Genetically modified human pluripotent stem cell derived hepatocytes display adult characteristics and functionality and are suitable as toxicity reporter

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ABSTRACT

Hepatocytes derived from human pluripotent stem cells have the potential to provide relevant human in vitro model systems for toxicity testing and drug discovery. Introduction of a GFP reporter fused to proteins sensitive for different mode of toxicity to human pluripotent stem cells will allow the generation of a toolbox for studying mechanistic toxicity in specialized cell types derived from human pluripotent stem cells.

In the present study, we differentiated genetically modified human embryonic stem cells exhibiting a GFP reporter fused to the actin filament, to hepatocytes (GFP-actin hES-HEP). The GFP-actin hES-HEP display several adult hepatic features e.g functional activity of drug metabolizing enzymes relevant for toxicity testing (e.g., CYP1A, 2C9, 2C19, 2D6, 2E1, and 3A4).The GFP-actin hES-HEP were exposed to troglitazone to induce toxicity and metformin a non-toxic drug for 48h. The GFP-actin filament rearrangement was assessed as toxic read-out by high content analysis, HCA.

The study proves that GFP-actin hESC-line can be differentiated to functional hepatocytes and used as a toxicity reporter. Importantly, using actin filament rearrangment as a toxic read-out showed that the GFP-actin hES-HEP sensitivity to trogliatzone was in the same range as primary human hepatocytes *(Ainscow E. et al 2008, AstraZeneca).*

Due to the functional cytochrome P450 enzymes and the GFP-actin reporter, GFP-actin hES-HEP are highly suitable for toxicity testing as well as other applications relying on an unlimited source of functional human hepatocytes.

METHOD

GFP-actin hES-reporter line

GFP-actin hES-reporter line was generated by mono allelic introduction of GFP under the actin promoter.

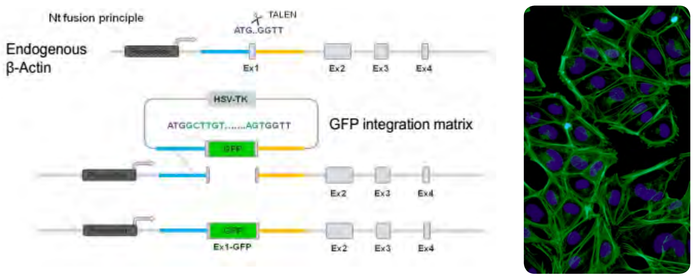


Figure 1. *Fusion of GFP to endogenous actin*

GFP-actin hES-derived Hepatocytes

GFP-actin hES-line was differentiated to hepatocytes (GFP-actin hES-HEP) for 30 days using Cellartis by Takara Bio Europe propriatory protocol. The protocol is robust and universal allowing differentiation of any hESC- or hiPSC-line to highly pure hepatocyte cultures (80-90% cells with hepatic morphology). The GFP-actin hES-HEP were characterised for functional hepatic phenotype by basal cytochrome P450 (CYP P450) activity by exposing cells for a substrate cocktail for 2h prior to metabolite formation measurement by LC/MS. Immunocytochemisty was performed to visualise expression and location of hepatic markers in the GFP-actin hES-HEP.

Toxicity Assay

GFP-actin hES-HEP were exposed to 8 doses (quater log) of the hepatotoxic drug troglitzone (top conc. 250µM) and the non-toxic drug metformin (1/2 log, top conc. 1000µM) for 48h prior to fixation and acquisition of images in a Scan R Olympus high content imaging microscope. GFP-actin rearrangement was used as toxicity read out measured by high content analysis.

GFP-actin rearrangement measurement:

- I_{nuc} , Intensity of green fluorescens in the inner mask (area between the inner circle and the nucleus)
- I_{cyto} , Intensity of green fluorescens in the outer mask (area between the outer and inner circle)

Read-out: Ratio $\frac{Inuc}{Icyto}$

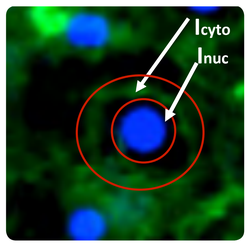


Figure 2. *Quantification of GFP-actin rearrangement in a cell by HCA*

RESULTS

Robust differentiation protocol generates CYP P450 competent hepatocytes

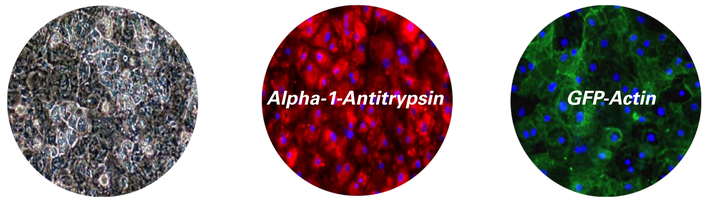


Figure 2. *GFP-actin hES-HEP express typical hepatic markers such as alpha-1-antitrypsin and the actin filaments are GFP-green.*

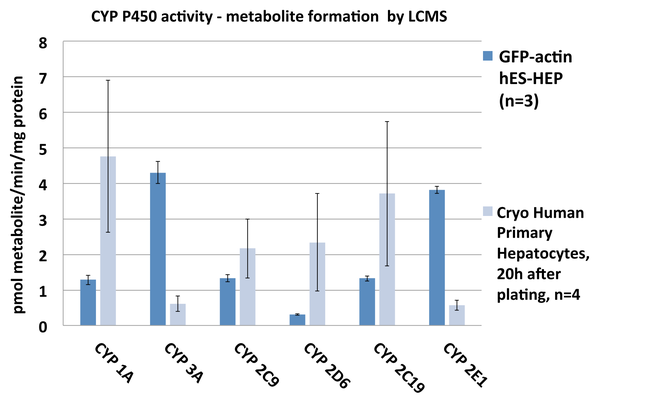


Figure 4. *CYP P450 activity of GFP-actin hES-HEP compared to cryopreserved human primary hepatocytes cultures 20h after plating. Data are presented as mean±SEM. Mean of 4 donors of human primary hepatocytes are presented, n=4.*

GFP-actin hESC line could be efficiently differentiated to hepatocyte cultures of which about 90% express hepatic markers such as HNF4alpha and alpha-1-antitrypsin and display CYP P450 activity in the same range as cryo human primary hepatocytes. Importantly, the variation is lower in GFP-actin hES-HEP compared to human primary hepatocytes.

GFP-actin hES-HEP can function as a toxicity reporter

GFP-actin hES-HEP responded to the drug troglitazone in a dose-dependant manner by GFP-actin rearrangement, while there was no response to the non-toxic drug metformin.The sensitivity of GFP-actin hES-HEP to troglitazone was in a similar range as for the golden standard, freshly isolated human primary hepatocytes, 72h in culture *(Ainscow E. et al 2008, AstraZeneca).*

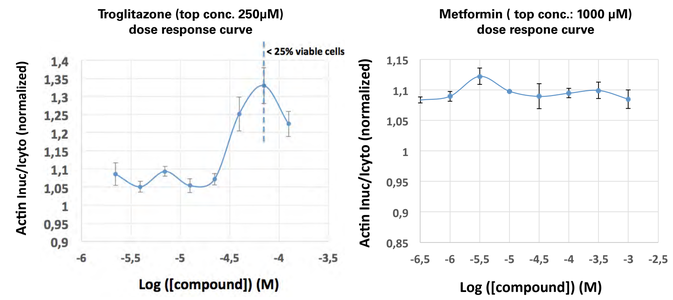
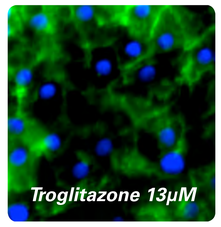
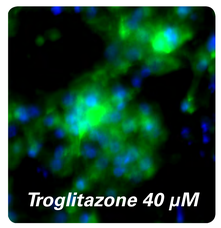


Figure 5. *Dose respons curves based on high conent analysis. GFP-actin hES-HEP day 30 of differentiation (n=4).*

GFP-actin hES-HEP





- Actin filament arrangment in GFP-actin hES-HEP at low dose of troglitazone (13 µM) and at toxic dose (40µM).
- Troglitazone increased the ratio (Inuc/Icyto) at 40µM in GFP-actin hES-HEP and at 63µM in human primary hepatocytes *(Ainscow E. et al 2008, AstraZeneca).*
- Data show that the sensitivity of GFP-actin hES-HEP to Troglitazone is in the same range as the golden standard, freshly isolated human primary hepatocytes, 72h in culture.

Figure 6. *High content images of GFP-actin hES-HEP at non-toxic and toxic doses.*

CONCLUSIONS

- Robust protocol allow efficient differentiation of GFP-actin hESC to hepatocyte cultures of high purity.
- GFP-actin hES-HEP display CYP P450 activity in the same range as cryo human primary hepatocytes.
- GFP-actin hES-HEP can function as a toxicity reporter, revealing GFP-actin rearrangement upon troglitazone induced toxicity.
- GFP-actin hES-HEP show sensitivity to troglitazone in the same range as human primary hepatocytes.

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