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Lab resource: Stem Cell Line

Generation and characterization of a human iPSC line (UAMi004-A) from a patient with propionic acidemia due to defects in the *PCCB* gene

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A B S T R A C T

A human induced pluripotent stem cell (iPSC) line was generated from fibroblasts of a patient with propionic acidemia that has a homozygous mutation (c.1218_1231del14ins12 (p.G407 fs)) in the *PCCB* gene. Reprogramming factors *OCT3/4*, *SOX2*, *KLF4* and *c-MYC* were delivered using a non-integrative method based on the Sendai virus. Once established, iPSCs have shown full pluripotency, differentiation capacity and genetic stability. The generated iPSC line represents a useful tool to study the pathomechanisms underlying the deficiency.

Resource table.

Unique stem cell lines identifier	UAMi004-A
Alternative name of stem cell line	PCCB10-FiPS4F-1
Institution	Centro de Biología Molecular Severo Ochoa UAM-CSIC, Universidad Autónoma de Madrid, CIBERER, IDIPaz, Madrid, Spain.
Contact information of distributor	Eva Richard, erichard@cbm.csic.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 30 Sex: Female Ethnicity if known: Caucasian
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Propionic acidemia
Gene/locus	*PCCB* / 3q22
Method of modification	Non applicable
Name of transgene or resistance	Non applicable
Inducible/constitutive system	Non applicable
Date archived/stock date	March 2019
Cell line repository/bank	Spanish National Bank of Cell Lines http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml
Ethical approval	Patient informed consent obtained. Ethics Review Board-competent authority approval obtained (CEI 71–1278)

1. Resource utility

The pathophysiology of propionic acidemia is not completely understood and its treatment currently is not satisfactory. In order to investigate underlying cellular pathological mechanisms and to test potential therapeutic compounds, we have established an iPSC line with a homozygous mutation in the *PCCB* gene.

2. Resource details

Propionic acidemia (PA, MIM#606054, ORPHA:35) is one of the most frequent life-threatening organic acidemias, caused by mutations

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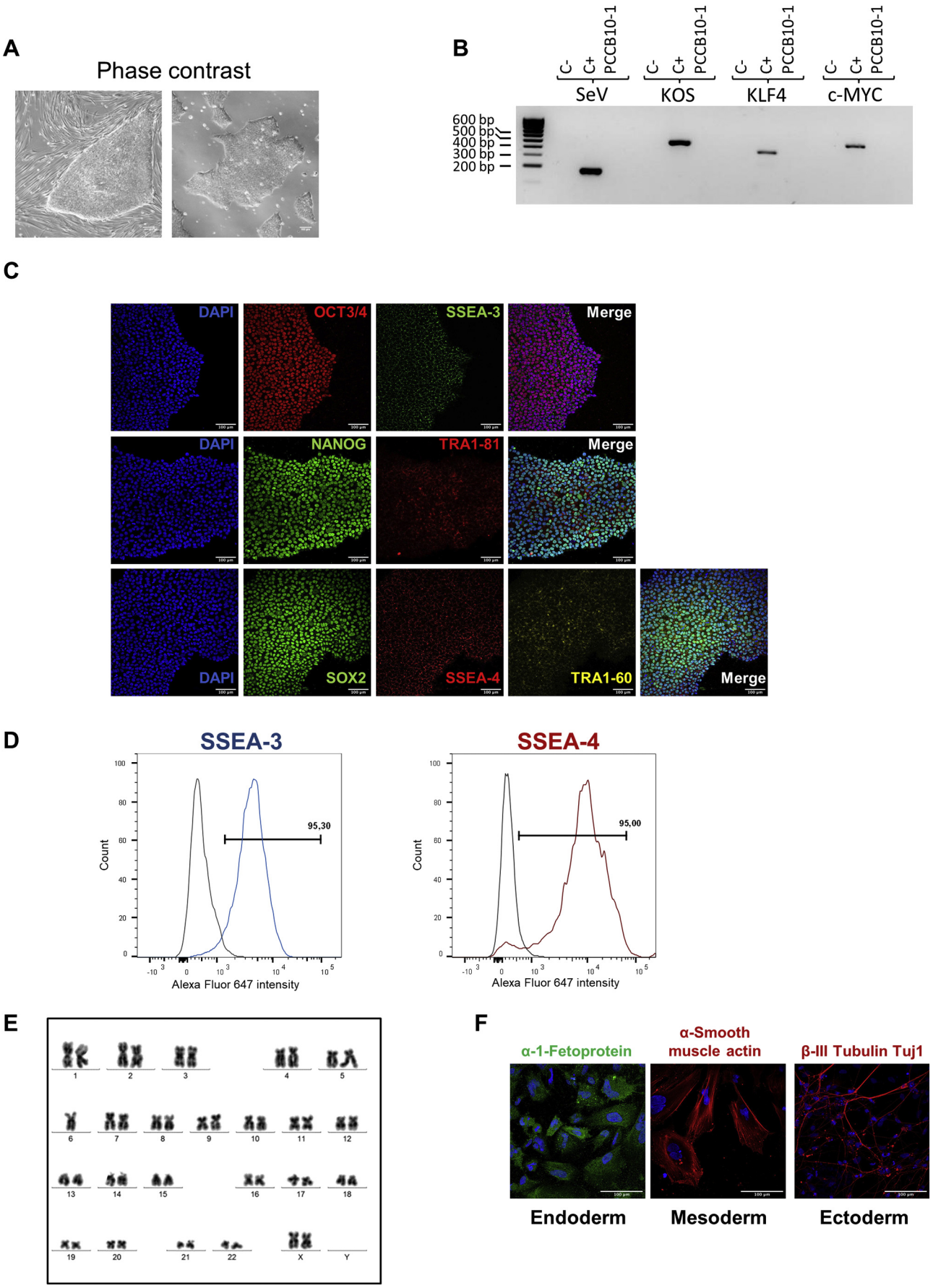

Fig. 1. Characterization of the iPSC line PCCB10-FiPS4F1 (UAMi004-A).

in either the *PCCA* or *PCCB* genes, encoding both subunits of the mitochondrial propionyl-CoA carboxylase (PCC) enzyme, that catalyzes the carboxylation of propionyl-CoA to D-methylmalonyl-CoA, which eventually enters the Krebs cycle as succinyl-CoA (Richard et al., 2015). Fibroblasts from a homozygous PA patient carrying the previously described c.1218_1231del14ins12 (p.G407 fs) mutation in the *PCCB* gene (Tahara et al., 1990) were reprogrammed using the CytoTune™ iPS Reprogramming kit delivering the four human reprogramming factors *OCT3/4*, *SOX2*, *c-MYC* and *KLF4* (Takahashi et al., 2007). The iPSC line PCCB10-FiPS4F1 (UAMi004-A) (PCCB10–1 in figures for short) displayed a typical round shape ESC-like morphology and growth behaviour on feeder layers and on feeder-free layers (Fig. 1A, Table 1). Clearance of the vectors and of the exogenous reprogramming factor genes was observed by RT-PCR after 8 culture passages (Fig. 1B). Expression of key pluripotency genes was observed at protein level by immunocytochemistry (transcription factors OCT4, NANOG and SOX2, and surface markers SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81) (Fig. 1C, Table 1) and flow cytometry analysis (Fig. 1D, Table 1). The iPSC line displayed a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1E). The cells had the capacity to form derivatives of all three germ layers (endoderm, mesoderm and ectoderm) upon embryoid body differentiation (Fig. 1F, Table 1). Mycoplasma testing by PCR revealed a negative result (Supplementary Fig. S1A). We confirmed the presence of the mutation in the iPSC line in homozygous fashion by Sanger sequencing (Supplementary Fig. S1B); and we also confirmed by DNA fingerprinting analysis that the line was derived from the patient fibroblasts.

3. Materials and methods

3.1. Non-integrative reprogramming of mutant PCCB fibroblasts into iPSC

The present study included available fibroblasts from a PA patient with defects in the *PCCB* gene. Experimental protocols were approved by the Institutional Ethical Committee of the Universidad Autónoma de Madrid according to Spanish and European Union legislation, and the informed consent was obtained from the legal care-givers. Fibroblasts were reprogrammed using the CytoTune™ iPS Reprogramming kit (ThermoFisher Scientific) following the manufacturer's instructions. iPSCs were maintained and expanded both on feeder layers and on feeder-free layers as previously described (Alonso-Barroso et al., 2017).

3.2. Detection of Sendai virus genome and transgenes

After 8 passages, iPSC line was tested for Sendai virus (SeV) residues as described (Alonso-Barroso et al., 2017). PCR was performed using the primers indicated in Table 2 and following the instructions as recommended by the manufacturer. In Fig. 1 panel B: C+: transduced cell pool at passage zero; C–: non-template control.

3.3. Immunofluorescence analysis

iPSC were grown on feeder-free layers on 15 μ-Slide 8 well culture plates (Ibidi) and fixed with Formaline Solution 10% (Sigma-Aldrich). Immunofluorescence analysis was performed as previously described (Alonso-Barroso et al., 2017). In Fig. 1 scale bars: 100 μm.

3.4. Flow cytometry analysis

We analysed the pluripotency-associated markers SSEA-3 and SSEA-4 by flow cytometry as described (Alonso-Barroso et al., 2017). Unstained iPSCs and the corresponding isotype antibodies were used as negative controls to exclude data from non-specific fluorescence.

3.5. In vitro differentiation

iPSCs were detached with Accutase™ into a single cell suspension and resuspended in mTESR™1 medium (StemCell™ Technologies) with 10 μM Rock inhibitor (StemCell™ Technologies). Embryoid body formation was induced by seeding 20,000–30,000 iPS cells in 120 μl of mTESR™1 medium in each well of 96-well v-bottom, low attachment plates (Deltalab). *In vitro* differentiation was performed as described (Alonso-Barroso et al., 2017).

3.6. Mycoplasma detection

Cells were screened for mycoplasma contamination by PCR (Uphoff & Drexler, 2014). A positive sample with mycoplasma was used as a control. In Supplementary Fig. S1A: positive control (C+).

3.7. Mutation analysis

Genomic DNA from patient-derived fibroblasts and iPSCs was isolated using MagNA Pure Compact DNA Isolation kit and MagNA Pure Compact instrument (Roche). Subsequently, amplification by PCR of the *PCCB* region containing the mutation was carried out using the primers indicated in Table 2, FastStart Taq DNA Polymerase (Roche), the Veriti Thermal Cycler (ThermoFisher Scientific) and the PCR program: 94 °C 5 min; 94 °C 25 s, 55 °C 25 s and 72 °C 40 s for 38 cycles; and 72 °C 7 min. Amplified PCR fragments were sequenced in an ABI3730 sequencer (Applied Biosystems).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for: OCT4, NANOG, SOX2, SSEA-3, TRA-1-81, SSEA-4 and TRA-1-60	Fig. 1 panel C
	Quantitative analysis (Flow cytometry)	SSEA-3 and SSEA-4: 95%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XX Resolution 450–500	Fig. 1 panel E
Identity	STR analysis	16 sites tested and all of them matched	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	c.1218_1231del14ins12 (p.G407fs)	Supplementary Fig. S1 panel B
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Supplementary Fig. S1 panel A
Differentiation potential	Embryoid body formation	Expression of α-1-fetoprotein (endoderm), α-smooth muscle actin (mesoderm) and β-III-tubulin Tuj1 (ectoderm)	Fig. 1 panel F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	No
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	No
	HLA tissue typing	Not performed	No

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse IgG anti-OCT4	1:60	Santa Cruz Cat# sc-5279, AB_628051
	Rat IgM anti-SSEA-3	1:3	Hybridoma Bank Cat# MC-631, AB_528476
	Rabbit IgG anti-SOX2	1:100	Fisher Thermo Scientific Cat# PA1–16968, AB_2195781
	Mouse IgG anti-SSEA-4	1:3	Hybridoma Bank Cat# MC-813-70, AB_528477
	Mouse IgM anti-TRA-1-60	1:200	Millipore Cat# MAB4360, AB_2119183
	Goat IgG anti-NANOG human	1:25	R&D Cat# AF1997, AB_355097
	Mouse IgM anti-TRA-1-81	1:200	Millipore Cat# MAB4381, AB_177638
Differentiation Markers	Rabbit IgG anti-α-Fetoprotein	1:400	Dako Cat# A0008, AB_2650473
	Mouse IgG anti-β-III-Tubulin Tuj1	1:500	Covance Cat# MMS-435P, AB_231377
	Mouse IgG anti-α-smooth muscle actin	1:400	Sigma-Aldrich Cat# A5228, AB_262054
Secondary antibodies	Alexa 555 Donkey anti-Mouse IgG	1:200	Thermo Fischer Cat# A-31570, AB_2536180
	Alexa 488 Goat anti-Rat IgM	1:200	Thermo Fischer Cat#A-21212, AB_2535798
	Alexa 488 Donkey anti-Rabbit IgG	1:200	Thermo Fischer Cat# A-31572, AB_162543
	Alexa 555 Donkey anti-Mouse IgG	1:200	Thermo Fischer Cat# A-31570, AB_2536180
	Alexa 647 Goat anti-Mouse IgM	1:200	Thermo Fischer Cat# A-21238, AB_2535807
	Alexa 647 Donkey anti-Goat IgG	1:200	Thermo Fischer Cat# A-21447, AB_2535864
	Cy3 Donkey anti-Mouse IgM	1:200	Jackson Cat# 715-165-140, AB_2340812
	Alexa 647 Goat anti-mouse IgG	1:600	Thermo Fischer Cat# A- 21235, AB_2535804
	Alexa 647 Donkey anti-rat IgM	1:600	Thermo Fischer Cat# A-21248, AB_2535816

Primers		
	Target	Forward/Reverse primer (5′-3′)
Reverse Transcription-PCR	*SeV* genome (181 pb)	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS transgene (528 bp)	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
	KLF4 transgene (410 bp)	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
	c-MYC transgene (532 bp)	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
Targeted mutation analysis/sequencing (PCR)	*PCCB-exons 11 and 12* (601 bp)	GGATGGCTGCTGAGGACAAA/TCCCACCACGGCTATGCTGTAG
Mycoplasma detection (PCR)	Mycoplasma species (986 bp: internal control band; and 520 bp: mycoplasma specific band)	Forward primers: CGCCTGAGTACGTTCGC CGCCTGAGTACGTACGC TGCCTGGGTAGTACATTCGC TGCCTGAGTAGTACATTCGC CGCCTGAGTAGTATGCTCGC CACCTGAGTAGTATGCTCGC CGCCTGGGTAGTACATTCGC Reverse primers: GCGGTGTGTACAAGACCCGA GCGGTGTGTACAAAACCCGA GCGGTGTGTACAAACCCCGA

3.8. Karyotype analysis

Karyotype analysis of the iPSC line was carried out using cells with more than twenty culture passages which were processed using standard cytogenetic techniques as described (Alonso-Barroso et al., 2017). At least 35 metaphases were karyotyped.

3.9. DNA fingerprinting analysis

DNA fingerprinting analysis was performed as previously described (Alonso-Barroso et al., 2017).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101469.

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