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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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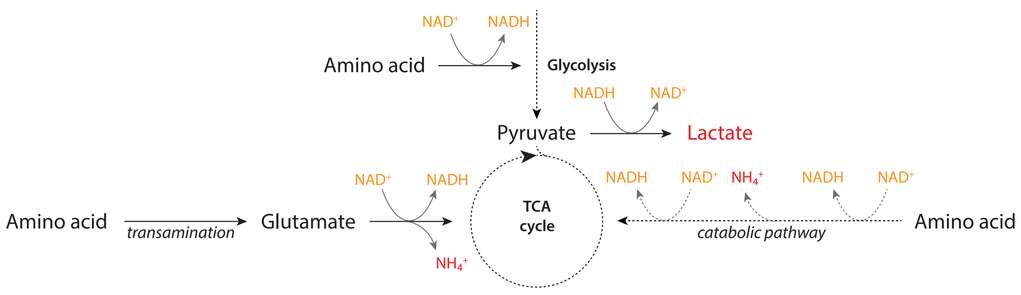
Key message

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[1], amongst these ammonium and lactate. Ammonium is produced by transamination and deamination reactions[2], 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[3]. Disruption of amino acid catabolic pathways may reduce production of growth-inhibiting metabolic by-products.



Overview of experiments

Target genes were identified using a metabolic network reconstruction of amino acid catabolism[4]. 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.

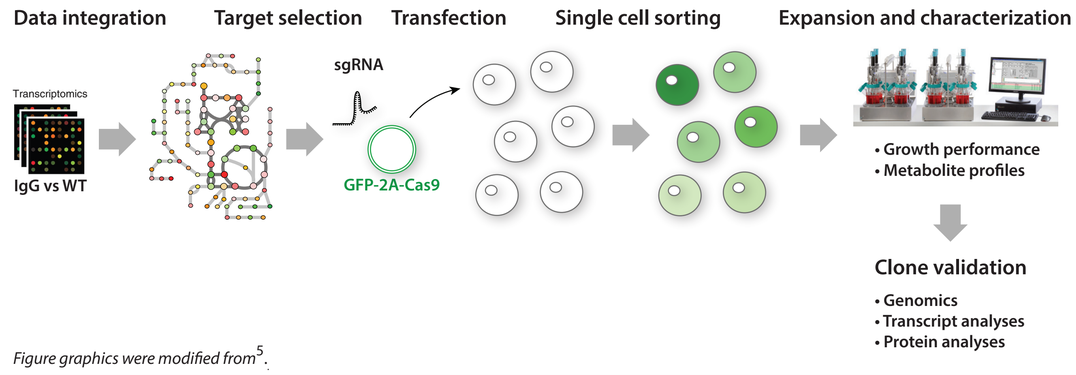
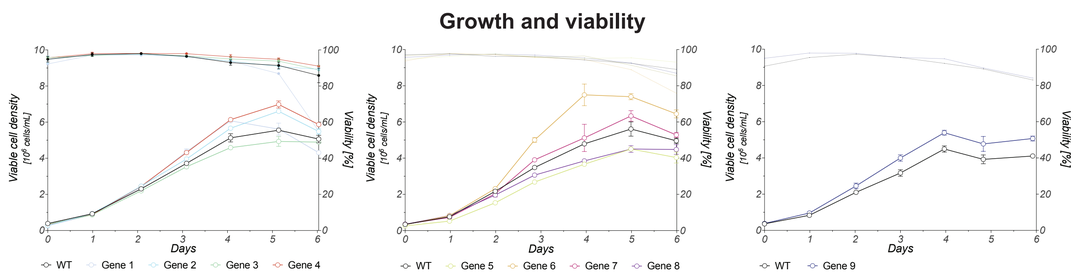


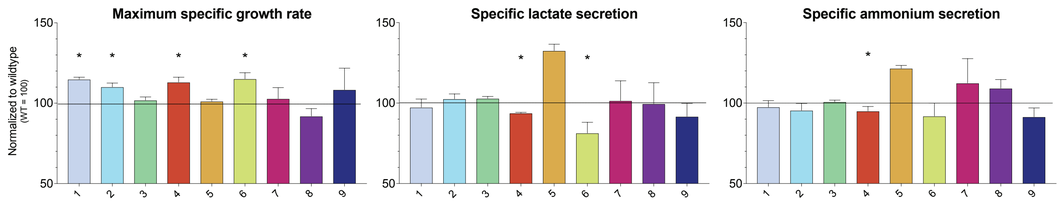
Figure graphics were modified from[5].

Physiology of single gene disrupted CHO cells

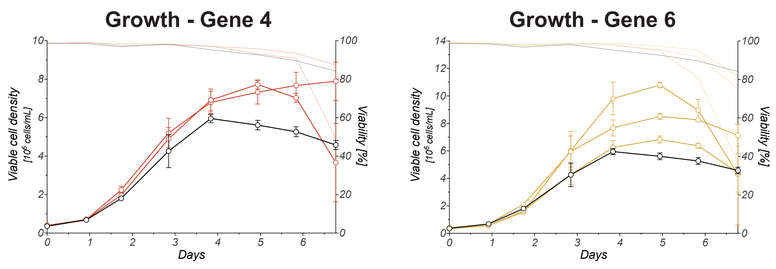
To study the physiological impact of disrupting single amino acid catabolic pathways, we characterized single gene disrupted clones in triplicate shake flask cultures in batch mode. We monitored physiological changes in terms of maximum specific growth rate (μ_{max}), integral of viable cell density (IVCD) and secretion of lactate and ammonium.



Single gene disrupted clones generally showed an increased growth phenotype with 8 of 9 clones displaying increased μ_{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).



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.

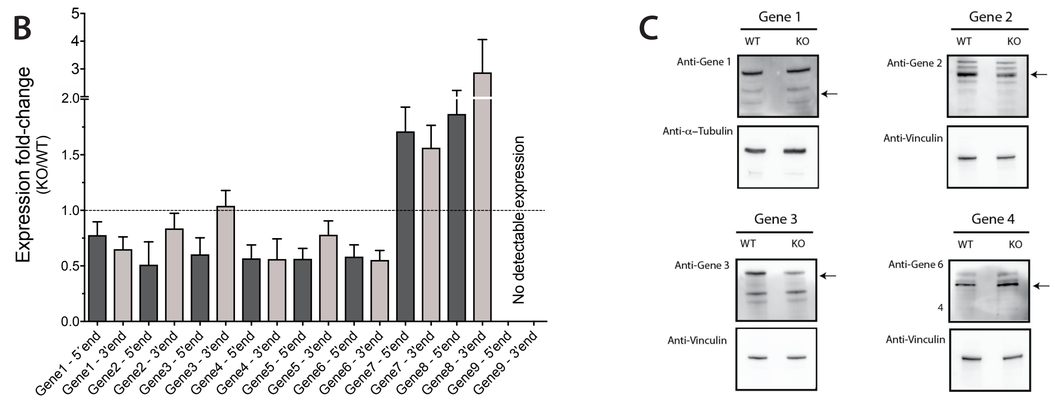


Validation of functional gene knock-out

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 type proteins in some clones (C), so proteomic analysis and mRNA sequencing was applied to verify functional knock-out of target genes (ongoing work).

A

Target gene:	1	2	3	4	5	6	7	8	9
Indel sizes:	-1	+1	-29/-16	+1	+28	-13	+1	-22	-20



Physiology of multiple gene disrupted CHO cells

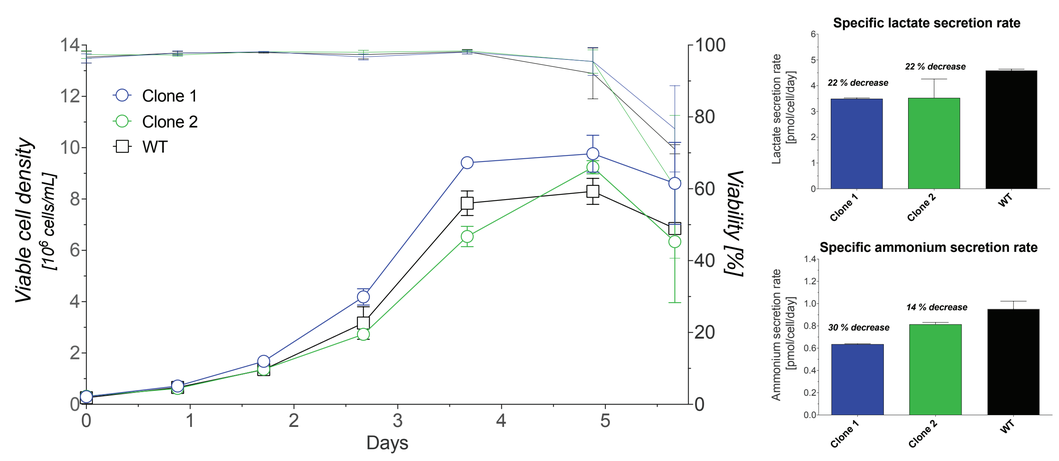
To explore potential synergistic effects of disrupting multiple pathways, we targeted gene 1-4 for knock-out, but did not achieve full knock-out of all genes. Still, we isolated two clones with interesting genotypes. Clones were characterized in duplicated bioreactor cultures and showed further reduced lactate and ammonium secretion, but no growth benefit.

Clone 1

Target gene:	1	2	3	4
Indel sizes:	-1/-10	-9/WT	+98/+105	+1

Clone 2

Target gene:	1	2	3	4
Indel sizes:	-1/-10	WT	+98/+105	+1



Conclusion

Disruption of single amino acid catabolic pathways in CHO cells reduces specific production of lactate and ammonium, while increasing μ_{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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Acknowledgements

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