# BLUEPRINT FROM NATURE: MULTI-OMICS COMPARISON OF CHO AND PLASMA CELLS UNVEILS NOVEL CELL ENGINEERING TARGETS TO IMPROVE PRODUCTIVITY

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Chinese hamster ovary (CHO) cells are the predominantly used workhorse for the biotherapeutic industry to efficiently express multiple recombinant proteins including monoclonal antibodies (mAbs) as well as difficult to express (DTE) artificial molecules. However, due to the epithelial-like, ovarian origin they are not naturally specialized for a massive secretion of recombinant proteins. In mammals, terminally differentiated B-cells, so called plasma cells, are responsible for the secretion of high amounts of immunoglobulins (IgGs) after an infection and have been evolutionarily optimized for this task. Plasma cells are known to feature an extended endoplasmic reticulum (ER) and Golgi apparatus and are capable for efficient glycosylation. Therefore, these cells represent an ideal blueprint from nature to explore molecular differences compared to CHO production cells. Identified molecules and pathways may then serve as targets for engineering CHO cells towards the naturally optimized plasma cell phenotype.

The presented study compared two industrial IgG producing CHO with four immortalized plasma cell lines using a comprehensive multi-omics analysis comprising transcriptome, proteome, miRNome, surfaceome and secretome analyses. By the comparison of all data sets and their evaluation via DESeq analysis followed by appropriate statistical testing, we identified gene ontology (GO) terms related to ER, Golgi apparatus, unfolded protein response and the secretory pathway to be significantly enriched in plasma cells. This analysis in addition to manual curation revealed a subset of over 300 promising engineering target genes involved in protein processing to be used for overexpression in CHO cells. On the other hand, GO terms related to cell adhesion, extracellular matrix and proteolysis were enriched in CHO cell surfaceome and secretome data sets, and lead to the identification of over 100 CHO host cell proteins (HCPs) to be interesting knock-out targets to eliminate potential adverse effects.

In order to generate an engineered and improved CHO-plasma hybrid cell line with enhanced productivity, we focused on the identified differentially expressed genes and cellular pathways between both cell types. Therefore, a set of potentially effective target genes was selected from the above described evaluation and used for transient experimental approaches. We both overexpressed 28 plasma cell specific genes in a CHO-mAb1 expressing cell line with functions in the ER, Golgi apparatus and the secretory pathway, as well as knocked down 18 potentially adverse CHO cell-specific genes identified in the surfaceome or secretome data sets using a siRNA mediated approach to eliminate potential negative effects as aggregation or proteolysis. These modifications lead to the identification of multiple significantly effective genes to enhance titer and specific productivity of CHO-mAb1 cells. A final set of most effective genes was selected for transposase mediated overexpression in two industrial CHO cell lines expressing a DTE artificial molecule and a classical IgG revealing effectiveness of selected genes to improve titer for more than 50% also in an already highly optimized industrial setting.

In conclusion, we could confirm the suitability of the approach to use plasma cells as a blueprint from nature for targeted CHO cell line engineering and established a comprehensive database comprising more than 400 differentially expressed genes between CHO and plasma cells, which may serve as a community resource for further analysis and engineering of industrial CHO production cells.