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Data in Brief

The HER2 amplicon includes several genes required for the growth and survival of HER2 positive breast cancer cells — A data description

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ABSTRACT

A large number of breast cancers are characterized by amplification and overexpression of the chromosome segment surrounding the *HER2* (*ERBB2*) oncogene. As the HER2 amplicon at 17q12 contains multiple genes, we have systematically explored the role of the HER2 co-amplified genes in breast cancer cell growth and their relation to trastuzumab resistance. We integrated array comparative genomic hybridization (aCGH) data of the HER2 amplicon from 71 HER2 positive breast tumors and 10 cell lines with systematic functional RNA interference analysis of 23 core amplicon genes with several phenotypic endpoints in a panel of trastuzumab responding and non-responding HER2 positive breast cancer cells. In this Data in Brief we give a detailed description of the experimental procedures and the data analysis methods used in the study (1).

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Specifications	
Organism/cell line/tissue	Human/BT474, HCC202, SKBR3, UACC812, HCC1954, HCC1569, JIMT1, SUM190, SUM225, KPL4, MCF7/breast cancer tumor tissue
Sex	Female
Sequencer or array type	Agilent Human Genome CGH 244K microarrays, plate based siRNA screen, protein lysate microarrays
Data format	aCGH microarrays raw- and PCF segmented data from tumors and cell lines, siRNA raw- and normalized data from cell lines, protein lysate microarray raw- and normalized data from cell lines
Experimental factors	HER2 positive breast cancer patients, HER2 positive cell lines, siRNA screens, drug treatments
Experimental features	Determination of the HER2 amplicon size from patient samples; siRNA screens and protein lysate microarrays of breast cancer cell line panel to discover significant HER2 co-amplified genes; validation of their importance in cancer cell survival
Consent	Patients have given informed consent and/or the studies are approved by regional ethical review boards
Sample source location	Norway, Finland and France

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Direct link to deposited data

Deposited data can be found here:

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34236
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17907
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32291
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20394

Material, methods and experimental design

Cell lines and primary tumors

11 breast cancer cell lines were grown and cultured following recommended conditions. Of these 10 were HER2 positive (HER2+); BT474, HCC202, SKBR3, UACC812, HCC1954 and HCC1569 were obtained from the American Type Culture Collection (ATCC, USA) and JIMT1 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). Both ATCC and the DSMZ authenticate all human cell lines by DNA-typing using short tandem repeats. SUM190 and SUM225 were kindly given by Stephen Ethier from Karmanos Cancer Institute in Michigan USA, whereas KPL4 was kindly provided by Junichi Kurebayashi from Kawasaki Medical School in Japan. The HER2 negative (HER2-) MCF7 cells were obtained from the Interlab Cell Line Collection (ICLC, Italy) and used as the control. Cells were cultured for a maximum of 30 passages prior to use.

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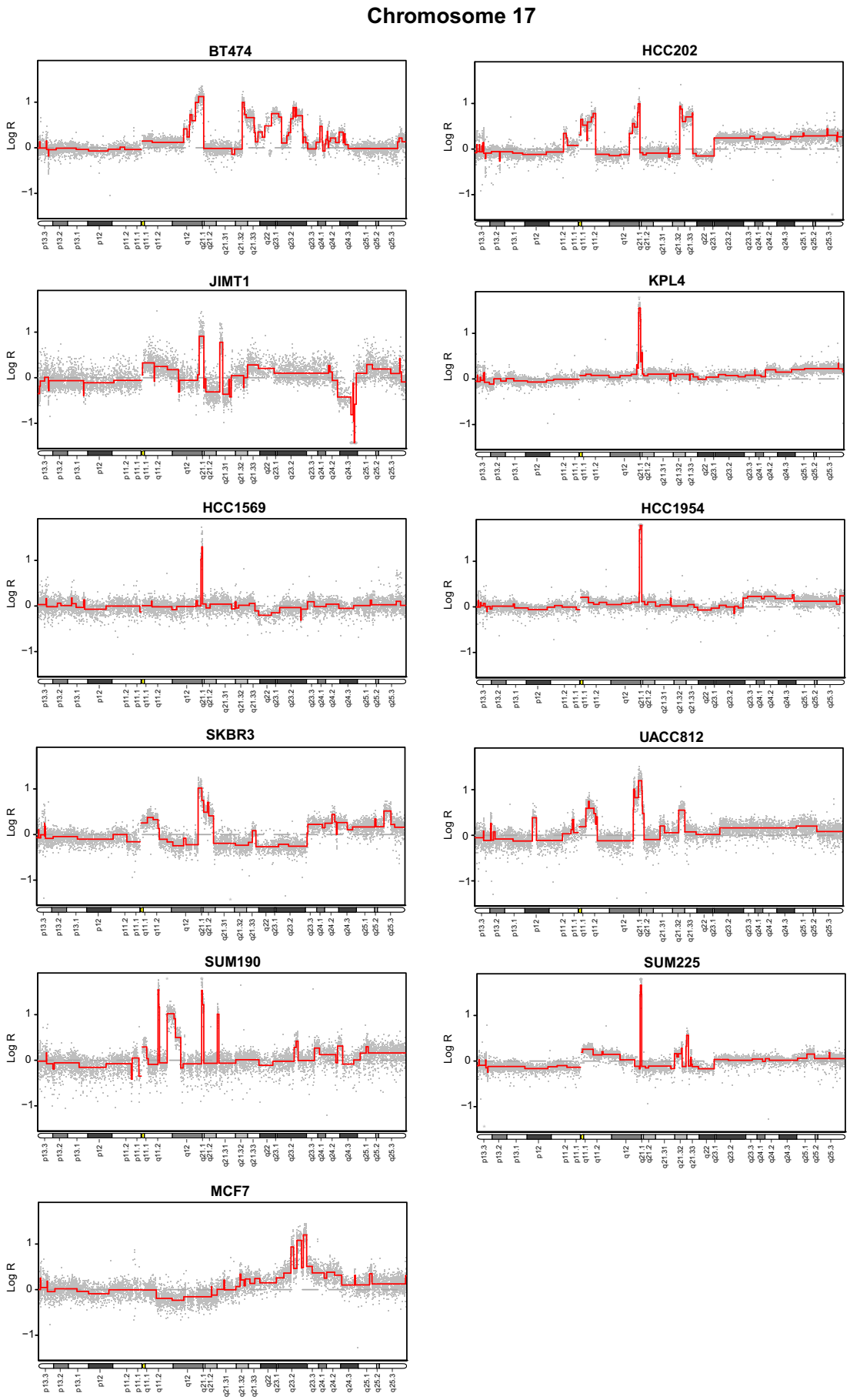
Chromosome 17
BT474
HCC202
JIMT1
KPL4
HCC1569
HCC1954
SKBR3
UACC812
SUM190
SUM225
MCF7
Log R
1
0
−1
p13.3 p13.2 p13.1 p12 p11.2 p11.1 q11.1 q11.2 q12 q21.1 q21.2 q21.31 q21.32 q21.33 q22 q23.1 q23.2 q23.3 q24.1 q24.2 q24.3 q25.1 q25.2 q25.3

Copy number changes from Agilent Human Genome CGH microarray 244K from 71 tumors were used to describe the *HER2* amplicon. Data from 54 breast tumors were retrieved from GEO (GSE17907) [2] and 17 additional tumors from (GSE32291, GSE20394) [3,4]. Only HER2 + samples were selected. Copy number changes for the 11 cell lines were submitted to GEO (GSE34236) [1].

aCGH analysis

We used copy number data from Agilent 244K arrays to study the size of the HER2 amplicon in detail in tumors and cell lines. Array-based CGH was carried out using Agilent Human Genome CGH 244K microarrays accordin'g to the protocol provided by the manufacturer (Agilent Technologies, Santa Clara, CA). Genomic DNA pooled from healthy female donors was used as reference in all hybridizations.

Briefly, 1 μg of digested and purified tumor and reference DNA was labeled with Cy5-dUTP and Cy3-dUTP (Perkin-Elmer, Wellesley, MA), respectively, in a random priming reaction using BioPrime DNA Labelling System (Invitrogen, Carlsbad, CA). Labeled tumor and reference samples were pooled and hybridized onto the arrays according to the protocol. After hybridization arrays were washed and scanned with a laser scanner (G2565 Scanner, Agilent Technologies). Feature Extraction software version 7.5.1 (Agilent Technologies) was used for the preprocessing of the signal intensities to obtain normalized intensity ratios. Data were log2 transformed. aCGH data were segmented using the Piecewise Constant Fit algorithm [5,6]. A penalty parameter, gamma, is introduced that can be tuned towards returning many or few breakpoints. The level of gamma controls the trade-off between high sensitivity and high specificity. A low gamma results in a higher number of breakpoints, whereas a high gamma results in fewer breakpoints and longer segments. Here, we chose a relatively low gamma ($\gamma = 15$), as we were interested in the breakpoints at gene levels to identify the size of the 17q12-21 amplicon in HER2 + breast cancer patients and cell lines. Segmented plots for chromosome 17 for the cell lines are shown in Fig. 1, and genome-wide segmented Log R values for cell lines and tumors can be found in Supplementary Tables 1–4. Using the ASCO guidelines for FISH for HER2 + (ratio of *ERBB2* gene signals to chromosome 17 signals of more than 2.2) [7], we defined a tumor with copy number gain >0.66 (log2 scale) to be HER2 +. All 71 tumors and 10 HER2 + cell lines included had copy number gain >0.9 for *HER2*.

The cell lines showed an amplification window similar to the tumors, with breakpoints mimicking those seen in the tumors. The average size of the amplicon seen in the cell lines was 1.47 Mbp (range: 0.37 Mbp–3.29 Mbp).

Proliferation assays

Five cell lines, two trastuzumab responsive; BT474 and SKBR3, two trastuzumab non-responsive; JIMT1 and KPL4, and MCF7 as a control, were subjected to small interfering RNA (siRNA) screening in 384 well plates with cell viability as an endpoint. SiRNAs were available for 23 of the 27 genes found amplified in over 60% of the tumors, and 2–4 siRNAs per gene were screened. Before screening, cell number was titrated for each cell line separately to ensure that cell proliferation remained in a linear-exponential range throughout the experiment. The siRNA oligonucleotides against the genes in the HER2 amplicon were obtained from Qiagen (2–4 siRNAs/gene according to availability). Validated siRNAs were preferred when available. AllStars Negative Control, vehicle control (transfection lipid), AllStars Cell Death Control and siRNAs against PLK1 and KIF11 were used as controls in all experiments (Qiagen, Hilden, Germany). For cell viability screening, siRNAs were robotically printed (Hamilton Bonaduz AG, Switzerland) in black, clear bottom 384-well plates (Greiner Bio-One, Germany) in a final concentration of 13 nM. SilentFect™ (BioRad Laboratories, CA) (BT474: 0.06 μl/well, KLP4: 0.05–0.06 μl/well, SKBR3: 0.05 μl/well, JIMT1: 0.056–0.07 μl/well, MCF7: 0.07–0.08 μl/well), DharmaFECT 1 (Thermo Fisher Scientific, MA, USA) (BT474: 0.06 μl/well) or HiPerfect (Qiagen, Hilden, Germany) (SKBR3: 0.02 μl/well) transfection agent was diluted in OptiMEM (Gibco Invitrogen, CA), incubated for 10 min at room temperature, and aliquoted into wells using Multidrop 384 Microplate Dispenser (Thermo Labsystems, Thermo Electron Corporation, MA). Plates were incubated for 1 h at RT. 35 μl of cell suspension with 1500–2000 cells per well depending on the cell line (BT474: 2000, SKBR3: 2000, KPL4: 1500, JIMT1: 1500 and MCF7: 2000 cells, respectively) was added to the plates and further incubated for 72 h at 37 °C, 5% CO_2. For cell viability assessment 25 μl of CellTiter-Glo assay reagent (Promega Corp, Madison, WI) was added directly to cell cultures and the plates were shaken for 30 min before signal detection. The EnVision Multilabel Plate Reader (PerkinElmer, Waltham, MA) was used for signal quantification. SiRNA screening data was normalized plate-wise for row/column effects using the formula *Well* / (*row median* × *column median*) × *plate median*. The results from replicate screens were merged (correlation between replicate screens: KPL4: 0.915, JIMT1: 0.915, BT474: 0.852, MCF7: 0.673, and SKBR3: 0.643) (Fig. 2). Method-based bias, like edge effects that are typically seen at the outer rows and columns of the plate, is taken care of by the chosen normalization. Co-silencing of genes and drug treatment experiments were done in three biological replicates. Two siRNAs per gene were pooled to a total of 13 nM and co-silencing with *HER2* was done by adding 13 nM of pooled *HER2* siRNA. The doubling of the siRNA amount was tested not to influence cell growth upfront. Transfection efficiency was optimized beforehand separately for each cell line by testing different transfection lipids and by titrating lipid amounts. SiRNA silencing efficiency was controlled by comparing decrease in cell viability of AllStars Negative Control siRNA (which does not kill the cells) to AllStars Cell Death control, KIF11 and PLK1 siRNAs which efficiently decrease the cell viability. In both SKBR3 and KPL4 cells a 80% decrease in cell viability was observed, indicating a similar knock-down in the responsive and the non-responsive cell lines. 10 μg/ml trastuzumab (Roche, Basal, Switzerland) and 100 nM lapatinib (Selleck Chemicals, TX, USA) were used in the validation assays. The screening data was validated by fluorescence-activated cell sorting (FACS) based cell cycle analysis, and knock-down with the individual siRNAs and their combination were validated for *HER2*, *GRB7*, *STARD3*, *PERLD1*, *PSMD3* and *PPP1R1B* genes using TaqMan quantitative RT-PCR (Applied Biosystems, CA, USA) in SKBR3 and KPL4 cells. Cells only and AllStars Negative Control siRNA (Qiagen) were used as controls.

Protein lysate microarrays

In order to look at several endpoints at the same time, additional siRNA screens in 384-well plates, with the same siRNA annotation as for the proliferation assay screens, were performed and samples were prepared for protein lysate microarrays (LMA) from BT474, SKBR3, JIMT1, KPL4, and MCF7 cell lines. The lysates were printed onto glass slides, and stained with HER2, cPARP, Ki67, phospho-AKT, phospho-p70-S6K, and p27 antibodies. Antibodies were selected based on their importance in breast cancer and HER2 + pathways. HER2 staining showed that *HER2* siRNA was equally effective in knocking down HER2 protein in all the cell lines. Protein lysate microarrays (LMA) [8–11] of the siRNA transfected cells from 384 well plates was performed 72 h after transfection (Fig. 3). Cells were lysed by adding 15 μl of lysis buffer (100 mM Tris, pH 8.0; 0.2% SDS; 25 mM DTT) directly onto the 384-well plates. Thereafter, the lysates were denatured in 95 °C

Fig. 1. PCF segmentation of chromosome 17 in HER2 + cell lines. The segmented value is shown in red, whereas the log ratio (Log R) probe values are shown in gray from chromosome 17 for the 11 cell lines. All HER2 + cell lines show a high amplification at 17q12-21.

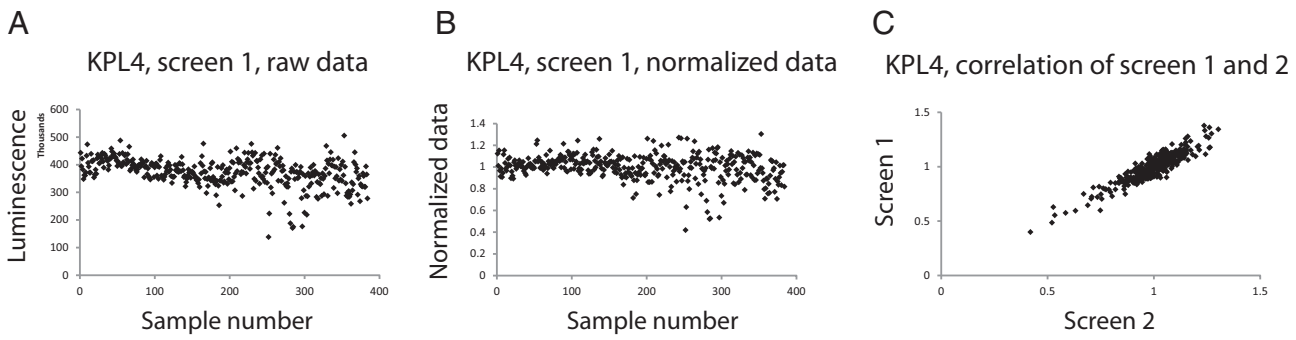

Fig. 2. Examples of CTG screening data distribution and normalization. A. Raw CellTiterGlo results from KPL4 screen 1. B. Plate-wise normalized KPL4 screening data corrected for row/column effects. C. Correlation of KPL4 screens 1 and 2 ($R^2 = 0.915$).

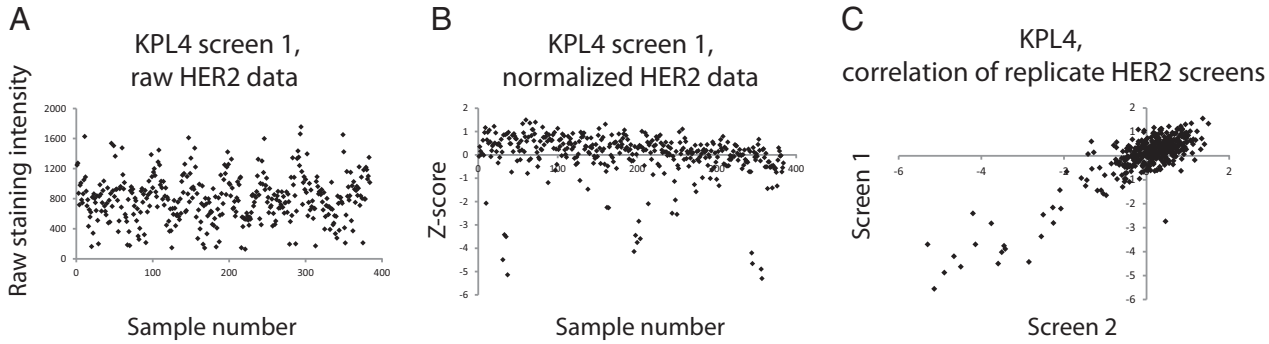

Fig. 3. Examples of lysate microarray (LMA) screening data distribution and normalization. A. Raw LMA results from KPL4 screen 1 lysates stained with HER2 antibody. B. Z-score normalized KPL4 LMA screening data for HER2 staining. Samples were expressed as a ratio to total protein staining from the same spot (Sypro Ruby staining), log2 transformed, and Z-score standardized within the screen. C. Correlation of KPL4 HER2 replicate screens. Correlation coefficient (R^2) between the screens was 0.808.

for 15 min, and stored at −20 °C prior printing. Microarray printer QArray2 with 32 split pins (Molecular Devices Ltd, UK) was used to print arrays on nitrocellulose-coated microarray slides (Whatman Inc., Florham Park, NJ, USA). Four drops of the cell lysates were stamped to each spot position, in total 3072 spots (~300 μm in diameter) per slide. To normalize the signal to total protein amount, the arrays were first fixed by immersion in 7% acetic acid, and 10% methanol for 15 min, washed, and stained with SYPRO® Ruby protein Blot stain (Invitrogen Inc., Carlsbad, CA) for 15 min, washed, dried, and scanned with a Tecan LS400 (Tecan Inc., Durham, NC, USA) microarray scanner at 488/670 nm to detect total protein. Each slide was then blocked with near-infrared blocking buffer (Rockland Immunochemicals Inc., Gilbertsville, PA) for 1 h at room temperature. The expression of cPARP (Abcam ab32064) (1:500), Ki67 (Dako M7240) (1:1000), HER2 (DakoA0485) (1:1000), phospho-Akt (Cell Signaling Technology, Beverly, MA, USA) (1:200), phospho-p70-S6K (Cell Signaling Technology) (1:200) and p27 (Cell Signaling Technology) (1:200) were detected by staining the slides with antibodies overnight at 4 ºC in a humidified chamber to prevent the slides from drying. The slides were washed with TBS + 0.1% Tween-20 three times for 5 min and exposed to Alexa Fluor 680-tagged and IRDye 800CW tagged secondary antibodies (Invitrogen Inc., Carlsbad, CA, USA and Rockland Immunochemicals) at a dilution of 1:5000 for 45 min. After washing off excess secondary antibody the arrays were scanned using an Odyssey LI-COR IR-scanner (LI-COR Biosciences, Lincoln, NE, USA) at 700 and 800 nm to detect the specific antibody signals. Array-Pro Analyzer microarray analysis software (Median Cybernetics Inc., Bethesda, MD, USA) was used to measure median pixel intensities of each spot and the slide background from each channel. After background subtraction, spot intensity was centered cell line-wise to the median intensity of pin to correct pin-to-pin variation on the array. In the normalization procedure implemented in analysis software, each median centered spot intensity was divided by the value of the point in the biquadratic normalization surface at that coordinate to eliminate regional dye bias on slide. Net signal from antibody staining was acquired after normalization to the respective Sypro signal. Finally, normalized values were log2 transformed and converted to Z-scores.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2014.06.025.

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