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REVIEW TOPIC OF THE WEEK

Induced Pluripotent Stem Cells for the Study of Cardiovascular Disease

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ABSTRACT

Groundbreaking advances in stem cell research have led to techniques for the creation of human cardiomyocytes from cells procured from a variety of sources, including a simple skin biopsy. Since the advent of this technology, most research has focused on utilizing these cells for therapeutic purposes. However, recent studies have demonstrated that stem cell-derived cardiomyocytes generated from patients with inherited cardiovascular disorders recapitulate key phenotypic features of disease in vitro. Furthermore, these cells can be maintained in culture for prolonged periods of time and used for extensive biochemical and physiological analysis. By serving as models of inherited cardiac disorders, these systems have the potential to fundamentally change the manner in which cardiovascular disease is studied and new therapies are developed. (J Am Coll Cardiol 2014;64:512–9) © 2014 by the American College of Cardiology Foundation.

The ability to translate basic scientific discovery into clinically-useful technology requires model systems that faithfully reflect the pathophysiology of human disease. Although animal models have contributed greatly to the study of cardiovascular disorders, experimental findings in these models often correlate poorly with outcomes in human studies (1–4). Ideally, the study of cardiac diseases would involve cardiomyocytes (CMs) obtained directly from patients, but difficulties in procuring and maintaining primary CMs in culture limits experimentation with human cardiac tissue.

Given the technical difficulties of working with direct human cardiac tissue, an enticing alternative for studying cardiovascular diseases (CVDs) has emerged: CMs generated from pluripotent stem cells (PSCs) (**Central Illustration**). This approach provides a new means for studying a wide spectrum of human diseases previously impervious to rigorous scientific investigation. Herein, we review how PSC-CMs are created as well as the various CVDs that have been studied using these systems. We then describe how PSC-CMs can be utilized for both drug discovery and toxicity studies, and we comment on the limitations of this potentially disruptive technology.

GENERATION OF CMs FROM PSCs

Mouse embryonic stem cells (ESCs) were first established in culture in 1981 by Evans et al. (5); 7 years later, Thomson et al. (6) developed the first human embryonic stem cell (hESC) line. In 2006, Takahashi and Yamanaka (7) demonstrated in mice that adult skin fibroblasts could be reprogrammed into ESC-like cells by using a retroviral vector to force the expression of the original 4 transcription factors: *Oct3/4*, *Sox2*, *Klf4,* and *c-Myc*. Further research reproduced these findings using human fibroblasts and showed that these cells, known as human-induced pluripotent stem cells (hiPSC), were capable of self-renewal, could be maintained in an undifferentiated state for extended periods, and could give rise to all somatic cell types (8–13).

Investigators employ several techniques to produce CMs from hESCs and hiPSCs (hereafter abbreviated as hESC-CMs and hiPSC-CMs when specifically

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referring to CM generation). The first widely-used method for developing PSC-CMs involved coculturing hESCs with the mouse endodermal cell line END-2, which stimulates differentiation toward a CM-like phenotype (14). However, this technique proved relatively inefficient and remains infrequently used in laboratory practice (15–18).

More commonly, human pluripotent stem cell (hPSC)-CMs are generated by either the "embryoid body" or monolayer techniques. With the former, hPSCs are removed from conditions supporting pluripotency and are suspended in droplets or plated on low-adherency plates. This promotes the formation of cell aggregates, termed embryoid bodies, which spontaneously differentiate into all 3 germ layers (19). Some of these differentiated cells will develop a "cardiomyocyte-like" phenotype and contract spontaneously. Although this technique demonstrated limited initial success (20,21), the stepwise addition and inhibition of signaling factors that replicate known steps in cardiac development has greatly improved efficiency (22–27). Current protocols typically result in the production of >70% to 90% hPSC-CMs. Further modifications, such as growing hPSCs as a monolayer, have allowed researchers to generate vast quantities of these cells entirely in chemically-defined media (28–32).

LIMITATIONS OF hiPSCS. Although hESCs were the first cells differentiated into CMs, the revolutionary discovery that adult cells could be reprogrammed to a pluripotent state greatly expanded the research uses for human stem cells. Most importantly, hiPSCs contain the complete genetic information of the individual from which they derive, providing the possibility of recreating and studying genetically-based diseases in vitro. Yet, despite their vast theoretical potential, current hiPSC systems have key limitations that restrict their widespread application to translational research.

At present, most laboratories employ protocols that utilize retroviral vectors to reprogram adult cells to a pluripotent state. Although reprogramming silences the transcription of viral transgenes, there is an inherent risk that the use of retroviral expression systems can cause events that could alter the host-cell phenotype, such as viral transgene reactivation, insertional mutagenesis, and host-cell gene removal. hiPSCs produced by these systems require intensive screening to exclude such events.

Current differentiation protocols produce admixtures of cells that have pacemaker-, atrial-, and ventricular-like phenotypes (33). Although research is ongoing to develop protocols that generate only the desired cell type, currently it is difficult to isolate homogenous cell populations. Of greater concern, however, is that gene expression patterns of hPSC-CMs more closely resemble fetal cardiac tissue than adult cells and that they act mechanically and electrophysiologically like immature CMs (34–37).

Compared with adult CMs, hPSC-CMs are smaller and circular in morphology, with greater myofibril disorganization and a shorter sarcomere length (13,14,37,38). These differences alter cell membrane

ABBREVIATIONS AND ACRONYMS

APD = action potential duration

ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy

CM = cardiomyocyte

CVD = cardiovascular disease

DCM = dilated cardiomyopathy

ESC = embryonic stem cell

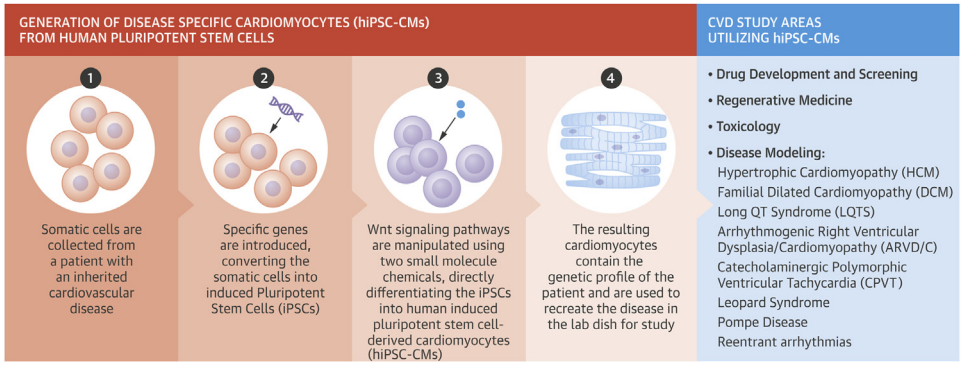
HCM = hypertrophic cardiomyopathy

hiPSC = human-induced pluripotent stem cell

hPSC = human pluripotent stem cell

LQTS = long QT syndrome

PSC = pluripotent stem cell



CENTRAL ILLUSTRATION **The Generation and Application of hiPSC-CMs**

Cardiomyocytes can be generated from patient-specific induced pluripotent stem cells through stage-specific activation and inhibition of the Activin, Wnt/B-catenin, and BMP signaling pathways. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a platform for modeling of cardiovascular disorders, drug screening, regenerative medicine, and many other experimental applications.
CVD = cardiovascular disease.

TABLE 1 Summary of Published hiPSC Models of Cardiovascular Disease

First Author (Ref. #)	Year	Summary
Arrhythmogenic right ventricular dysplasia/cardiomyopathy		
Caspi et al. (59)	2013	Heterozygous frameshift mutation in PKP2 (A324fs335X)
Kim et al. (57)	2013	Homozygous silent mutation in PKP2 resulting in cryptic splicing (c.2484C>T)
Ma et al. (58)	2013	Heterozygous missense mutation in PKP2 (L614P)
Catecholaminergic polymorphic ventricular tachycardia		
Zhang et al. (62)	2013	Heterozygous missense mutation in RYR2 (F2483I)
Di Pasquale et al. (65)	2013	Heterozygous missense mutation in RYR2 (E2311D)
Novak et al. (66)	2012	Homozygous missense mutation in CASQ2 (D307H)
Kajula et al. (64)	2012	Heterozygous missense mutation in RYR2 (P2328S)
Itzhaki et al. (63)	2012	Heterozygous missense mutation in RYR2 (M4109R)
Jung et al. (61)	2012	Heterozygous missense mutation in RYR2 (S406L)
Fatima et al. (60)	2011	Heterozygous missense mutation in RYR2 (F2483I)
Dilated cardiomyopathy		
Siu et al. (45)	2012	Heterozygous nonsense mutation in LMNA (R225X)
Sun et al. (46)	2012	Heterozygous missense mutation in TNNT2 (R173W)
Ho et al. (44)	2011	Heterozygous frameshift mutation in LMNA (S17fs40X)
Hypertrophic cardiomyopathy		
Lan et al. (43)	2013	Heterozygous missense mutation in MYH7 (R663H)
LEOPARD syndrome		
Carvajal-Vergara et al. (42)	2010	Heterozygous missense mutation in PTPN11 (T468M)
Long QT syndrome		
Matsa et al. (52)	2013	Heterozygous missense mutation in KCNH2 (A561T)
Ma et al. (55)	2013	Heterozygous missense mutation in SCN5A (V1763M)
Bellin et al. (53)	2013	Heterozygous missense mutation in KCNH2 (N996I)
Terrenoire et al. (54)	2012	Heterozygous missense mutation in SCN5A (F1473C) and polymorphism in KCNH2 (K897T)
Egashira et al. (38)	2012	Heterozygous deletion mutation in KCNQ1 (P631fs/33)
Lahti et al. (51)	2012	Heterozygous missense mutation in KCNH2 (R176W)
Itzhaki et al. (49)	2011	Heterozygous missense mutation in KCNH2 (A614V)
Matsa et al. (50)	2011	Heterozygous missense mutation in KCNH2 (A561T)
Yazawa et al. (56)	2011	Heterozygous missense mutation in CACNA1C (G406R)
Moretti et al. (47)	2010	Heterozygous missense mutation in KCNQ1 (R190Q)
Pompe syndrome		
Huang et al. (66)	2011	Specific mutations not published

capacitance, the rate of action potential depolarization, and maximum contractile force of hPSC-CMs (37). These hPSC-CMs also possess uniform distribution of the gap junction protein connexin 43 on the cell membrane (34,39)—which in adult cells is concentrated at the intercalated discs—and, like fetal CMs, they lack well-developed T-tubule (or transverse tubule) structures (38,40,41). Developing new techniques to make hPSC-CMs more "mature" will be critical for the technology to reach its full potential.

Despite these limitations, hiPSCs represent a new frontier in translational research, bringing the idea of recreating diseases "in the dish" closer to reality. Already, several genetically-based CVDs have been studied using hiPSCs. These models offer new insights into the molecular mechanisms underlying these diseases and hold exciting potential for the development of new diagnostic, prognostic, and therapeutic tools. In the following text, we review cardiac disease models that have been published and the potential for these models to advance translational research (Table 1).

CARDIOVASCULAR DISEASE MODELS

THE LEOPARD SYNDROME. Investigators created the first hiPSC model of a CVD using skin fibroblasts from a patient with LEOPARD (lentigines, electrocardiogram conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness) syndrome, an autosomal-dominant disorder caused by a mutation in the *PTPN11* gene encoding the protein-tyrosine phosphatase Shp2 that also results in myocardial hypertrophy. Compared with control subjects, LEOPARD hiPSC-CMs exhibit an increased median surface area and gene expression profiles consistent with HCM. Investigators have now demonstrated for the first time that hiPSC-CMs retain key phenotypic features of the patients from which they were derived (42).

HYPERTROPHIC CARDIOMYOPATHY. Characterized by an abnormal thickening of the ventricular wall, HCM can predispose affected individuals to aortic outflow obstruction, heart failure, and malignant ventricular arrhythmias. To better understand its pathophysiology, Lan et al. (43) recently developed a model of HCM from 5 related patients with a missense mutation in the beta-myosin heavy chain gene (*MYH7*). The HCM hiPSC-CMs produced were enlarged and multinucleated and had increased sarcomeric disorganization. Genetic analysis performed on hiPSC-CMs 40 days after cardiac induction showed an up-regulation of hypertrophy-related genes. They also exhibited arrhythmogenic waveforms and irregular Ca^{2+} transients. At 30 days post-induction, cytosolic Ca^{2+} concentration was approximately 30% higher in HCM hiPSC-CMs versus control subjects. Caffeine stimulation in diseased hiPSC-CMs caused smaller Ca^{2+} releases from the sarcoplasmic reticulum into the cytoplasm than in control subjects, suggesting that there is worse contractile function. Administration of calcineurin inhibitors, which block hypertrophy-related signaling pathways, reduced hypertrophy in HCM hiPSC-CMs. Stimulation of diseased hiPSC-CMs with isoproterenol caused a 2-fold increase in cell size and exacerbated irregular Ca^{2+} transients; coadministration of a beta-blocker abrogated these effects. Overall, these findings implicated abnormal Ca^{2+} handling as the central mechanism in the pathophysiology of HCM (43).

FAMILIAL DILATED CARDIOMYOPATHY. A leading cause of nonischemic heart failure, familial dilated cardiomyopathy (DCM) results in ventricular dilation and systolic dysfunction. Two groups have published hiPSC models of DCM with mutations in the lamin A/C gene, which encodes 2 isoforms of A-type lamins found in the nuclear lamina. By immunofluorescence, DCM hiPSCs displayed significant nuclear abnormalities; electron microscopy showed nuclear pore dilation, heterochromatin aggregation, and mitochondrial accumulation around the nuclear envelope (44). Electrical stimulation of DCM hiPSC-CMs increased nuclear senescence and apoptosis (44,45). Further investigation implicated the activation of MEK1, a component of the mitogen-activated protein kinase signaling pathway, as a key mediator in these events. Applying a MEK1 inhibitor diminished the apoptotic effects of electrical stimulation (45).

Sun et al. (46) published an hiPSC model of DCM from patients with a mutation in the troponin T type 2 gene (*TNNT2*). Compared with control subjects, DCM hiPSC-CMs demonstrated an abnormal distribution of sarcomeric alpha-actinin and underdeveloped mitochondria and sarcoplasmic reticulum. DCM hiPSC-CMs also had smaller cytosolic Ca^{2+} transients and weaker contraction as measured by atomic force microscopy. Treatment of DCM embryoid bodies with norepinephrine eliminated spontaneous contractions and increased sarcomeric disorganization and myofilament degeneration. Mechanical cell stretching produced similar results. Metoprolol treatment decreased the number of cells with disorganized alpha-actinin staining, and overexpression of Serca2 increased Ca^{2+} transients and strengthened measured contractions. The investigators also identified 191 genes with differing expression levels in diseased hiPSC-CMs compared with control subjects, several of which had not been previously linked to DCM and may be future targets for investigation.

LONG QT SYNDROME. Long QT syndrome (LQTS) is characterized by prolonged cardiac repolarization that can result in fatal ventricular arrhythmias. More than a dozen different types of inherited LQTS have been described, and hiPSCs have been used to study LQTS types 1, 2, 3, and 8. Long QT syndrome type 1 (LQTS1) is caused by a mutation in the *KCNQ1* gene, which encodes a voltage-gated potassium channel, $K_v7.1$, responsible for the slow component of the delayed rectifier potassium current. Moretti et al. (47) and Egashira et al. (48) showed that LQTS1 hiPSC-CMs had prolonged action potential duration (APD) on patch-clamping and a prolonged field potential duration on multielectrode array (MEA) analysis, findings that correlate with QT prolongation on electrocardiogram. Immunohistological testing showed that, in LQTS1 hiPSC-CMs, the diseased KCNQ1 protein localized to the endoplasmic reticulum rather than the cell surface (47,48). Treatment with an inhibitor of the fast component of the delayed rectifier potassium current (I_{Kr}) induced polymorphic ventricular tachycardia (VT)-like arrhythmias in LQTS1 hiPSC-CMs (48). Furthermore, isoproterenol stimulation caused VT-like arrhythmic events in diseased hiPSC-CMs, which could be ameliorated by propranolol pre-treatment (47,48).

Long QT syndrome type 2 (LQTS2) results from a mutation in the *KCNH2* gene encoding the alpha-subunit, $K_v11.1$ of the I_{Kr} or human ether-à-go-go related gene (hERG) channel. LQTS2 hiPSC-CMs exhibited prolonged APD and reduced repolarization compared with control subjects. MEA mapping demonstrated early afterdepolarizations (EADs) that gave rise to premature beats (49–53). Administration of beta-adrenergic agonists induced EADs, which then responded to beta-adrenergic blockers (50). Treatment with nifedipine and pinacidil, for example, completely eliminated EADs and triggered beats, but stopped contraction in some embryoid bodies. Ranolazine demonstrated antiarrhythmic effects, but did not alter APD in these cells (49).

Type 3 long QT syndrome (LQTS3) springs from persistent Na^+ influx during depolarization due to mutations in the *SCN5A* gene, which encodes the $Na_v1.5$ sodium channel. Terrenoire et al. (54) developed an hiPSC model from a patient with an *SCN5A* mutation and a polymorphism in *KCNH2*. They demonstrated that the Na^+ channel defect was the causal mutation in this patient with complicated genetics. Mexiletine treatment improved Na^+ channel function, but concomitant inhibition of I_{Kr} limited its use. Ma et al. (55) also developed an hiPSC-CM model of LQTS3 and showed that diseased CMs had a prolonged APD, correlating clinically with QT prolongation on electrocardiogram.

Yazawa et al. (56) modeled Timothy syndrome, a rare autosomal dominant disorder caused by a mutation in the *CACNA1C* gene encoding the voltage-dependent L-type calcium channel, $Ca_v1.2$., which is characterized by long ST syndrome type 8 (LQTS8), autism, immunodeficiency, and syndactyly. Compared with control subjects, LQTS8 hiPSC-CMs contracted more slowly and irregularly. $Ca_v1.2$ Ca^+ current in diseased hiPSC-CMs significantly reduced voltage-dependent inactivation, and ventricular-like LQTS8 hiPSC-CMs had APDs 3 times longer than control subjects. Numerous depolarizing events failed to trigger a full action potential in the LQTS8 hiPSC-CMs,

suggesting that defects in ventricular CMs may underlie the disease (56).

ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA/CARDIOMYOPATHY. Characterized by fibrofatty replacement of CMs in the right ventricle, arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) results from mutations in the cardiac desmosome. Kim et al. (57) developed a model of ARVD/C with hiPSC-CMs from 2 patients with homozygous and heterozygous mutations in the plakophilin-2 gene (*PKP2*). Treatment with adipogenic factors increased expression of peroxisome proliferator-activated receptor-alpha, a transcriptional regulator of fatty acid metabolism. Additional administration of activators of the peroxisome proliferator-activated receptor-gamma pathway, which may be hyperactivated in ARVD/C patients, increased lipogenesis and apoptosis and further shifted energy metabolism toward glucose utilization, an effect also seen in end-stage heart failure (57).

Additionally, Ma et al. (58) and Caspi et al. (59) published hiPSC models of ARVD/C from patients with heterozygous *PKP2* mutations. ARVD/C hiPSC-CMs demonstrated reduced transcription and immunofluorescent staining of *PKP2* and plakoglobin as well as reduced expression of gap-junction proteins (58,59). Transmission electron microscopy showed darker lipid drops and increased desmosomal abnormalities in diseased hiPSC-CMs (58,59). Lipogenic stress applied to ARVD/C hiPSC-CMs resulted in extensive lipid droplet accumulation (59).

CATECHOLAMINERGIC POLYMORPHIC VT. A stress-induced ventricular arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT) can produce polymorphic VT, syncope, and sudden cardiac death. Type 1 (CPVT1) is caused by an autosomal dominant mutation in the cardiac ryanodine receptor gene (*RYR2*), type 2 (CPVT2) by an autosomal recessive mutation in the calsequestrin gene. Both types trigger aberrant diastolic Ca^{2+} release from the sarcoplasmic reticulum, and several hiPSC models have been developed for both conditions.

Under basal conditions, control and CPVT1 hiPSC-CMs had similar resting, systolic, and diastolic sarcoplasmic reticulum Ca^{2+} concentrations (60,61). But, diseased hiPSC-CMs showed higher amplitudes and longer durations of spontaneous local Ca^{2+} release. Ca^{2+} release from internal stores also continued after repolarization in diseased hiPSC-CMs. These findings were consistent with aberrant sarcoplasmic reticulum Ca^{2+} release. Electrical pacing and beta-adrenergic stimulation exacerbated Ca^{2+} handling abnormalities in CPVT1 hiPSC-CMs and resulted in arrhythmogenic abnormalities on electrophysiological testing (60–64).

Several groups have analyzed various drugs to further elucidate properties of CPVT1 and evaluate treatment options. Propranolol and flecainide reversed the observed abnormalities induced by stress-like stimulation (63), whereas dantrolene, an inhibitor of the skeletal ryanodine receptor, restored normal Ca^{2+} spark properties and abolished triggered arrhythmias in diseased hiPSC-CMs (61). CPVT1 hiPSC-CMs treated with KN-93, a calcium/calmodulin-dependent serine-threonine protein kinase II inhibitor, demonstrated blunting of isoproterenol-induced electrical abnormalities. Treatment also stabilized calcium activation, resulting in a single initiation site in a beating cell cluster (65).

CPVT2 was studied with an hiPSC model derived from 2 family members with mutations in the calsequestrin gene. No differences were seen in gene expression or calsequestrin protein levels in diseased hiPSC-CMs compared with control subjects, but transmission electron microscopy showed large glycogen deposits, lipid droplets, roughened endoplasmic reticulum, reduced number of caveolae, and abnormally dilated and fragmented sarcoplasmic reticulum cisternae in CPVT2 hiPSCs (66).

POMPE DISEASE. This autosomal recessive disorder is caused by mutations in the acid alpha glucosidase gene (*GAA*), a lysosomal glycogen-degrading enzyme. Huang et al. (67) developed an hiPSC model of Pompe disease with a doxycycline-inducible expression of acid alpha glucosidase. Pompe hiPSC-CMs demonstrated depressed acid alpha-glucosidase activity and increased glycogen content, which was reversed with doxycycline treatment.

Additionally, diseased hiPSC-CMs produced lower oxygen consumption rates and lower extracellular acidification rates, suggestive of mitochondrial dysfunction. Human recombinant acid alpha-glucosidase and an autophagy inhibitor significantly reduced glycogen content in Pompe hiPSC-CMs. By comparing the expression of 16 genes related to glycogen metabolism and lysosomal and mitochondrial function in doxycycline-treated and untreated cells, they identified 6 genes as potential targets for in vivo drug testing.

CLINICAL APPLICATIONS

Recent advances in PSC technology are changing the landscape of translational research. CVD models based on this technology have the potential to help dissect the molecular pathways responsible for disease pathology and facilitate the rational design of targeted drugs toward these pathways.

Screening for drug toxicities represents an area of research potentially wide open to the use of hPSC models. More than one-third of medication withdrawals from 1990 to 2006 were related to cardiotoxicity (68). Currently, all developmental compounds must pass an hERG screen for QT-prolonging effects prior to clinical-use approval, but this screen relies on the use of nonhuman, noncardiac cells overexpressing a single ion channel. The drug alfuzosin famously passed hERG testing, but was later found to cause QT prolongation when used clinically. It was subsequently determined that alfuzosin does not bind the hERG channel, instead prolonging the QT interval through modulations in sodium currents (69), highlighting a critical weakness of this screening modality. Equally worrisome is that verapamil, a widely-used medication with no clinically-relevant QT-prolonging effects, yielded false positives on hERG screening. Given this tool's inadequacies, one could imagine that a screen utilizing hiPSC-CMs would perform better. Indeed, using MEA technology, Navarette et al. (70) demonstrated improved accuracy of an hiPSC-CM screening system over traditional hERG screening. They showed that hiPSC-CMs treated with medications known to cause QT prolongation recorded a comparable response to hERG screening. However, unlike hERG screening, their hiPSC-CM system predicted the correct clinical response to alfuzosin and verapamil (70).

The hiPSCs may have a role for individualized drug toxicity screening as well. Liang et al. (71) compared single-cell recordings in hiPSC-CMs from patients with LQTS and HCM to hESC-CMs and traditional hERG screening. The LQTS and HCM hiPSC-CMs treated with cisapride, a known QT-prolonging agent, developed QT prolongation at lower doses than control cells. This suggests hiPSC-CMs can be used for individualized screening. As the production of hiPSC-CMs becomes cheaper and faster, it may eventually be feasible to screen high-risk patients using assays derived completely from their own cells (71).

Potentially, hiPSCs may improve development of new therapeutics for inherited cardiac diseases, particularly by applying high-throughput technologies. With the continuing refinement of powerful computational software, we can now use hiPSC-CMs to recreate a disease phenotype in vitro and screen hundreds or thousands of novel and U.S. Food and Drug Administration–approved compounds in just hours or days. This technology may dramatically improve the rate at which we discover new experimental compounds (72).

CONCLUSIONS AND FUTURE DIRECTIONS

Although most existing hiPSC technology is not yet ready for clinical translation, the studies that we reviewed offer proof of concept that hiPSCs can generate disease-specific CMs that respond in physiologically meaningful ways to drug treatment. As differentiation protocols and high-throughput techniques improve, hiPSC systems also may play an integral role in developing new pharmacologic agents for genetically-based diseases and in screening compounds for potential cardiotoxicities. Further refinements in these models are needed to produce hiPSC-CMs that better replicate the physiology of adult cells. Research is ongoing to develop differentiation protocols that more faithfully mimic cardiac embryogenesis, and laboratories are studying the use of 3-dimensional structures, electrical pacing, and mechanical strain to determine if these stimuli can improve differentiation toward a more "adult-like" state. Additionally, development of viable systems that incorporate multiple cell types, noncell autonomous signaling events, and environmental factors may allow the study of an even broader range of cardiac diseases.

More than 30 years have passed since ESCs were first described, and many scientists have theorized that this technology would transform cardiovascular medicine. We firmly believe that the use of stem cells as models for CVD will serve as a stepping stone toward that transformation.

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