Supplemental Data 1

Endoglycosidase digestion. Sub-confluent COS-7 cells were transiently transfected with 200 ng of DNA (5 ng of Myc-tagged WT or mutated FGFR1 cDNA and 195 ng of empty vector (EV)) in 24-well plates using FuGene6 reagent (Roche Diagnostics). Forty-eight hours post transfection, cells were washed with PBS and then lysed with 100 ul of RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxylate and 0.1% SDS) containing 1X Halt protease inhibitor cocktail (Pierce, Rockford IL). For deglycosylation analysis, 4 µl of cleared lysate was subjected to PNGasef and EndoHf digestion according to manufacturer's recommendations (New England Biolab).

Western analysis. Untreated or endoglycosidase treated samples were resolved on NuPAGE 3-8% Tris-Acetate Gels (Invitrogen) under reducing conditions and then subjected to western analysis using an anti-Myc primary antibody (clone 4A6, 1:150; Upstate Biotechnology, Inc., Lake Placid, NY) and a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:15000, Upstate Biotechnology, Inc., Lake Placid, NY). Immunoreactivity was visualized using Western Lighting chemiluminescence reagent (PerkinElmer, Boston, MA). To control for equal loading, blots were stripped using Restore Western Blot Stripping Buffer (Pierce, Rockford, IL) and reprobed with horseradish peroxidase-conjugated anti-β-actin antibody (1:5000, Abcam). FGFR1 and β-actin immunoreactivity were quantified by densitometry using Eagle Eye imaging system (Stratagene, La Jolla, CA). Overall expression levels of WT and mutant receptor were determined from the PNGase treated samples and were normalized to their respective β-actin levels. Results were expressed as the ratio between mutant and WT.

For receptor maturation studies, the upper (mature) and lower (immature) band densities were determined individually from the EndoH treated samples and the percent of the mature fraction was calculated with results expressed as a ratio to WT expression. Endoglycosidase experiments were repeated two times.

Cell-surface expression. Expression of WT or mutant FGFR1 at the cell surface was quantified in COS-7 cells using an antibody binding assay (40). In brief, COS-7 cells were transfected as described above and 48 h later the cells were rinsed with binding buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM KCl, 2 mM CaCl2, 5% heat-inactivated horse serum, 0.5% fetal bovine serum, adjusted to pH 7.7 with HCl) and then incubated in binding buffer containing an anti-myc antibody (at 1:1000 dilution) at room temperature for 4 h. Following the first antibody incubation, the cells were rinsed and then incubated with [^{125}I] - rabbit anti-mouse IgG (300,000 cpm/well; PerkinElmer, Waltham, MA), in binding buffer, for an additional 2 h. The cells were then rinsed and lysed in 1 M NaOH and the entire lysate was counted in a gamma-counter. Experiments were performed in quadruplicate and repeated three times.