In vitro approach to citrin deficiency: cellular modeling for characterization and therapeutic screening

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Background

Citrin deficiency (CD) is a complex metabolic condition caused by pathogenic variants in the *SLC25A13* (citrin) gene. This gene encodes a hepatic mitochondrial carrier involved in transporting aspartate to the cytosol, where it is utilized by argininosuccinate synthetase (ASS) in the urea cycle. Citrin defects affect primarily the malate–aspartate shuttle but also other pathways including glycolysis, *de novo* lipogenesis, and the urea cycle. Given the involvement of multiple metabolic pathways, a comprehensive *in vitro* disease modeling approach that incorporates these pathways is crucial for elucidating the complex pathophysiology of CD.

Methods

We evaluated hepatoma-derived HepaRG cells after citrin knock-out (KO) using CRISPR/Cas9 targeting *SLC25A13* locus and primary liver samples obtained from CD patients after liver transplantation as model systems for disease characterization. Particularly, the ureagenesis capacity using stable isotopes, malate-aspartate shuttle and mitochondrial function were analyzed and gene expression profiles were evaluated by performing qPCR and RNA-sequencing.

Results

Citrin KO HepaRG clones were produced successfully and showed poor growth in comparison to controls. Further, cells produced less urea and accumulated more citrulline compared to controls, consistent with urea cycle impairment in CD. Additionally, elevated NADH/NAD^+ ratios and decreased ATP production indicated an impairment of malate-aspartate shuttle and mitochondria function, respectively. Gene expression profiles of citrin KO HepaRG cells, validated with comparison to the expression profiles of CD patient liver samples, showed downregulation of several lipid metabolism genes, particularly PPARα and SREBP, potentially related to hepatic steatosis development. Further analysis of CD patient liver samples revealed variable but consistently reduced ASS protein levels compared to controls. Interestingly, this decrease in protein occurred despite unchanged *ASS1* transcript levels.

Conclusion

For advancing CD research and therapy development, clinically relevant cellular models are essential. In this study, we have established and characterized citrin KO HepaRG cells, a faithful *in vitro* model that recapitulates metabolic defects observed in CD patients, consistent with findings from patient liver samples. This cellular model allows us to explore the interplay between metabolic pathways in the disease and it is currently being used to screen therapeutic compounds with the goal of identifying candidates that can improve metabolic function in CD patients.