RESCUE OF DRUG-INDUCED LONG QT SYNDROME TYPE 2 USING A HERG CHANNEL ACTIVATOR IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Vincenzo Macri[1], Jessica Norberg[1], Mark Hills[1], Terry Thomas[1], Allen Eaves[1,2], Stephen Szilvassy[1], Sharon Louis[1]

[1]STEMCELL Technologies, Vancouver, BC, Canada [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

Introduction

An important step in drug development is the evaluation of cardiac toxicity. Candidate drugs must have minimal effects on the hERG current that is essential for cardiac repolarization. Drugs that block the hERG channel can prolong the QT interval, leading to lethal ventricular arrhythmias. The hERG channel is prone to promiscuous interactions with drugs due to easy access to the channel pore. hERG channel activators can shorten repolarization in human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). One such activator, ginsenoside Rg_3, was recently shown to interact with the voltage-sensing domain of the hERG channel to stabilize the activated state[1]. Ginsenoside Rg_3 may change the conformation of the open channel and potentially limit subsequent drug blockade.

Hypothesis

E-4031-induced long QT syndrome type 2 can be rescued by ginsenoside Rg_3 in hPSC-CMs.

Methods

Three hPSC lines were differentiated into cardiomyocytes using an optimized cardiomyocyte differentiation medium and protocol. Flow cytometry and quantitative PCR (qPCR) were used to assay efficiency of hPSC differentiation to cardiomyocytes and cardiomyocyte subtype identity on day 15 and day 30. On day 18 of differentiation, two hPSC-CM lines were harvested and replated on microelectrode array (MEA) culture plates, and spontaneous electrical recordings were acquired between days 22 and 25. Spontaneous recordings consisted of 1) an initial 5-minute baseline measurement, 2) followed by 10 minutes with 10 nM E-4031, and 3) followed by 10 minutes with 10 µM ginsenoside Rg_3. A total of 25 minutes of continuous spontaneous electrical recordings were acquired and then analyzed to measure field potential duration (FPD), repolarization, and depolarization spike amplitudes.

Results

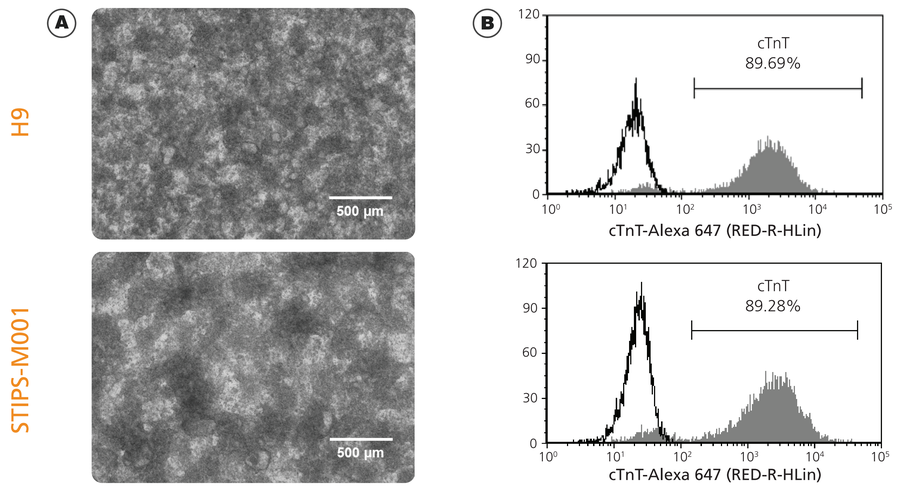

FIGURE 1. Robust and Equivalent Differentiation of Two hPSC Lines to Cardiomyocytes

(A) Bright-field images of two hPSC-CM lines (H9 and M001) that formed confluent monolayers on day 15. **(B)** Flow cytometry plots showing the two hPSC lines produced cells with > 89% cTnT expression on day 15.

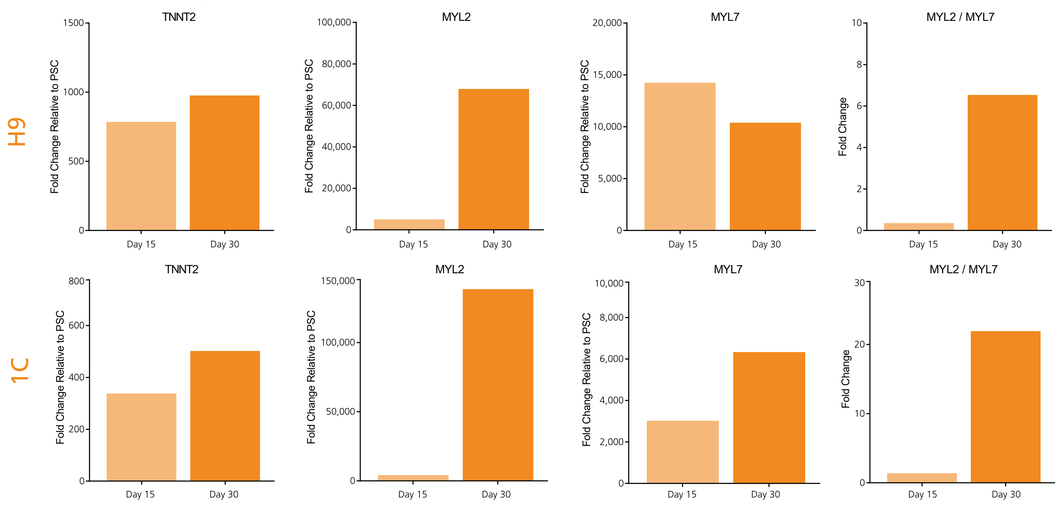

FIGURE 2. Transcript Analysis Shows Enrichment of hPSC-Derived Ventricular-Like Cardiomyocytes

Bar graphs showing qPCR analysis for *TNNT2*, *MYL2*, and *MYL7* with two hPSC-CM lines (H9 and 1C) on day 15 and day 30. The relative fold change in *TNNT2* and *MYL7* were similar between day 15 and day 30. The relative fold change in *MYL2* was substantially larger on day 30 compared to day 15. The *MYL2/MYL7* ratio increased 7- to 15-fold on day 30 compared to day 15 for both hPSC-CM lines. These values are similar to adult human primary ventricular cardiomyocytes (*MYL2/MYL7* = 9.8, Encode Project).

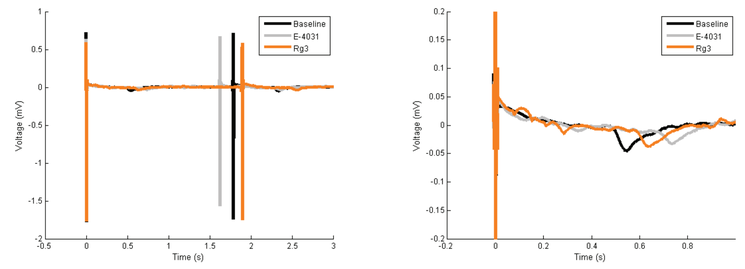

FIGURE 3. Ginsenoside Rg_3 Rescues Field Potential Duration Prolongation Induced by E-4031

Spontaneous field potential recordings of hPSC-CMs without drugs **(black)**, with 10 minutes of 10 nM E-4031 (grey), followed by the sequential addition of 10 µM ginsenoside Rg_3 (orange) for 10 minutes. The addition of E-4031 (grey) lengthened the FPD compared to baseline **(black)** and the addition of ginsenoside Rg_3 to the hPSC-CMs treated with E-4031 shifted the FPD toward baseline.

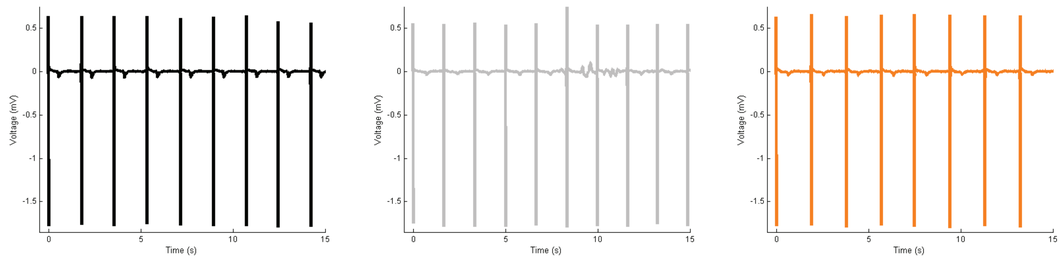

FIGURE 4. E-4031 Produces Electrical Instability and Arrhythmias That Can be Restored With the Addition of Ginsenoside Rg_3

15-second spontaneous field potential recordings in absence **(black)** or presence (grey) of 10 nM E-4031 for 10 minutes, followed by the addition of 10 µM ginsenoside Rg_3 (orange) for 10 minutes. The baseline recording had a stable electrical profile, and the addition of E-4031 lengthened the FPD and caused arrhythmias. The sequential treatment with ginsenoside Rg_3 of the hPSC-CMs exposed to E-4031 restored the stable electrical profile similar to baseline with no observed arrhythmias.

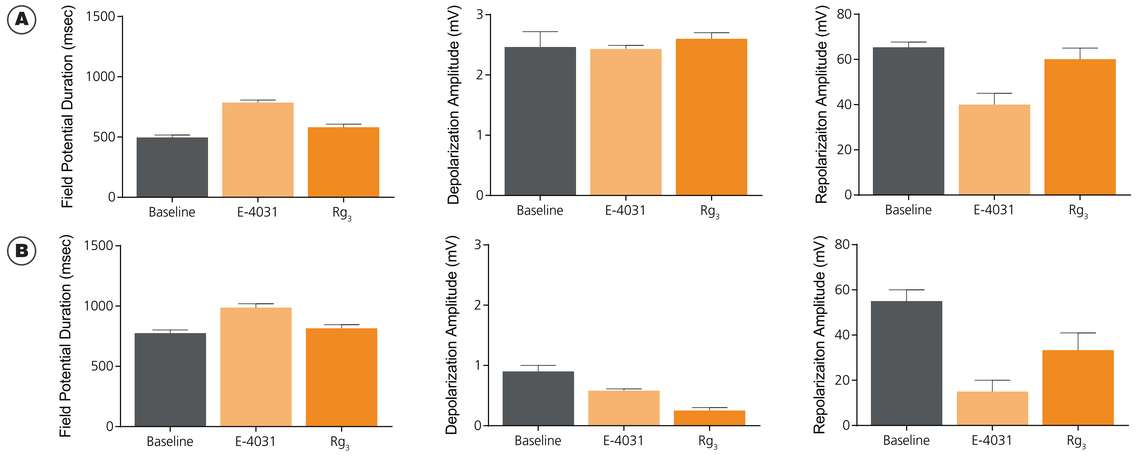

FIGURE 5. Deleterious Effects of E-4031 on hPSC-Derived Cardiomyocyte Repolarization Parameters Can be Rescued by Ginsenoside Rg_3

(A) Bar graphs of FPD, depolarization, and repolarization amplitudes measured from cardiomyocytes differentiated from the H9 hPSC line. Ginsenoside Rg_3 restored FPD and repolarization amplitude back to baseline values. **(B)** Bar graphs of FPD, depolarization, and repolarization amplitudes measured from cardiomyocytes differentiated from the M001 hPSC line. Ginsenoside Rg_3 restored FPD and repolarization amplitude back to baseline values. The depolarization amplitude for the H9 hPSC-CMs were not changed with the addition of either E-4031 or ginsenoside Rg_3; however, a stepwise reduction was observed for the M001 hPSC-CMs.

Summary

- Robust and equivalent differentiation to cardiomyocytes with similar morphology was observed in two hPSC lines (> 89% cTnT).
- Transcript analysis showed an enrichment of ventricular-like cardiomyocytes on day 30 compared to day 15 with the *MYL2/MYL7* ratio being significantly larger on day 30 in two hPSC lines.
- Ginsenoside Rg_3 rescued both field potential duration and electrical instability induced by E-4031.
- Ginsenoside Rg_3 can restore alterations in cardiomyocyte repolarization caused by E-4031 to values similar to baseline recordings.
- Ginsenoside Rg_3 rescued drug-induced LQTS2 in hPSC-CMs, suggesting that hERG activators that target the voltage-sensing domain may be used to offset cardiac safety issues of promising candidate drugs.

Reference:
1. Gardner, A., Wu, W., Thomson, S., Zangerl-Plessl, EM., Stary-Weinzinger, A., Sanguinetti, MC. Molecular Basis of Altered hERG1 Channel Gating Induced by Ginsenoside Rg_3. Molecular Pharmacology, 2017