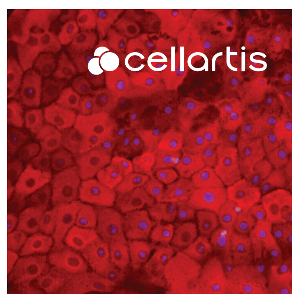

Stem Cell Products

Expression of CYP enzymes in Cellartis® Enhanced hiPS-HEP

Introduction

Hepatocytes derived from human induced pluripotent stem cells (hiPSC) have the potential to serve as an alternative human *in vitro* cell-based system for early drug discovery studies, provided that they display relevant levels of hepatic functions. However, until recently, the functionality of stem cell-derived hepatocytes has been insufficient for applications requiring high expression of multiple drug metabolizing enzymes.

Cellartis Enhanced hiPS-HEP are hepatocytes derived from hiPSC which display several adult hepatic features and have a substantially improved Phase I enzyme expression compared to previously established hepatocytes derived from hiPSC. In the current application note, data is presented on the expression of drug metabolizing enzymes in Cellartis Enhanced hiPS-HEP.

Materials and methods

Cell-culture

Cellartis Enhanced hiPS-HEP were thawed, plated and maintained according to the technical manual.

CYP activity assay

The CYP activities of Cellartis Enhanced hiPS-HEP were analysed at day 6 and 11 after thawing. LC/MS (performed at Pharmacelsus GmbH) was used for measuring the formation of specific metabolites, Acetaminophen (CYP1A), 4-OH-Diclofenac (CYP2C9), 4-OH-Mephenytoin (CYP2C19), OH-Bufuralol (CYP2D6) and 1-OH-Midazolam (CYP3A). Cells were incubated for 2h with a probe substrate cocktail (Table 1) diluted in WME containing 0.1% PEST, 25 mM HEPES, and 2 mM L-Glutamine. The metabolite concentrations measured by LC/MS are normalized to the amount of protein per well (determined using the Pierce™ BCA Protein Assay Kit) and the assay duration (120 min).

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TABLE 1 PROBE SUBSTRATE COCKTAIL

CYP	Substrate	Assay concentration
1A	Phenacetin	10μM
2B6	Bupropion	10μM
2C19	Mephenytoin	50μM
2C9	Diclofenac	10μM
2D6	Bufuralol	10μM
3A	Midazolam	5μM

Western Blot

Cellartis Enhanced hiPS-HEP were thawed and plated in T75 flasks and maintained in culture for 8 days before being harvested as cell pellets for western blot analysis. The following antibodies and dilutions were used for detection of CYP450 enzymes and P450 reductase: CYP3A4 (1:1000, PAP 011 Cypex), CYP2C rabbit anti-human CYP2C C-terminal peptide (1:1000, kindly provided by Dr R Edwards), CYP2C19 (1:1000, HPA015066 SigmaAldrich), P450 reductase (1:1000, ab13513 Abcam, SigmaAldrich). Western blot analyses were performed using 800 x g supernatant from two batches of Cellartis Enhanced hiPS-HEP and 15 ug of protein per lane. Quantification was done against a pool of human liver microsomes.

ICC

Cellartis Enhanced hiPS-HEP were fixed 7 days after plating and stained with the following primary and secondary antibodies: rabbit anti-CYP1A2 (1:100, BML-CR3130, Enzo), rabbit anti-CYP2C9 (1:2500, PAP091, CYPEX), and rabbit anti-CYP3A4 (1:200, PAP011, CYPEX), donkey anti-rabbit Alexa Fluor 5948 IgG (1:1000, A21207, Invitrogen).

qRT-PCR

Total RNA from Cellartis Enhanced hiPS-HEP day 7 and 11 post plating was extracted using the MagMAX™-96 Total RNA Isolation Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA was synthesized and qRT-PCR amplification reactions were carried out in an Applied Biosystems 7500 Real-Time PCR System. Gene expression was analyzed using TaqMan® Gene Expression Assays (Applied Biosystems) according to the manufacturer's recommendations. Each sample was analyzed in duplicate and data was captured using the 7500 Real-Time PCR System Sequence Detector Software v1.4.0 (Applied Biosystems). Gene product measured by qRT-PCR were: CYP3A4 (Hs00604506_m1), CYP3A5 (Hs00241417_m1), and CYP3A7 (Hs00426361_m1).

Results

Cryopreserved Cellartis Enhanced hiPS-HEP express levels of CYP1A, 3A 2D6, 2C19, 2B6 and 2C9 activity within ranges also identified for cryoplateable human primary hepatocytes (cryo hphep) cultured for 20hr. CYP activities in Cellartis Enhanced hiPS-HEP are stable for 6 days between day 6 and 11 after thawing.

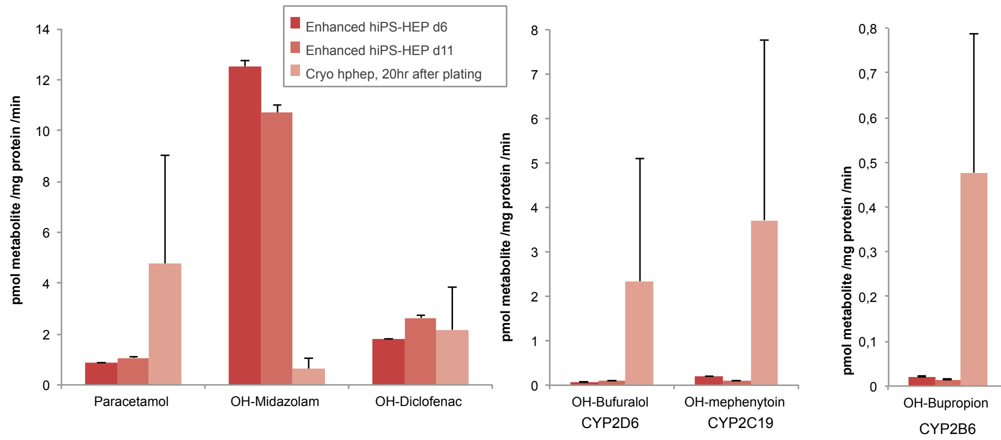

Figure 1. CYP activities in Cellartis Enhanced hiPS-HEP (6 and 11 days after thawing) and cryo hphep. Cellartis Enhanced hiPS-HEP: n=5 batches. hphep: n=4 different donors. Results are presented as mean ± SEM.

In Cellartis Enhanced hiPS-HEP, the adult enzymes CYP3A4 and 3A5 are expressed at levels comparable to cryopreserved human hepatocytes, whereas the fetal enzyme CYP3A7 is expressed at lower levels in Cellartis Enhanced hiPS-HEP than in cryopreserved human hepatocytes.

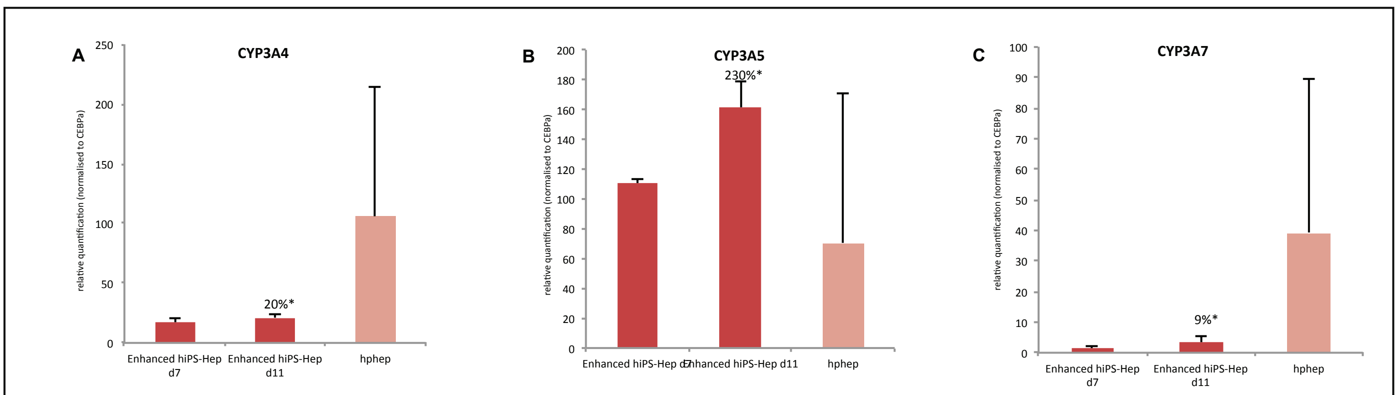

Figure 2. qRT-PCR analysis of CYP3A4, 3A5 and 3A7 mRNA expression in Cellartis Enhanced hiPS-HEP (7 and 11 days after thawing) compared to cryo hphep directly after thawing (0hr). Enhanced hiPS-HEP: n=3 batches. cryo hphep: n= 5 donors. * = % of hphep (0hr, directly after thawing).

Western blot analysis reveals expression of CYP1A, 3A4, 2C, 2C19, and P450 reductase in Cellartis Enhanced hiPS-HEP comparable to expression levels in human liver microsomes.

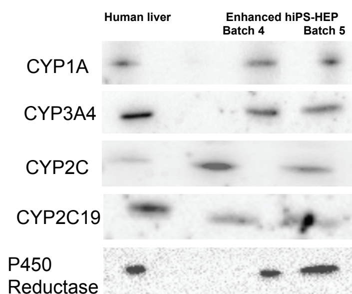

Figure 3. Western Blot analysis of CYP expression in Cellartis Enhanced hiPS-HEP and human liver microsomes. Two different batches of Enhanced hiPS-HEP were analyzed. Western Blot analyses were performed at Karolinska Institutet (Stockholm, Sweden).

Immunocytochemical stainings confirm CYP1A, 2C9 and 3A expression in Cellartis Enhanced hiPS-HEP.

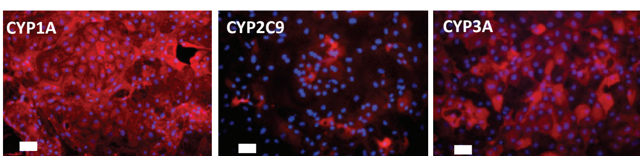

Figure 4. Immunocytochemical stainings for CYP1A, 2C9 and 3A in Cellartis Enhanced hiPS-HEP (7 days after thawing). All CYP immunostainings are merged with a nuclear counter-staining (DAPI). Scale bar = 50µm.

Batches of Cellartis Enhanced hiPS-HEP are consistent in homogeneity (Figure 5) and CYP function (Figure 6).

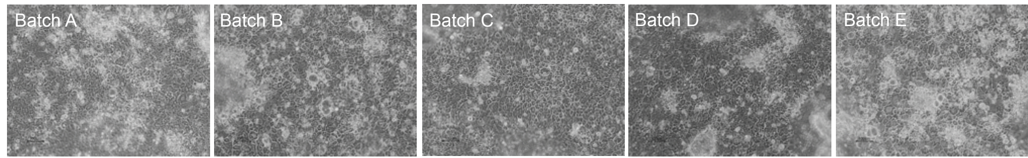

Figure 5. . Morphology pictures of 5 batches of Cellartis Enhanced hiPS-HEP (6 days after thawing).

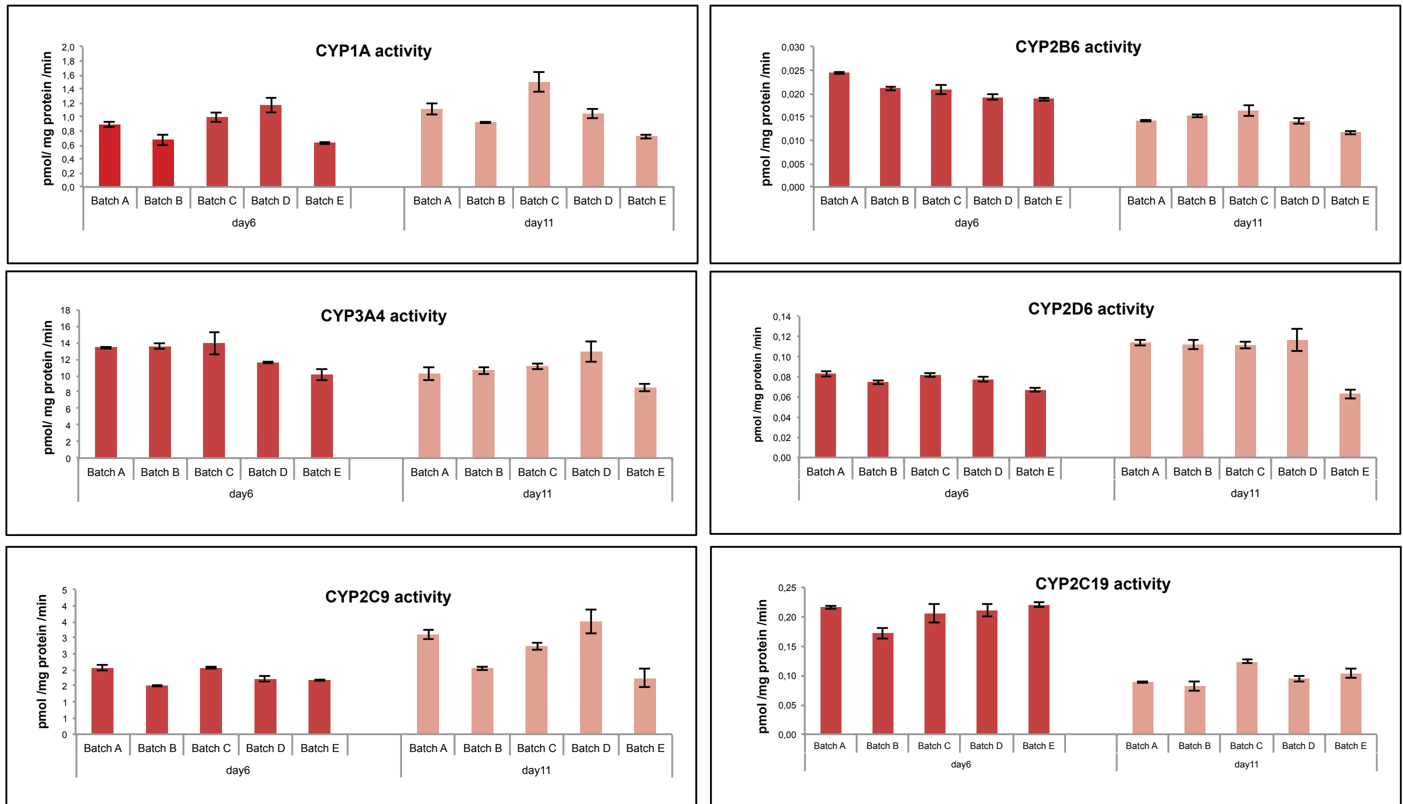

Figure 6. CYP activities in 5 batches of Cellartis Enhanced hiPS-HEP (6 and 11 days after thawing). For each batch, analyses were performed in triplicate. Data is presented as mean ± SEM.

Conclusions

Cellartis Enhanced hiPS-HEP express functional and relevant CYP enzymes detected both by enzyme activity, Western blot, immunocytochemistry and qPCR. CYP activity levels and cell homogeneity (> 90% pure hepatocytes) are consistent between batches. Cellartis Enhanced hiPS-HEP can serve as an unlimited source of human hepatocytes useful for applications that demand relevant hepatic CYP activity, a reproducible platform and continuous supply of material from the same genetic background.

Acknowledgement

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These data are part of ongoing activities in MIP-DILI to compare the quantitative evaluation of all test systems for drug safety evaluation in line with its primary objective (http://www.mip-dili.eu/).

Karolinska Institutet

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