HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Network 4: From Contaminant Molecules to Cellular Response

Subproject 4: Post-transcriptional regulation

Proteome expression analysis of Benzo(a)pyrene exposed cells

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The carcinogenic contaminant Benzo(a)pyrene (BaP) belongs to the substance class of polycyclic aromatic hydrocarbons (PAH), that are proven to cause diseases like lung cancer. For the first time, a BaP- time-and concentration dependent protein expression analysis with the DIGE (Difference Gel Electrophoresis)-technology was performed. Hepa 1c1c7-cells were exposed to 5 µM (toxic) and 50 nM BaP (subtoxic) and DMSO (control) for 2, 4, 12 and 24h to differentiate between an acute and chronic response. Our results show that BaP alters proteins involved in various pathways. For the confirmation and expansion of the DIGE-results, SILAC (stable incooperation of labeled amino acids in cell culture) was established. The protein expression data will then be correlated with gene expression data within the network. Thus, reproducible quantitative protein and gene expression data showing the effects of BaP on murine Hepa1c1c7-cells will be generated for the later model development predicting the effects of structurally related chemicals on cellular systems.

Methods

Experimental design for protein expression analysis

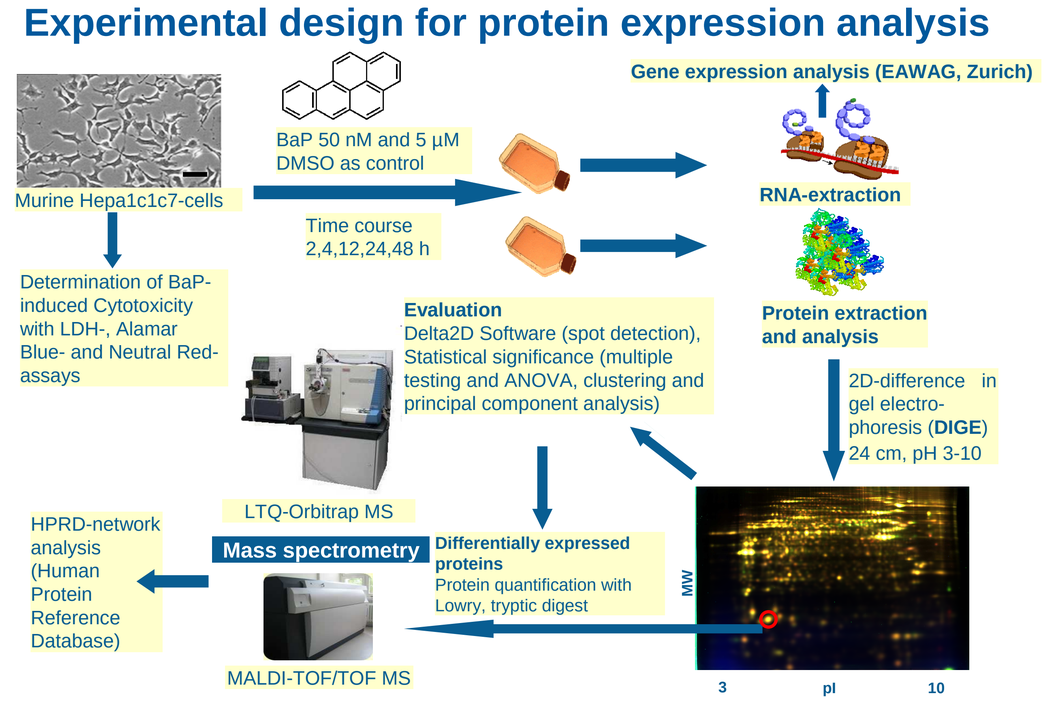

Figure 1: Experimental workflow of the BaP- time- and concentration dependent protein expression analysis of murine Hepa1c1c7-cells using the DIGE-technology and HPRD network analysis.

Establishment of SILAC

(stable isotope labeling by amino acids in cell culture)

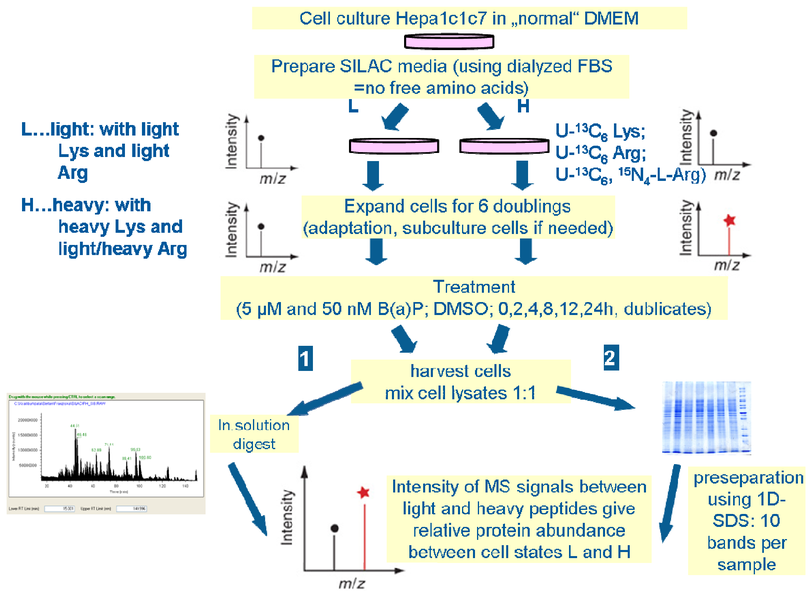

Figure 2: Experimental setup of the performed SILAC-experiment to detect more BaP-dependent differentially expressed proteins and to validate the DIGE-results and correlate protein expression data with gene expression data within the network 4. Furthermore, more timepoints could be integrated for further modelling analysis in Subproject 5.

Protein/mRNA-correlation

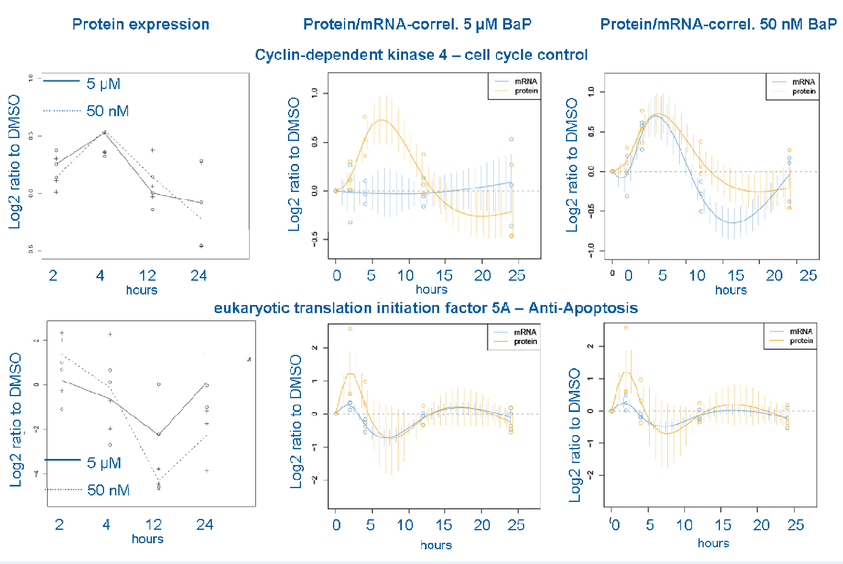

Figure 3: The correlation between the BaP-altered time- and concentration dependent expression curves of identified proteins and the corresponding mRNA-abundance (Subproject 3) revealed 70 % simularity.

Results

Applying the DIGE-technology, we detected 1227 spots of which 120 were differentially regulated over time and 95 were identified. 77 proteins respond before 2 hours of BaP-exposure, thus indicating an immediate response to BaP on the protein level. BaP alters the expression of proteins involved in oxidative stress (Fig. 4), protein degradation, cytoskeleton remodelling, growth signaling pathways (Fig. 5), cell cycle control (Fig. 6) and others (data not shown).

BaP: induces oxidative stress response

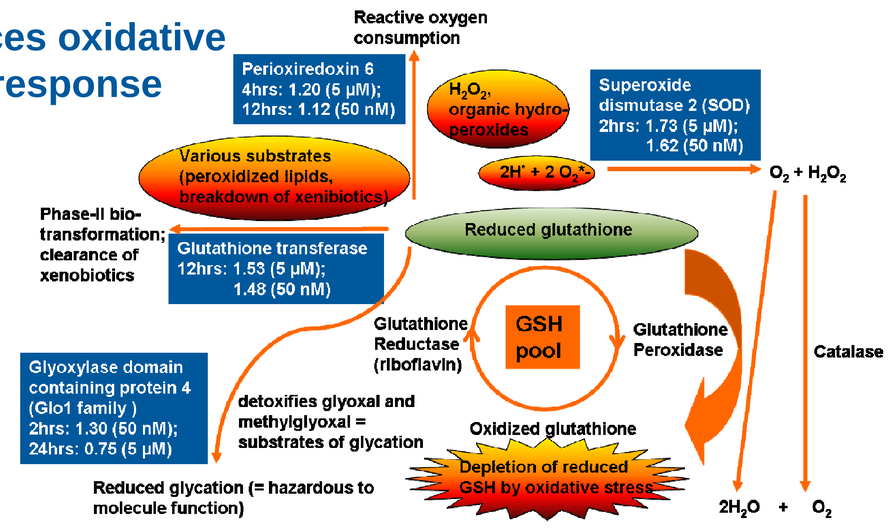

Figure 4: BaP alters the expression of proteins involved in reducing the cellular effects of oxidative stress.

HPRD network analysis

(Human Protein Reference Database)

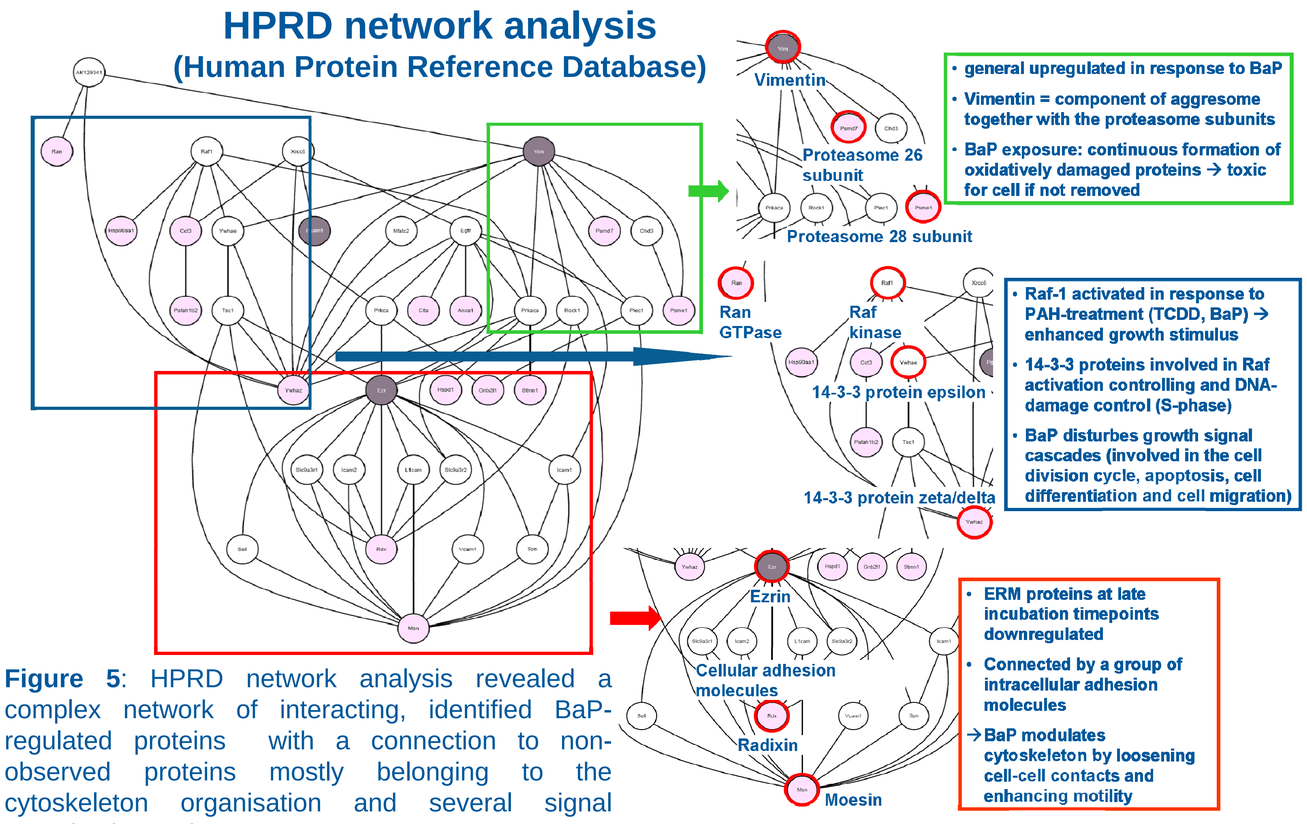

Figure 5: HPRD network analysis revealed a complex network of interacting, identified BaP-regulated proteins with a connection to non-observed proteins mostly belonging to the cytoskeleton organisation and several signal transduction pathways.

BaP: Influence on cell cycle control

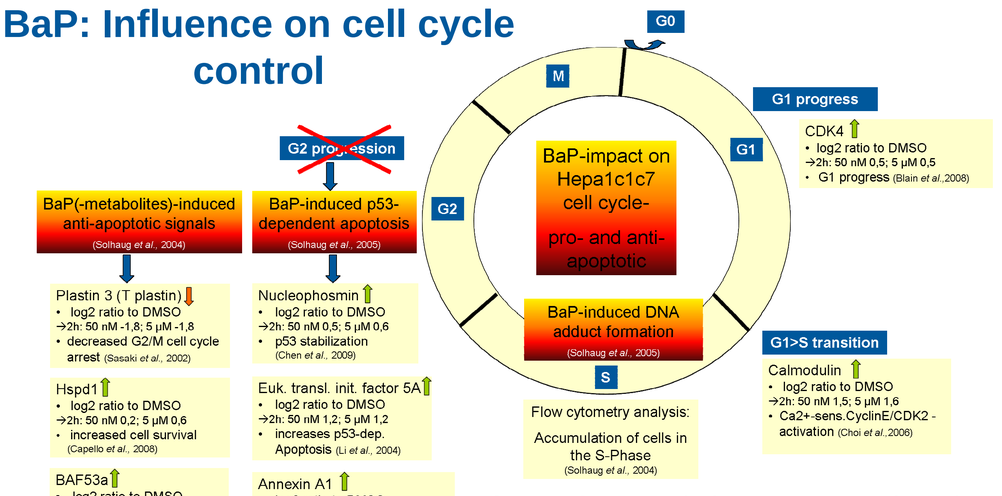

Figure 6: BaP induces pro- and anti-apoptotic stimuli resulting in a BaP-induced G1-progression, an accumulation of cells in the S-Phase and consequently inhibited G2-progression.

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