

Commented Summary from Current Medical Literature

Human Cardiomyocytes with Long-QT Syndrome in Dish

Summary: Background: Long-QT syndromes are heritable diseases associated with prolongation of the QT interval on an electrocardiogram and a high risk of sudden cardiac death due to ventricular tachyarrhythmia. In long-QT syndrome type 1, mutations occur in the *KCNQ1* gene, which encodes the repolarizing potassium channel mediating the delayed rectifier I(Ks) current.

Methods: We screened a family affected by long-QT syndrome type 1 and identified an autosomal dominant missense mutation (R190Q) in the *KCNQ1* gene. We obtained dermal fibroblasts from two family members and two healthy controls and infected them with retroviral vectors encoding the human transcription factors OCT3/4, SOX2, KLF4, and c-MYC to generate pluripotent stem cells. With the use of a specific protocol, these cells were then directed to differentiate into cardiac myocytes.

Results: Induced pluripotent stem cells maintained the disease genotype of long-QT syndrome type 1 and generated functional myocytes. Individual cells showed a “ventricular,” “atrial,” or “nodal” phenotype, as evidenced by the expression of cell-type-specific markers and as seen in recordings of the action potentials in single cells. The duration of the action potential was markedly prolonged in “ventricular” and “atrial” cells derived from patients with long-QT syndrome type 1, as compared with cells from control subjects. Further characterization of the role of the R190Q-*KCNQ1* mutation in the pathogenesis of long-QT syndrome type 1 revealed a dominant negative trafficking defect associated with a 70 to

80% reduction in I(Ks) current and altered channel activation and deactivation properties. Moreover, we showed that myocytes derived from patients with long-QT syndrome type 1 had an increased susceptibility to catecholamine-induced tachyarrhythmia and that beta-blockade attenuated this phenotype. **Conclusions:** We generated patient-specific pluripotent stem cells from members of a family affected by long-QT syndrome type 1 and induced them to differentiate into functional cardiac myocytes. The patient-derived cells recapitulated the electrophysiological features of the disorder. (Funded by the European Research Council and others.) Copyright 2010 Massachusetts Medical Society.

Source: Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, et al. Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome. *N Engl J Med*. 2010 Jul 21. [Epub ahead of print]

Comment: Although animal models and cell cultures of diseases are invaluable, they do not always faithfully mimic human diseases, particularly those for human contiguous gene syndromes. For instance, none of the available animal models of the long-QT syndrome generate the main cardiac repolarizing currents.¹ Additionally, the majority of human cell lines in wide use today for disease research carry genetic and epigenetic artifacts.² Recently, generation of pluripotent stem cells with characteristics similar to embryonic stem (ES) cells [termed induced pluripotent

stem (iPS) cells] has led to heightened excitement regarding the potential of these cells for improving the understanding of pathogenesis and mechanisms of diseases, as well as cell replacement therapies. Several groups have successfully derived a wide range of iPS cells from patients with neurological, immune, endocrine, skeletal muscle, hematological, and various genetic disorders thus far,³ among which only a few^{4,5} have been used for *in vitro* analysis of disease phenotypes.

Recently, a study by Moretti and colleagues⁶ have provided clear evidence to recapitulate the long-QT syndrome type 1 phenotype, *in vitro*. This disorder occurs by a mutation in the *KCNQ1* gene which encodes the repolarizing potassium channel mediating the delayed rectifier I_{Ks} current. Using an ectopic expression of Yamanaka transcription factors-*Oct4*, *Sox2*, *c-Myc*, and *Klf4*, they converted the dermal fibroblasts of a long-QT syndrome patient into iPS cells. In addition to displaying human ES cell-like characteristics, the produced iPS cells also kept the genotype of the disease inherited from their parental fibroblasts. Functional cardiomyocytes generated from these cells formed spontaneous contracting foci and cardiomyocyte markers' expressions were detected in individual differentiated cells. The differentiation efficiency of iPS cells into the cardiomyocyte lineage showed no major variations among clones derived from fibroblasts obtained from different subjects, and this made them reliable tools for further research. The informative *in vitro* cardiac and disease-related phenotypic analysis of differentiated myocytes, and the discovery of some of the pathogenic mechanisms of this syndrome are the strong points of this study.

For assessment of disease recapitulation, the authors performed action potential recordings and RT-PCR analysis in single cells. Their data demonstrated that differentiated cardiomyocytes showed ventricular, atrial, or nodal phenotype. In cells derived from the long-QT patient, only the action potential of nodal myocytes was similar to the control subject, whereas the action potentials of ventricular and atrial myocytes were significantly longer than the control. By having demonstrated the role of R190Q-KCNQ1, the mutation underlying the long-QT syndrome, and investigating the subcellular localization of KCNQ1, they have shown that these cells are defective in plasma membrane targeting of channel subunits in a domi-

nant negative manner, but not in their co-assembly. Moreover, this abnormality results in improper channel formation and consequently the action-potential rate adaptation and reduction of I_{Ks} currents by approximately 75% in defective cells. They have also shown that the defective cells are more susceptible to catecholamine-induced tachyarrhythmia than controls.

This strong iPS cell-based work by Laugwitz team⁶ is a very informative disease modeling study that has investigated most of the phenotypic characteristics of the disease at a single cell level. Considering there are ten types of this syndrome, by the help from this study and the generation of iPS cells from all types of the long-QT syndrome, it would be possible to shed light on the unknown areas of this syndrome as well as the study of heart arrhythmias from different dimensions. In the biological context of iPS cells, several points may be of interest: 1) based on recent studies^{7,8} iPS cells, particularly at lower passages, harbor a residual epigenetic memory of their original parental cell and this issue favors their differentiation toward the lineage from which they have arisen. This issue should be considered when choosing the best cell source for the generation of iPS cell models of the disease in question. 2) Regarding gene therapy trials for this disease,⁹ and possible gene correction of iPS cells,^{10,11} it would be desirable to analyze the phenotype of corrected long-QT iPS cells. 3) Finally, recent breakthroughs^{12,13} regarding the direct trans-differentiation of terminally differentiated cells into other cell types (e.g., fibroblasts into neurons or cardiomyocytes) by defined factors with higher efficiencies relative to iPS cells make researchers consider trans-differentiation as a possible new approach for modeling of diseases. Thus the question arises as to whether this approach could be better for disease modeling trials as these studies have investigated most of the characteristics of their produced functional cells.

Taken together, this study with its interesting approach in the phenotypic analysis of a disease in a dish has shown the great potential of iPS cells as a tool for diseases modeling, drug discovery, and the study of pathogenesis, disease mechanism(s), and possible cures for inherited disorders once the safety issue is overcome. This study has provided an informative model for a single gene disease, however, modeling multifactorial diseases still remains challenging.

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