Transcription Profiling of Different Recombinant CHO Clones Based on Cross-species Microarray Analysis

Evelyn Trummer[1,3], Wolfgang Ernst[1,3], Dethardt Müller[1,3], Kornelia Schriebl[1,3], Christine Lattenmayer[1,3], Karola Vorauer-Uhl[1,3], Renate Kunert[1,3], Hermann Katinger [1,2] and Friedemann Hesse[1,3]

[1] Institute of Applied Microbiology, Muthgasse 18, 1190 Vienna, Austria [2] Polymun Scientific, Immunbiologische Forschung, Nussdorfer Lände 11, 1190 Vienna, Austria [3] Austrian Center of Biopharmaceutical Technology, Muthgasse 18, 1190 Vienna, Austria

Abstract: Cross-species analysis was used to monitor the global transcription profile of CHO production cell lines. Mouse and rat-oligoarrays served to compare gene expression signatures of different recombinant CHO clones as well as different conditions in order to point out correlations between the observed phenotype and the transcriptome.

Key words: CHO cells, clonal variability, microarray analysis, cross-species hybridisation, mouse-oligo-DNA array

1. INTRODUCTION

Since different CHO-clones have been shown to exhibit a large degree of variability regarding recombinant protein production performance, the development of more efficient screening and selection methods is obligatory. Therefore, we combined the phenotypic characterisation of cell cultures with transcription profile analysis using microarray technology to understand the cellular mechanisms that control the phenotype of animal cells and to identify new potential selection parameters.

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2. MATERIAL AND METHODS

Experiments were carried out with clones of a serum-free adapted recombinant CHO-dhfr$^-$ cell line producing the fusion protein Epo-Fc. Different CHO clones were cultivated in the multireactor system Sixfors (Infors) during clone development in order to investigate their applicability for technological relevant production systems. The bioreactor cultivation was performed in repeated batch mode (5 batches), whereby total RNA of cells was isolated at the end of each batch. Preparation of RNA samples, reverse transcription, fluorescent dye labeling, hybridization and further array processing was done using the Agilent Low RNA input linear amplification kit. The fluorescently labeled targets were hybridized to mouse and rat 22k Oligo-60mer-Microarrays (Agilent). Agilent´s feature extraction software and Gene Spring 6.2 were used for data analysis. Differentially expressed genes were classified by an experimental threshold, which was determined by self versus self hybridization of equimolar mixtures and alternating dye labeling of all RNAs used. Based on a 99% confidence interval of the dataset, the following thresholds were determined: Mouse (>1.47/<0.64); Rat (>1.33/<0.64). The datasets were further filtered on confidence of replicate data. Changes in gene expression with $p<0.05$ was used as a cut-off to produce preliminary lists of genes. The gene lists were linked to GeneBank accession numbers, GO-terms, Biocarta and KEGG using MASI (Insilico Bioinformatics).

3. RESULTS AND DISCUSSION

3.1 Experiment 1: Phenotypic characterisation and transcriptional profiling of one clone at two different conditions

In the course of bioreactor cultivation the clone 2G4 showed altered growth characteristics, as the specific growth rate was reduced from 0.5 (d^{-1}) (1st and 2nd batch) to 0.37 (d^{-1}) (3rd, 4th and 5th batch). In the fifth batch reduced growth was followed by the induction of cell death, as viability declined from 90% to 65%. Specific recombinant protein production rates and degree of product sialylation remained constant in the course of bioreactor cultivation. RNA samples from the 2nd and 5th batch were analyzed for differential gene expression using microarrays. The results obtained from GO-and pathway analysis indicated a strong activation of MAPK-signaling pathway playing an important role in transcriptional activation, cell cycle arrest and apoptosis. These findings were underlined by the induction of genes like Gadd45a (3.08), CHOP (1.71), ATF-2 (1.66),

Tgfbr (1.66), Hmg-14 (1.60) and Mef2C (1.52) in the 5th batch. The overexpression of GADD45 genes is known to result in substantial activation of p38MAP Kinase and apoptosis (Kyriakis *et al*., 2001), whereas CHOP leads to growth arrest and apoptosis and is known to be responsive to perturbations that culminate in the induction of ER stress (Zinsner *et al*. 1998). The upregulation of several stress responsive genes and UPR signaling mediators was observed, as follows: (1) BIP (1.68) and several co-chaperones (Dnajb9 (2.0), Sec 63 (1.78) and Dnajc1 (1.6)). (2) CHOP (1.71), ATF-2 (1.66), ATF-4 (2.0), GCN-2 (1.79). (3) The ubiquitin dependent protein catabolism and members of the proteasome were tightly regulated (Psma6, Psma4, Psmc2, Pmsd5). (4) The induction of phospholipid biosynthesis could be a consequence of UPR activation, as this correlates with the presence of an elaborate ER [Sriburi *et al*., 2004]. To verify data obtained with microarrays, a quantitative RT-PCR analysis of four selected genes from clone 2G4 at two different stages was performed. Relative quantification was done by calculating the ratios of the target gene copy number to that of the reference house keeping gene beta-actin. Out of four selected genes, the expression level of three genes could be verified. qRT-PCR results showed a remarkable correlation to microarray data: Gadd45a (3.1-3.08); BIP (1.58-1.68); Gstm5 (0.99-0.96) (-fold induction qRT-PCR-microarray).

3.2 Experiment 2: Phenotypic characterisation and transcriptional profiling of two different clones

Transcription profiles of two clones with different production rates and post-translational modification capacities were analyzed to gain insight into their recombinant protein production performance. Clone 2C10 showed ten-fold higher specific Epo-Fc production rates than clone 8C6. The lowproducer 8C6, however, held potential for higher levels of terminal sialylation, as the secreted product contained 50% more sialic acids compared to clone 2C10. Genes involved in glycerolipid and sterol biosynthesis were highly represented in clone 8C6. The upregulation of genes involved in dolichol synthesis (Hmgcr, Hmgcs, Idi-1) could be an indictator for the higher product quality (sialylation of Epo-Fc) of the low producer 8C6. The concentration of dolichol phosphate, the immediate precursor for synthesis of the lipid linked oilgosaccharides used for N-glycosylation, is believed to be an important factor in determining the amount of glycosylation that occurs [Rosenwald *et al*., 1990]. In addition, a strong induction of BIP (2.04) was observed, what could be responsible for the observed low specific protein production rate, as enhanced BIP expression results in enhanced protein binding to BIP and, therefore, in blocking of protein secretion (Borth *et al*., 2005). Genes involved in protein

biosynthesis and protein metabolism were overrepresented in the high producer clone 2C10 and represented several cellular components involved in protein processing. Ribosomal upregulation, as well as well as the pronounced activation of translation (translation initiation and translation factor activity) may correlate with the almost 10-fold higher specific production rate of clone 2C10 compared to clone 8C6. At least four different proteins which are structural constituents of the ribosome or participate in ribosome biogenesis were found to be up-regulated (Rpl26, Rps19, RpI7a, Rps16).

4. CONCLUSION AND PERSPECTIVES

The data reveal, that cross-species transcription analysis of CHO cells using mouse and rat microarrays allows the identification of key genes responsible for the phenotypic behaviour and point out correlations between the phenotype and the transcriptome. The goal of the future work is to generate a dataset which emphasises those genes relevant to the use of CHO cells as a host for recombinant protein production.

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