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Short communication

A distribution analysis of action potential parameters obtained from patch-clamped human stem cell-derived cardiomyocytes

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ABSTRACT

We investigated electrophysiological properties of human induced-pluripotent-stem-cell-derived and embryonic-stem-cell-derived cardiomyocytes, and analyzed action potential parameters by plotting their frequency distributions. In the both cell lines, the distribution analysis revealed that histograms of maximum upstroke velocity showed two subpopulations with similar intersection values. Sub-populations with faster maximum upstroke velocity showed significant prolongation of action potential durations by application of E-4031, whereas others did not, which may be partly due to shallower maximum diastolic potentials. We described electrophysiological and pharmacological properties of stem-cell-derived cardiomyocytes in the respective sub-populations, which provides a way to characterize diverse electrical properties of stem-cell-derived cardiomyocytes systematically.

Considerable attention has been paid to the potential of human induced pluripotent stem cells (hiPSC) as an unlimited cell source for *in vitro* screening assays, because the production of hiPSC production possess less legal and ethical issues than that of human embryonic stem cells (hESC) (1–3). As a conventional assay for toxicological/pharmacological evaluations, field potentials measurement from multi-cellular preparations with micro-electrode array (MEA) platform (4,5) is utilized to reduce cell-to-cell variation of action potential (AP) dynamics (2,6,7), some reports revealed that considerable variation exists in commercially available cell-lines (8,9). Indeed, mixture of non-myocyte or different types changes excitability of multicellular preparations (10). This indicates necessity of precise information on cell-to-cell variation to understand mechanistic insights on results with conventional MEA assay. However, in previous single-cell analysis, cells were classified into arbitrary three types (i.e. nodal, atrial and ventricular) without applying distribution analysis (6,7,11), mainly because of small cell numbers. Thus, we collected significant numbers of action potential data from patch-clamped hSC-CMs, and analyzed frequency distribution of AP parameters to characterize the cell properties in respective sub-populations.

Cell cultures and isolation of the both cardiomyocytes were performed according to the company's protocols. In brief, frozen hiPSC-CMs (iCell cardiomyocytes, lot#1131800, #1791676, #1341341, iPS PORTAL, Kyoto, Japan) were thawed, and embryoid bodies of hESC-CMs (SA002, Cellartis AB, Gotebörg, Sweden) were shipped. Then, cells were isolated by trypsinization (0.25% trypsin-EDTA) from cell sheets or embryoid bodies respectively, and plated onto 0.5% gelatin/10 μg/ml laminin-coated plasma-etched glass bottom dishes, and used within two weeks. Isolated hiPSC-CMs were maintained in the CDI maintenance medium at 37 °C and at 7% CO_2. Isolated hES-derived cardiomyocytes were maintained in DMEM supplemented with 5 mM GlutaMax™, 20% FBS, 1% Pen/Strep, 1% non-essential amino acids, 0.2% 2-mercaptoethanol, and were kept at 37 °C and 5% CO_2. E-4031 (4′-[[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidinyl]carboxyl]methanesulfonanilide dihydrochloride dehydrate, Eisai Co., Tokyo) was kept as 10 mM stock in

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distilled water. All other materials were reagent quality and obtained from standard sources.

Action potentials were recorded at 36 ± 1 °C with the perforated patch configuration as described previously (12). External solution contained (in mM): NaCl (135), NaH_2PO_4 (0.33), KCl (5.4), $CaCl_2$ (1.8), $MgCl_2$ (0.53), glucose (5.5), HEPES (5), pH 7.4. To achieve patch perforation (<20 MΩ; series resistances), amphotericin B (0.3 μg/ml) was added to the internal solution composed of (in mM): potassium-aspartate (110), KCl (30), $CaCl_2$ (1), adenosine-5′-triphosphate magnesium salt (5), creatine phosphate disodium salt (5), HEPES (5), EGTA (11), pH 7.25. We adopted the AP data only when more than 35 stable AP traces were recorded. Data were acquired and analyzed using pClamp10 software (Molecular Devices, Sunnyvale, CA, USA). In quiescent cells, 2-ms duration current pulses (120% of the threshold intensity) were applied to elicit APs. All values are presented as mean ± S.E.M. Statistical significance was assessed with Student's *t*-test for simple comparisons and non-parametric Kolmogorov–Smirnov test for frequency distributions by using OriginPro 8 software (Origin Lab Co, Northampton MA, USA). Differences at $P < 0.05$ were considered to be significant.

In Fig. 1 and Table 1, APs recorded from 54 hiPSC-CMs and 80 hESC-CMs were summarized. Although the beating rate (BR) in hiPSC-CMs was significantly slower than that in hESC-CMs with comparing two averages (Table 1), it is difficult to conclude that these cells have different phenotypes. Because the distribution analysis revealed that hiPS-CM in itself would have at least more than two phenotypes, which is consistent with significantly larger variations of beat-to-beat times shown as bigger coefficient of variation (CV) in hiPS-CM (Fig. 1A and Table 1).

In terms of AP parameters, there was no statistical difference in average values of AP parameters (Table 1): AP amplitude (APA), AP duration at 50% and 70% of repolarization (APD_{50}, APD_{70}), maximum upstroke velocity (dV/dt_{max}), APD ratio during phase 2 ($APD_{20–40}/APD_{50–70}$); an indicative of AP plateau, and APD ratio (APD_{90}/APD_{50}); an indicative of phase 4 depolarization, except of slight but significant differences in mean values of maximum diastolic potential (MDP) (Table 1). Relatively large

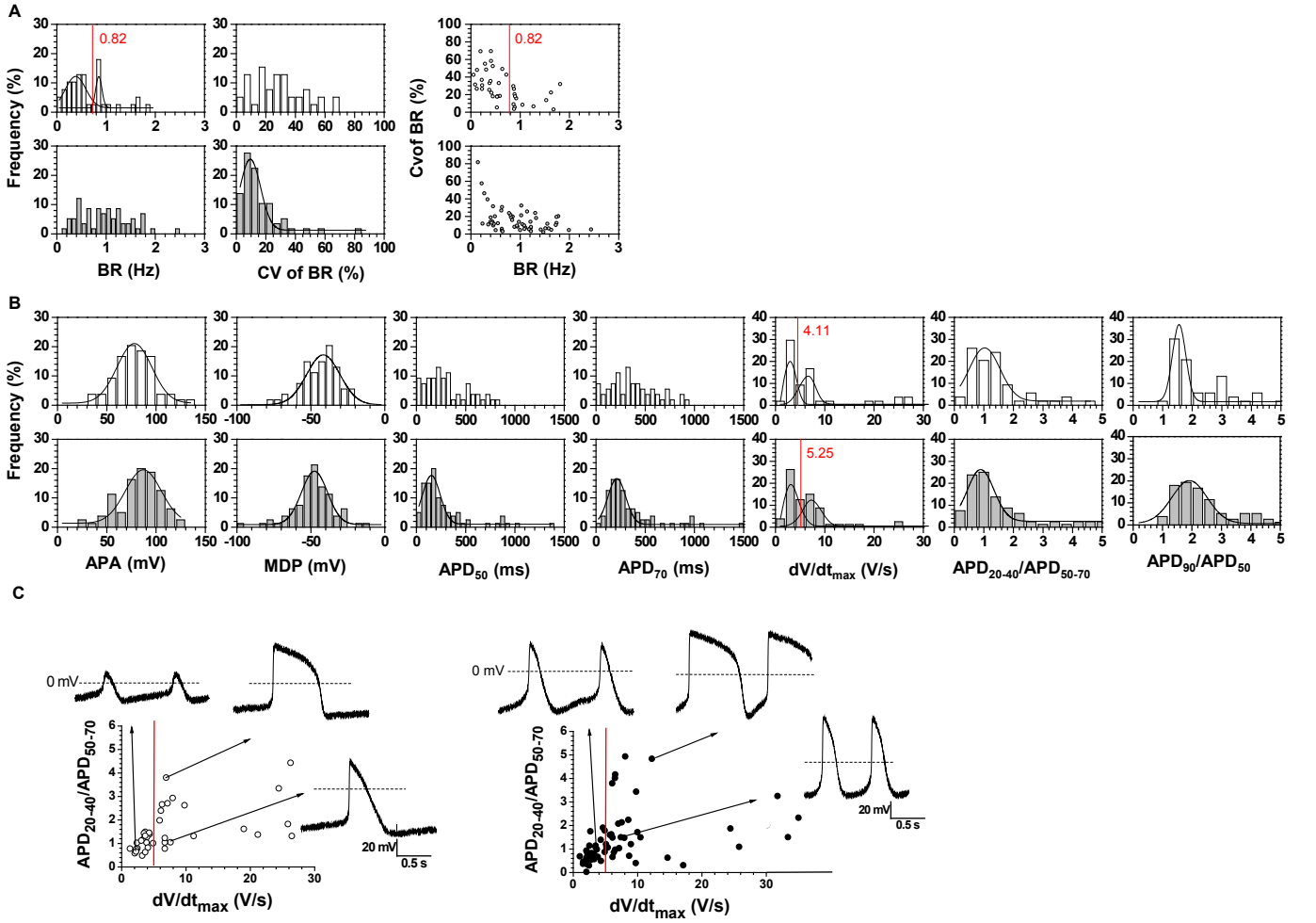

Fig. 1. Distributions of electrophysiological parameters in hiPSC- and hESC-derived cardiomyocytes (CMs). Histograms for AP (**A**) and beating rate (**B**) parameters were fitted with single or multiple Gaussian distributions. Multiple Gaussian functions were applied when coefficient of determination (R^2) on single Gaussian fitting fell below 0.81. Rate (%) of each fraction (i.e. peak) in multiple fitting was calculated from ratio of each peak area. **(A)** Frequency distribution of BR and its coefficient of variation (CV) in spontaneously AP firing 39 hiPSC-CMs (white bars, upper panels) and 54 hESC-CMs (gray bars, lower panels). The distribution on the BR of hiPSC-CMs showed two major peaks at 0.38 Hz (69.8%) and 0.88 Hz (30.2%) of Gaussian distribution (intersection 0.82 Hz, $R^2 = 0.88$). **(B)** Frequency distribution of AP parameters in 54 hiPSC-CMs (white bars, upper panels) and 80 hESC-CMs (gray bars, lower panels). Frequency peaks (% fraction for multiple distributions) of dV/dt_{max} are as follows: hiPSC-CMs; 2.6 V/s (50.8%) and 6.4 V/s (49.2%) (intersection 4.11 V/s, $R^2 = 0.927$), and hESC-CMs; 3.3 V/s (50.8%) and 7.4 V/s (49.2%) on hESC-CMs (intersection 5.25 V/s, $R^2 = 0.941$), respectively. The intersections of bimodal distributions for dV/dt_{max} were shown in red dotted lines. **(C)** Scatterplot representation of upstroke velocity (dV/dt_{max}) against $APD_{20–40}/APD_{50–70}$ ratio in hiPSC-CMs (white dots, left panel) and hESC-CMs (black dots, right panel). Representative AP waveforms at precincts are shown in insets.