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

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

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

Daniel Ley1,2, Sara Pereira3, Lasse Ebdrup Pedersen2, Johnny Arnsdorf3, Hooman Hefzi3,4, Anne Mathilde Lund1, Tae Kwang Ha2, Tune Wulff2, Helene Faustrup Kildegaard2, Mikael Rørdam Andersen1.

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 or deamination reactions, whereas lactate is produced by either amino acid catabolic pathways fueling glycolysis or by NADH producing catabolic pathways, which forces the cell to regenerate 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 flask 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.

Single gene disrupted clones generally showed an increased growth phenotype with 8 of 9 clones displaying increased µmax (up to 115% of WT), while 6 of 9 clones had increased IVCD (up to 136% of WT). Specific secretion of lactate was reduced in 4 of 9 clones (down to 81% of WT), while specific secretion of ammonium was reduced in 5 of 9 clones (down to 91% of WT). Monoclonal antibody titers increased proportionally to IVCD (data not shown).

Conclusion
Disruption of single amino acid catabolic pathways in CHO cells reduces specific production of lactate and ammonium, while increasing µmax and IVCD, leading to increased titers of recombinant proteins. Disruption of multiple catabolic pathways further reduces secretion of lactate and ammonium, but does not increase growth. Thus, we recommend combinatorial disruption of multiple amino acid catabolic pathways, to identify a set of disruptions that increase growth, while reducing secretion of lactate and ammonium.

References

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Overview of experiments
Target genes were identified using a metabolic network reconstruction of amino acid catabolism. Gene knock-out was performed with CRISPR-Cas9. Single cells expressing GFP-linked Cas9 were enriched on FACs. Physiology of gene edited clones was assessed in shake flasks and bioreactors. Phenotypes were validated by targeted genome sequencing, qRT-PCR, western blot and proteomic analysis.

Validation of functional gene knock-out
Functional gene disruptions were validated using deep sequencing of the targeted genomic loci, gene expression analysis, western blots and proteomics. All genes displayed out-of-frame mutations (A) and generally reduced transcription (B). Western blots indicated potential wild type proteins in some clones (C), to 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.

Data integration Transfection Single cell sorting Expansion and characterization
• Growth performance • Metabolic profiles
• Clone validation
• Genomic
• Transcriptome analysis
• Protein analysis

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Figure here!