not make (4). Our results with endogenous *MET*Δ14 expression levels achieved through genomic editing of a relevant human cell type argue that the exon 14 encoded portion of MET negatively regulates the kinase activity as well as the half-life of the receptor.

Case series have demonstrated variable responses to crizotinib treatment in patients with genomic *MET* alterations including but not limited to *MET*Δ14. We show for the first time that expression of the analogous MetΔ15 is oncogenic in adult mice and that its inhibition leads to demonstrable therapeutic benefit *in vivo*, proving MET itself (rather than an off-target kinase) as the most likely therapeutic target in the aforementioned clinical responses seen in patients. The use of this novel, preclinical model clarifies the likely target of a drug in routine use through directed interrogation of aberrations seen in actual patients. Variations of this flexible system could be deployed for evaluating numerous additional candidate driver oncogenes in a reliable, economically efficient and clinically relevant timeline.

Finally, we report the first case of acquired resistance to MET inhibition through multiple secondary missense mutations clustered in the activation loop of MET. This case emphasizes several important concepts in the targeted therapy of solid tumors. Most importantly, this is the first clinical documentation of multiple secondary mutations in the *MET*Δ14 allele acquired upon clinical progression, definitively proving that *MET*Δ14 is a therapeutic target. Secondly, it underscores the clinical relevance of tumor heterogeneity, as evidenced by multiple missense mutations *in trans* to one another, converging on residues critical for crizotinib binding. Finally, this case stresses the importance of unbiased reassessment of tumor genotype at progression, which in this case nominates available MET inhibitors with distinct modes of binding for treatment of progressive disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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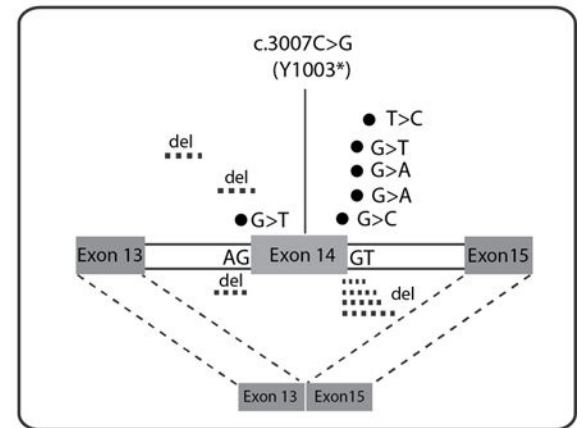
Abbreviations list

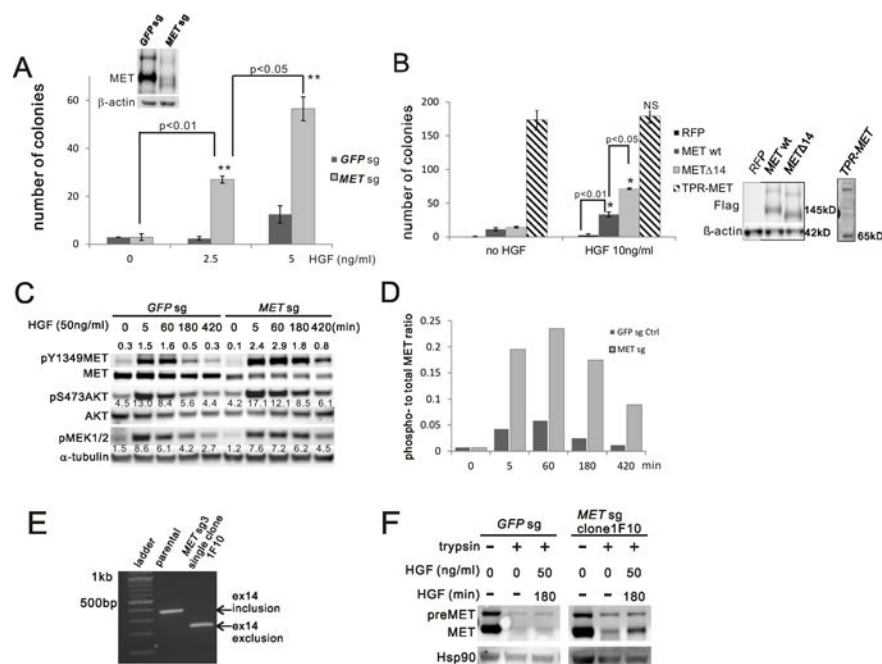
MET	mesenchymal-epithelial transition
HGF	Hepatocyte growth factor

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**Figure 2.**

METΔ14 mutants transform AALE cells in a HGF-dependent manner and are hyperactive. A) Anchorage-independent growth was assessed by soft-agar assay comparing AALE cells targeted with GFP (GFPsg) or *MET* intron/exon 14 skipping sgRNA (METsg) under the indicated HGF concentrations. Expression of MET wt and *METΔ14* was confirmed by immunoblotting. The sum of colonies from 5 random fields at week 3 is reported as the mean of duplicates ($\pm$ SD). ** $p<0.01$ B) Anchorage-independent growth was assessed by soft-agar assay comparing AALE cells with stable ectopic expression of indicated constructs. Immunoblotting confirms expression of FLAG-tagged constructs. The sum of colonies from 5 random fields at week 3 is reported as the mean of duplicates ($\pm$ SD). * $p<0.05$ C) AALE cell lysates were collected from GFPsg or METsg cells with HGF stimulation at the indicated concentration and time. Expression and phosphorylation of MET (pY1349), AKT (pS473) and MEK1/2 (pS217/221) were measured by immunoblotting. α -tubulin is a loading control. Values next to each band are quantified band intensity. D) Ratio of phospho- to total-MET band intensity from (C) at the indicated time points. E) *MET* exon 14 status (inclusion or exclusion) was determined by reverse transcription PCR from AALE parental cells and single clone(1F10) selected from *MET* sgRNA targeted cells (MET sg3). F) Trypsin assay: EGF starved AALE cells were induced by HGF (50ng/ml) for indicated time and then washed and cooled down on ice followed by a treatment with trypsin for 30min. Cell lysates were immunoblotted with MET and HSP90 as loading control.

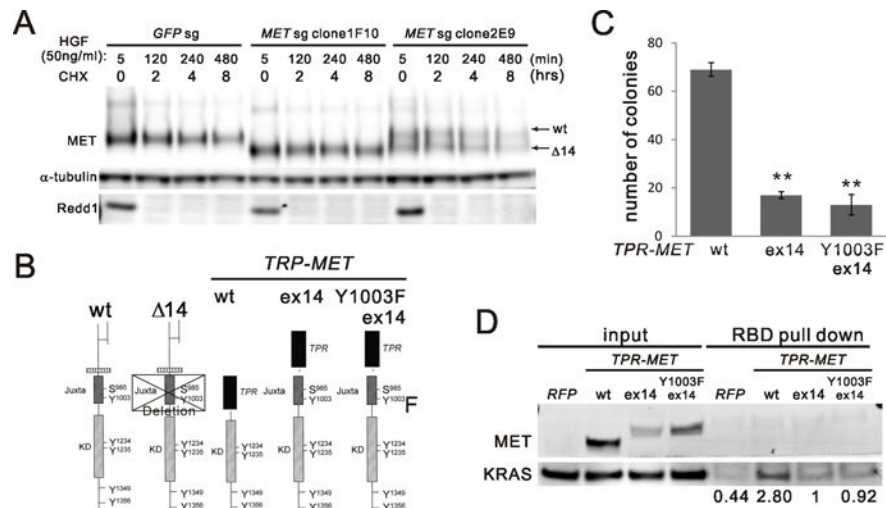


Figure 3.

A) MET protein half-life. AALE GFPsg cells or METsg single clone 1F10 and 2E9 were treated with cycloheximide (CHX) for indicated time after 5 min of HGF stimulation and washout. Cell lysates were immunoblotted with MET antibody and α-tubulin antibody and Redd1 antibody for CHX control. B) a schematic panel of MET protein domains in wild type (wt) and mutants. Exon 14 skipping (Δ14), exon 14 (ex14). C) Comparing AALE cells with stable ectopic expression of V5 tagged TPR-MET wt (wt) and mutants including wt exon14 insertion (ex14) and Y1003F exon14 insertion (Y1003F ex14). Anchorage-independent growth was assessed as described in (Fig. 2A). **p<0.01. D) RAS-binding domain (RBD) pull down assay comparing RAS activation in AALE cells described in (C).

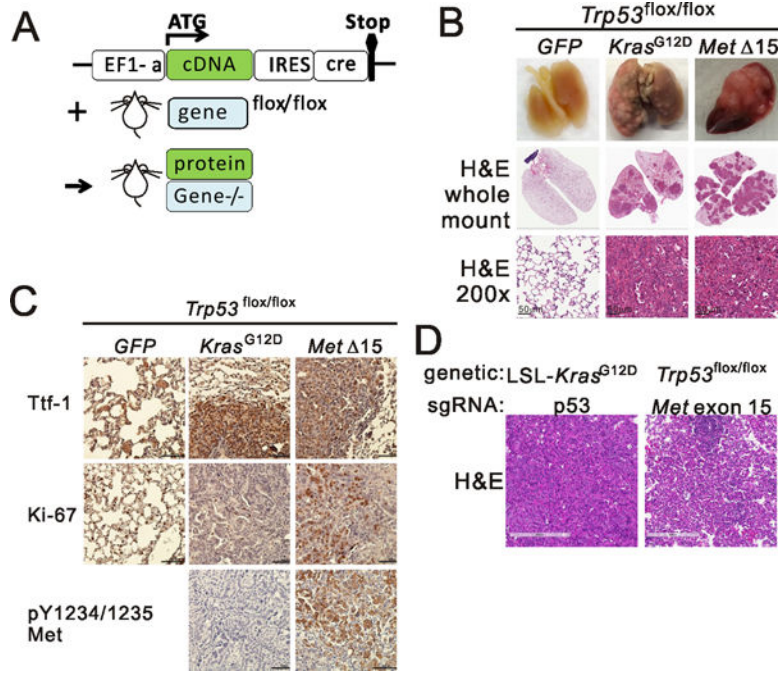
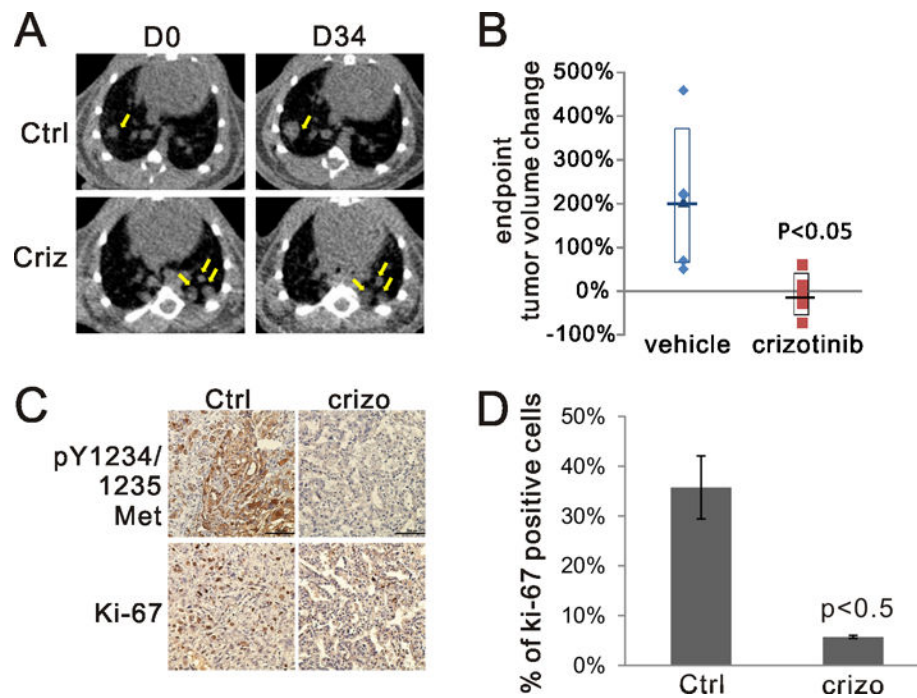


Figure 4.

A new mouse model of MetΔ15-driven Lung Cancer. A) Schematic diagram of the combination mouse model. B) Whole mount lungs from *Trp53*^{flox/flox} mice intranasal infected with indicated lentivirus. Top, macroscopic appearance of representative lungs; middle, Haematoxylin–eosin (H&E) staining of lungs, whole mount, bottom, H&E staining of lungs at high magnification. C) Representative immunohistochemistry of lungs from (B) stained with antibodies against the indicated proteins. D) Hematoxylin–eosin (H&E) staining of tumors from mice from indicated genetic background infected with the indicated sgRNA and cre encoding lentivirus.

**Figure 5.**

A) Representative microCT scans of mice receiving vehicle or crizotinib at the beginning (D0) and the end of treatment (D34). Yellow arrows point to representative tumor areas. B) The change of tumor volume reconstructed from microCT. % volume change = $(D34 - D0)/D0 \times 100\%$. C) Representative immunohistochemistry of lungs from vehicle or crizotinib treated groups stained with antibodies against the indicated proteins. Ctrl: vehicle treated, crizo: crizotinib treated. D) Quantification of Ki-67 positive cells from (C). Tumors from two mice for each group were evaluated.

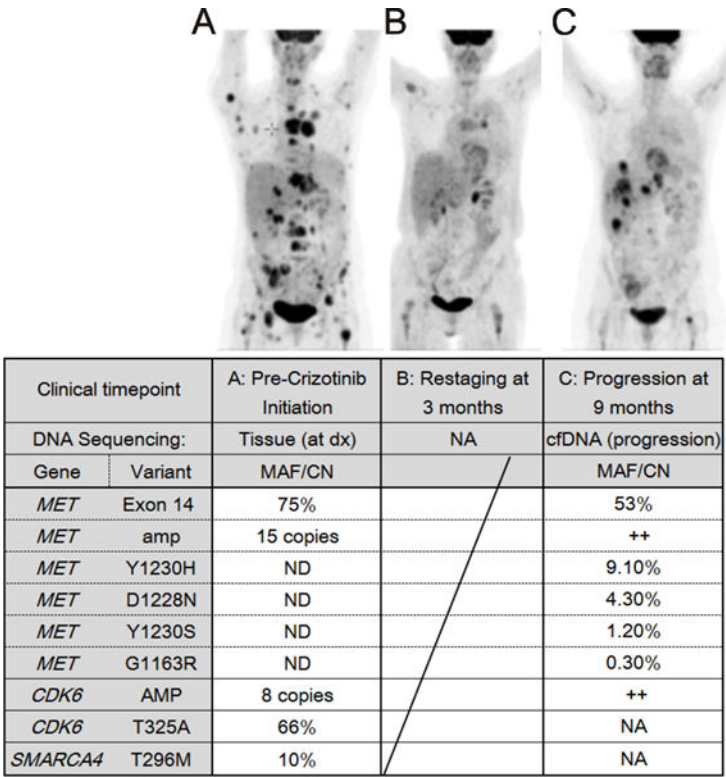


Figure 6. Acquired resistance to crizotinib in a 63-year-old NSCLC patient with METΔ14 mutation. Positron emission tomography of metastatic NSCLC in a 63-year-old female patient. A) Before treatment. B) After one month of crizotinib. C) Upon clinical progression. Inserted Table: Amp=amplification, Dx= diagnosis, cfDNA= cell free DNA, ND = Tested and Not Detected, NA= not assessed. MAF: Mutant Allele Fraction, CN: Copy Number, ++: amplified.