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

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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 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 ($\mu_{max}$), 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 $\mu_{max}$ (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).

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), so proteomic analysis and miRNA 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
4. Ley & Kazemi et al. (2015), Biotechnology and Bioengineering, 112(11), pp. 2373-2387.

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