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Poster Presentation

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Identifying key signatures of highly productive CHO cells from transcriptome and proteome profiles

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Background

One of the key challenges in biotherapeutics production is the selection of a high-producing animal cell line to maximize protein yield in cell culture. Clone selection is often a tedious process, involving rounds of selection and single cell cloning which is costly in both money and time. In an effort to increase the throughput of clone selection, we seek to identify key signatures of a highly productive cell line using an integrated genomic and proteomic platform. In our study, we analysed microarray and proteomics data generated from a characterization of two populations of CHO cells stably expressing high and low levels of green fluorescent protein (GFP). The high producer cells (HP) make 6x more GFP than the low producer cells (LP) as determined by ELISA. Comparison of transcript levels between HP and LP in the mid-exponential phase was performed using a proprietary 15k CHO cDNA microarray chip, of which 7559 genes are unique [1], while proteomic analysis on samples in the mid-exponential and stationary phases was performed using iTRAQ quantitative protein profiling technique [2]. Although there was a general lack of correlation between mRNA levels and quantitated protein abundance, results from both datasets concurred on groups of proteins/genes based on functional categorization.

Results

From microarray analysis, 84 genes had a change in relative abundance of ≥ 1.5-fold, either up or down, with p-value of ≤ 0.05. A significant number (23%) was involved in protein metabolism, transcription and RNA processing. Other major groups of genes include cell cycle regulation, signal transduction and transport. 50% of the genes had unknown functions and this could serve as a source of discovery for new and novel genes. Proteomic analysis gave 20 and 26 proteins that satisfied the cut-off criteria (≥ 1.2-fold change, 95% confidence) for the mid-exponential and stationary phase respectively. Proteins identified were mainly involved in protein metabolism, carbohydrate metabolism and transport (Figure 1). Proteome and transcriptome profiles of HP showed an up-regulation of biological processes related to protein metabolism such as protein folding (PPIB and Hyou1) and translation (Eef1a1, EIF2S3). With more protein production, genes involved in ubiquitylation (Arih1, Nedd4, Psma4, Psmc5 and Usp10) were also up-regulated to regulate misfolded proteins. Interestingly, a few of the identified genes involved in ubiquitylation have also been implicated in transcription. In particular Psmc5, a subunit of the 19S proteasome, interacts with TADs (Transcriptional Activation Domain) and general transcription factors TBP and TFIIH [3]. Key molecular chaperone genes of the UPR (unfolded protein response) pathway did not show significant differential expression, except for GRP78, an endo-

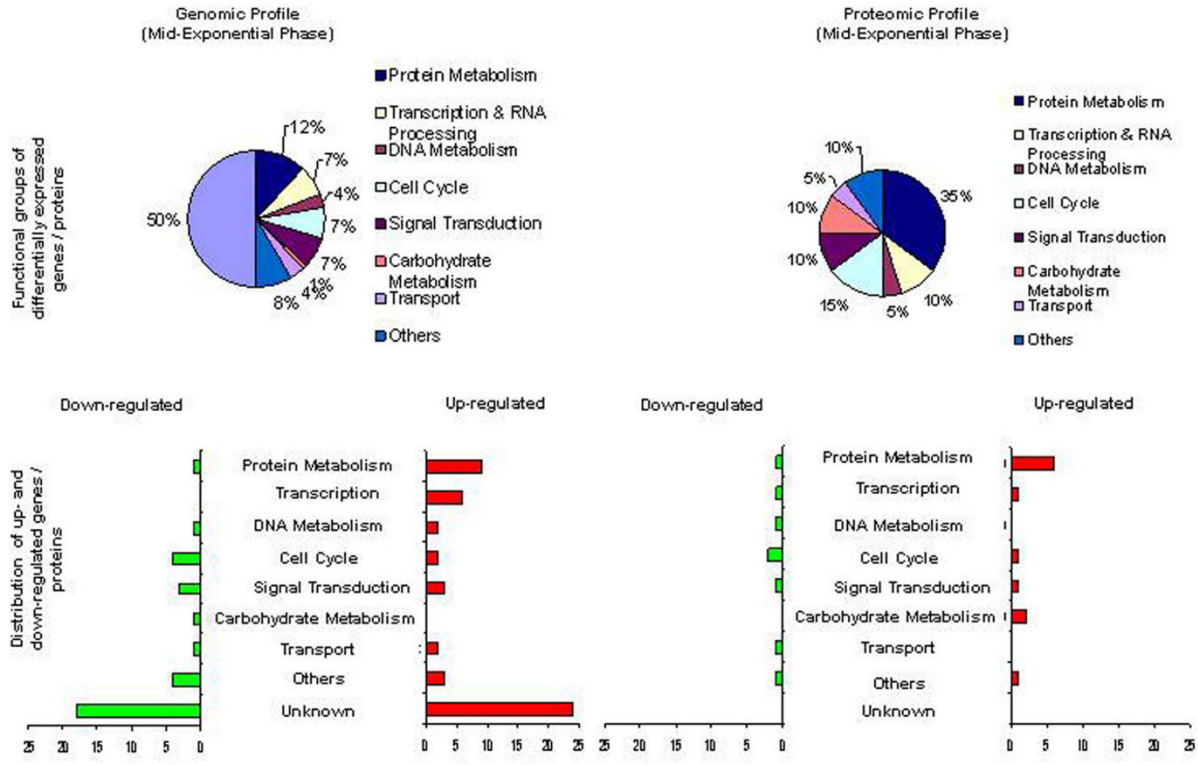

Figure 1
Genomic and proteomic profiles of differentially expressed genes in high producing cell line.

plasmic reticulum molecular chaperone gene implicated in ER overload response, which was down-regulated in HP. We also found differential expression in transcription and splicing factors, which give rise to a more active transcription and more efficient mRNA processing. Enzymes responsible for opening up chromatin, Hmgn3 and Hmgb1, were up-regulated while enzymes that condense chromatin, histone H1.2, were down-regulated. Both Hmgn3 and Hmgb1 bind to nucleosomes and reduce the compactness of the chromatin fiber, thus enhancing transcription from chromatin templates [4,5]. Genes and proteins that promote cell growth (Igfbp4, Ptma, S100a6 and Lgals3) were down-regulated while those that deter cell growth (Ccng2, Gsg2 and S100a11) were up-regulated, in agreement with the growth kinetics of HP compared to LP in our study. Mitochondrial and mitochondrial biogenesis genes and proteins (Cox7a2, Hspd1 and Mdh2) were up-regulated, indicating perhaps, more mitochondria. There was also a general up-regulation of proteins involved in carbohydrate metabolism (Pkm2, Gpd2, Idh1 and Gapd). This seems to point towards more energy generation in HP and hence a higher capacity for protein biosynthesis.

Conclusion

Our results show that an integrated approach using microarray and proteomics platform can be effectively utilized as tools to monitor transcriptional and post-transcriptional events of mammalian cells in culture, enabling us to identify distinctive changes in cells caused by recombinant protein expression. This information, together with changes in other important cellular processes, would be valuable in a rational approach for engineering cell-lines as well as for the designing of media and cell culture parameters to enhance product yield in CHO cells.

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