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. 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. Since then, more than 15 genes have been found to cause LQTS. In addition to LQTS, several other IADs have been described, including arrhythmogenic cardiomyopathy (ACM), catecholaminergic polymorphic ventricular tachycardia (CPVT) and Brugada syndrome (BrS). Each of these disorders is caused by mutations in an ever-growing number of genes (Table 1).

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 for 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. 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. These analyses have been augmented by structural homology modeling and by computational models that integrate individual channel properties into virtual action potentials (APs). Genetically modified mouse, and more recently rabbit and pig models have been useful to assess the effect of mutations within intact animals. 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. Lack of appropriate physiological context in heterologous systems, non-comprehensive protein structural information or differences in ion channel expression and cardiac electrophysiological properties 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 which promises to enhance mechanistic studies and potentially provide individualized information on risk and optimal therapy. Using a cocktail of reprogramming factors, this group...
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, genetic diseases. New method to study both monogenetic and complex polygenic disorders including models of cardiomyopathies derived from patients yield patient-specific disease models, which underlies IK1, normalized RMP, improved upstroke velocity, and reduced the percentage of spontaneously beating CMs in human embryonic stem cell-derived cardiomyocytes (hESC-CMs). 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.

Electrophysiologic Characteristics of hiPSC-CMs and Adult CMs

Several protocols for directed cardiac differentiation exist in the literature, often based on timed inhibition of GSK3β and WNT signaling. These protocols for generation of hiPSC-derived cardiomyocytes (hiPSC-CMs) are robust, straightforward, and highly efficient, 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. Methods for directed differentiation and selection of atrial, ventricular and even cardiac Purkinje 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. It is this area where iPSC technology is uniquely poised to provide an entirely new method to study both monogenetic and complex polygenic diseases.

### Table 1. Genetics of Inherited Arrhythmia Disorders

<table>
<thead>
<tr>
<th>Disorder / Causative Arrhythmia Disorders</th>
<th>Cellular phenotype</th>
<th>Clinical phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1 (LQT1), KCNE1</td>
<td>Decreased IKs</td>
<td>TdP</td>
</tr>
<tr>
<td>KCNH2 (LQT2), KCNE2</td>
<td>Decreased IKs</td>
<td>TdP</td>
</tr>
<tr>
<td>SCN5A (LQT3), SCN4B, SNTA1</td>
<td>Increased INa</td>
<td>TdP, bradycardia</td>
</tr>
<tr>
<td>ANK2</td>
<td>Decreased Na/K-ATP activity</td>
<td>Bradycardia, conduction block, TdP</td>
</tr>
<tr>
<td>KCNJ2 (Andersen-Tawil syndrome)</td>
<td>Decreased IKs</td>
<td>Facial anomalies, periodic paralysis, stress-induced VT</td>
</tr>
<tr>
<td>CACNA1C (Timothy syndrome)</td>
<td>Decreased IKs</td>
<td>TdP, autism, synaustyly</td>
</tr>
<tr>
<td>CAV3</td>
<td>Decreased INa</td>
<td>TdP</td>
</tr>
<tr>
<td>CALM1, CALM2</td>
<td>Altered calcium handling</td>
<td>VT</td>
</tr>
<tr>
<td>CPVT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RYR2</td>
<td>Increased calcium leak, altered calcium handling</td>
<td>Stress and exercise-induced VT/VF</td>
</tr>
<tr>
<td>CASQ2</td>
<td>Decreased SR calcium buffering</td>
<td>Stress and exercise-induced VT/VF</td>
</tr>
<tr>
<td>CALM1</td>
<td>Abnormal Ca2+ signaling</td>
<td>Stress and exercise-induced VT/VF/long QT</td>
</tr>
<tr>
<td>Triadin</td>
<td>Altered release of Ca2+</td>
<td>Stress and exercise-induced VT/VF</td>
</tr>
<tr>
<td>BrS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A, SCN10A, SCN1B, SCN2B, SCN3B, GPDL1, MOG1, SLMAP, PKP2, HEY2</td>
<td>Decreased INa+</td>
<td>ECG changes, VT/VF at rest or with fever</td>
</tr>
<tr>
<td>CACNA1C, CACNB2, CACNA2D1</td>
<td>Decreased IKa, IK+</td>
<td>VT/VF at rest</td>
</tr>
<tr>
<td>HCN4, KCNE3, KCNE5, KCND3, ABCC9, KCNJ4, KCNH2, PKP2</td>
<td>Increased IKr, IKc, IKs</td>
<td>VT/VF</td>
</tr>
<tr>
<td>ACM</td>
<td>Decreased INa and gap junctions; apoptosis</td>
<td>Fibrosis, cardiac dysfunction, VT</td>
</tr>
</tbody>
</table>

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.
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 V1/2 (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

| Table 2. iPSC-CM Models of Inherited Arrhythmia Disorders |
|----------------|----------------|----------------|----------------|
| IAD model | Gene mutation | Experimental methods | Findings |
| LQT1 | KCNQ1 (R190Q) | Patch-clamp | Prolongation of AP, inappropriate AP adaptation at higher pacing frequencies |
| LQT2 | KCNH2 (A614V) | Patch-clamp, MEA | Prolongation of AP and induction of EADs; decreased \( I_{Na} \); potential improvement with pinacidil |
| LQT2 | KCNH2 (G1681A) | Voltage-clamp | Comparison of symptomatic and asymptomatic carrier; AP prolongation and EADs; stronger phenotype in symptomatic carrier |
| LQT2 | KCNH2 (R176W) | Voltage-clamp, MEA | AP prolongation; differences in between voltage-clamp and MEA recordings |
| BrS/LQT3 | E1784K | Patch-clamp, MEA | LQT3 phenotype, but with SCN3B knockdown, BrS phenotype unmasked |
| LQT8 | CACNA1C (G1216A) | Patch-clamp, calcium imaging | AP prolongation; Slower contraction rates in mutant cells; prolonged \( Ca^{2+} \) transients; Possible therapeutic effect of rosvitine |
| CPVT | RYR2 (F243I) | Voltage-clamp, calcium imaging | Increased DADs with isoproterenol; increased spontaneous calcium sparks |
| CPVT | RYR (E2311N) | Voltage-clamp, calcium imaging | Increased DADs; increased spontaneous calcium sparks; normalization with CaMKII inhibition |
| CPVT | RYR (P23285) | Voltage-clamp, calcium imaging | Altered calcium signaling; DADs and EADs at baseline and with isoproterenol |
| CPVT | RYR2 (S406L) | Voltage-clamp, calcium imaging | Increased DADs with isoproterenol; improvement with dantrolene |
| CPVT | RYR2 (L3741P) | Calcium imaging, MEA | Increased DADs; increased spontaneous calcium sparks; reversal of phenotype with flecainide |
| CPVT | CASQ2 (D307H), RYR2 (R420Q) | Voltage-clamp, calcium imaging | Increased DADs with isoproterenol; increased spontaneous calcium sparks |
| ACM | PKP2 (L614P) | Patch-clamp, calcium imaging, IF, EM | Reduction of both PKP2 and plakoglobin by IF |
| ACM | PKP2 G784Af*, PKP2 K628Rfs | IF, EM | No definitive changes in \( I_{Na} \), \( I_{Ks} \) or \( I_{Ca,L} \) consistent with BrS |
| ACM | PKP2 A324fs | MEA, IF, EM | Decreased \( I_{Na} \) density and larger late current |
| BrS/LQT3 | SCN5A (1795insD) | Patch-clamp | Decreased \( I_{Na} \) density and larger late current |

*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.
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), lack T-tubules. 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. 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. 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 handling.

There are multiple potassium currents that contribute to the cardiac AP (see review by Nerbonne and Kass for a more comprehensive analysis). The delayed rectifier potassium current that is responsible for repolarization consists primarily of rapid (IKr) and slow (IKs) components, in addition to the aforementioned IK1 that contributes to RMP as well as the terminal phase of the AP. Both IKr and IKs are present in hiPSC-CMs, and these share similar biophysical properties and expression with human CMs. The expression of IKr is comparable between hiPSC-CMs and native ventricular CMs, with similar current densities and voltages of activation and inactivation. Functional expression of IKr has also been demonstrated by AP prolongation and early afterdepolarizations (EADs) with the potassium channel E4031. In contrast, the current density for IKs appears to be much more variable, as 2 different studies have reported vastly different average current densities varying from 0.31 pA/pF to as high as 2.5 pA/pF. In either case, the current density for IKs 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. One possibility for this variability may be the relative expression of KCNE1, which encodes an IKs subunit and has been demonstrated to have a significant effect on IKs current density in hESC-CMs.

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 IK1 expression; INa 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. 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.40,42,75,43 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 LQT34 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 new 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.80 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.81 High-throughput platforms to measure APD and other electrophysiologic parameters for drug screening have been reported.82 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 They are one 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.8,54,55,57 CPVT cell models recapitulate 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 cardiac 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.58,84 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 arrhythmogenic 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.

There are several reported hiPSC-CM models of ACM. These models recapitulate the desmosomal abnormalities that are central to the disease. ACM hiPSC-CMs have an increased propensity to accumulate lipid droplets under adiogenic culture conditions. 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.

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. BrS is an IAD characterized by stereotypical ECG findings such as a prolonged QT interval and tall T waves. 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.

BrS. One of the challenges in diagnosis is that a number of conditions and potentially reveal novel relevant mechanisms of disease.

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. 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 differences or unknown genetic modifiers. 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.

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. 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. Preininger and colleagues identified a
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. To accurately model IADs, it will be necessary to assemble CMs into annulus 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 and are being extended to arrhythmias. Incorporation of optogenetic techniques will permit spatially precise optical stimulation and recording from the engineered tissues. 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.

Acknowledgments

W.T.P. was supported by grants from the American Heart Association (16CSA28750006) and the National Institutes of Health (R01HL128694), and by charitable donations from the Department of Cardiology of Boston Children’s Hospital.

References

iPSC-CM Models of Inherited Arrhythmias

19

22. Maltese VA, Kyle JW, Undrovinas A. Late Na+ current pro-

iPSC-CM Models of Inherited Arrhythmias

2011; 191 – 201.
tent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation 2012; 125: 3079 – 3091.
27. Sakakibara Y, Furukawa T, Singer DH, Jia H, Backer CL, Arenzen CE, et al. Sodium current in isolated human ventricu-
29. Mews T, Ravenus U. Type calcium currents of human myo-
ocytes from ventricle of non-failing and failing hearts and from atrium. J Mol Cell Cardiol 1994; 26: 1307 – 1320.
42. Jost N, Virág L, Orpichini M, Szécsi J, Varró A, Papp JG.


