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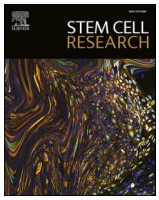

Generation of a heterozygous *TPM1*-E192K knock-in human induced pluripotent stem cell line using CRISPR/Cas9 system

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A B S T R A C T

E192K missense mutation of *TPM1* has been found in different types of cardiomyopathies (e.g., hypertrophic cardiomyopathy, dilated cardiomyopathy, and left ventricular non-compaction), leading to systolic dysfunction, diastolic dysfunction, and/or tachyarrhythmias. Here, we generated a heterozygous *TPM1*-E192K knock-in human induced pluripotent stem cell (iPSC) line using CRISPR/Cas9-based genome editing system. The cells exhibit normal karyotype, typical stem cell morphology, expression of pluripotency markers and differentiation ability into three germ layers. Accordingly, this cell line could provide a useful cell resource for exploring the pathogenic role of *TPM1*-E192K mutation in different types of cardiomyopathies.

1. Resource table

Unique stem cell line identifier	YUCMi019-A-1
Alternative name(s) of stem cell line	hiPSC-*TPM1*_KI
Institution	Yonsei University College of Medicine
Contact information of the reported cell line distributor	Boyoung Joung, cby6908@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Age: N/A Sex: Male
Cell Source	Cord blood cell
Method of reprogramming	N/A
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Cell culture system used	mTeSR™1 medium on matrigel-coated plate
Type of Genetic Modification	CRISPR/Cas9-mediated knock-in
Associated disease	Hypertrophic cardiomyopathy, dilated cardiomyopathy, left ventricular non-compaction
Gene/locus	*TPM1*, 15q22.2
Method of modification/site-specific nuclease used	CRISPR/Cas9 Electroporation
Site-specific nuclease (SSN) delivery method	
All genetic material introduced into the cells	Cas9 protein, single guide RNA (sgRNA), single strand oligo donor (ssODN)
Analysis of the nuclease-targeted allele status	Sanger sequencing of the targeted allele
Method of the off-target nuclease activity surveillance	N/A
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	N/A
Inducible/constitutive system details	N/A
Date archived/stock date	March 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/YUCMi019-A-1
Ethical/GMO work approvals	The hiPSC line, CMC-hiPSC-011 was provided by Korea National Stem Cell Bank originally provided from Catholic University. This study was approved by the Institutional Review Board/Ethics Committee of Severance Hospital, Yonsei University Health System (approval no. 4-2021-1626)
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

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2. Resource utility

The generated heterozygous *TPM1*-E192K knock-in iPSC line can be differentiated into targeted cells (e.g., cardiomyocytes), thereby providing a relevant model for studying the pathogenic role of *TPM1*-E192K mutation in different types of cardiomyopathies (see Table 1).

3. Resource details

Tropomyosin 1 (TPM1), a sarcomeric actin-binding protein, plays a key role in contractile regulation (Redwood and Robinson, 2013). A E192K missense mutation of *TPM1* has been implicated in different types of cardiomyopathies (e.g., hypertrophic cardiomyopathy, dilated cardiomyopathy, and left ventricular non-compaction), leading to systolic dysfunction, diastolic dysfunction, and/or tachyarrhythmias (Probst et al., 2011; Sewanan et al., 2021; Martinez et al., 2021; Cha et al., 2022). However, its functional and molecular consequences remain largely unexplored. Thus, we generated a heterozygous *TPM1* mutant (p.E192K, c.574G > A) knock-in iPSC line (hiPSC-*TPM1*_KI) using CRISPR/Cas9-based genome editing system. hiPSCs were electroporated with preassembled Cas9/sgRNA ribonucleoprotein (RNP) complexes and a single strand oligonucleotide donor (ssODN) which would create *TPM1*-E192K mutation. Among several colonies, we selected *TPM1*-E192K mutant colony by Sanger sequencing on the genomic level (Fig. 1A). The cells maintained normal karyotype (46, XY) without structural or numerical chromosomal abnormalities (Fig. 1B). In addition, the cells showed a typical iPSC-like colony morphology and high alkaline phosphatase (AP) activity (Fig. 1C). Immunofluorescence staining indicated that hiPSC-*TPM1*_KI cells express pluripotency markers, such as Sox-2, Oct-3/4, SSEA-4, and TRA-1-81 (Fig. 1D). The cells also possessed the ability to differentiate into three germ layers *in vitro*, as shown by the presence of OTX2-positive (ectoderm), Brachyury-positive (mesoderm), and Sox-17-positive (endoderm) cells (Fig. 1E). The cells were free of mycoplasma contamination, as measured by polymerase chain reaction (PCR) (Fig. 1F).

Table 1
Characterization and validation

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Pluripotency status evidence for the described cell line	Alkaline Phosphatase staining	Positive	Fig. 1 panel C
	Qualitative analysis *(Immunocytochemistry)*	Positive for Sox-2, Oct-3/4, SSEA-4, and TRA-1-81	Fig. 1 panel D
	Quantitative analysis *(RT-qPCR)*	Not performed	N/A
Karyotype	Karyotype (G-banding) and resolution	46XY, Resolution 525	Fig. 1 panel B
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	Heterozygous knock-in	Fig. 1 panel A
	Transgene-specific PCR	Not performed	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	Not performed	N/A
Parental and modified cell line genetic identity evidence	STR analysis	All sites matched	Submitted in the archive with journal
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR)	Heterozygous knock-in	Fig. 1 panel A
	PCR-based analyses	Not performed	N/A
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	Not performed	N/A
Off-target nuclease analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	Not performed	N/A
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by RT-PCR, Negative	Fig. 1 panel F
Multilineage differentiation potential	Directed differentiation	Positive for OTX2 (ectoderm), Brachyury (mesoderm), and Sox-17 (endoderm)	Fig. 1 panel E
Donor screening *(OPTIONAL)*	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype – additional histocompatibility info *(OPTIONAL)*	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

4. Materials and methods

4.1. Cell culture

The hiPSC line, CMC-hiPSC-011 was provided by Korea National Stem Cell Bank originally provided from Catholic University. Cells were cultured in TeSR™-E8™ medium (STEMCELL Technologies) on vitronectin (Gibco)-coated plates at standard culture conditions (37 °C, 5% CO_2, and 21% O_2) and subcultured every 4–6 days.

4.2. CRISPR/Cas9-mediated knock-in

hiPSC were dissociated with Gentle Cell Dissociation Reagent (STEMCELL Technologies) and single cells were centrifuged at 300*g* for 5 min. 8×10^4 single cells were electroporated with a mixture (1.5 μg Cas9 protein, 10 pmoles sgRNA, and 20 pmoles ssODN) under the condition of 1200 V, 20 ms, and 2 pulses by Neon™ Transfection System (Thermo Fisher Scientific). To acquire single-cell colonies, electroporated cells were seeded using the serial dilution method on matrigel (Corning)-coated 96-well plates containing mTeSR™1 medium (STEMCELL Technologies) with 10 μM Y-27632 (Tocris Bioscience). Briefly, 10 μL of electroporated cell suspension containing 8×10^4 cells were sequentially diluted until reaching a population of 1×10^2 cells. Then, the cells were supplied with 10 mL of medium and every 100 μL derived from the final step would roughly contain one cell, which was further transferred into 96-well plates. Y-27632 was removed next day and the medium was changed every other day. The single colonies were manually picked up 12–14 days later when the colonies were large enough, and transferred to matrigel-coated 24-well plates. When the cells reach 70–90% confluency, the cells were plated in matrigel-coated 12-well plates and a small portion of each cell was lysed for screening.

4.3. Sanger sequencing

Genomic DNA was isolated using G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology). The sequence containing knock-in region was amplified with PCR using AccuPower® PCR PreMix (BIONEER). Then, PCR products were sequenced by sanger sequencing at BIONICS Co., Ltd (Korea) and the results were aligned using SnapGene software.

4.4. AP staining

The cells were stained using Alkaline Phosphatase Live stain

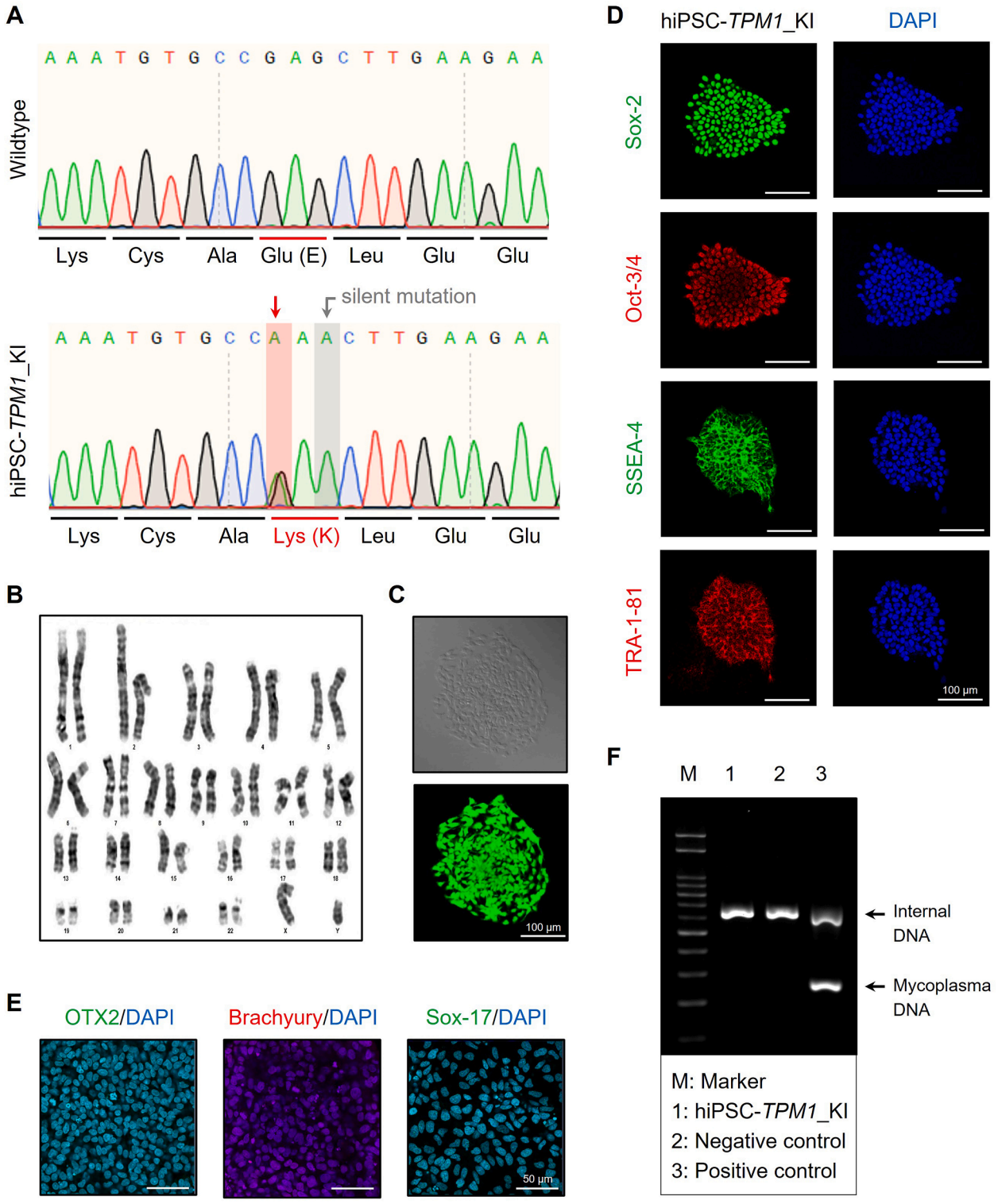

Fig. 1. Generation and characterization of YUCMi019-A-1 (hiPSC-*TPM1*_KI).

(Thermo Fisher Scientific) according to the manufacturer's instruction, and visualized by confocal microscopy (Zeiss LSM 710).

4.5. Immunocytochemistry

The cells were fixed with 4% paraformaldehyde for 15 min, washed three times with PBS, and permeabilized with 0.3% Triton-X100/PBS for 30 min at room temperature (RT). After blocking with 1% bovine serum albumin(BSA)/PBS for 30 min at RT, the cells were incubated overnight at 4 °C with fluorescence-conjugated primary antibodies in 1% BSA/PBS. Then, the cells were washed five times with PBS and nuclei were stained with Hoechst 33342 (Thermo Fisher Scientific). The cells were observed using confocal microscope (Zeiss LSM 710). All antibody details are listed in Table 2.

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Sox-2 (E-4) Alexa Fluor® 488	1:200	Santacruz Cat# sc-365823 AF488, RRID: AB_10842165
	Oct-3/4 (C-10) Alexa Fluor® 594	1:200	Santacruz Cat# sc-5279 AF594, RRID: AB_628051
	SSEA-4 Alexa Fluor® 488	1:200	Santacruz Cat # sc-21704 AF488, RRID: AB_628289
	TRA-1-81 Alexa Fluor® 647	1:200	Santacruz Cat# sc-21706 AF647, RRID: AB_628386
Differentiation Markers	OTX2 (D-8) Alexa Fluor® 488	1:200	Santacruz Cat# sc-514195 AF488
	Brachyury (A-4) Alexa Fluor® 594	1:200	Santacruz Cat# sc-374321 AF594, RRID: AB_10990301
	Sox-17 (3.5CH) Alexa Fluor® 488	1:200	Santacruz Cat# sc-130295 AF488, RRID: AB_2286667
Nuclear stain	Hoechst33342	1:2000	Thermo Fisher Scientific Cat # H3570
Site-specific nuclease			
Nuclease information	Cas9	Thermo Fisher Scientific Cat # A36498	
Delivery method	Electroporation	Neon transfection system (Thermofisher Scientific)	
Selection/enrichment strategy	N/A	N/A	
Primers and Oligonucleotides used in this study			
	Target	Forward/Reverse primer (5′-3′)	
Genotyping	TPM1	F: GATTTGGTCACCCTGCCTTCR: AAGTGTTGCTTTCTGGCAGG	
Targeted mutation sequencing	TPM1	Sanger sequencing chromatograms in Fig. 1A.	
sgRNA	TPM1	TTTCAATTCTTCTTCAAGCT	
ssODN		TTCTGCCTCTGATCGAAAACATTAGCAAATGTGCCAAACTTGAAGAAGAATTGAAAACTGTGACGAACAACTTG	

4.6. In vitro trilineage differentiation

The cells were cultured and differentiated using STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) according to the manufacturer's instructions. All antibody details are listed in Table 2.

4.7. Karyotyping

G-banding karyotype analysis was performed at Samkwang Medical Lab (Korea) using standard protocols for GTG banding. Total 20 metaphases were analyzed at 525 band resolution.

4.8. Short tandem repeat (STR) analysis

STR analysis was performed with detection of 17 loci and 1 gender marker by Cosmogenetech (Korea).

4.9. Mycoplasma detection

SafeDry™ Mycoplasma PCR Detection Kit (CellSafe) was used to assess mycoplasma contamination according to the manufacturer's instructions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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