

Genomic correction of familial cardiomyopathy in human engineered cardiac tissues

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In this study, we used three-dimensional human engineered cardiac tissue technology to directly show that phospholamban (PLN) R14del mutation impairs cardiac contractility and to demonstrate restoration of contractile properties with targeted genetic correction of this inheritable form of dilated cardiomyopathy.

Keywords

Dilated cardiomyopathy • Engineered heart tissue • Gene editing • Induced pluripotent stem cells • Phospholamban

Dilated cardiomyopathy (DCM) is a common cause of heart failure associated with ventricular dilation and deterioration of heart performance. Some forms of DCM are hereditary, and one of the genes found to be associated with inherited DCM is the one encoding phospholamban (PLN),^{1,2} a protein that regulates the sarcoplasmic reticulum Ca²⁺ ATPase (SERCA2a).³ Several PLN mutations, including a deletion of arginine 14 codon (R14del), have been linked to hereditary DCM; heterozygous patients exhibit ventricular dilation, contractile dysfunction, episodic ventricular arrhythmias, and heart failure.¹ To understand the molecular mechanism underlying the pathogenesis of R14del-induced DCM, we recently examined the functional characteristics of human cardiomyocytes (hCMs) obtained by directed differentiation of induced pluripotent stem cells (iPSCs) derived from a patient with cardiomyopathy associated with PLN R14del mutation.⁴ Compared with iPSC-CMs derived from a healthy patient expressing wild-type PLN, the R14del-CMs exhibited Ca²⁺ handling abnormalities, irregular electrical activity, abnormal intracellular distributions of PLN, and increased mRNA levels for molecular markers associated with cardiac hypertrophy. Moreover, targeted correction of the PLN mutation in R14del-iPSCs, performed with transcription activator-like effector nucleases (TALENs), mitigated the disease phenotypes in iPSC-CMs derived from these isogenic iPSC clones.⁴ However, due to limitations of

traditional cell culture methods, the effects of the PLN R14del mutation on cardiac muscle contractility were not previously tested. In this study, we used three-dimensional human engineered cardiac tissue (hECT) technology to show that PLN R14del mutation impaired cardiac contractility and to demonstrate that genetic correction restored contractile function.

Patient-specific iPSCs were corrected by gene editing using a TALEN method. As previously described,⁴ we developed a TALEN vector pair designed to introduce a double-strand break adjacent to the R14del mutation of the PLN gene and validated its efficiency by the surveyor assay (estimated modification efficiency 25.6%). Using low-density replating, puromycin selection, and PCR screening, we obtained homogeneous isogenic iPSC clones that differed only in the corrected R14del mutation compared with the parent clone. The corrected clones were expanded and the correction of the R14del mutation was confirmed by Sanger sequencing. Directed differentiation was then used to create human iPSC-CMs that recapitulate characteristics of healthy cardiomyocytes, DCM cardiomyocytes (PLN mutant), and TALEN-edited isogenic corrected cardiomyocytes⁴ (Figure 1A). After 15 days of differentiation, iPSC-CMs were harvested, suspended in culture medium, and mixed with collagen type-I and Matrigel solution. The cell-matrix suspension was transferred into polydimethylsiloxane (PDMS)

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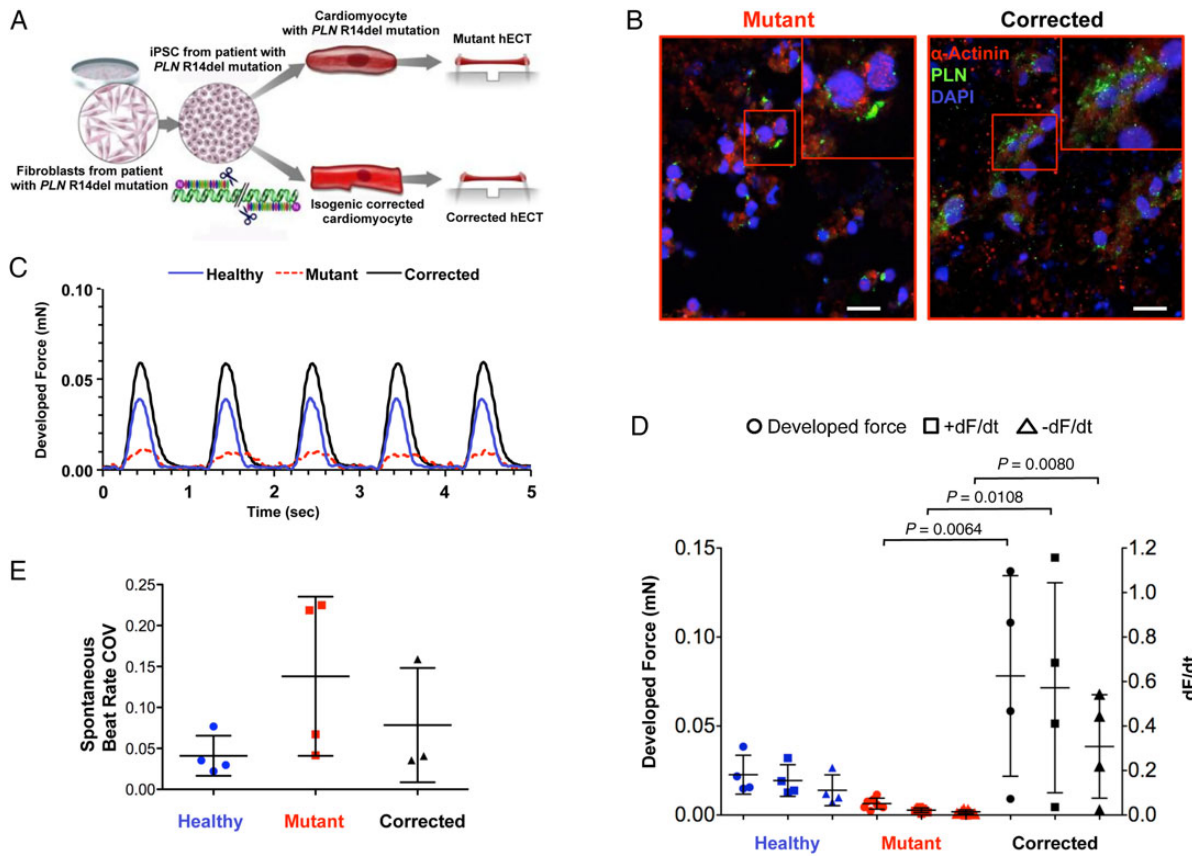


Figure 1 Effect of *phospholamban* R14del mutation on cardiac muscle contractility in three-dimensional human engineered cardiac tissues. (A) Schematic of the methods. (B) Representative immunofluorescence images of mutant and corrected human engineered cardiac tissues stained for α -actinin (red), phospholamban (green) and DAPI (blue), scale bar = 25 μ m. (C) Example twitch tracings from healthy (solid blue line), mutant (dashed red line), and corrected (solid black line) human engineered cardiac tissues during pacing at 1 Hz. (D) Twitch force parameters: developed force, +dF/dt, and -dF/dt (symbols indicate individual measurements, bars represent mean \pm SD) analysed by ANOVA with Scheffe's *post hoc* test (corrected for multiple pairwise comparisons); $n = 4$ healthy, $n = 7$ mutant, $n = 4$ corrected human engineered cardiac tissues. (E) Spontaneous beat rate variability expressed as coefficient of variation, recorded from: $n = 4$ healthy, $n = 4$ mutant, and $n = 3$ corrected human engineered cardiac tissues ($P = 0.209$ by ANOVA). COV, coefficient of variation; hECTs, human engineered cardiac tissues.

moulds, with integrated cantilever end-posts for non-invasive measurement of contractile function.⁵

After 1 week in culture, the hECTs from all three conditions beat spontaneously and resembled thin trabecular muscles. In mutant hECTs, the intracellular distribution of PLN within hCMs was polarized at one side of the cell, similar to observations in cardiac tissue sections from R14del patients,⁴ whereas corrected hECTs showed a more homogeneous distribution throughout the hCM cytoplasm (Figure 1B). These findings suggest that the 3D environment of hECTs preserves the disease-dependent distribution of PLN observed in native tissue samples and monolayer cultures of the corresponding cell types.⁴ All hECTs achieved 1:1 capture during electrical field stimulation at 1 Hz (Figure 1C), but the excitation threshold potential to initiate pacing was significantly lower in mutant hECTs (85 ± 59 mV/mm) compared with corrected (239 ± 100 mV/mm, $P = 0.011$), possibly suggesting an increased susceptibility to arrhythmogenesis in mutant hECTs. This was corroborated by a trend toward higher intrinsic variability of the

spontaneous beating rate in mutant vs. corrected (1.8-fold) and vs. healthy (3.4-fold) hECTs (Figure 1E), though the effect was not statistically significant by ANOVA ($P > 0.2$). In terms of contractile performance, the amplitude of the developed twitch force was dramatically lower in mutant compared with corrected hECTs ($P = 0.0064$) (Figure 1D). Similarly, the maximum rates of contraction and relaxation (+dF/dt and -dF/dt, respectively) were significantly slower in mutant vs. corrected hECTs ($P = 0.0108$ and $P = 0.0080$), a direct functional manifestation of the abnormal calcium handling observed in cultured R14del-CMs.⁴ Interestingly, the developed force, +dF/dt and -dF/dt values from corrected hECTs tended to exceed the corresponding values from healthy hECTs ($P = 0.055$, $P = 0.085$ and $P = 0.113$ respectively), partly reflecting the intrinsic genetic variability between individuals and/or differences in the epigenetic state among iPSC lines.⁶ This comparison illustrates the advantage of genome editing and tissue engineering technologies to study the effects of specific mutations within the context of a common genetic background. This study

demonstrates that the *PLN R14del* mutation interferes with intrinsic muscle contractility in patient-specific hECTs, and lays the groundwork for targeted gene therapy for treating patients with hereditary forms of cardiomyopathy.

Conflict of interest: none declared.

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CARDIOVASCULAR FLASHLIGHT

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Periaortic to right atrial shunt for severe aortic root bleeding

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A 33-year-old man previously underwent replacement of a stenotic bicuspid aortic valve with a pulmonary autograft (Ross procedure) as a teenager with subsequent development of severe neo-aortic valve regurgitation and ascending neo-aorta aneurysm. During performance of a modified Bentall procedure, adhesions of the aneurysmal ascending neo-aorta prevented safe dissection and re-resection of the neo-aortic root (Panel A). Following separation from cardiopulmonary bypass, severe posterior aortic root bleeding was present and unamenable to surgical repair. The unresected aneurysmal pulmonary autograft (prior neo-aorta) was sutured distally to the ascending composite valve graft (CVG), and a separate 8 mm Hemashield graft (*) was placed to shunt bleeding from the aortic perigraft space to the right atrial appendage (RAA) as seen in Panel B.

A perigraft to right atrial shunt (*) may be employed in situations where aortic root bleeding is inaccessible or unamenable to surgical repair due to poor tissue, as encountered in this case. Creation of this left-to-right shunt is a viable option to treat bleeding, as it serves to redirect peri-aortic bleeding to the right atrium, thereby mitigating the necessity of repeated transfusions for ongoing bleeding, prolonged unsuccessful surgical attempts at haemostasis, or extended cardiopulmonary bypass use. Coronal (Panel C) and 3D (Panel D) contrast-enhanced computerized tomography obtained 4 days post-operatively demonstrated a widely patent shunt between the pulmonary autograft-CVG junction and the right atrium. Perigraft to right atrial shunts typically close spontaneously days to weeks following implantation, as was the case with this patient. Operative closure is rarely required.

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