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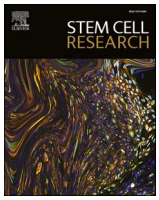

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line derived from a patient with dilated cardiomyopathy carrying *LMNA* nonsense mutation

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A B S T R A C T

Dilated cardiomyopathy (DCM) is a refractory heart disease characterized by dilation of the left ventricle and systolic dysfunction. *LMNA*, the gene encoding lamin A/C (a nuclear envelope protein), is the second leading causative gene associated with familial DCM. *LMNA*-related DCM is likely to develop severe heart failure, various types of arrhythmias, and poor prognosis. We established a human induced pluripotent stem cell line, derived from a patient with DCM carrying a nonsense mutation in *LMNA*. This line should be a useful resource for elucidating disease mechanisms and developing fundamental treatments for *LMNA*-related DCM.

1. Resource utility

This human induced pluripotent stem cell (hiPSC) line would contribute to elucidating how *LMNA* mutations induce dilated cardiomyopathy (DCM) and to the subsequent development of new therapies for it. In particular, iPSC-derived cardiomyocytes (iPSC-CMs) can be used to recapitulate *LMNA*-related DCM *in vitro*.

2. Resource table

Unique stem cell line identifier	BRCi021-A
Alternative name(s) of stem cell line	hiPSC-TUH001
Institution	Department of Cardiology, Faculty of Medicine, University of Tsukuba, Japan
Contact information of distributor	Nobuyuki Murakoshi (n.murakoshi@md.tsukuba.ac.jp)
Type of cell line	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age: 49, Sex: Male, Ethnicity: Japanese
Cell source	Peripheral blood mononuclear cells
Clonality	Clonal
Associated disease	Dilated cardiomyopathy
Gene/locus	*LMNA*/1q22 Heterozygous *LMNA* c.801 T > A (p.Y267X)

(*continued on next column*)

(*continued*)

Date archived/stock date	October 2020
Cell line repository/bank	University of Tsukuba, Japan / RIKEN BioResource Research Center (BRC), Japan https://hpscreg.eu/cell-line/BRCi021-A
Ethical approval	Approved by the Clinical Research Ethics Review Committee of University of Tsukuba Hospital (No. R02-078)

3. Resource details

DCM is an intractable myocardial disorder characterized by dilation of the left ventricle and low contractile force (Weintraub et al., 2017). DCM increases the risk of developing heart failure and arrhythmias, with the 5-year survival rate in patients with DCM being approximately 50%. Although some causative genes have been identified, the underlying mechanism of DCM pathogenesis remains largely elusive. Hence, fundamental therapies for DCM have not been developed, except heart transplantation.

LMNA is a gene that encodes lamin A/C, a nuclear envelope protein, and accounts for 10–15% of hereditary DCM. It is the second leading causative gene, after titin (*TTN*), in patients with familial DCM. DCM patients with *LMNA* mutations exhibit a poorer prognosis than those with *TTN* mutations (Tobita et al., 2018). In addition, truncating

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1, panel A
Phenotype	Qualitative analysis (immunocytochemistry)	Positive for OCT3/4, NANOG, and SOX2	Fig. 1, panel B
	Quantitative analysis (flow cytometry)	TRA-1–60: 92.3%, SSEA-4: 98.7%	Fig. 1, panel C
Genotype	Virtual karyotyping (KaryoStat Assay)	46XY	Fig. 1, panel E
Identity	STR analysis	10 loci tested; all matched respective donor profile	Available with authors
Mutation analysis (IF APPLICABLE)	Sequencing and western blotting	c.801 T > A p.Tyr267*	Fig. 1, panel F and Fig. 1, panel G
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Expression of TUJ1 for ectodermal marker, SMA for mesodermal marker, and AFP for endodermal marker	Fig. 1, panel D
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer**	Ectoderm: TUJ1, Mesoderm: SMA, Endoderm: AFP	Fig. 1, panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

mutations in *LMNA* are associated with lower cardiac function and more severe symptoms, including malignant arrhythmia and cardiac conduction defects, than missense mutations in *LMNA* (Nishiuchi et al., 2017).

In this study, we established a DCM-specific hiPSC line from a 49-year-old male patient with an *LMNA*-truncating mutation. We generated this hiPSC line from the patient's peripheral blood mononuclear cells (PBMCs) using an episomal vector mix for reprogramming. The generated hiPSC line showed self-renewal, pluripotency (Table 1), and typical embryonic stem cell (ESC)-like morphologies (Fig. 1A). The expression of self-renewal markers, including OCT3/4, NANOG, and SOX2, was confirmed via immunocytochemistry (Fig. 1B). Flow cytometric analysis revealed high expression levels of SSEA-4 and TRA-1–60 (Fig. 1C). Pluripotency of the line was assessed using an embryoid body (EB) formation assay. The spontaneously differentiated EBs contained positive cells for ectodermal (tubulin beta III, TUJ1), mesodermal (smooth muscle actin, SMA), and endodermal (alpha-fetoprotein, AFP) markers (Fig. 1D). The virtual karyotyping assay did not detect any copy number changes in the whole genome of this hiPSC line (Fig. 1E). To examine the persistence of episomal vectors used for hiPSC generation, the EBNA1 included in episomal vectors was quantified via quantitative polymerase chain reaction (PCR). This line was demonstrated to contain low copy numbers of EBNA1 in genomic DNA. We confirmed the presence of the mutation by identifying exon 4 of the *LMNA* gene via sequencing (Fig. 1F). The same nonsense mutation, c.801 T > A (p.Tyr267*), was identified in this hiPSC line as the donor blood sample. The cell line was authenticated by STR analysis, and no mycoplasma contamination was detected via PCR analysis (Supplementary Fig. 1). Finally, we differentiated hiPSCs into cardiomyocytes (hiPSC-CMs) and examined the expression level of lamin A/C via western blotting. Two specific bands of lamin A/C were detected in blots corresponding to hiPSC-CMs derived from healthy individuals; however, such bands were absent in blots corresponding to hiPSC-CMs derived from TUH001. (Fig. 1G). These results indicated that the generated hiPSC line possessed proper hiPSC characteristics with an *LMNA* nonsense mutation.

4. Materials and methods

4.1. Reprogramming of PBMC and hiPSC culture

PBMCs were isolated from 10 mL of whole blood of the patient using Ficoll-Paque Plus (GE Healthcare) and cultured in EBM-2 Endothelial Cell Growth Medium supplemented with EGM-2 MV Microvascular Endothelial SingleQuots Kit (Lonza) by incubating them at 37 ℃ with 5% CO_2. After a few passages, the cells were reprogrammed using Human iPS Cell Generation Episomal Vector Mix (TaKaRa) and Neon Transfection System (Thermo Fisher Scientific). The PBMC suspension (100 μL, 1×10^7 cells/mL) was mixed with 3 μL of episomal vector mix, and the following condition of electroporation was performed: pulse voltage 1650 V, pulse width 10 msec, and pulse number 3. The cells were seeded on a 6-well plate coated with iMatrix-511 (Matrixome) and cultured in StemFit AK02N medium (Ajinomoto) supplemented with 10 μM Y-27632 (FUJIFILM Wako). After two weeks, individual ESC-like colonies were picked and seeded into new wells. Cells were passaged every 5–7 days with 0.5x TrypLE Select (Thermo Fisher Scientific), and the medium was changed every other day from the day after the passage. HPS3386, which was derived from a healthy individual and provided by the Cell Engineering Division, RIKEN BioResource Research Center, was used as a control hiPSC line.

4.2. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde (FUJIFILM Wako) at passage number 5. They were permeabilized with 0.3% Triton X-100 (Sigma-Aldrich) in PBS and blocked with 1% bovine serum albumin (BSA; Sigma-Aldrich). SOX2, OCT-3/4, and NANOG were detected with a pluripotency marker kit (R&D systems, listed in Table 2).

4.3. Flow cytometry

HiPSCs were fixed and permeabilized using the Foxp3/transcription factor staining buffer set (Thermo Fisher Scientific) at passage number 9. They were then incubated with antibodies for 45 min on ice. The antibodies used in this study are listed in Table 2. The stained cells were analyzed using FACSVerse (BD) and FlowJo software (v.10.6.1, Tree Star).

4.4. In vitro differentiation assay

The *in vitro* differentiation assay was performed as described in our previous study (Kuramochi et al., 2021) at passage number 9. The germ markers used are listed in Table 2.

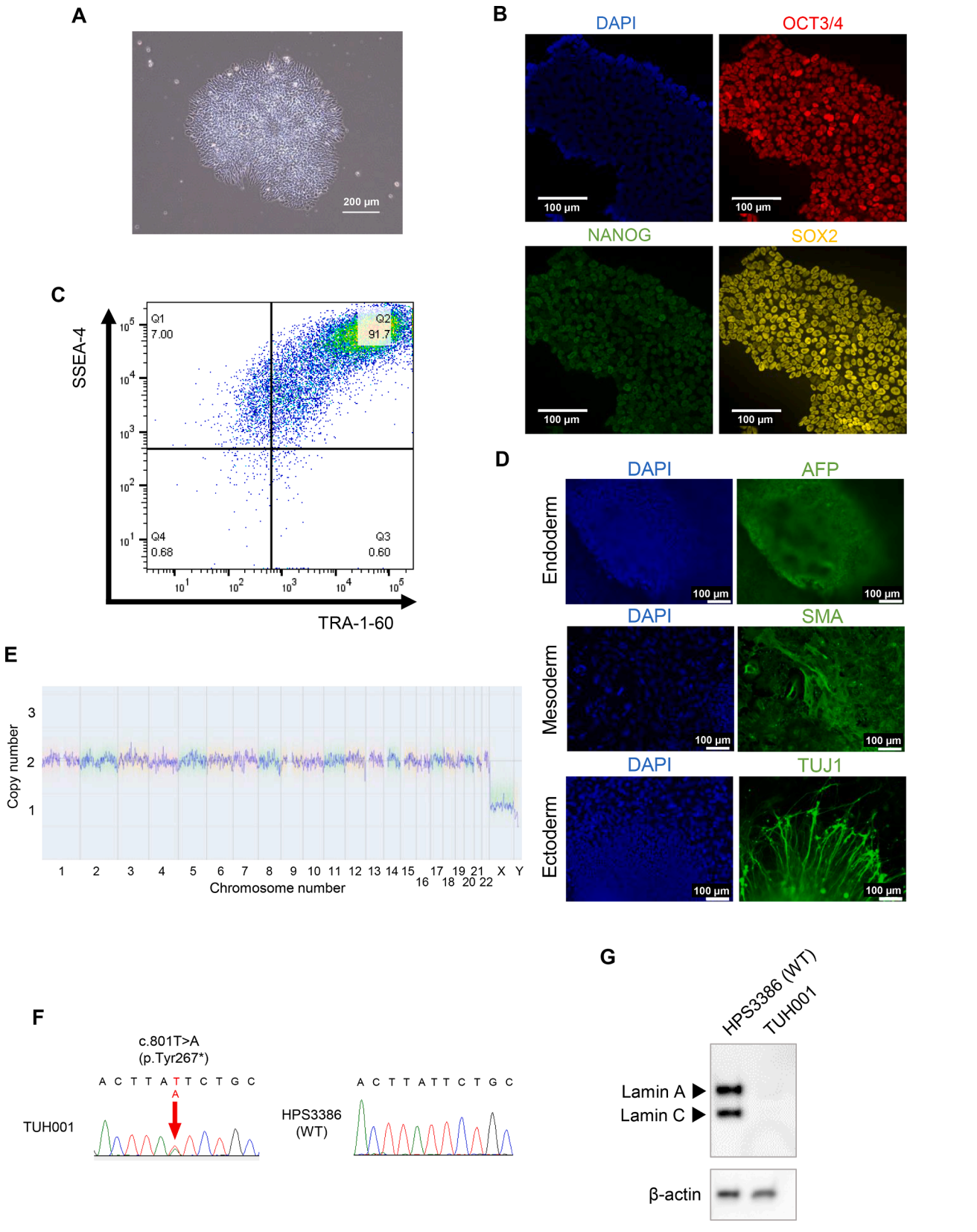

Fig. 1. The characterization of hiPSC line from a patient with LMNA-related dilated cardiomyopathy.

4.5. *Differentiation of hiPSC-CMs*

We modified the differentiation protocol, "GiWi method", (Lian et al., 2013) (Schemes in Supplementary Fig. 2 and Supplementary Table 1) for the differentiation of hiPSC-CM. In our protocol, the inhibitors, CHIR99021 (Sigma-Aldrich) and Wnt-C59 (Cayman Chemical), were used.

Table 2
Reagent details.

	Antibodies used for immunocytochemistry/flow cytometry/western blotting			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency marker	Human Pluripotent Stem Cell 3-Color Immunocytochemistry Kit (NL557-conjugated Goat anti-Human SOX2, NL637-conjugated Goat anti-Human OCT-3/4, and NL493-conjugated Goat anti-Human NANOG)	1:10	R&D systems Cat# SC021	N/A
Pluripotency marker	Vio488 anti-TRA-1–60	1:50	MiltenyiBiotec Cat# 130-106-872	RRID: AB_2654228
Pluripotency marker	PE anti-human SSEA-4	1:50	BioLegend Cat#330405	RRID: AB_1089207
Differentiation marker (ectoderm)	Mouse anti-TUJ1	1:250	R&D systems Cat# MAB1195	RRID: AB_357520
Differentiation marker (mesoderm)	Mouse anti-SMA	1:250	R&D systems Cat# MAB1420	RRID: AB_262054
Differentiation markers (endoderm)	Mouse anti-AFP	1:200	R&D systems Cat# MAB1368	RRID: AB_357658
Western blotting of lamin A/C	Rabbit anti-lamin A/C	1:1,000	Abcam Cat# ab108922	RRID: AB_10860619
Western blotting of beta-actin	Mouse anti-beta-actin	1:1,000	Santa Cruz Biotechnology Cat# sc-47778	RRID: AB_2714189
Secondary antibody for western blotting	Goat anti-rabbit IgG	1:10,000	Abcam Cat# ab6721	RRID: AB_955447
Secondary antibody for western blotting	Horse anti-mouse IgG	1:10,000	Cell Signaling Technology Cat# 7076	RRID: AB_330924
Secondary antibody for immunocytochemistry	Donkey anti-Mouse IgG Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21202	RRID: AB_141607

	Primers		
	Target	**Size of band**	**Forward/reverse primer (5′-3′)**
Episomal vector detection	EBNA1	542 bp	ATCAGGGCCAAGACATAGAGATG / GCCAATGCAACTTGGACGTT
Target mutation analysis/sequencing	*LMNA* Exon4	334 bp	GGCCTCCCAGGAACTAATTCTG / CTCCCTGCCACCATCTGC
Nested-PCR, 1st step PCR (MCGpF11/ MCGpR1)	Mycoplasma detection	350–850 bp	ACACCATGGGAG(C/T)TGGTAAT / CTTC(A/T)TCGACTT
Nested-PCR, 2nd step PCR (R16–2/ MCGpR21)	Mycoplasma detection	200–750 bp	(C/T)CAGACCCAAGGCAT GTG(C/G)GG(A/C)TGGATCACCTCCT / GCATCCACCA(A/T)A(A/T)AC(C/T)CTT

4.6. Western blotting

Proteins were extracted from hiPSC-CMs using PRO-PREP protein extraction solution (iNtRON Biotechnology). The proteins were loaded with Mini-PROTEAN TGX precast gels (4–20%, BIO-RAD) and transferred onto membranes using Trans-Blot Turbo PVDF transfer packs (BIO-RAD). The antibodies used for the detection of the respective proteins are listed in Table 2.

4.7. Mycoplasma tests

Mycoplasma tests, comprising DNA fluorescence staining and nested PCR, were performed as described in our previous study (Kuramochi et al., 2021). The cells were tested at passage number 9. The primers used are listed in Table 2.

4.8. Karyotyping

Genomic DNA was extracted from hiPSCs using the QIAamp DNA tissue kit (Qiagen) at passage number 5. KaryoStat assay arrays (Thermo Fisher Scientific) were used to detect copy number variation (CNV) in the whole genome, as described our previous study (Kuramochi et al., 2021).

4.9. STR analysis

Genomic DNA was extracted from hiPSCs and from the patient's blood samples using the QIAamp DNA blood midi kit (Qiagen) at passage number 5. They were subjected to STR analysis using the GenePrint10 system (Promega).

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102793.

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