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

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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 inhibitory 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 NADPH 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 understand 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.

Validation of functional gene knock-out

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.

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