Assessing functional and structural cardiotoxicity in cultured human iPSC-cardiomyocytes in a single plate format

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Abstract

A comprehensive profiling of cardiotoxicity early in drug discovery and development can aid in reducing late-stage attrition and establishing risk-mitigation strategies during clinical development. In most cases, multiple assay platforms and instrument-specific plate formats are required for this type of approach. In this study, we evaluated both functional and structural endpoints associated with cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) cultured in a single 384-well plate. We measured intracellular Ca²⁺ transits, caspase 3/7 activity, and plasma membrane permeabilization sequentially in the same plate via a series of assay readouts. A set of cardiac ion channel modulators (dofetilide, sotalol, nilutamide, and mexiletine) and chemotherapeutics (tamoxifen, doxorubicin, and vincristine) was tested at clinically relevant concentrations for effects on intracellular Ca²⁺ transits after a short-term (30 minutes) exposure, and plasma membrane permeabilization and caspase 3/7 activation after a long-term (72 hours) exposure. Intracellular Ca²⁺ transits were monitored by fluorescent images taken with a high-speed camera in beating cardiomyocytes loaded with Fluo-3 AM, and plasma membrane permeabilization and caspase 3/7 activation after a long-term (72 hours) exposure. 

Introduction

Cardiotoxicity is frequently a dose-limiting toxicity associated with many chemotherapeutics, which include both classic cytotoxic or cytostatic agents, such as doxorubicin or other anthracycline analogs, and newly developed targeted anti-cancer molecules such as protein kinase inhibitors (i.e. sunitinib, dasatinib and nilotinib). As this adverse effect can be manifested by either structural damage (i.e. cardiomyopathy and heart failure) or functional alteration (i.e. arrhythmia and sudden cardiac death), evaluation on risk to induce both structural and functional cardiotoxicity should be included in preclinical safety profiling of new anti-cancer drug prior to the first dose in human.

Human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) represent a novel cellular model system to test for cardiotoxicity and are being used increasingly with a wide variety of analytic platforms in study of cardiac biology and drug safety testing. In this study, we developed an image-based, multiplex assay that enables interrogation of both functional and structural toxicity endpoints in a single plate format.

Methods & Materials

Cells:

Cryopreserved PSC-cardiomyocytes were provided by Dr. Joseph C. Wu and Stanford Cardiovascular Institute (SCVI) Biobank.

Reagents:

RPMI 1640, 20% Matrigel (Fisher/Corning), B27-insulin, DMEM/F12 (Gibco/Life Sciences); Accutase (Sigma); Cal-520® Ca²⁺ dye (AAT Bioquest); DRAQ™ DNA dye (abcam), Caspase-Glo 3/Assay kit (Promega); Doxilide, sotalol, nilutamide, mexiletine, tamoxifen, nilotinib, sunifonib and abtinabin (NCI Compound Repository)

Biomarkers:

Ca²⁺ transits: contractile function, repolarization-delay, arrhythmia DNA stain: permeabilization of plasma membrane (cell death) 

Caspase 3/7 activity: apoptosis activation

Results

Ca²⁺ transits of beating cardiomyocytes: Figure 1. Ca²⁺ cycling imaged at 31 fps from a single view-field

Figure 2. Ca²⁺ transits analyzed by CYBERnano i-Cardo

Figure 3. Representative traces of typical effects on Ca²⁺ transits

Figure 4. Beat variability analysis on Poincare plots of CTD90

Table 1. Summary of effects on Ca²⁺ transit parameters

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Figure 5. Representative images of nuclear stains

Figure 6. Caspase 3/7 activity

Discussion & Conclusion

Ca²⁺ transits were sensitive to ion channel modulators, with changes in beat rate and Ca²⁺ transits amplitude; EADs, beat-to-beat variability and triangulation of CTD were more specific than Ca²⁺ lengthening to predict arrhythmogenesis

Caspase 3/7 activity was a sensitive indicator of insults to hPSC-CMs but increased nuclear stains of impermeable DNA dye was more robust to label structural cardiotoxicity

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