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Cardiomyopathy in a dish: Using human inducible pluripotent stem cells to model inherited cardiomyopathies

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Abstract

Inherited cardiomyopathies including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathies (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and other inherited forms of heart failure represent a unique set of genetically defined cardiovascular disease processes. Unraveling the molecular mechanisms of these deadly forms of human heart disease has been challenging, however recent groundbreaking scientific advances in stem cell technology has allowed for the generation of patient-specific human inducible stem cell (hiPSC)-derived cardiomyocytes (hiPSC-derived CMs). hiPSC-derived CMs retain the genetic blueprint of the patient, they can be maintained in culture, and they recapitulate the phenotypic characteristics of the disease *in vitro*, thus serving as a disease in a dish. This review provides an overview of *in vitro* modeling of inherited cardiomyopathies using patient-specific hiPSC-derived CMs.

Keywords

cardiomyopathy; stem cells; disease models

Introduction

Heart failure is common and deadly. While many factors contribute to heart failure progression, genetic causes are receiving intense interest. In recent years, research advances have enhanced our understanding of the genetic causes of cardiomyopathies. These genetic causes include inherited forms of hypertrophic cardiomyopathy (HCM), dilated

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cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), inherited restrictive cardiomyopathies (RCM), and several other genetic cardiomyopathies [1] While the genetic basis of several of these diseases has been identified, the definition of the molecular mechanisms, the response to therapeutic interventions and the development of novel therapies have been limited [2].

Our current understanding of molecular mechanisms and cellular pathophysiology of cardiomyopathies has been elucidated from genetic animal models, such as the mouse. These animal models, however, do not always accurately recapitulate the human cardiomyopathic phenotype, which may be due to the cardiovascular differences between the divergent species. Furthermore, the acquisition and isolation of human cardiomyocytes from patients, and especially those with cardiomyopathies, are invasive and yield a relatively small and non-renewable amount of tissue. Moreover, the adult human cardiomyocyte has a limited survival in standard cell culture conditions. Given the difficulties in securing diseased human cardiomyocytes and the inability of mouse models to fully recapitulate the human cardiomyopathic phenotype, there has been a clear need for human *in vitro* models of genetic cardiomyopathies that more closely recapitulates the human phenotype.

Significance of hiPSCs

Recent groundbreaking scientific advances have demonstrated the capacity of adult somatic cells to be reprogrammed using defined transcription factors into pluripotent stem cells, known as human induced pluripotent stem cells (hiPSCs) [3-5]. In 2006, the Yamanaka laboratory identified 24 transcription factors that were differentially enriched in mouse embryonic stem cells [6]. Analysis of these transcription factors revealed that the combination of Oct4, Sox2, Klf, and C-Myc when virally transduced into mouse fibroblasts, reprogrammed the fibroblasts into embryonic like stem cells with pluripotent potential. Hence, these cells were named induced pluripotent stem cells [6]. In 2007, this technology was translated to the development of human iPSCs independently by the Yamanaka and Thompson laboratories [4, 5]. The Yamanaka laboratory isolated human dermal fibroblasts and generated hiPSCs by delivering the reprogramming factors Oct4, Sox2, Klf4, and C-myc via a retroviral vector system [4]. The Thompson laboratory also used human dermal fibroblasts however these investigators used the reprogramming factors Oct4, Sox2, Nanog, and LIN28 and a lentiviral vector delivery system to generate hiPSCs [5].

Currently, human somatic tissue including dermal fibroblasts, blood, urine, and organ-specific tissues, such as cardiac biopsies, can be isolated to generate hiPSCs. The isolated human somatic tissue is cultured *in vitro* and the reprogramming factors are introduced into the cells. The initial hiPSCs were generated using integrating systems which included retroviral and lentiviral systems, where the transcription factors integrated into the host genome. Currently, non-integrating systems, including sendai virus, episomal reprogramming factors and microRNAs can also be used to generate hiPSCs without the integration of the reprogramming factors into the genome [7, 8]. After introduction of the reprogramming factors, the cells are cultured to allow for the expression of the pluripotency factors. In approximately 10-14 days, hiPSC colonies can be identified by their

characteristic compact round features with cells having large nucleoli, which appear comparable to human embryonic stem cells (Figure 1). These hiPSC colonies are selected and further propagated. Subsequently, the hiPSC colonies are characterized by their expression of pluripotency markers including Oct4, SSEA-4, and alkaline phosphatase. The hiPSC colonies can also be introduced into a murine host and give rise to teratomas with all 3 germ layers demonstrating the pluripotency of the hiPSCs. hiPSC technology provides a number of advantages in biomedical research. First, hiPSCs are generated from somatic human tissues and avoids the ethical concerns of human embryonic stem cell research. Second, hiPSCs are self-renewing and provide an unlimited source of human cells. Importantly, the hiPSCs and their derivatives retain the genetic blueprint of the patient and can be differentiated into a variety of cell types. This innovative technology allows for an unprecedented opportunity to study human diseases *in vitro* and to develop and test novel therapies.

Differentiation of CMs from hiPSCs

The ability to generate high purity, functional cardiomyocytes is a transformative step in the development of cardiac disease models. The current understanding of embryonic cardiac development and differentiation of embryonic stem cells has been applied to the development of several differentiation protocols that reliably direct hiPSCs to the cardiac lineage [9-12]. These differentiation protocols include the temporal modulation of the BMP, TGF- β /activin/nodal, WNT/ β -catenin, and FGF pathways to specify hiPSCs to a cardiac fate in a multistep process involving epithelial to mesenchymal transition, mesodermal and cardiac specification resulting in functional cardiomyocyte-like cells.

Maturity of hiPSC-derived CMs

While cardiomyocytes can be derived from hiPSCs, the gene expression profiling and physiological characterization indicate that they are immature. Compared to adult human cardiomyocytes the hiPSC-derived cardiomyocytes are smaller, circular rather than rod-like and have a less organized sarcomeric structure resulting in decreased force generation [13-15]. hiPSC-derived CMs develop more organized cardiac sarcomeric structure during differentiation, but have a morphology that is more comparable to fetal cardiomyocytes than rod shaped adult cardiomyocytes (Figure 1). Additionally, hiPSC-derived CMs express genes such as smooth muscle actin, connexin 45, and MLC2a, which are expressed in immature cardiomyocytes. Similar to immature or fetal cardiomyocytes, hiPSC-derived CMs do not have well-developed T-tubules, rely primarily on glucose for metabolism and have less mature calcium handling [16, 17]. Strategies to generate more mature hiPSC-derived CMs are a research focus and will be essential for future advancement of the field.

hiPSC Cardiac models

Efficiently differentiating hiPSCs to the cardiomyocyte lineage is a major feat for the field and has for the first time allowed for a renewable source of human cardiomyocytes that can be maintained *in vitro*. Despite the concerns regarding the maturity of hiPSC-derived CMs, this technology has numerous applications including: drug development, drug toxicity analysis, and as a source for regenerative medicine. One of the most powerful uses for

hiPSC-derived CMs is to interrogate the molecular and cellular mechanisms of human cardiac diseases in a dish and the ability to test novel therapies (Figure 2). While there are many advantages associated with hiPSC-derived CMs, there have been concerns regarding the ability to fully model adult human cardiovascular diseases. Nevertheless, recent studies support the notion that hiPSC-derived CMs are important cellular models for cardiac diseases. The initial hiPSC-based cardiovascular models were recently published and focused on inherited arrhythmias such as long QT Syndrome, Timothy Syndrome, and LEOPARD Syndrome [18-20]. These studies provided a proof-of-principle for these technologies and emphasized that genetic mutations elicited a clear phenotype, recapitulated the disease, and allowed for the testing of pharmacologic therapies. Subsequently, numerous studies on Long QT and catecholaminergic polymorphic ventricular tachycardia (CPVT) have been published [21-35]. Modeling inherited channelopathies using hiPSC-derived CMs have been successful given that the phenotype is more easily elicited by interrogating the channel of interest through electrophysiological studies.

hiPSC models have been utilized to examine inherited cardiomyopathies with complex phenotypes and varied phenotypes including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), Freiderich's Ataxia, Barth Syndrome, and Pompe Disease. This review highlights the impact of hiPSC-derived CM technologies and our understanding of inherited cardiomyopathies.

hiPSC Models of Inherited Cardiomyopathy (Table 1)

Dilated Cardiomyopathy

Non-ischemic dilated cardiomyopathy is characterized by dilatation of the left ventricle with associated reduced systolic function in the absence of ischemic heart disease. Inherited DCM accounts for over one third of the cases of nonischemic dilated cardiomyopathies [36]. Inherited DCM has an autosomal dominant inheritance pattern and currently over 40 genes have been identified and shown to contribute to familial DCM. These familial cardiomyopathies largely involve sarcomeric and cytoskeletal protein encoding genes [37] (Figure 3).

Sun et al. derived hiPSCs from a family cohort with dilated cardiomyopathy due to a point mutation in the gene encoding cardiac troponin T (TNNT2 R173W) [38]. Compared to hiPSC-derived CMs obtained from unaffected patients in the same family, the DCM hiPSC-derived CMs demonstrated an abnormal distribution of sarcomeric α -actinin, altered regulation of calcium transients, and decreased contractility as measured by atomic force microscopy. When DCM hiPSC-derived CMs were exposed to norepinephrine, an α and β -adrenergic agonist, the phenotype was exacerbated and the DCM hiPSC-derived CMs had cessation of beating, less organized sarcomeric α -actinin, and decreased contractility. Treating the DCM hiPSC-derived CMs with metoprolol, a β 1-adrenergic blocker or overexpressing the ATPase SERCA2a, which is downregulated in DCM, rescued the phenotype.

Mutations in genes encoding nuclear membrane proteins, such as LMNA which encodes for lamin A/C have also been identified to cause DCM and conduction abnormalities [39]. Siu

et al. derived hiPSCs from a patient with a mutation in lamin A/C (LMNA R225X) [40]. Lamin A/C mutant hiPSC-derived CMs demonstrated increased nuclear bleb formation and micronucleation (identified by immunofluorescence) and nuclear pore dilation (observed using electron microscopy). When LMNA hiPSC-derived CMs were exposed to electrical stimulation (pacing), they demonstrated increased apoptosis and nuclear senescence. The phenotype was also replicated when LMNA was knocked down in control hiPSC-derived CMs using a shRNA strategy. Activation of MEK1, a component of the mitogen activated protein kinase signaling pathway, was found to be a central mediator of the phenotypic response and inhibitors of MEK1 were protective against apoptosis during electrical stimulation.

hiPSC disease modeling was also utilized in a patient with inherited DCM having an unknown genetic etiology by Tse et al. [41]. The patient underwent whole exome sequence analysis to identify a novel mutation in desmin (DES A285V). These investigators utilized hiPSC modeling to confirm that the mutation was responsible for the phenotype. The DCM hiPSC-derived CMs demonstrated decreased desmin co-localization with sarcomeric proteins, abnormal aggregates of desmin, and disorganized sarcomeric structure. DCM hiPSC-derived CMs also demonstrated decreased calcium uptake and an inability to respond appropriately to isoproterenol. These phenotypic changes were also observed when the mutation was introduced into control hiPSCs and subsequently differentiated to the cardiac lineage.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is an inherited cardiomyopathy, which is characterized by severe hypertrophy of the left ventricle leading to left ventricular outflow obstruction, heart failure, ventricular arrhythmias and sudden cardiac death [42]. HCM is inherited in an autosomal dominant fashion with variable and age-related penetrance. HCM is a result of mutations in genes encoding sarcomeric proteins and the most common mutations account for over 70% of mutations include those in β -myosin heavy chain (MYH7) and cardiac myosin binding protein C (MYBPC3) [43] (Figure 3). While the sarcomeric genes have been identified as the cause of HCM, the molecular and cellular pathways that result in myocyte hypertrophy, heart failure, and arrhythmias have not been well defined. Lan and colleagues derived hiPSCs from a 10 member family, in which several members had the HCM due to an autosomal dominant missense mutation in the MYH7 gene (R663H), which encodes β -myosin heavy chain [44]. hiPSC-derived CMs from patients with HCM at 40 days of differentiation demonstrated cellular hypertrophy and disorganized sarcomeres. Additionally, genes associated with cardiac hypertrophy were upregulated in HCM hiPSC-derived CMs, including NFAT and calcineurin, and by blocking the pathway with calcineurin inhibitors cellular hypertrophy was reduced. Patch clamp electrophysiological studies of HCM hiPSC CM demonstrated frequent delayed after depolarizations (DAD) associated with arrhythmogenic waveforms and irregular calcium transients. The phenotype was worsened with inotropic stress and was partially rescued with beta-blocker treatment. Calcium imaging of HCM hiPSC-derived CMs at 30 days after differentiation demonstrated elevated intracellular calcium stores. Since calcium handling abnormalities appeared to precede the hypertrophic phenotype, it was hypothesized that calcium handling may be

central to the phenotype. To test this hypothesis, HCM hiPSC-derived CMs were treated with the calcium channel blockers verapamil, nifedipine, and diltiazem; and the results demonstrated prevention of myocyte hypertrophy, arrhythmias, and calcium handling abnormalities. This study demonstrated that aberrant calcium handling is a central mechanism in HCM. While calcium blockers have not been effective in treating patients with overt HCM the benefits of prophylactic treatment requires more investigation.

Arrhythmogenic right ventricular cardiomyopathy (ARVC)

ARVC is largely an inherited form of cardiomyopathy which is characterized by the replacement of right ventricular myocardium with fibro-fatty tissues. Clinically, patients develop severe right ventricular failure, arrhythmias originating from the right ventricle, and sudden cardiac death [45]. ARVC is typically inherited in an autosomal dominant pattern with variable expression and results from a mutation in genes which encode components of the desmosomes, such as plakoglobin, desmoplakin, plakophilin-2, desmoglein-2, and desmocollin-2 (Figure 3). Mouse knockout models of various ARVC genes have been generated, but have not fully recapitulated the human cardiomyopathy [46, 47]. Thus far three studies have utilized hiPSC for modeling ARVC and have focused on the mutations in the gene PKP2, which encodes the plakophilin-2 protein [48-50].

Kim et al. generated hiPSC-derived CMs from 2 patients with a homozygous (c.2484C > T) or a heterozygous (c.2013delC) mutation in the PKP2 gene [49]. These investigators demonstrated abnormal nuclear translocation of plakoglobin and low β -catenin activity in ARVC hiPSC-derived CMs, however the phenotype was not fully recapitulated in this standard cell culture conditions. Exposure to an adipogenic culture medium resulted in increased lipogenesis and apoptosis. In the adipogenically stimulated ARVC hiPSC CM, PPAR- γ , which is a regulator of fatty acid metabolism, was abnormally activated. The phenotype was rescued with inhibitors of PPAR- γ during adipogenic stimulation, thus supporting the hypothesis that lipid metabolism and energetics have a central role in the pathophysiology of ARVC.

Ma et al. generated hiPSC-derived CMs from a patient with a heterozygous mutation in PKP2 (c.1841T>C) [50]. Similarly, the ARVC hiPSC-derived CMs demonstrated reduced plakoglobin using immunofluorescence, but normal desmoplakin, N-Cadherin, and connexin 43 staining in comparison to control hiPSC-derived CMs. Electron microscopy demonstrated increased lipid droplets in ARVC undifferentiated hiPSCs. After 2 weeks of adipogenic stimulation ARVC hiPSC-derived CMs demonstrated significantly more lipid accumulation compared to control CM, thus recapitulating the ARVC phenotype.

Similar findings were noted in a study by Caspi et al. where hiPSC-derived CMs were derived from 2 patients with ARVC due to a heterozygous PKP2 mutation (A324fs335X) [48]. The investigators verified that ARVC hiPSC-derived CMs had reduced expression of PKP2 using quantitative polymerase chain reaction and reduced plakoglobin and connexin-43 by immunostaining techniques. Electrophysiological assessment demonstrated a prolonged field potential rise time in ARVC hiPSC-derived CMs, which may be associated with reduced gap junctions and also seen in PKP2 animals. Additionally, they demonstrated that ARVC hiPSC-derived CMs had higher lipid accumulation in CMs that had more severe

desmosomal distortion, suggesting a link between desmosomal malfunction and lipid accumulation. Similar to Kim et al., Caspi et al. also identified abnormal PPAR- γ activation in ARVC hiPSC CM and worsening of the phenotype with adipogenic stimulation. The ARVC hiPSC-derived CMs phenotype during adipogenic stimulation was prevented by application of the glycogen synthase kinase 3b inhibitor (6-bromoindirubin-3'-oxime, BIO), which may suggest a role for the canonical Wnt signaling pathway in ARVC pathogenesis. All of these studies partially recapitulated the ARVC phenotype, provided mechanistic insights into the pathogenesis of ARVC as well as potential treatment strategies.

Friedreich's ataxia

Friederich's ataxia is an autosomal recessive disorder characterized by neurodegeneration leading to severe ataxia, dysarthria and cardiac dysfunction. These cardiac abnormalities include the development of cardiomyopathy and arrhythmias; and over 50% of patients have a cardiac cause of death [51]. Friederich's ataxia is caused by an intronic GAA triplet repeat in the FXN gene leading to reduced expression of frataxin. Frataxin is a mitochondrial protein involved in iron-sulfur cluster biosynthesis (figure 3). Mouse and cell models of frataxin deficiency have not fully recapitulated the human disease, thus limiting the development of emerging therapies [52]. Hick et al. generated hiPSCs from two patients with Friederich's ataxia and then differentiated the hiPSCs to neurons and cardiomyocytes [53]. The Friederich's ataxia hiPSC-derived neurons and CMs demonstrated a decrease in frataxin expression and impaired mitochondrial function, but no other biochemical differences compared to control hiPSCs. The investigators assessed for arrhythmias using imaging technologies of beating CMs which did not reveal any differences in Friederich's ataxia hiPSC-derived CMs however electrophysiological studies were not performed.

Barth Syndrome

Barth syndrome is an x-linked mitochondrial myopathy characterized by dilated cardiomyopathy, skeletal myopathy, growth retardation and neutropenia [54]. Barth syndrome is caused by a mutation in TAZ1, a gene encoding tafazzin, an acyltransferase that is critical in the remodeling of the mitochondrial phospholipid cardiolipin (figure 3) [55]. A tafazzin knock down mouse was generated but has not demonstrated the significant cardiac phenotype observed in patients with Barth syndrome [56]. Dudek et al. derived hiPSCs from fibroblasts from 3 unrelated patients with Barth Syndrome [57]. The undifferentiated patient-derived hiPSCs demonstrated decreased remodeling of cardiolipin, decreased mitochondrial oxygen consumption, structural changes in the respiratory chain supercomplex, increased reactive oxygen species generation and recapitulated the biochemical features of Barth syndrome. Importantly, this study established hiPSCs as a model for Barth Syndrome. Subsequently, cardiomyopathy associated with Barth syndrome was also modeled by Wang et al. using hiPSC-derived CMs generated from 2 patients with Barth syndrome [58]. The mitochondrial function of Barth syndrome patients were investigated and in comparison to controls demonstrated high basal oxygen consumption, depleted ATP, and the unique "heart-on-a-chip" platform demonstrating irregular sarcomeric structure, decreased contractile force development, and increased reactive oxygen species generation in comparison to control hiPSC-derived CMs. These phenotypic findings were confirmed to be secondary to the TAZ1 mutation through genome editing

utilizing CRISPR/Cas9. Reintroduction of wild type TAZ in Barth hiPSCs resulted in normalization of mitochondrial function in the genetically engineered Barth hiPSC-derived CMs and the introduction of TAZ1 mutation in control hiPSC resulted in mitochondrial dysfunction in the control hiPSC-derived CMs. Small molecule interventions were also undertaken and linoleic acid, a precursor of cardiolipin demonstrated attenuation of mitochondrial dysfunction and improved contractility in Barth hiPSC-derived CMs [58].

Pompe Disease

Pompe disease is an autosomal recessive glycogen storage disorder that is caused by a mutation in the gene encoding alpha-glucosidase (GAA), a lysosomal glycogen-degrading enzyme (figure 3) [59]. The absence of alpha-glycosidase leads to abnormal glycogen accumulation in skeletal, cardiac, and respiratory muscles. The disease is classified based on the onset of symptoms such as infantile-onset or late-onset. The infantile-onset disease is characterized by hypotonia and cardiomyopathy before 6 months of age, and if untreated results in death by the age of 18 months [60]. GAA knockdown mice have been generated however with much milder symptoms and a normal lifespan in comparison to patients with Pompe disease [61]. Huang et al. derived hiPSCs from two patients with Pompe disease, introduced a doxycycline-inducible GAA into the hiPSCs, and differentiated them into CMs [62]. Pompe hiPSC-derived CMs demonstrated decreased GAA activity, high glycogen content, altered respiration, and mitochondrial dysfunction. The phenotype of the Pompe hiPSC-derived CMs was rescued with human recombinant GAA and partially rescued with L-carnitine. The investigators identified 16 genes involved in glycogen metabolism, lysosomal, and mitochondrial function that were differentially expressed in whole transcriptome analysis of Pompe hiPSC-derived CMs and in Pompe hiPSC-derived CMs with induced GAA. These candidate genes have the potential to be used for *in vitro* gene testing.

Clinical Applications

hiPSC technologies provide a major breakthrough in heart failure basic and translational research through the generation of disease models of cardiomyopathy that define molecular and cellular pathways for disease pathogenesis. These models provide a platform for drug discovery to generate novel therapies for the inherited cardiomyopathies, however hiPSC-derived cardiomyocytes also have clinical applications that extend more broadly to cardiovascular disease and heart failure. One significant application includes pharmacologic cardiotoxicity screening. Currently, pharmaceutical drugs must undergo assays to screen for QT prolonging effects where the drug is applied to a non-human non-cardiac cell over expressing a human hERG channel. However, from 1990 to 2006 over a third of medications on the market were withdrawn due to cardiotoxicity, which highlights the limitations of the current cardiotoxicity testing assays [63]. hiPSC-derived CMs are a human-based cardiac model that can be harnessed for cardiotoxicity screening. Navarette et al., demonstrated that control hiPSC-derived CMs treated with drugs known to prolong QT more accurately predicted the clinical drug response than standard drug testing [64]. However, the majority of drug-induced cardiotoxicity occurs in patients with heart failure or structural heart disease who are at an increased risk of fatal arrhythmias. Thus Liang et al., examined hiPSC-derived CMs from patients with familial DCM, HCM, long QT syndrome

and identified that HCM and long QT syndrome derived hiPSC CMs developed QT prolongation with lower doses of cisapride, a known QT prolonging agent, than healthy control hiPSC-derived CMs [65]. Potentially, panels of hiPSC-derived CMs with various cardiomyopathic phenotypes could be used to predict untoward cardiac effects of established and novel therapies prior to expensive clinical testing. Additionally, as hiPSC technology becomes more cost-effective it may be possible to use the technology for personalized medicine, by deriving hiPSC CMs from patients who are at high risk for cardiotoxicity and screening drug therapies on their own cardiomyocytes.

Conclusions

Disease modeling using hiPSC-derived CMs is a unique and powerful technology to model inherited cardiomyopathies and provide a human cell based platform to study disease mechanisms and develop novel therapies. Thus far, hiPSC-derived CMs have been used to model a number of inherited cardiomyopathies including: DCM, HCM, ARVC, Frederick's ataxia, Barth syndrome, and Pompe disease (Table 1). These studies have established hiPSC disease modeling for inherited cardiomyopathies and have provided a unique molecular insight into disease mechanisms and potential treatment strategies. While there are limitations of hiPSC-derived CMs including developmental immaturity in comparison to an adult cardiomyocyte, hiPSC-derived CMs represent the only current renewable human cardiac tissue source and platform to elucidate disease pathogenesis. The field is rapidly advancing and promising research to promote mature hiPSC-derived CMs for the development of functional multi-cell type organ models is actively being conducted, which will impact research focused on heart failure.

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Highlights

- Human inducible pluripotent stem cells (hiPSC) are from a somatic source and retain the patient genotype
- hiPSC-derived cardiomyocytes serve as a disease model in a dish
- Inherited cardiomyopathies including dilated cardiomyopathy, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular dysplasia have been modeled
- hiPSC-derived CM models of inherited cardiomyopathies have allowed for understanding disease mechanism and testing therapies

Figure 1. Cardiac differentiation of human inducible pluripotent stem cells

A) Phase contrast image of an undifferentiated hiPSC colony; B) hiPSC cardiac progenitor at day 15 of differentiation immunostained for cardiac troponin T (green) and DAPI (blue); C) hiPSC-derived cardiomyocyte at d30 of differentiation immunostained for cardiac troponin T (green) and DAPI (blue) demonstrating organized cardiac sarcomeric striations; D) hiPSC-derived cardiomyocyte at d90 of differentiation immunostained for cardiac troponin T (green) and DAPI (blue) demonstrating robust and organized sarcomeric striations; E) Neonatal murine cardiomyocytes expressing the sarcomeric protein MYH6 tagged with mCherry (red) and co-stained with α -actinin (green) and DAPI (blue); F) Mature murine cardiomyocytes with a classic rod-shaped morphology expressing the sarcomeric protein MYH6 tagged with mCherry (red) and costained with α -actinin (green) and DAPI (blue).

Figure 2. Modeling cardiomyopathies using disease-specific human induced pluripotent stem cells

Adult human somatic tissue from patients with inherited cardiomyopathies due to genetic mutations are obtained to generate patient specific hiPSC lines. hiPSCs are differentiated into cardiomyocytes using directed differentiation protocols. These hiPSC-derived CMs retain the patient's specific genetic mutation and serve as an *in vitro* disease model in a dish. The hiPSC-derived CMs can be used to interrogate the molecular and cellular mechanisms of disease and test novel therapies, which eventually can be used to treat patients.

Figure 3. Cellular localization of proteins encoded by genes associated with inherited cardiomyopathies that have been modeled using human inducible pluripotent stem cell derived cardiomyocyte models

Sarcomeric proteins such as β -Myosin heavy chain are implicated in HCM, while mutations in cardiac troponin T have been associated with DCM. Mutations in desmosomal proteins such as plakophilin 2 have been associated largely with ARVC. Mutations in lysosomal enzymes, mitochondrial proteins, and nuclear membrane proteins are also associated with specific forms of inherited cardiomyopathies.

Table 1

Published hiPSC models of inherited cardiomyopathies

Cardiomyopathy	Mutated Gene	Mutations studies	Phenotypic analysis methods	Phenotype in hiPSC CM	Rescue	Reference
DCM	TNNT2	Heterozygous missense mutation, R173W	IF, EM, Ca ²⁺ imaging, EP, atomic force microscopy	↑ Myofibril disarray, ↓ contraction, calcium handling changes, phenotype worse with catecholamine exposure	Metoprolol, Serca2A overexpression	Sun 2012
DCM	LMNA	Heterozygous nonsense mutation, R225X	IF, electrical stimulation, EM	↑ nuclear senescence, ↑ apoptosis with electrical stimulation, ↑ MEK1 activation	MEK1 inhibitor	Siu 2012
DCM	DES	Heterozygous mutation, A285V	IF, EM, Ca ²⁺ imaging	↑ abnormal DES aggregations, ↓ sarcomere organization, ↓ calcium handling	None	Tse 2013
HCM	MYH7	Heterozygous missense mutation, R663H	IF, EM, Ca ²⁺ imaging, EP	↑ Sarcomere hypertrophy, ↑ disorganized sarcomeres, ↑ Ca ²⁺ handling abnormalities	Propranolol Calcium channel blockers Calcineurin blockers	Lan 2013
ARVC	PKP2	Homozygous silent mutation leading to cryptic splicing, c.2484C > T	IF, mitochondrial studies, Ca ²⁺ imaging	↑ excessive lipogenesis and apoptosis with PPAR-γ activation	PPAR-γ antagonist GW9662	Kim 2013
ARVC	PKP2	Heterozygous missense mutation, c.1841T>C	IF, EM, EP	↓ Plakoglobin staining, ↑ accumulation of larger lipid droplets upon adipogenic stimulation	None	Ma 2013
ARVC	PKP2	Heterozygous frameshift mutation A324fs335X	IF, EM, EP	↑ lipid accumulation, ↑ apoptosis, ↑ desmosome distortion, ↑ field potential rise time,	GSK-3β inhibitor (BIO)	Caspi 2013
Frederich's Ataxia	FNX	GAA repeats 900, 800	IF, EM	↑ Abnormal mitochondrial structure, ↑ sarcomere disarray	none	Hick 2011

Cardiomyopathy	Mutated Gene	Mutations studies	Phenotypic analysis methods	Phenotype in hiPSC CM	Rescue	Reference
Barth Syndrome	TAZ	Frameshift c.517delG, missense c.328T>C	mitochondrial assays, IF, EM, lipid mass spectroscopy, heart-on-a-chip, genome editing	↑ Sarcomere disarray, ↓ contractility, ↓ mitochondrial function, ↑ ROS	Linoleic acid, Gene rescue	Wang G, 2014
Pompe Disease	GAA	Specific mutation unpublished	IF, EM, mitochondrial studies	↑ Glycogen content, ↓ mitochondrial function	Recombinant human α-glucosidase, L-carnitine, 3-MA	Huang 2011

Abbreviations: Immunofluorescence imaging IF, electron microscopy EM, electrophysiology EP, Multielectrode array MEA