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Full paper

Overexpression of KCNJ2 in induced pluripotent stem cell-derived cardiomyocytes for the assessment of QT-prolonging drugs

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ABSTRACT

Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes hold great potentials to predict pro-arrhythmic risks in preclinical cardiac safety screening, although the hiPSC cardiomyocytes exhibit rather immature functional and structural characteristics, including spontaneous activity. Our physiological characterization and mathematical simulation showed that low expression of the inward-rectifier potassium (I_{K1}) channel is a determinant of spontaneous activity. To understand impact of the low I_{K1} expression on the pharmacological properties, we tested if transduction of hiPSC-derived cardiomyocytes with *KCNJ2*, which encodes the I_{K1} channel, alters pharmacological response to cardiac repolarization processes. The transduction of KCNJ2 resulted in quiescent hiPSC-derived cardiomyocytes, which need pacing to elicit action potentials. Significant prolongation of paced action potential duration in KCNJ2-transduced hiPSC-derived cardiomyocytes was stably measured at 0.1 μM E-4031, although the same concentration of E-4031 ablated firing of non-treated hiPSC-derived cardiomyocytes. These results in single cells were confirmed by mathematical simulations. Using the hiPSC-derived cardiac sheets with KCNJ2-transduction, we also investigated effects of a range of drugs on field potential duration recorded at 1 Hz. The KCNJ2 overexpression in hiPSC-derived cardiomyocytes may contribute to evaluate a part of QT-prolonging drugs at toxicological concentrations with high accuracy.

1. Introduction

Technologies of human induced pluripotent stem cells (hiPSCs) can significantly improve the robustness of *in vitro* assays designed to predict toxicities associated with drug-induced cardiac ventricular arrhythmia.[1] Prolongation of the QT interval is a manifestation of delayed repolarization of ventricular action potential (AP). Because the repolarization process is a delicate and highly regulated process, accurate pre-clinical prediction of drug-induced pro-arrhythmic risks remains challenging.[1]

Because hiPSC-cardiomyocytes (hiPSC-CMs) exhibit immature gene expression patterns and functional variation in cell-to-cell,[1] multicellular hiPSC-CM preparations have been used for

Abbreviations: AP, action potential; APD, action potential duration; CM, cardiomyocyte; EAD, early after depolarization; hiPSCs, human induced pluripotent stem cells; hSC-CMs, human stem cell-derived cardiomyocytes; MDP, maximum diastolic membrane potential; MEA, microelectrode array.

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evaluating the effects of QT-prolonging drugs. There are marked differences between adult human cardiomyocytes and hiPSC-CMs. For example, most hiPSC-CMs retain spontaneous activity and display relatively depolarized maximum diastolic membrane potentials (MDP).[1,2] In the heart, the cardiac inward rectifier K^+ (Kir2.1) channel current (I_{K1}) is responsible for stabilizing resting membrane potentials close to the reversal potential of K^+, and contributes to the repolarization process of ventricular APs.[3] Consistent with these observations, expression of the Kir2.1 channel gene (*KCNJ2*) is reportedly much lower in hiPSC-CMs than in adult mammalian ventricular myocytes, and KCNJ2 expression in hiPS-CMs hyper-polarizes the MDP.[4,5] Recently, patch-clamp studies using functionally-matured hiPSC-CMs have revealed that a selective hERG channel blocker, E-4031, prolonged AP duration (APD) and induced early after depolarization (EAD), suggesting that I_{Kr} is functional in iPS-CMs.[6,7] However, there has been no systematic study to understand impacts of the low KCNJ2 expression hiPS-CMs on multi-electrode array (MEA) assays for evaluation of QT-prolonging drugs such as K^+ channel blockers.

To address this issue, we firstly measured APs with the patch-clamp assay, and found that a submaximal concentration of E-4031 depolarized the resting membrane potentials in some single beating hiPSC-CMs; this precluded robust evaluation the effects that drugs had on the cardiac repolarization processes. The *KCNJ2*-transduction in hiPSC-CMs made the cell totally quiescent, but they were excitable with electrical stimulations.[8] In these *KCNJ2*-transduced hiPSC-CMs, E-4031 prolonged APD in a concentration-dependent manner and did not affect the resting potential. To understand the pharmacological impact of the KCNJ2 overexpression in hiPSC-CMs, we employed a mathematical model of human ventricular APs[9] with some modifications based on both the literature and our experimental data. The simulation of E-4031 treatment in this cell model recapitulated the E-4031-dependent depolarization. Finally, we also investigated effects of a range of drugs which alter cardiac repolarization processes (E-4031, chromanol 293B, isoproterenol) on field potential duration of the paced KCNJ2-transduced hiPSC cardiac sheets. These results provide new insights into the evaluation of drug efficacies or toxicities of QT-prolonging drugs in hiPSC-CMs.

2. Materials and methods

Details are shown in the Supplementary Information.

2.1. Cell culture

As a commercially available hiPSC-CM, iCell-CM (CDI Fujifilm, Japan) was used. The cells were prepared according to manual. HEK293 cells were cultured as previously described.[10] Cultures could be maintained until 30 days post transduction.

2.2. Molecular biology

Virapower™ Adenoviral expression System (Invitrogen) was used to generate adenoviral expression constructs. The adenovirus was amplified in 293A cells and then used to infect HEK293 cells and iCell-CMs. Adenoviral infection was achieved by applying adenoviral extract at 100 MOI for 24 h.

2.3. Biochemistry

This study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committees on the Ethics of Animal Experiments of Tokyo Medical and Dental University (0140103A).

We have carried out western blot analysis and immunocytochemistry according to the previous methods as described in the Supplemental Information.

2.4. Electrophysiology

APs and membrane currents were recorded with the perforated configuration of the patch-clamp technique, and spontaneous and paced field potentials (FPs) were recorded with a MED64 MEA system (AlphaMED Science, Osaka, Japan) as described the Supplemental Information.

2.5. Chemicals

E-4031, a selective I_{Kr} blocker, was a kind gift from Eisai Corporation (Japan). All other materials were reagent quality and obtained from standard sources.

2.6. Data analysis and statistics

All values are presented as mean ± S.E.M, and the details are described in the Supplemental Information.

3. Results

It has been known that the shapes of APs recorded from patch-clamped hiPSC-CMs vary from cell to cell, and most cells beat spontaneously.[1] This means that the electro-activity of hiPSC-CMs is distinct from that of adult ventricular myocytes.[7] In comparison with adult ventricular myocytes, typical features of hiPS-CMs are enhancement of I_f channel currents (I_f) and reduction of I_{K1} channel currents (I_{K1}). In Fig. 1, to understand how these functional differences in hiPS-CMs contribute to their electrophysiology, we quantified I_f channel activities in iCell-CMs, and evaluated contribution of I_{K1} by conducting simulations with a modified O'Hara-Rudy model[9] which shows improved APD dependence on pacing rate. Patch-clamp experiments revealed that 70% of iCell-CMs exhibited a robust I_f conductance. From these cells, voltage-dependent activities of I_f channel were investigated (Fig. 1A–C).

A mathematical model of the data obtained from hiPSC-CMs was developed using the following 5 steps.

(1) Membrane capacitance (C_m) was set to 50 pF based on our experimental data in iCell-CMs, and transverse tubules were removed according to previous reports in hiPSCs.[11,12]
(2) Electrophysiological properties of hiPSC-CMs are similar to those of embryonic CMs, and therefore, we referred to the developmental changes in individual ionic currents of CMs between the early embryonic and neonatal stages.[13–16] Thus, we set the maximum conductances of I_{NaF}, I_{NaL}, I_{to}, I_{Kr}, I_{Ks}, I_{pCa}, and I_{NaCa} of the O'Hara-Rudy model[9] to 112.5 mS/μF, 0.01125 mS/μF, 0.0025 mS/μF, 0.046 mS/μF, 0.0017 mS/μF, 0.000025 mS/μF, and 0.004985 μCoul/μF, respectively. The relative ratio of I_{NaK} was set to 0.7, and the permeability of Ca^{2+} of I_{CaL} to 0.00005 cm/s. Additionally, relative ratios of sarcoplasmic reticulum Ca^{2+} fluxes, I_{rel}, I_{up}, I_{tr}, and I_{leak}, were set to 1.7, 1.0, 0.3, and 0.3, respectively.
(3) Because the I_f is not included in the original O'Hara-Rudy model,[9] we developed the following equations based on our experimental patch-clamp data in iCell-CMs (Fig. 1G–I):

$$I_f = 0.07(V_m - 0.3833.\ E_{Na} - 0.6167.\ E_K) \times y,$$

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