Europace (2016) **18**, iv67–iv76
doi:10.1093/europace/euw352

SUPPLEMENT PAPER

Human-induced pluripotent stem cell-derived cardiomyocytes from cardiac progenitor cells: effects of selective ion channel blockade

Claudia Altomare[1], Enea Pianezzi[1,2], Elisabetta Cervio[1], Sara Bolis[1], Vanessa Biemmi[1], Patrizia Benzoni[3], Giovanni G Camici[2], Tiziano Moccetti[1], Lucio Barile[1†], and Giuseppe Vassalli[1,4†]

[1]Cellular and Molecular Cardiology Laboratory, Swiss Institute for Regenerative Medicine (SIRM), 6807 Taverne Switzerland and Cardiocentro Ticino, 6900 Lugano, Switzerland; [2]Department of Molecular Cardiology, University of Zürich, 8001 Zürich, Switzerland; [3]Fibroblast Reprogramming Unit, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy; and [4]Department of Cardiology, University of Lausanne Medical Center (CHUV), 1011 Lausanne, Switzerland

Received 20 May 2016; accepted after revision 29 September 2016

Aim Human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are likely to revolutionize electrophysiological approaches to arrhythmias. Recent evidence suggests the somatic cell origin of hiPSCs may influence their differentiation potential. Owing to their cardiomyogenic potential, cardiac-stromal progenitor cells (CPCs) are an interesting cellular source for generation of hiPSC-derived cardiomyocytes. The effect of ionic current blockade in hiPSC-derived cardiomyocytes generated from CPCs has not been characterized yet.

Methods and results Human-induced pluripotent stem cell-derived cardiomyocytes were generated from adult CPCs and skin fibroblasts from the same individuals. The effect of selective ionic current blockade on spontaneously beating hiPSC-derived cardiomyocytes was assessed using multi-electrode arrays. Cardiac-stromal progenitor cells could be reprogrammed into hiPSCs, then differentiated into hiPSC-derived cardiomyocytes. Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin showed higher upregulation of cardiac-specific genes compared with those of fibroblastic origin. Human-induced pluripotent stem cell-derived cardiomyocytes of both somatic cell origins exhibited sensitivity to tetrodotoxin, a blocker of Na^+ current (I_{Na}), nifedipine, a blocker of L-type Ca^{2+} current (I_{CaL}), and E4031, a blocker of the rapid component of delayed rectifier K^+ current (I_{Kr}). Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin exhibited sensitivity to JNJ303, a blocker of the slow component of delayed rectifier K^+ current (I_{Ks}).

Conclusion In hiPSC-derived cardiomyocytes of cardiac origin, I_{Na}, I_{CaL}, I_{Kr}, and I_{Ks} were present as tetrodotoxin-, nifedipine-, E4031-, and JNJ303-sensitive currents, respectively. Although cardiac differentiation efficiency was improved in hiPSCs of cardiac vs. non-cardiac origin, no major functional differences were observed between hiPSC-derived cardiomyocytes of different somatic cell origins. Further studies are warranted to characterize electrophysiological properties of hiPSC-derived cardiomyocytes generated from CPCs.

Keywords Induced pluripotent stem cell • Cardiac progenitor cell • Cardiomyocyte • Ion current • Arrhythmia

* Corresponding author. Tel: +41918053359; fax: +41918053150. *E-mail address: giuseppe.vassalli@cardiocentro.org*; Tel: +41918053384; fax: +41918053150. *E-mail address: lucio.barile@cardiocentro.org*

† The last two authors contributed equally to the study.

What's new?

- Human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes can be generated from adult cardiac-stromal progenitor cells (CPCs).
- Human-induced pluripotent stem cell-derived cardiomyocytes generated from adult CPCs exhibit sensitivity to an adrenergic stimulus and to selective pharmacological blockade of Na^+ current (I_{Na}), L-type Ca^{2+} current (I_{CaL}), and both the rapid and slow component of delayed rectifier K^+ current (I_{Kr} and I_{Ks}, respectively).
- Although hiPSC-derived cardiomyocytes generated from adult CPCs may serve as a valuable *in vitro* model of arrhythmic disorders associated with several cardiac ionic channels, their electrophysiological properties are not clearly improved compared with those of hiPSC-derived cardiomyocytes generated from skin fibroblasts.

Introduction

Personalized medicine aims to develop predictive and therapeutic approaches that take into consideration individual variability. With respect to arrhythmic disorders, personalized medicine addresses the fundamental questions as to why some patients with specific genetic mutations or single nucleotide polymorphisms are at increased risk of developing arrhythmias, and why others with the same genetic modification respond in a different way to the same drug.[1] Answering these questions will require a clear comprehension of arrhythmogenic processes at the organ and tissue level, as well as at the cellular and molecular level. Human-induced pluripotent stem cell (hiPSC) technology provides a unique tool for *in vitro* modelling of human diseases with their genetic and epigenetic backgrounds.[2] With respect to cardiac electrophysiology, this technology represents a constant source of patient-specific cardiomyocytes that mimic endogenous cardiomyocytes in many respects,[3] thus circumventing the inability of primary cardiomyocytes to be maintained in culture for extended periods of time. The use of animal cardiomyocytes is limited by differences in the expression profile of ionic channels compared with human cardiomyocytes, which influence electrophysiological properties including pharmacological responses. Over the past few years, hiPSC technology has emerged as a unique investigational platform in cardiac electrophysiology. Human-induced pluripotent stem cell-derived cardiomyocytes express cardiac-specific genes and proteins including a panel of cardiac ion channels associated with arrhythmic disorders. In addition, they display atrial-, nodal-, and ventricular-like cardiac action potentials (APs), contract spontaneously and rhythmically, and mimic functional excitation–contraction coupling characteristics of adult cardiomyocytes.[1,4,5] Being donor-specific, hiPSC-derived cardiomyocytes carry the genetic information of the patient. At the same time, these cells have several limitations including structural and functional immaturity[6] as well as fibroblast contamination, clearly indicating that they are not a surrogate of adult ventricular myocytes. Yet, it has been proposed that hiPSC-derived cardiomyocytes can be used as an investigational platform, provided that the following criteria are met: (i) the gene of interest must be functionally expressed, (ii) the phenotype observed must be reliably measured, and (iii) the phenotype measured must be comparable with adult cardiomyocytes to provide adequate clinical correlation or predictive ability.[4] Currently available data support Criteria 1 and 2 but not definitively Criterion 3. Prospective assessment in population-level studies is required to evaluate the latter.

Human-induced pluripotent stem cell-derived cardiomyocyte technology has been used to investigate the mechanistic relationships between patients' genotypes and their electrophysiological phenotypes. Long-QT syndrome type 1 was the first genetic arrhythmia modelled using hiPSC-derived cardiomyocytes in patients with gene mutations in cardiac ion channels associated with the disease.[7] Subsequently, these cells were used to model long QT syndrome type 2 and 3, catecholaminergic polymorphic ventricular tachycardia, ventricular arrhythmias associated with familial hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia, and a number of other arrhythmic disorders (for reviews, see Refs 1, 3, and 4).

Human-induced pluripotent stem cells have been derived from adult skin fibroblasts and other somatic cell sources. It has been suggested that the somatic cell origin may influence the differentiation potential of iPSCs and the functional maturity of the re-differentiated cells, a phenomenon referred to as somatic cell memory.[8] For instance, iPSCs generated from neonatal mouse cardiomyocytes[9] or foetal cardiac-stromal progenitor cells (CPCs)[10,11] exhibited enhanced capacity towards cardiomyogenic re-differentation. Here, we report on the generation of hiPSC-derived cardiomyocytes using donor adult CPCs compared with donor skin fibroblasts from the same patients. Selective pharmacological blockade of cardiac ionic currents that are associated with arrhythmic disorders was analysed in spontaneously contracting hiPSC-derived cell clusters using multi-electrode arrays (MEAs). Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin exhibited sensitivity to selective blockade of several ionic currents.

Methods

Generation of human-induced pluripotent stem cells and human-induced pluripotent stem cell-derived cardiomyocytes

Protocols used in this study were approved by local Ethics Committee for Clinical Research. Right cardiac atrial appendage and sternal skin specimens were procured from 2 patients with heart valve disease (without ischaemic heart disease) who underwent heart surgery and gave informed consent. Cardiac-stromal progenitor cells were isolated as the cellular outgrowth of *ex vivo* cultured tissue explants, as described previously.[12] To generate hiPSCs, CPCs and skin fibroblasts were reprogrammed through Sendai virus infection using *OCT3/4, SOX2, KLF4*, and *MYC* (CytoTune™-iPS 2.0 Sendai Reprogramming Kit; ThermoFisher). To assess pluripotency, clusters exhibiting embryonic stem cell (ESC)-like were immunostained for the pluripotency markers Tra-1-60, Nanog, and SSEA-4 (antibodies were from Abcam). Moreover, expression of the ESC-associated genes *Nanog*, *Lefty*, and *SSEA-1* was measured at varying time points post-infection using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analyses. Ribonucleoprotein extraction, reverse transcription, and real-time PCR were carried out essentially as described previously.[12] In addition, the ability of reprogrammed cells to give rise to cell derivatives of the three germ layers was assessed using

Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems). Cells cultured in diverse differentiation media, as per manufacturer's instructions, were immunostained using antibodies against the ectodermal markers Otx-2 (R&D) and PGP9 (Abcam), the mesodermal markers T-Bry (R&D) and GATA4 (Santa Cruz), and the endodermal markers Sox17 and α-smooth muscle actin (α-SMA; Santa Cruz). Cardiac differentiation of hiPSCs was induced using Gibco™ PSC Cardiomyocyte Differentiation Kit (ThermoFisher). Cells cultured in cardiac differentiation medium were immunostained using antibodies against cardiac α-sarcomeric actinin (Abcam) and connexin 43 (Santa Cruz). In addition, expression of cardiac-specific genes (*HCN1*, *RyR2*, *αMHC*, *cTnI*, *Cx43*) was measured using real-time RT-PCR. The threshold cycle (Ct) of each gene was automatically defined and normalized to control genes *GAPDH* and *Rpl13* (ΔCt value). Reverse transcriptase-polymerase chain reaction data are shown as $2^{-\Delta\Delta Ct}$ values for the indicated population of interest with respect to the indicated reference population.

Electrophysiological recordings

Multi-electrode arrays recordings were performed on microdissected, spontaneously beating cell clusters formed by hiPSCs of CPC or skin fibroblast origin at ∼15 days of differentiation. Multi-electrode arrays chips were coated with Synthemax (Corning) 5 μLin 200 μL of gelatine 0.02%, and incubated overnight at 4 °C. Clumps (∼200–300 μm) of beating cells were microdissected using surgical scissors and positioned on the electrodes of standard 60 electrode MEAs at high spatial (200 μm) resolution (60MEA-200/30iR-Ti; Multi Channel Systems, Reutlingen, Germany). Extracellular field potentials (FPs) were recorded 72 h after plating to allow for cell attachment. During recording, the temperature was maintained at 37.0 °C. Field potential duration was analysed offline by ClampFit (Molecular Devices), as reported.[13] Corrected FP (cFP) duration was calculated using standard Bazett's correction. Spike amplitude of the instantaneous depolarizing component of the trace was measured to evaluate the effect of Na^+ channel blockade. Following baseline recording, 20 μL of stock solution of the test drug were added to 2 mL of culture medium (RPMI with B27 supplementation). Test drugs included norepinephrine (dose range: 10 nM–10 μM; Sigma), tetrodotoxin (TTX; 10 μM; Tocris, Bristol, UK), nifedipine (20 nM; Sigma), E4031 (1 μM; Alomone Laboratories, Jerusalem, Israel), and JNJ303 (2 μM; Tocris).

Statistics

Student's *t*-test was applied and data are expressed as mean ± SE; $P < 0.05$ defines significance, sample size is reported in figure legends.

Results

Human-induced pluripotent stem cell-derived cardiomyocytes can be generated using donor adult cardiac-stromal progenitor cells

Cardiac-stromal progenitor cells and skin fibroblasts formed cell clusters exhibiting ESC-like morphology at 25–30 dpi (*Figure 1A*) and 15–20 dpi, respectively. Cell clusters were microdissected and transferred to new dishes. Cells expressed the pluripotency markers Tra-1-60, Nanog and SSEA-4, as assessed by immunohistochemistry (*Figure 1B*). Human-induced pluripotent stem cells of cardiac origin showed higher upregulation of the ESC-associated genes *Nanog*, *Lefty*, and *SSEA-1* compared with hiPSCs of fibroblastic origin, as assessed by real-time RT-PCR at varying time points post-infection (*Figure 1C*). They were able to give rise to cell derivatives of the three germ layers under appropriate differentiation conditions, as demonstrated by immunostaining for ectodermal (Otx-2, PGP9), mesodermal (T-Bry, GATA4), and endodermal markers (Sox17, α-smooth muscle actin; *Figure 1D*). Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin exhibited spontaneous, rhythmic beating activity at ∼12 days of differentiation. They stained positive for cardiac α-sarcomeric actinin and connexin 43 (*Figure 2A*). Human-induced pluripotent stem cell-derived cardiomyocytes of dermal fibroblastic origin exhibited spontaneous activity at a similar time point as those of cardiac origin. The latter exhibited higher upregulation of cardiac-specific genes (*HCN1*, *RyR2*, *αMHC*, *cTnI*, *Cx43*), relative to the respective hiPSC populations, as compared with hiPSC-derived cardiomyocytes of fibroblastic origin (*Figure 2B*).

Multi-electrode arrays electrophysiological parameters of human-induced pluripotent stem cell-derived cardiomyocytes under basal conditions and responses to norepinephrine

Multi-electrode arrays recordings were performed on microdissected, spontaneously beating clusters of hiPSC-derived cells of both somatic cell origins at ∼15 days of differentiation. Beating frequencies of hiPSC-derived cardiomyocytes from CPCs tended to be lower than those of cell clusters from skin fibroblasts, although the difference was not statistically significant (64.8 ± 9.5 vs. 85.8 ± 18.0 bpm). Corrected FP durations likewise tended to be lower in cell clusters of CPC origin compared with those of skin fibroblast origin (208 ± 60 vs. 304 ± 28 ms; NS). Responses to norepinephrine (an α-adrenergic agonist at low concentrations and a β-adrenergic agonist at high concentrations) were recorded. Human-induced pluripotent stem cell-derived cardiomyocytes of both somatic cell origins responded to norepinephrine with a dose-dependent increase in beating frequencies (*Figure 3A*). No major changes in cFP duration were observed after 10 nM of norepinephrine (data not shown). These results indicate that hiPSC-derived cardiomyocytes of both somatic cell origins are sensitive to (β-) adrenergic stimulation.

Human-induced pluripotent stem cell-derived cardiomyocytes of both cell origins are tetrodotoxin- and nifedipine-sensitive

The spontaneous beating activity suggested that high-density inward current via voltage-activated channels may be present. Thus, we first tested the effects of voltage-gated Na^+ channel and Ca^{2+} channel blockers on the excitable properties of hiPSC-derived cell clusters. Multi-electrode arrays recordings under basal condition and after application of the selective Na^+ channel blocker TTX are shown in *Figure 3B*. Tetrodotoxin (10 μM) reduced peak amplitude (Peak Ampl) by 51% in hiPSC-derived cardiomyocytes of cardiac origin and totally abolished the instantaneous component of depolarization in those of fibroblastic origin. The spontaneous beating frequencies were slightly diminished in both populations, with no major changes

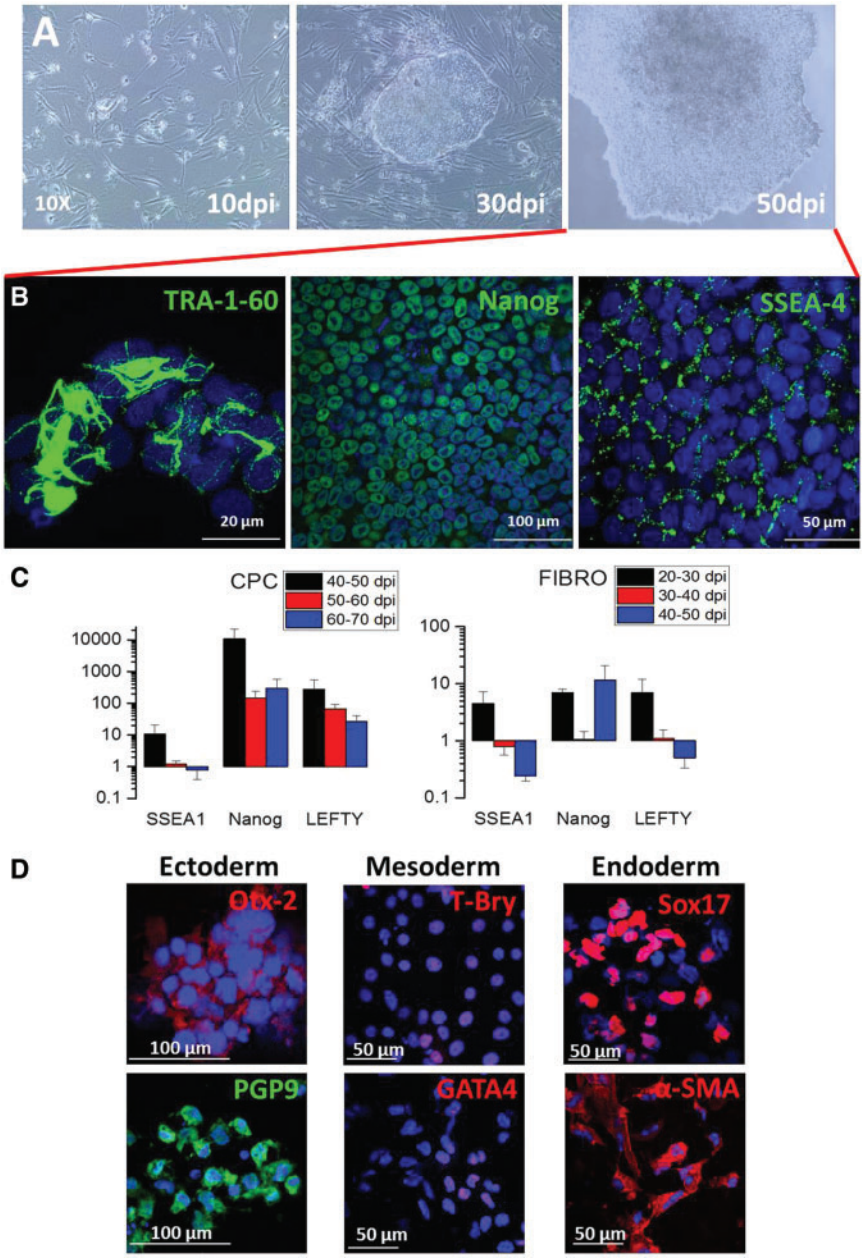

Figure 1 Generation and characterization of hiPSC-derived cardiomyocytes. (*A*) Bright-field photomicrographs of CPCs at 10, 30, and 50 days post-infection (dpi). A cell cluster showing ESC-like morphology at 30 dpi is shown. (*B*) Immunostaining of hiPSCs generated from CPCs showing expression of the pluripotency markers TRA1-60, Nanog, and SSEA-4. (*C*) Real-time RT-PCR data on ESC-associated gene expression (*SSEA-1, Nanog, Lefty*) in hiPSCs of both somatic cell origins, CPCs and dermal fibroblasts (FIBRO), at the indicated time points. With the exception of the shared 40–50 dpi time point, time points of RT–PCR analyses differed for the two groups as a result of different growth rates and culture passages. (*D*) Immunostaining of hiPSCs generated from CPCs and cultured in various differentiation media showing expression of the indicated ectodermal, mesodermal, and endodermal markers in cells cultured in the respective differentiation media.

in cFP durations. These results indicate presence of functional Na^+ channels that are essential for initiation of APs in hiPSC-derived cardiomyocytes of both somatic cell origins. The L-type Ca^{2+} channel blocker nifedipine (20 nM) slowed the spontaneous beating rates while also shortening cFPs in hiPSC-derived cardiomyocytes from both somatic cell types (*Figure 4A*). These results demonstrate presence of functional Ca^{2+} channels in hiPSC-derived cardiomyocytes of both somatic cell origins.

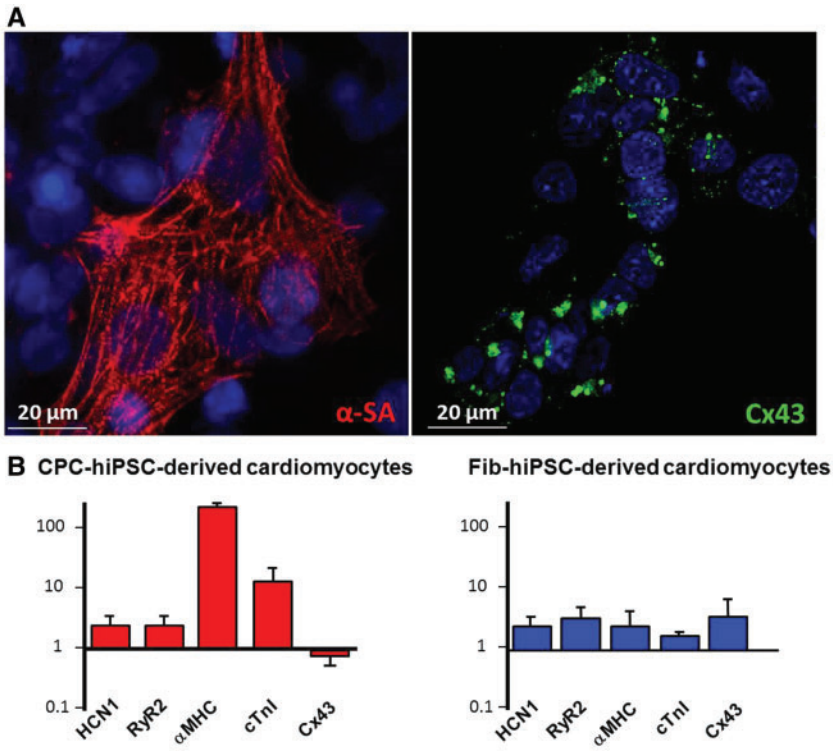

Figure 2 Cardiac differentiation of hiPSCs. (*A*) Immunostaining of hiPSC-derived cardiomyocytes generated from CPCs, demonstrating expression of α-sarcomeric actinin (α-SA) and connexin 43 (Cx43). (*B*) Real-time RT–PCR data on expression of the indicated cardiac-specific genes in hiPSC-derived cardiomyocytes of both somatic cell origins (CPC- and Fib-hiPSC-derived cardiomyocytes).

Human-induced pluripotent stem cell-derived cardiomyocytes are E4031-sensitive

E4031 (1 µM), a selective blocker of the human Ether-à-go-go Related Gene (hERG) K^+ channel that conducts the rapid component of the delayed rectifier K^+ current (I_{Kr}), markedly slowed the spontaneous beating frequencies and prolonged cFPs in hiPSC-derived cardiomyocytes of both somatic cell origins (*Figure 4B*). These results indicate prominent expression of functional hERG K^+ channels in both cell populations.

Human-induced pluripotent stem cell-derived cardiomyocytes derived from cardiac-stromal progenitor cells are JNJ303-sensitive

JNJ303 (2 µM), a selective and potent blocker agent of the slow component of the delayed rectifier K^+ current (I_{Ks}), slightly slowed beating frequencies and marginally prolonged cFPs in hiPSC-derived cardiomyocytes generated from CPCs, but not in those generated from dermal fibroblasts (*Figure 5*). These observations suggest that functional I_{Ks} currents may be present in hiPSC-derived cardiomyocytes of cardiac origin. However, quantitative differences between hiPSC-derived cardiomyocytes of cardiac vs. non-cardiac origin with respect to JNJ303-sensitivity were modest, and therefore no definitive conclusions on this issue can be drawn.

Discussion

Human-induced pluripotent stem cell-derived cardiomyocytes offer a unique patient-specific investigational platform in cardiomyocyte physiology. However, relative immaturity of these cells compared with primary adult ventricular myocytes remains a significant limitation. As residual somatic cell memory influences the differentiation potential of hiPSC-derived cells, we evaluated adult CPCs as a somatic cell source of hiPSC-derived cardiomyocytes. Here, we show that four different ionic currents associated with arrhythmic disorders are present in hiPSC-derived cardiomyocytes generated from adult CPCs. Using MEAs, multicellular electrophysiological recordings were performed on hiPSC-derived cardiomyocytes of cardiac vs. non-cardiac origin. Results are summarized in *Table 1*. Norepinephrine caused a dose-dependent increase in beating frequencies without major changes in extracellular cFP durations in cardiomyocytes of both somatic cell origins. Tetrodotoxin (10 µM) reduced total spike amplitude and slightly slowed beating frequencies in hiPSC-derived cardiomyocytes of cardiac origin, and totally abolished the instantaneous component of depolarization in those of dermal origin, indicating presence of TTX-sensitive Na^+ current (I_{Na}) in

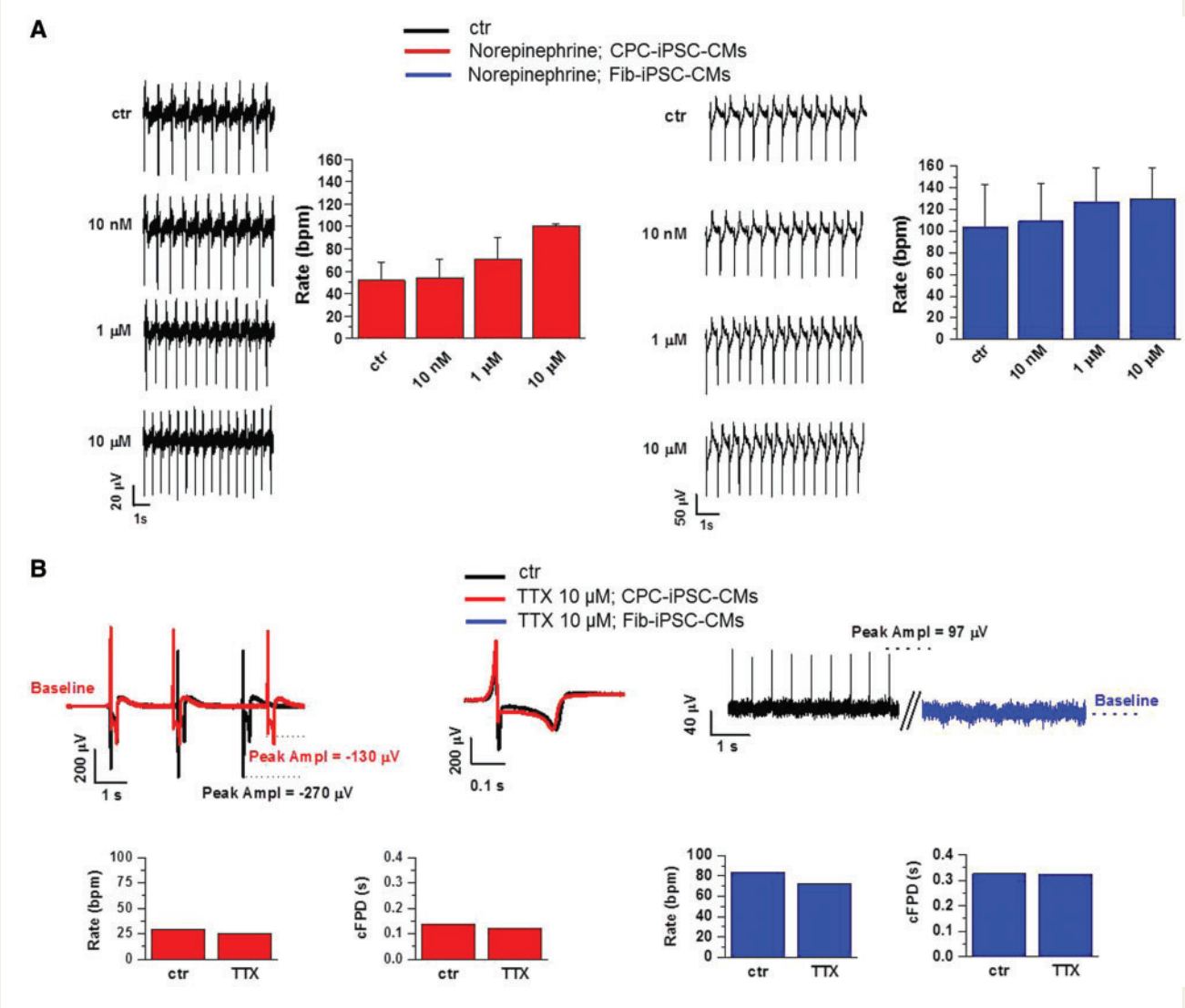

Figure 3 Multi-electrode arrays electrophysiological analyses of the effects of norepinephrine or I_{Na} blockade in hiPSC-derived cardiomyocytes generated from CPCs or skin fibroblasts (CPC- and Fib-iPSC-CMs, respectively). (*A*) Norepinephrine caused a dose-dependent increase in spontaneous beating rates in hiPSC-derived cardiomyocytes of both somatic cell origins. (*B*) Tetrodotoxin (TTX; 10 µM) reduced peak voltage amplitude by 51% in hiPSC-derived cardiomyocytes of cardiac origin and totally abolished the instantaneous component of depolarization in those of fibroblastic origin. Tetrodotoxin slowed spontaneous beating frequencies without major changes in cFP durations in both groups.

both groups. These results are in general agreement with previous data in human ESC-derived cardiomyocytes[14] and hiPSC-derived cardiomyocytes generated from a fibroblast cell line.[5] Nifedipine (20 nM) slowed beating frequencies and shortened extracellular cFPs in both cell groups, indicating prominent presence of nifedipine-sensitive L-type Ca^{2+} current (I_{CaL}). These findings are in general agreement with previous data in hPSC-CMs.[15] Cardiomyocytes formed during early iPSC *in vitro* differentiation exhibit spontaneous APs and a relatively depolarized resting membrane potential, presumably as a result of the momentary lack of the inward rectifier K^+ current (I_{K1}). In this study, E4031 (1 µM), a selective blocker of the rapid component of the delayed rectifier K^+ current (I_{Kr}), slowed spontaneous beating frequencies and prolonged extracellular cFPs, consistent with expression of functional hERG channels, in hiPSC-derived cardiomyocytes of both somatic cell origins. These results are in general agreement with previous data using different hERG blockers, such as dofetilide and D,L-sotalol, in hPSC-derived cardiomyocytes,[15] and using E4031 in high-purity hiPSC-derived cardiomyocytes.[5]

JNJ303 (2 µM), a potent and selective blocker of the slow component of the delayed rectifier K^+ current (I_{Ks}), slightly slowed spontaneous beating frequencies and marginally prolonged extracellular cFPs in hiPSC-derived cardiomyocytes of cardiac somatic origin, consistent with presence of I_{Ks}, but not in those of non-cardiac origin. In principle, these observations would suggest a possible functional improvement in hiPSC-derived cardiomyocytes of cardiac vs. non-

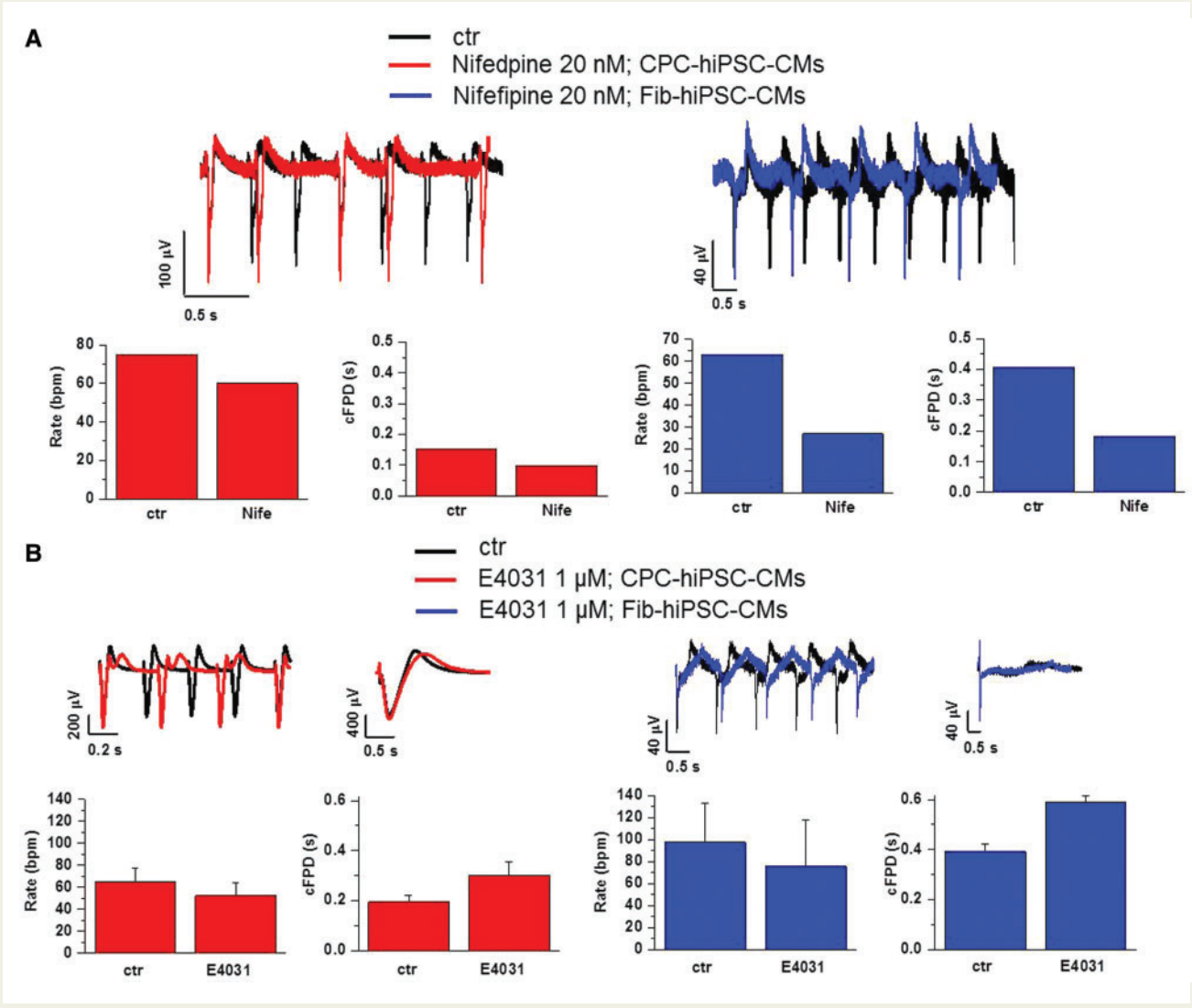

Figure 4 Multi-electrode arrays electrophysiological analyses of the effects of I_{CaL} and I_{Kr} blockade in hiPSC-derived cardiomyocytes generated from CPCs or skin fibroblasts (CPC- and Fib-iPSC-CMs, respectively). (*A*) Nifedipine (Nife; 20 nM) slowed spontaneous beating rates and shortened cFPs in hiPSC-derived cardiomyocytes of both somatic cell origins. (*B*) E4031 (1 µM) slowed spontaneous beating frequencies and prolonged cFPs in both groups.

cardiac origin; however, quantitative differences between the two groups in terms of JNJ303 effects were modest and their statistical significance could not be tested due to the small sample size. Thus, we have provided evidence of the presence of I_{Ks} in hiPSC-derived cardiomyocytes of cardiac origin. However, our data do not rule out presence of I_{Ks} in hiPSC-derived cardiomyocytes of non-cardiac origin, despite the fact that we were unable to detect it. A few previous reports support presence of I_{Ks} in the latter. In a recent study, JNJ303 and HMR1556, a distinct I_{Ks} blocker, exerted only minor effects on the extracellular FPs in hPSC-derived cardiomyocytes despite evidence of functional I_{Ks} channels.[15] Nevertheless, JNJ303 strongly prolonged FP duration in models of diminished 'repolarization reserve', a concept that reflects the capacity of cardiomyocytes to repolarize, and a strong risk factor for the development of ventricular arrhythmias. In another study using AP recordings, presence of I_{Ks} as a 3R4S-chromanol 293B-sensitive current (in the continuous presence of 500 nmol/L E4031 to block I_{Kr}) was detected in 5 of 16 spontaneously beating hiPSC-derived cardiomyocytes of fibroblastic origin.[5]

Our findings in hiPSC-derived cardiomyocytes using donor adult CPCs are in general agreement with recent data using donor foetal CPCs.[10,11] Human-induced pluripotent stem cells generated from foetal CPCs exhibited higher cardiac differentiation efficiency compared with those obtained from skin fibroblasts, although this was not associated with functional improvement. Selective ionic current blockade was not tested in these studies. We likewise observed a higher upregulation of cardiac-specific genes in hiPSC-derived cardiomyocytes of cardiac vs. non-cardiac origin, without major functional

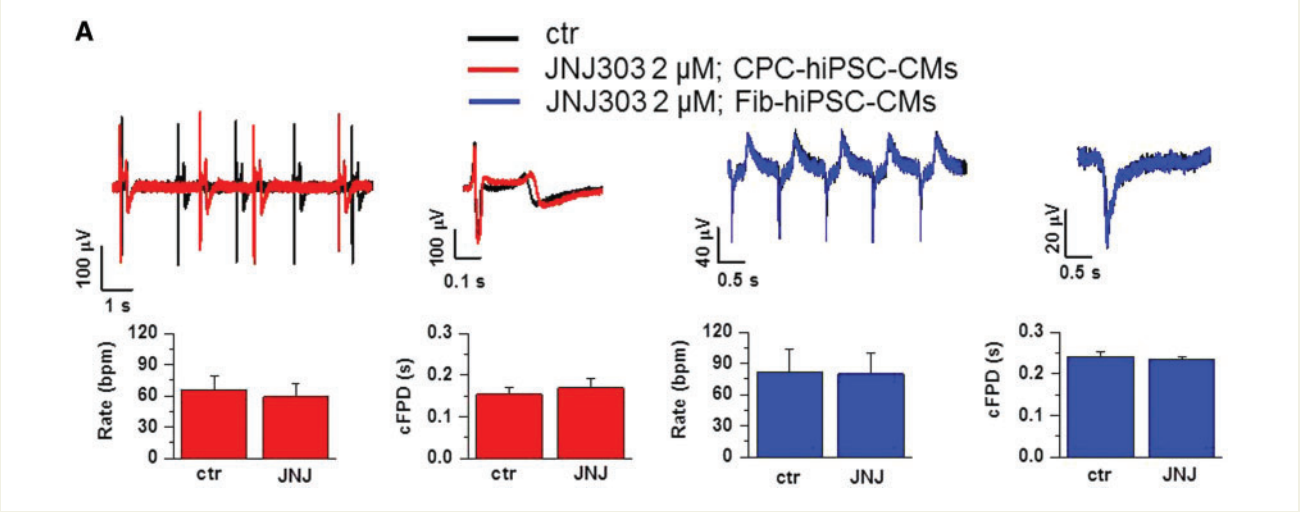

Figure 5 Multi-electrode arrays electrophysiological analyses of the effects of I_{Ks} blockade in hiPSC-derived cardiomyocytes generated from CPCs or skin fibroblasts (CPC- and Fib-iPSC-CMs, respectively). JNJ303 (2 μM) slightly slowed beating frequencies and marginally prolonged cFPs in hiPSC-derived cardiomyocytes from CPCs, but not in those from fibroblasts.

Table 1 Summary of the predicted and observed effects of the indicated drugs on spontaneous beating frequencies and FP duration in hiPSC-derived cardiomyocytes generated from CPCs or skin fibroblasts (CPC- and Fib-iPSC-CMs, respectively)

			Observed effect on beating rates			Observed effect on repolarization	
Compound class	**Name**	**Predicted effect on beating rates**	**CPC-hiPS cardiomyocytes**	**Fib-hiPS cardiomyocytes**	**Predicted effect on repolarization**	**CPC-hiPS cardiomyocytes**	**Fib-hiPS cardiomyocytes**
Adrenergic agonist	Norepinephrine	Increase	Increase	Increase	FP shortening	Minor shortening	Unchanged
I_{Na} blocker	TTX	Unchanged	Decrease	Decrease	Unchanged	Observed	Observed
Calcium (I_{CaL}) blocker	Nifedipine	Decrease	Decrease	Decrease	FP shortening	Observed	Observed
hERG (I_{Ks}) blocker	E4031	Decrease	Decrease	Decrease	FP prolongation	Observed	Observed
I_{Ks} blocker	JNJ303	Decrease	Minor decrease	Unchanged	Minor FP prolongation	Observed	Not observed

differences between the two populations, as discussed above. Further studies are needed to address potential advantages of donor CPCs over non-cardiac somatic cell types for generation of hiPSC-derived cardiomyocytes.

Human-induced pluripotent stem cell-derived cardiomyocytes for disease modelling in cardiac electrophysiology

Human-induced pluripotent stem cell-derived cardiomyocytes enable researchers to elucidate the mechanistic relationships between patients' genotypes and their electrophysiological phenotypes, including the functionality of ion-channel mutations within their individual genetic backgrounds. Examples of hiPSC-based applications to the study of genetic arrhythmias are mentioned above.[1,3,4,7] An exciting application field is patient-specific drug testing. During the early stages of drug development, the selection of early hit-molecules for further development towards pre-clinical drug candidates relies on predictive assays to identify efficacy and safety. There is an unmet need for testing cardiac responses of candidate drugs in a predictable model that recapitulates the complex ion-channel interactions of cardiomyocytes in the heart of an individual patient. Owing to the observation that hERG blocking compounds can induce ventricular tachyarrhythmias known as *torsades de pointes*, early efforts at evaluating pharmacological risks of lethal arrhythmias were based on electrophysiological assays measuring I_{Kr} blockade. However, hERG blockade alone does not detect all molecules that can induce delayed repolarization and *torsades de pointes*, as AP repolarization does not

solely depend on I_{Kr} but also on other K^+ currents including I_{Ks}. In a recent study, hERG or calcium blocking properties could be reliably identified among a set of compounds using MEA recordings in hPSC-derived cardiomyocytes.[15] The safety profiles that emerged from these analyses were comparable with those using current assays based on primary cardiomyocytes. In addition, more complex genetic and compound interactions could be detected using this model. Reduced repolarization reserve affected sensitivity to I_{Ks} blockade as pharmacological inhibition of I_{Kr} predisposed to enhanced sensitivity to I_{Ks} blockade. Genetic inhibition caused by a mutation in hERG in LQT2 patients, from whom hPSC-derived cardiomyocytes were obtained, induced similar effects.

Human-induced pluripotent stem cell-derived cardiomyocyte models are amenable to high-throughput analyses in drug discovery,[16] which can provide a general prediction of a compound before launching the high-cost preclinical studies. Genetically encoded fluorescent voltage sensors that accurately measure transmembrane potentials in hiPSC-derived cardiomyocytes can be used in rapid cardiomyocyte phenotyping and screening for drug-induced cardiotoxicity. Whether or not hiPSC-derived cardiomyocytes, despite their promises, will actually play a significant role in arrhythmia risk assessment remains unclear. Several limitations of these cells including relative immaturity and heterogeneous electrophysiological phenotypes must be taken into account.[6] The latter aspect relates to differences in PSC induction and cardiomyocyte differentiation protocols.

In silico electrophysiological models for pharmacological safety assessment

Platforms used to investigate arrhythmia and the effect of drug action on the electrical activity of the heart range from cell-based to organism-based and computer-based platforms. Although our study did not address *in silico* electrophysiological models, is evident the potential of strategies that combine both cellular and *in silico* approaches.

A recent study compared *in silico* AP models of ventricular-like and atrial-like hiPSC-derived cardiomyocytes and human adult ventricular cardiomyocytes for simulation of the main effects of four selective current blockers.[17] Qualitatively, *in silico* hiPSC-derived cardiomyocyte and human adult ventricular cardiomyocyte APs showed similar responses to current blockade consistent with results from experiments. Quantitatively, however, the *in silico* hiPSC-derived cardiomyocyte model was more sensitive to block of I_{CaL} due to overexpression of the Na^+/Ca^{2+} exchanger, as well as to block of I_{K1} due to reduced repolarization reserve. Thus, *in silico* hiPSC-derived cardiomyocytes exhibited greater sensitivity to block, a feature that could potentially facilitate the detection of drug-induced effects.

A general caveat, however, is that the prediction accuracy of an *in silico* model relies mainly on the precision of the underlying experimental data used to build the model. Both *in vitro* and *in silico* models need to be validated through testing panels of drugs with pro-arrhythmic activity and drugs lacking this activity. Another concern is patient background, with respect to the definition of a normal individual, as well as for the possibility of incorporating hiPSC-derived cardiomyocytes from patients harbouring genetic mutations or polymorphisms that increase susceptibility to drug-induced arrhythmia.[16]

Conclusion

We have generated hiPSC-derived cardiomyocytes from adult CPCs and skin fibroblasts, providing electrophysiological evidence of presence of I_{Na}, I_{CaL}, and I_{Kr} currents in hiPSC-derived cardiomyocytes of both somatic cell origins, as well as of I_{Ks} in those of cardiac origin. Thus, hiPSC-derived cardiomyocytes express a panel of functional ion channels that play important roles in arrhythmic disorders. By recapitulating the complex ion-channel interactions of cardiomyocytes in the heart of an individual patient, hiPSC-derived cardiomyocytes provide a unique tool in cardiac electrophysiology and personalized medicine. Both *in vitro* and *in silico* models for functional assessment of ion channels are of great interest to potential applications in cardiomyocyte physiology and preclinical drug safety screening.

Acknowledgements

Patrizia Dell'Era, Ph.D. (Fibroblast Reprogramming Unit, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy) provided expert advice on cell reprogramming.

Conflict of interest: none declared.

Funding

The study was supported by a grant of the Cecilia-Augusta Foundation, Lugano, Switzerland. Hartman Müller Foundation, Zurich Switzerland; Swiss Heart Foundation, Bern, Switzerland.

References

1. Chen IY, Matsa E, Wu JC. Induced pluripotent stem cells: at the heart of cardiovascular precision medicine. *Nat Rev Cardiol* 2016;**13**:333–49.
2. Yamanaka S. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 2012;**10**:678–84.
3. Karakikes I, Ameen M, Termglinchan V, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes: insights into molecular, cellular, and functional phenotypes. *Circ Res* 2015;**117**:80–8.
4. Sallam K, Li Y, Sager PT, Houser SR, Wu JC. Finding the rhythm of sudden cardiac death: new opportunities using induced pluripotent stem cell–derived cardiomyocytes. *Circ Res* 2015;**116**:1989–2004.
5. Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ, January CT. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am J Physiol Heart Circ Physiol* 2011;**301**:H2006–17.
6. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res* 2014;**114**:511–23.
7. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, Seyfarth M, Sinnecker D, Schömig A, Laugwitz KL. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 2010;**363**:1397–409.
8. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, Huo H, Loh YH, Aryee MJ, Lensch MW, Li H, Collins JJ, Feinberg AP, Daley GQ. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat Biotechnol* 2011;**29**:1117–9.
9. Rizzi R, Di Pasquale E, Portararo P, Papait R, Cattaneo P, Latronico MV, Altomare C, Sala L, Zaza A, Hirsch E, Naldini L, Condorelli G, Bearzi C. Postnatal cardiomyocytes can generate iPS cells with an enhanced capacity toward cardiomyogenic re-differentation. *Cell Death Differ* 2012;**19**:1162–74.
10. Sanchez-Freire V, Lee AS, Hu S, Abilez OJ, Liang P, Lan F, Huber BC, Ong SG, Hong WX, Huang M, Wu JC. Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells. *J Am Coll Cardiol* 2014;**64**:436–48.
11. Meraviglia V, Wen J, Piacentini L, Campostrini G, Wang C, Florio MC, Azzimato V, Fassina L, Langes M, Wong J, Miragoli M, Gaetano C, Pompilio G, Barbuti A, DiFrancesco D, Mascalzoni D, Pramstaller PP, Colombo GI, Vincent Chen HS, Rossini A. Higher cardiogenic potential of IPSCs derived from cardiac versus skin stromal cells. *Front Biosci (Landmark)* 2016;**21**:719–43.
12. Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM, Torre T, Siclari F, Moccetti T, Vassalli G. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. *Cardiovasc Res* 2014;**103**:530–41.

13. Lian X, Hsiao C, Wilson G, Zhu K, Hazeltine LB, Azarin SM, Raval KK, Zhang J, Kamp TJ, Palecek SP. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc Natl Acad Sci* 2012;**109**:E1848–57.
14. Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I, Gepstein L. Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol* 2004;**559**(Pt 2):479–96.
15. Braam SR, Tertoolen L, Casini S, Matsa E, Lu HR, Teisman A, Passier R, Denning C, Gallacher DJ, Towart R, Mummery CL. Repolarization reserve determines drug responses in human pluripotent stem cell derived cardiomyocytes. *Stem Cell Res* 2013;**10**:48–56.
16. del Alamo JC, Lemons D, Serrano R, Savchenko A, Cerignoli F, Bodmer R, Mercola M. High throughput physiological screening of iPSC-derived cardiomyocytes for drug development. *Biochim Biophys Acta* 2016;**1863**(7 Pt B):1717–27.
17. Paci M, Hyttinen J, Rodriguez B, Severi S. Human induced pluripotent stem cell-derived versus adult cardiomyocytes: an *in silico* electrophysiological study on effects of ionic current block. *Br J Pharmacol* 2015;**172**:5147–60.