Supplemental experimental procedures

Antibodies

Antibodies against tMEK (#9122), pMEK1/2 (#9121), tERK1/2 (#4695), pERK1/2 (#9101), pAKT (S473, #4060), pAKT (T308, #4056), tAKT (#9272), pEGFR (Y1068, #3777), HER2 (#2165), pHER2 (Y1221/1222, # 2243), tHER3 (#4754), pHER3 (Y1289, #4791), pHER3 (Y1197, #4561), p85 (#4292), tPDGFRB (#3169), pPDGFRB (Y751, #3166) and pTyr (#9411) were from Cell Signaling Technology. Antibodies against GRB2 (#610111) was obtained from BD Biosciences, anti-HER2 (04-1127) for immunoprecipitation from Millipore, anti-EGFR (sc-03) and anti-NRG1 from Santa Cruz (sc-28916) and R&D Systems (MAB377).

Expression profiling analysis

SW1736 and SK-MEL-18 cells were treated with or without 2 μM PLX4032 for 1, 6, or 48 h in triplicate. RNA integrity was determined using a Bioanalyzer 2100. Samples were processed and hybridized on Illumina HumanHT-12 v4 bead arrays by the MSKCC Genomic core facility. Gene expression data of all samples were $\log_2$-transformed and quantile normalized with Partek GS software. To identify differentially expressed genes between SW1736 and SK-MEL-28 cells, we used the maSigPro (microarray significant profiles) regression method (1), where the categorical variable was the cell type and the continuous variable the time of treatment. This approach is based on two regression steps, a regression model for gene selection and a regression step for modeling expression profile patterns. The threshold for gene selection was a FDR-adjusted p-value < 0.01. A polynomial degree of 2 and a significant level for model variable <0.01 was applied for modeling profile patterns. Filtered genes with similar expression patterns were clustered together and visualized as the average of the expression value for the cluster by cell line. Those gene clusters with a biologically meaningful profile were compared with the rest of the genome for simple functional enrichment analysis using FatiGO tool (2). The most significant KEGG (Kyoto encyclopedia of genes and genomes) terms related to signaling pathways were listed by level of significance. All analysis tools are implemented in the web-based software Babelomics v4 (3).

PCR primer sequences

Name	Sequence (5´-3´)
HER2 Forward	ACACAGCGGTGTGAGAAGTG
HER2 Reverse	AACACTTGGAGCTGCTCTGG
HER3 Forward	GATGGGGAACCTTGAGATTG
HER3 Reverse	GGCAAACTTCCCATCGTAGA
EGFR Forward	TGCAAATAAAACCGGACTGA
EGFR Reverse	GGTGGTTCTGGAAGTCCATC
PDGFRβ Forward	TATATCATCCCCCTGCCTGA
PDGFRβ Reverse	GGCTGTCACAGGAGATGGTT
EPHB2 Forward	AGTACACGGACAAGCTGCAA
EPHB2 Reverse	CCTCGTAGGTGAAAGGATCG
CtBP1 Forward	GGAACCCTTCAGCTTTAGCC
CtBP1 Reverse	GGCTGTCAGATGGTCCTTGT
CtBP2 Forward	TTCAAGGCCCTGAGAGTGAT
CtBP2 Reverse	GAGTCCGCTGTCTCTTCCAC
GAPDH Forward	CATGGCCTTCCAACTTGACT
GAPDH Reverse	CATCTGCCGTCACATTGTTC
HER3 promoter-ChIP Forward	TACCCTTCCCTGGATCTGG
HER3 promoter-ChIP Reverse	GATGGGGCTCACCCTAATTT

Retroviral HER3 shRNA

Retrovirus production was carried out by co-transfecting pSIREN-RetroQ-HER3-shRNA plasmid (HER3 target sequence: CACTGTACAAGCTCTACGA) and pVSV-G envelop vector into GP2-293 cells as previously

described (4). SW1736 cells were plated in 10 cm plates (2 x 10^5 cells) and following adherence overnight, cells were infected in the presence of hexadimethrine bromide (Sigma-Aldrich). Twelve hours post-infection, virus-containing medium was replaced by fresh growth medium. Selective medium with 1 μg/ml puromycin was added 48 h post-infection and cells were refed 3 days later. After 2 puromycin selection cycles, cells were harvested and plated for cell growth assays.

Supplemental reference list

(1) Conesa A, Nueda MJ, Ferrer A, Talon M. maSigPro: a method to identify significantly differential expression profiles in time-course microarray experiments. Bioinformatics 2006;22:1096-102.

(2) Al-Shahrour F, Diaz-Uriarte R, Dopazo J. FatiGO: a web tool for finding significant associations of Gene Ontology terms with groups of genes. Bioinformatics 2004;20:578-80.

(3) Medina I, Carbonell J, Pulido L, Madeira SC, Goetz S, Conesa A, et al. Babelomics: an integrative platform for the analysis of transcriptomics, proteomics and genomic data with advanced functional profiling. Nucleic Acids Res 2010;38:W210-W213.

(4) Swift S, Lorens J, Achacoso P, Nolan GP. Rapid production of retroviruses for efficient gene delivery to mammalian cells using 293T cell-based systems. Curr Protoc Immunol 2001;Chapter 10:Unit 10.17C.:Unit.