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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 compounds1, amongst these ammonium and lactate. Ammonium is produced by transamination and deamination reactions2, 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 synthesis3. 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).

To exclude that the improved phenotypes are caused by clonal variation, we characterized multiple clones with different mutations in gene 4 and 6, and found a strong link between genotype and phenotype.

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
1. Madsen N. E. et al. (2017), Biotechnology and Bioengineering 114(6), pp. 1779-1790.