

Analysis of the mitochondrial subproteome of the human cell line AGE1.HN – a contribution to a Systems Biology approach

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Introduction

In Systems Biology approaches a good amount of reliable data is essential for effective modelling of distinct biological processes. Hence the focus of the SysLogics-Project was on modelling of the central metabolism using different functional genomic techniques. For displaying the involved proteins, it is crucial to enrich them using subproteomic fractions. As many enzymes being involved in central metabolism are located in the mitochondria (see fig. 1), we investigated the expression dynamics of mitochondrial proteins during batch cultivations of human AGE1.HN cells (ProBioGen, Berlin, Germany).

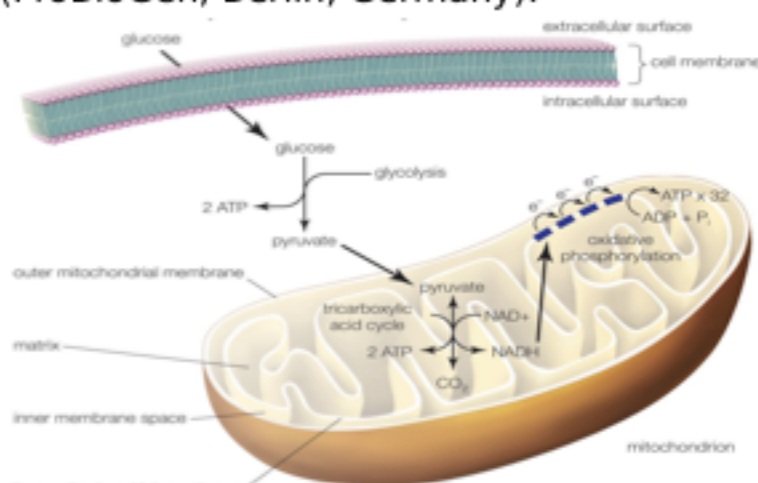


Fig. 1: Location of central metabolism pathways in eukaryotic cells

Methods

Cultivation and isolation of mitochondria:

Cultivations were performed as batch-processes with chemically-defined and animal-component-free media 42-MAX-UB (Teutocell, Bielefeld, Germany) with addition of 5 mM glutamine. The processes were performed in a 20 L-stainless steel vessel (Sartorius-Stedim, Germany). After disintegration of cells by addition of glass beads (0,1 mm diameter) and vortexing, the isolation of mitochondria was performed with sucrose density-centrifugation in an ultracentrifuge (Beckman Coulter, Krefeld, Germany). Protein extraction from mitochondria was performed with lysis-buffer, which contains Tris-HCl, NaCl, EDTA, SDS, PMSF and NP40.

Proteomics:

The first dimension of 2D-electrophoresis was performed with Ettan IPGPhor3-system*, using pH 3-11 (NL) IPG-strips*. For generating the mitochondrial master-map, six gels with 0.45 mg protein extract were prepared. For DIGE-approaches 0.15 mg were applied, each sample with four replicates. Second dimension was accomplished with Ettan Dalt six Electrophoresis System*. 2DE-gels with DIGE-staining were processed with Ettan Dige Imager*. Software evaluation was implemented with Delta2D (Decodon, Germany). After isolation and tryptic digest protein-identification was performed with MS/MS using MALDI-ToF/ToF (UltrafleXtreme, Bruker, Germany), followed by database-searching. *(GE Healthcare, Sweden)

Results

Two standardised batch cultivations in a 20 L-stirred tank stainless steel vessel were carried out with daily sampling for metabolomics, transcriptomics, metabolic flux and proteomics, in order to generate reliable and comparable samples. Batch cultivation in 20 L-scale of AGE1.HN.AAT shows cell densities and duration as expected. Glucose- and lactate-concentrations developed also as estimated (see fig. 2a and 2b).

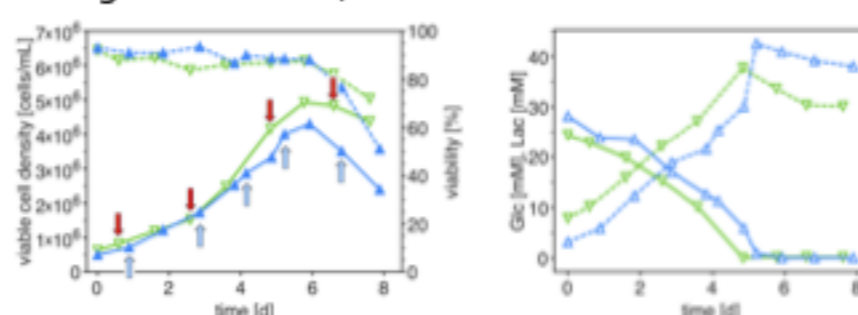


Fig. 2a (left): VCD and viability during batch process 1 (green) and 2 (blue), arrows show particular sample points. **Fig. 2b** (right): Concentrations of glucose (solid) and lactate (dashed) during batch process 1 (green) and 2 (blue).

Isolation of mitochondria was successfully controlled by staining with Rhodamine 123, a mitochondria-specific fluorescent dye.

2DE-gels of mitochondrial fraction led to 519 separated spots. 280 proteins could be reliably identified (see fig. 3), out of which more than 50 % are exclusively mitochondrial.

This 2D-proteome map is our basis for approaches of analysing different protein expression in mitochondria.

Subproteomic DIGE-analysis of mitochondrial proteins resulted in 114 proteins that were significantly differently expressed in the first and 206 proteins in the second process during cultivation time ($p < 0.05$, factorial Anova). More than 40 proteins were found to be significantly regulated in both experiments.

DIGE-approaches of mitochondrial fraction of both batch cultivations led to expression profiles of identified proteins. Four examples of typical proteins of TCA and respiratory chain are shown below in fig. 4.

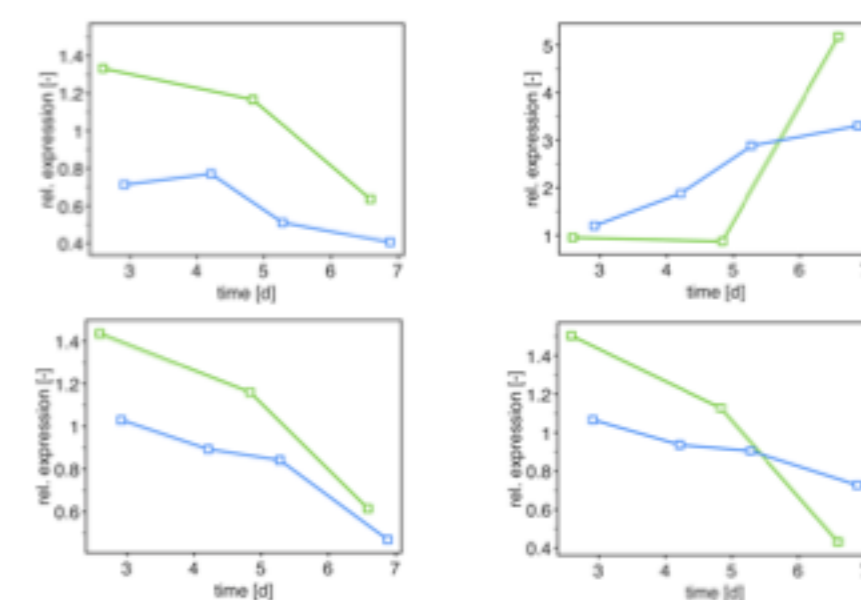


Fig. 4: Examples of expression profiles of TCA and respiratory chain enzymes. Batch process 1: green, batch process 2: blue line. Expression normalised on sample point 1.

Fig. 4a (upper left): Pyruvate dehydrogenase E1b

Fig. 4b (upper right): L-lactate dehydrogenase B chain

Fig. 4c (lower left): Dihydropyrimidinase 5-succinyltransferase

Fig. 4d (lower right): Electron transfer flavoprotein alpha

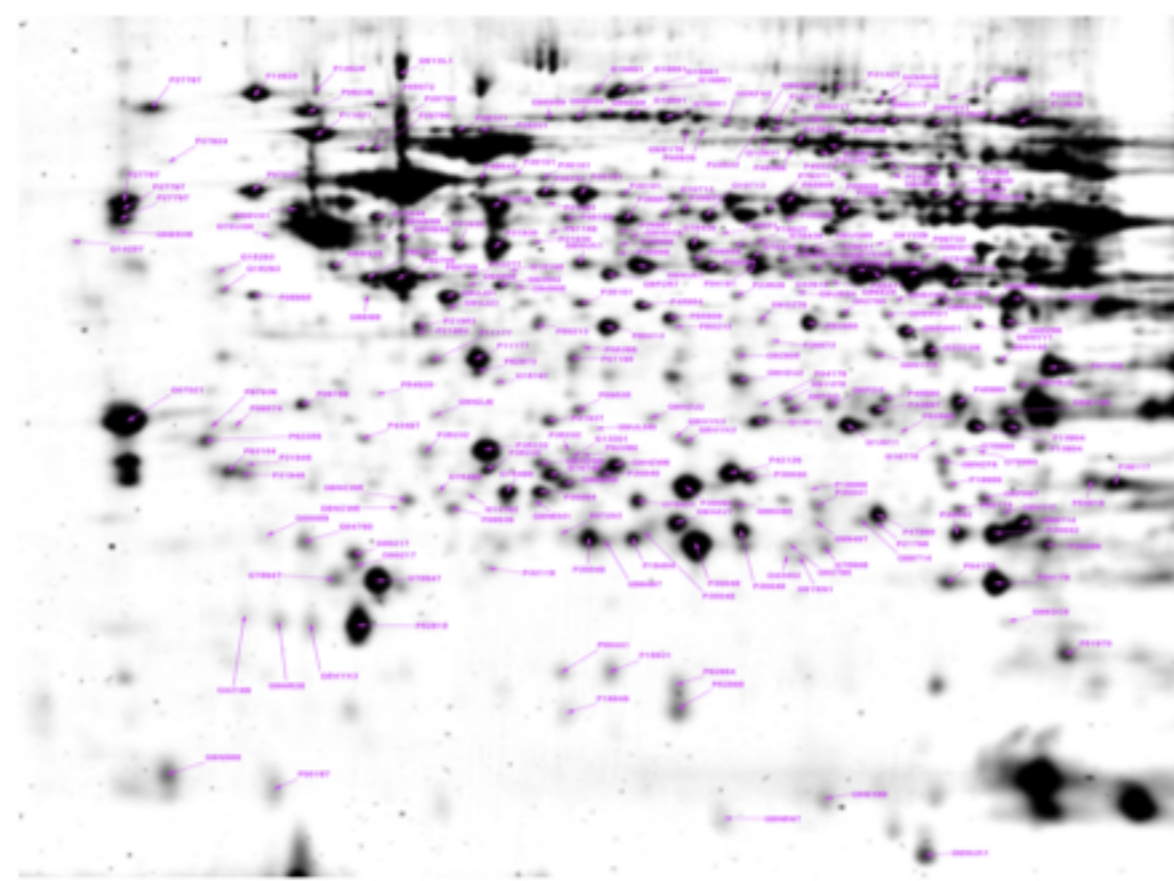


Fig 3: 2DE-map of mitochondrial proteins. pH ranges from pH 3 (left) to pH 10 (right) nonlinear. Molecular weight ranges from 150 kDa to 10 kDa (top-down). Identified proteins are labelled with uniprot-accessions.

With our 2DE-approach it was possible to observe important biological processes, as for instance:

- tricarboxylic acid cycle
- respiratory chain
- anti-apoptosis and apoptosis
- signal transduction
- chaperone/protein folding and processing
- fatty acid/lipid-metabolism
- amino acid-degradation
- inhibition of DNA-synthesis
- ketone-metabolism
- protein-biosynthesis
- redox-regulation
- ubiquinone-biosynthesis

Hence, we see this data as a valuable contribution to the Systems Biology approach for modelling central metabolism events.

Conclusions

- Reproducible methods for isolation of mitochondrial proteins from cultured cells for further analysis, such as proteomic 2DE-approaches, have been established.
- DIGE-analysis of mitochondrial subproteome of AGE1.HN.AAT cells result in the identification of more than 280 identified proteins, of which more than 40 are regulated during batch cultivation.
- Proteomics data in conjunction with the other "omics"-results offer a promising basis for characterisation of central metabolism during Systems Biology approaches (see poster 5.43).