Molecular Cardiology

Cardiomyocyte Differentiation of Human Induced Pluripotent Stem Cells

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Background—The ability to derive human induced pluripotent stem (hiPS) cell lines by reprogramming of adult fibroblasts with a set of transcription factors offers unique opportunities for basic and translational cardiovascular research. In the present study, we aimed to characterize the cardiomyocyte differentiation potential of hiPS cells and to study the molecular, structural, and functional properties of the generated hiPS-derived cardiomyocytes.

Methods and Results—Cardiomyocyte differentiation of the hiPS cells was induced with the embryoid body differentiation system. Gene expression studies demonstrated that the cardiomyocyte differentiation process of the hiPS cells was characterized by an initial increase in mesoderm and cardiomesoderm markers, followed by expression of cardiac-specific transcription factors and finally by cardiac-specific structural genes. Cells in the contracting embryoid bodies were stained positively for cardiac troponin-I, sarcomeric α-actinin, and connexin-43. Reverse-transcription polymerase chain reaction studies demonstrated the expression of cardiac-specific sarcomeric proteins and ion channels. Multielectrode array recordings established the development of a functional syncytium with stable pacemaker activity and action potential propagation. Positive and negative chronotropic responses were induced by application of isoproterenol and carbamylcholine, respectively. Administration of quinidine, E4031 (I.Kr blocker), and chromanol 293B (I.Ks blocker) significantly affected repolarization, as manifested by prolongation of the local field potential duration.

Conclusions—hiPS cells can differentiate into myocytes with cardiac-specific molecular, structural, and functional properties. These results, coupled with the potential of this technology to generate patient-specific hiPS lines, hold great promise for the development of in vitro models of cardiac genetic disorders, for drug discovery and testing, and for the emerging field of cardiovascular regenerative medicine. (Circulation. 2009;120:1513-1523.)

Key Words: differentiation  myocytes  stem cells

The derivation of human embryonic stem cells (hESCs)1 and their proven ability to differentiate into a plurality of cell lineages, including cardiomyocytes,2-7 have provided researchers with a unique tool for several research areas, including the emerging field of cardiovascular regenerative medicine. A major obstacle for the use of hESC derivatives for myocardial regeneration, however, is the expected immune rejection of such allogenic cell grafts. Similarly, the possibility of deriving patient-specific hESC lines as models to study disease-specific processes has thus far proved to be largely impractical.

In 2006, Takahashi and Yamanaka8 were able to reprogram mouse fibroblasts by ectopic retroviral expression of 4 transcription factors (Oct4, Sox2, c-Myc, and Klf4), yielding cells with characteristics similar to those of mouse ESCs. The resulting mouse induced pluripotent stem (iPS) cells were demonstrated to be able to differentiate into cell derivatives of all 3 germ layers, including functional cardiomyocytes.9,10 Application of this approach to human somatic cells followed shortly, yielding human iPS (hiPS) cell lines with characteristics similar to hESCs.11,12 More recently, the ability to generate patient-specific hiPS cell lines was also reported.13,14

Whereas a number of studies described the general in vitro differentiation capabilities of various hiPS cell lines into cell derivatives of the 3 germ layers, the data are limited with regards to the ability to establish specific differentiation toward the cardiac lineage.15 Therefore, the objective of the present study was to characterize the cardiomyocyte differentiation potential of hiPS cells, to define the developmental steps involved in this differentiation process, and to study the...
molecular, structural, and functional properties of the generated hiPS cell–derived cardiomyocytes (hiPS-CMs).

Methods

Culture of hiPS and Cardiomyocyte Differentiation

The hiPS cell lines used in the present study (hFib2-iPS cells; kindly provided by G.Q. Daley, Boston, Mass) were generated by retroviral transduction of human fibroblasts with OCT4, SOX2, c-MYC, and KLF4, together with hTERT and SV40-large T.16 These lines were shown to display all the defining parameters of iPS cell lines.16 The undifferentiated hiPS cell colonies were cultured on a mitotically inactivated mouse embryonic fibroblast (MEF) feeder layer (Figure 1A) as previously described.3 The culture medium consisted of 80% knockout high-glucose glutamine-free DMEM with sodium pyruvate supplemented with 20% serum replacement, 1 mmol/L L-glutamine, 0.1 mmol/L mercaptoethanol, 4 ng/mL human recombinant basic fibroblast growth factor, and 0.1% nonessential amino acid stock (Invitrogen, Carlsbad, Calif). To induce differentiation, hiPS cells were dispersed into small clumps with collagenase IV (1 mg/mL for 20 minutes; Life Technologies, Carlsbad, Calif). The cells were then transferred to plastic Petri dishes, where they aggregated to form embryoid bodies (EBs; Figure 1B) and were cultured in suspension for 10 days. The EBs were then plated on 0.1% gelatin–coated culture dishes and examined daily for the appearance of spontaneous contractions (Figure 1C).

Immunostaining Studies

Whole EBs were fixed with 4% paraformaldehyde, permeabilized with 1% Triton X-100 (Sigma, St Louis, Mo), blocked with 5% horse serum, and incubated overnight at 4°C with primary antibodies targeting the following: Tra-1-60 at 1:400 dilution, Oct-4 (1:150), connexin-43 (Cx43; 1:20) (all from Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), Nanog (1:400; Abcam, Cambridge, Mass), cardiac troponin-I (1:200; Chemicon, Temecula, Calif), SSEA-1 (1:100), SSEA-4 (1:200) (both from R&D Systems, Minneapolis, Minn), and sarcomeric α-actinin (1:200; Sigma). The preparations were incubated with secondary antibodies: donkey anti-mouse Cy2-labeled immunoglobulin G (IgG), donkey anti-goat Cy5-labeled IgG, donkey anti-rabbit Cy3-labeled IgG, donkey anti-goat Cy5-labeled IgG, and donkey anti-mouse Cy3-labeled IgM (all from The Jackson ImmunoResearch Laboratories, West Grove, Pa) at a dilution of 1:200 for 1 hour. Nuclei were counterstained with DAPI (1:500, Sigma). Preparations were examined with a laser scanning confocal microscope (Zeiss LSM-510-PASCAL, Carl Zeiss Microimaging, Thornwood, NY).

Reverse-Transcription Polymerase Chain Reaction

For reverse-transcription (RT) polymerase chain reaction (PCR) and real-time PCR analysis, undifferentiated hiPS cells (day 0) and differentiating EBs (4, 12, and 20 to 21 days of differentiation) were frozen in liquid nitrogen. RNA was isolated with the RNeasy plus mini kit (Qiagen GmbH, Hilden, Germany). RT into complementary DNA was conducted with Superscript II first-strand synthesis system (Invitrogen). The PCR-related primers are detailed in the Table. Each RT-PCR included 30 seconds at 95°C, 30 seconds at 56°C, and 1 minute at 72°C with AccuPower PCR premix (Bioneer, Daejeon, Korea). cDNA template (2.5 ng) was used from each sample.

SYBR green real-time PCR studies were performed with Absolute blue QPCR SYBR mix ROX (Thermo-Fisher, Erembodegem, Belgium) and primers (the Table). All experiments were conducted in
The Bazzet correction formula: 

\[ cFPD = \frac{FPD}{cFPD} \]

where FPD is the field potential duration (FPD) to the beating rate of the contracting areas with the local T wave. FPD measurements were normalized (corrected between the initial deflection of the FP to timing of the maximum of the measured FP to baseline (QTend) and as the time interval as the time interval between the initial deflection of the FP to return the local QT interval.\(^{18,21–23}\)

The estimated FPD was defined 2 ways: shown to correlate with the action potential duration and to reflect field potential (FP) duration (FPD). This parameter was previously derived from the constructed maps as previously described.\(^{20,21,23}\)

The recorded electrograms were also used to determine the local field potential (FP) duration (FPD). This parameter was previously shown to correlate with the action potential duration and to reflect the local QT interval.\(^{18,21–23}\) The estimated FPD was defined 2 ways: as the time interval between the initial deflection of the FP to return of the measured FP to baseline (QTend) and as the time interval between the initial deflection of the FP to timing of the maximum of the local T wave. FPD measurements were normalized (corrected FPD [cFPD]) to the beating rate of the contracting areas with the Bazzet correction formula: 

\[ cFPD = \frac{FPD}{\sqrt{RR \text{ interval}}} \]

This formula is routinely used in patients\(^{24}\) and animal models\(^{25}\) to standardized QT measurements. More recently, we have used the Bazzet formula to demonstrate predictable, dose-response, drug-induced changes in the cFPD of hESC-derived cardiomyocyte tissue.\(^{25}\)

To assess the effects of different drugs on the electrophysiological properties, 20 µL tested drug stock solution was added to 2 mL medium. The tested drugs included quinidine, isoproterenol hydrochloride, carbamylcholine, chromanol 293B, verapamil (all from Sigma), tetrodotoxin, and E4031 (Alomond Labs, Jerusalem, Israel).

Extracellular recording were performed for 60 seconds at baseline and at 5 minutes after drug application.

**Statistical Analysis**

Results are reported as mean±SEM. Drug effects on the cells were analyzed by paired t test (when a single dose was evaluated) or by repeated-measures ANOVA, followed by posthoc analysis with the Holm-Sidak test (when multiple doses of the test drug were evaluated). Values of \(P<0.05\) were considered statistically significant.

**Results**

**In Vitro Cardiomyocyte Differentiation of hiPS Cells**

The hiPS colonies were propagated in the undifferentiated state on top of the MEF feeder layer (Figure 1A). The morphology of the undifferentiated colonies closely resembled that of the hESCs, and they were stained positively for the pluripotent markers Nanog, Oct-4, TRA-I-60, and SSEA-1, which is not expressed by undifferentiated hiPS cells and SSEA-4 (Figure 1D through 1G, left) but not for SSEA-1, which is not expressed by undifferentiated hiPS cells and SSEA-4 (Figure 1H). The expression of these pluripotent markers eventually disappeared as the hiPS cells began to differentiate as EBs (Figure 1D through 1G, right).
To induce cardiomyocyte differentiation, the hiPS cells were removed from the MEF feeder and cultivated in suspension, where they formed 3-dimensional differentiating cell aggregates (EBs) (Figure 1B). After 10 days in suspension, the EBs were placed on gelatin-covered plates. Rhythmically contracting areas appeared as early as 6 days after plating (Figure 1C and Movie I of the online-only Data Supplement). The contracting areas had a diameter ranging from 0.2 to 1.5 mm and continued to beat vigorously for several weeks in culture.

We next evaluated the temporal gene expression pattern associated with this in vitro differentiation process. As can be seen in the real-time PCR experiments in Figure 2, differentiation was characterized by an initial decrease in the expression of undifferentiated pluripotent markers (OCT4 and NANOG). The first event related to cardiomyogenesis was a peak in the expression of primitive streak, mesoderm, and cardiomesoderm markers (Brachyury and MESP1) at 4 days of differentiation. This was followed by expression of cardiac progenitor markers (such as Isl-1, a marker of secondary heart field progenitors) and cardiac-associated transcription factors (Nkx2.5 [Nkx2.5], Mef-2c [MEF2C], and Gata-4 [GATA4]). The last markers to be expressed were cardiogenic-specific structural genes, including sarcomeric-related proteins (α-myosin heavy chain [MYH6], β-myosin heavy chain [MYH7], and cardiac troponin-I [CTNNI]) and ion channel proteins (Ca,1.2 [CACNA1C] and Ca,1.3 [CACNA1D]) and ion channel proteins (Ca,1.2 [CACNA1C] and Ca,1.3 [CACNA1D]) encoding the α-1C and α-1D subunits of the L-type calcium channel; KCNH2 [hERG] mediating the rapid delayed rectifier potassium current [I_{K1}]; and the hyperpolarization-activated cyclic nucleotide-gated potassium channel [HCN-2] responsible for the If pacemaker current).

**Molecular and Structural Characterization of the hiPS-CMs**

Immunostaining studies were performed to determine the presence of cardiac-specific proteins in cells within the contracting EBs and to define their spatial organization. As can be seen in Figure 3, the hiPS-CMs were stained positively for the sarcomeric proteins cardiac troponin-I and sarcomeric α-actinin. The cardiomyocytes within the beating areas were arranged as an isotropic tissue (with cells projecting in various orientations). Positively stained cardiomyocytes demonstrated an immature striated pattern, typical of early-stage myocytes. We next assessed whether the generated hiPS-CMs can form cell-to-cell structural connections. To this end, co-staining experiments were performed, focusing on the major gap junction protein Cx43 (although other connexins were also demonstrated to contribute to developing gap junctions). As can be seen in Figure 3C, a punctuate Cx43 immunosignal (red) pattern that seemed to be localized to the junctions between the hiPS-CMs (green cells positively stained for sarcomeric α-actinin) was identified, suggesting the development of gap junctions and intact electric coupling.

We next used semiquantitative RT-PCR to assess the expression of cardiac-related genes by the contracting areas. These studies (Figure 3D) demonstrated the expression of several cardiac-related transcription factors (Nkx2.5, MEF-2c, and GATA-4) and structural genes encoding for sarcomeric-related proteins (myosin light chain-2V [MLC-2v], MYH6, MYH7, and cTNNI) and cardiac ion channels (such as the L-type calcium channel [Ca,1.2 (CACNA1C) and Ca,1.3 (CACNA1D)] and KCNH2 and KCNQ1, which are responsible for the rapid [I_{kr}] and slow [I_{ks}] components of the delayed rectifier potassium current).

**Functional Characterization of hiPS-CMs**

We next sought to determine whether the hiPS-CMs display functional electrophysiological properties and specifically whether they can generate a functional syncytium. To this end, we microdissected the beating areas and plated them on top of MEA plates (Figure 4A). The extracellular electrograms, recorded from several electrodes underlying the beating EB (Figure 4B), were analyzed and used to construct detailed electric activation maps (Figure 4C). These maps demonstrated the development of a functional syncytium in the hiPS cell–derived cardiac tissue with stable pacemaker activity and synchronized action potential propagation (with the wave front propagating from the earliest [red] to the latest [dark blue] activation sites; Figure 4C). Interestingly, both pacemaker position and the conduction patterns were relatively stable and reproducible within each EB.

**Chronotropic Responses and Pharmacological Studies**

One of the most important properties of cardiac tissue physiology is the ability to respond appropriately to neuro-hormonal regulation. To address this issue, we assessed the ability of the hiPS-CMs to adequately respond to adrenergic and muscarinic stimuli. Specifically, we evaluated the possible chronotropic changes induced by administration of the β-agonist isoproterenol (Figure 5A) and the muscarinic agonist carbamylcholine (Figure 5B). Isoproterenol administration resulted in a dose-dependent increase in the spontaneous beating frequency of the hiPS cardiac tissue (by 14±5% and 46±5% at 1 and 10 μmol/L, respectively; n=7; Figure 5D). In contrast, carbamylcholine administered resulted in a dose-related decrease in the spontaneous beating frequency (a 21±4% and 34±5% decrease from its baseline value at doses of 1 and 10 μmol/L, respectively; n=9; Figure 5D). Interestingly, pacemaker position remained rather stable during both muscarinic and adrenergic stimulation. Thus, in 80% of the EBs studied, the earliest activation site remained stable despite application of escalating doses of these agonists.

We next evaluated whether the hiPS cell–derived cardiac tissue can also serve as a platform for drug testing and specifically whether it can be used to evaluate drug effects on repolarization (QT screening). To this end, we used the MEA system to measure the cFPD from hiPS-CMs. These measurements were previously shown to predict the effects of known chemicals on the repolarization properties of neonatal rat ventricular myocytes and mouse ESC- and hESC-derived cardiac tissue.

We first evaluated the effects of quinidine (100 μmol/L), a class IA antiarrhythmic agent (Figure 6A). Quantitative assessment with 2 different measuring algorithms (from the initial deflection of the extracellular potential [vertical line in Figure 6A] to either the peak of the local T wave [arrow] or its return to baseline [arrowhead]) demonstrated a significant
Figure 2. Gene-expression patterns during hiPS cardiomyogenesis. Real-time PCR studies showing that cardiomyocyte differentiation was characterized by a continuous decrease in the expression of pluripotent markers (OCT4 and NANOG) coupled with an initial increase in mesoderm and cardiomesoderm markers (Brachyury and MESP1). This was followed by the expression of secondary heart field progenitor marker (Isl-1), by cardiac-related transcription factors (NKX2.5, MEF-2c, and GATA-4), and finally by cardiac-specific structural genes (MYH7, MYH6, cTNNI, and MLC-2A) and ionic channels (CACNA1C, CACNA1D, KCNH2, and HCN-2). Depicted is the mean±SEM of the relative gene expression at each time point as normalized by baseline values.
increase in the cFPD after quinidine treatment (Figure 6B). Interestingly, the effect of quinidine (a sodium channel blocker) on the amplitude of the first negative peak of the FP was variable, with a reduction in some EBs (as noted previously\(^{18,22}\)) and an increase in others (Figure 6A), a phenomenon that is probably due to the multichannel blocking properties of this agent. Similarly, the effect of quinidine on chronotropy was also variable, slightly increasing the rate...
is some EBs and decreasing it in others. This effect may also stem from the multichannel blocking properties of quinidine and perhaps from the variability in adrenergic receptor expression.

Quinidine is known to modulate a number of ionic currents; therefore, its effect on the local FP morphology may be multifactorial. To dissect the relative contribution of the different ionic currents to the electrophysiological signature of the hiPS-CMs, we evaluated some more specific ion channel blockers. Application of chromanol 293B (n=8), a specific blocker of the slow component of the delayed rectifier potassium current (\(I_{Kr}\)), resulted in a dose-dependent increase in the cFPD (Figure 6C). Similarly, application of E4031 (n=7), a specific blocker of the rapid component of the delayed rectifier potassium current (\(I_{Kr}\)), also resulted in significant prolongation of the cFPD (Figure 6D). This latter finding is important because the HERG-encoded \(I_{Kr}\) current is the most common target of pharmacological compounds known to be associated with QT prolongation and proarrhythmia.

We next studied the effects of verapamil, an L-type calcium channel blocker. As expected from an inward current blocker, verapamil (1 \(\mu\)mol/L; n=8) significantly shortened the cFPD (Figure 6E). Interestingly, verapamil also affected the chronotropic properties of the hiPS-CMs. At 1 \(\mu\)mol/L, verapamil significantly reduced the beating frequency of the hiPS-CMs (Figure 5C and 5E), whereas at 5 \(\mu\)mol/L, spontaneous beating was completely arrested (Figure 5C).

Finally, the potential to evaluate drug effects on conduction was studied by analyzing MEA recordings from the hiPS cell–derived cardiac tissue. As an example for an intervention that slows conduction, we evaluated the effects of tetrodotoxin, a sodium channel blocker. As the example in Figure 7A shows, application of tetrodotoxin (10 \(\mu\)mol/L) resulted in significant slowing of conduction, manifested by an increase in the total activation time of the culture from 39 to 60 milliseconds and localized crowding of isochrones. This phenomenon was observed in all the preparations examined (n=7), with a significant increase in the mean total activation time (Figure 7B) and a significant decrease in both the global conduction velocity and the average local vectorial conduction velocity (Figure 7C).

**Discussion**

The ability to generate pluripotent hiPS cell lines by reprogramming of somatic cells introduces a powerful new research tool that can potentially be used to develop cell replacement strategies that can evade the immune system, to study basic disease mechanisms, and to establish screens for drug discovery. Yet, to fulfill the potential of this unique technology, strategies have to be developed to allow the differentiation of well-characterized functional cell derivatives. In the present study, we aimed to assess the ability of hiPS cells to differentiate into the cardiac lineage. Our results show (1) that the hiPS cell–based EB differentiating system can give rise (in a manner similar to the hESC system) to Figure 5. Chronotropic responses to pharmacological interventions. A through C, Chronotropic changes induced by increasing doses of isoproterenol (A), carbamylcholine (B), and verapamil (C). D, Summary of the positive and negative chronotropic changes induced by the adrenergic (isoproterenol) and muscarinic (carbamylcholine) agonists, respectively. E, Summary of the effects of verapamil on beating frequency. *\(P<0.05\), **\(P<0.01\) vs baseline.
cardiomyocytes with the appropriate molecular, structural, and functional properties; (2) that the temporal gene expression pattern associated with hiPS cardiomyogenesis involves the expression of mesoderm- and cardiomesoderm-specific markers, followed by expression of cardiac-specific transcription factors and structural genes; (4) that this differentiating system is not limited to the generation of isolated cardiomyocytes but rather a functional cardiac syncytium is formed; and (5) that the hiPS cell–derived cardiac tissue can respond appropriately to adrenergic and muscarinic signals and can serve as a unique platform for drug testing.

Our results showing the ability to achieve cardiomyocyte differentiation of hiPS cells (established by retroviral reprogramming of fibroblasts with OCT4, SOX2, c-MYC, and KLF4 and supplemented by hTERT and SV40 large T) are in agreement with 2 recent studies showing similar cardiomyocyte differentiation potential in the mouse iPS cell system and with a very recent report showing the ability to derive cardiomyocytes from hiPS cell lines generated by lentiviral-mediated transduction of 4 transcription factors (OCT4, SOX2, NANOG, and LIN28). This is important because it shows the potential to achieve cardiomyocyte differentiation from hiPS cells generated with different sets of transcriptional factors and viral vectors. Nevertheless, whether other hiPS cell lines derived from nonfibroblast somatic cell sources, from fibroblasts taken from patients with different disease states, with a reduced number of reprogramming factors, with nonviral vectors, or by subsequent removal of the reprogramming genes have the same cardiogenic differentiation potential is still unknown. Similarly, in the present study, we used hiPS cell lines with no or limited exogenous expression of the reprogramming factors. It is not known, however, whether continuous exogenous expression of the reprogramming genes, as noted in some hiPS cell lines, may influence their differentiating potential or the phenotype of their differentiated progeny.

Another finding of the present study is that the temporal expression pattern of genes associated with the cardiomyogenesis process parallels that observed in the hESC differentiation system and is in general agreement with other cardiac developmental models. The earliest developmental steps were a decrease in the expression of pluripotent markers (OCT4 and NANOG) coupled with an initial increase in the expression of primitive streak and cardiomesoderm markers (Brachyury and MESP1). This was followed by the expression of cardiac-specific transcription factors and finally by the expression of cardiac-specific structural genes, including sarcomeric-related proteins and ion channels. These findings may have important implications for future developmental studies and suggest that this differentiation system may be amenable to manipulations of known cardiomesoderm signaling pathways in an attempt to augment cardiomyocyte yield.

One of the most exciting prospects of the iPS technology is the potential for the development of personalized autologous cell therapy procedures for myocardial repair. Although there is no direct evidence at the moment proving the ability of hiPS cell derivatives generated by retroviral reprogramming to remain immunoprivileged after transplantation, it is hypothesized that such cells, which can be generated from the patient’s own fibroblasts, will be able to circumvent the problem of immune rejection, as already suggested by some murine transplantation studies. Nevertheless, several obstacles have to be overcome before this strategy can...
become a clinical reality. These obstacles include issues similar to those raised with regard to the potential clinical use of hESC-CMs\textsuperscript{38} such as the need to derive purified cultures of relatively homogeneous populations of cardiomyocytes, the need to scale up the differentiation process to derive clinically relevant number of cells, the need to increase graft size and the requirement for in vivo maturation and functional integration of the hESC-CMs, and the need to minimize the risk of teratoma formation and proarrhythmia. A specific concern relating to the iPS technology is the potential risk of tumor formation resulting from the use of potential oncogenic factors and of viruses that randomly integrate into the genome. Recent work in this rapidly advancing field has established the ability to produce iPS cell lines without the oncogenic factor c-Myc\textsuperscript{27,39} without the use of retroviral expression vectors\textsuperscript{28,29} or even by subsequent removal of the reprogramming genes\textsuperscript{30} potentially addressing some of the key safety questions surrounding this groundbreaking technology.

Finally, although significant attention has been paid to the prospects of using the iPS technology in the emerging field of regenerative medicine, these special cells hold great promise for other disciplines. Some examples include the ability to establish patient-specific in vitro models of disease\textsuperscript{13,14} and the ability to use these in vitro models for pathophysiological and developmental studies and for drug development and testing. To address the last issue, we performed proof-of-concept studies assessing the ability of the hiPS-CMs to serve as a model for electrophysiological drug testing. By using a high-resolution MEA mapping technique, we were able to show that the hiPS-CMs can respond appropriately to pharmacological compounds known to affect chronotropy (β-adrenergic and muscarinic agonists and calcium blockers), conduction (sodium channel blockers), and repolarization (potassium and calcium channel blockers and antiarrhythmic agents).

The ability to predict the effects of pharmacological agents on myocardial repolarization, as demonstrated by the prolongation of the cFPD after application of specific blockers of the delayed rectifier potassium channels, may bring a unique value to the field of QT drug screening. Drug-induced QT prolongation (resulting from inhibition of the HERG-encoded \( I_{Ks} \) current), which can predispose to life-threatening ventricular arrhythmias, has become the single most common reason for the withdrawal or restriction of the use of drugs that have already been marketed.\textsuperscript{26} In this respect, the ability to provide
human cardiomyocytes for in vitro QT screening may be of great value. Moreover, because the iPS technology has the potential to derive diverse hiPS cell lines from different patients (reflecting the population’s diverse genetic background heterogeneity) and perhaps in the future even to establish personalized, patient-specific, in vitro tissue models for drug screening, this technology may also bring unique value to the emerging field of pharmacogenomics.

The hiPS-derived cardiomyocyte tissue also has some important limitations with regard to its potential use as an in vitro electrophysiological model system. These include the fact the electrophysiological properties of the hiPS-CMs may be relatively immature, that the generated tissue is isotropic and differs from that of the adult heart, and that the phenotype of the cardiomyocytes within the contracting EBs may be diverse (comprised of atrium-like, ventricle-like, and node-like cells) and may vary from 1 hiPS cell line to another. These potential cellular electrophysiological differences may be reduced, however, within the functional cardiac syncytium because of electrotonic interactions. In addition, the microarchitecture of the contracting tissues is unpredictable, ranging in size and shape, in a manner similar to the hESC and mouse ESC systems. Hence, although some EBs were characterized by a broad cardiomyocyte area and relatively homogenous and fast conduction, others contained more complex structures with narrow tissue strands, leading to relatively slow conduction. This structural drawback is counterbalanced by the ability to use each EB as its own control and to follow its electrophysiological properties longitudinally under different interventions. The final drawback of the system is that it provides a 2-dimensional representation of the spread of electric activation in a 3-dimensional structure (although the contracting areas used was rather thin, usually containing <8 layers of cells).

Conclusions
Our study shows that hiPS cells are capable of differentiating into cardiomyocytes and even a functional cardiac tissue with the appropriate molecular, structural, and functional properties and that this differentiating system recapitulates key cardiogenic developmental steps. The ability to generate a reproducible hiPS-CM differentiation system may bring unique value to the emerging field of personalized medicine: for the establishment of patient- and disease-specific disease models, for drug screening and target validation, and for the establishment of future autologous myocardial cell-replacement therapies.

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Disclosures
None.

References
Cell-replacement therapy is emerging as a novel experimental therapeutic paradigm for myocardial repair/regeneration but is hampered by the limited sources of autologous cardiomyocytes. The breakthrough technology, allowing the reprogramming of adult human fibroblasts with a set of transcription factor into pluripotent stem cells (iPS cells), may provide a possible solution to the aforementioned cell-sourcing problem. In the present study, we describe the in vitro differentiation of human iPS (hiPS) cells into the cardiac lineage and demonstrate that the molecular events characterizing this differentiation process parallel previously reported in vitro and in vivo cardiomyogenic models. The generated hiPS-derived myocytes displayed molecular, structural, and functional properties of early-stage human cardiomyocytes and responded appropriately to adrenergic and cholinergic signaling. In addition, the hiPS differentiating system was not limited to the generation of isolated cardiomyocytes; rather, a functional cardiomyocyte syncytium was established with stable pacemaker activity and action potential propagation. Finally, proof-of-concept pharmacological studies demonstrated the potential of the hiPS cell–derived cardiac tissue to serve as a unique platform for individualized drug testing and specifically for “QT screening.” In conclusion, the ability to generate a reproducible hiPS-cardiomyocyte differentiation system may bring unique value to the emerging field of personalized medicine: for the establishment of patient/disease-specific disease models, for drug screening and target validation, and for the establishment of future autologous myocardial cell-replacement therapies.
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