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Lab Resource: Multiple Cell Lines

Generation and characterization of a human iPSC line (UAMi005-A) from a patient with nonketotic hyperglycinemia due to mutations in the *GLDC* 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 nonketotic hyperglycinemia bearing the biallelic changes c.1742C > G (p.Pro581Arg) and c.2368C > T (p.Arg790Trp) in the *GLDC* 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. This cellular model provides a good resource for disease modeling and drug discovery.

Resource table

Unique stem cell lines identifier	UAMi005-A
Alternative name of stem cell line	GLDC27-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: died at 1 month of age Sex: Male Ethnicity if known: Caucasian
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Nonketotic hyperglycinemia
Gene/locus	*GLDC*/9p23-p24
Method of modification	Non applicable
Name of transgene or resistance	Non applicable
Inducible/constitutive system	Non applicable
Date archived/stock date	May 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-75-1372)

Resource utility

The establishment of an iPSC line with missense changes in the *GLDC* gene of a nonketotic hyperglicinemia patient will allow getting insights into the pathomechanisms of this genetic disease that provokes severe neurological alterations, and exploring new therapeutic options.

Resource details

Nonketotic hyperglycinemia (NKH, OMIM # 605899) is a rare genetic defect in glycine metabolism caused by a defective glycine

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cleavage activity primarily due to mutations in the *GLDC* gene encoding for the GCS P-protein, one of the three catalytic components of the mitochondrial multienzymatic complex. The deficiency provokes a considerable accumulation of glycine in all tissues, especially in central nervous system, leading a severe neurological phenotype whose pathomechanism is not completely understood, but probably involves an aberrant neurotransmission and proliferation disturbance. Here, we accomplish the reprogramming of fibroblasts from a previously described NKH-patient (Bravo-Alonso et al., 2017), bearing the *GLDC* mutations c.1742C > G (p.Pro581Arg) and c.2368C > T (p.Arg790Trp) 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 GLDC27-FiPS4F-1 (UAMi005-A) (GLDC27–1 in figures for short) exhibited classical embryonic stem cell morphology and growth behaviour on feeder layers and on feeder-free layers (Fig. 1A, Table 1). Clearance of the vectors and exogenous reprogramming factor genes was verified with reverse transcription polymerase chain reaction (RT-PCR) using specific primers with no virus present at passage 9 (Fig. 1B). Immunofluorescent staining (Fig. 1C, Table 1) and flow cytometry (Fig. 1D, Table 1) showed that the iPSCs expressed high levels of pluripotency associated markers (transcription factors OCT4, NANOG and SOX2, and surface markers SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81). The three-germ-layer differentiation capacities of the iPSCs were revealed with an *in vitro* assay based on embryoid bodies (EB) formation (Fig. 1E, Table 1). Mycoplasma testing by PCR revealed a negative result (Supplementary Fig. S1A). The iPSC line retained the *GLDC* mutations (Supplementary Fig. S1B) and normal karyotype (46, XY) after 22 passages in culture (Supplementary Fig. S1C, Table 1). We also confirmed by DNA fingerprinting analysis that the line was derived from patient-derived fibroblasts (Supplementary Fig. S1D, Table 1).

Materials and methods

Non-integrative reprogramming of mutant GLDC fibroblasts into iPSC

The present study included available fibroblasts from a NKH patient with defects in the *GLDC* gene, that were reprogrammed using the CytoTune™ iPS Reprogramming kit (ThermoFisher Scientific) following the manufacturer's instructions. The study was approved by the Institutional Ethical Committee (Universidad Autónoma de Madrid). Experimental methods were performed in accordance with the relevant guidelines and regulations, and the informed consent was obtained from the legal care-givers.

Cell culture

iPSCs were maintained and expanded on feeder layers as previously described (Alonso-Barroso et al., 2017). iPSCs were also adapted and cultured in feeder-free conditions on Matrigel® (Corning) using mTESR™1 medium (StemCell™ Technologies). In this case for the iPSC propagation, ReleSR™ (StemCell™ Technologies) was used when colonies were 70–80% confluent. In some cases, cells were detached with Accutase™ (StemCell™ Technologies) into a single cell suspension and resuspended in mTESR™1 medium with 10 μM Rock inhibitor (StemCell™ Technologies). iPSCs were maintained in a humidified incubator at 37 °C with 5% CO_2.

Detection of Sendai virus genome and transgenes

RT-PCR was performed using total RNA from the transduced cell pool at passage zero (positive control, C+ in Fig. 1 panel B) and from the iPSC line at passage 9 as described (Alonso-Barroso et al., 2017). Primers indicated in Table 2 and manufacturer's instructions were used to perform the PCR technique. In Fig. 1 panel B: C+: transduced cell pool at passage zero; C–: non-template control.

Immunofluorescence analysis

iPSC were seeded onto matrigel-coated 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) and images were obtained using a confocal laser scanning microscope Nikon A1R+. In Fig. 1 scale bars: 100 μm.

Flow cytometry analysis

We analysed the pluripotency-associated markers SSEA-3 and SSEA-4 by flow cytometry as described (Alonso-Barroso et al., 2017) in a BD FACSCanto™ A instrument (Becton Dickinson) using FACSDiva 8.0 software. Unstained iPSCs and the corresponding isotype antibodies were used as negative controls to exclude data from non-specific fluorescence.

In vitro differentiation

iPSCs were detached with Accutase™ into a single cell suspension and resuspended in mTESR™1 medium with 10 μM Rock inhibitor. EB 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). The generated EBs were collected on day 5, seeded onto matrigel-coated 15 μ-Slide 8 well culture plates (Ibidi) and differentiated into the three-germ layers for 2–3 weeks using the differentiation mediums as described (Alonso-Barroso et al., 2017).

Mycoplasma detection

PCR was used to test mycoplasma contamination (Uphoff and Drexler, 2014). A positive sample with mycoplasma was used as a control. In Supplementary Fig. S1A: positive control (C+).

Mutation analysis

Genomic DNA was extracted from patient-derived fibroblasts and iPSCs using MagNA Pure Compact DNA Isolation kit and MagNA Pure Compact instrument (Roche) followed by PCR amplification using FastStart Taq DNA Polymerase (Roche) and the specific primers indicated in Table 2. Amplifications were carried out on Veriti Thermal Cycler (ThermoFisher Scientific) using the following program: 94 °C 5 min; 94 °C 25 s, 55 °C 25 s and 72 °C 40 s for 38 cycles; 72 °C 7 min and 4 °C indefinite. Amplified PCR fragments were sequenced in an ABI3730 sequencer (Applied Biosystems).

Karyotype analysis

Karyotype analysis of the iPSC line was carried out using cells at 22 culture passage which were processed using standard cytogenetic

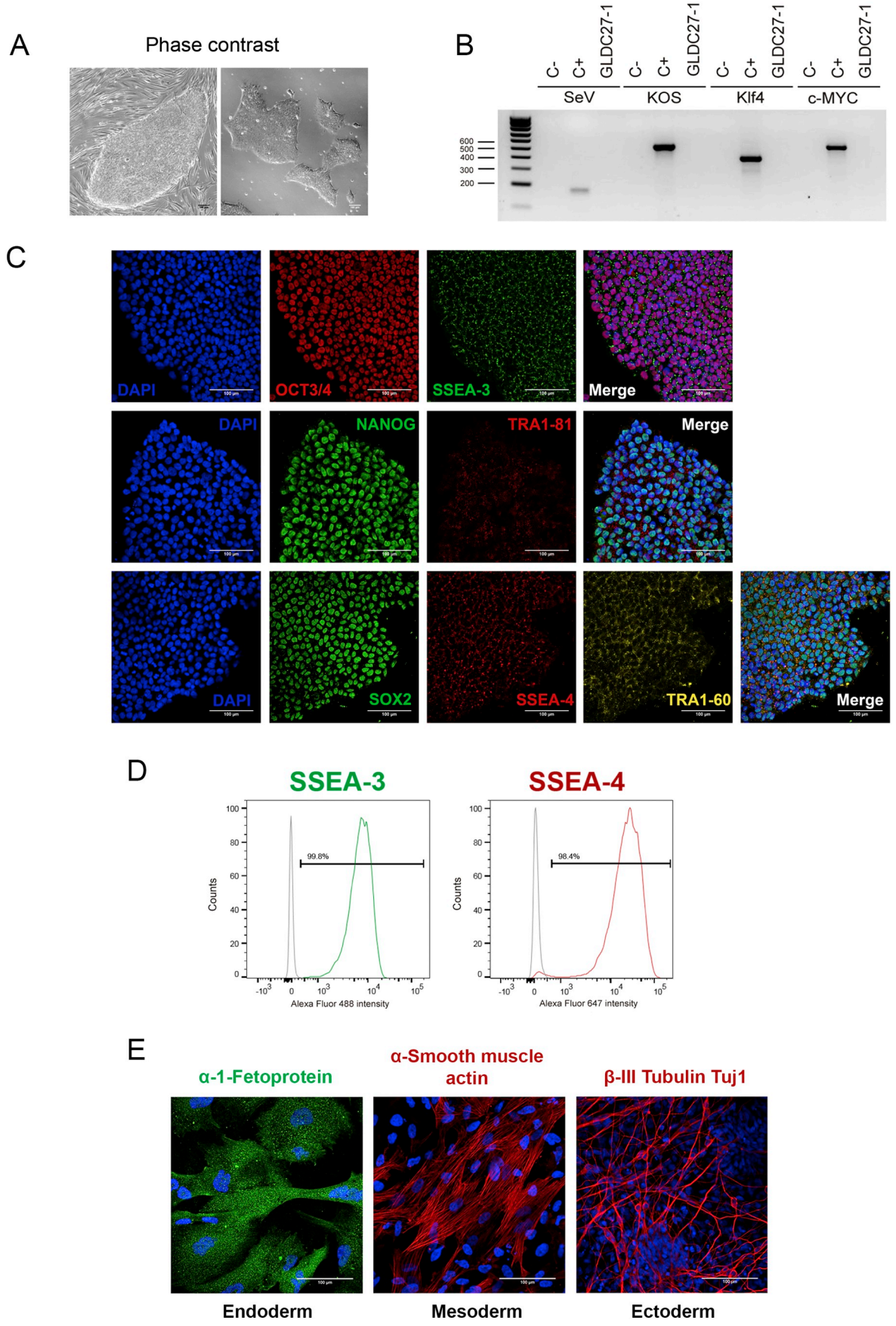
A
Phase contrast
B
C-
C+
GLDC27-1
SeV
KOS
Klf4
c-MYC
600
500
400
300
200
C
DAPI
OCT3/4
SSEA-3
Merge
NANOG
TRA1-81
SOX2
SSEA-4
TRA1-60
D
SSEA-3
SSEA-4
99.8%
98.4%
Counts
Alexa Fluor 488 intensity
Alexa Fluor 647 intensity
E
α-1-Fetoprotein
α-Smooth muscle actin
β-III Tubulin Tuj1
Endoderm
Mesoderm
Ectoderm

Fig. 1.

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: 99.8% SSEA-4: 98.4%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XY Resolution 450–500	Supplementary Fig. S1 panel C
Identity	STR analysis	16 sites tested and all of them matched	Supplementary Fig. S1 panel D
Mutation analysis (IF APPLICABLE)	Sequencing	c.1742C > G (p.Pro581Arg) c.2368C > T (p.Arg790Trp)	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 E
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

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)	*GLDC-exon 15* (500 bp)	TGTCCCACTTTTAAATGAAGACAG/ACACGATGCACCTTCTAGCC
	GLDC-exon 20 (373 bp)	AAGAACAAGGCTTTCTGGGAG/CATCAGCAATATTCTTTGAACCAC
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

techniques as described (Alonso-Barroso et al., 2017). At least 20 metaphases were karyotyped.

DNA fingerprinting analysis

DNA fingerprinting analysis was performed as previously described at Parque Científico de Madrid, Campus Moncloa, UCM, Madrid, Spain. (Alonso-Barroso et al., 2017).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101503.

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