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USE OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES TO UNDERSTAND MECHANISMS OF CARDIOTOXIC COMPOUNDS

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Human pluripotent stem cell technology has the potential to overcome inefficiencies in early drug screening and clinical testing by allowing researchers to use human cells that reflect a variety of genetic backgrounds in assays that require minimal sample and test compound. Here we provide information about current practices and brief examples of the use of pluripotent stem cells in cytotoxicity testing using assays to detect mechanisms of cytotoxicity, such as apoptosis.

Introduction

The discovery and development of novel therapeutics continues to be an arduous and expensive process that is wrought with inefficiencies due to lack of appropriate tools that accurately predict the human physiological response. We see at least three barriers to increasing the efficiency of the drug discovery and development process. First, ascertaining potential off-target toxicity is often accomplished through the use of nonhuman animal models, and translating the results of such experiments to potential human toxicity is a challenging and imperfect science. A second hurdle is that, early in the screening process (prior to scale up), test compounds are available only in relatively small amounts for performing the important safety studies that are necessary before proceeding to expensive preclinical animal models. Last, the relatively small sample size of early clinical trials (usually between 20–50 patients) typically does not include rare but potentially relevant genetic backgrounds. Human pluripotent stem cell technology offers the potential to reduce the burden of each of these factors and thus decrease both the time and cost of bringing new therapeutics to market.

The advent of induced pluripotent stem cell (iPS) technology increases the utility of stem cell-derived experimental models, and negates many of the ethical concerns surrounding embryonic stem cells.

Stem cells are pluripotent cells that give rise to all the cells of the body and in the laboratory provide the potential to generate any cell type of interest in vitro. Embryonic stem cells have already been used to generate fully differentiated cells suitable for both drug discovery and toxicity testing (1). Furthermore, stem cells are amenable to automated cell culture and can provide a potentially limitless supply of any cell type, reducing the need for animals as test material. The advent of induced pluripotent stem cell (iPS) technology, whereby fully differentiated cells are induced to return to a progenitor state, makes it possible to generate any cell type from any genetic background, increases the utility of stem cell-derived experimental models, and negates many of the ethical concerns surrounding embryonic stem cells. This article will provide brief examples of current practices and future directions for the use of stem cells in cytotoxicity testing.

Screening for Cardiotoxicity Using hPSC-Derived Cardiomyocytes

Cardiovascular disease is a leading cause of death worldwide and thus is an important therapeutic area. However, off-target cardiac toxicity is also the most common cause of regulatory delay in approval and market withdrawal of new pharmaceuticals. Human pluripotent stem cell (hPSC)-derived cardiomyocytes display normal cardiac characteristics, survive under cell culture conditions for extended periods and thus hold the potential to serve as a human-based model for both drug development and cardiotoxicity screening (2). The electrical activity and controllable environmental conditions of hPSC-cardiomyocytes provide an ideal model for both drug development and toxicity testing. While the use of hPSC-cardiomyocytes in drug development is on the near horizon, their use as a cardiotoxicity screening tool is occurring now.

Action potentials are the rhythmic electrical oscillation of cardiac myocyte membrane potential that underlie the heartbeat and basic cardiac function. The action potential waveform results from ions crossing the plasma membrane through a variety of ion channels, the dysfunction of which can lead to altered cardiac rhythm. Cardiac ion channel dysfunction arises from genetic mutations and/or interactions with drug compounds. Dysfunction resulting in action potential prolongation is of particular concern as it can lead to ventricular arrhythmias and sudden death. The most common cause of cardiac action potential prolongation is drug block of the human ether a'go-go (hERG) channel.

Different species express different types of cardiac ion channels as well as differing relative levels of each channel type. Therefore different toxicity assessments could be drawn from applying the same compound to cardiomyocytes from different species. As shown in Figure 1, hPSC-cardiomyocytes allow for an early indicator of drug-induced ion channel dysfunction in a human cardiomyocyte. Single hPSC-cardiomyocytes were subjected to perforated voltage clamp analysis. Action potentials were recorded from single

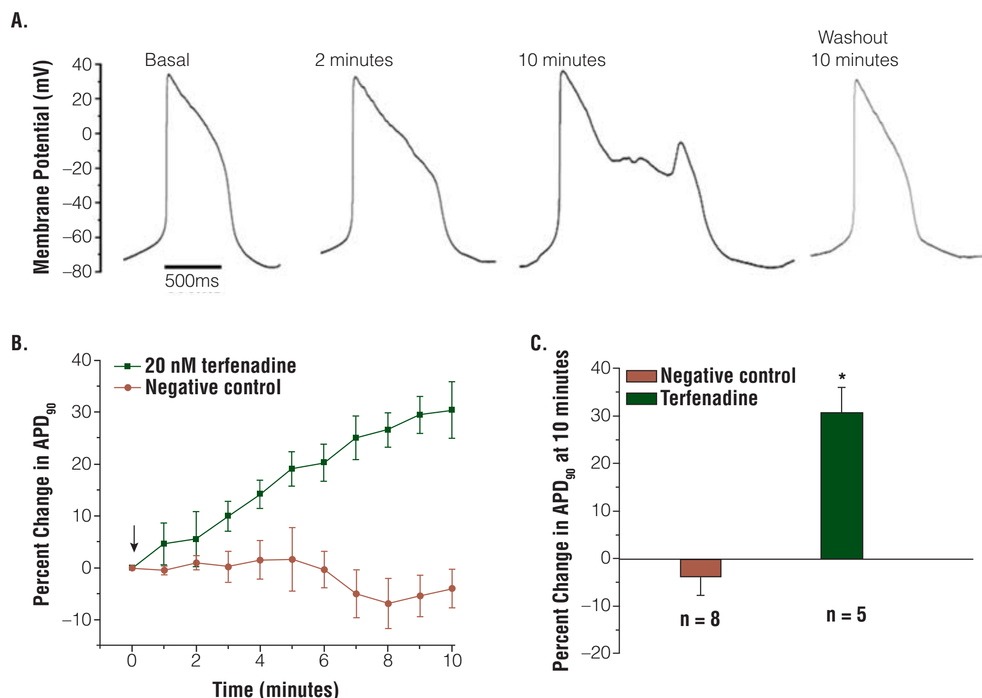

Figure 1. The effects of terfenadine on hPSC-derived cardiomyocyte action potential duration. Representative perforated voltage clamp recordings from a single hPSC-derived cardiomyocyte before, during, and following washout of terfenadine (**Panel A**). The mean percent increase in APD90 throughout perfusion with terfenadine (**Panel B**) and at 10 minutes of perfusion (**Panel C**).

cells under basal conditions and in the presence of terfenadine, a nonsedating antihistamine that was pulled from the market in 1998 for its potent hERG block and potential to cause sudden cardiac death. Figure 1, Panel A shows representative voltage clamp recordings of cardiac action potentials under basal conditions, during perfusion with 20 nM terfenadine, and following compound washout. Myocytes show action potential waveforms typical of ventricular hPSC-cardiomyocytes under basal conditions. Addition of 20 nM terfenadine prolongs action potential duration (2 minutes post drug addition) and can lead to secondary premature depolarizations termed early-after depolarizations (EADs; 10 minutes post drug addition), which are considered a potential trigger for life-threatening cardiac arrhythmias. Upon drug washout the action potential returns to its basal phenotype. The mean increase in action potential duration at 90% of repolarization (APD90) as a function of drug exposure duration is shown in Figure 1, Panel B, while the mean increase in APD90 at ten minutes of drug exposure is shown in Figure 1, Panel C.

Assessing Other Toxicities Using hPSC-Derived Cardiomyocytes

Recent events have demonstrated that cardiac cells are also susceptible to other types of toxicities (3). hPSC-cardiomyocytes are readily amenable to assessing additional or alternative toxic endpoints such as cell viability, apoptosis,

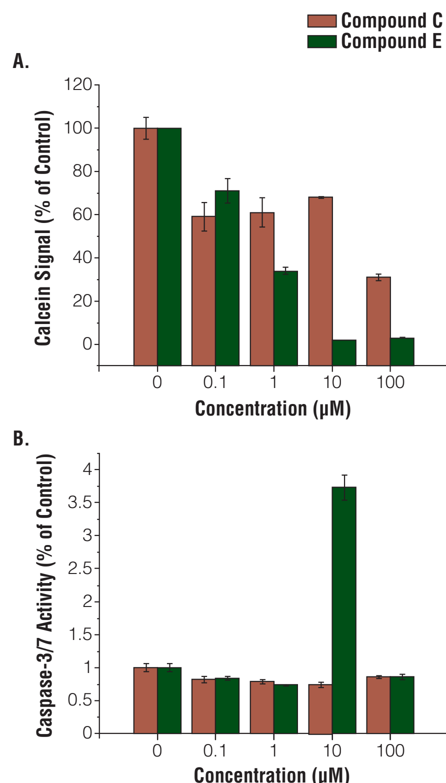

Figure 2. The effects of two experimental compounds on hPSC-derived cardiomyocyte viability and apoptosis. Cell viability and apoptosis were measured with the Live/Dead Viability/Cytotoxicity Kit (Invitrogen) and the Caspase-Glo® 3/7 Assay (Promega), respectively. Values are expressed as a percent of control.

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ATP production/metabolism, and mitochondrial dysfunction through readily available test kits. Figure 2 illustrates an example where hPSC-cardiomyocytes were used in basic cell viability and apoptosis assays. Cells were incubated for approximately 96 hours in the presence of increasing concentrations of two experimental compounds, and cell viability was assessed with the Live/Dead Viability/Cytotoxicity Kit (Invitrogen Cat. #: L-3224-1000). Both compounds significantly reduced cell viability (Figure 2, Panel A), while the negative control did not. Results from additional experiments examining the underlying toxic mechanism are shown in Figure 2, Panel B. Cells were incubated in increasing concentrations of experimental compound or positive control for approximately 24 hours, and apoptosis was assessed by measuring caspase-3/7 activities with the Caspase-Glo® 3/7 Assay (Promega Cat.# G8091). Incubation with Compound C did not significantly change caspase activity while Compound E caused a large increase in activity at 10 µM, indicative of apoptosis. The observed decrease in activity at 100 µM of Compound E is presumed to be a result of nearly complete cell death. In this example, both experimental compounds showed decreases in cell viability; however, only one compound resulted in apoptotic events. Here relatively simple experiments from an abundant supply of human cells enabled the rapid elucidation of compound-specific toxic mechanisms. These two examples show how hPSC-cardiomyocytes can provide a single, human-based model that is capable of assessing electrophysiological, biochemical, and viability cytotoxic endpoints under acute and longer-term chronic exposures.

Future Directions and Summary

Manual techniques are currently employed for the majority of stem cell culture methods. New automated methodologies for cell maintenance, screening, and directed differentiation are in development. Automated splitting and feeding of undifferentiated cells is expected to produce nearly unlimited amounts of pluripotent stem cells. Coupled with the nearly unlimited differentiation potential of these cells, automated directed differentiation protocols will generate sufficient amounts of cell types for use in large-scale screening, toxicology testing and drug discovery processes.

There are currently a limited number of stem cell lines available for the in vitro generation of differentiated cell types. The onset of iPS cell technology overcomes this obstacle and removes nearly all the ethical implications associated with using embryonic tissue. iPS cell technology offers the promise of generating stem cells from any individual and ultimately can provide differentiated cell types of any genotype. The developing iPS cell technology and automated culturing techniques will enable in vitro testing of pharmaceuticals for both efficacy and toxicity over any individual genetic makeup and truly bring the potential of personalized medicine to the clinic.

References

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3. Chu T.F. *et al.* (2007) *Lancet* **370**, 2011–9.

Ordering Information

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	2.5 ml	G8090
	10 ml	G8091
	10 × 10 ml	G8093
	100 ml	G8092

For Laboratory Use.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information. Caspase-Glo is a registered trademark of Promega Corporation.

PROMEGA PRODUCTS IN STEM CELL RESEARCH

Induction of pluriopotent stem cells from adult human fibroblasts by defined factors

Takahashi, K. *et al.* (2007) *Cell* **131**, 861–72.

The authors of this paper describe the generation of induced pluripotent stem (iPS) cells from human dermal fibroblasts. STR analysis using the PowerPlex® 16 System showed that patterns of 16 STRs in the clones matched the parent cell line. Luciferase assays to assess activity of the OCT3/4 and Rex1 promoters were performed using the Dual-Luciferase® Assay System.

Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts

French, A.J. *et al.* (2008) *Stem Cells 2008*, 485–93 .

Somatic cell nuclear transfer technique was used to generate human blastocyst-stage embryos using nuclei from adult male fibroblasts cell lines and enucleated oocytes. Genomic DNA was analyzed using the PowerPlex® 16 system to confirm the genetic identity of the blastocyst cells.

High-throughput identification of genes promoting neuron formation and lineage choice in mouse embryonic stem cells

Falk, A. *et al.* (2007) *Stem Cells* **25**, 1539–45.

These authors developed a strategy for screening large numbers of genes that influence the pluripotency and differentiation of embryonic stem cells to specific fates. A plasmid expression library was grown in deep-well plates, 32 clones were pooled and the plasmid isolated using the PureYield™ Plasmid Midiprep System. The purified plasmid DNA was used to transfect E14 ES cells and measure expression of a specific cell fate reporter construct.