Generate Better Hypertrophic Cardiomyopathy Model Using Induced Pluripotent Stem Cells and Crispr/Cas9

Zhe Han

La Sierra Academy, 4900 Golden Ave, Riverside, CA 92505, USA

ABSTRACT

Hypertrophic cardiomyopathy (HCM) has been a heart disease causing the most sudden cardiac death among young people in US, and this problem still remains unsolved. In this paper, we will reintroduce HCM and discuss the methods that could be used to treat it. Many other studies, involving animal models; Induced pluripotent stem cells (iPSCs); CRISPR/Cas9, would also be mentioned to understand the disease better. Many techniques are useful in curing HCM. One of the helpful mechanisms would be iPSCs, which helps reprogramming a patient’s somatic cells, differentiating them into cells needed for the study. Due to different background issues, CRISPR/Cas 9 would then be added to generate isogenic iPSCs. Combining both mechanisms would help in comparing patient’s HCM iPSCs and control iPSCs, which provides us more information about pathogenic background of HCM and further assists in the process of curing HCM. (Int J Biomed Sci 2019; 15 (3): 79-83)

Keywords: HCM; CRISPR/Cas9; iPSCs; heart disease and isogenic

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a cardiac disorder characterized by left ventricular hypertrophy which predominantly occurs in interventricular septum (1). HCM is the most common genetic heart defect with prevalence of 1:500 in the general population, and its annual mortality rate is 1-5% (2). Over 400 autosomal mutations have been identified in genes encoding sarcomeric or sarcomere-related proteins in HCM patients (2). Despite much efforts in HCM research, studying the molecular mechanism underlying HCM has been hampered by a number of factors including difficulties to obtain human cardiac samples and propagate human heart tissue in cell culture systems. Although several murine HCM models have been generated and become a valuable tool in the disease study (3-5), they may not truly recapitulate human HCM as mouse heart is mainly constituted of α-myosin whereas the human heart mainly consists of β-myosin (6).

Induced pluripotent stem cells (iPSCs) reprogrammed from patient somatic cells have widely been used for disease modeling (7). In those studies, phenotypic differences observed in the iPSCs-derived cells of the patients and healthy individuals were usually claimed to be relevant to the disease pathophysiology. However, this traditional approach overlooks the impact of genetic background between healthy control and patients, which may result in misleading outcomes (8). Recently, isogenic iPSCs gen-
erated by CRISPR/CAS9 can eliminate the genetic back-
ground difference associated with the traditional IPSCs-
ated disease modeling, offering more reliable research
outcomes (9, 10). In this article, I review the current
methodologies in HCM studies and discuss the potential
of isogenic iPSCs-based HCM disease modeling by using
CRISPR/CAS9.

Genetic Causes of HCM
HCM was the first inherited cardiovascular disorder in
which a genetic basis was identified. To date, over 400 au-
tosomal mutations have been identified in at least 13 genes
encoding sarcomeric or sarcomere-related proteins in
HCM patients (Table 1). Of these genes, β-myosin heavy
chain (β-MHC) and myosin-binding protein C (MyBP-C)
are the two most common sarcomeric proteins that harbor
approximately 70% of all mutations in HCM.

Recently, multiple mutations have been identified in ap-
approximately 5% of HCM patients (2). In contrast to HCM
patients with a single heterozygous causative mutation,
HCM patients with the multiple mutations are clinically
more severe including earlier age of disease onset, more
severe left ventricular hypertrophy, and more frequent
and rapid progression to significant HCM complications
of HCM (2). The severity of the phenotype is believed to
directly correlate with the inherent protein dysfunction
caused by the accumulation of multiple genetic mutations.

The identification of multiple mutations in individual pa-
tients drastically changes genetic diagnosis, counseling,
and treatment. Rather than single-gene testing, whole pan-
els of genes should be tested in new families presenting
with disease. Particularly, with multiple mutations, the
likelihood of passing down the disease is even greater.

Methodologies in HCM studies
Over the past years, large achievements have been
made in the management of HCM disease. However, de-
finite treatment to HCM is still lacking, and the mo-
lecular mechanism of HCM is poorly understood. Animal
models and human iPS cells-based disease modeling have
been widely used to study HCM molecular mechanism.

Animal Models
Genetically engineered animals have been very useful
to study human HCM. Transgenic mice and rabbits that
over-express mutant forms of myosin heavy chains exhib-
ted the histopathological features seen in patients with
enlarged left ventricles (11, 12). Similar studies of cardiac
troponin-T and MyBP-C have been studied (4, 13, 14). As
MyBP-C is one of main sarcomeric proteins implicated in
HCM. Yang et al generated a mouse line that expresses a
murine cardiac isoform of MyBP-C lacking both the ti-
tin and myosin binding sequences in attempt to mimic a
class of MyBP-C HCM mutations (4). It was shown that
the transgenically encoded, truncated MyBP-C protein is
stable but not inserted efficiently into the sarcomere, re-
sulting in a leftward shift in the pCa²⁺ force curve and a
reduction of the power output (4). The researchers further
noted that expression of the mutant MyBP-C protein leads
to decreased levels of endogenous MyBP-C, resulting in
a striking pattern of sarcomere disorganization and dys-
genesis (4). Additionally, one of the most extensively stud-
ied mouse model of HCM was generated by introducing
an Arg403Gln, a well characterized mutation in human
HCM, into the α-cardiac myosin heavy chain. The
heterozygous mouse genetically recapitulates the human
HCM phenotype, and analysis showed features similar to
the human disease (15).

In summary, the animal models of human HCM have
demonstrated that a sarcomeric gene mutation is indeed
the primary cause of HCM, and levels of mutant sarco-
meric protein expressed within the heart show some corre-
lation with the severity of myocyte dysfunction. Although
murine HCM models have become a valuable tool in the
HCM disease study (3-5), they may not faithfully reca-
pitulate human HCM. Indeed, several HCM studies have

| Table 1. Identified genes that are causative to human HCM |
|-----------------|-----------------|-----------------|
| Gene            | Symbol          | Frequency       |
| β-Myosin heavy chain | MYH7            | ~30%            |
| Myosin binding protein-C | MYBPC3         | ~20%            |
| Cardiac troponin T     | TNNT2           | ~20%            |
| α-tropomyosin          | TPM1            | ~5%             |
| Cardiac troponin I     | TNN13           | ~5%             |
| Myosin light chain, essential | MYL3    | <5%             |
| Myosin light chain regulatory | MYL2   | <5%             |
| Cardiac α-actin         | ACTC            | <5%             |
| K voltage gated channel | KCNQ4          | Rare            |
| Titin               | TTN             | <5%             |
| Protein Kinase A, β subunit | PRKAR2B | ?* |
| α-Myosin heavy chain | MYH6            | Rare            |
| Mitochondrial DNA*     | MTTI            | Rare            |

*"?" indicates that the frequency of such gene is still unknown.
yielded conflicting results (16-20). For instance, it was suggested that mutant sarcomeric proteins impaired cardiac myocyte contractility, providing an impetus for compensatory hypertrophy (16, 20). In contrast, other studies found that various causative mutations produced inconsistent cardiac myocyte contractility, with some mutations reducing contractility and some mutations enhancing contractility (21, 22). In addition, some other molecular mechanisms have been proposed including perturbations in calcium cycling and sensitivity (23). Therefore, better approaches to elucidate the HCM molecular mechanisms are highly sought after.

Traditional iPSC-HCM Studies
The recent development of iPSC reprogrammed from patient skin or blood cells, which are then differentiated into cardiomyocytes, can help our understanding of HCM disease and develop treatment strategies (23-29) (Figure 1). To dates, several iPSC-based HCM studies have been reported. In most of these reports, iPSC-derived cardiomyocytes are larger in size than those derived from healthy human iPSCs. For instance, Lan et al showed cellular enlargement in patient-specific iPSC-cardiomyocytes from a ten-member family cohort carrying a hereditary HCM missense mutation (Arg663His) in the MYH7 gene (23). In addition, Ojala et al studied two patients with HCM caused by Gln1061X mutation in myosin-binding protein C (MYBPC3) gene and Asp175Asn mutation in α-tropomyosin TPM1 gene (27). The cardiomyocytes derived from HCM patient-specific iPSC carrying either MYBPC3-Gln1061X or TPM1-Asp175Asn mutation displayed larger cellular size than those derived from healthy control iPSC (27). In addition, the myosin regulatory light chain (MYL2) mutation Arg58Gln is known to be associated with severe HCM, and cardiomyocytes derived iPSC of a HCM patient with MYL2-Arg58Gln were nearly 30% larger than the control iPSC-cardiomyocytes at day 60 (29). Other than larger cardiomyocyte cell size, myofibrillar disarray and abnormal electrophysiological properties were also observed in cardiomyocytes derived from HCM. For example, Han et al reported a HCM study by using iPSC reprogrammed from the dermal fibroblasts of a HCM patient with a single mutation (Arg442Gly) in the MYH7 gene (26). By Comparison to cardiomyocytes derived from healthy human iPSCs, they showed that the cardiomyocytes differentiated from the HCM patient iPSC displayed disorganized sarcomeres and irregularities in electrophysiology (26). Similar results were also observed in other studies. Zhou et al demonstrated that the percentage of myofibrillar disarray and cells with irregular beating in iPSC-cardiomyocytes of HCM patient with MYL2-Arg58Gln mutation was significantly higher than that in control cells (29). Moreover, Ca²⁺ plays a fundamental role in regulation of excitation-contraction coupling and electrophysiological signaling in the heart, and changes in Ca²⁺ handling was also seen in HCM iPSC-cardiomyocytes (23, 29).

Though iPSCs-based HCM modeling offer important insight in the understanding of disease mechanism. However, this traditional approach overlooks impacts of confounders such as genetic background, which may result in misleading outcomes. In the iPSCs-based dyskeratosis congenita disease modeling, for instance, two independent groups reported conflicting results with one showing re-growth of telomere and the other showing telomere decay (30, 31). In addition, Reinhardt et al. recently showed that neurons derived from iPSCs reprogrammed from different patients with Parkinson’s disease could display distinct phenotypes. In particularly, one patient iPSC-derived neurons exhibited phenotypes similar to healthy control iPSCs-derived neurons (8). Such misleading results are mainly attributed to confounders of iPSC lines generated from patients and individual controls with different genetic background. Therefore, to ensure precise comparative analysis in iPSCs-based disease studies, it is imperative to eliminate the genetic and other variabilities between iPSC lines reprogrammed from patients and healthy individuals.

Isogenic iPSCs generated by CRISPR/Cas9 for HCM modeling
Recent rapid development in genomic editing technology CRISPR/Cas9 makes it feasible to generate isogenic iPSC lines which only differ in the disease causative muta-

Figure 1. Reprogramming somatic cells (such as dermal fibroblasts and blood cells) into iPSCs which are then differentiated into cardiomyocytes (CM) for disease modeling, drug screening and cell therapy.
tions, would circumvent differences in genetic background and other variabilities among the cell lines. The CRISPR/ Cas9 system is derived from a variation of prokaryotic defense which protects them from foreign genetic elements. CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) come together to complex with Cas9 to identify and cut out these foreign sequences (32). A single-guide RNA (sgRNA) conjugated from the crRNA and tracrRNA can specifically introduce targeted double-stranded breaks (DSB) in human genome (32). The DSB then initiates cellular machinery to repair the DNA breaks typically by two repairing approaches, the non-homologous end-joining (NHEJ) repair which frequently results in nonspecific insertions and deletions (indels), and homology-directed repair (HDR) which utilizes DNA repair templates to generate knock-in of specific mutations (33).

There are two approaches to generate isogenic iPSC lines, correcting causative mutations in disease iPSCs or introducing disease causative mutations into health control iPSCs (Figure 2). Isogenic iPSC lines generated with either approach yield identical study outcomes, and recapitulate true disease mechanisms without genetic background variability (8, 34, 35). The first approach, to correct causative mutations in the disease-iPSCs, typically requires patient donor tissues for disease-iPSC reprogramming first. In addition, it is also associated with creation of multiple isogenic control lines with distinct genetic backgrounds when disease-iPSC lines of different patients are involved in a study, thus complicating isogenic iPSCs-based disease modeling (Figure 1). In contrast, the second approach, to directly introduce disease causative mutations into one control WT-iPSC line, is much simpler. It neither requires patient biopsies for iPSC reprogramming nor requires a different control line for a new disease-causative mutation (Figure 1). To date, there are only two studies of the isogenic iPSCs-based disease modeling have been reported for HCM disease (36, 37). Smith and colleagues reprogrammed hiPSC lines from patients carrying the E99K mutation and a healthy non-carrier relative, and then generated isogenic iPSCs lines by correcting the mutation in the diseased iPSCs or introduce the mutation in the healthy non-carrier iPSCs using CRISPR/Cas9. Their study recapitulated HCM disease phenotypes including abnormal contractility, Ca²⁺ sensitivity/handling, arrhythmogenesis, and hypertrophic signaling (36).

CONCLUSION

HCM, the most common genetic heart defect, is the leading cause of sudden cardiac death in young people in the United States. Current therapy can only relieve symptoms and prevent life-threatening complications, mainly due to unclear pathogenic mechanisms in HCM. Although animal models have offered some insight to HCM mechanisms, they may not faithfully recapitulate human HCM. In addition, all the traditional iPSCs-based HCM disease studies did not consider influence of the individuals’ genetic background difference and other variables which could result in misleading outcomes. This problem can be potentially overcome in isogeneic iPSCs generated by CRISPR/Cas9 which eliminate genetic background difference. Indeed, the recent two isogenic iPSCs-based HCM studies faithfully recapitulated HCM phenotypes (36, 37). Further comparative studies of isogenic disease HCM iPSC-CM with the control iPSC-CMs will help us understand the authentic pathogenic mechanisms underlying HCM as well as represent a valuable tool to screen the urgently needed drugs that can attenuate or reverse cardiac hypertrophy or fibrosis in HCM.

CONFLICT OF INTERESTS

The authors declare that no conflicting interests exist.
REFERENCES