FGFR1 mRNA and Protein Expression, not Gene Copy Number, Predict FGFR TKI Sensitivity Across All Lung Cancer Histologies

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Supplementary Figure Legends

Figure S1. Immunoblot analysis of FGFR1 in lung cancer cell lines. A, Extracts from lung cancer cell lines were submitted to immunoblot analysis for FGFR1. The filter was stripped and reprobed for the α-subunit of the NaK-ATPase as a loading control. Multiple FGFR1 polypeptides were variably, but reproducibly, detected in the cell lines and all immunoreactive bands between 100 and 150 KDa were quantified by densitometry. The quantified FGFR1 protein expression corrected for the NaK-ATPase α-subunit loading control and normalized to the FGFR1 expression level in Colo699 is shown below the immunoblots. B, FGFR1 immunoblots from representative lung cancer cell lines and the NaK-ATPase α-subunit loading controls are shown. H157, SK-MES-1, H1299, H726, H1385 and H596 cells express detectable FGFR1 protein, but exhibit ponatinib IC₅₀ values >50 nM.
Figure S2. Inhibition of clonogenic growth in Colo699 and H1581 cells by shRNA-mediated silencing of FGFR1. Lentiviral-encoded shRNAs targeting GFP as a control or two independent shRNAs against FGFR1 were packaged and transduced into the indicated lung cancer cell lines or mouse embryo fibroblasts (MEFs; to determine relative virus titer) and then selected for puromycin resistance. Following 10-14 days, the cultures were fixed, stained with crystal violet and photographed. The colony density in the different wells was quantified by Metamorph imaging and the resulting values are presented in the individual panels as a percent of the GFP controls. Levels of FGFR1 mRNA in replicate Colo699 and H1581 cell cultures following transduction of GFP, FGFR1-0417 and FGFR1-1185 shRNAs were measured by QPCR and graphed relative to GAPDH mRNA levels.

Figure S3. Growth inhibition by the ligand trap, FP-1039. Colo699 cells were submitted to anchorage-independent growth assays in the presence of the indicated concentrations of the ligand trap, FP-1039. After 14 days of culture, the wells were incubated with NBT for 12 hours and the colony area was measured.

Figure S4. Overlap of FGFR1, FGF2 and FGF9 mRNA positivity in ponatinib sensitive lung cancer cell lines and FGFR2 isoform expression and activation. A, The Venn diagram shows the overlap of FGF2 and/or FGF9 mRNA expression with FGFR1 mRNA expression in the 14 ponatinib-sensitive lung cancer cell lines and is derived from the data in Table S1. B, FGFR2 cDNA sequences encompassing the third extracellular immunoglobulin loop were amplified as previously described (Marek et al. Mol Pharmacol. 2009, 75:196-207) and digested with restriction enzymes that discriminate between the FGFR2 IIIb (Ava I) and FGFR2 IIIc (Hinc II) sequences. The data reveal that H1048 cells express FGFR2 IIIb while H1581 cells express FGFR2 IIIc. C, H1581 and H1048 cells were incubated for 2 hrs in HITES medium and then treated for 10 minutes with the indicated FGFs at 10 ng/ml. Cell extracts were prepared and immunoblotted for phospho- and total ERK. The findings demonstrate that H1581 cells respond exclusively to FGF2 and FGF9 while H1048 cells also respond to FGF7, consistent with the expression of FGFR2 IIIb.
Figure S5. ROC analysis of FGFR1 GCN and mRNA expression in a lung cancer cell line TMA. A TMA comprised of 21 lung cancer cell lines processed as FFPE tissues was stained for FGFR1 GCN by SISH and FGFR1 mRNA by ISH. The performance of the biomarkers at a ponatinib sensitivity cut-point of 50 nM was determined by ROC analysis.

Figure S6. Kaplan-Meier analysis of overall survival of lung cancer patients with increased FGFR1 GCN or high mRNA. The surgical lung cancer cohort analyzed for overall survival based on FGFR1 GCN (>4 vs. <4) or FGFR1 mRNA (4+ vs. <4). No significant association of FGFR1 gene amplification or increased mRNA expression is observed.

Figure S7. Overlap of FGF2 or FGF9 expression with FGFR1 mRNA positivity in primary lung cancers. FGF2 and FGF9 mRNA expression levels were extracted from lung SCC and adenocarcinoma TCGA RNAseq datasets and the degree of overlap with tumors with high FGFR1 mRNA is shown in the Venn diagrams.
Figure S1
Figure S2
Figure S3

Colo699 Cells

Growth (% control)

IC$_{50}$ = 3 nM

FP-1039, μM
Figure S4
Figure S5
Figure S6
Figure S7