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

Combining the Chinese hamster ovary (CHO) - K1 draft genome\(^1,2\), identified CHO glycosyltransferases\(^3\) and the power of multiplexing gene knock-outs with CRISPR/Cas9\(^4\) via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO-S cell lines differing in amount and combination of insertions or deletions (indels) in the targeted genes. Clones harboring 9, 6 and 4 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-glycan structures 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.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, CHO in Silico Engineering of Glycosylation and Protein Quality (CiSe), CHO Core, iLoop, Department of Biotechnology and Biomedicine, Network Engineering of Eukaryotic Cell factories
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Humanizing recombinant glycoproteins from Chinese hamster ovary cells

With new tools for gene-editing like zinc-fingers, TALENS and CRISPR, it is now feasible to tailor-make the N-Glycoforms for therapeutic glycoproteins that have previously been almost impossible. We here demonstrate a case of humanizing a recombinant human glycoprotein that in Wild type (WT) Chinese hamster ovary (CHO) cells are making a very heterogeneous mixture of N-Glycans. We speculate that the CHO pattern of N-Glycans would affect half-life and/or efficacy of the glycoprotein in the bloodstream making it unsuitable for human intravenous use, whereas our humanized version would be identical to the native human glycoprotein.

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Authors: Hansen, A. H. (Intern), Amann, T. (Intern), Kol, S. (Intern), Kildegaard, H. F. (Intern)
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Projects:

**Design of optimal CHO glycosylation profiles**
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Department of Systems Biology
Network Engineering of Eukaryotic Cell Factories
Period: 01/09/2015 → 31/08/2018
Number of participants: 3
Phd Student: Amann, Thomas (Intern)
Supervisor: Kildegaard, Helene Fastrup (Intern)
Main Supervisor: Andersen, Mikael Rørdam (Intern)

**Design of optimal CHO glycosylation profiles**
Technical University of Denmark
Period: 01/09/2015 → 31/08/2018
Number of participants: 4
Phd Student: Amann, Thomas (Intern)
Supervisor: Bolt, Gert (Ekstern)
Kildegaard, Helene Fastrup (Intern)
Main Supervisor: Andersen, Mikael Rørdam (Intern)

**Financing sources**
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)

**Relations**
Activities:
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Project: PhD

**Enhancing CHO by Mammalian Systems Biotechnology**
Innovative Training Network (ITN) under Horizon 2020
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Department of Systems Biology
Network Engineering of Eukaryotic Cell Factories
Period: 01/01/2015 → 31/12/2018
Number of participants: 7
synthetic biotechnology, cell factory optimization, systems biology, industrial biotechnology, biopharmaceuticals
Acronym: eCHO Systems
Contact person:
Lohmann, Ricarda (Intern)
Phd Student: Pristovsek, Nusa (Intern)
Financing sources
Source: EU research programme (public)
Name of research programme: Horizon 2020 MSCA ITN
Web address: http://www.echo-systems.eu/home.html

Relations
Activities:
CRISPR meets CHO cell factories. Club Biotech at University of Natural Resources and Life Sciences (BOKU). Vienna, Austria.
Publications:
Improving the secretory capacity of Chinese hamster ovary cells by ectopic expression of effector genes: Lessons learned and future directions
Project

Activities:

The 11th Danish Conference on Biotechnology and Molecular Biology: Glycobiology and Carbohydrate Biotechnology.
Period: 26 May 2016
Thomas Amann (Participant)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related event
The 11th Danish Conference on Biotechnology and Molecular Biology: Glycobiology and Carbohydrate Biotechnology.
26/05/2016 → 27/05/2016
Vejle, Denmark
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.