Bioreactor cultivation of CHO DP-12 cells under sodium butyrate treatment – comparative transcriptome analysis with CHO cDNA microarrays

Sandra Klausing*, Oliver Krämer†, Thomas Noll

From 22nd European Society for Animal Cell Technology (ESACT) Meeting on Cell Based Technologies Vienna, Austria. 15-18 May 2011

Background
Sodium butyrate (NaBu) is not only known to inhibit proliferation but also to increase the specific productivity in cultivation of Chinese hamster ovary (CHO) cells – the most commonly used mammalian cell line for pharmaceutical protein production [2]. So far, little is known about the underlying mechanisms and genes that are affected by butyrate treatment. Besides the proteomic approach to unravel proteins involved in the processes, the analysis of transcriptomes presents another promising method. Here we show an application of our CHO cDNA microarray to identify genes associated with increased productivity during cultivation of CHO cells under sodium butyrate treatment.

Materials and methods
Four batch cultivations of CHO DP-12 cells (clone # 1934, ATCC CRL-12445) were performed in 2 L bioreactor systems under pO2- and pH-controlled conditions. In the exponential growth phase, 67 hours after inoculation, 2 mM sodium butyrate was added to three processes. The fourth was left untreated to function as control culture. Samples were taken before and then repeatedly after the addition of butyrate. RNA was isolated from cell pellets of 5·10⁶ cells using TRIzol® Reagent (Invitrogen). For subsequent cDNA labeling, the Agilent Low-Input QuickAmp Labeling Kit (Agilent Technologies) was used. The custom designed 2 x 105 k cDNA microarray (Agilent Technologies) was spotted with 94,580 probes designed from CHO cDNA sequenced in-house. 38,310 of 41,039 sequenced contigs were used for the microarray, each covered by 2-4 probes [3]. Data analysis was done with ArrayLims, EMMA, and SAMS, three CeBiTec based software tools [4]. The raw data gathered by the microarray experiments were processed by standard Agilent background normalization and subsequent lowess normalization.

Results
The control culture reached a maximum viable cell density of 1·10⁷ cells/mL while NaBu treated cells reached a plateau at about 6·10⁶ cells/mL and retained a viability above 90 % four days longer than untreated cells (Figure 1A). The three biological replicates of NaBu cultures yielded results with similar general trends. The maximum antibody concentration of the control culture was 110 mg/L whereas cells treated with NaBu reached a maximum of 175 mg/L antibody. 72 hours after addition of NaBu the specific antibody production rate was increased by a factor of 3.6 (NaBu culture: 4.5 pg/(cell·d)) compared to control culture (1.2 pg/(cell·d)).

Of this time point, samples were analyzed in microarray experiments. A significance test with FDR control (α=0.05) was carried out for the four technical replicates (including two dye-swaps) of the microarray. For analysis, the following filtering settings were chosen to identify differentially expressed genes: adjusted p-value ≤ 0.05, log-ratio < -1 or > 1 (equals fold change < -2 or > 2) and log-intensity ≥ 6 (equals ≥ 64 raw intensity). From a total of 1461 genes found to be differentially expressed under NaBu treatment, 771 genes were

* Correspondence: skl@zellkult.techfak.uni-bielefeld.de
† Contributed equally
Institute of Cell Culture Technology, Bielefeld University, 33615 Bielefeld, Germany

© 2011 Klausing et al; licensee BioMed Central Ltd. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
upregulated and 690 genes were downregulated (derived from EC numbers in KEGG pathways, Figure 1B). Many differentially expressed genes from pathways involved in carbohydrate, lipid, amino acid and glycan metabolism are upregulated which is most likely linked to higher productivity. A large portion of genes from pathways associated with cell growth and death are downregulated and most of these genes originate from cell cycle processes. This correlates with reports of cell cycle arrest under NaBu treatment [1]. Some examples of regulated genes are shown in Table 1.

Conclusions

Microarray analysis revealed a high number of regulated genes under sodium butyrate treatment in pathways like carbohydrate metabolism, cell cycle and signal transduction. Some of the regulated genes are promising targets for overexpression or knockdown/knockout experiments and we will further investigate the knockdown effect of selected genes using a siRNA approach in CHO cells. Our in-house microarray is suitable for further transcriptomic analysis of CHO cells under various conditions.

### Table 1 Fold change of selected genes from microarray analysis.

| KEGG pathway category | Gene symbol | Description | Mean fold change in NaBu culture compared to control culture | # of probes |
|-----------------------|-------------|-------------|-------------------------------------------------------------|-------------|
| **Transcription & Translation** | CA150 | Transcription factor CA150b | -3.31 (↓) | 3 |
| | Ccdc12 | Coiled-coil domain containing 12 | -2.01 (↓) | 1 |
| | Y14 | RNA-binding protein 8A | 2.14 (↑) | 2 |
| **Cell Cycle** | Pkmyt1 | Protein kinase, membrane associated tyrosine/threonine 1 | -2.04 (↓) | 2 |
| | c-Myc | Myc proto-oncogene protein | -3.44 (↓) | 3 |
| | Ink4c | Cdkn2c cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4) | 3.04 (↑) | 2 |
| | CycD | Cyclin D1 (Ccdn1) | 2.49 (↑) | 1 |
| **Apoptosis** | Pdcd4 | Programmed cell death 4 | 3.89 (↑) | 2 |
| | Casp6 | Caspase 6 | 3.06 (↑) | 3 |
| | PI3K | Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 | 2.44 (↑) | 2 |
Acknowledgements

We would like to thank E. Schulte-Berndt (Institute for Genome Research and Systems Biology, CeBiTec, Bielefeld) for help with microarray hybridization and O. Rupp (Bioinformatics Resource Facility, CeBiTec, Bielefeld) for help with microarray data analysis.

Published: 22 November 2011

References

1. Kumar N, Gammell P, Clynes M: Proliferation control strategies to improve productivity and survival during CHO based production culture: A summary of recent methods employed and the effects of proliferation control in product secreting CHO cell lines. Cytotechnology 2007, 53(1-3):33-46.

2. Jayapal K, Wlaschin K, Yap M, Hu W-S: Recombinant protein therapeutics from CHO cells - 20 years and counting. Chem. Eng. Prog. 2007, 103(10):40-47.

3. Becker J, Hackl M, Jakobs T, Rupp O, Timmermann C, Szczepanowski R, Borth N, Goessmann A, Grillari J, Noll T, Pühler A, Tauch A, Brinkrolf K: Next-Generation Sequencing of the CHO cell transcriptome. BMC Proceedings 2011, 5(Suppl 8):P6.

4. Center for Biotechnology, IFB - Institute for Bioinformatics: Computational Genomics, Software.[http://www.cebitec.uni-bielefeld.de/cebitec/computational-genomics/software.html].

doi:10.1186/1753-6561-5-S8-P98

Cite this article as: Klausing et al.: Bioreactor cultivation of CHO DP-12 cells under sodium butyrate treatment – comparative transcriptome analysis with CHO cDNA microarrays. BMC Proceedings 2011 5(Suppl 8): P98.