Pharmacological Evaluation of Disease Relevant hiPSC-derived Cardiomyocyte Models

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Introduction

Cardiomyopathies and arrhythmia, have been successfully modeled in cell culture dishes using subject-specific iPSC-derived cardiomyocytes (SS-iPSC CMs). These model systems are promising tools for understanding disease mechanism and can potentially be used in drug discovery as well as cardiac liability assessment in specific populations which may carry genetic anomalies. Here, we performed pharmacological evaluation of a disease relevant iPSC-cardiomyocyte line derived from a subject who has an LMNA-Related Congenital Muscular Dystrophy. Firstly, the functional profile of the disease cardiomyocytes (LMNA CM), including contractility and electrophysiology, were determined using the xCELLigence RTCA CardioECR system. Compared to its isogenic control, LMNA CMs showed 36% faster beating rate and 10% smaller beating amplitude, which is a surrogate for force of contraction. In addition, LMNA CMs had a significant shorter field potential duration (FPD) compared to control at 1Hz pacing frequency. We additionally investigated the pharmacological responses of these cells. Several well-known tool compounds, E4031, flecainide, isoproterenol (ISO) and BayK 8644, were tested in both lines. LMNA CMs displayed higher sensitivity to BayK8644 treatment but less sensitivity to E4031 as indicated by the degree of prolongation of FPD compared to the CTRL group. However, flecainide and ISO treatment lead to similar responses in both disease and CTRL lines. Flecainide increased FPD at low doses and arrhythmia at high concentration. ISO, a positive chronic and inotropic compound, increased beating rate but slightly decreased beating amplitude. It has been repeatedly reported that chronic electrical pacing can improve maturity of hiPSC CMs and therefore we investigated if long-term pacing could have an impact on the phenotypical differences between LMNA CMs and CTRL. The response of CTRL cells to ISO was reversed after 15-day of continuous pacing as demonstrated by an increase in beating amplitude. However, the response of LMNA CMs to ISO still remained the same indicating an inherent deficiency in excitation-contraction coupling leading to force generation. In summary, our data suggests that the LMNA CMs have different baseline property of contraction and electrophysiology and different pharmacological profile from CTRL. Additionally, long-term electrical pacing may be a useful tool to distinguish disease phenotypes and recapitulate *in vivo* results.

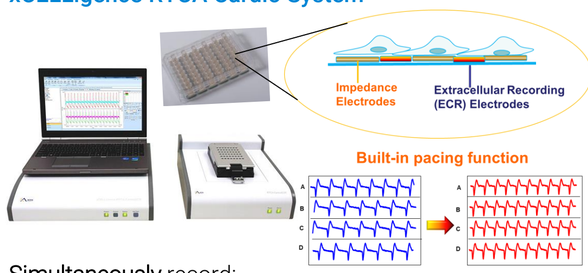
Experimental

Cardiomyocytes used in the study

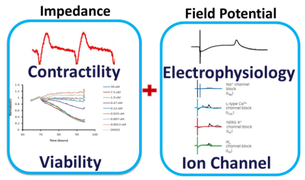
MyCell Cardiomyocytes (ID# 01016.105), LMNA-Related Congenital Muscular Dystrophy (Clone 2), which are derived from disease donor, and isogenic CTRL line (ID # 01016.420) were purchased from Fujifilm Cellular Dynamics (FCDI).

Experimental

xCELLigence RTCA Cardio System

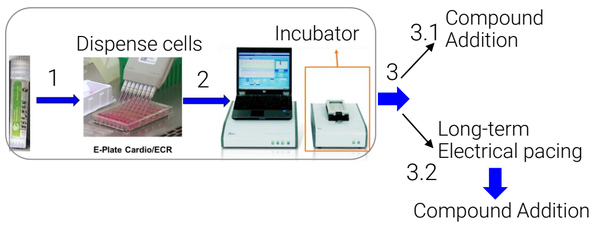


Simultaneously record:
Impedance (IMP) + Field Potential (FP)



- Contractility
- Cellular toxicity
- Ion channels modulation (Ca2+, Na+, hERG)
- Arrhythmic events

Experimental Workflow

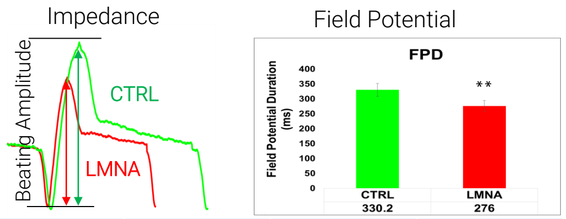


1. Directly seed cells on the E-Plate CardioECR 48
2. Real-time monitor cell performance (viability, contractile and electrical activity) in the incubator.
3. Compound addition

3.1 compound addition after cells generate consistent and robust contractile and electrical signals (Standard/non-paced CMs).
3.2 Compound addition after applying long-term electrical pacing at progressively increased pacing frequencies (Paced CMs)

Results and Discussion

Result 1. Functional profile of LMNA and isogenic CTRL CMs



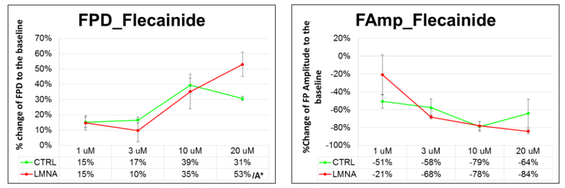
	CTRL	LMNA	% to CTRL
BR[a] (beats/min)	40 ± 2	55 ± 2	136%
BAmp[b]	0.19 ± 0.02	0.15 ± 0.01	78%
FPDc (ms)	330	276	83%

Results and Discussion

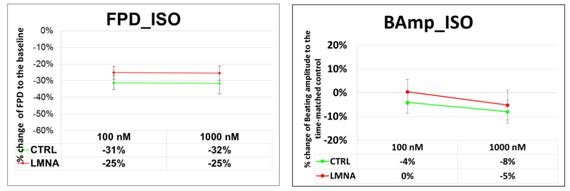
The contractile and electrical activities of LMNA and CTRL cardiomyocytes were evaluated 12 days post-seeding, when the cells generated consistent and robust contractile and electrical signals. The data presented in the table was mean ± STDEV; N= 24. BR[a]: Beating Rate; Bamp[b]: Beating Amplitude; **: P < 0.01; FPDc: FPD values were obtained while LMNA and CTRL cardiomyocytes were electrically paced at 1Hz

Result 2. Compound test in standard LMNA and CTRL CMs

Flecainide: hERG channel and Na+ channel inhibitor

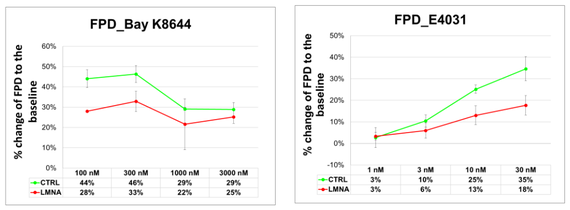


Isoproterenol (ISO): β adrenergic receptor activator



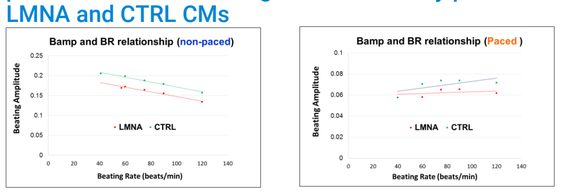
BayK 8644: Ca2+ channel activator

E4031: hERG channel inhibitor



LMNA and CTRL cardiomyocytes were treated with flecainide, isoproterenol (ISO), BayK 8644 and E4031 after 14 days post-seeding. The field potential duration (FPD), FP spike amplitude (FAmp) from FP readout and beating amplitude (BAmp) from IMP readout were calculated 30 min post-drug. The data was presented as mean ± STDEV (N=3).
A* : arrhythmia

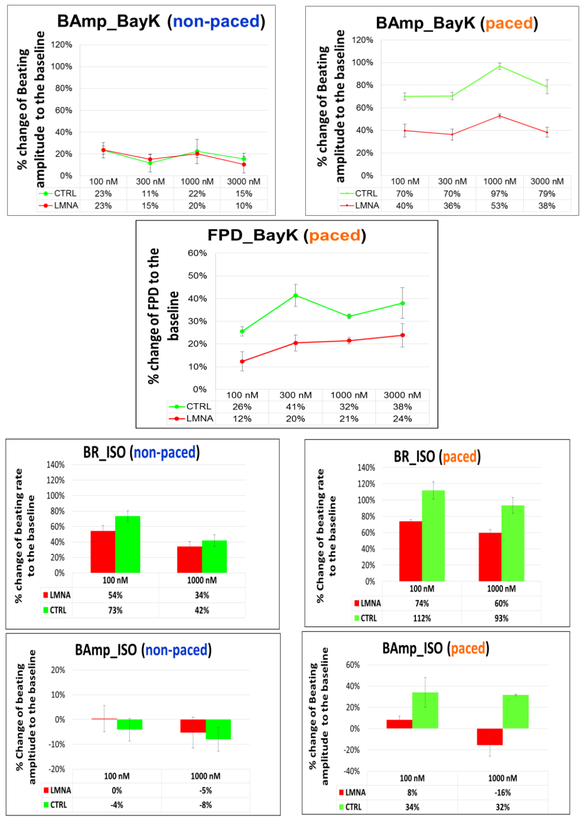
Result 3. Force-frequency relationship in non-paced/Standard and long-term electrically paced LMNA and CTRL CMs



LMNA and CTRL CMs were cultivated (non-paced) or continuously paced for additional 15 days after cells established consistent contractile and electrical activity 12 days post-seeding. BAmp was calculated at different BRs, which were controlled by electrical pacing.

Results and Discussion

Result 4. Compound test in non-paced and long-term electrically paced LMNA and CTRL CMs



Non-paced and chronically paced LMNA and CTRL CMs were treated with BayK8644 and ISO. BAmp, BR and FPD were calculated 30 min post-drug.

Conclusions

1. The differences of the functional profile between disease cardiomyocytes (LMNA CMs) and isogenic CTRL CMs including contractility and electrophysiology can be determined using the xCELLigence RTCA CardioECR system.
2. LMNA CMs displayed higher sensitivity to BayK8644 but less sensitivity to E4031 as indicated by the degree of prolongation of FPD compared to the CTRL group. However, flecainide and ISO treatment lead to similar responses in both disease and CTRL lines.
3. Long-term pacing facilitated the phenotypical differences between LMNA CMs and CTRL. After ISO addition, CTRL CMs started to show increase in beating amplitude, while LMNA CMs remained the same as before chronic pacing, displaying no change in beating amplitude.