A new generation of human pluripotent stem cell-derived hepatocytes displays adult characteristics and a substantially improved functionality

Barbara Küppers-Munther[1,2], Annika Asplund[1,2], Mariska van Giezen[1], Petter Björquist[1]

[1] Cellectis AB, Arvid Wallgrensbacke 20, 41346 Gothenburg, Sweden

[2] University of Skövde, Systems Biology Research Center, School of Bioscience, 54128 Skövde, Sweden

Introduction

Hepatocytes derived from human pluripotent stem cells (hPSC) have the potential to provide relevant human in vitro model systems for toxicity testing and drug discovery studies. However, until recently, the functionality of stem cell-derived hepatocytes has remained on a level that renders the cells sub-optimal for drug metabolism studies as well as for some toxicity testing requiring high activity of multiple CYP enzymes.

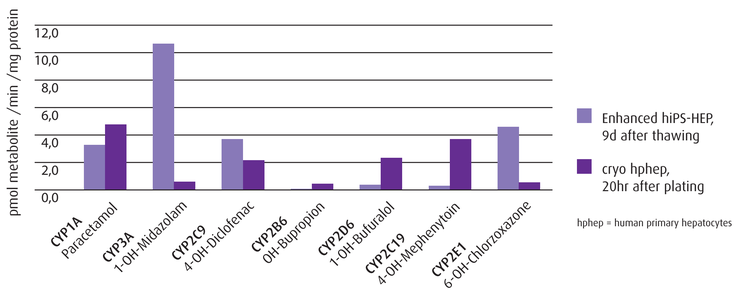
We have developed a new generation of human induced pluripotent stem cell-derived hepatocytes, Enhanced hiPS-HEP, which display several adult hepatic features and an improved functionality.

To the best of our knowledge, this is the first time such improved functionality is described for stem cell-derived hepatocytes in a 2D culture system.

Results and discussion

The cryopreserved Enhanced hiPS-HEP:

* display high CYP1A, 3A, 2C9, and 2E1 activities and lower but detectable CYP2B6, 2C19, and 2D6 activities (Fig. 1).
* show stable CYP activity between day 6 and 11 after thawing (data not shown).
* express substantial mRNA levels of the adult enzymes CYP2C9, 2C19, 3A4, and 3A5, and low mRNA levels of the fetal genes CYP3A7 (Fig. 2) and AFP (data not shown).
* display mRNA expression of the phase II enzymes UGT1A1 and 2B7, as well as the transporter proteins MRP2, NTCP, and OATP1B1 (Fig.2).
* have a typical hepatic morphology like a polygonal cell shape and the presence of binucleated cells (Fig. 3A, arrowheads).
* are immuno-positive for Albumin, α1-Antitrypsin, Cytokeratin 18 (CK18), CYP1A2, 2C9, 3A4, and GSTA1-1 (Fig. 3B-H). Interestingly, for all markers except for CK18 only distinct subgroups are immuno-positive for different markers reminiscent of the metabolic zonation found in the liver lobe.
* The improved differentiation protocol is highly robust resulting in homogeneous populations of more than 90 % pure hepatocytes and low batch-to-batch variation.



cryopreserved Enhanced hiPS-HEP, n=2
cryopreserved hphep, n=4 (4 different donors)

Figure 1: CYP activities of Enhanced hiPS-HEP compared to cryopreserved human primary hepatocytes.

Cryopreserved Enhanced hiPS-HEP (9 days after thawing) have higher or similar activities of CYP1A, 3A, 2C9 and 2E1 compared to cryoplateable human primary hepatocytes (cultured for 20 hr in total, average of 4 donors). CYP2B6, 2D6 and 2C19 activities are lower in Enhanced hiPS-HEP than in hphep, but detectable.

Selected references:

Ohtsuki *et al*, 2012, Drug metabolism and disposition, 40: 83–92.

Ulvestad *et al*, 2013, Biochemical Pharmacology, 86: 691–702.

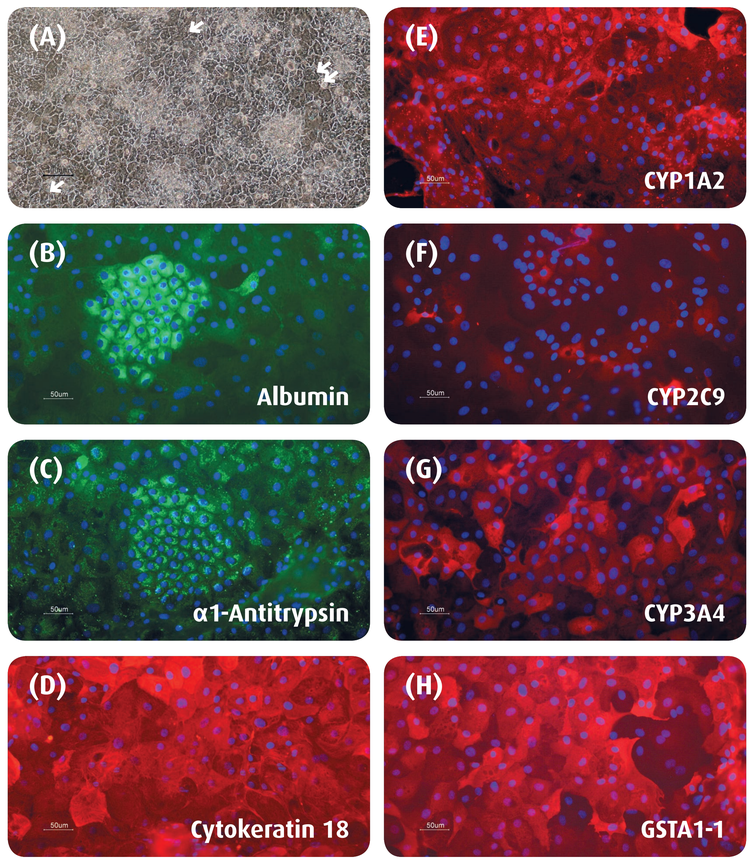


Figure 2: Morphology and immunocytochemical stainings of cryopreserved Enhanced hiPS-HEP (7 days after thawing).

The Enhanced hiPS-HEP display a typical hepatic morphology (A) with polygonal cell shape and binucleated cells (arrow heads). Subgroups of the Enhanced hiPS-HEP are immuno-positive for Albumin, α1-Antitrypsin, Cytokeratin 18, CYP1A2, 2C9, 3A4, and GSTA1-1 (B-H; all overlayed with nuclear counterstain with DAPI). Scale bar: 100 µm in A and 50 µm in B-H.

Conclusions

The Enhanced hiPS-HEP:

* have substantially improved functionality and high activity levels of enzymes relevant for toxicity testing and drug metabolism studies.
* have many adult features, e.g. substantial expression of the adult enzymes CYP2C9, 2C19, 2E1 and 3A4, and low expression of the fetal genes CYP3A7 and AFP.
* are highly homogeneous hepatocyte cultures with low batch-to-batch variation due to a highly robust differentiation protocol.
* can serve as an inexhaustible source of functional human hepatocytes.
* are ideal for applications that demand a highly reproducible platform and continouos supply of material from the same genetic background.
* are available both as fresh and cryopreserved cells.
* will be available in near future from several hiPSC-lines showing different CYP profiles (e.g. lower CYP3A or higher 2C19 activity) reflecting inter-individual variation observed in the population.

Contact details:

Barbara Küppers-Munther

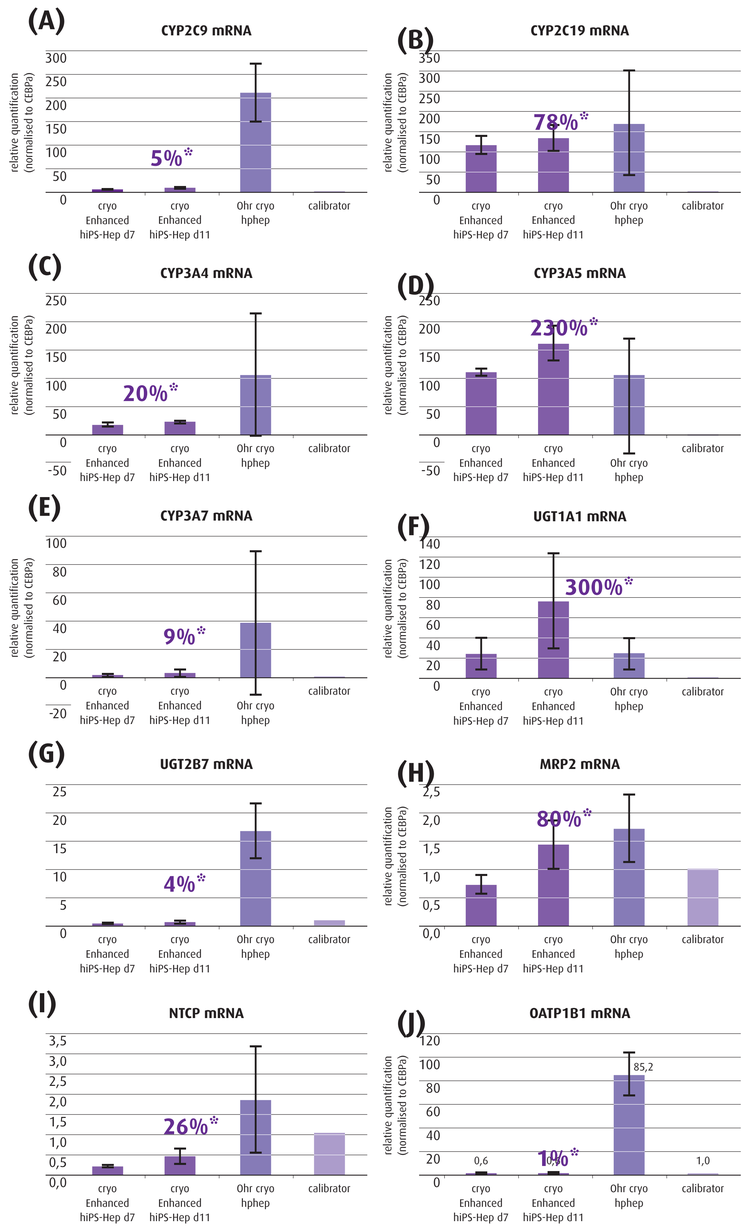
Cellectis AB

Arvid Wallgrensbacke 20, 41346 Göteborg, Sweden

email: barbara.kuppers_munther@cellectis.com

phone: +46 31 75 80 948

http://www.cellectis-bioresearch.com



Cryo Enhanced hiPS-HEP, n=3; cryo hphep, n=5 (5 different donors)
***= % of average of 0 hr cryopreserved hphep (5 donors)**

Figure 3: qPCR analysis of cryopreserved Enhanced hiPS-HEP (7 and 11 days after thawing, respectively) compared to 0 hr cryopreserved human primary hepatocytes (hphep).

Enhanced hiPS-HEP show substantial mRNA expression of the adult phase I enzymes CYP2C9, 2C19, 3A4 and 3A5 (A-D) and low expression of the fetal enzyme CYP3A7 (E). In addition, mRNA expression of the phase II enzymes UGT1A1 (F) and UGT2B7 (G) and the transporters MRP2 (H), NTCP (I) and OATP1B1 (J) could be observed. Note that the correlation between CYP2C9 and 2C19 mRNA and activity levels is poor as also described by Ohtsuki et al (2012). Expression data are presented as relative quantification normalized to CEBPα serving as house-keeping gene and a calibrator consisting of a mix of cDNA from hphep, HepG2, HEK293 cells, undifferentiatied hPSC as well as hPSC-derived definitive endoderm, embryoid bodies and cardiomyocytes.

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Cryopreserved Enhanced hiPS-HEP are available from Cellectis Bioreseach.