



Modeling Inherited Arrhythmia Disorders Using Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Vassilios J. Bezzerides, MD, PhD; Donghui Zhang, PhD; William T. Pu, MD

Inherited arrhythmia disorders (IADs) are a group of potentially lethal diseases that remain diagnostic and management challenges. Although the genetic basis for many of these disorders is well known, the pathogenicity of individual mutations and the resulting clinical outcomes are difficult to predict. Treatment options remain imperfect, and optimizing therapy for individual patients can be difficult. Recent advances in the derivation of induced pluripotent stem cells (iPSCs) from patients and creation of genetically engineered human models using CRISPR/Cas9 has the potential to dramatically advance translational arrhythmia research. In this review, we discuss the current state of modeling IADs using human iPSC-derived cardiomyocytes. We also discuss current limitations and areas for further study.

Key Words: Arrhythmogenic cardiomyopathy; Brugada syndrome; Catecholaminergic polymorphic ventricular tachycardia; Induced pluripotent stem cells; Long QT syndrome

Even before the first recordings of the ECG by Willem Einthoven in the early 1900s the notion that the heart was coordinated by electrical currents was well recognized. Indeed, the first case of the inherited arrhythmia syndrome long QT syndrome (LQTS) was described in 1856 when a deaf girl died after her teacher yelled at her and the family had a previous child who had died after a fright.^{1,2} However it was not until the late 1990s when the first genes for congenital LQTS were cloned by Keating and colleagues, leading to the broad understanding that inherited arrhythmia disorders (IADs) were often caused by mutations in ion channels or related proteins.³⁻⁵ Since then, more than 15 genes have been found to cause LQTS.⁶ In addition to LQTS, several other IADs have been described, including arrhythmogenic cardiomyopathy (ACM),⁷ catecholaminergic polymorphic ventricular tachycardia (CPVT)⁸ and Brugada syndrome (BrS).⁹ Each of these disorders is caused by mutations in an ever-growing number of genes (Table 1).¹⁰⁻¹²

Key questions that arise when evaluating a patient with a potential IAD are: Is the mutation truly pathogenic? What is the patient's risk for catastrophic events, including sudden cardiac death (SCD)? What is the optimal therapy for the patient? What are the implications of the patient's diagnosis for family members? Unfortunately, these questions can be difficult to answer prospectively. Obtaining DNA sequencing information can be helpful to establish a diagnosis and assess family members, but often does not help to stratify risk or direct optimal therapy. One major reason is that these disorders can have variable penetrance or expressivity, ranging from no overt clinical manifestation to mild ECG abnormalities to frequent life-threatening

arrhythmias and SCD.¹³ The genetic or cellular mechanisms that underlie variable expressivity for cardiac channelopathies remain poorly understood, making it difficult to predict clinical outcomes based solely on the genetic abnormality present.

Model systems have been essential for understanding how mutations in IAD genes cause arrhythmias. Expression of mutant genes in non-cardiac expression systems has allowed the characterization of the effect of mutations on individual channel function.^{14,15} These analyses have been augmented by structural homology modeling¹⁶ and by computational models that integrate individual channel properties into virtual action potentials (APs).^{16,17} Genetically modified mouse, and more recently rabbit and pig models have been useful to assess the effect of mutations within intact animals.¹⁸⁻²⁰ Although these methods can provide evidence about the pathogenicity and mechanism of action of particular mutations, there is uncertainty in their ability to predict clinical outcomes.²¹ Lack of appropriate physiological context in heterologous systems,²² non-comprehensive protein structural information²³ or differences in ion channel expression²⁴ and cardiac electrophysiological properties²⁵ between animal models and humans all limit the ability of traditional disease models to predict human clinical phenotype, no less predict the interindividual variation that arises from the interaction of the mutation with genetic modifiers.

Recently, Takahashi et al developed a groundbreaking platform for human disease modeling,^{26,27} which promises to enhance mechanistic studies and potentially provide individualized information on risk and optimal therapy. Using a cocktail of reprogramming factors, this group

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Department of Cardiology, Boston Children's Hospital, Boston, MA (V.J.B., D.Z., W.T.P.); Harvard Stem Cell Institute, Cambridge, MA (W.T.P.), USA

Mailing address: William T. Pu, MD, Department of Cardiology, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115, USA. E-mail: wpu@pulab.org

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Table 1. Genetics of Inherited Arrhythmia Disorders		
Disorder / Causative genes	Cellular phenotype	Clinical phenotype
LQTS		
KCNQ1 (LQT1), KCNE1	Decreased I_{Ks}	TdP
KCNH2 (LQT2), KCNE2	Decreased I_{Kr}	TdP
SCN5A (LQT3), SCN4B, SNTA1	Increased I_{Na}	TdP, bradycardia
ANK2	Decreased Na/K-ATP activity	Bradycardia, conduction block, TdP
KCNJ2 (Andersen-Tawil syndrome)	Decreased I_{K1}	Facial anomalies, periodic paralysis, stress-induced VT
CACNA1C (Timothy syndrome)	Decreased I_{Ca}	TdP, autism, syndactyly
CAV3	Decreased I_{Kr}	TdP
CALM1, CALM2	Altered calcium handling	VT
CPVT		
RYR2	Increased calcium leak, altered calcium handling	Stress and exercise-induced VT/VF
CASQ2	Decreased SR calcium buffering	Stress and exercise-induced VT/VF
CALM1	Abnormal Ca^{2+} signaling	Stress and exercise-induced VT/VF/long QT
Triadin	Altered release of Ca^{2+}	Stress and exercise-induced VT/VF
BrS		
SCN5A, SCN10A, SCN1B, SCN2B, SCN3B, GPD1L, MOG1, SLMAP, PKP2, HEY2	Decreased I_{Na+}	ECG changes, VT/VF at rest or with fever
CACNA1C, CACNB2, CACNA2D1	Decreased I_{Ca2+}	VT/VF at rest
HCN4, KCNE3, KCNE5, KCND3, ABCC9, KCNJ8, KCNH2, PKP2	Increased I_{K+} ; I_{to}	VT/VF
ACM		
PKP2, DSG2, DSC2, DSP, JUP	Decreased I_{Na} and gap junctions; apoptosis	Fibrosis, cardiac dysfunction, VT

ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; LQTS, long QT syndrome; Tdp, torsades de pointes; SR, sarcoplasmic reticulum; VF, ventricular fibrillation; VT, ventricular tachycardia.

showed that adult somatic cells such as skin fibroblasts could be converted into pluripotent stem cells (“induced pluripotent stem cells” or iPSCs), which can then be directed to differentiate into other cell types, including cardiomyocytes. Since its original description, generation of iPSCs from a wide range of tissue sources (skin, blood or urine) has become a routine procedure. Because iPSCs carry the genetic material of the cell donor, human iPSCs (hiPSCs) derived from patients yield patient-specific disease models, including models of cardiomyopathies^{28–30} and IADs (Table 2; Figure). Theoretically, these patient-specific models should be able to recapitulate genetically determined phenotypic differences between patients, although empirical validation of this concept is sparse.^{31,32} It is this area where iPSC technology is uniquely poised to provide an entirely new method to study both monogenetic and complex polygenetic diseases.

Electrophysiologic Characteristics of hiPSC-CMs and Adult CMs

Several protocols for directed cardiac differentiation exist in the literature, often based on timed inhibition of GSK3 β and WNT signaling. These protocols for generation of hiPSC-derived cardiomyocytes (hiPSC-CMs) are robust, straightforward, and highly efficient,^{33,34} often yielding cultures in which 70–90% of cells express the cardiomyocyte marker TNNT2. Without positive or negative selection, 50–70% of these hiPSC-CMs are ventricular or ventricular-like cells, with the remaining cells being either atrial or nodal subtypes.³⁵ Methods for directed differentiation and

selection of atrial,³⁶ ventricular³⁷ and even cardiac Purkinje³⁸ subtypes of iPSC-CMs have been described, but these have not been applied in most reported studies. Therefore, cellular heterogeneity must be considered when interpreting data from hiPSC-CM experiments.

Using single-cell patch-clamp technique, a great deal of work has already been done to define the electrophysiologic properties of hiPSC-CMs. In general, hiPSC-CMs have less mature physiology than adult CMs from human patients or animal models. The resting membrane potential (RMP) of hiPSC-CMs is more depolarized (–50 to –75 mV) than adult CMs (–82 to 87 mV; in this review CM is used to indicate cardiomyocytes obtained from animals or humans), consistent with a more immature phenotype.³⁹ This elevated RMP is caused by reduced expression of the constitutive inward potassium current I_{K1} in iPSC-CMs compared with mature CMs (0.9 pA/pF³⁹ vs. 3.6 pA/pF⁴⁰). Forced expression of KCNJ2 (also known as Kir2.1), which underlies I_{K1} , normalized RMP, improved upstroke velocity, and reduced the percentage of spontaneously beating CMs in human embryonic stem cell-derived cardiomyocytes (hESC-CMs).⁴¹ These data correlate with comparative gene expression studies of long-term cultures of hiPSC-CMs and adult CMs, which demonstrate a gradual increase in the expression of KCNJ2 as well as a relative decrease in the amount of the pacemaker channel HCN4, responsible for a slow depolarizing current that contributes to increased automaticity of iPSC-CMs.⁴²

The expression and relative levels of ion channel proteins and their associated subunits play a major role in shaping the cardiac AP and calcium oscillation.⁴³ The car-

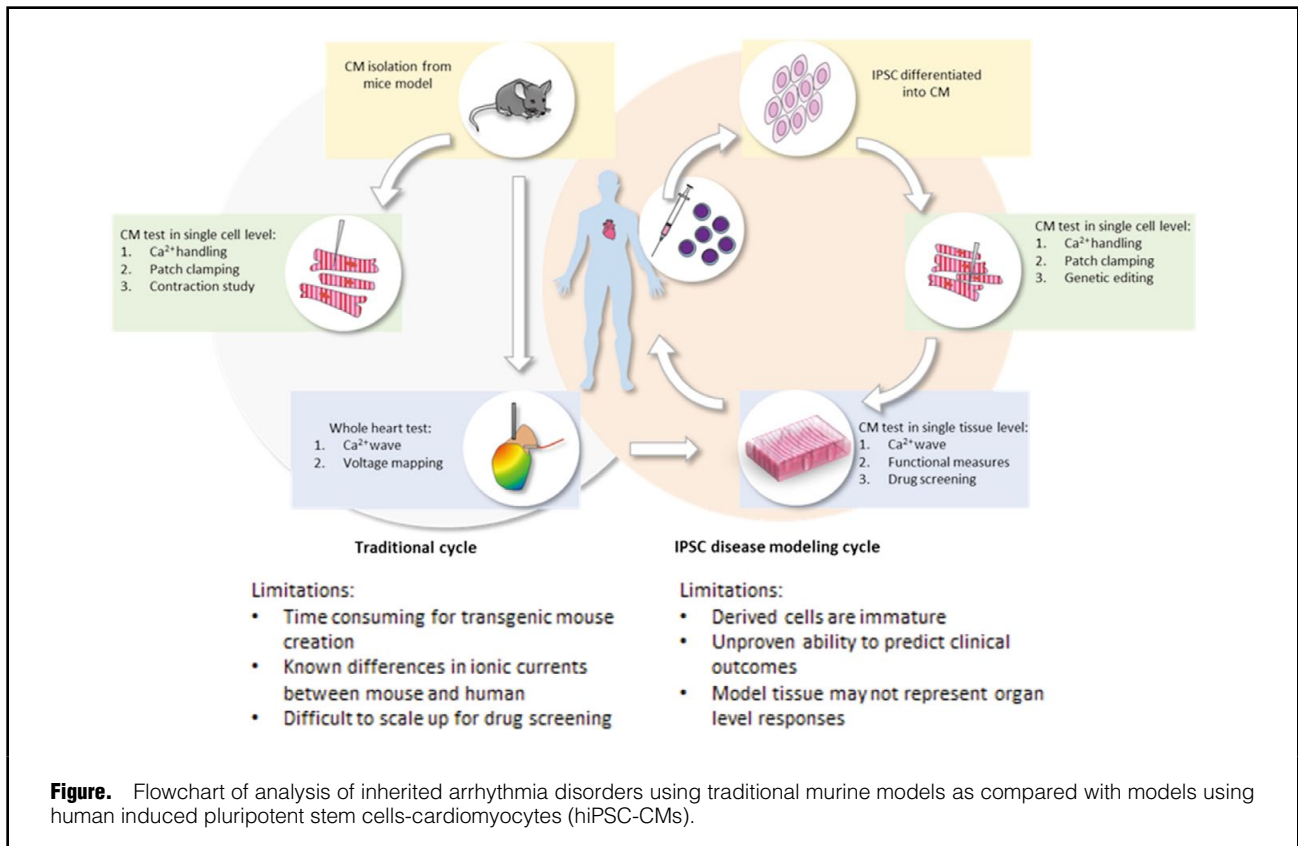
Table 2. iPSC-CM Models of Inherited Arrhythmia Disorders				
IAD model	Gene mutation	Experimental methods	Findings	Ref.
LQT1	KCNQ1 (R190Q)	Patch-clamp	Prolongation of AP, inappropriate AP adaptation at higher pacing frequencies	62
LQT2	KCNH2 (A614V)	Patch-clamp, MEA	Prolongation of AP and induction of EADs; decreased I_{Kr} ; potential improvement with pinacidil	48
LQT2	KCNH2 (G1681A)	Voltage-clamp	Comparison of symptomatic and asymptomatic carrier; AP prolongation and EADs; stronger phenotype in symptomatic carrier	61
LQT2	KCNH2 (R176W)	Voltage-clamp, MEA	AP prolongation; differences in severity between voltage-clamp and MEA recordings	59
BrS/LQT3	E1784K	Patch-clamp, MEA	LQT3 phenotype, but with SCN3B knockdown, BrS phenotype unmasked	46
LQT8	CACNA1C (G1216A)	Patch-clamp, calcium imaging	AP prolongation; Slower contraction rates in mutant cells; prolonged Ca^{2+} transients; Possible therapeutic effect of rosovitine	73
CPVT	RYR2 (F243I)	Voltage-clamp, calcium imaging	Increased DADs with isoproterenol; increased spontaneous calcium sparks	54
CPVT	RYR (E2311N)	Voltage-clamp, calcium imaging	Increased DADs; increased spontaneous calcium sparks; normalization with CaMKii inhibition	84
CPVT	RYR (P2328S)	Voltage-clamp, calcium imaging	Altered calcium signaling; DADs and EADs at baseline and with isoproterenol	85
CPVT	RYR2 (S406L)	Voltage-clamp, calcium imaging	Increased DADs with isoproterenol; improvement with dantrolene	55
CPVT	RYR2 (L3741P)	Calcium imaging, MEA	Increased DADs; increased spontaneous calcium sparks; reversal of phenotype with flecainide	57
CPVT	CASQ2 (D307H), RYR2 (R420Q)	Voltage-clamp calcium imaging	Increased DADs with isoproterenol; increased spontaneous calcium sparks	56
ACM	PKP2 (L614P)	Patch-clamp, calcium imaging, IF, EM	Reduction of both PKP2 and plakoglobin by IF	90
ACM	PKP2 G784Afs*, PKP2 K628Rfs	IF, EM		89
ACM	PKP2 A324fs	MEA, IF, EM		91
BrS	Unknown, SCN5A 1795insD	Patch-clamp	No definitive changes in I_{Na} , I_{to} or $I_{Ca,L}$ consistent with BrS	99
BrS/LQT3	SCN5A (1795insD)	Patch-clamp	Decreased I_{Na} density and larger late current	44

*Homozygous mutation. AP, action potential; DAD, delayed afterdepolarization; EAD, early after depolarization; EM, electron microscopy; IF, immunofluorescence; MEA, multiple electrode array. Other abbreviations as in Table 1.

diac sodium channel, which produces the cardiac Na^+ current (I_{Na}), is responsible for the AP upstroke in chamber (atrial or ventricular) CMs. I_{Na} has relatively comparable biophysical properties in hiPSC-CMs compared with isolated adult CMs, including voltage-dependent activation and inactivation characteristics. However, hiPSC-CM peak currents are considerably larger (≈ -216 pA/pF vs. -20 pA/pF).^{39,44,45} Interestingly, while the upstroke velocities (dV/dt_{max}) of hiPSC-CMs are generally slower than adult CMs (9–40 V/s vs. 215–234 V/s), the sodium channel density does not appear to be the limiting factor. Rather, reduced upstroke velocity likely reflects partial sodium channel inactivation secondary to the relatively more depolarized RMP. Consistent with this hypothesis, quiescent hiPSC-CMs exhibit both a more depolarized RMP and increased upstroke velocity than their spontaneously beating counterparts, consistent with relatively higher maturity of the quiescent compared with spontaneously beating hiPSC-CMs.⁴⁴ Despite the reduced AP upstroke velocity in hiPSC-CMs, patient-derived hiPSC-CMs harboring *SCN5A* or *SCN3B* mutations in large part recapitulate the abnormalities observed in CMs derived from transgenic mice with similar genetic anomalies.^{44,46}

The L-type ($I_{Ca,L}$) calcium current, the major calcium current expressed in the mammalian heart, is present in hiPSC-CMs. However, its biophysical properties are more comparable to those observed in adult atrial than ventricular CMs;^{39,47} the $V_{1/2}$ (the voltage when 50% of the channels are active or inactive) of activation and of inactivation is much lower (left shift) in hiPSC-CMs than in native ventricular CMs. The pharmacology of the $I_{Ca,L}$ in hiPSC-CMs appears similar to that in adult CMs, as application of nifedipine shortens the AP without significant effects on upstroke velocities.^{39,48}

Although hiPSC-CMs recapitulate many features of the $I_{Ca,L}$, these cells lack T-tubules, an extensive network of plasma membrane invaginations that are key components of the calcium release units of CMs. T-tubules, a hallmark of mature ventricular CMs, permit rapid propagation of APs into the center of the cardiomyocytes, thereby coordinating calcium release and contraction despite the large size of these cells. The calcium release unit is composed of “dyads”, in which junctional domains of the sarcoplasmic reticulum (jSR) are closely juxtaposed with the T-tubules. As a result, ryanodine receptor 2 (RYR2), the major CM intracellular calcium release channel located on the jSR, is



positioned close to the L-type calcium channels, located on the T-tubules, thereby facilitating cardiomyocyte calcium-induced calcium release. hiPSC-CMs, like embryonic and neonatal CMs (and mature rodent atrial CMs),^{49,50} lack T-tubules.⁵¹ In these cells, RYR2 within the SR continues to align with sarcomere Z-lines, as it does in mature CMs, but L-type calcium channels are limited to the cell periphery, resulting in sequential and less synchronous calcium release.⁵⁰ In addition, the inositol-3-phosphate receptor contributes to a much greater proportion of calcium transients that in adult CMs, another feature consistent with immaturity.^{52,53} Despite these differences in calcium handling between hiPSC-CMs and adult ventricular CMs, hiPSC-CMs have been productively used to model diseases such as CPVT that are caused by mutations affecting calcium handling⁵⁴⁻⁵⁷ (Table 2).

There are multiple potassium currents that contribute to the cardiac AP (see review by Nerbonne and Kass⁵⁸ for a more comprehensive analysis). The delayed rectifier potassium current that is responsible for repolarization consists primarily of rapid (I_{Kr}) and slow (I_{Ks}) components, in addition to the aforementioned I_{K1} that contributes to RMP as well as the terminal phase of the AP. Both I_{Kr} and I_{Ks} are present in hiPSC-CMs, and these share similar biophysical properties and expression with human CMs.^{39,59} The expression of I_{Kr} is comparable between hiPSC-CMs and native ventricular CMs, with similar current densities and voltages of activation and inactivation.^{48,60} Functional expression of I_{Kr} has also been demonstrated by AP prolongation and early afterdepolarizations (EADs) with the potassium channel E4031.^{48,61} In contrast, the current den-

sity for I_{Ks} appears to be much more variable, as 2 different studies have reported vastly different average current densities varying from 0.31 pA/pF³⁹ to as high as 2.5 pA/pF.⁶² In either case, the current density for I_{Ks} is significantly higher in hiPSC-CMs than what has been reported for native left ventricular human CMs in which the average current density is approximately 0.18 pA/pF.⁶³ One possibility for this variability may be the relative expression of KCNE1, which encodes an I_{Ks} subunit and has been demonstrated to have a significant effect on I_{Ks} current density in hESC-CMs.⁶⁴

Overall, in several respects hiPSC-CMs are more similar to immature neonatal myocytes than to adult CMs. Although some aspects of hiPSC-CM channel expression and physiology are well developed, others (RMP and I_{K1} expression; I_{Na} upstroke velocity; calcium release kinetics and calcium release unit ultrastructure) are not under current culture conditions. Three-dimensional tissue engineering, mechanical loading, modulation of substrate stiffness, phasic electrical stimulation, hormonal treatment (e.g., thyroid hormone), and long-term culture have all been used to enhance hiPSC-CM maturity, with some success.⁶⁵⁻⁷² Further progress will require improved understanding of the natural mechanisms that regulate CM maturity. Caution must be applied when using hiPSC-CMs to model IADs, particularly those involving physiologic parameters that are divergent between hiPSC-CMs and mature CMs. Nevertheless, hiPSC-CMs have proven to be useful IAD models (see next section), just as genetically engineered rodents have been imperfect but valuable models for understanding IAD pathogenesis.

Use of hiPSC-Derived CMs to Model IADs

LQTS

One of the first examples of using patient-specific hiPSCs to model human disease was for LQTS,^{48,62,73} an IAD caused by mutations that prolong repolarization by either decreasing repolarizing inward currents or by increasing depolarizing outward currents. In these studies, fibroblasts isolated from patients with clinical and genetic evidence for congenital LQTS were reprogrammed into hiPSCs. Subsequent differentiation into CMs (hiPSC-CMs) then provided a platform to study LQTS mutations in an appropriate cellular context as compared with the more traditional heterologous expression systems such as HEK cells.

Several groups have demonstrated expected electrophysiologic abnormalities in hiPSC-CM LQTS models, including LQT1,^{48,62} LQT2,⁷⁴ LQT3⁴⁴ and LQT8.⁷³ Those studies have focused on the phenotypes of single cells or small clusters of cells, as measured by patch-clamp recordings. Extracellular field potential duration, recorded by multiple electrode arrays, or whole-cell calcium transients, recorded using fluorescent calcium-sensitive dyes, have been used as higher-throughput assays.⁷⁵ Collectively these studies have shown that LQTS hiPSC-CMs exhibit increased AP duration (APD), an in vitro correlate of prolongation of the QT interval. Voltage-clamp studies of hiPSC-CMs developed from LQTS patients exhibit prolonged APDs and frequent EADs, cellular abnormalities hypothesized to initiate the hallmark arrhythmia of LQTS, torsades de pointes.⁴⁸ That hiPSC-CMs accurately reproduce known electrophysiologic abnormalities provides evidence supporting the use of hiPSC-CMs to model LQTS and by extension other IADs.

hiPSC-CM models can provide new insights into patient disease pathogenesis. For example, hiPSC-CMs from patients with complex or previously unreported mutations, which are difficult to study by more traditional methods, have been informative. Terrenoire et al and colleagues, systematically dissected the relative importance of abnormal sodium and potassium currents in iPSC-CMs from a patient with variants in both *SCN5A* (LQT3) and *KCNH2* (LQT2).⁷⁶ Those authors elegantly demonstrated by patch-clamp recordings that the observed clinical phenotype of severe QT prolongation is likely dominated by dysfunction in the late sodium current without significant contribution from the more common variant in *KCNH2*. It is this type of analysis that clearly demonstrates the promise of patient-specific hiPSC-CMs as a platform to determine the potential pathogenicity of specific mutations or variants of unknown significance as compared with traditional methods.⁷⁷

Another important aspect of hiPSC-CMs is the ability to test drug responses in vitro. The potential for a nearly unlimited supply of hiPSC-CMs to screen for novel drugs or test the side effects of existing compounds has garnered significant interest from the drug development industry.⁷⁸ Use of human PSC-CMs is one of the 3 pillars of the Comprehensive In vitro Pro-arrhythmia Assay initiative, intended to become a mainstay for regulatory evaluation of drugs for their potential to prolong the QT interval and thereby potentially cause lethal arrhythmias.⁷⁹ hiPSC-CMs accurately modeled the protective effect of β -blockers, an established therapy for LQTS.⁶² Several groups have used hiPSC-CMs to show that already approved medications have efficacy in single-cell assays, suggesting the possibility that they could be repurposed as novel therapies for inherited arrhythmia syndromes. Yazawa and colleagues

developed a model of LQT8 (Timothy syndrome) and demonstrated that the cyclin-dependent kinase inhibitor roscovitine (also known as seliciclib) could partially rescue the abnormal phenotypes observed in Timothy syndrome hiPSC-CMs.⁷³ High-throughput platforms to measure APD and other electrophysiologic parameters for drug screening have been reported.⁸⁰ Whether these methods have the phenotypic sensitivity and/or specificity to screen for novel therapeutics is yet to be determined.

CPVT

CPVT is characterized by recurrent ventricular or atrial arrhythmias during times of stress or exercise.⁸ Although most CPVT patients suffer from RYR2 mutations, pathologic variants have also been reported in the calsequestrin (CASQ2),⁸¹ and calmodulin (CALM1) genes⁸² and in triadin (TRDN).⁸³ These mutations generally increase SR calcium release through RYR2, resulting in elevated intracellular calcium that can drive the sodium calcium exchanger (NCX1) in reverse.¹⁰ This generates a depolarizing current that trigger EADs or DADs, which are thought to underlie the polymorphic or bidirectional ventricular tachycardia that are hallmarks of CPVT.

To date, 5 hiPSC-CM models of RYR2 mutations have been described.^{54,55,57,84,85} They cover 3 of the 4 regions of RYR2 where the vast majority of pathogenic mutations have been described.⁸ Using primarily voltage-clamp and calcium imaging of small cell clusters, it has been demonstrated that CPVT hiPSC-CMs have an increased frequency of atypical calcium transients, spontaneous calcium release events (Ca²⁺ sparks), and single-cell voltage membrane depolarization abnormalities, resulting in EADs and DADs.^{54,55,57,84} Consistent with the clinical phenotype of arrhythmia precipitated by catecholaminergic stimulation, these abnormalities are exacerbated by administration of the β -adrenergic agonist isoproterenol. Several groups have also demonstrated that the duration of spontaneous Ca²⁺ sparks are prolonged in CPVT-hiPSC-CMs compared with controls.^{57,84} Preininger et al further investigated calcium homeostasis in CPVT-derived hiPSC-CMs by using tetracaine to inhibit RYR2 Ca²⁺ release from the SR and demonstrated increased diastolic Ca²⁺ leak as well as lower SR Ca²⁺ loading after tonic stimulation.⁵⁷ These data suggest that enhanced diastolic calcium release (Ca²⁺ leak), and possibly impaired SR calcium loading, may cause the observed abnormalities in CPVT-hiPSC-CMs, rather than SR Ca²⁺ overload, which has been proposed as a mechanism in studies of isolated CMs from transgenic mice.⁸⁶

hiPSC-CM models of CPVT have been used to test new therapeutic strategies. Dantrolene is used in malignant hyperthermia to stabilize the closed state of the skeletal ryanodine receptor (RYR1). In a single-cell CPVT model, dantrolene was effective in suppressing excessive calcium sparks, EADs, and triggered beats caused by RYR2 mutation.^{55,84} Although CPVT occurs under conditions of high adrenergic stimulation, the downstream targets of adrenergic signaling that interact with RYR2 mutation to induce arrhythmogenesis are not known. Treatment of hiPSC-CM and mouse models of CPVT with inhibitors of CaMKII, a major kinase-activated downstream of the β -adrenergic receptor, blocked arrhythmic phenotypes,^{84,87} suggesting that CaMKII phosphorylation of downstream target(s) is central to disease pathogenesis.

These data suggest that hiPSC-CMs recapitulate the cardinal features of CPVT, which makes them useful to

investigate arrhythmia mechanisms and to expedite drug development.

ACM

ACM, also known as arrhythmogenic right ventricular cardiomyopathy, is a set of disorders caused by mutation of desmosome genes. Through uncertain mechanisms, ACM causes cardiomyocyte loss, cardiac fibrosis, and fatty infiltration, resulting in cardiac dilatation, dysfunction, and arrhythmia. Although cardiac dysfunction and fibrosis undoubtedly contribute to the arrhythmias in this disease, some patients present with arrhythmia early in the disease that is out of proportion to the extent of cardiac involvement, suggesting primary arrhythmogenic mechanisms.⁸⁸

There are several reported hiPSC-CM models of ACM.⁸⁹⁻⁹² These models recapitulate the desmosomal abnormalities that are central to the disease.⁸⁹⁻⁹² ACM hiPSC-CMs have an increased propensity to accumulate lipid droplets under adipogenic culture conditions.⁸⁹⁻⁹² Although interesting, it is important to note that the ACM myocardium is infiltrated by bona fide adipocytes, whereas lipid-laden CMs are not a frequently noted feature of clinical ACM. These metabolic derangements have been linked to increased hiPSC-CM apoptosis.^{89,91,92} There have only been 2 studies that have examined the characteristics of ACM hiPSC-CMs directly relevant to conduction and arrhythmia. One study reported no electrophysiologic abnormalities or differences in response to calcium-channel blockade (nifedipine) or adrenergic stimulation (isoproterenol).⁹⁰ The other study found that field potential rise time was prolonged in ACM hiPSC-CMs, suggesting reduced conduction or excitability.⁹¹ hiPSC-CMs also exhibit decreased Cx43 gap junction density and wider desmosomes, which interestingly has been correlated with the extent of intracellular lipid accumulation. The interaction between desmosomes, metabolism, and conduction are intriguing, but more research will be necessary to determine if hiPSC-CMs can serve as effective models for arrhythmia in ACM.

BrS

BrS is an IAD characterized by stereotypical ECG findings without significant structural heart disease and is associated with a high incidence of SCD, especially in males.^{93,94} The gene most frequently mutated in BrS is SCN5A, encoding the cardiac sodium channel, but SCN5A mutations only account for approximately 20% of all BrS patients.¹² Interestingly, SCN5A mutations also cause LQT3, and some specific “overlap” mutations have been implicated in both BrS and LQT3. It is generally accepted that depolarization abnormalities, especially in the right ventricular outflow tract, underlie the pathophysiology of BrS. One of the challenges in diagnosis is that a number of asymptomatic patients present only with incidental ECG changes,⁹⁵ but the first symptoms are often SCD or aborted cardiac arrest.⁹⁶ Although various diagnostic and clinical criteria have been studied, few parameters have been found that reliably identify patients at greatest risk.

Davis and colleagues⁴⁴ studied patient-derived hiPSC-CMs with heterozygous *SCN5A* 1795insD mutation, an overlap mutation associated with both BrS and LQT3.^{97,98} By patch-clamp, these cells had decreased I_{Na} density (BrS phenotype) as well as increased late sodium current (LQT3 phenotype), consistent with CMs from mice with the equivalent mutation that were studied in parallel.⁴⁴ However, a second study of hiPSC-CMs from 3 patients

with clinically well-defined BrS, but with undefined genetics, as well as hiPSC-CMs from a patient with 1795insD did not reach the same conclusions.⁹⁹ Using very detailed single-cell electrophysiologic analysis, the 2nd study's authors did not find the characteristic abnormalities in I_{Na} typically observed in CMs harboring pathogenic BrS mutations.⁹⁹ They also did not detect any consistent changes in the other currents proposed to contribute to BrS (I_{to} or $I_{Ca,L}$).⁹⁵ A 3rd study of hiPSC-CMs from a patient with E1784K SCN5A mutation and a mixed LQT3 and BrS phenotype found that the hiPSC-CMs manifested electrophysiological abnormalities consistent with LQT3 but not BrS.⁴⁶ SCN3B, the embryonic β -subunit of the cardiac sodium channel, was found to be expressed in hiPSC-CMs at higher levels than in mature CMs. Knockdown of SCN3B unmasked the BrS phenotype. Thus gene expression changes related to maturity affect the phenotypic expression of the mutant genotype in hiPSC-CMs compared with adult CMs and perhaps also account for the adult onset of BrS.

Genome Editing in Disease Modeling

Designer meganucleases such as TALENs and in particular CRISPR/Cas9 have enabled precise genome editing with minimal off-target mutagenesis.¹⁰⁰ These nucleases can be used to inactivate genes with targeted insertions or deletions, or make precise changes through homology-directed repair. Genome editing is an essential addition to the hiPSC-based disease modeling toolbox for 3 reasons. First, the ability to study different mutations in the same genetic background reduces the potential variability in phenotype that might be introduced by epigenetic differences¹⁰¹ or unknown genetic modifiers.^{102,103} By maintaining a well-defined genetic background, the introduction of isogenic mutations can also improve the specificity of the quantifiable phenotypes attributable to particular mutations and potentially reveal novel relevant mechanisms of disease.^{104,105} Second, the reciprocal approach is to use genome editing to correct the known mutations from patient-derived iPSC lines to create the proper controls for comparison, rather than using unrelated samples or unaffected family members.¹⁰⁶ This method can also provide information about the role of the genetic background or the contribution of a single mutation in complex multi-gene disorders.⁴⁴ Finally, genome editing will likely be a powerful tool for exploring complex signaling pathways and the contributions of individual signaling events on cellular phenotypes.

Prediction of Clinical Phenotypes

As reviewed here, it is clear that hiPSC-CMs have applications for studying disease mechanisms and for identifying potential therapies. The potential for making patient-specific models that capture the differences between patients is perhaps the most exciting and disruptive feature of this technology, but at this point there is scant empiric evidence that hiPSC-CMs reproduce phenotypic differences between patients.³² Proof-of-concept will require correlating phenotypes across a panel of patients to their cellular equivalents, measured on the hiPSC-CMs derived from these patients. Some studies have done this anecdotally. For example, some CPVT patients respond to flecainide but others do not.¹⁰⁷ Preininger and colleagues identified a

patient with CPVT who responded to flecainide and then they demonstrated that the abnormalities in calcium handling at a single-cell level, including increased spark duration and frequency, with isoproterenol administration were greatly mitigated by pre-incubation with flecainide.⁵⁷ However, systematic studies of many patients are needed to determine in practice how well hiPSC-CM models capture interindividual differences, a prerequisite if information from these models will be practically useful to prospectively guide clinical decision making.

Single-Cell vs. Tissue-Level Phenotypes

To date, hiPSC-CM IAD models have focused almost exclusively on the cellular phenotypes that arise from mutation of IAD genes. These phenotypes have primarily been studied in individual cells or small islands of cells. However, in the heart CMs are integrated into a highly connected network of cells, and this electrical coupling of excitable cells profoundly influences expressed phenotypes. When individual cells are electrically coupled, aberrant activity of a single cell is buffered by dissipation of its electrical activity across the remaining cells. This “source-sink mismatch” can suppress asynchronous aberrant activity, such as EADs and DADs, so that single-cell phenotypes may not be the same as phenotypes recorded from cells integrated into tissues.

Clinical arrhythmias are not cellular phenotypes, but rather are the emergent properties of tissues. In many cases the link between cellular phenotypes, such as EADs/DADs, and clinically relevant arrhythmias such as ventricular tachycardia remain cryptic.¹⁰⁸ To accurately model IADs, it will be necessary to assemble CMs into anisotropic sheets that are rhythmically paced in defined geometries. Achieving this goal will require combining patient-derived or genetically engineered iPSC-CMs with bioengineering to assemble patterned, 2D and 3D tissues. Examples of these approaches have been used to model cardiomyopathies^{29,30} and are being extended to arrhythmias. Incorporation of optogenetic techniques¹⁰⁹ will permit spatially precise optical stimulation and recording from the engineered tissues.¹¹⁰ Combining iPSC-CMs with bioengineered cardiac tissues and optogenetics will certainly enhance IAD models and make it more likely that these models will be useful for predicting clinical outcomes.

Future Perspectives

In a short period of time, IAD modeling with hiPSC-CMs has advanced rapidly. Despite challenges and limitations arising from the immaturity of iPSC-CMs, the pioneering studies summarized in this review have established the utility of hiPSC-CMs to faithfully recapitulate core features of IADs and to advance the current state of knowledge by revealing new disease mechanisms or facilitating drug discovery. Ongoing work will improve the hiPSC-CM models by enhancing cellular maturity and by yielding pure preparations of relevant hiPSC-CM subtypes. Expanding the use of genome editing will allow a greater range of clinically relevant mutations to be studied, and will allow for rigorous experimental designs that appropriately control for genetic background. Incorporation of tissue engineering and optogenetics will establish tissue-level disease models, which are necessary to understand how molecular defects cause tissue-level arrhythmia. A major area for future

work will be to establish the extent to which hiPSC-CM models will be able to fulfill their promise of providing individualized disease models to stratify patient risk, guide therapy, and facilitate genetic counseling for family members. Undoubtedly, fulfilling this vision will require successful development of methods to enhance iPSC-CM maturation and integrate them into tissue-level models.

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