Supplementary Information

Preclinical Modeling of KIF5B-RET Fusion Lung Adenocarcinoma

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Supplementary Information 1: Supplementary methods

Generation of transgenic mice

tetO-KIF5B-RET transgenic mice were generated in the Transgenic and Gene Targeting Core at Georgia State University (Atlanta, GA). To generate tetO-KIF5B-RET transgenic mice, we injected circular pCAG-Cre plasmid (Addgene plasmid #13775) (1) either with circular L3/L2-tetO-KIF5B-RET plasmid or with a tetO-KIF5B-RET transgene DNA fragment (Fig. 1A) into fertilized zygotes, which were derived by mating a mixture of homozygous and heterozygous tetO-SHP2^{E76K} (2) or tetO-SHP2^{CSDA} (3) stud male mice with superovulated FVB/N mice (Harlan Laboratories). A tetO-KIF5B-RET-positive/tetO-SHP2-negative transgenic pup (A1) was obtained from a co-injection experiment using the linear tetO-KIF5B-RET fragment (3ng/µl total DNA) and tetO-SHP2^{CSDA} (line 389) stud male.

tetO-KIF5B-RET lines A2 and G6 transgenic mice were generated using conventional procedure by microinjection of the tetO-KIF5B-RET transgene cassette into fertilized eggs from wildtype FVB/N mice. Briefly, mice were housed in a specific pathogen free vivarium with a 12-

h light/dark cycle. Wild type FVB/N female mice (4 weeks olds) were superovulated by IP injection of 5 IU PMSG followed by IP injection of 5 IU hCG 47 hours later and then were mated with stud males to prepare embryo donors. CD-1 female mice (>6 weeks old) were mated overnight with CD-1 vasectomized males to prepare pseudopregnant recipients for embryo transfer. Embryos were harvested from the oviducts of donor females and were incubated in KSOM medium (EMD Millipore, Billerica, MA) at 37°C in 5% CO_2 before microinjection. The pronuclear microinjection was performed in M2 medium (EMD Millipore, Billerica, MA) using a microinjection system (Sutter Instrument, Novato, CA) and microscope (Olympus Corporation). The tetO-KIF5B-RET transgene (2 ng/µl) was injected into pronuclei of embryos. The injected embryos were incubated in KSOM medium at 37°C in 5% CO_2 before embryo transfer. The CD-1 recipients were anesthetized with 2.5% avertin (2,2,2-tribromoethanol, Sigma, St. Louis, MO) by IP injection at a dose of 0.016 ml/g of body weight. The injected embryos were transferred to CD-1 recipients via oviduct transfer. The recipients were supplied with high protein diets during pregnancy. The pups were delivered 19 days after embryo transfer. When the pups were at least 7 days old, their tails were clipped for genotyping.

Supplementary Information 2: Radiologist's reading of CT images

Vehicle-treated mice

Mouse #1

Pre-treatment: Lesions in the upper lobe of the left lung. Diffused tumor all over right lung

Post-treatment: Tumor left lung large. Right lung looks worse

Mouse #2

Pre-treatment: Left lung diffuse tumor. Lung base has inflammation. Multi-focal opacities at the base of the right lung.

Post-treatment: Left lung almost opaque. Right lung more opacities.

Mouse #3

Pre-treatment: Diffused infiltration in both lungs. Right lung is worse than the left lung. Possible post-obstructive pneumonia.

Post-treatment: Visible progression of the disease. Both lungs look worse. Especially lower lobe sections of the lungs.

Mouse #4

Pre-treatment: Right lung is worse than the left lung. Bilateral diffused tumor.

Post-treatment: Progression of disease in both lungs.

Ponatinib-treated mice

Mouse #5

Pre-treatment: Right lung has tumors in the upper, middle and lower regions.

Left lower lobe has opacities.

Post-treatment: Medial right lung has less opacities. In a lower lobe anterior shows improve ventilation

Mouse #6

Pre-treatment: Right lung upper, right lung medial and anterior lower left lung have opacities. Left lung base has opacities.

Post-treatment: Right lung looks much better. Marked improvement. Left lung base opacities had improved. Upper part left lung improved.

Mouse #7

Pre-treatment: Posterior right lung from diffused tumor middle extended to upper lung.

Post-treatment: Marked improvement.

Mouse #8

Pre-treatment: Left lung completely opaque, Right lung has diffused tumor at lower lobe.

Post-treatment: Left lung is open, slight signs of inflammation. Right lung base became better.

Supplementary Information 3: Supplementary figure legends

sFigure 1. Histological examination of mouse lungs. A, C/KR (line G6) mouse was induced with Dox for 2 M. A lung section was stained with H&E. The right panel shows an area with desmoplastic reaction. B, C/KR (line A2) mouse was induced with Dox for 5 M. Lung section was stained with H&E or Trichrome. The right panels show an area with desmoplastic reaction. C, a H&E stained section of the lungs from a 6 month old $KRas^{LA1}$ mouse. Multiple Lung tumors were present in the lungs without visible desmoplastic stroma. Arrows indicate areas shown on the right panels.

sFigure 2. Tissue sections from human lung adenocarcinoma. A, Tissue images from two cases of TCGA RET fusion lung adenocarcinoma (4, 5). Arrowhead points to area of pleural thickening. B, an invasive lung adenocarcinoma with desmoplasia.

sFigure 3. Cabozantinib (CBT) and vandetanib (VDT) resistant cells. A, Five cell clones were randomly selected from CBT resistant cells cultured in the presence of 0.85 µM CBT and 5 cell clones were randomly selected from VDT resistant cells cultured in the presence of 4.5 µM VDT. Cell lysates were analyzed for the presence of active KIF5B-RET by immunoblotting with an anti-RET pY905 antibody. B/KR were used as the positive control. CBT and VDT, respectively, treated B/KR cells (CBT and VDT sensitive cells) were used as the negative controls. Genomic DNA was isolated from these cells and the KIF5B-RET kinase domain was sequenced from both strands. * indicates RET^{V804L} or RET^{G810A} mutation was identified in these cell clones. No RET kinase domain mutation were detected in cell clones V1 and V4. The mechanism of resistance to VDT inhibition in these two cell clones are unknown. B, comparison

of CBT and VDT sensitivity *in vitro*. KIF5B-RET, KIF5B-RET^{V804L}, and KIF5B-RET^{G810A} were immunoprecipitated from cells and in vitro kinase assay was performed in the presence of indicated concentrations of the drugs using a GST-Gab1 protein as the substrate similar to that described (6). C, comparison of KIF5B-RET and KIF5B-RET^{G810A} protein levels in B/KR cells (WT), B/KR^{G810A} cells derived from the B/KR cells by culturing B/KR cells with vandetanib (G810A), and B/KR^{G810A} cells generated by infecting BaF3 cells with lentiviral KIF5B-RET^{G810A} (G810A-REC). D, comparison of vandetanib resistance between B/KR^{G810A} and B/KR$^{G810A\text{-}REC}$ cells. The data were from three triplicate experiments (n = 9). The nonlinear regression curve fitting was accomplished with GraphPad Prism 6 using variable slope (four parameters).

sFigure 4. Comparison of *in vitro* inhibition of RET, RET^{V804L} and RET^{V804M} by ponatinib, cabozantinib, vandetanib, and lenvatinib. The experiment was performed by Reaction Biology (Malvern, PA) as a commercial service in parallel 10-dose IC_{50} singlet assay, with 3-fold serial dilution starting at 10 μM. Ponatinib displayed the most potent inhibitory activities against RET, RET^{V804L}, and RET^{V804M} among these four compounds.

sFigure 5. Ponatinib treatment schedule and body weight measurements. After Dox induction for 5 months, C/KR mice with MRI-detectable lung tumors were treated with vehicle or ponatinib for 1 month. X-axis indicate the dates of body weight measurement and drug delivery. V and dash line, vehicle treated; P and solid line, ponatinib treated; F, female mouse; M, male mouse.

sFigure 6. Lung section histology from Dox-induced C/KR mice and treated with vehicle or ponatinib for 1 months. H&E stained lung tissue images and the same sections analyzed by Genie® v1 histology pattern recognition software are shown. White, not lung tissue, area excluded; pink, background; blue, normal; purple: hyperplasia/neoplasia.

sFigure 7. µCT examination of dox-induced C/KR mice with lung tumors before and after one month treatment with vehicle or ponatinib. Mice were treated and analyzed by µCT as described in the main text. A, a representative vehicle-treated mouse. B, a representative ponatinib-treated mouse. Left panel: before treatment. Right panel, after treatment. See sMovie for the complete µCT scan data.

sMovie. µCT scans. Top tracks: before (left) and after (right) vehicle treatment of mouse #2 in Supplementary Information 2. Bottom tracks: before (left) and after (right) ponatinib treatment of mouse #8 in Supplementary Information 2.

Supplementary Information 4: Supplementary References

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