1. ARRAY DESIGN DESCRIPTION

A brief description of the array design, feature location, information on the cDNA collection and the spotting protocols can be found on the producer´s website at
http://www.microarray.org/sfgf/jsp/home.jsp

Protocols for the pre-hybridisation procedures (post-processing) of the arrays can be downloaded from our website at http://www.microarray.at

2. EXPERIMENT DESCRIPTION

2.1. Experimental design

2.1.1.Laboratory, authors, contact

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2.1.2. Type of experiment

We obtained gene-expression profiles of HK-2 cells (a human proximal tubular cell line) incubated with 50 nM of bortezomib, and we compared these gene expression profiles to unstimulated cells (controls).

2.1.3. Experiment factors

Human proximal tubular cells (hPTC) HK-2 (human kidney-2) were cultured in Keratinocyte-Serum Free Medium (KSFM) containing 10 % fetal bovine serum (FBS), 5 ng/ml recombinant epidermal growth factor (rEGF), 0.05 mg/ml bovine pituitary extract (BPE), 100 units/ml penicillin, and 100 µg/ml streptomycin. The cells were grown at 37° C in a humidified 5 % CO_2 atmosphere and, after growth to subconfluent state, cells were washed once, made quiescent by incubation in serum- and supplement-free medium for 48 hours, and then used for experiments. Stimulations with 50 nM bortezomib were performed in the absence of serum and any other growth supplements. Four time points were examined (4, 8, 16, 32 hours).

2.1.4. Microarray hybdridizations

HK-2 RNA from each time point from stimulated and control experiments were co-hybridized to one array. Bortezomib-stimulated samples were labelled with Cy-5 (red) and control samples were labelled with Cy-3 (green). Microarray hybridizations were performed in biological duplicates (i.e. in total 8 array hybridizations).

2.1.5. Reference

RNA from the unstimulated control experiments served as reference RNA.

2.1.6. Quality control

To test for reproducibility we calculated the intra-array variability of the duplicate arrays. In addition SMD's array quality tools (Sector ANOVA and Printing-plate ANOVA) were used to calculate the quality of the hybridizations. Duplicate arrays were combined before statistical analysis.

2.2. Samples used, extract preperation, amplification and labelling

2.2.1. Bio-source properties

Organism: Homo sapiens. Cell line: HK-2 (human kidney-2) cell line is a immortalized human proximal tubular cell line. Further information can be obtained from the ATCC-webpage (ATCC number: CRL-2190) at www.atcc.org.

2.2.2. Biomaterial manipulations, amplification and labelling protocol

Total cellular RNA was extracted using TRI Reagent (molecular Research Center, Cincinnati, OH). RNA quantity was estimated by spectrophotometric analysis. RNA was reverse transcribed using CyScribe cDNA Post Labelling Kit (GE Healthcare life sciences, Piscataway, NJ, USA). 20 ug RNA from bortezomib-stimulated cells was labelled with Cy-5 (red) and 20 ug RNA from unstimulated control experiments was labelled with Cy-3 (green). For each of the four different time points (4, 8, 16, and 32 hours) a co-hybridization of Cy-5- and Cy-3-labelled reverse transcribed RNA was performed in biological duplicates. The protocols for RNA labelling, hybridization and washing of microarrays can be downloaded from our website (http://www.microarray.at).

2.3. Hybridization procedures and parameters

Date	**Array-ID** batch, no. (Stanford)	**Experiment** **Name** user - defined	**optical control** 1=ok,2=moderate, 3=bad	**SMD-Array-color-tool** Sector-ANOVA R-squared values	 Printing-Plate-ANOVA R-squared values
01.03.07	HOL042	B4h 1 vs C4h 1	2	0,088	0,043
	HOL055	B8h 1 vs C8h 1	2	0,140	0,725
	HOL041	B16h 1 vs C16h 1	1-2	0,144	0,036
	HOL048	B32h 1 vs C32h 1	1-2	0,130	0,060
13.03.07	HOL054	B4h 2 vs C4h 2	1-2	0,045	0,263
	HOL046	B8h 2 vs C8h 2	1	0,071	0,115
	HOL057	B16h 2 vs C16h 2	1-2	0,028	0,134
	HOL043	B32h 2 vs C32h 2	1-2	0,029	0,058

2.4. Measurement data and specification of data processing

2.4.1. Raw data description

Scan hardware: GenePix 4000 B (Axon Instruments, Union City, CA)

Scan software: GenePix Pro 4.1 (Axon Instruments, Union City, CA)

Raw data can be found in the data section of our website (http://www.microarray.at).

2.4.2. Background subtraction

The “local feature” algorithm was used for individual background subtraction.

2.4.3. Image analysis and quantitation

Image gridding and calculation of spot intensity was performed with GenePix Pro 4.1 software.

2.4.4. Normalized and summarized data

Normalization was done through the default computed normalization by SMD (at http://genomewww5.stanford.edu/help/results_normalization.shtml). For data retrieval the log2 red/green normalized ratio was used. Signals with a signal intensity in the red channel (patient sample) < 1.5 fold over background were excluded from analysis. 32083 of the 36293 retrieved spots were mapped to HGNC Gene Symbols. The other 4210 spots represented ESTs and hypothetical proteins. Functional gene categorization was based on gene ontology terms which were assigned to all annotated genes on the chip using the GO package from the Bioconductor R module. The set of genes associated with apoptosis was extracted and gene expression values of those genes further analyzed. Values of different clones assigned to a single gene cluster were averaged and genes showing a fold-change of at least two in either direction in both replicate arrays at any time point were defined as differentially expressed.