Phenotypic Recapitulation and Correction of Desmoglein-2-deficient Cardiomyopathy using Human Induced Pluripotent Stem Cell-derived Cardiomyocytes

志波幹夫[1]、肥後修一朗[1,2]、近藤匠巳[1]、李俊君[3,4]、劉莉[3,4]、池田善彦[5]、小濱康明[1]、亀田聡士[1]、田端智香[1]、井上裕之[1]、中村聡希[2,6]、武田真季[3]、高島成二[6]、宮川繁[3]、澤芳樹[3]、彦惣俊吾[1]、坂田泰史[1]

1: 大阪大学大学院医学系研究科　循環器内科学、2: 大阪大学大学院医学系研究科　重症心不全内科治療学寄附講座、3: 大阪大学大学院医学系研究科　心臓血管外科学、4: 大阪大学大学院医学系研究科　組織再生デザイン学、5: 国立循環器病研究センター　病理部、6: 大阪大学大学院生命機能研究科　医化学

Background

Mutations in desmosome genes cause arrhythmogenic cardiomyopathy, mainly in an autosomal dominant manner. Rare cases of recessive inheritance manifest as severe biventricular dilatation and contractile dysfunction due to loss of desmosome protein, requiring the human disease model for therapeutic development.

Methods

We identified a homozygous stop-gain mutation in *DSG2* (c.C355T, p.R119X) that led to complete desmoglein-2 deficiency in a patient with severe biventricular heart failure initially diagnosed as idiopathic dilated cardiomyopathy. The patient's ventricular myocardium was analyzed histologically. Phenotype analysis was performed on cardiomyocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs) with or without restored desmoglein-2 expression mediated by CRISPR/Cas9 genome editing.

Results

Immunohistochemical and electron microscopic analysis revealed abnormal deposition of desmosome proteins, disrupted intercalated disc structures, and aggregated cytoplasmic desmosomes in the patient's left ventricular myocardium lacking desmoglein-2 expression. iPSCs were generated from the peripheral blood mononuclear cells of the patient (R119X-iPSC), and the mutated *DSG2* gene locus was heterozygously corrected to the normal allele via homology-directed repair (HDR-iPSC), which restored desmoglein-2 expression. Both isogenic iPSCs were differentiated into cardiomyocytes (iPSC-CMs). Multiple electrode array analysis detected abnormal excitation in R119X-iPSC-CMs but not HDR-iPSC-CMs. Micro force testing of 3-dimensional self-organized tissue rings (SOTRs) detected tissue fragility and weak maximum forces in those from R119X-iPSC-CMs. Notably, these phenotypes were significantly recovered in SOTRs from HDR-iPSC-CMs. The myocardial fiber structures in R119X-iPSC-CMs were severely aberrant, and electron microscopic analysis revealed disrupted desmosomes in R119X-iPSC-CMs. Unexpectedly, the absence of desmoglein-2 in R119X-iPSC-CMs led to the decreased expression of desmocollin-2 but no other desmosome proteins (plakophilin-2, plakoglobin, or desmoplakin).

Conclusions

We identified a desmoglein-2-deficient cardiomyopathy among the clinically diagnosed idiopathic dilated cardiomyopathies. The recapitulation and correction of the disease phenotype using iPSC-CMs provide evidence to support the development of precision medicine targeting intractable cardiomyopathy.