

82365_Auto_Edited.docx

Name of Journal: *World Journal of Stem Cells*

Manuscript NO: 82365

Manuscript Type: MINIREVIEWS

Disease modeling of desmosome-related cardiomyopathy using induced pluripotent stem cell-derived cardiomyocytes

Higo S. Disease modeling of desmosome-related cardiomyopathy

Shuichiro Higo

Shuichiro Higo, ³ Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita 565-0871, Japan

Abstract

Cardiomyopathy is a pathological condition characterized by cardiac pump failure due to myocardial dysfunction and the major cause of advanced heart failure requiring heart transplantation. Although optimized medical therapies have been developed for heart failure during the last few decades, some patients with cardiomyopathy exhibit advanced heart failure and are refractory to medical therapies. Desmosome, which is a dynamic cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause arrhythmogenic cardiomyopathy (AC), a rare inheritable disease, and predispose patients to sudden cardiac death and heart failure. Recent advances in sequencing technologies have elucidated the genetic basis of cardiomyopathies and revealed that desmosome-related cardiomyopathy is concealed in broad cardiomyopathies. Among desmosomal genes, mutations in *PKP2* (which encodes PKP2) are most frequently identified in patients with AC. *PKP2* deficiency causes various pathological cardiac phenotypes. Human cardiomyocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows the precise arrangement of the targeted genome, are powerful experimental tools for studying disease. This review summarizes the current issues associated with practical medicine for advanced heart failure and the recent advances in disease modeling using iPSC-derived cardiomyocytes targeting desmosome-related cardiomyopathy caused by *PKP2* deficiency.

Key Words: Cardiomyopathy; Advanced heart failure; Induced pluripotent stem cell-derived cardiomyocytes; Desmosome; Genome editing; Gene therapy

Higo S. Disease modeling of desmosome-related cardiomyopathy using induced pluripotent stem cell-derived cardiomyocytes. *World J Stem Cells* 2023; In press

Core Tip: Prevention of advanced heart failure caused by cardiomyopathy is an urgent unmet need in the field of cardiovascular medicine. Desmosome, a cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause desmosome-related cardiomyopathy, an intractable disease refractory to standard medical therapies. This review introduces the recent advances in disease modeling of desmosome-related cardiomyopathy caused by *PKP2* mutations using induced pluripotent stem cell-derived cardiomyocytes.

INTRODUCTION

Heart failure is a clinical syndrome characterized by dyspnea, malaise, swelling, and/or decreased exercise capacity owing to impaired cardiac pumping function^[1]. The established optimal medical therapies for heart failure have increased the survival rates of patients in the last few decades^[2-4]. However, some patients are refractory to medical therapies and develop symptoms that are diagnosed as advanced heart failure. Currently, the therapeutic strategies

available for these patients are heart transplantation and implantation of the ventricular assisting device^[1,5]. Cardiomyopathy is a disease of cardiac pump failure due to myocardial dysfunction and is the major cause of advanced heart failure requiring heart transplantation^[6-11]. Cardiomyopathies are differentially diagnosed mainly by using imaging modalities, including echocardiography, scintigraphy, computed tomography, magnetic resonance imaging, and cardiac catheterization. Based on the findings of these modalities, ⁵cardiomyopathies are classified into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), or other rare cardiomyopathies, such as arrhythmogenic right ventricular cardiomyopathy (ARVC)^[12]. Among 36,883 heart transplantation recipients registered ⁴in the International Society for Heart and Lung Transplantation Thoracic Organ Transplant Registry between 2010 and 2018, the major primary diagnoses were non-ischemic DCM (50.8%), ischemic cardiomyopathy (ICM) (32.4%) with coronary artery disease, RCM (3.5%), and HCM (3.4%)^[13]. In Japan, cardiomyopathies [DCM (64%), end-stage HCM with left ventricular systolic dysfunction (12%), and ICM (9%)] account for more than three-quarters of underlying diseases among heart transplant recipients^[14]. ARVC, a rare inherited disease, is ²characterized by the risk of life-threatening arrhythmias, myocardial dysfunction, and fibrofatty replacement of myocardial tissue, predisposing the patients to sudden cardiac death and heart failure^[9,11]. The prevalence of ARVC among the registrants for heart transplantation is rare (0.3% and 1%-2% in the United Network for Organ Sharing registry^[15] and Japan Organ Transplant Network^[14], respectively).

DESMOSOME-RELATED CARDIOMYOPATHY IS CONCEALED IN ADVANCED HEART FAILURE

Recent clinical studies utilizing high-throughput sequencing technologies have elucidated the genetic basis of cardiomyopathies, identified various causative genetic variants, and revealed the correlation between genetic factors and clinical phenotypes or cardiac morphologies in patients with cardiomyopathies^[16-20]. ARVC is an inherited disease caused by mutations in desmosomal genes (*PKP2*, *JUP*, *DSC2*, *DSG2*, and *DSP*) (Figure 1)^[11,21,22]. These genes encode the structural components of the desmosome, a dynamic junction between cells that maintain the structural integrity of heart tissues^[23,24]. The original disease phenotypes of ARVC are characterized by predominant right ventricular enlargement and contractile dysfunction. However, recent studies have reported left ventricular or biventricular involvement in patients with ARVC, resulting in the use of a broad phrase [arrhythmogenic cardiomyopathy (AC)]^[9,11]. Although the prevalence of AC in patients with advanced heart failure is rare, recent genetic analyses in large cohorts have demonstrated an increased incidence of desmosomal gene mutations in patients with DCM^[18,25,26], which is the most frequent basal disease among heart transplantation registrants. Furthermore, homozygosity and compound or digenic heterozygosity of desmosomal genes are not rare, and patients with combined mutations exhibit a severe phenotype^[27-30]. Recently, we identified *DSG2*-deficient cardiomyopathy caused by a rare homozygous stop-gain mutation in a patient initially diagnosed with idiopathic sporadic DCM^[30]. *Dsg2* deficiency is associated with embryonic lethality in mice. Additionally, *Dsg2*-

depleted embryonic stem cells do not proliferate^[31]. However, a human male patient with a complete lack of *DSG2* expression did not exhibit pathological phenotypes at birth but developed advanced heart failure during the teenage years. Immunohistochemical and transmission electron microscopy analyses of left ventricular heart tissues revealed that the loss of *DSG2* leads to aberrant deposition of desmosomal proteins and disruption of intercalated discs in cardiomyocytes. These findings suggest that desmosome-related cardiomyopathy is concealed in patients with advanced heart failure who are diagnosed with idiopathic DCM. As desmosome impairment is the most upstream molecular change in these patients, experimental studies must focus on elucidating the molecular mechanisms underlying the instability of cell-to-cell junctions to overcome advanced heart failure caused by desmosome-related cardiomyopathy. For disease modeling, patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows precise genomic modification of the targeted mutations, are powerful experimental tools to recapitulate pathological phenotypes based on the molecular factors of inherited cardiomyopathies^[30,32-35].

PHENOTYPIC RECAPITULATION OF CARDIOMYOPATHY CAUSED BY PKP2 DEFICIENCY USING PATIENT-DERIVED IPSC-CMS

PKP2, which is encoded by *PKP2*, is a desmosomal protein localized to the outer dense plaque and functions as a scaffold for the other desmosome proteins *DSG2*, *DSC2*, *JUP*, and *DSP*^[23,36] (Figure 1). Among the desmosomal genes, mutations in *PKP2* are most frequently identified in patients with

AC^[11,37-39], and have been extensively studied using patient-derived iPSC-CMs compared to other desmosomal genes (*DSG2*^[30,40,41], *DSP*^[42,43], and *DSC2*^[44,45]). Various clinical phenotypes and pathological characteristics observed in patients with AC harboring *PKP2* mutations, downregulated desmosomal protein expression, upregulated lipogenesis, and increased apoptosis in heart tissues have been recapitulated using genetically engineered mouse models^[11] and human cardiomyocytes differentiated from iPSCs^[46-54] (Table 1). Most known mutations of *PKP2* are heterozygous and are missense, nonsense, and frameshift mutations. Studies on patient-derived iPSCs have identified that *PKP2* variants are heterozygous missense^[48], heterozygous frameshift^[46,47,49,50,54], homozygous frameshift^[47,51], compound heterozygous, and frameshift^[52] mutations. Disease-specific iPSCs are generated from fibroblasts^[46-48,51], keratinocytes^[49], adipose tissue-derived stromal cells^[52], and peripheral blood mononuclear cells^[54], whereas control iPSCs are generated from healthy subjects^[46-49,51,52], human embryonic stem cells^[50], or isogenic cells engineered from patient-derived iPSCs using genome editing^[54]. Genome editing allows disease modeling by introducing heterozygous and homozygous frameshift mutations in wild-type iPSC lines^[53]. Decreased expression of desmosomal proteins, aberrant lipogenesis, and apoptosis of cardiomyocytes are observed in the heart tissues of patients with AC^[9,55,56]. These pathological phenotypes are recapitulated in iPSC-CMs with *PKP2* mutations as determined using immunostaining^[46-54], lipid staining^[47-50], electron microscopy^[46,54], terminal transferase dUTP nick end labeling staining^[46,47], and cleaved-CASP3 expression analysis^[54]. Lethal arrhythmia is a hallmark of patients with AC.

Arrhythmia phenotypes are recapitulated using iPSC-CMs with *PKP2* mutations as evidenced by the results of patch-clamp^[48,52], multielectrode array^[46], calcium imaging^[47], and optical voltage recording^[53]. In clinical settings, global or regional ventricular contractile dysfunction is defined as a major criterion for the diagnosis of ARVC in modified Task Force criteria^[21] and Padua criteria^[57]. However, the functional consequence in cardiomyocyte contractility caused by *PKP2* mutations has not been fully studied in human iPSC-CMs.

PKP2 DEFICIENCY AND CONTRACTILE DYSFUNCTION

We established iPSCs from a patient with AC harboring a heterozygous frameshift *PKP2* mutation (c.1228dupG, p.D410fsX425) and generated an isogenic set of iPSC clones harboring three genotypes [heterozygous mutation (Hetero), homozygously corrected with homology-directed repair (HDR), and homozygously introduced frameshift mutations *via* non-homologous end joining (NHEJ)] using genome editing^[54] (Figure 1). These isogenic sets of iPSCs comprise patient-derived Hetero-iPSCs, HDR-iPSCs with two-fold higher *PKP2* expression relative to Hetero-iPSCs, and NHEJ-iPSCs, which do not express *PKP2*, recapitulating both haploinsufficiency and complete loss of *PKP2*. After cardiomyocyte differentiation using the monolayer protocol with chemically defined medium^[58], NHEJ-iPSC-CMs lacking *PKP2* expression exhibit lipid droplet accumulation, increased apoptosis, and decreased propagation rate (Table 1). However, patient-derived Hetero-iPSC-CMs with half-dose *PKP2* expression do not exhibit these pathological phenotypes, suggesting that the

haploinsufficiency of *PKP2* is not sufficient to induce the above pathological phenotypes within 28 days after differentiation. In contrast, haploinsufficiency of *PKP2* decreased contractility, which was evaluated using motion vector analysis, within 14 days of differentiation. As the monolayer protocol confers strong contraction to iPSC-CMs on culture plates immediately after differentiation^[58,59], continuous tensile overload may facilitate the contractile phenotype among isogenic iPSC-CMs. A recent study used isogenic iPSC-CMs in which heterozygous or homozygous frameshift mutation was introduced into wild-type iPSC-CMs^[53]. The authors reported that *PKP2* deficiency decreased systolic force in three-dimensional cardiac microtissues. This further supported the functional relationship between *PKP2* deficiency and contractile dysfunction. An experimental study using cardiac tissue-specific *Pkp2* knockout mice demonstrated that the loss of *Pkp2* increased the distance between the cell periphery and DES, an intermediate filament protein in cardiomyocytes^[60]. As DES connects Z-discs of sarcomeres to sarcolemmal costameres, desmosomes, and nuclear envelope^[11,61], further experimental studies focusing on these cellular networks are required to elucidate the pathogenesis of desmosome-related cardiomyopathy.

DESMOSOME IMAGING USING THE ISOGENIC IPSC-CMS AND AAV-MEDIATED GENE REPLACEMENT

In the isogenic background, the haploinsufficiency of *PKP2* did not affect the localization or expression levels of desmosomal proteins in iPSC-CMs as evidenced by the results of immunostaining or western blotting analyses.

However, the desmosome area represented by dot distribution on the cell periphery in Hetero-iPSC-CMs was significantly lower than that in HDR-iPSC-CMs^[54], suggesting that desmosome assembly is impaired by *PKP2* haploinsufficiency. The impaired assembly of desmosomal proteins in human iPSC-CMs is supported by another study using isogenic iPSC-CMs. Fluorescence recovery after photobleaching experiments combined with lentivirus-mediated expression of fluorescent protein-tagged N-cadherin provided evidence that molecular stability of junctional N-cadherin is impaired by *PKP2* deficiency^[53]. To trace the molecular behavior of endogenous proteins in cardiomyocytes, fluorescent tagging of the structural proteins through genome editing is a powerful tool^[62,63]. However, fluorescent tagging of endogenous desmosomal genes might affect desmosome structures or cell-to-cell integrity in iPSCs or iPSC-CMs. We previously identified a patient with *DSG2*-deficient cardiomyopathy due to a rare homozygous stop-gain mutation and demonstrated that complete loss of *DSG2* in human iPSCs does not affect the differentiation or cellular morphology in iPSC-CMs^[30]. These findings prompted us to use *DSG2* as the target of endogenous tagging by fluorescent protein to trace desmosome dynamics in live human iPSC-CMs. Genome editing targeting *DSG2* alleles was performed to establish the isogenic iPSC-CMs harboring identical two *DSG2* alleles comprising intact and knocked-in tdTomato alleles under the adjusted *PKP2* expression levels (Figure 2). The desmosome area (represented by desmoglein-2-tdTomato fusion protein) was significantly downregulated due to *PKP2* haploinsufficiency. Adeno-associated virus (AAV), ⁶ a small, nonenveloped virus with a linear, single-stranded DNA,

is widely used for gene therapy targeting human diseases, including heart failure^[64,65]. AAV-mediated gene replacement of *PKP2* significantly restored the decreased contractility in Hetero-iPSC-CMs and NEHJ-iPSC-CMs, demonstrating the proof-of-concept for *PKP2* gene therapy in human cells. Furthermore, time-lapse imaging using NHEJ-iPSC-CMs captured the recovery of desmosomes, which gradually assembled at the cell periphery after AAV-mediated *PKP2* replacement (Figure 2). The established isogenic iPSCs harboring knocked-in tdTomato alleles allowed desmosome-imaging in living cells and provided distinct readouts for therapeutic development.

GENE REPLACEMENT THERAPY TARGETING HEART FAILURE

Several clinical trials using AAV-mediated gene replacement have been designed targeting cardiovascular disease^[65,66]. A large-scale clinical trial was conducted as a randomized, multinational, double-blind, placebo-controlled phase 2 study targeting up to 250 patients with moderate-to-severe heart failure and reduced contractile function (CUPID2 trial)^[67]. The study aimed to deliver sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) into heart tissues *via* intracoronary injection. ¹ SERCA2a regulates cardiomyocyte contraction and relaxation by transporting Ca^{2+} from the cytosol into the sarcoplasmic reticulum during diastole^[68]. The deficiency of SERCA2a is associated with heart failure progression^[69,70]. Although promising results were achieved in preceding preclinical and clinical studies^[71-73], gene replacement of SERCA2a ¹ did not improve the clinical course of patients with heart failure^[74]. The two clinical trials of gene therapy targeting patients with heart failure conducted in the

same period (AGENT-HF^[75] and SERCA-LVAD^[76]) were terminated due to the neutral result of the CUPID2 trial and the lack of functional benefit. The amount of vector DNA in heart tissues obtained from patients who received gene therapy and subsequently underwent heart transplantation or mechanical circulatory support device implantation was low, suggesting ¹that only a small proportion of cardiomyocytes expressed AAV-delivered SERCA2a in the myocardium. Although these clinical trials demonstrate the difficulty of gene delivery targeting human heart tissues, they provide the evidence for the safety of cardiac gene therapy and a basis for the design of future gene therapy trials. Recent genetic analysis clarified a large number of genetic mutations that cause cardiomyopathies with advanced heart failure in a loss-of-function manner and can be targeted by specific gene replacement therapy^[77,78]. In desmosome-related cardiomyopathy, most of the identified mutations in *PKP2* are heterozygous^[22,37,79,80]. However, in extremely rare cases, homozygous mutations of *PKP2* cause lethal infantile heart failure with left ventricular non-compaction or hypoplastic left heart syndrome^[81-83]. No effective therapies are available for these patients who require a novel therapeutic approach for desmosome-related cardiomyopathy. Proof-of-concept studies for structural and functional recovery using both human iPSC-CM models and *in vivo* models are required for future clinical application.

CONCLUSION

Although human iPSC-CMs are immature and do not fully recapitulate *in vivo* heart tissues^[59], tissue engineering approaches^[84,85] will promote the maturation

of iPSC-CMs and provide a useful tool in combination with genome editing. The isogenic iPSC-CMs that we established represent a human disease model that recapitulates reduced contractility and impaired desmosome assembly and provides a convenient cellular platform for therapeutic screening to examine upstream molecular targets of desmosome-related cardiomyopathy.

ORIGINALITY REPORT

4%

SIMILARITY INDEX

PRIMARY SOURCES

1	Barry Greenberg, Javed Butler, G Michael Felker, Piotr Ponikowski et al. "Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial", The Lancet, 2016 <small>Crossref</small>	51 words — 2%
2	pubmed.ncbi.nlm.nih.gov <small>Internet</small>	16 words — 1%
3	www.nature.com <small>Internet</small>	14 words — 1%
4	journals.lww.com <small>Internet</small>	13 words — < 1%
5	www.preprints.org <small>Internet</small>	13 words — < 1%
6	circres.ahajournals.org <small>Internet</small>	10 words — < 1%

EXCLUDE QUOTES ON

EXCLUDE BIBLIOGRAPHY ON

EXCLUDE SOURCES < 10 WORDS

EXCLUDE MATCHES < 10 WORDS