Detection of Xenobiotic-Induced Hepatotoxicity in Human iPSC-derived Hepatocytes 2.0

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Abstract

Hepatotoxicity is a leading cause of drug withdrawal from the market, and current preclinical models are not sufficiently predictive of drug effects in humans. Causes of hepatotoxicity range from intrinsic toxic effects to the enzymatic production of toxic metabolites. Development of more predictive in vitro model systems to identify hepatotoxicity early in the drug development is critical for decision making and to avoid Drug Induced Liver Injury (DILI) in the clinic. Human induced pluripotent stem cell (iPSC)-derived hepatocytes (iCell® Hepatocytes 2.0) that exhibit high purity and sustained biologically relevant functions help to address some of the needs of hepatotoxicity studies.

Here, we demonstrate the functional utility of iCell Hepatocytes 2.0 (HC 2.0) in assessing drug-induced hepatotoxicity. By evaluating HC 2.0 responses to a set of known hepatotoxins (i.e., amiodarone, acetaminophen (APAP), troglitazone, nefazodone, chlorpromazine, and FCCP) across a number of cell death readouts highlighting their capacity for mechanistic toxicology studies. In addition, the prolonged viability also enables chronic dosing in vitro affording the potential to detect the effects of slow to form metabolites and also perform analyses at physiologically relevant concentrations over protracted exposure periods. The short term high concentration sensitivities observed were comparable to those seen with primary human hepatocytes. However, effects seen over 48 hr and 7 day dosing are illustrative of the potential of HC 2.0 for predictive in vitro toxicity correlation. With the ability to routinely access patient specific genotypes and also culture in 3D spheroids and in coculture with other hepatic stellate cells, iPSC-derived Hepatocytes provide a biologically relevant human model system for investigating hepatotoxicity in preclinical drug development.

Hepatocyte 2.0 Characterization Data

iCell Hepatocytes 2.0 display characteristic hepatocyte morphology, including polygonal shape, evidence of polynucleation (blue circles) and the formation of bile canalicular channels (green arrows). HC 2.0 have been characterized for expression of the liver cell markers including albumin, α1AT, and HNF4α. These cells form tight junctions in culture as evidenced by the expression pattern for occludin staining. Basal P450 functions have been detected in the range of primary human hepatocytes and also express functional transporters (not shown). HC 2.0 are currently employed in ADME-T applications, organotypic culture systems, hepatitis infectivity modeling (HCV and HBV), malaria and NASH disease modeling.

The iCell Family of Hepatocytes

The current iCell Hepatocytes family of products derived from human iPSC cells offers three different stages of hepatocyte differentiation and maturity as cryopreserved cells ready to thaw and plate. iCell Hepatoblasts represent a bi-potential cell capable of differentiating into either a hepatocyte or cholangiocyte cell population. iCell Hepatocytes 2.0 and iCell Hepatocytes 1.0 are terminally specified hepatocyte populations. Functionally, HC 1.0 and 2.0 are very similar, with the main difference being that HC 1.0 is a more immature cell type that fully expresses albumin at the time of thaw, whereas HC 2.0 requires 5 to 7 days of culture to express both albumin and alpha-1-antitrypsin (α1AT) at high levels. All data in this poster was generated with Hepatocytes 2.0.

Hepatocyte 2.0 Characterization Data

iCell Hepatocytes 2.0 can be stably cultured for more than 2 weeks, which enables their use for evaluation of liver toxicity in vitro over short and longer term chronic drug exposure times. In this experiment, cells were treated with compounds and then assayed at earlier (day 7) or later (day 12) time-points. Cell viability was measured using CellTiter-Glo (Promega). Two key observations were made. First, iCell Hepatocytes could tolerate up to 0.5% DMSO (vehicle control) with 90% viability remaining at day 12. Second, longer exposure times usually resulted in more compound toxicity effects (lower EC50 values).

Summary and Conclusions

- The iCell Hepatocyte family of products provides multiple options for the study of hepatic biology
- iCell Hepatocytes 2.0 have characteristic hepatocyte morphology, marker expression and function
- iCell Hepatocytes 2.0 are useful for short and longer-term in vitro hepatotoxicity studies
- Three-dimensional (3D) spheroid cultures can be generated from iCell Hepatocytes 2.0 and miniaturized
- 3D cultures provide useful liver tissue-like model systems with increased metabolite function and drug sensitivities versus traditional 2D cultures