Supplementary Table 1 The following primers were used to introduce mutations

(sense, 5’ to 3’ with mutagenic changes underlined and silent substitutions in lower case):

E219K	CACTGGAGCCTCATTATG<u>A</u>AAAGTGTGGTaCCATCTGA
G227E	GGTCCCATCTGACAAGG<u>A</u>AAAcTATACaTGTGTGGTGGAG
V248D	ACACGTACCACCTGGATG<u>AC</u>GTcGAGCGATCGCCTCAC
R251Q	CTGGAcGTTGTcGAGC<u>A</u>ATCGCCTCACCGG
S252W	GTTGTGGAGCGcT<u>G</u>GCCTCACCGGC
G271E	CCTCCACAGTcGTCG<u>A</u>AGGAGACGTcGAGTTTG
C278F	TGGTCGGAGGAGACGTcGAGTTTGTCT<u>T</u>CAAGGTTTACAG
E475K	CCAGAGGAtCCAAAATGG<u>A</u>AGTTTCCAAGAGATAAGCTG
D530N	ACAGAGAAAGACCTTTCT<u>A</u>ATCTGGTcTCAGAGATGG
I642V	CAGAAAACAATGTcATGAAA<u>G</u>TAGCAGACTTTGGACTCG
A648T	TAGCAGACTTTGGtCTC<u>A</u>CCAGAGATATCAACAATATAGAC
N549K	CACAAGAATATCATAAA<u>g</u>CTTCTTGGAGCCTGCACAC

Supplemental Methods- Mutation Analysis

PCR .

The initial mutation screen included the majority of exons in FGFR1 and FGFR2. As virtually no mutations had even been reported in the N-terminal regions of the FGFRs, exons 1 and 2 were not screened for FGFR3 and exons 1-6 were not screened for FGFR4 (Summarized in Supplemental Table 2). Notably all exons in which the majority of activating mutations had previously been identified in the germline of FGFR1-3 were screened across all four genes. PCR primers were M13 tailed and sequences are available on request. New PCR primers were designed for FGFR2 for amplification from FFPE tissue.

Supplemental Table 2.

Gene Name	Reference Seqence	Exons Screened
FGFR1	NM_023110	Exons 2-18 (ATG in exon 2)
FGFR2	NM_00141	Exons 2-18 (ATG in exon 2)
FGFR2	NM_022970	Exon 8 (epithelially spliced exon)
FGFR3	NM_000142	Exons 3-17 (ATG in exon 1)
FGFR4	NM_213647	Exons 7-18 (ATG in exon 1)

dHPLC. 40 additional cell lines were screened for mutations in exons 6-18 of FGFR2 by DHPLC using the Transgenomic WAVE Nucleic Acid Fragment Analysis System. The base-pair sequence for each FGFR2 exon was imported in to the WAVEMAKER software to identify suitable denaturing conditions (conditions available on request). Prior to the samples being loaded, each sample was spiked 50:50 with a CEPH control to ensure the detection of both heterozygous and homozygous variants. At the completion of DHPLC, chromatograms were printed out and individually analyzed for the presence of a

potential variation. In those cases where identical DHPLC peak profiles were present in many samples, several cases were chosen for sequencing and in all cases a common SNP was present and not a mutation. PCR exons were either cleaned up using a PCR purification kit (Qiagen) and the BIOROBOT 9600 dual vacuum system (Qiagen) or purified after thermocycling using solid phase reversible immobilization (SPRI)-based technology (AMPure®; Agencourt Biosciences Corp., Beverly, MA), resulting in the removal of unincorporated dNTPs, primers, and salts. PCR products were eluted in 30µl distilled H_2O

Sequencing: Both strands of each PCR product were sequenced as follows: Sequencing reactions were performed using 3µl (approximately 25ng) of purified PCR product in a 6µl reaction containing 0.33µl BigDye Terminator v3.1 premix, 3.2 pmol of either M13 forward (TGTAAAACGACGGCCAGT) or M13 reverse (CAGGAAACAGCTATGACC) primer, and 1.03µl 5X BigDye sequencing buffer. Cycle-sequencing was performed for 35 cycles following the manufacturers recommendations on GeneAmp 9700 PCR machines (Applied Biosystems, Foster City, CA). Sequencing reactions were purified using CleanSEQ® (Agencourt Biosciences Corp., Beverly, MA) to remove unincorporated dye-terminators, and analyzed on 3730xl DNA analyzers (Applied Biosystems, Foster City, CA).

Sequence Analysis and Confirmation. We aligned and analyzed sequence chromatograms using Sequencher Version 4.1 (Gene Codes). All mutations were verified in an independent PCR amplification. For the primary tumors, DNA was extracted as

previously described [39]. Due to cccasional PCR failure and limiting DNA quantity, we were unable to sequence all 18 exons of FGFR2 for every tumor sample. Overall, about 85% of the coding region of FGFR2 was sequenced in the panel of primary tumors. PCR dropouts were repeated once and samples were included if they had less than 5 exons dropout. For three of these tumors, there was sufficient normal tissue from which to extract DNA and in all cases the mutation was only present in the tumor, confirming the somatic origin of these changes.