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Short Communication

Genome Editing in Human Pluripotent Stem Cells: A Multidisciplinary Approach to Dissecting Cellular Mechanism of Cardiomyopathy

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Abstract

Rapid advances in genome editing technology allow the targeted genes to be modified both in vitro and in vivo. In this short communication, we will summarize the development and progress in genome editing techniques and highlight their application in human induced pluripotent stem cells, which have been utilized to investigate the pathophysiological mechanisms of gene mutation - induced cardiomyopathy.

INTRODUCTION

Cardiomyopathy is a disorder of heart muscle in which cardiac contractility is reduced and weakened, often associated with ventricular remodeling and heart tissue fibrosis. This disease causes gradual deterioration, consequently leading to heart failure or arrhythmias that compromise the quality of life and ultimately lead to death. Several etiological risk factors including ischemia, viral infection, and metabolic disorders are responsible for the pathophysiological development of cardiomyopathy. Among them, genetic factors likely play an important role in determining the occurrence and progress of this disease. Actually, the correlation between a number genetic mutations and cardiomyopathy has been revealed through research on human subjects [1], and a variety of mutations in the coding region of myofilament proteins and sarcoplasmic reticulum (SR) proteins have been identified in families with hereditary heart failure [2-5]. In past decades, the casual mechanisms involved in genetic mutation -related cardiomyopathy have been extensively investigated using murine models in both *in vitro* and *in vivo* conditions. Exogenous introduction of problematic mutant genes into the isolated cardiomyocytes can lead to weakened contractility and irregular electrophysiological responses. Correspondingly, the typical features of cardiomyopathy such as myofibrillar loss, interstitial fibrosis, and arrhythmia have been observed when these mutant genes were constitutively expressed in mouse hearts. Yet, despite making significant advances using a mouse model system, the molecular mechanisms of gene mutation-induced cardiomyopathy are not completely elucidated due to epigenetic variations and epigenomic differences that occur between mice and humans.

Induced pluripotent stem cell technology provides an opportunity to understand the pathogenesis of cardiomyopathy using human autologous cells. Human somatic cells acquire pluripotency post transduction of Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc). This genetic reprogramming progress enables these cells to proliferate and develop tri - lineage differentiation potential. Specifically, human dermal fibroblasts have been used to perform genetic reprogramming through transfection with Yamanaka factors [6] or other small molecules [7]. After exposure to cardiac differentiation culture conditions, these pluripotent cells are committed to execute a cardiomyocyte terminal differentiation program. The cardiac differentiation efficacy can be significantly improved when culture media is supplemented with certain morphogens and growth factors, such as active in A [8], bone morphogenic protein 4 (BMP-4) [9], basic fibroblast growth factor (bFGF) [10], and vascular endothelial growth factor (VEGF) [11]. Although obtaining adult cardiomyocyte phenotypes is still demanding, typical morphological features such as cardiac troponin and sarcomere structure have been demonstrated in iPSC - derived cardiomyocytes (iPSC-CM). Interestingly, cardiomyocyte purity can be enhanced to a greater degree when iPSC - derived cells are sorted using membrane proteins [12] and mitochondrial membrane potential [13] as indicating markers. Rhythmic contraction, calcium handling,

and electrical activity have observed in these iPSC - derived CMs, indicating that human - derived cells can be employed to investigate the pathophysiological genesis of cardiomyopathy. Recently, an iPSC model of hereditary cardiomyopathy has been established from patients with a mutation in TNNT2, a gene encoding the thin -filament contractile protein cardiac troponin T [14]. These gene-mutant iPSC - derived cardiomyocytes displayed a blunt β-adrenergic response compared with normal iPSC - derived cardiomyocytes. Further study on these problematic cells revealed that phosphodiesterases (PDEs) 2A and PDE3A were up - regulated in response to the TNNT2 mutation, while pharmacologic blockade of PDE2A and PDE3A restored cAMP levels and mitigated the damaged to β-adrenergic signaling. Another human iPSC colony has been established to elucidate the pathophysiology underlying the cardiomyopathy of patients suffering from Barth syndrome (BTHS) [12], a monogenic mitochondrial cardiomyopathy triggered by a mutation of the tafazzin (TAZ) - encoding gene. Energetic metabolic, mitochondrial structure and functional defects were observed in BTHS iPSCs - derived cardiomyocytes, associated with excessive levels of reactive oxygen and disruption of sarcomere assembly. Interestingly, intervention with linoleic acid effectively rectifies the aberrant metabolic phenotype of BTHS iPSC - derived cardiomyocytes. These promising studies not only offer insightful clarifications into hereditary cardiomyopathies, but also provide a platform for screening and identifying protocols that produce more precise individualized therapeutic treatments [15].

Genome editing is a technology in which engineered nucleases (or molecular scissors) are utilized to slice a specific site within an organism's genome, through which the functional gene can be deleted, inserted, or replaced by other DNA fragments [16]. Three successful genome editing approaches that have been developed and widely used are zinc finger nucleases (ZFN), transcription activator - like effectors nucleases (TALEN), and the CRISPR - Cas9 system [17]. These methods can yield specific double - strand breaks (DSBs) at desired sites within the genome that can be identified and processed through non - homologous end-joining (NHEJ) or homology - directed recombination (HDR). Particularly, experiments that employ NHEJ will lead to the loss of gene function while HR provides an opportunity to introduce exogenous DNA fragments, resulting in the targeted mutation or gene correction.

The first generation of genome editing tools included ZFNs, or chimeric proteins containing repeated DNA - target recognition domains and FokI endonucleases in which each domain is engineered to recognize 3-4 base pair nucleotides and multiple domains can jointly target DNA sequences of 9-18 base pairs. Although ZFNs were successfully induced for site - specific mutations in *Drosophila* [18], *Zebrafish* [19], and human stem cells [20], their wide applications are limited due to low recombination efficiency and an excess of off - target effects. Editing efficiency and specificity were considerably improved in the second generation of genome editing tools in which DNA - target recognition domains were replaced by transcription activator - like effectors (TALE), the naturally secreted proteins from bacteria *spp* [21]. It is worth mentioning that correction of human phospholamban (PLN) R14del mutations have been achieved using the TALENs approach [22]. This mutation, which can lead to the deletion of arginine 14 in the coding region of PLN gene, was detected through genetic screening of dilated cardiomyopathy patients and finally identified in a large family with hereditary heart failure [23]. Interestingly, this gene mutation - induced calcium handling disorder, electrical instability, and pathological hypertrophic response was entirely displayed in cardiomyocytes that were derived from iPSCs with the PLN R14del mutation, the same phenotype as observed in corresponding gene - mutant mouse models. Rectifying the R14del mutation was attained using patient - derived iPSCs through co-transfection with PLN - specific TALEN pair together with the gene correction matrix without compromising pluripotency, differentiation potential, and karyotype integrity of the reprogrammed - cell colonies. After cardiac differentiation, TALEN - corrected cardiomyocytes exhibited a normal Ca^{2+} cycling phenotype and a significantly reduced resting diastolic Ca^{2+} level when compared with iPSC - derived cardiomyocytes. However, there are limitations in the use of TALENs, such as the huge size of TALEN - encoding plasmid that may complicate assembly or maintenance after introduction into cells and consequently compromise the efficacy of genetic editing. The CRISPR/Cas9 system was developed to overcome those limitations and has arisen as the principle genome-editing tool since the discovery of its key components: guide RNA (gRNA) and the Cas9 endonuclease. The uncomplicated approaches of construction and assembly permit CRISPR/Cas9 vectors to avoid the time-consuming and cumbersome process. Most importantly,

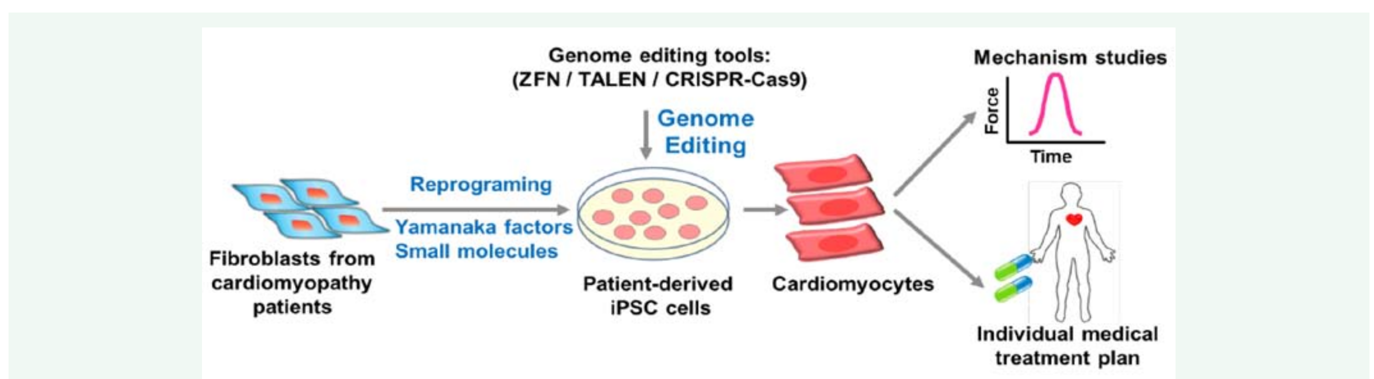

Figure 1 Genome editing in patient - derived iPSCs can provide a platform to investigate cardiomyopathy mechanisms and make individual medical treatment plan.

the introduction of gRNA enables endonuclease to recognize the target DNA with a highly specific pattern that allows multiple sites to be edited simultaneously. Currently, mutant genes in iPSC - derived cardiomyocytes cannot be totally cleared or replaced by a correct gene using CRISPR/Cas9 system. Such a limitation may be a result of HDR - induced correct gene expression that occurs only during the DNA - synthesis phase of the cell cycle in proliferated cells, and cardiomyocyte proliferation is unfortunately quiescent in mammals.

The advent of iPSCs technology has made it feasible to simulate cardiomyopathy *in vitro* using large quantities of human cardiomyocytes free from ethical issues, and the development of genome editing allows comprehensive investigation into the genetic basis and molecular mechanisms of cardiomyopathy. Such a multidisciplinary approach provides optimism for researchers who are further dissecting the molecular mechanisms of gene mutation related heart disease, making individual treatment plans that offer more precisely targeted therapeutics possible for patients in a routine clinical setting (Figure 1).

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