CHO glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous N-glycan profiles

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CHO-glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous N-glycan profiles

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1. KEY MESSAGE
Combining the Chinese hamster ovary (CHO) - K1 draft genome1,2, identified CHO glycosyltransferases3 and the power of multiplexing gene knock-outs with CRISPR/Cas9 via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO cell lines differing in amount and combination of insertions or deletions (indels) in the targeted genes. Clones harboring 9, 8 and 6 indels were further investigated for growth, Rituximab productivity and secretome N-glycosylation.

This resulted in clones with prolonged viabilities, no changes in N-glycan galactose contents but an increase of matured and sialylated N-glycans in the secretome. Additionally we point out, that multiplexing an increasing amount of genes most likely results in clones only revealing a few of all possible combinations of the targets and is highly driven by the sgRNA efficiency which can differ from each other by factor 4, even after FACS sorting.

2. Introduction: N-glycan engineering

A. Background information
Although CHO cells’ strength is the production of similar human proteins4, non-engineered CHO cell lines display a broad variety of N-glycans which often includes N-glycan structures, that have an undesired effect on e.g. efficacy, antibody-dependent cell cytotoxicity (ADCC) or leak mediated clearance of the glycoprotein. In this work, we investigated the limitations of targeting up to ten gene targets via multiplexing in a Rituximab producing CHO cell line. The targets include N-glycosyltransferases, enzymes involved in nucleotide sugar synthesis, N-glycosyltransferase modulation, apoptosis and glutamine synthesis.

5. Results: Growth, Rituximab titers and secretome N-glycosylation

A. Growth and Viability in Batch Experiment

Figure 6: The three top KO clones display higher-genezen titers and productivity, where the 6x KO has the lowest and the 4x KO the highest titer. Within the control group, the two non-engineered clones reveal similar titers and specific Rituximab productivities.

B. Rituximab quantification

Figure 7: Absolute product concentration, (A) the non-engineered clones and (B) the 6x KO clones with clones in Box. Bax, GLUL. Target 4, BgGal2, BgGal2-F, BgGal2-T, Target 10.

C. Secretome N-glycan analysis

Figure 8: The purity of the secretome from (a) the non-engineered clones and (b) the 6x KO clones with clones in Box, Bax, GLUL. Target 4, BgGal2, BgGal2-F and target 10 (SA = sialic acid).

References:

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