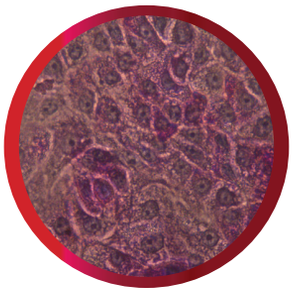
DefiniGEN

DefiniGEN human iPS-derived GSD1a hepatocytes



DefiniGEN's GSD1a human hepatocytes effectively model glycogen storage diseasae type 1a the most common of the glycogen storage diseases. This genetic disease results from a deficiency in the glucose-6-phosphatase (G6P) enzyme which impairs the ability of the liver to produce free glucose from glycogen and gluconeogenesis. These cell products display the disease phenotype in combination with general hepatocyte functions in a similar manner to human primary hepatocytes.

Phenotypic analysis of the cells using periodic acid/diastase staining has demonstrated that the cells excessively accumulate glycogen as the glucose-6-phosphatase enzyme is dysfunctional. Accordingly these cell products can offer disease modelling and drug discovery researchers a novel tool for dissecting the underlying mechanisms of this and similar lysosomal storage diseases.

Functional analysis

Period acid-Schiff (PAS) staining has revealed Def-HEP GSD1a hepatocytes accumulated substantially greater amounts of intracellular glycogen compared with iPSC derived hepatocytes from control subjects (Figure 1a); in addition BODIPY staining showed excessive production of intracellular lipids in Def-HEP GSD1a hepatocytes (Figure 1B); and Def-HEP GSD1a hepatocytes secrete more lactate compared with iPSC derived hepatocytes from control subjects, as assessed by ELISA analysis of a 24-hour collection of cell culture medium.

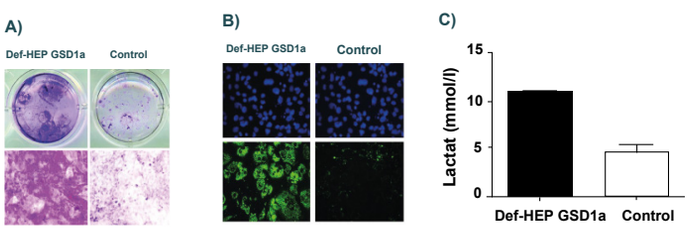


Figure 1. A) Period acid-Schiff (PAS) staining revealed Def-HEP GSD1a hepatocytes accumulated substantially greater amounts of intracellular glycogen than did those of controls (Figure 1a) and showed excessive production of lipid (Figure 1B) and lactic acid (Figure 1C) confirming the cellular disease phenotype.

PAS/Diastase functional test

Period acid-Schiff (PAS) is a staining method used to detect glycogen storage in tissue and in the diagnosis of GSDs. Diastase is an alpha-amylase enzyme that breaks down glycogen and is used in combination with a PAS stain to specifically identify glycogen granules. PAS staining revealed that Def-HEP GSD1a hepatocytes accumulated significantly higher amounts of intracellular glycogen than the negative control (Figure 2).

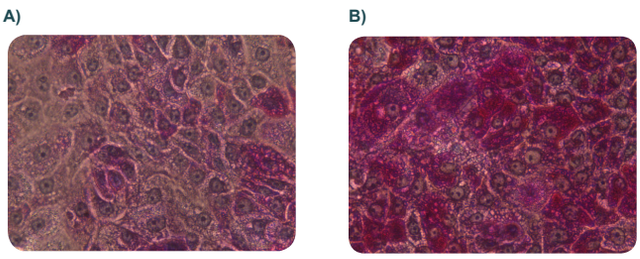


Figure 2. A) PAS/Diastase staining of cryopreserved Def-HEP GSD1a. Magnification level: x400. (A) PAS/Diastase staining showing breakdown of accumulated glycogen in the cells. (B) PAS staining showing the accumulation of glycogen in the cells.

DefiniGEN

For the production of the Def-HEP A1ATD cells, A1ATD patient fibroblasts are first reprogrammed into iPSC using the Nobel Prize winning technology developed by Yamanaka and colleagues. These iPSC are then differentiated into liver hepatocytes using the OptiDIFF protocol developed at the University of Cambridge Laboratory for Regenerative Medicine. Def-HEP A1ATD patient derived hepatocytes represent an optimized disease model for drug discovery applications and are an effective tool for elucidating the underlying mechanisms of the disease.

Detection of disease markers via ELISA

The A1AT disease marker can be quantified using an ELISa for mutant polymer and wild-type secreted A1AT. The assay utilizes antibodies specific for A1AT polymers (2C1mAb, top) or all conformers of A1AT (bottom) (Figure 3).

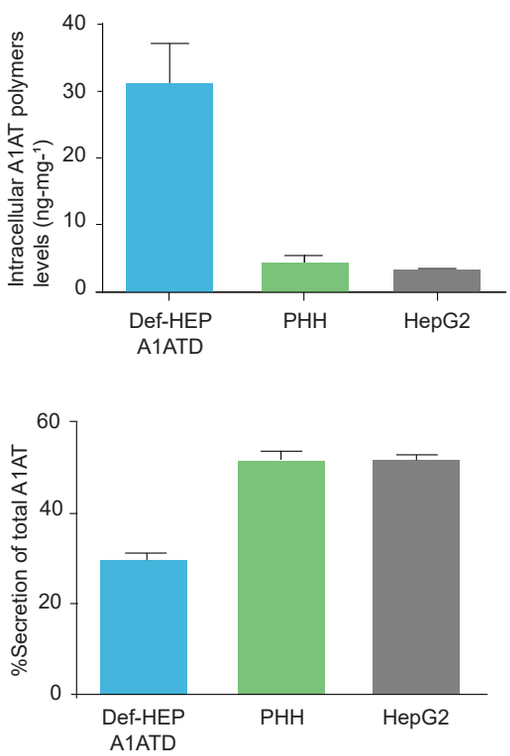


Figure 3. Quantification of intracellular A1AT polymers and intercellular total A1AT secretion in Def-HEP A1ATD cells. Primary human hepatocytes (PHH) and hepatocellular liver carcinoma cells (Hep G2) are used as internal controls.

Immunocytochemistry analysis of A1AT mutant polymer

Previous studies have shown that the Z allele (Glu342Lys) results in the formation of ordered polymers of a1-antitrypsin that are retained within the ER. This pathway of a1-antitrypsin polymerization is central to the clinical phenotype. We therefore used the 2C1 polymer specific monoclonal antibody to detect polymers within Def-HEP A1ATD hepatocytes. Polymers were detected by immunostaining (Figure 4). The immunocytochemistry data anlysis show that accumulation of a1-antitrypsin polymers only occurs in disease-specific human Def-HEP hepatocytes from individuals with A1ATD; no polymers are present in human iPS cell-derived hepatocytes from control subjects.

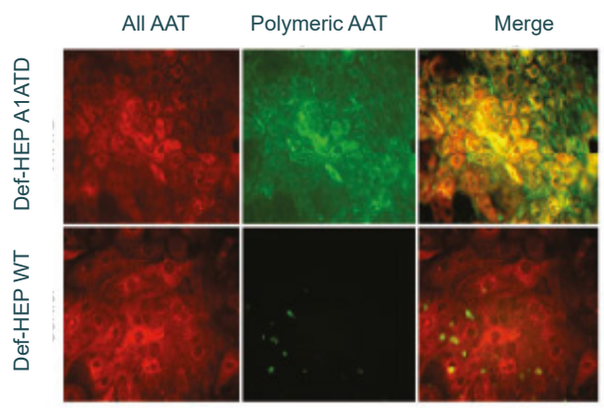


Figure 4. Immunostaining analyses for expression of misfolded polymeric a1-antitrypsin using the polymer- specific 2C1 antibody (green) or an antibody that detects all forms a1-antitrypsin (red) in Def-HEP A1ATD disease modelled cells and control human iPS cell-derived hepatocytes. Merged images are shown at right.

Email: info@definigen.com
Phone: 617-674-3260
Phone: +44 (0) 1223 497 106
www.definigen.com