

EDITORIAL COMMENT

Can Patient Pluripotent Stem Cell-Derived Cardiomyocytes Provide Useful Modeling on Arrhythmias of DMD Cardiomyopathy?*



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Duchenne muscular dystrophy (DMD) is a rare, life-threatening genetic disorder that is the most common and severe form of muscular dystrophy in children (1). It is caused by mutations in the dystrophin gene, which encodes a protein that helps strengthen muscle fibers and protect them from injury (2). Currently, cardiomyopathy is the main cause of mortality in patients with DMD. Most patients with DMD develop cardiomyopathy features between the ages of 10 and 15 years. All patients age >18 years develop cardiomyopathy, which ultimately leads to advanced heart failure (2,3). Currently, the knowledge concerning DMD cardiomyopathy is limited. There are multiple causes of DMD cardiomyopathy, and clinical recognition is often challenging due to a long subclinical phase of ventricular dysfunction. Additionally, it is difficult to assess cardiovascular symptomatology in these patients, who usually lose ambulation during their early adolescence (3). As such, it is crucial to better understand the pathophysiology of DMD cardiomyopathy to improve the survival of patients with DMD.

In vitro modeling of DMD cardiomyopathy is important to advance our understanding of the disease and allow the development of new drug therapies. The challenge remains to grasp the complexity of the pathogenesis to design efficient models of

heart failure to aid us in current research. Other challenges arise from the ability to obtain the adequate human heart tissue samples needed to isolate primary cardiomyocytes (CMs) and work toward drug testing. In recent studies, single CMs derived from induced pluripotent stem cells (iPSCs) of humans (hiPSC-CMs) have been used to mimic models of human heart diseases. As the technology continues to evolve, hiPSC-CMs of patients with various inherited heart diseases have been developed to model certain medical processes, including familial hypertrophic cardiomyopathy (4,5), dilated cardiomyopathy (6,7), Barth syndrome (8), long QT syndrome (9), catecholaminergic polymorphic ventricular tachycardia (8), and arrhythmogenic right ventricular cardiomyopathy (10). Also, DMD, with major manifestations of cardiomyopathy, has been the subject of research using hiPSC-CMs. hiPSC-CMs could be extremely useful in testing developmental progression of the disease, calcium handling, mitochondrial and myocardial functions during differentiation, and CM maturation. Several studies have shown that hiPSC-CMs with diverse mutations of the dystrophin gene make it feasible either to reproduce the pathogenesis of DMD or to evaluate new therapeutics as an in vitro DMD model (11-13).

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In this issue of the *Journal*, Kamdar et al. (14) present 3 fundamental discoveries in their study of DMD patient-specific hiPSC-CMs: defining temporal protein expression profiles of dystrophin-glycoprotein complex components, replicating the phenotype observed in patients with DMD cardiomyopathy, and defining molecular mechanisms in

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both human and rodent DMD that overall contribute to dystrophic cardiomyopathy. To model and identify physiologic changes for future drug therapies in DMD cardiomyopathy, Kamdar et al. (14) used 3 different systems: hiPSC-CMs derived from patients with DMD; hiPSC-CMs with mutated *DMD* engineered by Cas9/clustered regularly interspaced short palindromic repeats; and *mdx*, *DMD* gene-null mouse. They also replicated certain clinical phenotypes, such as an increased incidence of arrhythmic calcium traces, in hiPSC-CMs derived from patients with DMD with no apparent cardiac defect at the time. Further investigations showed that the frequency of cardiac arrhythmia and arrhythmic death are significantly decreased with a propranolol treatment in all 3 models. These results provide a platform for future studies that will examine both the short- and long-term impacts of β -blocker agents in DMD and Becker muscular dystrophy cardiomyopathy. To further dissect the mechanisms underlying these alterations, the authors performed single-cell and bulk RNA-sequencing analyses to define pathways with perturbed sub-baseline conditions and observe if a propranolol treatment would normalize the conditions in *mdx* mouse models. Kamdar et al. found similar abnormal molecular and biological features in a comparison conducted among 3 models and human DMD left ventricular transcriptomes, which supports the feasibility of patient-derived hiPSC-CM models of DMD cardiomyopathy. The transcriptome analysis provided a clue to examining the benefits of β -blocker therapy in patients with dystrophinopathies.

However, several concerns still remain, including whether hiPSC-CMs would be functional in immaturity and fetal-like phenotypes (15). This immaturity of hiPSC-CMs may affect their physiological properties and molecular features. Kamdar et al. (14) describe the involvement of metabolic changes and cardiac remodeling in mouse hearts, with a normalization of the abnormal molecular program under β -blockers. However, these molecular events are not further explored in hiPSC-CMs (notably, calcium dysregulation as a mechanism of arrhythmias). Given the onset time for DMD cardiomyopathy (mostly in adolescence), a study on matured iPSC-CMs that most closely resemble adult CMs is needed. Additionally, more research is needed at the organ level, such as engineered heart tissue, for future clinical applications. Through advances of technology in transcriptome analysis, it is now easier to understand the molecular and cellular pathways in a single cell.

However, the changes in transcriptome alone are not enough to detail the adverse cardiac events seen in DMD hiPSC-CMs. There is growing evidence that diverse post-translational modifications (PTMs) can contribute to the functional regulation of key arrhythmia-related calcium-handling proteins such as SERCA2a (16-18), RyR2 (19-21), phospholamban (22,23), and NCX (24). This study has, in fact, demonstrated that there was no difference in SERCA2a, phospholamban, RyR2, or NCX gene expression levels between patients with DMD and control subjects in human DMD cardiac tissues or iPSC-CMs. A study of changes of post-translational modifications in DMD hiPSC-CMs in response to internal and environmental stimuli should be considered as factors in the goal of better understanding DMD cardiomyopathy. Patients with DMD have a high clinical heterogeneity due to the >1,000 different mutations in the dystrophin gene, giving rise to differential effects in inpatient clinical manifestations. As Kamdar et al. (14) describe in the study limitations, the analytical region and number of human tissue samples ($n = 2$) are limited. Furthermore, although DMD is an X-linked recessive disorder, female patients who inherit a single copy of the diseased gene for DMD can express classic symptoms of DMD cardiomyopathy. Further hiPSC-CMs studies on a large number of patients with DMD but also female patients would be informative in investigating a beneficial DMD disease treatment.

The patient-specific hiPSC-derived CMs were given as a possible new platform to model recapitulating complex human diseases. These results represent an important expansion of previous studies on inherited cardiac disorders, presenting structural, functional, and biochemical properties. This study indicates how the development of patient-specific hiPSCs can offer valuable insight into the modeling of inherited human diseases. Furthermore, beyond modeling, iPSC-CM has also been used as a platform to test genome-editing therapies. In summary, this patient-specific hiPSC strategy is quickly expanding in research fields such as modeling and is becoming a powerful tool for the treatment of human diseases, thereby warranting careful consideration in the field of precision medicine.

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