Reprogramming amino acid catabolism in CHO cells with CRISPR-Cas9 genome editing improves cell growth and reduces by-product secretion

Ley, Daniel; Domingues Pereira, Sara Isabel; Pedersen, Lasse Ebdrup; Arnsdorf, Johnny; Hefzi, Hooman; Lund, Anne Mathilde; Kwang Ha, Tae; Wulff, Tune; Kildegaard, Helene Fastrup; Andersen, Mikael Rørdam

Publication date: 2017

Reprogramming Amino Acid Catabolism in CHO Cells with CRISPR-Cas9 Genome Editing Improves Cell Growth and Reduces By-Product Secretion

Daniel Ley1,2, Sara Pereira2, Lasse Ebdrup Pedersen2, Johnny Arnsdorff2, Hooman Hefzi3,4, Anne Mathilde Lund1, Tae Kwang Ha2, Tune Wulf2, Helene Faustrup Kildegaard2, Mikael Rørdam Andersen1.

(1) The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark. (2) Novo Nordisk Foundation Centre for Biosustainability, Technical University of Denmark. (3) Department of Biotechnology, University of California, San Diego, United States. (4) Novo Nordisk Foundation Center for Biosustainability. (5) Department of Biotechnology and Biomedicine, University of California, San Diego, School of Medicine, United States.

Key message
CHO cells primarily utilize amino acids for three processes: biomass synthesis, recombinant protein production and catabolism. In this work, we disrupted 9 amino acid catabolic genes participating in 7 different catabolic pathways, to increase synthesis of biomass and recombinant protein, while reducing production of growth-inhibiting metabolic by-products from amino acid catabolism.

Background
Amino acid catabolism produces a wide range of growth inhibiting compounds, amongst these ammonium and lactate. Ammonium is produced by transamination and deamination reactions, whereas lactate is produced by either amino acid catabolic pathways feeding glycolysis or by NAD+ producing catabolic pathways, which forces the cell to reorganize NAD+ through lactate synthesis. Disruption of amino acid catabolic pathways may reduce production of growth-inhibiting metabolic by-products.

Physiology of single gene disrupted CHO cells
To study the physiological impact of disrupting single amino acid catabolic pathways, we characterized single gene disrupted clones in triplicate shake flasks cultures in batch mode. We monitored physiological changes in terms of maximum specific growth rate (μmax), integral of viable cell density (IVCD) and secretion of lactate and ammonium.

Validation of functional gene knock-out
Functional gene disruptions were validated using deep sequencing of the targeted genomic regions and qPCR analysis. All genes displayed out-of-frame mutations (A) and generally reduced transcription (B). Western blots indicated potential wild type proteins in some clones (C), as the proteomic analysis and mRNA sequencing was applied to verify functional knock-out of target genes (ongoing work).

Physiology of multiple gene disrupted CHO cells
To explore potential synergistic effects of disrupting multiple pathways, we targeted gene 1-4 for knock-out, but did not achieve full knock-out of all genes. Still, we isolated two clones with interesting genotypes. Clones were characterized in duplicated bioreactor cultures and showed further reduced lactate and ammonium secretion, but no growth benefit.

References
1. Markkola et al. (2017), Biotechnology and Bioengineering, 114(6), pp. 1579-1590.