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Lab resource: Stem Cell Line

Generation and characterization of the human iPSC line CABi001-A from a patient with retinitis pigmentosa caused by a novel mutation in PRPF31 gene

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A B S T R A C T

PRPF31 gene codes for a ubiquitously expressed splicing factor but mutations affect exclusively the retina, producing the progressive death of photoreceptor cells. We have identified a novel PRPF31 mutation in a patient with autosomal dominant retinitis pigmentosa. A blood sample was obtained and mononuclear cells were reprogrammed using the non-integrative Sendai virus to generate the cell line CABi001-A. The iPSC line has been characterized for pluripotency and differentiation capacity and will be differentiated toward photoreceptors and retinal pigment epithelium cells to study the molecular mechanism of the disease and test possible therapeutic strategies.

Resource table

Unique stem cell line identifier	CABi001-A
Alternative name(s) of stem cell line	OF0081-PRPF31c3
Institution	Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain.
Contact information of distributor	Berta de la Cerda; berta.delacerda@cabimer.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 70 Sex: Female Ethnicity: European descent
Cell Source	Peripheral blood mononucleated cells
Clonality	Clonal
Method of reprogramming	hOCT3/4, hSOX2, hc-MYC, and hKLF4 *via* non-integrative Sendai virus.
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Autosomal Dominant Retinitis pigmentosa
Gene/locus	PRPF31 c.165G > A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	05.04.2017
Cell line repository/bank	hpsreg.eu/cell-line/CABi001-A
Ethical approval	Cellular Reprogramming Board of Andalucía. Ethical Approval number: PR_01_2015

Resource utility

PRPF31 codes for a splicing factor, but mutations in this gene only affect the retina, causing autosomal dominant retinitis pigmentosa (Waseem et al., 2007). Differentiation to retinal cell types from CABi001-A will provide appropriate cellular models for the study of the molecular mechanism of the disease.

Resource details

A patient was diagnosed with retinitis pigmentosa and the associated genetic defect was identified as PRPF31 heterozygous mutation c.165G > A by molecular inversion probes (Weisschuh et al., 2018); and then verified by Sanger sequencing. After informed consent, a

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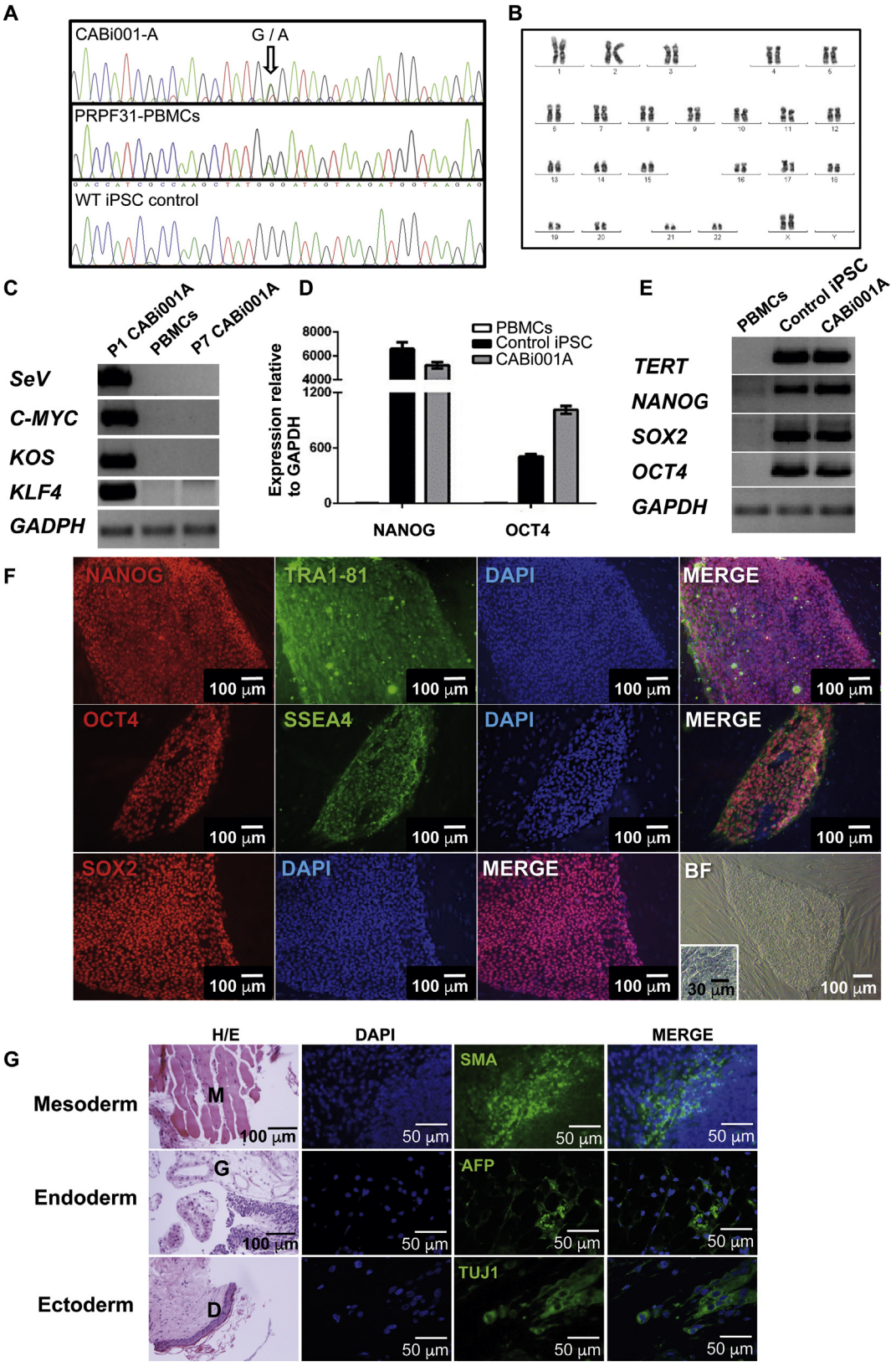

Fig. 1. Characterization of CABi001-A. A) Sanger sequencing of the mutation region in the iPSC line, primary PBMCs and a healthy control. B) Normal human karyotype. C) Clearance of viral genes on passage 7 compared to passage 1 and to PBMCs. D) Quantification of expression of NANOG and OCT4 pluripotency genes compared to an iPSC control and to PBMCs. E) Gene expression of pluripotency markers. F) Immunofluorescence staining for pluripotency markers and bright field image of a typical colony. G) Identification of derivatives of the three germ layers generated in a teratoma. H/E images show muscle (M), gland (G) and dermal tissue (D) and IF shows staining of actin smooth muscle (ASM), alpha fetoprotein (AFP) and beta 3 tubulin (TUJ1) as markers of mesoderm, endoderm and ectoderm, respectively.

blood sample was obtained and primary culture of peripheral blood mononuclear cells (PBMCs) was established. The reprogramming factors hOCT3/4, hSOX2, hc-MYC, and hKLF4 (Takahashi et al., 2007) were transduced into the primary PBMCs *via* the non-integrative Sendai virus following manufacturer instructions. We obtained the induced pluripotent stem cell (iPSC) line OF0081-PRPF31c3 (registered as CABi001-A at www.hPSCreg.com) with the typical ES-like growth and morphological features, including polygonal shape, refractive edges and high nuclear/cytoplasmic ratio (Fig. 1F). To characterize the cell line, we performed Sanger sequencing of the genomic region covering the mutation in PRPF31 gene, shown in Fig. 1A compared to a healthy control and primary PBMCs; checked for normal female karyotype (Fig. 1B) and for identity of the cell line by DNA fingerprinting matching with the gDNA of the parental PBMCs (available with the authors). Seven passages after reprogramming, clearance of the viral particles was checked by absence of gene expression corresponding to the viral capsid and the ectopic reprogramming factors compared to iPSC in passage 1 as positive control for viral expression and PBMCs as negative control (Fig. 1C). To check pluripotent state of CABi001-A, the expression level of endogenous pluripotency markers NANOG and OCT4 was quantified by qPCR relative to the parental PBMCs. An already characterized WT iPSC line was used as positive control (Fig. 1D). Qualitative assessment of a panel of pluripotency markers was analyzed by rt-PCR, using the same positive and negative control samples as for the qPCR (Fig. 1E). Immunostaining for pluripotency markers and a bright field image to assess the morphology of the colonies are also shown (Fig. 1F). Differentiation capacity was tested *in vivo* by teratoma assay (Fig. 1G) showing histological features of the three germ layers, labeled as M for muscle, D for dermal tissue and G for gut-like epithelium in the haematoxilin-eosin images (H/E). Additionally, Fig. 1G shows positive immunostaining for endodermal marker α-fetoprotein (AFP), ectodermal marker III β-tubulin (TUJ1) and mesodermal marker actin smooth muscle (ASM). Characterization is summarized in Table 1.

Materials and methods

Mutation sequencing

Genomic DNA from peripheral blood mononuclear cells (PBMCs) and iPSCs was isolated using the QIAamp DNA Blood mini kit (Qiagen). Primers used for amplification and Sanger sequencing of PRPF31 flanking the mutation site are described in Table 2.

Peripheral blood mononuclear cells (PBMCs) primary culture

Ficoll-Plaque method was used to purify PBMCs from 4 mL of peripheral blood using Vacutainer CPT tubes (BD) and cultured in expansion medium (EM: QBSF-60 Stem Cell (Quality Biological) with 50 μg/ml of ascorbic acid (Sigma-Aldrich); 50 ng/ml of SCF (Stemcell Technologies); 10 ng/ml IL-3 (Stemcell Technologies); 2 U/mL of EPO (Stemcell Technologies); 40 ng/ml IGF-1 (Stemcell Technologies); 1 μM Dexamethasone (Sigma-Aldrich) and 1% Pen/strep (Gibco)).

Reprogramming PBMCs to iPSC

PBMCs were transduced with reprogramming factors using the CytoTune®-IPS Reprogramming Kit (Life Technologies) by 24 h incubation with the virus. After washing, cells re-suspended in EM were cultured for 48 h. 10^5 cells/well were plated on feeders (human fetal foreskin fibroblasts (ATCC CRL2429) inactivated by 45 Gy irradiation) in QBSF-60 Stem Cell (Quality Biological) with 50 μg/ml of ascorbic acid (Sigma-Aldrich) and 1% pen/strep (Gibco). After appearance of the first colonies on day 15, half the medium was replaced with hESC medium (Knock Out DMEM-F12 (Gibco); 20% Knock Out serum (Gibco); 1% glutamine (Gibco); 1% non-essential amino acids (Gibco); 0.23 mM β-mercaptoethanol (Gibco); 1% pen-strep (Gibco) and 10 ng/ul bFGF (Peprotech)). Colonies, including clone 3, which has been named CABi001-A, were picked on day 21. Clones were seeded on feeders and maintained in hESC medium with change of medium three-times a week. IPSCs were mechanically passaged once a week with a 1:3 split ratio. All cell culture was performed in standard cell incubators at 37 °C, 5% CO_2, 20% O_2.

RT-PCR and qPCR for detection of viral clearance and pluripotency markers

Total RNA was isolated from cultured iPSCs with RNeasy Mini Kit (Qiagen). 1 μg of total RNA was used as template to obtain cDNA, using QuantiTect Reverse Transcription Kit (Qiagen). RT-PCR reaction was performed using MyTaq DNA Polymerase (Bioline GmbH) with the primers listed in Table 2 using a MyCycler™ Thermal Cycler System 1709703 (Bio-Rad) with the program: 35 cycles of 94 °C for 15 s, 58 °C for 45 s and 72 °C for 45 s (for GAPDH, 30 cycles). For qPCR a CFX96 Realtime PCR detection system and C1000 thermal cycler (Biorad) was used with a program: 95 °C for 1 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Triplicate reactions were prepared with TaqMan Gene expression Master Mix and TaqMan probes: NANOG (Hs02387400g1), OCT4 (Hs01654807s1) and GADPH (Hs02786624g1), all from Applied Biosystems.

Immunofluorescence analysis

Cells grown in glass coverslips were washed with PBS, fixed in 4% PFA, for 15 min, washed twice in PBS and placed in blocking solution (2% donkey serum in TPBS: 0.2% Triton-X100/PBS) for 1 h at room temperature. Primary antibody incubation was for 1 h at room temperature in 1% BSA in TPBS, then washed 3 times in TPBS, and incubated with secondary antibody diluted in 1% BSA in TPBS for 1 h at room temperature. After 3 washes, preparations were finished with mounting medium with DAPI. Imaging was performed in a confocal microscope Leica TCS SP5. Antibodies are listed in Table 2.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Visual record of the line: normal*	*Fig. 1F*
Phenotype	Qualitative analysis	*Staining for pluripotency markers: OCT4, NANOG, SOX2, TRA 1–81, SSEA-4. Expression of TERT, NANOG, SOX2, OCT4*	*Fig. 1E and1F*
	Quantitative analysis	*qPCR for NANOG and OCT4*	*Fig. 1D*
Genotype	Karyotype (G-banding) and resolution	*46XX, Resolution 450–500*	*Fig. 1B*
Identity	STR analysis	*10 sites tested. All matched*	*Available with the authors*
Mutation analysis	Sequencing	*Heterozygous PRPF31. Chr:19: 542183 c.165G > A*	*Fig. 1A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by PCR. Negative*	*Supplementary Fig. 1*
Differentiation potential	Teratoma formation	*Finding of muscle (mesoderm), dermal tissue (ectoderm) and gut-like epithelium (endoderm) in HE stained sections and positive immunostaining for mesodermal muscle actin, endodermal α-feto protein and ectodermal β-tubulin.*	*Fig. 1G*

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	*Rabbit anti-OCT4*	*1:400*	*Cell Signaling Technology Cat# 2840, RRID: AB_2167691*
	Rabbit anti-NANOG	*1:400*	*Cell Signaling Technology Cat# 4903, RRID:AB_10559205*
	Rabbit anti-SOX2	*1:400*	*Cell Signaling Technology Cat# 3579, RRID:AB_2195767*
	Mouse anti-SSEA4	*1:100*	*BD Biosciences Cat# 560073, RRID: AB_1645601*
	Mouse anti-TRA-1-81	*1:100*	*Stemgent Cat# 09-0069, RRID: AB_2119069*
Differentiation Markers	*Rabbit anti-PAX6*	*1:500*	*Covance Cat# PRB-278P, RRID: AB_291612*
	Mouse anti-TUJ1	*1:2000*	*Covance Cat# MMS-435P, RRID :AB_2213773*
	Mouse anti-SMA	*1:300*	*Sigma-Aldrich Cat# A5228, RRID: AB_262054*
	Mouse anti- AFP	*1:20*	*R & D Systems Cat# MAB1368, RRID: AB_357658*
	Rabbit anti-DESMIN	*1:100*	*Lab Vision Cat# RB-9014-P0, RRID: AB_149768*
Secondary antibodies	*Donkey Anti-Rabbit 594*	*1:500*	*Thermo Fisher Scientific Cat# A-21207, RRID: AB_141637*
	Donkey Anti-Mouse 488	*1:500*	*Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607*
Primers			
	Target, size (bp)	Forward/Reverse primer (5′-3′)	
Pluripotency Markers	*NANOG, 260*	*CCAAATTCTCCTGCCAGTGAC/CACGTGGTTTCCAAACAAGAAA*	
(rt-PCR)	*OCT4, 165*	*AAGCCCTCATTTCACCAGG/CTTGGAAGCTTAGCCAGGTC*	
	SOX2, 181	*TCACATGTCCCAGCACTACC/CCCATTTCCCTCGTTTTTCT*	
	TERT, 259	*GCGTTTGGTGGATGATTTCT/GGCATAGCTGGAGTAGTCGC*	
House-Keeping Gene (rt-PCR)	*GAPDH, 86*	*ACGACCCCTTCATTGACCTCAACT/ ATATTTCTCGTGGTTCACACCCAT*	
Pluripotency markers (qPCR)	*NANOG*	Hs02387400g1	
	OCT4	Hs01654807s1	
House-keeping gene (qPCR)	*GADPH*	Hs02786624g1	
SeV genome silencing	*SeV, 181*	*GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC*	
	KOS, 528	*ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG*	
	KLF4, 410	*TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA*	
	cMYC, 532	*TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG*	
Targeted mutation analysis/sequencing	*PRPF31, 355*	*ATGTCTCTGGCAGATGAGCTCTT/CTT CTGAAG CTTTGGCTTGCTTG*	

Differentiation potential

For one teratoma assay, approximately 10^5 iPSCs were mechanically picked, dissociated in small clumps, collected in 50 µL of Matrigel (BD) and kept on ice until injection. A hypodermic insulin syringe was used to inject the cells subdermally in the dorsal area of an adult SCID nude mouse. After 6 weeks, animal was sacrificed and the teratoma was dissected, subjected to PFA fixation and histological processing into 20 µm cryosections. Eosin-haematoxylin staining was used to identify structures corresponding to the three germ layers. Images were taken on a Leica DM6000B microscope. Immunostaining for markers of the three germ layers was also performed with antibodies listed in Table 2 and imaged in a Leica DM6000B fluorescence microscope.

Karyotype analysis

On passage 12, 30 metaphases were counted and genome integrity was analyzed by G-banding at 400–550 band resolution in Biobanco del SSPA, Granada, Spain.

Fingerprinting

Genomic DNA from PBMCs and iPSCs were extracted using QIAamp DNA Blood mini kit (Qiagen) with RNAse (Roche). STR analysis was performed in Biobanco del SSPA, Granada, Spain using the GenePrint® 10 System (Promega) to check STRs for AMEL, CSF1PO, D13S317, D16S539, D21S11, D5S818, D7S820, TH01, TPOX and vWA.

Mycoplasma detection

The presence of mycoplasma was tested by PCR using the EZ-PCR™ Mycoplasma Detection Kit (Bioline) in which contamination is seen as the amplification of a band of approximately 500 bp, and absence of mycoplasma as absence of amplification (Supplementary Fig. 1).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101426.

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