Assessment of the Mechanism of Cytotoxicity Using iPSC-derived Cardiomyocytes and Bioluminescent Assays

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1. Abstract
Assessment of the mechanism of cytotoxicity in vitro requires the measurement of multiple parameters such as cell viability, apoptosis, and necrosis. Here we present data generated using a system consisting of human induced pluripotent stem cell derived cardiomyocytes (iCell® Cardiomyocytes, Cellular Dynamics International) and bioluminescent assays. iPSC-derived cells are an excellent experimental model for assessing cytotoxicity as these stem cells can generate many differentiated cell types as well as genetically diverse cells. There are multiple bioluminescent assays that address mechanisms of cytotoxicity: i) reporter gene assays for pathway analysis, ii) ATP concentration assays for viable cell counts, iii) enzymatic activity assays based on pro-luciferin substrates, and iv) live-cell biosensors based on engineered luciferases. The power of bioluminescence is realized in robust, dynamic, and simple to use assays that reflect complex biological events. Such assays can be miniaturized to a 384-well format. The iCell® Cardiomyocytes provide high purity, uniform layers, and ease of handling with a simple and fast (3 – 7 days) protocol. The data presented show that bioluminescent assays for viability, apoptosis, oxidative stress, HDAC state and proteasome function, used with iPSC-derived cardiomyocytes, are an ideal mammalian cell model system for sensitive analysis of the mechanisms of cytotoxicity.

2. Biomarkers and the Cytotoxic Response
The cytotoxic phenotype is shaped by: 1. Dosage 2. Exposure time 3. Cellular susceptibility
No single parameter assay can fully characterize cytotoxicity

3. Bioluminescent Assays from Firefly Luciferase

Measure (luciferase)
- Reporter gene assays

Measure (ATP)
- Cell proliferation, viability
- Kinase Assays

Measure (luciferin)
- Enzymes coupled to proluciferin-luciferin conversion

Measure activities of engineered luciferases
- Live cell biosensors

4. Cell-based Assays using iPSC-derived Cardiomyocytes

Day 1: Thaw and Plate iCell® Cardiomyocytes

Day 2-3: Change Medium

Day 2-6: Begin Drug Incubation

Day 3-7: Perform Assay

5. iPSC-derived Cardiomyocytes: Receptive Test System

6. CellTiter-Glo Assay Miniaturized to 384-well plates

Measure [luciferase]
- Reporter gene assays

Measure [ATP]
- Cell proliferation, viability
- Kinase Assays

Measure [luciferin]
- Enzymes coupled to proluciferin-luciferin conversion

Measure activities of engineered luciferases
- Live cell biosensors

7. iPSC-derived Cells Are Responsive Across Assays

8. iCell® Cardiomyocytes & Mechanistic Insights

9. Summary and Conclusions
- iCell® Cardiomyocytes are an ideal mammalian cell model system for analysis of mechanism of cytotoxicity
- Cardio toxicity assays can be miniaturized to 384-well format
- Promega provides easy to use assays for mechanistic assessments of toxicity including:
  - Viability
  - Cytotoxicity
  - Apoptosis
  - Oxidative Stress
  - HDAC State
  - Proteasome Function

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