Predictive High-Content/High-Throughput Assays for Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Hepatocytes

Oksana Sirenko, Jayne Hesley, Emile Nuwaysir, Ivan Rusyn, and Evan F Cromwell

Molecular Devices, LLC, 1311 Orleans Drive, Sunnyvale, CA 94089, 1Cellular Dynamics International, 525 Science Drive, Madison WI 53711, 2University of North Carolina, Chapel Hill NC 27599

Introduction

Human iPSC-derived hepatocytes have been developed as a replacement for primary cells and show promise with respect to liver-like phenotypes, unlimited capacity, and a potential to establish cells from individuals who are prone/resistant to adverse drug reactions. Accordingly, there is great interest in using iPSC-derived hepatocytes as tools for screening in drug development. While unlimited supply of such cells from multiple donors addresses one common bottleneck (i.e., availability of cells), it is yet to be shown that iPSC-derived hepatocytes are amenable to high-throughput and high-content screening analyses. In this paper, we tested several automated screening approaches for assessing general and mechanism-specific hepatotoxicity using iPSC-derived hepatocytes. We found that multi-parametric automated image analysis greatly increases assay sensitivity while also providing important information about possible toxicity mechanisms. Specifically, for the in vitro screening of 240 compounds with a similar sensitivity of 60% with a specificity of 93% and predictivity of 75%. This superior to evaluation of cell viability endpoint only. We conclude that the high-throughput and high-content automated screening assays using iPSC-derived hepatocytes is feasible and can facilitate safety assessment or drugs and chemicals.

Methods

Cell Preparation

iPSC-Derived hepatocytes (iCell HEPATOCYTES) from Cellular Dynamics International (CDI) were plated according to their recommended protocol. Cells were plated at a density of 40K/well (96-well plates) or 100K/well (24-well plates) for 3 days. Then cells were treated with appropriate compounds for 72 hr.

Figure 1. Characterization of iCell Hepatocytes.

Multi-Parameter Cytotoxicity Assay

Multi-parametric image analysis can be used to monitor changes in cell viability (Calcein AM), nuclear shape (Hoechst), and mitochondrial integrity (MitoTracker Orange) associated with different types of toxicities. The ImageXpress Micro system allows automatic analysis on a cell-by-cell basis using the MetaXpress 5 software Multi-Well/A pitching (MWSC) module.

Figure 2. Top: iCell hepatocytes treated with Antimycin for 48 hours. Images taken with I0s objective and analysis with MWSC program. Response of various compounds of target mechanism of action analysis using valid cells. All EC50 values given in µM.

Phenotypic characterization of hepatotoxicity by quantification of the average and total stained cell areas.

Figure 3. Images of iCell Hepatocytes treated with compounds for 72 hr. Then stained with Calcein AM and Hoechst 33342. Exemplifies presented msop impact of different compounds on total positive cell and area stained compared to control.

Nuclear Characterization

Nuclear condensation is characterized by "decreased nuclear area" + "increased average intensity". This can provide additional sensitivity to toxicity.

Specific Toxicity Assays

Mitochondria Potential Assay

Mitochondria depolarization is an early signal for hypoxic damage or oxidative stress. Mitochondria membrane potential was monitored with the mitochondrial active dye JC-10. Data was analyzed using the MetaXpress 5 software Granularity module. This assay can be used either as an end-point or live-cell real-time assay.

Figure 5. Cell Hepatocytes treated with compounds for 72 hr. Cells stained with Hoechst (nuclei), and JC-10 (mitochondrial integrity). Images taken with I0s objective and analyzed with MetaXpress software Granularity module. Assays are shown in the bottom three. Concentration response curves and IC50 values are graphed on right.

Cytoskeleton Integrity

Cytoskeleton integrity was assessed by phalloidin staining. The parameters measured in this assay were total number of cells positive for actin staining, total positive cell area, or integrated fluorescence intensity. These measurements can be used for the characterization of concentration dependent hepatotoxicity response. The cytotoxicity integrity assay had an excellent assay window (W10-1, and low variance (S-value: 0.2).

Figure 6. Top: Images of Cell Hepatocytes treated with 30 µM of Atalocin B1 for 72 hr, then fixed and stained with Alexa Fluor 488 - Phalloidin and Hoechst 33342. Bottom: Concentration-dependent response for several compounds, showing number of actin positive cells as a read-out.

Autophagy & Phospholipidosis

Selection degradation of intracellular targets, such as misfolded proteins and damaged organelles is an important homeostatic function of the cell. In disease, autophagy may function as a survival mechanism by removing damaged organelles and toxic metabolites to maintain viability during periods of stress. Autophagic machinery can be manipulated to treat human diseases.

Phospholipidosis is a systemic storage disorder characterized by the excess accumulation of phospholipids in tissues. Many cytotoxic aromatic drugs, including anti-depressants, antimalarial, and cholesterol-lowering agents, are reported to cause drug-induced phospholipidosis (DIPL) in humans.

Figure 7. A: Autophagy Assay. Images of Cell Hepatocytes treated with compounds for 72 hr and stained with Cyto-ID Autophagy detection kit. Analysis with Granularity module is shown in the top row. B: Phospholipidosis Assay. Images of Control and Cell Hepatocytes treated with propylthiouracil for 48 hr. Phospholipidosis and autophagy detected using Oil Red O reagent showing phospholipid and neutral fats as an indicated. C: Concentration-dependent response using Total Granular Area output and corresponding EC50 values for phospholipidosis.

Compound Library Screening

Screen-Well™ Hepatotoxicity Library (ENDO) contains 240 compounds including anti-cancer, anti-inflammation, neuroleptic, antibiotics, and other classes. Compounds represent different mechanisms of hepatotoxicity: AT elevation, statins, phospholipidosis, mitochondria damage, etc. A multi-parameter hepatotoxicity assay (Calcein AM, MitoTracker, Hoechst) 72 hr for 5 compounds was used to screen toxicity of compounds in the library. In addition, mitochondrial potential/oxidative stress assay (JC-10) 60min was also used to increase overall predictivity.

Figure 8. Compound Library Screening.

Analysis Sensitivity by Compound Class

• Assay Sensitivity “hits” based on number of parameters deviating more than twice from DMSO controls.
• Number of compounds in group shown in parentheses.

• High predictivity was found for many classes of compounds, e.g. neuroleptic, anti-cancer, cardiac drugs, toxins.
• Low predictivity was observed for anti-inflammatory, antibiotics, and anti-viral drugs.

Summary

• Live-cell assays using the ImageXpress Micro XL High Content Imaging System with human iCell Hepatocytes can measure the impact of pharmacological compounds on hepatocyte viability and intrinsic hepatocyte functions.
• Multi-parametric read-outs allow simultaneous assessment of viability, membrane permeability, lipid accumulation, cytoskeleton integrity, and mitochondrial depolarization in live cells and increased assay sensitivity.
• We demonstrate utility of these in vitro assay models for toxicity screening and understanding potential hepatotoxic effects early in the drug development process.