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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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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 NAD+ production, 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 ammonium was reduced in 5 of 9 clones (down to 91% of WT), while specific secretion of lactate was reduced in 4 of 9 clones (down to 81% of WT), and increased µ max and IVCD, leading to increased titers of recombinant protein.

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.

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 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 loci, gene expression analysis, western blots and proteomics.

Conclusion

Disruption of single amino acid catabolic pathways in CHO cells reduces specific production of lactate and ammonium, while increasing $\mu_{max}$ 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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