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

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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.

**Key message**

CHO cells utilize amino acids for three processes: biomass synthesis, recombinant protein production and catabolism. Disruption of single amino acid catabolic pathways in CHO cells reduces specific production of by-products, while increasing growth and viability.

**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.

**References**

1. Madsbad et al. (2019), Biotechnology and Bioengineering, 114(8), pp. 1779-1790.