iPSC-Derived Cardiomyocytes and Luciferase Reporters: A Robust Reporting Platform for Monitoring Cardioprotection and Pathway Biology in Endogenous Human Tissue Cells

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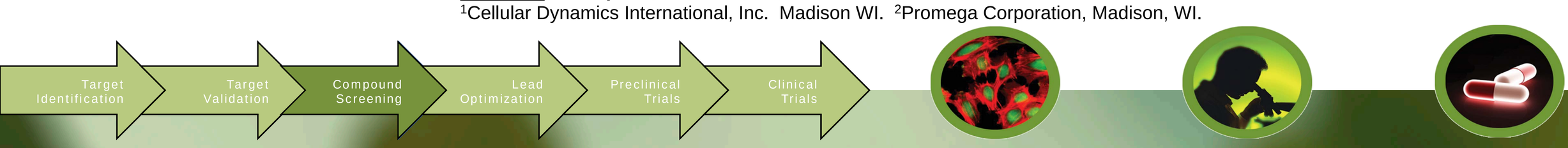


Abstract

Pathophysiological conditions, medical interventions, and off-target toxicities can all result in cellular oxidative stress. In cardiac myocytes, prolonged and/or excessive oxidative stress can lead to cardiotoxicity: a primary cause of developmental delays, black-box warnings, and post-launch withdrawal of pharmaceuticals. Many protective responses to oxidative stress are mediated at the transcriptional level through antioxidant response elements (AREs). iCell® Cardiomyocytes (Cellular Dynamics International) are fully functional cardiomyocytes derived from human induced pluripotent stem cells (iPSCs). The ability to monitor ARE activity in this relevant tissue cell would provide an excellent tool for both interrogating and enhancing cardioprotective processes through basic experimental and screening paradigms, respectively. To that end, a novel ARE luciferase reporter construct, pGL4[luc2P/NRF2/Hygro] (ARE-luc: Promega) was transiently transfected into iCell Cardiomyocytes and functionally validated by inducing oxidative stress through application of tertiary butylhydroquinone (tBHQ) and monitoring increases in luciferase activity. Control experiments with GFP demonstrated that lipid-based methodologies could introduce the plasmid into the terminally differentiated iCell Cardiomyocytes with greater than 40% transfection efficiency with minimal optimization. Transfection of ARE-luc into iCell Cardiomyocytes and subsequent incubation with tBHQ produced increases in luciferase activity in a dose dependent manner. Similarly, luciferase-based CRE and NFAT reporters acted as a surrogate readout for GPCR modulation by generating dose dependent increases in luciferase activity upon transfection into iCell Cardiomyocytes and stimulation with isoproterenol. Together, these data demonstrate that a variety of luciferase-based reporters are easily transfected into iCell Cardiomyocytes. The combination of a nearly-limitless supply of relevant human tissue cells and a robust reporter system promises great utility for pathway analysis for both basic and applied research endeavors.

Introduction

iCell Cardiomyocytes are human iPSC-derived cardiomyocytes that recapitulate the biochemical, electrophysiological, mechanical and pathophysiological properties of native human cardiac myocytes. Due to their human origin, high purity, functional relevance, and ease of use, iCell Cardiomyocytes represent an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

Aims

- To assess the transfection efficiency of iCell Cardiomyocytes at different times in culture.
- To demonstrate the feasibility of using luciferase reporter vectors with iCell Cardiomyocytes.
- To assess the reporter activity at different times in culture in iCell Cardiomyocytes.
- To demonstrate the induction and suppression of ARE-driven gene expression in iCell Cardiomyocytes.

Methods

Cell Preparation:

iCell Cardiomyocytes were plated in iCell Cardiomyocytes Plating Medium at 20K cells/well in 96-well cell culture plates coated with 0.1% gelatin per manufacturer instructions. Cells were cultured in iCell Cardiomyocytes Maintenance Medium at 37°C, 7% CO_2. Medium was changed every 48 hours.

Transfection:

Medium was changed on the day of transfection. Lipofectamine® LTX & Plus Reagent was used. Plus reagent and pDNA were combined at a ratio of 1:1 in Opti-MEM® and incubated for 5 minutes at room temperature. Lipofectamine LTX reagent was added to the mixture at a ratio of 2:1 (Lipofectamine LTX:pDNA) and incubated for 30 minutes at room temperature. 25 µl Reagent:pDNA mixture was added to the cardiomyocytes in 100 µl maintenance medium for a final concentration of 0.4 µl LTX reagent, 0.2 µl Plus reagent, and 0.2 µg pDNA per well.
Cardiomyocytes were incubated at 37°C, 7% CO_2 for 18 hours. Medium was changed to a serum-free DMEM-based medium for 6 hours prior to induction.

Luciferase Reporter Vectors:

Pathway	Product
ARE	pGL4[luc2P/NRF2/Hygro] Vector
CRE	pGL4.29[luc2P/CRE/Hygro] Vector
NFAT	pGL4.30[luc2P/NFAT-RE/Hygro] Vector
CMV	pGL4.51[luc2/CMV/Neo] Vector

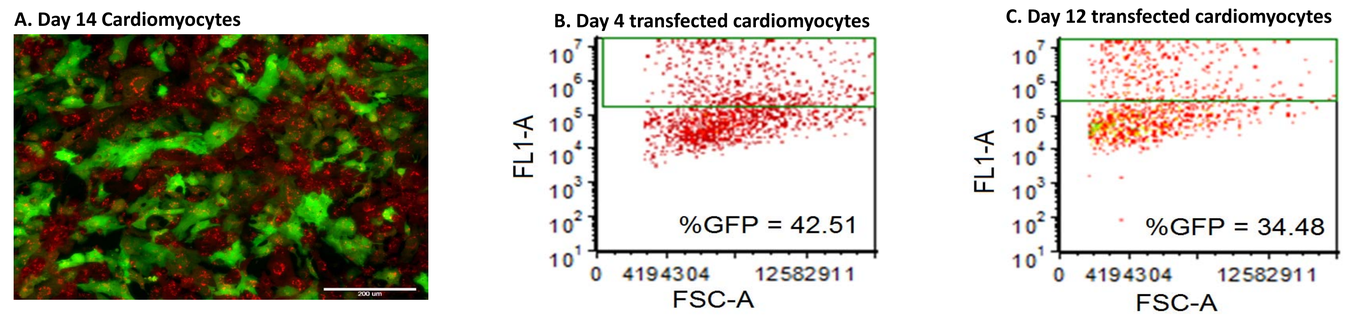
Summary

iCell Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes suitable for a wide variety of applications. Monitoring cell-signaling pathways in a human-based in vitro environment allows for extensive cellular analysis and increased understanding of drug toxicity.

iCell Cardiomyocytes are amenable to DNA transfection and exhibit the expected responses to the induction and suppression of various cell-signaling pathways.

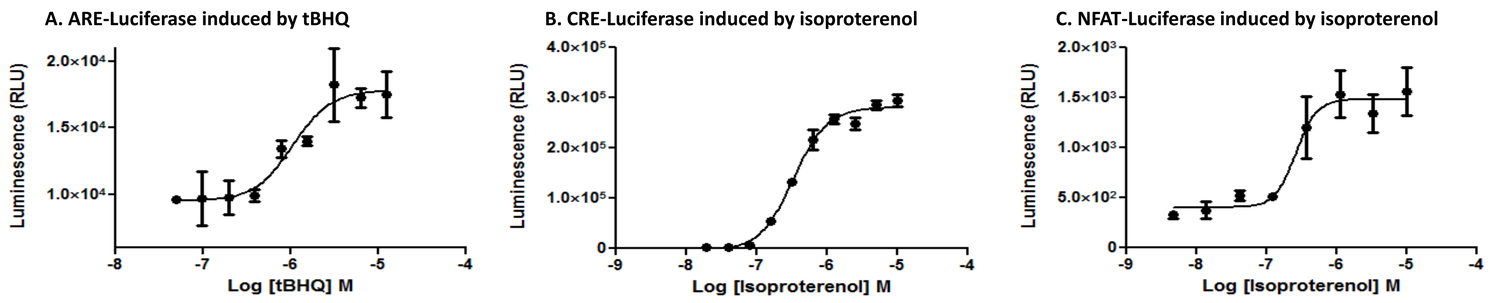
The use of luciferase reporter assays with iCell Cardiomyocytes in preclinical testing provides a means for detecting and isolating relevant pathways and targets in drug discovery as well as early detection of toxicity.

iCell Cardiomyocytes display similar levels of transfection efficiency when transfected at different times in culture



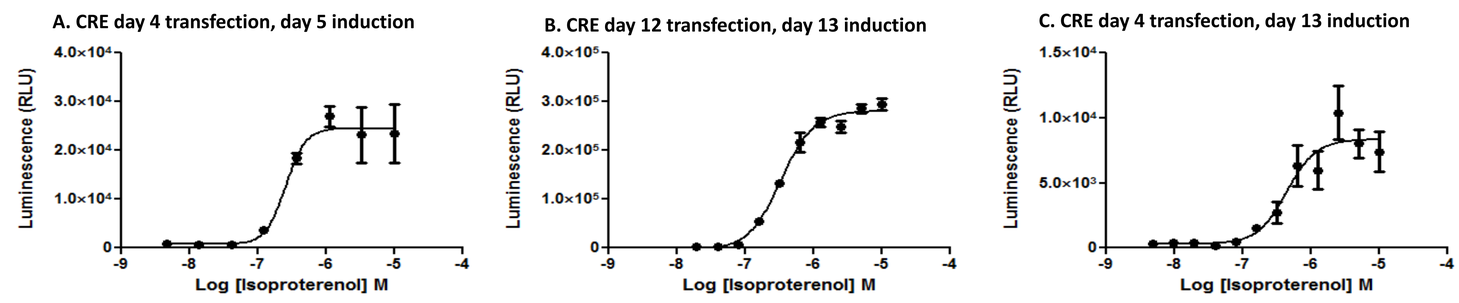
GFP transfection efficiency measured in iCell Cardiomyocytes transiently transfected on multiple days in culture. A: iCell Cardiomyocytes endogenously expressing RFP were transiently transfected with GFP 12 days after thaw and imaged after an additional 48 hours. GFP-positive cells expressed both RFP and GFP expression. **B-C:** GFP transfection efficiency was measured by FACS analysis 48 hours after transfection. GFP expression was gated off of live dead staining with propidium iodide. iCell Cardiomyocytes were transfected with GFP 4 (B) or 12 (C) days after thaw.

Transiently transfected iCell Cardiomyocytes exhibit reporter-driven luciferase activity in response to compound induction



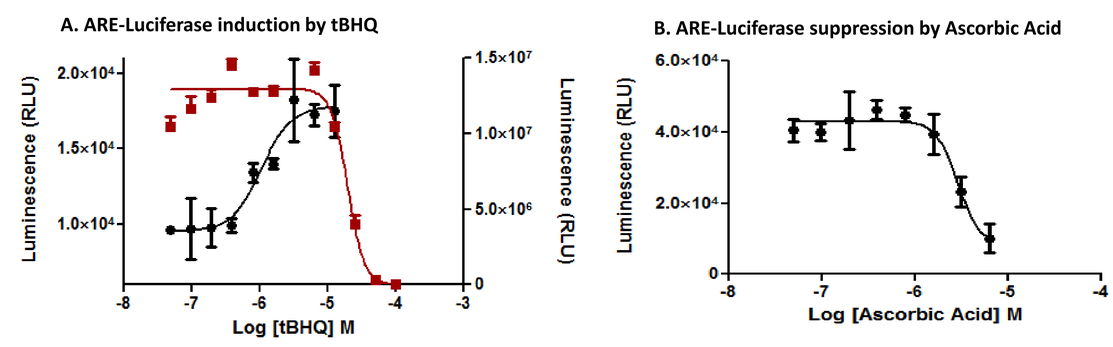
Compound-induced reporter-driven gene expression in transiently transfected iCell Cardiomyocytes. iCell Cardiomyocytes were transiently transfected with luciferase reporter vectors 12 days after thaw and 24 hours later compound s were added to induce reporter gene expression. Luciferase activity was assayed using the ONE-Glo™ Luciferase Assay System. **A:** iCell Cardiomyocytes were incubated with 1:2 serial dilutions of tBHQ for 6 hours to induced ARE-driven gene expression. **B:** iCell Cardiomyocytes were incubated with 1:2 serial dilutions of isoproterenol overnight to induce CRE-driven gene expression. **C:** iCell Cardiomyocytes were incubated with 1:3 serial dilutions of isoproterenol overnight to induce NFAT-driven gene expression.

Luciferase reporter vectors transiently transfected in iCell Cardiomyocytes remain active over prolonged culture durations



Isoproterenol-induced CRE-driven gene expression in iCell Cardiomyocytes transiently transfected and induced on multiple days in culture. A: iCell Cardiomyocytes were transiently transfected with CRE-Luciferase at 4 (A and C) or 12 days (B) after thaw. **A-B:** Isoproterenol was added 24 hours after transfection, cardiomyocytes were incubated overnight, and luciferase activity was measured. **C:** Isoproterenol was added 9 days after transfection, cardiomyocytes were incubated overnight, and luciferase activity was measured. Reporter activity remained viable in iCell Cardiomyocytes after prolonged culture.

Antioxidants suppress tBHQ-induced ARE-driven gene expression in iCell Cardiomyocytes



Induction and suppression of ARE-Luciferase activity in iCell Cardiomyocytes. A: iCell Cardiomyocytes transfected 12 days after thaw with ARE (black) or CMV (red) reporter vectors and treated 24 hours later with 1:2 serial dilutions of tBHQ for 6 hours. Higher concentrations of tBHQ had a toxic effect, demonstrated by a decreased luminescence in the CMV positive controls. **B:** iCell Cardiomyocytes were transfected with ARE-Luciferase reporter vector 12 days after thaw. 24 hours later, the cardiomyocytes were incubated with 25 µM tBHQ for 6 hours followed by 1:2 serial dilutions of ascorbic acid for an additional 12 hours. ARE-driven gene expression was dose-dependently suppressed by ascorbic acid.