Advancements in the Use of iPS Cell-Derived Systems for In Vitro Disease Modeling and Phenotypic Screening

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Abstract

Cellular Dynamics International (CDI) is the world’s largest producer of fully functional, terminally differentiated cell types derived from human induced pluripotent stem (iPS) cells. The quality, quantity, and purity of iCell® Cardiomyocytes, Endothelial Cells, Hepatocytes, and Neurons have been the driving force for adoption of this technology in the scientific community. The use of iCell products has helped to overcome many limitations of current in vitro cellular models, including limited supply, culture instability, poor representation of the disease state, and genetic background variability. Such advantages are illustrated in a rapidly growing list of publications highlighting the utility and predictivity of iCell products for various high throughput screening (HTS) applications.

To demonstrate the impact of iCell products in the drug discovery and development space, we present here examples of assay miniaturization, transfection optimization, and high content imaging-based phenotypic assays. With respect to disease modeling, we have utilized iCell Cardiomyocytes to simulate cardiac hypertrophy in vitro with a diverse array of endpoint readouts – including analysis of both gene expression and protein production of the biomarker, BNP. We have also optimized the delivery of siRNA oligonucleotides into iCell Neurons to develop unique systems for modulation studies at the gene-specific level.

Finally, we share a published case study where researchers at GlaxoSmithKline screened a focused chemical library against iCell Neurons for compounds that blocked Aβ1–42 toxicity (Xu, X., et al. 2013. Stem Cell Res. 10, 213–227). This example, in addition to the other work presented here, provides an excellent paradigm for how iPS cell-derived terminal cell types offer a high level of consistency from experiment-to-experiment that is both scalable and on par with the complex human biology for which they are able to recapitulate. Implementation of iCell products into routine workflows should both accelerate the understanding of and yield more predictive information on drug activity in the human body.

Phenotypic Screening and Cardiac Hypertrophy

The HTS community is constantly striving to combine new technologies with advanced cellular systems and simplified assay workflows. However, the availability of physiologically-relevant cell-based assays or disease-specific models that accurately represent the human condition in sufficient quantity, quality, and purity needed for a drug discovery campaign is severely lacking. Featured here is an in vitro disease model of cardiac hypertrophy using iCell Cardiomyocytes, as well as the development of a phenotypic assay in 384-well format that is suitable for screening. Understanding and manipulating the many complex mechanisms underlying cardiac hypertrophy has enormous therapeutic impact for cardiac dysfunction and heart disease.

Hallmark characteristics and widely-accepted biomarkers of the hypertrophic disease response in cardiac cells include:

1. Increased cell size
2. Enhanced protein synthesis
3. Structural reorganization of contractile proteins
4. Re-activation of the fetal gene program
5. Expression of B-type natriuretic peptide (BNP)

Monitoring Cell Size Changes in Real Time

Real-time impedance-based detection on the xCellLigand system (96-well format) can be used as a surrogate measurement for changes in cell size (or shape) following induction of hypertrophy with ET-1.

Re-expression of the fetal gene program occurs during an induced hypertrophic response. The levels of NPPB increase in an ET-1 dose-dependent manner.

HTS-Compatibility

Assay Miniaturization was demonstrated with iCell Hepatocytes seeded in a variety of cell culture plates, from 6-well down to 1536-well, and fluorescently stained to show equivalent cell morphology across the different formats. CDI has found that consistent cell handling protocols for the iCell Products translates to the expected viability, functionality and performance in downstream assays. Orange (MitoTracker) and Blue (Hoechst 33342). Scale bar = 50 μm.

Monitoring pathway signaling events in live cells was accomplished via transfection of plasmids (Promega) into iCell Cardiomyocytes after 4 days in culture, stimulated with isoproterenol (β2-adrenergic agonist) overnight, and then luciferase activity was measured. Robust responses were obtained in both proximal and reporter gene assay readouts. CRECUMP response element

iCell Products are amenable to many other key HTS applications

Ca2+ Handling FLIPR, 96- and 384-well (Molecular Devices)
Metabolism XF Analyzer, 96-well (Seahorse Biosciences)
Field Potential Maestro MEA, 48-well (Axion BioSystems)

Relevant Neuronal Model for Alzheimer’s Disease


Researchers at GSK used iCell Neurons to establish a cellular model of Alzheimer’s Disease. Neuronal loss was induced by exposure of the cells to an insult of Aβ1–42 aggregates. CDK2 was validated as an important signaling target for rescue of toxicity using known inhibitors and shRNA against CDK2. This model system was further utilized to identify novel modulators of this neurodegenerative disease in a focused drug screen.

Sensitivity of iCell Neurons to Aβ1–42 was demonstrated using a cell viability assay and high content imaging of neurite outgrowth.

RNAi as a Tool for Discovery

Genetic manipulation techniques in primary neuronal cultures are especially inefficient and often toxic. In fact, neurones are considered one of the most difficult and resistant cell types for introduction of siRNA oligos. We have successfully utilized the Accell siRNA reagents (Thermo Scientific) to knockdown target gene expressions measured by quantitative real-time PCR (qPCR).

These data lay the foundation for downstream investigation into the roles of specific genes in neuron development and functionality.

Summary

CDI’s core competencies are the reprogramming, engineering, and differentiation aspects of iPS cell technology. However, in order to help promote the use of iCell Products to routine laboratory workflows, the benefits and utility of using these human cell types must be demonstrated. The data presented in this poster illustrate such advantages, including a scalable and consistent cell source, compatibility with a variety of HTS formats and applications, high quality data with robust and reproducible results, and an ideal system for phenotypic screening. Furthermore, these examples highlight the potential new opportunities that iCell Products create for drug screening efforts in the future.

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