PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR

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Supplementary methods

SNP-Arrays and expression arrays

Copy number analysis was performed using 250K StyI Affymetrix SNP-arrays; data was analyzed using GenePattern software (www.broad.mit.edu/cancer/software/genepattern/). Expression data were obtained using Affymetrix U133A arrays from 53 of the cell lines. RNA extraction, hybridization to arrays and scanning of arrays were performed using standard procedures. Preprocession of CEL files from U133A arrays and hierarchical clustering were performed with dCHIP. All gene expression CEL-files are available at the Gene Expression Omnibus (GEO) database (serial number: #15590434).

Cell-based screening

All compounds were purchased from commercial suppliers. Cells were plated into sterile microtiter plates using a Multidrop instrument (Thermo Scientific, Germany) and cultured overnight. Compounds were then added in serial dilutions. Cellular viability was determined after 96h by measuring cellular ATP content using the CellTiter-Glo assay (Promega, Germany). Plates were measured on a Mithras LB940 plate reader (Berthold Technologies, Germany). Half-maximal inhibitory concentrations ("IC_{50}-values") were determined from the preimage under the growth inhibition curve, where the latter was smoothed according to the logistic function with the parameters appropriately chosen.

Western blotting

The following antibodies were used for immunoblotting: PTEN (N19, A2B1), ß-actin (Santa Cruz; Germany), ErbB3, p-ErbB3, STAT3, p-STAT3, AKT, p-AKT, p-ERK, ERK (Cell Signaling Technology; Beverly, MA), p-EGFR (Biosource; USA), Bim (Axxora, Germany), anti-rabbit-antibody, anti-mouse (Millipore; Germany). The Enhanced Chemiluminescence (ECL) system (Amersham-Pharmacia) was used to develop the blots.

Transfection and infection

Replication-incompetent retroviruses were produced from pBabe-based vectors by transfection into the Phoenix 293T packaging cell line (Orbigen, San Diego, California, United States) using the calcium precipitation method. Replication-incompetent lentiviruses were produced from pLKO.1-Puro based vectors containing the shRNA insert following standard procedures (http://www.broad.mit.edu/genome_bio/trc/). Cells were infected with viral supernatants in

the presence of polybrene. After 36h medium was changed and cell lines were selected with 1-2µg/ml puromycin, from which stable transduced clonal cell lines were derived.

Quantitative genomic real-time PCR (qPCR), long distance PCR and sequencing

We applied qPCR-based copy number analysis using 54 oligonucleotide pairs (5). Long-distance PCR was performed using the ABI GeneAmp XL Kit (www.appliedbiosystems.com). All oligonucleotide sequences are available on request.

Apoptosis assays

Cells were plated in 6-well plates, after 24h of incubation treated with erlotinib for 48-96h and finally harvested after trypsinization. Then cells were washed with PBS, resuspended in Annexin-V binding buffer and finally stained with Annexin-V-FITC and PI. FACS analysis was performed on a FACS Canto Flow Cytometer (BD Biosciences, Germany) and results were calculated using FACS Diva Software.

Statistical analysis

Statistical analysis was performed using a two-tailed *t*-test implemented in "R".

Figure legend

Supplementary figure 1

(A) Phosphorylation of AKT in H1650 cells after treatment with DMSO (0.1%) as control („control") or 1µM of erlotinib ("erlotinib") or erlotinib with an allosteric AKT inhibitor ("AKTi") are depicted. Actin was immunohistochemically detected as loading control. (B) Viability of H1650 cells after treatment with DMSO (0.1%) as control („control") or 1µM of erlotinib ("erlotinib") or erlotinib with an allosteric AKT inhibitor ("AKTi") are depicted. The p-value for the comparison of respective measurements is indicated above the columns.