Supplementary Experimental Procedures

Tumor dissociation and xenosphere generation

Xenopatients were obtained as described (8) and their tumors were mechanically dissociated, digested with Type-I Collagenase (1 mg/ml, Life Technologies-Invitrogen), filtered through a 70 μm cell strainer (BD Falcon), and cleared with histopaque-1077 (Sigma), according to manufacturer instructions. Cells were grown in ultra-low attachment plastics (Sigma-Corning) in standard stem-cell medium. This included human recombinant epidermal growth factor (EGF, 20 ng/ml; Sigma), basic fibroblast growth factor 2 (bFGF, 10 ng/ml; Peprotech), added to stem cell medium, i.e. DMEM/F-12 (Sigma) supplemented with 2 mM glutamine (Sigma), penicillin-streptomycin (1:100, EuroClone), N-2 supplements (Life Technologies-GIBCO), 0.4% BSA (Sigma), 4 μg/ml heparin (Sigma), CD Lipid Concentrate (Life Technologies-GIBCO). Cells were kept in humidified incubators at 37°C, with 5% O_2 and 5% CO_2.

Genomic DNA extraction and mutational screening

Genomic DNA was extracted with the Wizard® SV genomic DNA purification system (Promega) according to the manufacturer's instructions. The quality of nucleic acids was verified by measuring the 260/280 absorbance ratio with ND-1000 V3.7.1 Nanodrop (Thermo Scientific). Purified gDNA was analyzed for KRAS, NRAS, BRAF, PIK3CA, as previously described (8).

Microarray data generation and processing

RNA was extracted using the miRNeasy Mini Kit (Qiagen), according to the manufacturer's intructions. Synthesis of cDNA and biotinylated cRNA (from 500 ng total RNA) was performed using the IlluminaTotalPrep RNA Amplification Kit (Ambion), according to the manufacturer's instuctions. Quality assessment and quantitation of total RNA and cRNAs were performed with Agilent RNA kits on a Bioanalyzer 2100 (Agilent). Hybridization of cRNAs (750 ng) was carried out using Illumina Human 48k gene chips (Human HT-12 V4 BeadChip). Array washing was performed by Illumina High Temp Wash Buffer for 10' at 55°C, followed by staining using streptavidin-Cy3 dyes (Amersham Biosciences). Hybridized arrays were stained and scanned in a Beadstation 500 (Illumina).

Probe intensity data were extracted using the Illumina Genome Studio software (Genome Studio V2011.1) and subjected to Loess normalization using the Lumi R package. To minimize the noise due to cross-species hybridization of transcripts deriving from murine infiltrates in PDX tissues, two pure murine samples were hybridized on human arrays in a pilot experiment, and all probes that generated detectable signals in this assay were removed from further analyses. Probes were filtered

to select those that showed detectable signal (detection P value = 0) in at least one samples. For each of such genes, only the probe with the highest variance of signal was selected. Pearson's correlations were performed for any possible PDX/Xenosphere permutation. The dataset was uploaded on the GEO Database (GEO accession number GSE101792).

Generation of NRG1-expressing xenopheres

To generate stable xenospheres (M016 and M049) or murine fibroblasts expressing NRG1, cells were transduced with a lentiviral vector generated by transfecting 293-T cells with different plasmids: the packaging construct encodes the HIV-1 Gag and Pol precursors, the regulatory proteins Tat and Rev, pMDL, the VSV-G expressing construct, and the transfer construct OriGene's TrueORF clone RC220134L1V (Origene). The ORF cloned in this vector was expressed as a tagged protein with c-terminal Myc-DDK tags.

Real time RT-PCR

Total RNA was extracted using miRNAeasy mini Kit according to manufacturer's instructions (Qiagen). 1 µg of purified RNA was used as a template for cDNA synthesis with random and hexamer primers and high capacity reverse transcription kit (Applied Biosystem). To evaluate NGR1 expression Real-time PCR was performed using TaqMan® Universal Master Mix (Life Technologies) containing 200 ng of cDNA and a TaqMan® gene expression probe Hs00247620_m1 on ABI PRISM 7900HT sequence detection system. Relative quantification value for NGR1 gene expression was obtained by normalizing to ubiquitin and beta actin as endogenous controls. All samples were run in triplicate and the mean and standard deviation calculated.

Murine Fibroblast Conditioned Medium (mFIBR CM)

A murine fibroblast culture derived from a xenopatient was isolated and transduced to express human NRG1 as described above. Cells were plated in adhesive dishes in DMEM supplemented with 2 mM glutamine, penicillin-streptomycin, and 10% FBS. To obtain mFIBR CM, fibroblasts were grown up to confluence and then kept for 24h in basal stem-cell medium.

Western Blotting

Xenospheres protein expression were analyzed on whole-cell lysates, solubilized in boiling Laemmli buffer. For spheropatient protein expression, snap-frozen tissues were mechanically dissociated and solubilized with EB-extraction buffer (20 mMTris-HCl pH 7.4, 150 mM NaCl, 10% Glycerol, 1% TritonX-100, 5mM EDTA) in the presence of protease and phosphatase inhibitors.

Protein concentration was determined using a BCA Protein Assay Reagent kit (Pierce Biotechnology). Equal amounts of proteins were resolved by SDS-PAGE in reducing conditions and analyzed by immunoblotting. Antibodies were visualized with appropriate horseradish peroxidase-conjugated secondary antibodies (Amersham), and the enhanced chemioluminescence system (ECL, Amersham). Blot images were captured using the FujiFilm LAS-3000 digital imaging system.

Immunohistochemistry

Tumors explanted from mice were formalin-fixed and paraffin-embedded according to standard procedures. 4 μm tissue sections were dried in a 37°C oven overnight. Slides were de-paraffinized in xylene and rehydrated through graded alcohol to water. Endogenous peroxidase was blocked in 3% hydrogen peroxide for 30 minutes. Microwave antigen retrieval was carried out using a microwave oven (750 W for 10 minutes) in 10 mmol/L citrate buffer, pH 6.0. Slides were incubated with individual primary antibodies overnight at 4°C inside a moist chamber. After washings in TBS, anti-rabbit secondary antibody (DakoEnvision+System-horseradish peroxidase–labeled polymer, Dako) was added. Incubations were carried out for 1 hour at room temperature. Immunoreactivities were revealed by incubation in DAB chromogen (DakoCytomation Liquid DAB Substrate Chromogen System, Dako) for 10 minutes. Slides were counterstained in Mayer's hematoxylin, dehydrated in graded alcohol, cleared in xylene, and the coverslip was applied by using DPX. A negative control slide was processed with secondary antibody, omitting primary antibody incubation. Images were captured with the LEICA LAS EZ software with the use of a LEICA ICC50 HD microscope.