Stem Cell Research 16 (2016) 673–676

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of a human iPSC line from a patient with an optic atrophy 'plus' phenotype due to a mutation in the *OPA1* gene

Teresa Galera-Monge [a,b,c,d], Francisco Zurita-Díaz [a,b,c,d], Ana Moreno-Izquierdo [d,e], Mario F. Fraga [f], Agustin F. Fernández [g], C. Ayuso [c,h], Rafael Garesse [a,b,c,d], M. Esther Gallardo [a,b,c,d,*]

[a] Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
[b] Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Madrid, Spain
[c] Centro de Investigación Biomédica en Red (CIBERER), Madrid, Spain
[d] Instituto de Investigación Hospital 12 de Octubre ("i + 12"), Madrid, Spain
[e] Servicio de Genética, Hospital 12 de Octubre, Madrid, Spain
[f] Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Spain
[g] Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA), HUCA, Universidad de Oviedo, Oviedo, Spain
[h] Department of Genetics, Instituto de Investigación Sanitaria-University Hospital Fundacion Jimenez Diaz (IIS-FJD, UAM), Madrid, Spain

ARTICLE INFO

Article history:
Received 14 March 2016
Accepted 25 March 2016
Available online 13 April 2016

ABSTRACT

Human iPSC line Oex2054SV.4 was generated from fibroblasts of a patient with an optic atrophy 'plus' phenotype associated with a heterozygous mutation in the *OPA1* gene. Reprogramming factors OCT3/4, SOX2, CMYC and KLF4 were delivered using a non-integrative methodology that involves the use of Sendai virus.

Resource table

Name of stem cell line	Oex2054SV.4
Institution	Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Facultad de Medicina, Universidad Autónoma de Madrid and Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER) Madrid, Spain. Instituto de Investigación Hospital 12 de Octubre ("i+12"), Madrid, Spain.
Person who created resource	Teresa Galera-Monge
Contact person and email	M. Esther Gallardo, egallardo@iib.uam.es
Date archived/stock date	November 1, 2015
Origin	Human skin cells
Type of resource	Biological reagent: induced pluripotent stem cells (iPSC) from a patient with an optic atrophy 'plus' phenotype due to a mutation in the *OPA1* gene
Sub-type	Cell line
Key transcription factors	OCT3/4, SOX2, CMYC, KLF4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	None
Information in public databases	None

Resource details

The generation of the human iPSC line, Oex2054SV.4, was carried out using non-integrative Sendai viruses containing the reprogramming factors, *OCT3/4*, *SOX2*, *CMYC*, *KLF4* (Takahashi et al., 2007). For this purpose, fibroblasts from a patient with an optic atrophy 'plus' phenotype were obtained (Amati-Bonneau et al., 2008). The patient's fibroblasts carried a heterozygous mutations in the *OPA1* gene (c.1861C>T; p.Gln621Ter). The presence of this mutation in the iPSC line was evaluated and confirmed by Sanger sequencing (Fig. 1A). Oex2054SV.4 iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B) and they stained positive for alkaline phosphatase activity (Fig. 1C). We confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after eight culture passages (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors *OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1* was also evaluated by RT-PCR (Fig. 1E). Immunofluorescence analysis revealed expression of transcription factors *OCT4*, *NANOG*, *SOX2* and surface markers *SSEA3*, *SSEA4*, *TRA1-60* and *TRA1-81* characteristics of pluripotent ES cells (Fig. 1F). Promoters of the pluripotency associated genes, *OCT4* and *NANOG*, heavily methylated in the original fibroblasts were almost demethylated in the Oex2054SV.4 line suggesting an epigenetic reprogramming to pluripotency (Fig. 1G). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XY) after more than twenty culture passages (Fig. 1H). We also confirmed by DNA fingerprinting analysis that the line Oex2054SV.4 was derived from the patient's fibroblasts (Fig. 1I). Finally,

* Corresponding author at: Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid, Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Avda Arzobispo Morcillo s/n 28029, Madrid, Spain.
E-mail addresses: egallardo@iib.uam.es, esther.gallardo@ciberer.es (M.E. Gallardo).

http://dx.doi.org/10.1016/j.scr.2016.03.011

the capacity of the generated iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) was tested *in vitro* using an embryoid body based assay (Fig. 1J).

Materials and methods

Non-integrative reprogramming of mutant OPA1 fibroblasts into iPSC

All the experimental protocols included in the present study were approved by the Institutional Ethical Committee of the Autonoma University of Madrid according to Spanish and European Union legislation. Human fibroblasts from a patient presenting with a severe mitochondrial encephalopathy associated with mutations in the *OPA1* gene were used. For this purpose tissue fragments were obtained from a skin biopsy of this patient after informed consent. These fragments were directly plated in a dish with DMEM high glucose with 10% defined fetal bovine serum (Hyclone), 2 mM GlutaMAX and 1X Penicillin-Streptomycin (Life Technologies). After 3–4 weeks, fibroblasts outgrowing from the biopsy pieces will cover most of the dish and were expanded at 37 °C in 5% CO_2 until the second passage. Subsequently, these fibroblasts were reprogrammed using the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer. After eight passages of the iPSC line, silencing of the exogenous reprogramming factors genes and sendai virus genome was confirmed by RT-PCR following the manufacturer's instructions. Oex2054SV.4 was maintained and expanded both on feeder layers and on feeder-free layers. In the first case, irradiated human fibroblasts feeders with ES medium containing: Knockout DMEM (Life technologies), Knockout serum replacement 20%, (Life technologies), MEM non-essential amino acids solution 1X (Life technologies), GlutaMAX 1X (Life technologies), β-mercaptoethanol (100 μM), penicillin/streptomycin 1X (Life technologies) and bFGF (4ng/ml) (Miltenyi Biotec) were used. Subsequently, Oex2054SV.4 was adapted and cultured in feeder-free conditions on matrigel (354277, Corning) with mTeSR1 medium (StemCell) following the recommendations of the manufacturer. For the propagation of the line, both enzymatic (dispase, collagenase IV and accumax) and mechanical procedures have been used.

Phosphatase alkaline analysis

The iPSC line Oex2054SV.4 was seeded on a feeder layer plate. After seven days direct phosphatase alkaline activity was determined using the phosphatase alkaline blue membrane substrate solution kit (Sigma, AB0300) following the instructions of the manufacturer.

Mutation analysis

Total DNA from the patient's fibroblasts and iPSCs was extracted using a standard phenol-chloroform protocol. Subsequently, amplification by PCR of the *OPA1* gene region containing the c.1861C>T mutation was carried out using the following primers: (OPA1-20F: 5′-TATTATAA GTTAATGATACTTCAG-3′ and OPA1-20R: 5′-GTAGCAACATTAAGGTTA GTC-3′). Following PCR amplification, direct sequencing of amplicons was performed on both strands in an ABI 3730 sequencer (Applied Biosystems; Foster City, CA) using a dye terminator cycle sequencing kit (Applera, Rockville, MD).

qPCR analyses

Total mRNA was isolated using TRIZOL and 1 μg was used to synthesize cDNA using the Quantitect reverse transcription cDNA synthesis kit. One microliter of the reaction was used to quantify by qPCR the expression of the endogenous pluripotency associated genes (*OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1*). Primers sequences were described by Aasen et al., 2008. All the expression values were normalized to the *GAPDH* housekeeping gene. Plots are representative of at least three independent experiments.

Bisulfite pyrosequencing

Bisulfite modification of genomic DNA was performed with the EZ DNA Methylation-Gold kit (Zymo Research) following the manufacturer's instructions. The set of primers for PCR amplification and sequencing of *NANOG* and *OCT4* were designed using the software PyroMark Assay Design (version 2.0.01.15; Qiagen): Forward-*NANOG* (5′-TAT TGG GAT TAT AGG GGT GGG TTA-3′), Reverse-*NANOG* (5′-[Btn]-CCC AAC AAC AAA TAC TTC TAA ATT CAC-3′), and sequencing primer S-*NANOG* (5′-ATA GGG GTG GGT TAT-3′); Forward-*OCT4*_prox (5′-GGG GTT AGA GGT TAA GGT TAG TG-3′), Reverse-*OCT4*_prox (5′-[Btn]-ACC CCC CTA ACC CAT CAC-3′), and sequencing primer S-*OCT4*_prox (5′-GGG GTT GAG TAG TTT-3′); Forward-*OCT4*_dist (5′-TTT TTG TGG GGG ATT TGT ATT GA-3′), Reverse-*OCT4*_dist (5′-[Btn]-AAA CTA CTC AAC CCC TCT CT-3′), and sequencing primer S-*OCT4*_dist (5′-ATT TGT ATT GAG GTT TTG GA-3′). PCR was performed with primers biotinylated to convert the PCR product to single-stranded DNA templates, using the Vacuum Prep Tool. After PCR amplification, pyrosequencing reactions and methylation quantification were performed using PyroMark Q24 reagents, equipment and software (version 2.0.6; Qiagen), according to the manufacturer's instructions.

Karyotype analysis

Karyotype analyses of the iPSC line were carried out using cells with more than twenty culture passages. These cells were processed using standard cytogenetic techniques. Briefly, cells were treated with 10μg/ml of Colcemid (Gibco) for 90 min at 37 °C, trypsinized, treated with 0.075 M hypotonic KCl solution, and fixed with Carnoy's fixative. Cells were then dropped on a microscope glass slide and dried. Metaphase cells were G banded using Wright staining. At least 20 metaphases were karyotyped.

Immunofluorescence analysis

Cells were grown on 0.1% gelatin-coated P35 culture plates (81156, Ibidi) and fixed with 4% paraformaldehyde. The following antibodies for the staining were used: TRA-1-60 (Millipore; MAB4360; 1:150); TRA-1-81 (Millipore; MAB4381; 1:150); SOX2, (Thermo Scientific; PA116968; 1:100); NANOG (R&D Systems; AF1997; 1:25); SSEA-4 (Millipore; MAB4304; 1:10); SSEA-3 (Millipore; MAB4303; 1:10); OCT4 (Santa Cruz Biotechnology; Sc-5279; 1:100); neuron-specific class III beta-tubulin (Tuj1) (Sigma, T8660, 1:300), α-fetoprotein (AFP) (Sigma, WH0000174M1, 1:300), smooth muscle alpha actin (SMA) (Sigma, A2547, 1:400). Secondary antibodies used were all from the Alexa Fluor Series from Jackson Immunoresearch (all 1:500). Images were taken using a Zeiss confocal microscope.

In vitro differentiation assay

The *in vitro* pluripotency capacity of the iPSC line was tested by spontaneous embryoid body differentiation. For this purpose, iPSCs from a P100 plate with 80% of confluency were dissociated into a single cell suspension with accumax (SCR006,Millipore) and resuspended in 12 ml of mTeSR1 medium (Stemcell). Embryoid body formation was induced by seeding 120 μl of the iPSC suspension in each well of 96-well v-bottom low attachment plates and centrifuging the plates at 800 g for 10 min to aggregate the cells. After 2–3 days the embryoid bodies were transferred to an untreated P60 culture plate for 2–4 days. Subsequently, the embryoid bodies were transferred to 0.1% gelatin-coated P35 culture plates (81156, Ibidi) and cultured in differentiation medium (DMEM F12 supplemented with 20% fetal bovine serum, 2 mM glutamine, 0.1 mM β-mercaptoethanol, 1X non essential