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G. Tiscornia, N. Monserrat and J.C. Izpisua Belmonte
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Modelling Long QT Syndrome With iPS Cells: Be Still, My Beating Heart . . .

G. Tiscornia, N. Monserrat, J.C. Izpisua Belmonte

**Commentary**

**Modeling the Long QT Syndrome With Induced Pluripotent Stem Cells**

Itzhaki et al

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**Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome**

Moretti et al


Recent publications by Itzhaki et al and Moretti et al introduce the latest improvement for in vitro disease modelling of cardiac arrhythmias. iPS lines derived from patients with LTQ1 and LTQ2 can be differentiated into cardiomyocytes, showing the disease’s characteristic electrophysiologic signature, establishing a convenient and powerful system for studying mechanisms of pathogenesis and testing therapeutic compounds.

Long QT syndrome (LQTS) is characterized by prolonged QT interval on a surface electrocardiogram (ECG). Clinically, LQTS presents with sudden arrhythmia, which can lead to fainting or syncope and sudden death, typically in young or otherwise completely healthy individuals. The cardiac action to fainting or syncope and sudden death, typically in young or otherwise completely healthy individuals. For example, the particular potassium current that is reduced in LQT2, is essentially absent in a normal mouse heart.

In an ECG, the distance between the onset of Q to the end of the T waves (the QT interval) measures the time it takes for the heart to depolarize and repolarize in preparation for the next beat. A longer than normal QT interval can be caused by mutations in more than 10 potassium and sodium channels involved in cardiac electrophysiology. It can also be induced by certain medications, such as cisapride, haloperidol, or ziprasidone. The diagnosis can be problematic, as up to 2.5% of healthy individuals may show prolonged QT intervals, and up to 15% of mutation carriers may fail to show prolonged QT intervals in an electrocardiogram.

A delay in the repolarization of the heart can lead to palpitations typically caused by ventricular arrhythmias, such as torsade de pointes and eventually to fainting or sudden death. In a normal heart, excitation increases the activity of depolarizing pumps, which in turn is compensated by an increase in repolarizing activity. In LQTS, this link is disturbed, explaining why adrenergic stimuli, such as strenuous physical activity or intense emotions, can trigger arrhythmias. Perhaps the phrase “not for the faint of heart” originally referred to LQTS patients. An opportunity to develop drugs to treat the condition and a concern because drugs developed for other conditions may induce LQTS.

To date, mechanistic studies of ion channels have mostly relied on overexpressing the component of the pump in particular cell types, followed by more or less standard molecular and electrophysiologic approaches. Although these studies have provided much of what we know about ion channels, cellular pumps are complex macromolecular assemblies and their behaviour is remarkably cell-type and species specific. Human cardiomyocytes are typically not available, and animal models do not always faithfully reproduce the human phenotype. In mice, the ion channels that contribute to the cardiac action potential are remarkably different from that of humans in many respects. For example, I_{Kr}, the particular potassium current that is reduced in LQT2, is essentially absent in a normal mouse heart.

Enter iPS technology. The possibility of modelling human disease has been a potential in the field ever since the derivation of human ES cells in 1998, and with direct reprogramming, pluripotent cells have been derived for a growing number of diseases, bringing this promise a step further to realization. Two recent studies by Itzhaki et al and Moretti et al, published in *Nature* and the *New England Journal of Medicine*, respectively, provide powerful proof of principle demonstrations that modelling disease in iPS cells can lead to both insights on the mechanisms of pathogenesis and serve as assays for small molecule function.

Itzhaki et al took fibroblasts from a patient with LQT2, which arises from a missense mutation (A614V) in the KCNH2 gene encoding the hERG potassium channel, and reprogrammed them to iPS using the by now classic retroviral transduction approach of Oct4, Sox2, and Klf4, pioneered by the Yamanaka group.

They obtained clonal iPS which they characterized and subsequently differentiated to the cardiac lineage, obtaining embryoid bodies containing differentiated cardiomyocytes expressing the proper markers and capable of spontaneous beating. Electrophysiologic analysis demonstrated the 2 essential features of LQT2: longer QT intervals and early afterdepolarizations (EAD), spontaneous low-amplitude depolarizations thought to prelude ventricular arrhythmias in LQTS. Patch-clamp analysis of these beating embryoid bodies revealed several different types of action potentials typical of nodal, atrial, and ventricular cells and, importantly, demonstrated increased action potential duration (APD) when

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From the Center for Regenerative Medicine in Barcelona (G.T., N.M.), Barcelona, Spain; and The Salk Institute for Biological Studies (J.C.I.B.), La Jolla, CA.

Correspondence to J.C. Izpisua Belmonte, The Salk Institute for Biological Studies, La Jolla, CA 92037. E-mail belmonte@salk.edu

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compared with control cells, in which the phenotype could be induced by treating them with the $I_{Kr}$-specific blocker, E-4031. The $I_{Kr}$ current itself was also significantly lower in LQT2 cardiomyocytes, lower than would be expected from loss of a single allele, confirming the previous observation in a heterologous expression system that A614V acts as a dominant negative protein. The longer duration of the action potential and the EAD observed by patch-clamp were confirmed by a second method, the microarray electrode mapping technique, which analyzes the electrophysiologic properties at the multicellular level, essentially measuring the equivalent of the ECG QT interval. Both approaches showed a marked arrhythmogenicity in the affected cardiomyocytes. Taking the paradigm a step further, Itzhaki et al showed the utility of their system to evaluate potential pharmacologic compounds. First, they demonstrated that known $I_{Kr}$ blockers, such as E-4031 or cisapride (a serotonin receptor agonist, which increase gastric motility but was taken off the market because it led to ventricular arrhythmias resulting from its unintended $I_{Kr}$ blocking activity) caused longer action potentials and arrhythmogenicity in their LQT2 cardiomyocytes. Then, they tested three different compounds, each hypothesized to counter action potentials prolongation and myocardial arrhythmogenicity through their effects on different types of channels. Treatment with nifedipine, an inhibitor of $Ca^{2+}$ channels that are thought to contribute to APD and EAD, resulted in significant APD abbreviation and elimination of EAD, although prolonged exposure resulted in loss of beating embryoid bodies. A second hypothesis predicted that augmenting outward potassium currents, rather than inward calcium currents, might be an alternative approach, and thus pinacidil was tested. This compound also reduced APD and eliminated EAD, yet still induced arrhythmias, presumably generating an also proarrhythmic short QT syndrome. Finally, ranolazine, a $Na^{+}$ channel blocker, had no effect on APD but did abolish arrhythmia.

Moretti et al followed the same approach, albeit for studying LQT1, which is caused by a mutation in the $KCNQ1$ gene and also encodes a repolarizing potassium channel. Patient fibroblasts were similarly reprogrammed to iPSC and differentiated to cardiomyocytes that showed increased APD. Measurement of current reduction levels supported the hypothesis of a dominant negative protein trafficking defect as a mechanism of pathogenesis. These publications focusing on arrhythmic syndromes convincingly confirm our expectations that disease modeling will be one of the main contributions of iPSc technology to human health. Other recent articles have focused on other diseases, such as Leopard’s syndrome, Fanconi’s anemia, and Rett syndrome, to name a few, and we are bound to see a flurry of similar works in the near future. Despite potential shortcomings of the technology, such as lack of maturity or heterogeneity of the differentiated cells, these examples show that iPSc disease modelling provides a new system for the study of disease, particularly disorders for which there are no satisfactory animal models. It provides an accessible, convenient, and cost-effective approach for gaining insight into the mechanisms underlying disease phenotypes and for testing of therapeutic compounds. In the case of LQT2, the compounds tested do not seem to be obvious clinical candidates, and the laboriousness of electrophysiologic readings as an assay output does not bode well in terms of developing high-throughput screens. Perhaps its immediate use will be to test whether compounds intended for treating a given condition have arrhythmia as a secondary effect, introducing a new level of safety that must be pass before proceeding into phase 1 clinical trials. This is no small thing. The next few years promise to be very interesting.

References