Induced Pluripotent Stem Cells 10 Years Later
For Cardiac Applications

Yoshinori Yoshida, Shinya Yamanaka

Abstract: **Induced pluripotent stem cells (iPSCs) are reprogrammed cells that have features similar to embryonic stem cells, such as the capacity of self-renewal and differentiation into many types of cells, including cardiac myocytes. Although initially the reprogramming efficiency was low, several improvements in reprogramming methods have achieved robust and efficient generation of iPSCs without genomic insertion of transgenes. iPSCs display clonal variations in epigenetic and genomic profiles and cellular behavior in differentiation. iPSC-derived cardiac myocytes (iPSC cardiac myocytes) recapitulate phenotypic differences caused by genetic variations, making them attractive human disease models, and are useful for drug discovery and toxicology testing. In addition, iPSC cardiac myocytes can help with patient stratification in regard to drug responsiveness. Furthermore, they can be used as source cells for cardiac regeneration in animal models. Here, we review recent progress in iPSC technology and its applications to cardiac diseases. (*Circ Res*. 2017;120:1958-1968. DOI: 10.1161/CIRCRESAHA.117.311080.)**

Key Words: drug discovery ■ embryonic stem cells ■ heart failure ■ induced pluripotent stem cells ■ regeneration

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Discovery of Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) were first reported in 2006, but the foundation of reprogramming was made long before. In 1981, Evans et al[1] showed that embryonic stem cells (ESCs) could be derived by cultivating the inner cell mass of murine blastocysts. Human ESCs were successfully established from the inner cell mass of human blastocysts in 1998.[2] From the perspective of developmental biology, ESCs have 2 important properties: self-renewal and pluripotency. Decades earlier, in 1958, Gurdon et al[3] injected the nucleus of somatic cells from a Xenopus tadpole into an enucleated oocyte of the same species to produce a cloned frog. Wilmut et al[4] prepared the first mammal clone through similar means. These cloning studies demonstrated that nuclei in somatic cells can be reprogrammed into the pluripotent stem state by appropriate stimulation. Finally, another important finding crucial to the discovery of iPSCs is that each cell type has its own master regulator genes, which specifically work to maintain the cellular identity. The first proof of this feature came by showing that the expression of a single gene, MyoD, can convert mouse fibroblasts into skeletal muscle cells.[5] This finding led to the idea that individual master regulator genes can directly convert numerous cell types. Together, the above, abbreviated list of landmark discoveries paved the way to iPSCs.

The original mouse iPSCs were established by retrovirally introducing a set of 4 transcription factors (c-Myc, Oct3/4, Sox2, and Klf4)[6] into mouse fibroblasts. iPSCs were shown not only to contribute to chimera formation but also to give rise to germline transmission, making them comparable with mouse ESCs.[7–9] Human iPSCs were established similarly by introducing the same or another set of transcription factors.[10,11] Like mouse iPSCs, human iPSCs are comparable to human ESCs, which in this case means that they do not contribute to chimeric formation.

Oct3/4 is a homeodomain transcription factor that controls the maintenance and differentiation of pluripotent stem cells (PSCs). Sox2 plays a crucial role in controlling the expression of Oct3/4.[12] Together with Nanog, Oct3/4 and Sox2 constitute the key transcriptional network for pluripotency. c-Myc is a proto-oncogene associated with the cause of various cancers. It recruits chromatin-modifying proteins, leading to widespread transcriptional activation. It was previously shown that c-Myc is dispensable for reprogramming[13] and can be replaced with L-Myc which is deficient in transformation activity.[14] Klf4 acts as an oncoprotein or a tumor suppressor in a context-dependent manner, is a downstream target of leukemia inhibitory factor, and activates Sox2.[15] Although the precise mechanisms have not been fully elucidated, the coordination of these reprogramming factors leads to the reprogramming of somatic cells into pluripotency.

Retroviruses and lentiviruses were initially used to introduce these transgenes, risking the development of insertional mutations in the cells. Furthermore, although the transgenes are silenced after reprogramming to pluripotency, they can be unintentionally reactivated, which risks tumorigenicity. To avoid these drawbacks, nongenetic methods, including adenovirus,[16] plasmid vectors,[17–19] removable piggyBac transposons,[20–22] and Sendai virus,[23,24] were developed. It was recently reported that mouse embryonic fibroblasts can be reprogrammed into iPSCs by a combination of chemical compounds.[25]

From the Center for iPS Cell Research and Application, Kyoto University, Japan (Y.Y., S.Y.); and Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA (S.Y.).

Correspondence to Yoshinori Yoshida, MD, PhD, Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, 53, Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail yoshinor@cira.kyoto-u.ac.jp

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Nonstandard Abbreviations and Acronyms	
CRISPR/Cas9	clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9
ESC	embryonic stem cell
iPSC	induced pluripotent stem cell
LQTS	long QT syndrome
MHC	myosin heavy chain
PSC	pluripotent stem cell

The reprogramming efficiency to iPSCs was initially very low but has since been significantly improved. Chemical compounds, such as valproic acid, sodium butyrate, and histone deacetylase inhibitors, have been shown to enhance iPSC generation.[26–28] The culture environment, such as hypoxic cultivation, also improves the reprogramming efficiency.[29] The generation of iPSCs can be further facilitated by the inhibition of the p53 pathways[30–34] or the inhibition of Mbd3, a component of the NuRD (Mbd3/nucleosome remodelling and deacetylation repressor) complex,[35] although additional factors specifically expressed in oocytes, such as Glis1 and H1foo, also enhance the reprogramming efficiency.[36,37]

Characterization of iPSC Lines

In mouse iPSCs, pluripotency can be confirmed by the capacity to contribute to chimeras after blastocyst injection. The pluripotency of human iPSCs can be confirmed by the capacity to form teratomas after injection into immunodeficient mice. However, iPSCs are generated from several types of cells by various methods that can lead to different genetic aberrations and epigenetic profiles (Figure 1). This effect may explain why the presence of marked differences in the differentiation propensity of human iPSC lines was previously reported.[38,39] Since this realization, several factors that affect differentiation capacity have been reported. One factor is the residual DNA methylation signature transmitted from the parental cells, known as epigenetic memory.[38–42] Another factor is the genetic differences between individual donors.[43] A third factor is aberrations acquired during the reprogramming process, such as reprogramming-associated aberrant DNA methylation.[40,44–49] We recently reported that epigenetic variations influence the differentiation and maturation capacity of human iPSC lines.[49] These differences are important when using iPSCs for disease modeling. Investigation of the molecular mechanisms that cause clonal variations in the differentiation/maturation capacity is therefore critical.

Several studies have described the differences of ESCs and iPSCs especially with regard to epigenetic profiles.[38,39,50] These epigenetic differences are supposedly attributable in part to variations among iPSC clones. By comparing multiple human iPSC and ESC lines, we have shown that the methylation profiles of iPSC-specific differentially methylated regions differ markedly depending on the iPSC clone, with some iPSCs showing similar epigenetic profiles in iPSC-specific differentially methylated regions to those of human ESCs.[49] These findings underscore the importance of comprehensive profiling of iPSC lines to identify those suitable for biomedical application.

Generation of Cardiac Myocytes From PSCs

The generation of cardiac myocytes from PSCs was first reported using embryoid bodies with media containing serum[51]; however, the efficiency was 5% to 10%. Since then, several groups have revealed ways to elevate the efficiency. Mummery et al[52] reported that coculture with mouse endodermal-like cells (END2) enhances the differentiation efficiency. Efficient cardiac myocyte induction using cytokines, such as Activin A and BMP4 (bone morphogenetic protein 4), was achieved in 2-dimensional monolayer and embryoid body–based differentiation systems.[53,54] The addition of chemical compounds that inhibit Wnt signaling was also shown to enhance the cardiac myocyte differentiation efficiency markedly.[55] Burridge et al[56] more recently reported a 3-step differentiation system using culture conditions with only chemically defined factors and without the use of serum to generate cardiac myocytes.

The differentiated cells induced from PSCs are a heterogeneous mixture of cell types. Antibodies against SIRPα (signal regulatory protein α) or VCAM1 (vascular cell adhesion molecule 1) were reported effective in isolating cardiac myocytes.[57–59] By using media with glucose depletion and the supplementation of lactate, PSC-derived cardiac myocytes can be purified metabolically.[60] In addition, the combination of glutamine and glucose depletion was reported to further eliminate undifferentiated PSCs.[61] We recently reported that synthetic RNA capable of sensing cardiac myocyte-specific microRNAs can purify cardiac myocytes at unprecedented levels.[62]

PSC-derived cardiac myocytes, such as cardiac myocytes in the heart, are composed of ventricular-, atrial-, and nodal-like cells,[63] and each cardiac myocyte subtype has distinct electrophysiological properties. Blazeski et al[64] reported that current differentiation protocols are biased to generate ventricular-like cells, with only a small proportion of cells becoming atrial or nodal like. The manipulation of BMP signaling and retinoid acid signaling during the cardiac myocyte induction enhances the efficiency of nodal-like cell generation,[65] although treatment with retinoic acid was found to promote the specification of atrial cardiac myocytes.[66] PSC-derived cardiac myocytes should be mature when used for regenerative medicine or drug discovery. Ideally, the maturity will be similar to that of cardiac myocytes in the adult myocardium, such that the derived cells display similar contractility, electrophysiological performance, and responses to pharmacological stimulation. However, in reality, PSC-derived cardiac myocytes are immature and more consistent with cardiac myocytes in the embryonic state.[67] Immature cardiac myocytes show less-organized sarcomeric structures and calcium-handling machinery.[68] These characteristics are reflected by the low expression of maturation-related sarcomeric genes, such as *MYL2, MYH7, TCAP*, and *MYOM2,* and ion transport–related genes, such as *KCNJ2* and *RYR2*.[69]

Consequently, multiple efforts have been made to induce cardiac myocyte maturation. Several types of methods, including the addition of thyroid hormone,[70] a thick layer of matrigel,[71] or long cultivation,[72] have been reported to mature PSC-derived cardiac myocytes. In addition, mechanical conditioning in 3-dimensional cardiac tissue combined with electric stimulation was reported to mature PSC-derived cardiac myocytes.[73] Some miRNAs have also shown to induce the

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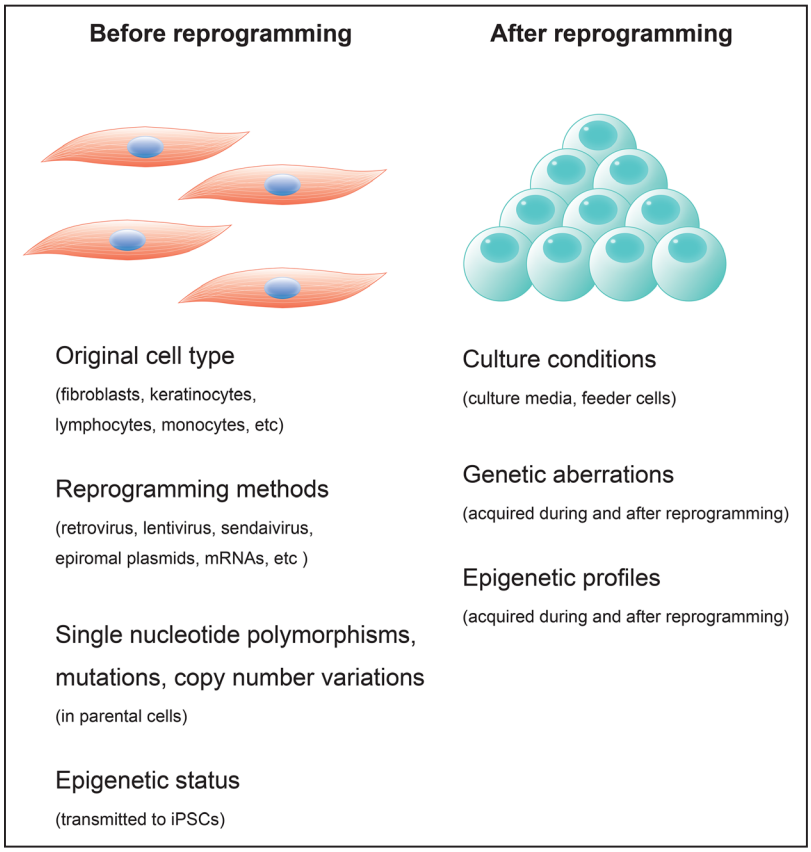

Figure 1. Factors which possibly cause clonal differences of induced pluripotent stem cells (iPSCs).

maturation of PSC-derived cardiac myocytes.[74,75] Kuppusamy et al[75] reported that let-7 induces cardiac myocyte maturation via the suppression of the phosphoinositide 3 kinase/AKT pathway and activation of fatty acid metabolism (Figure 2).

With maturation, the shape of the cardiac myocytes takes a prolonged and anisotropic form, and the sarcomeric structure becomes well organized and shows an increased sarcomeric length. In addition, some proteins in the sarcomere structure undergo an isoform switch. As an example, *TNNI1* is expressed in human embryonic cardiac myocytes, but *TNNI3* (slow skeletal troponin T) is expressed in adult hearts.[76,77] This isoform switch was also observed in human iPSC–derived cardiac myocytes.[78] Similarly, isoform switching of myosin heavy chain (MHC) occurs during development. In rodents, β-MHC (encoded by MYH7 gene) is expressed predominantly in fetal ventricle and replaced by α-MHC (encoded by MYH6) after birth.[79] On the other hand, in humans, β-MHC is the predominant isoform in ventricular myocardium,[80,81] and human PSC–derived cardiac myocytes show increased expression of β-MHC and decreased expression of α-MHC during maturation.[67,70] The maturation of the sarcomeric structure and myofibrillar isoform switch is essential for efficient force generation in cardiac myocytes. Further studies to induce these changes are required.

In addition, electrophysiological properties change during cardiac myocyte maturation. It was previously reported that the electrophysiological properties of cardiac myocytes derived from human PSCs resemble embryonic or fetal-like cardiac myocytes.[52,82,83] The resting membrane potential in adult cardiac myocytes in heart tissue is ≈−90 mV,[68] but that in human PSC–derived cardiac myocytes is less negative, probably because of the lower expression level of I_{K1} channel. I_{K1} and I_{to} currents increase during the maturation process.[84] Calcium handling and excitation–contraction coupling are important determinants of the contractile properties of cardiac myocytes. It was reported that cardiac myocytes derived from PSCs, like those from heart tissues, show calcium handling[85–89] and that the RYR-mediated sarcoplasmic reticulum calcium store increases during maturation.[88] Furthermore, transverse tubules play a key role in excitation–contraction coupling in adult cardiac myocytes. In rats, fetal cardiac myocytes show an absence of T-tubules, but T-tubules are formed after birth.[90] Human PSC–derived cardiac myocytes cultured in vitro were reported to have few or no T-tubules,[88,91,92] which may hinder the recapitulation of disease phenotypes or responses to pharmacological stimulation. Therefore, a protocol in which PSC-derived cardiac myocytes are matured is needed to ensure that cellular properties are consistent with the adult myocardium.

As explained above, PSC-derived cardiac myocytes are a heterogeneous mixture of different cardiac subtypes and maturation stages. Therefore, the selective generation of cardiac myocytes of specific subtypes and maturation stages will facilitate the application of PSC-derived cardiac myocytes for cardiac regeneration and disease modeling.

Disease Phenotype and Drug Discovery

The electrophysiological properties of human and mouse cardiac myocytes are different. Mouse cardiac myocytes have shorter action potential duration and faster heart rates (≈600 bpm). These differences are reasons why mouse models do not adequately recapitulate human disease. In addition,

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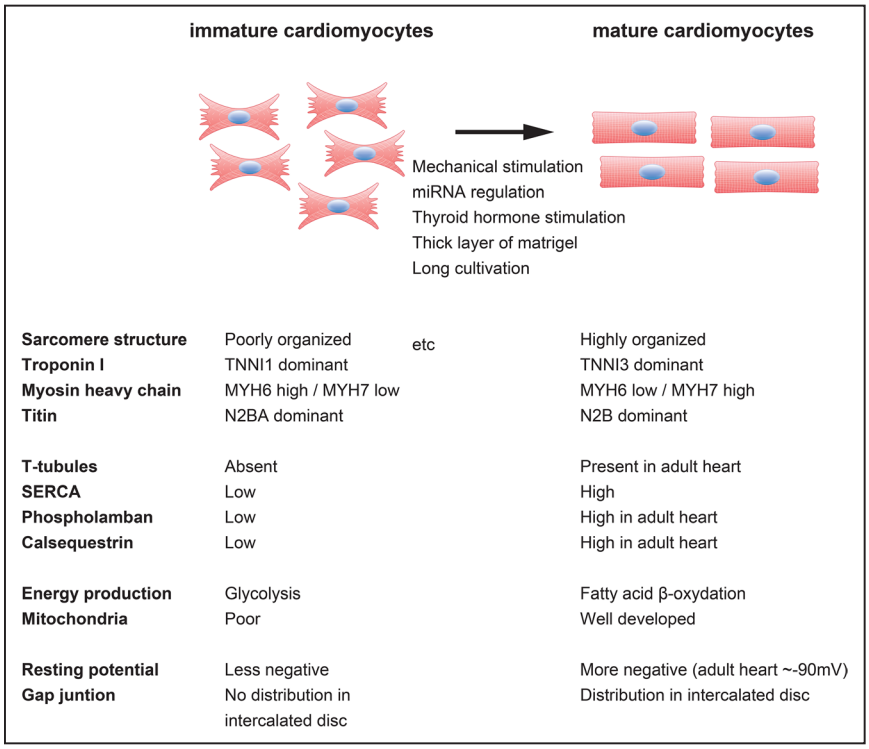

Figure 2. Cardiac myocyte maturation and differences in gene expression and cellular phenotypes.

human primary cardiac myocytes are difficult to sample and maintain stably in vitro. Furthermore, unlike mouse models, iPSC-derived cardiac myocytes can be created from patient cells. For these reasons, human iPSC–derived cardiac myocytes are intriguing disease models. Another advantage of iPSC-based disease modeling is the absence of compensatory mechanisms often observed in in vivo diseased conditions; disease phenotypes in vivo are presented as a mixture of disease-causing deficits that stimulate compensatory changes. Furthermore, the combination of gene-editing technologies, such as CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9), with iPSC technology allows for the investigation of mutations and SNPs (single nucleotide polymorphisms) under the same genetic background, enabling the precise analysis of disease phenotypes and drug responses under genetic conditions identical to those of the actual patient.

Moreover, iPSC-derived cells can recapitulate the cellular phenotypes of not only monogenic disorders but also polygenic/complex diseases. The penetration of most genetic disease caused by autosomal dominant mutations is <100% because of the presence of a modifier that affects the development and severity of the diseases. A comparison of iPSC-derived endothelial cells from patients with familial pulmonary arterial hypertension and unaffected carriers of diseases with *BMPR2* mutations revealed the presence of modifier pathways that protect against familial pulmonary arterial hypertension.[93] That report suggested that iPSC technology can help clarify development and progression of diseases caused by multiple genetic factors. Furthermore, iPSC technology provides a promising tool for investigating the correlation of differences in gene expression and genetic variations among individuals.[94]

Finally, recent iPSC studies have reported the recapitulation of individual susceptibility to cardiotoxicity caused by drugs, such as doxorubicin and sotalol.[95,96] iPSC-derived cardiac myocytes are therefore expected to be helpful for predicting the response of individual patients to new drugs, which may facilitate drug development through the identification of drug responders (Figure 3).

Cardiac Arrhythmias

One of the earliest reports of iPSC-based disease modeling was on long QT syndrome (LQTS), in which prolongation of the depolarization period is associated with an increased risk of lethal ventricular arrhythmias. iPSC-based studies of LQTS1,[97–99] LQTS2,[100–103] and LQTS3[104,105] have been reported. The first report focused on type 1 LQTS, which is caused by the mutation of *KCNQ1* (KCNQ1-R190Q).[99] iPSC-derived ventricular cardiac myocytes from patients with the *KCNQ1* mutation displayed prolonged action potential duration in a whole-cell patch-clamp analysis. A voltage clamp analysis revealed a decrease in the I_{Ks} current of LQTS1-iPSC–derived ventricular cardiac myocytes. Another article that modeled LQTS2 reported that iPSC-derived cardiac myocytes with the *KCNH2* mutation (KCNH2-A614V) showed a prolonged action potential duration and decreased I_{Kr} current.[103] That study further revealed an increased frequency of early afterdepolarization and triggered activity. Gain-of-function mutations in *SCN5A* are responsible for LQTS3, which was modeled using patient-specific iPSCs.[104,105]

Other channelopathies studied with iPSCs include Timothy syndrome, which is caused by a mutation in *CACNA1C* and presents with a variety of symptoms, including QT prolongation, syndactyly, autism, and immune deficiency.[106] Embryoid bodies composed of cardiac myocytes derived from Timothy

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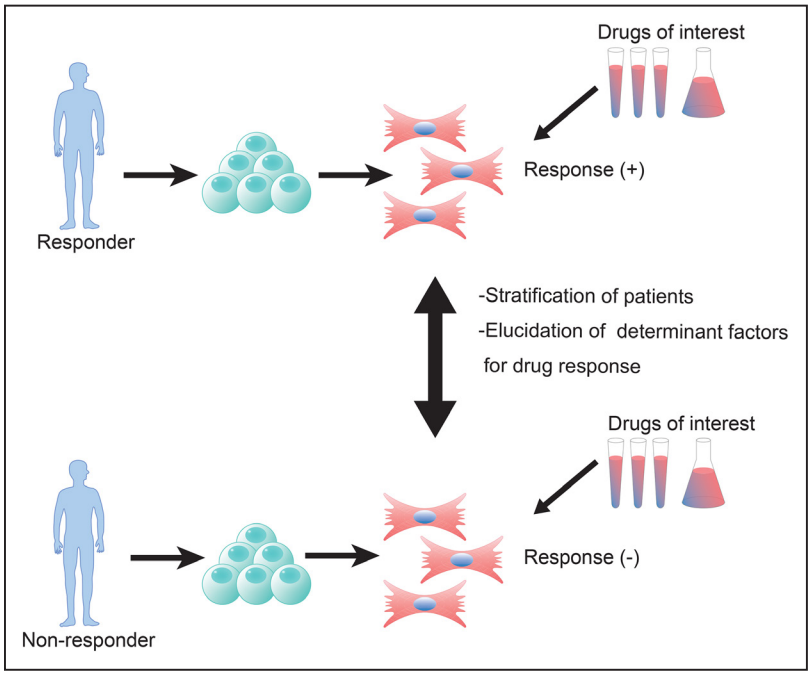

Figure 3. Patient stratification based on drug responsiveness using induced pluripotent stem cells–derived cardiac myocytes.

syndrome patients showed an increased rate of spontaneous beating. A whole-cell patch-clamp analysis using iPSC-derived ventricular cardiac myocytes with *CACNA1C* mutation showed a prolongation of action potential duration and frequent occurrence of delayed afterdepolarization. A voltage clamp analysis demonstrated impaired inactivation of the L-type calcium channel, resulting in hyperfunction of this channel. The same study also showed that roscovitine, which enhances the inactivation of CaV1.2, can shorten the action potential duration.

As alluded to above, the combination of CRISPR/Cas9 with iPSC technology can further the study of cardiac arrhythmias. Mutations in calmodulin genes cause early-onset severe LQTS (LQTS14, 15) by preventing Ca^{2+}/calmodulin-dependent inactivation of L-type Ca channels.[107,108] Recently, the disease phenotypes of cardiac myocytes derived from iPSCs with calmodulin gene mutations were corrected by allele-specific knockdown or interference using CRISPR technology.[109,110] These reports demonstrate the potential of using LQTS patient iPSC-derived cardiac myocytes to model disease phenotypes and provide insights onto new therapies.

Catecholaminergic polymorphic ventricular tachycardia, which is caused mainly by mutations in *RYR2* (catecholaminergic polymorphic ventricular tachycardia-1) or *CASQ2* (catecholaminergic polymorphic ventricular tachycardia-2), has also been investigated using patient-specific iPSCs.[111–118] Cardiac myocytes derived from catecholaminergic polymorphic ventricular tachycardia iPSCs with the *RYR2* mutation showed an increased concentration of intracellular calcium after the addition of isoproterenol, and catecholaminergic stimulation increased the frequency of calcium sparks.[115] Dantrolene,[115] thapsigargin (a intracellular calcium releaser), S107 (an RYR stabilizer),[115] CAMKII inhibitors, propranolol (a β blocker), and flecainide were all shown to ameliorate the disease phenotypes. These findings indicate the potential of cardiac myocytes derived from disease-specific iPSCs for drug screening.

Cardiomyopathy

Along with cardiac arrhythmias, patient-specific iPSCs have been used to model cardiomyopathies in vitro. One of the earliest reports studied LEOPARD syndrome.[119] Mutations in the *PTPN11* gene are responsible for LEOPARD syndrome, which show various clinical manifestations, including hypertrophic cardiomyopathy, lentigines, pulmonary stenosis, abnormal genitalia, retarded growth, and deafness. *PTPN11* is a protein tyrosine phosphatase SHP2 that plays an important role in the RAS/MAPK signal pathway. Cardiac myocytes derived from patient iPSCs showed increased cell size, developed sarcomere structure, and the nuclear translocation of NFATC4, all of which are consistent with changes in hypertrophic cardiac myocytes. In addition, the cardiac myocytes showed upregulated phosphorylation of ERK and MEK.

Other reports have used iPSCs to study pathologies associated with hypertrophic cardiomyopathy.[98,120,121] The resulting cardiac myocytes displayed higher frequencies of sarcomeric disorganization and increased cellular size than normal. Liang et al[98] reported that cardiac myocytes derived from hypertrophic cardiomyopathy iPSCs were more prone to drug-induced prolongation of the action potential duration and arrhythmias.

Disease models also exist for dilated cardiomyopathy.[98,122–125] Patient iPSC-derived cardiac myocytes showed cellular characteristics consistent with dilated cardiomyopathy, including sarcomere disorganization, a decreased contractile function, and calcium-handling abnormality. Sun et al[122] reported that the disease phenotypes of dilated cardiomyopathy iPSC–derived cardiac myocytes, which have a point mutation in *TNNT2*, are ameliorated by metoprolol. A report on LMNA-related cardiomyopathy showed enhanced nuclear senescence and apoptosis of patient iPSC-derived cardiac myocytes. For further disease modeling of cardiac disorders, 3-dimensional tissue engineering is advised. As an example, Hinson recently investigated the disease phenotypes of dilated cardiomyopathy caused by several types of *TTN* mutations by

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comparing the contractile performance of cardiac microtissues engineered from iPSC-derived cardiac myocytes.[124]

Arrhythmogenic right ventricular cardiomyopathy is another cardiomyopathy modeled with patient iPSCs.[126–128] iPSC-derived cardiac myocytes with the *PKP2* mutation showed no pathological phenotypes under normal culture conditions, but when the media were supplemented with defined factors to induce the activation of lipogenic pathways, the cardiac myocytes showed increased lipogenesis and apoptosis.[128] That study suggested that the induction of adult-like metabolic conditions could facilitate the manifestation of disease phenotypes in adult-onset diseases in vitro.

Other Types of Cardiomyopathies and Myocarditis

Disease models of the mitochondrial disease Barth syndrome[129] using iPSCs have been reported. Barth syndrome is caused by a mutation in the *TAZ* gene, which plays an important role on the mitochondria structure. Mutant *TAZ* causes an abnormal mitochondrial structure and function. Wang et al[129] reported that cardiac myocytes derived from Barth syndrome iPSCs show mitochondrial dysfunction, abnormal contractility, and elevated reactive oxygen species. In addition, abnormalities in the Barth syndrome iPSC cardiac myocytes were corrected by the addition of linoleic acid, a precursor of cardiolipin, or mitoTEMPO, a mitochondria-targeted antioxidant. Other types of cardiac myocyte diseases, including the glycogen storage disorder Pompe disease[130] and infectious myocarditis,[131] have been reported. Sharma et al[131] reported the applicability of iPSC-derived cardiac myocytes for antiviral drug screening against coxsackievirus B3-induced myocarditis.

Cardiac Safety Testing

During drug development, drug-induced proarrhythmias are a paramount concern. In the cardiovascular field, avoiding a drug-induced ventricular arrhythmia, torsades de Pointes, is particularly critical,[132–134] and drugs that can prolong ventricular repolarization in the preclinical phase have been intensely sought.[135–137] The association of blockade of I_{Kr} current with prolongation of ventricular repolarization was shown, and an hERG test, which uses cell lines stably expressing hERG (namely KCNH2, which encodes the I_{Kr} channel) to identify compounds with a propensity to block I_{Kr} current, was adopted for safety testing. However, because the actual risk of drug-induced cardiac toxicity is determined by multiple cardiac channels, the results of these tests may not adequately describe the actual risk. A lack of specificity may overestimate the cardiac toxicity, terminating the development of potentially effective drugs.[138,139] PSC-derived cardiac myocytes are expected to help resolve the current limitations in cardiotoxicity tests. Most of the currents seen in adult ventricular cardiac myocytes can be recapitulated in PSC-derived cardiac myocytes.[63,140] Electrophysiological profiling, including a patch-clamp analysis, microelectrode array, calcium indicator dye, and membrane potential dye using cardiac myocytes derived from PSCs, was used in in vitro pharmacological testing to demonstrate drug-induced proarrhythmic responses, including the prolongation of action potential duration.[82,141–145] As described above, PSC-derived cardiac myocytes are equivalent to embryonic cardiac myocytes, but for drug safety testing, cardiac myocytes are required to display drug responses consistent with adult heart tissues, so further maturation should improve the predictability of drug-induced cardiac toxicity. The Comprehensive in vitro Proarrhythmia Assay proposes that proarrhythmic risks be determined by integrating the nonclinical data of the effects of drugs on multiple human cardiac ion channels obtained by an exogenous expression system using patch-clamp methods with the findings from in silico analyses and subsequent confirmation using cardiac myocytes derived from human stem cells, such as PSCs.[146,147]

It has also been shown that PSC-derived cardiac myocytes can be used to predict cardiac toxicity caused by anticancer drugs, as Burridge et al[96] reported that patient-specific iPSC-derived cardiac myocytes can recapitulate individual propensities toward doxorubicin-induced cardiotoxicity.

Cardiac Myocyte Regeneration

Heart failure is one of the most common causes of death worldwide, and medical treatment for patients with severe heart failure still has only limited benefit. Surgeries, including cardiac transplantation and the implantation of ventricular assist devices, are available for only a limited number of patients. Cardiac regeneration using PSCs is therefore expected to be useful as a treatment for otherwise untreatable severe heart failure. Transplanted PSC-derived cardiac myocytes are expected to improve cardiac function via mechanistic contribution to the cardiac contraction and via trophic effects. For the former purpose, electrophysiological integration of the transplanted cells and host myocardium is important. Using a guinea pig model, Shiba et al[148] reported that transplanted cardiac myocytes were able to form gap junctions with the surrounding host myocardium and achieve 1:1 host graft coupling. Trophic effects were attributable to factors secreted from the grafted cells, such as growth factors. Cotransplantation of noncardiac myocytes may enhance the trophic effects.[149]

It was reported that transplanted human PSC–derived cardiac myocytes can engraft and form myocardium in rodents.[54,150,151] However, the survival of the transplanted cardiac myocytes is limited, compromising efficient regeneration of the injured myocardium. Hydrogel composed mainly of laminin, matrigel, and a prosurvival cocktail (including insulin-like growth factor 1 and cyclosporine A) along with heat shock pretreatment improved the survival of the transplanted cells through antiapoptotic effects.[54,152] We recently reported that the engraftability of iPSC-derived cardiac myocytes differs depending on the maturation stage.[69]

To overcome the poor survival of transplanted cells, sheet- or patch-form cardiac myocytes and aggregates of cardiac myocytes have been used.[153,154] Epicardial transplantation using stacked cell sheets between which gelatin hydrogel beads are loaded was also reported to improve the cardiac function.[155] Zimmermann et al[156] reported technology to generate engineered heart tissue that generates contractile force using neonatal rat cardiac myocytes. The engineered heart tissues engrafted efficiently after transplantation into immunosuppressed infarcted rat hearts and improved the cardiac function. This technology can be applied to cardiac myocytes derived from human PSCs.[157,158]

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Building on murine models, larger animal models have been reported more recently. Transplantation studies using a monkey model revealed that human PSC–derived cardiac myocytes were able to engraft in the infarcted hearts of monkeys treated with immunosuppressive agents.[159] Kawamura et al[160] reported the transplantation of cell sheets composed of cardiac myocytes derived from human iPSCs using a pig model of myocardial infarction. Intramyocardial transplantation of cardiac myocytes along with smooth muscle cells and endothelial cells, all derived from human iPSCs, with a 3-dimensional fibrin patch containing IGF-1 (insulin-like growth factor 1) was shown to increase the cardiac function in another porcine model of acute myocardial infarction.[161]

New evidence indicates that the outcomes of cell therapies will benefit from donor matching. In allogeneic transplantation experiments, cardiac myocytes derived from monkey iPSCs with major histocompatibility complex homozygosity were shown to engraft into infarcted hearts and improve the cardiac function of heterozygous major histocompatibility complex–matched monkeys.[162] The immune response of the heterozygous major histocompatibility complex monkeys was favorable when transplantation involved cardiac myocytes derived from homozygous major histocompatibility complex–matched monkey iPSCs than from monkeys without identical major histocompatibility complex alleles.[163] These findings support the clinical rationale of allogeneic transplantation using major histocompatibility complex homozygous PSCs.

Nevertheless, ventricular arrhythmias may occur after the transplantation of cardiac cells.[159,162] The transplantation of immature or dedifferentiated cells can result in heterogeneity of repolarization, leading to reentry and triggered activity. Paracrine factors secreted from the graft cells may also cause electrophysiological changes, resulting in arrhythmia generation through increased automaticity, triggered activity, and reentry.[164,165]

The first clinical transplantation of human ESC–derived cardiac progenitors was reported by Menasche et al.[166,167] They successfully transplanted cardiac progenitor-loaded fibrin patches into the hearts of patients with advanced ischemic heart failure. Considering the similarity between cardiac myocytes derived from human ESCs and those derived from iPSCs, a platform developed using human ESCs should be applicable to human iPSCs too.

Conclusions

iPSCs have been shown to be useful for investigating the phenotypes and disease mechanisms in cells of variable mutations and other genetic conditions. These properties of iPSCs are expected to make them a powerful tool for providing new therapeutic insights in the era of precision medicine. Furthermore, iPSCs have been applied to cell transplantation and are expected to function as source cells for cardiac regeneration. Before reaching this level, however, several issues, such as arrhythmias, should be addressed.

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Disclosures

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References

1. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981;292:154–156.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282:1145–1147.
3. Gurdon JB, Elsdale TR, Fischberg M. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*. 1958;182:64–65.
4. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997;385:810–813. doi: 10.1038/385810a0.
5. Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell*. 1987;51:987–1000.
6. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676. doi: 10.1016/j.cell.2006.07.024.
7. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*. 2007;1:55–70. doi: 10.1016/j.stem.2007.05.014.
8. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007;448:313–317. doi: 10.1038/nature05934.
9. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 2007;448:318–324. doi: 10.1038/nature05944.
10. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872. doi: 10.1016/j.cell.2007.11.019.
11. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917–1920. doi: 10.1126/science.1151526.
12. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov AA, Ko MS, Niwa H. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol*. 2007;9:625–635. doi: 10.1038/ncb1589.
13. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*. 2008;26:101–106. doi: 10.1038/nbt1374.
14. Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka S. Promotion of direct reprogramming by transformation-deficient Myc. *Proc Natl Acad Sci USA*. 2010;107:14152–14157. doi: 10.1073/pnas.1009374107.
15. Niwa H, Ogawa K, Shimosato D, Adachi K. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature*. 2009;460:118–122. doi: 10.1038/nature08113.
16. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science*. 2008;322:945–949. doi: 10.1126/science.1162494.
17. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008;322:949–953. doi: 10.1126/science.1164270.
18. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009;324:797–801. doi: 10.1126/science.1172482.

Downloaded from http://ahajournals.org by on November 11, 2018

19. Okita K, Matsumura Y, Sato Y, et al. A more efficient method to generate integration-free human iPS cells. *Nat Methods*. 2011;8:409–412. doi: 10.1038/nmeth.1591.
20. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*. 2009;458:771–775. doi: 10.1038/nature07864.
21. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*. 2009;458:766–770. doi: 10.1038/nature07863.
22. Yusa K, Rad R, Takeda J, Bradley A. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat Methods*. 2009;6:363–369. doi: 10.1038/nmeth.1323.
23. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc Jpn Acad Ser B Phys Biol Sci*. 2009;85:348–362.
24. Seki T, Yuasa S, Oda M, Egashira T, Yae K, Kusumoto D, Nakata H, Tohyama S, Hashimoto H, Kodaira M, Okada Y, Seimiya H, Fusaki N, Hasegawa M, Fukuda K. Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. *Cell Stem Cell*. 2010;7:11–14. doi: 10.1016/j.stem.2010.06.003.
25. Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, Ge J, Xu J, Zhang Q, Zhao Y, Deng H. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science*. 2013;341:651–654. doi: 10.1126/science.1239278.
26. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol*. 2008;26:1269–1275. doi: 10.1038/nbt.1502.
27. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol*. 2008;26:795–797. doi: 10.1038/nbt1418.
28. Mali P, Chou BK, Yen J, et al. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. *Stem Cells*. 2010;28:713–720. doi: 10.1002/stem.402.
29. Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S. Hypoxia enhances the generation of induced pluripotent stem cells. *Cell Stem Cell*. 2009;5:237–241. doi: 10.1016/j.stem.2009.08.001.
30. Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG, Hochedlinger K. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature*. 2009;460:1145–1148. doi: 10.1038/nature08285.
31. Li H, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*. 2009;460:1136–1139. doi: 10.1038/nature08290.
32. Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, Okita K, Yamanaka S. Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature*. 2009;460:1132–1135. doi: 10.1038/nature08235.
33. Marión RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernandez-Capetillo O, Serrano M, Blasco MA. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature*. 2009;460:1149–1153. doi: 10.1038/nature08287.
34. Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, Wahl GM, Izpisúa Belmonte JC. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*. 2009;460:1140–1144. doi: 10.1038/nature08311.
35. Rais Y, Zviran A, Geula S, et al. Deterministic direct reprogramming of somatic cells to pluripotency. *Nature*. 2013;502:65–70. doi: 10.1038/nature12587.
36. Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, Ichisaka T, Kawamura Y, Mochizuki H, Goshima N, Yamanaka S. Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. *Nature*. 2011;474:225–229. doi: 10.1038/nature10106.
37. Kunitomi A, Yuasa S, Sugiyama F, et al. H1foo has a pivotal role in qualifying induced pluripotent stem cells. *Stem Cell Reports*. 2016;6:825–833. doi: 10.1016/j.stemcr.2016.04.015.
38. 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;29:1117–1119. doi: 10.1038/nbt.2052.
39. Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010;467:285–290.
40. Lister R, Pelizzola M, Kida YS, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011;471:68–73. doi: 10.1038/nature09798.
41. Ohi Y, Qin H, Hong C, et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat Cell Biol*. 2011;13:541–549. doi: 10.1038/ncb2239.
42. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010;28:848–855. doi: 10.1038/nbt.1667.
43. Kajiwara M, Aoi T, Okita K, Takahashi R, Inoue H, Takayama N, Endo H, Eto K, Toguchida J, Uemoto S, Yamanaka S. Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. *Proc Natl Acad Sci USA*. 2012;109:12538–12543. doi: 10.1073/pnas.1209979109.
44. Huang K, Shen Y, Xue Z, Bibikova M, April C, Liu Z, Cheng L, Nagy A, Pellegrini M, Fan JB, Fan G. A panel of CpG methylation sites distinguishes human embryonic stem cells and induced pluripotent stem cells. *Stem Cell Reports*. 2014;2:36–43. doi: 10.1016/j.stemcr.2013.11.003.
45. Koyanagi-Aoi M, Ohnuki M, Takahashi K, et al. Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells. *Proc Natl Acad Sci USA*. 2013;110:20569–20574. doi: 10.1073/pnas.1319061110.
46. Nazor KL, Altun G, Lynch C, et al. Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. *Cell Stem Cell*. 2012;10:620–634. doi: 10.1016/j.stem.2012.02.013.
47. Ruiz S, Diep D, Gore A, et al. Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells. *Proc Natl Acad Sci USA*. 2012;109:16196–16201. doi: 10.1073/pnas.1202352109.
48. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, Kono T, Shioda T, Hochedlinger K. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature*. 2010;465:175–181. doi: 10.1038/nature09017.
49. Nishizawa M, Chonabayashi K, Nomura M, et al. Epigenetic variation between human induced pluripotent stem cell lines is an indicator of differentiation capacity. *Cell Stem Cell*. 2016;19:341–354. doi: 10.1016/j.stem.2016.06.019.
50. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, Herb B, Ladd-Acosta C, Rho J, Loewer S, Miller J, Schlaeger T, Daley GQ, Feinberg AP. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet*. 2009;41:1350–1353. doi: 10.1038/ng.471.
51. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest*. 2001;108:407–414. doi: 10.1172/JCI12131.
52. Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R, van der Heyden M, Opthof T, Pera M, de la Riviere AB, Passier R, Tertoolen L. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation*. 2003;107:2733–2740. doi: 10.1161/01.CIR.0000068356.38592.68.
53. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature*. 2008;453:524–528.
54. Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*. 2007;25:1015–1024. doi: 10.1038/nbt1327.
55. Ren Y, Lee MY, Schliffke S, Paavola J, Amos PJ, Ge X, Ye M, Zhu S, Senyei G, Lum L, Ehrlich BE, Qyang Y. Small molecule Wnt inhibitors enhance the efficiency of BMP-4-directed cardiac differentiation of human pluripotent stem cells. *J Mol Cell Cardiol*. 2011;51:280–287. doi: 10.1016/j.yjmcc.2011.04.012.
56. Burridge PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, Diecke S, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC. Chemically defined generation of human cardiomyocytes. *Nat Methods*. 2014;11:855–860. doi: 10.1038/nmeth.2999.
57. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, Gramolini A, Keller G. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. *Nat Biotechnol*. 2011;29:1011–1018. doi: 10.1038/nbt.2005.

Downloaded from http://ahajournals.org by on November 11, 2018

58. Uosaki H, Fukushima H, Takeuchi A, Matsuoka S, Nakatsuji N, Yamanaka S, Yamashita JK. Efficient and scalable purification of cardiomyocytes from human embryonic and induced pluripotent stem cells by VCAM1 surface expression. *PLoS One*. 2011;6:e23657. doi: 10.1371/journal.pone.0023657.
59. Elliott DA, Braam SR, Koutsis K, et al. NKX2-5(eGFP/w) hESCs for isolation of human cardiac progenitors and cardiomyocytes. *Nat Methods*. 2011;8:1037–1040. doi: 10.1038/nmeth.1740.
60. Tohyama S, Hattori F, Sano M, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell*. 2013;12:127–137. doi: 10.1016/j.stem.2012.09.013.
61. Tohyama S, Fujita J, Hishiki T, et al. Glutamine oxidation is indispensable for survival of human pluripotent stem cells. *Cell Metab*. 2016;23:663–674. doi: 10.1016/j.cmet.2016.03.001.
62. Miki K, Endo K, Takahashi S, et al. Efficient detection and purification of cell populations using synthetic microRNA switches. *Cell Stem Cell*. 2015;16:699–711. doi: 10.1016/j.stem.2015.04.005.
63. 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–H2017. doi: 10.1152/ajpheart.00694.2011.
64. Blazeski A, Zhu R, Hunter DW, Weinberg SH, Boheler KR, Zambidis ET, Tung L. Electrophysiological and contractile function of cardiomyocytes derived from human embryonic stem cells. *Prog Biophys Mol Biol*. 2012;110:178–195. doi: 10.1016/j.pbiomolbio.2012.07.012.
65. Protze SI, Liu J, Nussinovitch U, Ohana L, Backx PH, Gepstein L, Keller GM. Sinoatrial node cardiomyocytes derived from human pluripotent cells function as a biological pacemaker. *Nat Biotechnol*. 2017;35:56–68. doi: 10.1038/nbt.3745.
66. Devalla HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, Gkatzis K, Elliott DA, Chuva de Sousa Lopes SM, Mummery CL, Verkerk AO, Passier R. Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Mol Med*. 2015;7:394–410. doi: 10.15252/emmm.201404757.
67. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res*. 2014;114:511–523. doi: 10.1161/CIRCRESAHA.114.300558.
68. Ivashchenko CY, Pipes GC, Lozinskaya IM, Lin Z, Xiaoping X, Needle S, Grygielko ET, Hu E, Toomey JR, Lepore JJ, Willette RN. Human-induced pluripotent stem cell-derived cardiomyocytes exhibit temporal changes in phenotype. *Am J Physiol Heart Circ Physiol*. 2013;305:H913–H922. doi: 10.1152/ajpheart.00819.2012.
69. Funakoshi S, Miki K, Takaki T, Okubo C, Hatani T, Chonabayashi K, Nishikawa M, Takei I, Oishi A, Narita M, Hoshijima M, Kimura T, Yamanaka S, Yoshida Y. Enhanced engraftment, proliferation, and therapeutic potential in heart using optimized human iPSC-derived cardiomyocytes. *Sci Rep*. 2016;6:19111. doi: 10.1038/srep19111.
70. Yang X, Rodriguez M, Pabon L, Fischer KA, Reinecke H, Regnier M, Sniadecki NJ, Ruohola-Baker H, Murry CE. Tri-iodo-l-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. *J Mol Cell Cardiol*. 2014;72:296–304. doi: 10.1016/j.yjmcc.2014.04.005.
71. Feaster TK, Cadar AG, Wang L, Williams CH, Chun YW, Hempel JE, Bloodworth N, Merryman WD, Lim CC, Wu JC, Knollmann BC, Hong CC. Matrigel mattress: a method for the generation of single contracting human-induced pluripotent stem cell-derived cardiomyocytes. *Circ Res*. 2015;117:995–1000. doi: 10.1161/CIRCRESAHA.115.307580.
72. Kamakura T, Makiyama T, Sasaki K, Yoshida Y, Wuriyanghai Y, Chen J, Hattori T, Ohno S, Kita T, Horie M, Yamanaka S, Kimura T. Ultrastructural maturation of human-induced pluripotent stem cell-derived cardiomyocytes in a long-term culture. *Circ J*. 2013;77:1307–1314.
73. Nunes SS, Miklas JW, Liu J, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat Methods*. 2013;10:781–787. doi: 10.1038/nmeth.2524.
74. Fu JD, Rushing SN, Lieu DK, Chan CW, Kong CW, Geng L, Wilson KD, Chiamvimonvat N, Boheler KR, Wu JC, Keller G, Hajjar RJ, Li RA. Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. *PLoS One*. 2011;6:e27417. doi: 10.1371/journal.pone.0027417.
75. Kuppusamy KT, Jones DC, Sperber H, Madan A, Fischer KA, Rodriguez ML, Pabon L, Zhu WZ, Tulloch NL, Yang X, Sniadecki NJ, Laflamme MA, Ruzzo WL, Murry CE, Ruohola-Baker H. Let-7 family of microRNA is required for maturation and adult-like metabolism in stem cell-derived cardiomyocytes. *Proc Natl Acad Sci USA*. 2015;112:E2785–E2794. doi: 10.1073/pnas.1424042112.
76. Bhavsar PK, Dhoot GK, Cumming DV, Butler-Browne GS, Yacoub MH, Barton PJ. Developmental expression of troponin I isoforms in fetal human heart. *FEBS Lett*. 1991;292:5–8.
77. Hunkeler NM, Kullman J, Murphy AM. Troponin I isoform expression in human heart. *Circ Res*. 1991;69:1409–1414.
78. Bedada FB, Chan SS, Metzger SK, Zhang L, Zhang J, Garry DJ, Kamp TJ, Kyba M, Metzger JM. Acquisition of a quantitative, stoichiometrically conserved ratiometric marker of maturation status in stem cell-derived cardiac myocytes. *Stem Cell Reports*. 2014;3:594–605. doi: 10.1016/j.stemcr.2014.07.012.
79. Taegtmeyer H, Sen S, Vela D. Return to the fetal gene program: a suggested metabolic link to gene expression in the heart. *Ann N Y Acad Sci*. 2010;1188:191–198. doi: 10.1111/j.1749-6632.2009.05100.x.
80. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circ Res*. 2000;86:386–390.
81. Gorza L, Mercadier JJ, Schwartz K, Thornell LE, Sartore S, Schiaffino S. Myosin types in the human heart. An immunofluorescence study of normal and hypertrophied atrial and ventricular myocardium. *Circ Res*. 1984;54:694–702.
82. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res*. 2003;93:32–39. doi: 10.1161/01.RES.0000080317.92718.99.
83. 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;559:479–496. doi: 10.1113/jphysiol.2004.068213.
84. Sartiani L, Bettiol E, Stillitano F, Mugelli A, Cerbai E, Jaconi ME. Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. *Stem Cells*. 2007;25:1136–1144. doi: 10.1634/stemcells.2006-0466.
85. Zhu WZ, Santana LF, Laflamme MA. Local control of excitation-contraction coupling in human embryonic stem cell-derived cardiomyocytes. *PLoS One*. 2009;4:e5407. doi: 10.1371/journal.pone.0005407.
86. Itzhaki I, Rapoport S, Huber I, Mizrahi I, Zwi-Dantsis L, Arbel G, Schiller J, Gepstein L. Calcium handling in human induced pluripotent stem cell derived cardiomyocytes. *PLoS One*. 2011;6:e18037. doi: 10.1371/journal.pone.0018037.
87. Germanguz I, Sedan O, Zeevi-Levin N, Shtrichman R, Barak E, Ziskind A, Eliyahu S, Meiry G, Amit M, Itskovitz-Eldor J, Binah O. Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. *J Cell Mol Med*. 2011;15:38–51. doi: 10.1111/j.1582-4934.2009.00996.x.
88. Satin J, Itzhaki I, Rapoport S, Schroder EA, Izu L, Arbel G, Beyar R, Balke CW, Schiller J, Gepstein L. Calcium handling in human embryonic stem cell-derived cardiomyocytes. *Stem Cells*. 2008;26:1961–1972. doi: 10.1634/stemcells.2007-0591.
89. Liu J, Fu JD, Siu CW, Li RA. Functional sarcoplasmic reticulum for calcium handling of human embryonic stem cell-derived cardiomyocytes: insights for driven maturation. *Stem Cells*. 2007;25:3038–3044. doi: 10.1634/stemcells.2007-0549.
90. Ziman AP, Gómez-Viquez NL, Bloch RJ, Lederer WJ. Excitation-contraction coupling changes during postnatal cardiac development. *J Mol Cell Cardiol*. 2010;48:379–386. doi: 10.1016/j.yjmcc.2009.09.016.
91. Snir M, Kehat I, Gepstein A, Coleman R, Itskovitz-Eldor J, Livne E, Gepstein L. Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. *Am J Physiol Heart Circ Physiol*. 2003;285:H2355–H2363. doi: 10.1152/ajpheart.00020.2003.
92. Lieu DK, Liu J, Siu CW, McNerney GP, Tse HF, Abu-Khalil A, Huser T, Li RA. Absence of transverse tubules contributes to non-uniform Ca(2+) wavefronts in mouse and human embryonic stem cell-derived cardiomyocytes. *Stem Cells Dev*. 2009;18:1493–1500. doi: 10.1089/scd.2009.0052.
93. Gu M, Shao NY, Sa S, Li D, Termglinchan V, Ameen M, Karakikes I, Sosa G, Grubert F, Lee J, Cao A, Taylor S, Ma Y, Zhao Z, Chappell J, Hamid R, Austin ED, Gold JD, Wu JC, Snyder MP, Rabinovitch M. Patient-specific iPSC-derived endothelial cells uncover pathways that protect against pulmonary hypertension in BMPR2 mutation carriers. *Cell Stem Cell*. 2017;20:490.e5–504.e5.
94. Carcamo-Orive I, Hoffman GE, Cundiff P, et al. Analysis of transcriptional variability in a large human iPSC library reveals genetic and non-genetic determinants of heterogeneity. *Cell Stem Cell*. 2017;20:518.e9–532.e9. doi: 10.1016/j.stem.2016.11.005.

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95. Stillitano F, Hansen J, Kong CW, Karakikes I, Funck-Brentano C, Geng L, Scott S, Reynier S, Wu M, Valogne Y, Desseaux C, Salem JE, Jeziorowska D, Zahr N, Li R, Iyengar R, Hajjar RJ, Hulot JS. Modeling susceptibility to drug-induced long QT with a panel of subject-specific induced pluripotent stem cells. *Elife*. 2017;6:e19406.
96. Burridge PW, Li YF, Matsa E, et al. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med*. 2016;22:547–556. doi: 10.1038/nm.4087.
97. Egashira T, Yuasa S, Suzuki T, et al. Disease characterization using LQTS-specific induced pluripotent stem cells. *Cardiovasc Res*. 2012;95:419–429. doi: 10.1093/cvr/cvs206.
98. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, Diecke S, Sallam K, Knowles JW, Wang PJ, Nguyen PK, Bers DM, Robbins RC, Wu JC. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation*. 2013;127:1677–1691. doi: 10.1161/CIRCULATIONAHA.113.001883.
99. 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;363:1397–1409. doi: 10.1056/NEJMoa0908679.
100. Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C. Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *Eur Heart J*. 2011;32:952–962. doi: 10.1093/eurheartj/ehr073.
101. Matsa E, Dixon JE, Medway C, Georgiou O, Patel MJ, Morgan K, Kemp PJ, Staniforth A, Mellor I, Denning C. Allele-specific RNA interference rescues the long-QT syndrome phenotype in human-induced pluripotency stem cell cardiomyocytes. *Eur Heart J*. 2014;35:1078–1087. doi: 10.1093/eurheartj/eht067.
102. Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkelä E, Hyttinen J, Kontula K, Swan H, Conklin BR, Yamanaka S, Silvennoinen O, Aalto-Setälä K. Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. *Dis Model Mech*. 2012;5:220–230. doi: 10.1242/dmm.008409.
103. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L. Modelling the long QT syndrome with induced pluripotent stem cells. *Nature*. 2011;471:225–229. doi: 10.1038/nature09747.
104. Terrenoire C, Wang K, Tung KW, Chung WK, Pass RH, Lu JT, Jean JC, Omari A, Sampson KJ, Kotton DN, Keller G, Kass RS. Induced pluripotent stem cells used to reveal drug actions in a long QT syndrome family with complex genetics. *J Gen Physiol*. 2013;141:61–72. doi: 10.1085/jgp.201210899.
105. Ma D, Wei H, Zhao Y, Lu J, Li G, Sahib NB, Tan TH, Wong KY, Shim W, Wong P, Cook SA, Liew R. Modeling type 3 long QT syndrome with cardiomyocytes derived from patient-specific induced pluripotent stem cells. *Int J Cardiol*. 2013;168:5277–5286. doi: 10.1016/j.ijcard.2013.08.015.
106. Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature*. 2011;471:230–234. doi: 10.1038/nature09855.
107. Makita N, Yagihara N, Crotti L, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. *Circ Cardiovasc Genet*. 2014;7:466–474. doi: 10.1161/CIRCGENETICS.113.000459.
108. Crotti L, Johnson CN, Graf E, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation*. 2013;127:1009–1017. doi: 10.1161/CIRCULATIONAHA.112.001216.
109. Limpitikul WB, Dick IE, Tester DJ, Boczek NJ, Limphong P, Yang W, Choi MH, Babich J, DiSilvestre D, Kanter RJ, Tomaselli GF, Ackerman MJ, Yue DT. A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. *Circ Res*. 2017;120:39–48. doi: 10.1161/CIRCRESAHA.116.309283.
110. Yamamoto Y, Makiyama T, Harita T, et al. Allele-specific ablation rescues electrophysiological abnormalities in a human iPS cell model of long-QT syndrome with a CALM2 mutation. *Hum Mol Genet*. 2017;26:1670–1677. doi: 10.1093/hmg/ddx073.
111. Lodola F, Morone D, Denegri M, Bongianino R, Nakahama H, Rutigliano L, Gosetti R, Rizzo G, Vollero A, Buonocore M, Napolitano C, Condorelli G, Priori SG, Di Pasquale E. Adeno-associated virus-mediated CASQ2 delivery rescues phenotypic alterations in a patient-specific model of recessive catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis*. 2016;7:e2393. doi: 10.1038/cddis.2016.304.
112. Di Pasquale E, Lodola F, Miragoli M, Denegri M, Avelino-Cruz JE, Buonocore M, Nakahama H, Portararo P, Bloise R, Napolitano C, Condorelli G, Priori SG. CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis*. 2013;4:e843. doi: 10.1038/cddis.2013.369.
113. Itzhaki I, Maizels L, Huber I, Gepstein A, Arbel G, Caspi O, Miller L, Belhassen B, Nof E, Glikson M, Gepstein L. Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. *J Am Coll Cardiol*. 2012;60:990–1000. doi: 10.1016/j.jacc.2012.02.066.
114. Fatima A, Xu G, Shao K, et al. In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells. *Cell Physiol Biochem*. 2011;28:579–592. doi: 10.1159/000335753.
115. Jung CB, Moretti A, Mederos y Schnitzler M, et al. Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. *EMBO Mol Med*. 2012;4:180–191. doi: 10.1002/emmm.201100194.
116. Kujala K, Paavola J, Lahti A, Larsson K, Pekkanen-Mattila M, Viitasalo M, Lahtinen AM, Toivonen L, Kontula K, Swan H, Laine M, Silvennoinen O, Aalto-Setälä K. Cell model of catecholaminergic polymorphic ventricular tachycardia reveals early and delayed afterdepolarizations. *PLoS One*. 2012;7:e44660. doi: 10.1371/journal.pone.0044660.
117. Sasaki K, Makiyama T, Yoshida Y, et al. Patient-specific human induced pluripotent stem cell model assessed with electrical pacing validates S107 as a potential therapeutic agent for catecholaminergic polymorphic ventricular tachycardia. *PLoS One*. 2016;11:e0164795. doi: 10.1371/journal.pone.0164795.
118. Novak A, Barad L, Zeevi-Levin N, Shick R, Shtrichman R, Lorber A, Itskovitz-Eldor J, Binah O. Cardiomyocytes generated from CPVTD307H patients are arrhythmogenic in response to β-adrenergic stimulation. *J Cell Mol Med*. 2012;16:468–482. doi: 10.1111/j.1582-4934.2011.01476.x.
119. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang YS, Schaniel C, Lee DF, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature*. 2010;465:808–812.
120. Lan F, Lee AS, Liang P, et al. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell*. 2013;12:101–113. doi: 10.1016/j.stem.2012.10.010.
121. Tanaka A, Yuasa S, Mearini G, et al. Endothelin-1 induces myofibrillar disarray and contractile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-derived cardiomyocytes. *J Am Heart Assoc*. 2014;3:e001263. doi: 10.1161/JAHA.114.001263.
122. Sun N, Yazawa M, Liu J, et al. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. *Sci Transl Med*. 2012;4:130ra47. doi: 10.1126/scitranslmed.3003552.
123. Tse HF, Ho JC, Choi SW, et al. Patient-specific induced-pluripotent stem cells-derived cardiomyocytes recapitulate the pathogenic phenotypes of dilated cardiomyopathy due to a novel DES mutation identified by whole exome sequencing. *Hum Mol Genet*. 2013;22:1395–1403. doi: 10.1093/hmg/dds556.
124. Hinson JT, Chopra A, Nafissi N, et al. HEART DISEASE. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. *Science*. 2015;349:982–986. doi: 10.1126/science.aaa5458.
125. Siu CW, Lee YK, Ho JC, Lai WH, Chan YC, Ng KM, Wong LY, Au KW, Lau YM, Zhang J, Lay KW, Colman A, Tse HF. Modeling of lamin A/C mutation premature cardiac aging using patient-specific induced pluripotent stem cells. *Aging*. 2012;4:803–822. doi: 10.18632/aging.100503.
126. Ma D, Wei H, Lu J, Ho S, Zhang G, Sun X, Oh Y, Tan SH, Ng ML, Shim W, Wong P, Liew R. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J*. 2013;34:1122–1133. doi: 10.1093/eurheartj/ehs226.
127. Caspi O, Huber I, Gepstein A, Arbel G, Maizels L, Boulos M, Gepstein L. Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. *Circ Cardiovasc Genet*. 2013;6:557–568. doi: 10.1161/CIRCGENETICS.113.000188.
128. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature*. 2013;494:105–110. doi: 10.1038/nature11799.
129. Wang G, McCain ML, Yang L, et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and

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heart-on-chip technologies. *Nat Med*. 2014;20:616–623. doi: 10.1038/nm.3545.
130. Huang HP, Chen PH, Hwu WL, Chuang CY, Chien YH, Stone L, Chien CL, Li LT, Chiang SC, Chen HF, Ho HN, Chen CH, Kuo HC. Human Pompe disease-induced pluripotent stem cells for pathogenesis modeling, drug testing and disease marker identification. *Hum Mol Genet*. 2011;20:4851–4864. doi: 10.1093/hmg/ddr424.
131. Sharma A, Marceau C, Hamaguchi R, et al. Human induced pluripotent stem cell-derived cardiomyocytes as an in vitro model for coxsackievirus B3-induced myocarditis and antiviral drug screening platform. *Circ Res*. 2014;115:556–566. doi: 10.1161/CIRCRESAHA.115.303810.
132. Roden DM. Cellular basis of drug-induced torsades de pointes. *Br J Pharmacol*. 2008;154:1502–1507. doi: 10.1038/bjp.2008.238.
133. Kannankeril P, Roden DM, Darbar D. Drug-induced long QT syndrome. *Pharmacol Rev*. 2010;62:760–781. doi: 10.1124/pr.110.003723.
134. Fabiato A, Coumel P. Torsades de pointes, a quarter of a century later: a tribute to Dr. F. Dessertenne. *Cardiovasc Drugs Ther*. 1991;5:167–169.
135. Spector PS, Curran ME, Keating MT, Sanguinetti MC. Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K+ channel. Open-channel block by methanesulfonanilides. *Circ Res*. 1996;78:499–503.
136. Woosley RL, Chen Y, Freiman JP, Gillis RA. Mechanism of the cardiotoxic actions of terfenadine. *JAMA*. 1993;269:1532–1536.
137. Rampe D, Brown AM. A history of the role of the hERG channel in cardiac risk assessment. *J Pharmacol Toxicol Methods*. 2013;68:13–22. doi: 10.1016/j.vascn.2013.03.005.
138. Gintant G, Sager PT, Stockbridge N. Evolution of strategies to improve preclinical cardiac safety testing. *Nat Rev Drug Discov*. 2016;15:457–471. doi: 10.1038/nrd.2015.34.
139. Giorgi MA, Bolaños R, Gonzalez CD, Di Girolamo G. QT interval prolongation: preclinical and clinical testing arrhythmogenesis in drugs and regulatory implications. *Curr Drug Saf*. 2010;5:54–57.
140. Honda M, Kiyokawa J, Tabo M, Inoue T. Electrophysiological characterization of cardiomyocytes derived from human induced pluripotent stem cells. *J Pharmacol Sci*. 2011;117:149–159.
141. Yamazaki K, Hihara T, Taniguchi T, Kohmura N, Yoshinaga T, Ito M, Sawada K. A novel method of selecting human embryonic stem cell-derived cardiomyocyte clusters for assessment of potential to influence QT interval. *Toxicol In Vitro*. 2012;26:335–342. doi: 10.1016/j.tiv.2011.12.005.
142. Schaaf S, Shibamiya A, Mewe M, Eder A, Stöhr A, Hirt MN, Rau T, Zimmermann WH, Conradi L, Eschenhagen T, Hansen A. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. *PLoS One*. 2011;6:e26397. doi: 10.1371/journal.pone.0026397.
143. Nalos L, Varkevisser R, Jonsson MK, Houtman MJ, Beekman JD, van der Nagel R, Thomsen MB, Duker G, Sartipy P, de Boer TP, Peschar M, Rook MB, van Veen TA, van der Heyden MA, Vos MA. Comparison of the IKr blockers moxifloxacin, dofetilide and E-4031 in five screening models of pro-arrhythmia reveals lack of specificity of isolated cardiomyocytes. *Br J Pharmacol*. 2012;165:467–478. doi: 10.1111/j.1476-5381.2011.01558.x.
144. Tanaka T, Tohyama S, Murata M, et al. In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes. *Biochem Biophys Res Commun*. 2009;385:497–502. doi: 10.1016/j.bbrc.2009.05.073.
145. Spencer CI, Baba S, Nakamura K, et al. Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia. *Stem Cell Reports*. 2014;3:269–281. doi: 10.1016/j.stemcr.2014.06.003.
146. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. *Am Heart J*. 2014;167:292–300. doi: 10.1016/j.ahj.2013.11.004.
147. Chi KR. Revolution dawning in cardiotoxicity testing. *Nat Rev Drug Discov*. 2013;12:565–567. doi: 10.1038/nrd4083.
148. Shiba Y, Fernandes S, Zhu WZ, et al. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature*. 2012;489:322–325. doi: 10.1038/nature11317.
149. Masumoto H, Matsuo T, Yamamizu K, Uosaki H, Narazaki G, Katayama S, Marui A, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK. Pluripotent stem cell-engineered cell sheets reassembled with defined cardiovascular populations ameliorate reduction in infarct heart function through cardiomyocyte-mediated neovascularization. *Stem Cells*. 2012;30:1196–1205. doi: 10.1002/stem.1089.
150. Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, Gepstein L. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol*. 2007;50:1884–1893. doi: 10.1016/j.jacc.2007.07.054.
151. van Laake LW, Passier R, Monshouwer-Kloots J, Verkleij AJ, Lips DJ, Freund C, den Ouden K, Ward-van Oostwaard D, Korving J, Tertoolen LG, van Echteld CJ, Doevendans PA, Mummery CL. Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. *Stem Cell Res*. 2007;1:9–24. doi: 10.1016/j.scr.2007.06.001.
152. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol*. 2001;33:907–921. doi: 10.1006/jmcc.2001.1367.
153. Hattori F, Chen H, Yamashita H, et al. Nongenetic method for purifying stem cell-derived cardiomyocytes. *Nat Methods*. 2010;7:61–66. doi: 10.1038/nmeth.1403.
154. Shimizu T, Sekine H, Yang J, Isoi Y, Yamato M, Kikuchi A, Kobayashi E, Okano T. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. *FASEB J*. 2006;20:708–710. doi: 10.1096/fj.05-4715fje.
155. Matsuo T, Masumoto H, Tajima S, Ikuno T, Katayama S, Minakata K, Ikeda T, Yamamizu K, Tabata Y, Sakata R, Yamashita JK. Efficient long-term survival of cell grafts after myocardial infarction with thick viable cardiac tissue entirely from pluripotent stem cells. *Sci Rep*. 2015;5:16842. doi: 10.1038/srep16842.
156. Zimmermann WH, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med*. 2006;12:452–458. doi: 10.1038/nm1394.
157. Riegler J, Tiburcy M, Ebert A, et al. Human engineered heart muscles engraft and survive long term in a rodent myocardial infarction model. *Circ Res*. 2015;117:720–730. doi: 10.1161/CIRCRESAHA.115.306985.
158. Didié M, Christalla P, Rubart M, et al. Parthenogenetic stem cells for tissue-engineered heart repair. *J Clin Invest*. 2013;123:1285–1298. doi: 10.1172/JCI66854.
159. Chong JJ, Yang X, Don CW, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*. 2014;510:273–277. doi: 10.1038/nature13233.
160. Kawamura M, Miyagawa S, Fukushima S, Saito A, Miki K, Ito E, Sougawa N, Kawamura T, Daimon T, Shimizu T, Okano T, Toda K, Sawa Y. Enhanced survival of transplanted human induced pluripotent stem cell-derived cardiomyocytes by the combination of cell sheets with the pedicled omental flap technique in a porcine heart. *Circulation*. 2013;128:S87–S94. doi: 10.1161/CIRCULATIONAHA.112.000366.
161. Ye L, Chang YH, Xiong Q, et al. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. *Cell Stem Cell*. 2014;15:750–761. doi: 10.1016/j.stem.2014.11.009.
162. Shiba Y, Gomibuchi T, Seto T, et al. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. *Nature*. 2016;538:388–391. doi: 10.1038/nature19815.
163. Kawamura T, Miyagawa S, Fukushima S, Maeda A, Kashiyama N, Kawamura A, Miki K, Okita K, Yoshida Y, Shiina T, Ogasawara K, Miyagawa S, Toda K, Okuyama H, Sawa Y. Cardiomyocytes derived from MHC-homozygous induced pluripotent stem cells exhibit reduced allogeneic immunogenicity in MHC-matched non-human primates. *Stem Cell Reports*. 2016;6:312–320. doi: 10.1016/j.stemcr.2016.01.012.
164. Gouadon E, Moore-Morris T, Smit NW, Chatenoud L, Coronel R, Harding SE, Jourdon P, Lambert V, Rucker-Martin C, Pucéat M. Concise review: pluripotent stem cell-derived cardiac cells, a promising cell source for therapy of heart failure: where do we stand? *Stem Cells*. 2016;34:34–43. doi: 10.1002/stem.2205.
165. Pedrotty DM, Klinger RY, Kirkton RD, Bursac N. Cardiac fibroblast paracrine factors alter impulse conduction and ion channel expression of neonatal rat cardiomyocytes. *Cardiovasc Res*. 2009;83:688–697. doi: 10.1093/cvr/cvp164.
166. Menasché P, Vanneaux V, Hagège A, et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. *Eur Heart J*. 2015;36:2011–2017. doi: 10.1093/eurheartj/ehv189.
167. Menasché P, Vanneaux V, Fabreguettes JR, et al. Towards a clinical use of human embryonic stem cell-derived cardiac progenitors: a translational experience. *Eur Heart J*. 2015;36:743–750. doi: 10.1093/eurheartj/ehu192.

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