Induced Pluripotent Stem Cell Models for Arrhythmias

Induced Pluripotent Stem Cell-Derived Cardiomyocytes
A Versatile Tool for Arrhythmia Research

Daniel Sinnecker, Alexander Goedel, Karl-Ludwig Laugwitz, Alessandra Moretti

Abstract: Induced pluripotent stem cells offer the possibility to generate patient-specific stem cell lines from individuals affected by inherited disorders. Cardiomyocytes differentiated from such patient-specific induced pluripotent stem cell lines have been used to study the pathophysiology of arrhythmogenic heart diseases, such as the long-QT syndrome or catecholaminergic polymorphic ventricular tachycardia. Testing for unwanted drug side effects or tailoring medical treatment to the specific needs of individual patients with arrhythmogenic disorders may become future applications of this emerging technology. (Circ Res. 2013;112:961-968.)

Key Words: catecholaminergic polymorphic ventricular tachycardia ■ disease modeling ■ long-QT syndrome ■ reprogramming

Disease Modeling With Patient-Specific Induced Pluripotent Stem Cells

The development of human embryonic stem cell technology and the ability to differentiate these pluripotent stem cells into somatic cells, such as cardiomyocytes, have spawned the idea to use somatic cells generated from pluripotent stem cells as a novel type of in vitro disease model. The quest for pluripotent stem cell–based disease models was propelled in 2006 by the demonstration that murine somatic cells can be reprogrammed to pluripotent stem cells (termed “induced pluripotent stem cells” [iPSCs]) that closely resemble embryonic stem cells just by forcing them to overexpress a specific cocktail of transcription factors. Barely 1 year later, this method was successfully transferred to human cells. With iPSC technology, it became feasible to take somatic cells from a patient with a heritable disorder and generate patient-specific iPSCs, which carry the disease-causing genotype. These cells can be differentiated into somatic cells that exhibit the phenotype of the disease and may be studied in a culture dish (Figure 1).

iPSC Models for Arrhythmias: State of the Art

The first arrhythmogenic heart diseases for which iPSC-based model systems had been developed were Mendelian arrhythmogenic disorders, namely long-QT syndromes types 1, 2, 3, 5, and 8, as well as catecholaminergic polymorphic ventricular tachycardia (CPVT). In these studies, iPSCs lines were generated from somatic cells, usually skin fibroblasts, of patients and healthy controls. Aggregation of the iPSC in so-called embryoid bodies was applied to induce spontaneous differentiation, yielding (among other cell types) cardiomyocytes, which could be identified by spontaneously occurring rhythmic contractions. These cardiomyocytes were studied by single-cell methods, such as patch clamp electrophysiology or fluorometric calcium imaging. In patient-specific iPSC-derived cardiomyocytes, these studies could recapitulate...
altered physiological properties that corresponded well with already known aspects of the pathophysiology of the respective disorders.

Long-QT syndrome type 1 was modeled by reprogramming cells from patients with 2 different mutations of the potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) gene, R190Q and 1893delC. Both models showed prolonged action potentials attributable to a reduced $I_{Ks}$ current. By immunofluorescence analysis of iPSC-derived cardiomyocytes, it was shown that in both cases, a trafficking defect of the channel from the endoplasmic reticulum to the plasma membrane was the underlying disease mechanism. This led to additional experiments in heterologous systems, demonstrating a dominant-negative nature of this effect.

Long-QT syndrome type 2 was modeled by generating iPSCs from patients with 3 different potassium voltage-gated channel, subfamily H (eag-related), member 2 (KCNH2) mutations, namely A614V, G1681A, and R107W. Prolonged action potentials were present in all 3 model systems. In 1 of the studies, iPSC lines were derived from 1 symptomatic carrier and 1 asymptomatic carrier of the mutation and seemed to be able to reflect the severity of the disease as observed in the patients.

Nonstandard Abbreviations and Acronyms

<table>
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<th>Abbreviation</th>
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<tr>
<td>CPVT</td>
<td>catecholaminergic polymorphic ventricular tachycardia</td>
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<td>HERG</td>
<td>human ether-a-go-go related gene</td>
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<tr>
<td>iPSC</td>
<td>induced pluripotent stem cell</td>
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<tr>
<td>KCNH2</td>
<td>potassium voltage-gated channel, subfamily H (eag-related), member 2</td>
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Figure 1. Modeling cardiac diseases with patient-specific induced pluripotent stem cells (iPSC). Schematic representation of the steps necessary to model a human heart disorder by generating patient-specific iPSC lines, differentiating them to cardiomyocytes, and studying the disease phenotype in these cells.
Long-QT syndrome type 8 as part of Timothy syndrome, which also leads to syndactyly, immune deficiency, and autism, was modeled by reprogramming skin fibroblasts from 2 patients with a G1216A missense mutation of the calcium channel, voltage-dependent, L type, alpha 1 C subunit (CACNA1C) gene encoding Cav1.2. In this model, a marked action potential prolongation was observed. The cardiomyocytes displayed a delayed inactivation of the I\text{Na}	extsuperscript{v} current and abnormal intracellular calcium handling with larger and prolonged calcium transients.

CPVT1 caused by 2 different mutations of the cardiac ryanodine receptor, namely F243I and S406L, was modeled with patient-specific iPSC. In both models, the β-adrenergic agonist isoproterenol led to delayed afterdepolarizations and an increased frequency of elementary calcium release events from the sarcoplasmic reticulum as compared with control cardiomyocytes. CPVT2 caused by an autosomal-recessive D307H mutation of the sarcoplasmic reticulum calcium buffering protein CASQ2 was also modeled with patient-specific iPSC. The diseased cardiomyocytes displayed a reduced spontaneous beating frequency, and isoproterenol stimulation led to delayed afterdepolarizations, oscillatory arrhythmic prepotentials, and cytosolic calcium overload.

Patient-specific iPSC lines have also been generated from a patient with a 1795insD mutation of the sodium channel, voltage-gated, type V, alpha subunit (SCN5A) gene, leading to an overlap syndrome with features of both Brugada syndrome (a disorder characterized by ST-segment elevation in the right precordial ECG leads and a susceptibility to sudden cardiac death attributable to ventricular arrhythmias) and long-QT syndrome type 3. Cardiomyocytes derived from these iPSCs displayed a reduced peak I\text{Na}	extsuperscript{v} current density and an increase in persistent I\text{Na}	extsuperscript{v} along with prolonged action potentials, which is consistent with a combined gain-of-function and loss-of-function defect of the cardiac sodium channel.

In addition to improving our understanding of arrhythmogenic diseases, iPSC-based disease models hold the promise of providing new medical approaches to their treatment. β-blockers are the state-of-the-art therapy for different forms of long-QT syndrome, as well as for CPVT. The studies that used β-blockers in in vitro assays on iPSC-derived cardiomyocytes from long-QT syndrome patients were able to recapitulate the beneficial effect seen in numerous clinical trials and daily clinical routine. It was shown that β-blockers abolished the proarrhythmic effect of isoproterenol stimulation. Moreover, medications that are already in use for different indications but that seemed to be promising for the treatment of arrhythmogenic diseases were successfully tested. For example, dantrolene, a drug in clinical use for malignant hyperthermia, which is a disease of the skeletal muscle ryanodine receptor, rescued the phenotype seen in cardiomyocytes derived from iPSCs of a CPVT1 patient. It significantly reduced Ca\textsuperscript{2+} spark frequency in isoproterenol-treated cardiomyocytes and almost abolished delayed afterdepolarizations and triggered activity. Nifedipin partially restored electrophysiological properties of iPSC-derived cardiomyocytes from a long-QT syndrome type 2 patient by shortening the prolonged action potentials and also by reducing the number of early afterdepolarizations. In the iPSC-based model of Timothy syndrome, a new compound named roscovitine, which interferes with the voltage-dependent Cav1.2 calcium channel, was shown to improve the impaired inactivation of this channel. These results suggest that the iPSC-based disease models will be useful in experimental pharmacology.

**The Road Ahead: Chances and Challenges**

The studies conducted so far have been, at least to a large extent, proof-of-principal studies that paved the way for future research relying on patient-specific iPSC lines. The ongoing success of such research will depend largely on the generation of novel findings and new pathophysiological insight beyond the scope of conventional methodology, which might be expected in the areas described here.

**Drug Safety**

One of the major unwanted side effects of new drug candidates is QT interval prolongation in the electrocardiogram (ECG), which frequently led to use restrictions or even withdrawal from the market of pharmacological agents because of the concomitant risk of fatal arrhythmias. Drug-induced QT interval prolongation is commonly caused by inhibition of the human ether-a-go–go related gene (HERG) potassium channel, which may be quantified by measuring the drug effect on the HERG-mediated I\text{Kr} current in animal cardiomyocytes or even in HERG-overexpressing cell lines. However, such a test system might fail to predict QT interval prolongation if the drug prolongs the QT interval by an indirect HERG inactivation mediated by a factor not present in the test system, or even by a HERG-independent pathway. A test system consisting of iPSC-derived human cardiomyocytes might provide a preclinical means to become aware of such an arrhythmogenic potential early in the drug development process. Because a genetic predisposition for drug-induced QT interval prolongation seems to exist, such a test system might be based on a panel of iPSC lines derived from patients with a history of QT interval prolongation in response to different drugs.

**Personalized Medicine**

It is commonplace that patients respond differently to a specific therapy, and that the genetic background plays an important role in this variance. The emerging field of personalized medicine deals with tailoring therapies to the specific needs of single patient. Modern oncology represents a paradigm of successful realization of this concept, in which the specific molecular pathways that went astray, leading to malignant transformation in a specific tumor, can be targeted with drugs (eg, tyrosine kinase inhibitors) that specifically interfere with these pathways. Strikingly, characteristics of a specimen of the tumor (eg, expression of a specific marker) can predict whether an individual patient will benefit from such a highly specific therapy. Although it is tempting to translate this successful concept to cardiac diseases, the use of a myocardial biopsy to obtain cardiomyocytes to test different drugs in vitro is hampered by the invasiveness of such an approach. By contrast, patient-specific cardiomyocytes generated from iPSCs might...
provide a platform for such experiments. Although a plethora of antiarrhythmic drugs exists, their clinical use is restricted because of frequent side effects, especially their possible proarrhythmic potential. Cardiomyocytes generated from iPSCs might be used to evaluate the effect of different drugs and drug combinations on the arrhythmia of a specific patient, as well as to screen for proarrhythmic side effects. Similarly, electrophysiological characterization of these cells might provide a novel means of risk stratification, for example, to assess the risk of a specific patient experiencing sudden cardiac death and to provide a basis for the decision regarding whether this patient should have an internal defibrillator implanted. However, there is still a long way to go before such clinical applications of iPSC-derived cardiomyocytes become reality. It remains to be demonstrated whether favorable and unfavorable drug effects can be predicted in vitro using patient-specific iPSCs and whether risk stratification can be improved by such an experimental approach.

Defining a Functional Phenotype for Diseases of Unknown Genetics

Although most of the diseases studied so far using iPSC-derived somatic cells were monogenetic diseases, one of the strengths of the iPSC system is the ability to study a disease phenotype even if the genetic background is unknown. By using iPSC-derived neuronal cells from patients with schizophrenia, certain molecular aspects of the disease could be studied in vitro, providing new insight into this complex disorder, regardless of the underlying mutations.25 This could be transferred to the cardiac field by studying more complex arrhythmogenic disorders, such as atrial fibrillation or arrhythmias associated with dilated cardiomyopathy, in which the genetic background is often unknown. Similarly, the Brugada syndrome is known to be caused by mutations of the cardiac sodium channel, voltage-gated, type V, α-subunit.26 However, sodium channel, voltage-gated, type V, α-subunit mutations account for <30% of cases.26 Moreover, a highly varying degree of penetrance exists among patients with known mutation status, and the typical ECG pattern is present more often in men than in women,27 suggesting that other genetic factors might play an important role for the precipitation of the phenotype. Cardiomyocytes generated from iPSCs derived from patients with and without known mutations as well as from mutation carriers with and without clinical symptoms might provide a platform to define a functional Brugada syndrome phenotype and to identify novel molecular compounds involved in the pathophysiology of this disease.

Steps Still to Be Taken

iPSC technology is a relatively young field, and despite substantial progress that has already been made there is still much room for methodological improvements.

One important area of current research is to increase the efficacy of cardiac differentiation of iPSCs.28,29 Patient-specific iPSC-derived cardiomyocytes would provide an ideal platform for hypothesis-generating approaches, such as transcriptome or proteome analysis, which could be used to identify novel pathophysiological pathways, for example, in diseases leading to cardiac hypertrophy or heart failure. However, these techniques require substantial amounts of cardiomyocytes with low contamination of noncardiomyocyte cells. The development of protocols to drive cardiac differentiation of human iPSCs has been promoted by insights gained by similar work with human embryonic stem cells (reviewed recently).30 During embryoid body differentiation, key stages of embryonic heart development are recapitulated. Current research therefore aims at providing growth factors to the differentiating cells in a sequential fashion to mimic the cellular microenvironment during cardiogenesis. Alternatively, cardiac differentiation also can be achieved in monolayers rather than in embryoid bodies,31,32 which provides a more uniform access of applied growth factors to the differentiating cells. Another approach to promote cardiac differentiation is coculture with visceral endoderm-like cells,33,34 which are thought to provide a cardiogenic microenvironment to the differentiating cells. Another desirable goal is to direct differentiation toward specific subtypes of cardiomyocytes. In this respect, it could be shown that in a chemically defined culture system, differentiation of human embryonic stem cells could be directed either to atrial-like or to ventricular-like cardiomyocytes by exposing the cells either to retinoic acid or to a retinoic acid antagonist at a specific time point of differentiation.35

A current problem is that no single protocol exists that can be reliably used to generate sufficient amount of cardiomyocytes for physiological experiments from any available iPSC line. It is typically necessary to adjust the protocol or to try different types of protocols to optimize cardiac differentiation for each iPSC line. This is problematic if cardiomyocytes generated by different protocols are to be compared because part of the differences found in the cardiomyocytes might arise from differences in the differentiation protocols, rather than from the genomic differences between the iPSC lines.

An alternative or addition to developing highly efficient differentiation protocols would be to purify the cardiomyocytes from a heterogeneous cell population, which is also an area of intensive methodological investigation. This might be performed by cell sorting either based on the expression of specific cell surface markers36,37 or based on the fluorescence of a mitochondrial-specific live cell dye, taking advantage of the fact that cardiomyocytes have an exceptionally high number of mitochondria.38,39 Another approach, which has been successfully pursued to selectively purify embryonic stem cell–derived cardiomyocytes, is to genetically modify the pluripotent stem cells to express a selection marker under the control of a cardiomyocyte-specific promoter.40,41 By using appropriate promoters, even subpopulations of cardiomyocytes, such as ventricular-like or nodal-like cardiomyocytes, can be selectively labeled. However, the downside of this genetic approach is that the genetic modification has to be introduced into every single iPSC line, requiring a high amount of time and labor, especially if cardiomyocytes generated from several iPSC lines (eg, different patients and controls) are to be compared.

An important limitation of iPSC-based cardiac disease models is the lack of maturity of the cardiomyocytes generated by current differentiation protocols. Morphologically, by
gene expression and by electrophysiological properties, they are more similar to fetal than to adult cardiomyocytes. For example, the action potentials of iPSC-derived cardiomyocytes clearly differ from those of mature cardiomyocytes by exhibiting a more positive maximum diastolic potential and a slower upstroke velocity. Furthermore, iPSC-derived cardiomyocytes typically exhibit spontaneous activity, whereas mature ventricular cardiomyocytes normally remain electrically silent unless excited.44–46 Similarly, the intracellular calcium cycling system is immature in iPSC-derived cardiomyocytes, which may be exemplified by lack of T tubules15 and a low expression level of the sarcoplasmic reticulum calcium buffering protein calsequestrin.14 This immature phenotype of iPSC-derived cardiomyocytes might be aggravated by the common practice of selecting cells for analysis based on their spontaneous contractions,46 which may lead to analysis of a selection of the most immature cells. Action potentials recorded from nonspontaneously beating iPSC-derived cardiomyocytes displayed lower resting membrane potentials and higher maximal upstroke velocities than those of spontaneously contracting cells.17

Protocols to improve maturation of iPSC-derived cardiomyocytes would be highly valuable. To date, it cannot be foreseen whether techniques, such as 3-dimensional cell culture or electric stimulation during differentiation, will help to reach this goal. Moreover, the development of standards to assess the degree of maturity of a cardiomyocyte preparation also might help to compare results generated by different research groups by analyzing cardiomyocytes at different stages of maturation.

Most of the assays used in iPSC-based cardiac disease models so far examined cell-autonomous defects, such as changes in electric properties or calcium cycling. However, important cardiac diseases such as heart failure, developmental defects, and myocardial infarction cannot be sufficiently explained at the single-cell level. Tissue engineering, 3-dimensional cell culture, as well as coculture with other types of cells found in adult hearts might provide new tools to mimic a more

Figure 2. Investigating arrhythmias at different levels with patient-specific induced pluripotent stem cells (iPSCs). Schematic representation of the different levels at which iPSCs might help to unravel the pathophysiology of arrhythmogenic disorders. Examples of experimental approaches directed at the different levels are given.
physiological environment to study noncell-autonomous aspects of cardiac disorders.

A problem encountered whenever cardiomyocytes derived from patient-specific iPSCs are investigated is the appropriate choice of experimental controls. Because an inherent variability exists between different iPSC lines, even if they are derived from the same proband or patient, it is crucial to demonstrate that any phenotype found in iPSC-derived cardiomyocytes is caused by the specific genetic background of the cell donor and not just by this variability. Possible sources of the line-to-line variability are the genomic integration of reprogramming factors with possible disruption of endogenous genes, as well as clonal selection steps necessary during the reprogramming process that may lead to the selection of clones with accumulated genomic defects. It has been demonstrated that the reprogramming process may disrupt the genomic and epigenomic integrity of the cells and introduces mutations. The resulting genetic and epigenetic differences between different iPSC lines may also affect the differentiation potential into different lineages, for example, to cardiomyocytes. Until reprogramming methods free from these problems or until test systems to select genomically and epigenomically intact iPSC clones are available, it will be necessary to always derive and use several iPSC lines from each individual and to control for the phenotypic variability between these lines.

Recent studies indicate that it may be possible to directly reprogram cells, such as fibroblasts, to cardiomyocytes without the intermediate step of a pluripotent stem cell line. Whether this leads to fewer genetic and epigenetic alterations than iPSC generation remains to be investigated. Although this approach seems to be promising for applications, such as cell replacement after myocardial infarction, it cannot be foreseen right now whether it also will be helpful for disease modeling. A conceptual problem is that this approach leads only to the generation of a limited number of cardiomyocytes, which interferes with reproducibility of experiments. By contrast, an iPSC line can be propagated to other laboratories, where experimental key results may be reproduced.

The most common practice to date is to use iPSCs generated from healthy donors unrelated to the patient as control lines. However, the problem associated with this approach is that such patient and control iPSC lines differ not only in the disease-causing mutation (in case of a Mendelian disorder) but also in thousands of other mutations and polymorphisms, which also may account for phenotypic differences between patient and control cardiomyocytes. One way to at least limit this variability would be to derive control iPSC lines from close relatives of the patient who are not affected by the disease-causing mutation. Another possible approach is to use a panel of control iPSC lines derived from several genetically diverse individuals. The genetic variation between patient and control iPSCs could be further reduced if the control line is generated by genetically correcting the disease-causing mutation in the patient-derived iPSCs. With such a strategy, it could be unequivocally demonstrated that a phenotype is only caused by the mutation undergoing study.

Locating iPSC-Based Disease Models in the Research Landscape

The pathophysiology of an arrhythmogenic disorder, such as long-QT syndrome, manifests at and can be studied at different levels, ranging from the molecular level (eg, the exchange of an amino acid in a protein constituting a cardiac potassium channel altering the conductance of the channel pore for potassium ions) to the cellular level (eg, prolonged action potential duration of a single cardiomyocyte leading to susceptibility to delayed afterdepolarizations), to the level of cardiac tissue (eg, macro-reentries leading to ventricular tachycardia), to the level of the whole organism (eg, insufficient cardiac output during ventricular tachycardia leading to sudden cardiac death), and even up to the level of patient populations (eg, application of an antiarrhythmic drug in a randomized controlled trial leading to fewer arrhythmic episodes in the verum group). However, no model system, no matter how complex it may be, can faithfully replicate every single aspect of the part of the world it is supposed to represent; the best material model for a cat is another or preferably the same cat. Two major goals for those working with disease models are being aware of the strengths and weaknesses of each particular model and striving for always having several complementary models to use.

Disease models based on iPSCs will very likely add their share to the field of arrhythmogenic diseases (Figure 2) in the context of other well-established methodologies. Most notably, animal models, especially genetically modified mouse models, have been used to help in the past and continue to help us to understand many aspects of the pathophysiology of these disorders. However, because substantial differences exist in the cellular electrophysiology of cardiomyocytes from mice (with heart rates >400/min) and humans, the findings in mouse disease models cannot always be easily generalized to human physiology. The addition of iPSC-based patient-specific human cardiomyocytes to the electrophysiologist’s toolkit might broaden our understanding of the pathophysiology of these clinically important conditions and might help us transfer already established knowledge to the human system.

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Disclosures

None.

References


34. Zhu WZ, Xie Y, Moyes KW, Gold JD, Askari B, Laflamme MA. Neuregulin 1/ErbB signaling regulates cardiac subtype specification.


Response to Laugwitz and Coauthors

Bjorn C. Knollmann

I have been asked to prepare a response to the above article making a case for iPSC models of arrhythmia. Clearly, there is a consensus that iPSC offers the wonderful opportunity to create patient-specific disease models in the dish. I also agree with the authors that currently published iPSC arrhythmia models have been largely proof-of-principle studies of limited scope. Whether the published iPSC arrhythmia models "paved the way for future research relying on patient-specific iPSC lines," as the authors suggest, or rather represent the picking of low-hanging fruits, remains to be seen. Furthermore, I could not agree more with the authors’ statement, "future success of such research will depend on the generation of novel findings and new pathophysiological insight beyond the scope of conventional methodology." Unfortunately, the rapidly decreasing journal impact factor of the published iPSC arrhythmia models published during the past 2 years (Figure 2 in my review article) suggests that gaining such novel insight may be difficult using the iPSC methodology, at least in arrhythmia research. Although the authors may argue that 2 years is too short to uncover the full potential of the iPSC arrhythmia models, to me, such a rapid decrease of the impact factor in such a short time is very concerning and could indicate that the future impact of iPSC for arrhythmia research will be relatively modest. The authors further suggest that, in the future, iPSC models of arrhythmia will become particularly valuable in the following 3 areas: (1) drug safety studies; (2) personalized medicine; and (3) defining the functional phenotype for diseases of unknown genetics. I am somewhat less optimistic, given the formidable technical and conceptual barriers that need to be overcome before any of these 3 applications can be realized. I am particularly skeptical regarding the use of iPSC arrhythmia models to personalize antiarrhythmic drug therapy. For example, I would not consider the 14 odd antiarrhythmic drugs available to clinicians in the United States a plethora, as suggested by the authors. Rather, in sharp contrast to the many drugs available for personalizing anticancer therapy, the options for antiarrhythmic drug therapy are very limited to begin with, and there is no evidence that iPSC models have better predictive value than other existing models (ie, heterologous expression systems). Furthermore, the time delay (several months until a patient-specific iPSC line can be generated) and cost (>$15 000 per iPSC line only for reagents) will make routine clinical use difficult. Nevertheless, although other scientists may call iPSC arrhythmia models a mild case of irrational over exuberance, I believe that iPSC is a promising new technology that will make important contributions in arrhythmia research. How important remains to be seen.


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