DefiniGEN
DISEASE MODEL INNOVATION

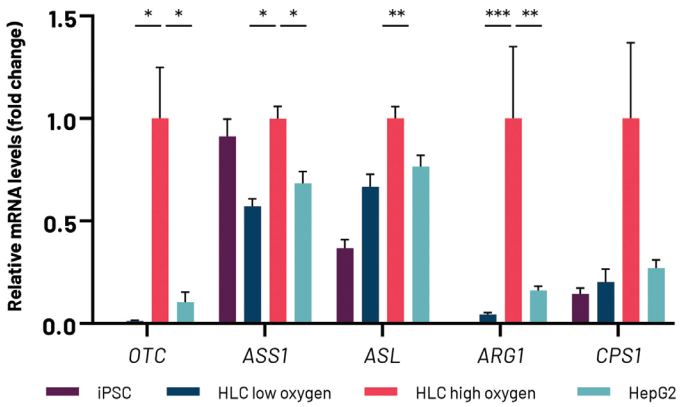
Disease Modeling

DefiniGEN human iPSC-derived Ornithine transcarbamylase (OTC) modelled hepatocytes

Description

Ornithine transcarbamylase (OTC) deficiency is an inherited, X-linked, recessive metabolic disorder and, currently, the most common UCD with a prevalence of one in 60-70,000 in humans. It is mainly caused by mutations on the OTC gene, which encodes the mitochondrial enzyme ornithine transcarbamylase. There are no prevalent mutations in the human population, and most of them are distributed throughout the gene. Due to difficulties in developing iPSC- derived hepatocytes with a functional urea cycle pathway, no *in vitro* disease models for OTC deficiency are available to date.

Functional urea cycle pathway



Advantages

Disease circuit verified carrying the D175V mutation (GAT>GTT) on the OTC gene

Display multiple key hepatocyte markers A1AT, Albumin, Glucose

Application a platform for primary screening activities

Standardized cell product containing iPSC-derived human hepatocytes producing reproducible and biologically relevant data

Figure 1. mRNA expression levels of key genes involved in urea cycle in iPSC, low oxygen-cultured hepatocyte-like cells (HLCs), high oxygen-cultured HLCs, and HepG2 cells. mRNA data were normalised to 18SrRNA and are presented as mean±SEM of n=3 biological replicates. *p<0.05, **p<0.01, ***p<0.001

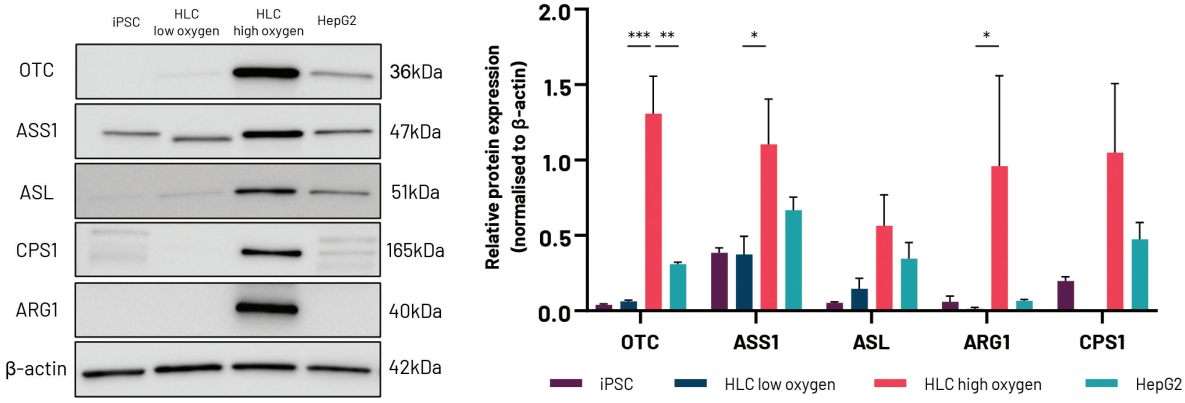


Figure 2. Protein expression levels of key enzymes involved in urea cycle in iPSC, low oxygen-cultured HLCs, high oxygen-cultured HLCs, and HepG2 cells. Data were normalised to beta actin and are presented as mean±SEM of n=3 biological replicates. *p<0.05, **p<0.01, ***p<0.001

DefiniGEN iPSC-derived wild-type hepatocyte-like cells (HEP-003) secrete urea in a time-dependent manner

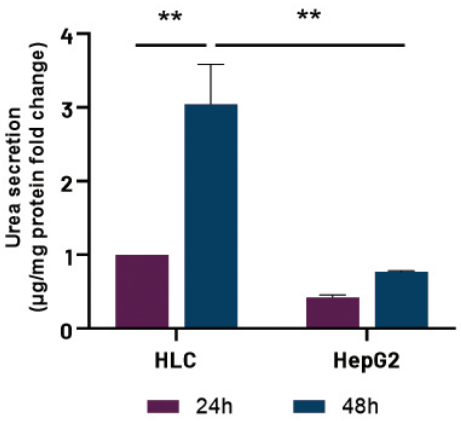


Figure 3. Media urea levels in high oxygen-cultured HLCs and HepG2 cells for 24h and 48h. Data were normalised to total protein levels and are presented as mean±SEM fold change of n=3 biological replicates. **p<0.01

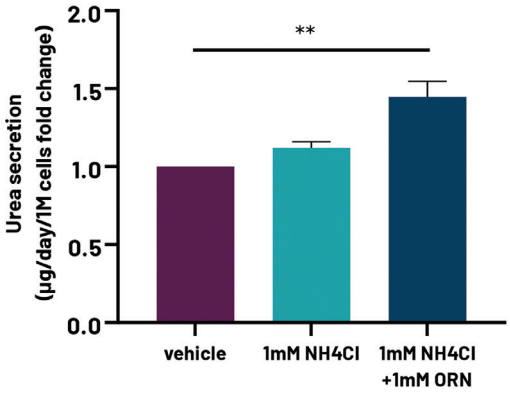


Figure 4. Media urea levels in high oxygen-cultured HLCs cultured in the presence of either vehicle, 1mM NH4Cl, or 1mM NH4Cl +1mM Ornithine for 24h, suggestive of functional OTC activity. Data were normalised to total cell number and are presented as mean±SEM fold change of n=3 biological replicates. **p<0.01

DefiniGEN CRISPR-derived OTC deficiency (OTCD) HLCs carry the D175V mutation

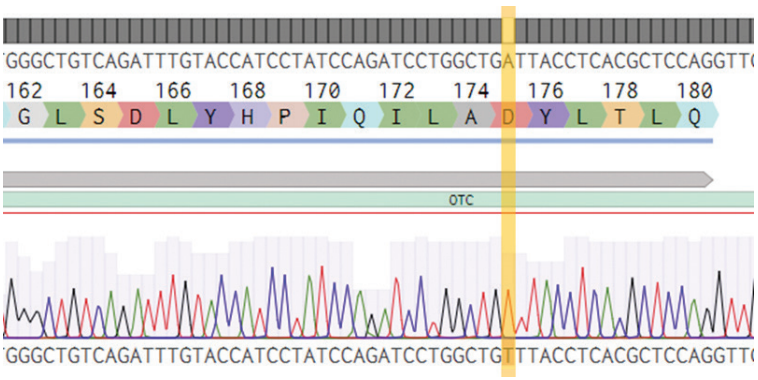


Figure 5. Sanger sequencing showing healthy wild-type (top sequence) as well as mutated iPSCs (bottom sequence) carrying the D175V mutation (GAT>GTT) on the OTC gene. The codon change is highlighted with yellow.

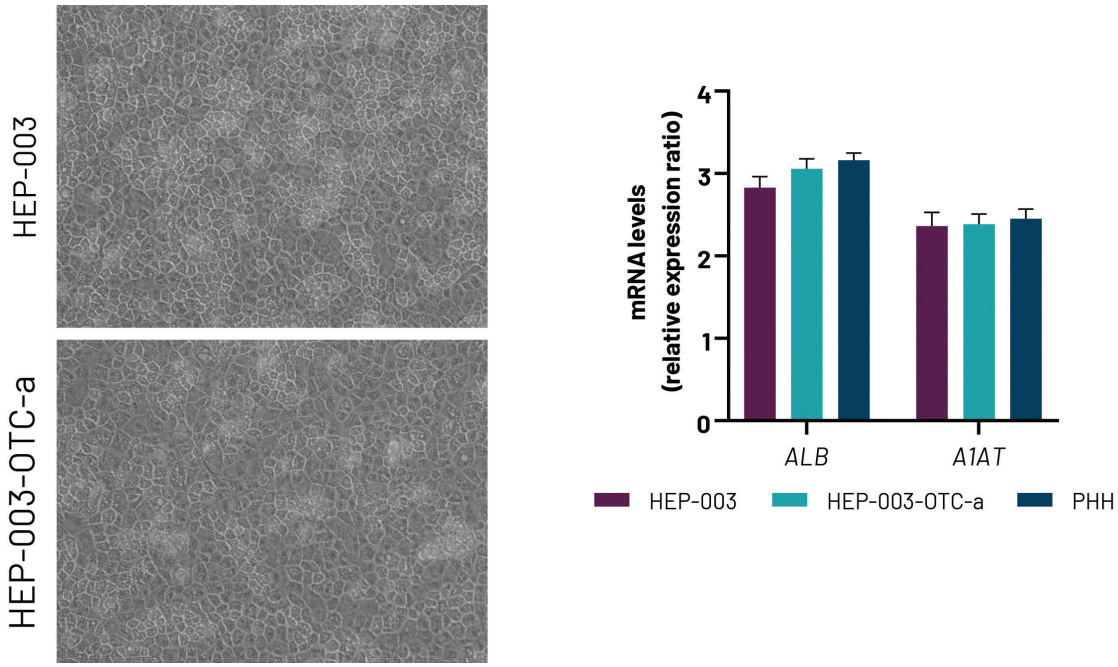


Figure 6. A) Representative images demonstrating the characteristic hepatocyte cobblestone morphology in wild-type (HEP-003) and OTCD (HEP-003-OTC-a) hepatocyte-like cells (HLCs). B) mRNA expression levels of the hepatocyte maturity markers albumin (ALB) and alpha-1-antitrypsin (A1AT) in wild-type HLCs (HEP-003), OTCD HLCs (HEP-003-OTC-a), and primary human hepatocytes (PHH). mRNA data were normalised to PPIA and are presented as mean±SEM of n=3 independent experiments. Objective: 10x.

DefiniGEN CRISPR-derived OTCD HLCs demonstrate decreased OTC protein expression and urea secretion compared to wild-type HLCs

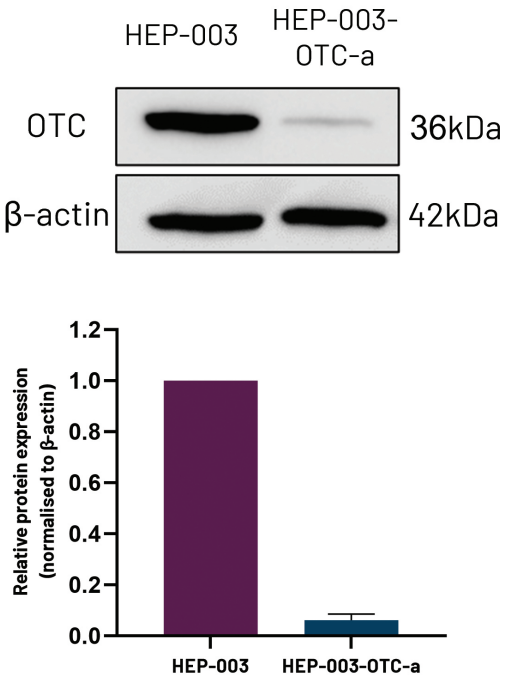


Figure 7. Protein expression levels of OTC in wild-type HLCs (HEP-003) and CRISPR-derived OTCD HLCs (HEP-003-OTC-a). Data were normalised to beta actin and are presented as mean±SEM of n=3 biological replicates.

DefiniGEN CRISPR-derived OTCD HLCs demonstrate decreased OTC protein expression and urea secretion compared to wild-type HLCs

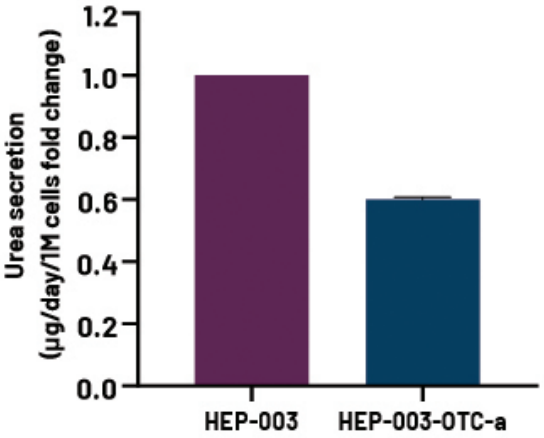


Figure 8. Media urea levels in wild-type HLCs (HEP-003) and CRISPR-derived OTCD HLCs (HEP-003-OTC-a). Data were normalised to total cell number and are presented as mean±SEM fold change of n=2 biological replicates.

Conclusion

This case study demonstrates, for the first time, a fully functional urea cycle pathway in wild-type iPSC-derived hepatocyte-like cells (HLCs) and reveals the superiority of DefiniGEN iPSC-derived HLCs compared to liver carcinoma HepG2 cells. Supporting these findings, we demonstrate functional OTC activity, with a >30% increase in urea secretion when cells are stimulated with 1 mM NH_4Cl and 1 mM ornithine (OTC substrate). Informed by these data, and by applying CRISPR gene editing on DefiniGEN wild-type iPSCs, we have successfully generated an OTC deficiency (OTCD) iPSC line carrying the pathogenic, missense mutation D175V. This CRISPR-engineering OTCD iPSC line can differentiate equally well towards HLCs, without any compromise in the expression of hepatocyte maturity markers (ALB, A1AT). Crucially, and upon differentiation, the OTCD HLCs reveal decreased protein expression of OTC and decreased urea formation compared to their isogenic controls, demonstrating their ability to serve as a platform for primary screening activities.

DefiniGEN Limited
Babraham Research Campus, Babraham,
Cambridge, CB22 3AT, United Kingdom

For more information please contact us:
+44 (0) 1223 497 106
info@definigen.com

www.definigen.com