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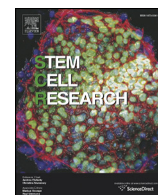
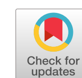


Lab Resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line (CSC-40) from a Parkinson's disease patient with a *PINK1* p.Q456X mutation



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A R T I C L E I N F O

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A B S T R A C T

Parkinson's disease (PD) is a neurodegenerative disease with unknown etiology. Here we show the generation of an induced pluripotent stem cell (iPSC) line, named CSC-40, from dermal fibroblasts obtained from a 59-year-old male patient with a homozygous p.Q456X mutation in the PTEN-induced putative kinase 1 *(PINK/PARK6)* gene and a confirmed diagnosis of PD, which could be used to model familial PD. A non-integrating Sendai virus-based delivery of the reprogramming factors OCT3/4, SOX2, c-MYC and KLF4 was employed. The CSC-40 cell line showed normal karyotyping and fingerprinting following transduction as well as sustained expression of several pluripotency markers and the ability to differentiate into all three germ layers.

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Resource table

Unique stem cell line identifier	ULUNDi001-A
Alternative name(s) of stem cell line	CSC-40F
Institution	Stem Cell Laboratory for CNS Disease Modeling, Department of Experimental Medical Science, Lund University
Contact information of distributor	Laurent Roybon; Laurent.Roybon@med.lu.se
Type of cell line	iPSC
Origin	Human
Additional origin info	Age of patient at onset: 36 Sex of patient: male Ethnicity: N/A
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus transduction with OCT3/4, SOX2, c-MYC, and KLF4
Genetic Modification	No modification
Type of Modification	No modification
Associated disease	Parkinson's disease
Gene/locus	*PINK1* (MIM # 608309) located on the chromosome 1p36.12 Genotype: c.1366C–T transition in exon 7 (p.Q456X substitution)
Method of modification	No modification
Name of transgene or resistance	No transgene or resistance
Inducible/constitutive system	Not inducible
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	Parkinson Institute Biobank (part of the Telethon Genetic Biobank Network http://biobanknetwork.telethon.it/): approved by Ethics Committee “Milano Area C” (http://comitatoeticoareac.ospedaleniguarda.it/) on the 26/06/2015, Numero Registro dei pareri: 370–062015. Reprogramming: 202,100–3211 (delivered by Swedish work environment Arbetsmiljöverket).

Resource utility

The mutation in the *PINK1* gene, encoding the phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1) protein, was

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identified in early-onset recessive Parkinson's disease (PD) in 2001 (Valente et al., 2001). The CSC-40 induced pluripotent stem cell (iPSC) line, with a mutation in the *PINK1* gene, allows investigations of familial PD cells for disease modeling and potential therapeutic explorations.

Resource details

The PINK1 protein together with PARKIN can initiate the ubiquitination and removal of the damaged mitochondrion (Geisler et al., 2010). Mutation in *PINK1* is linked to early-onset recessive PD

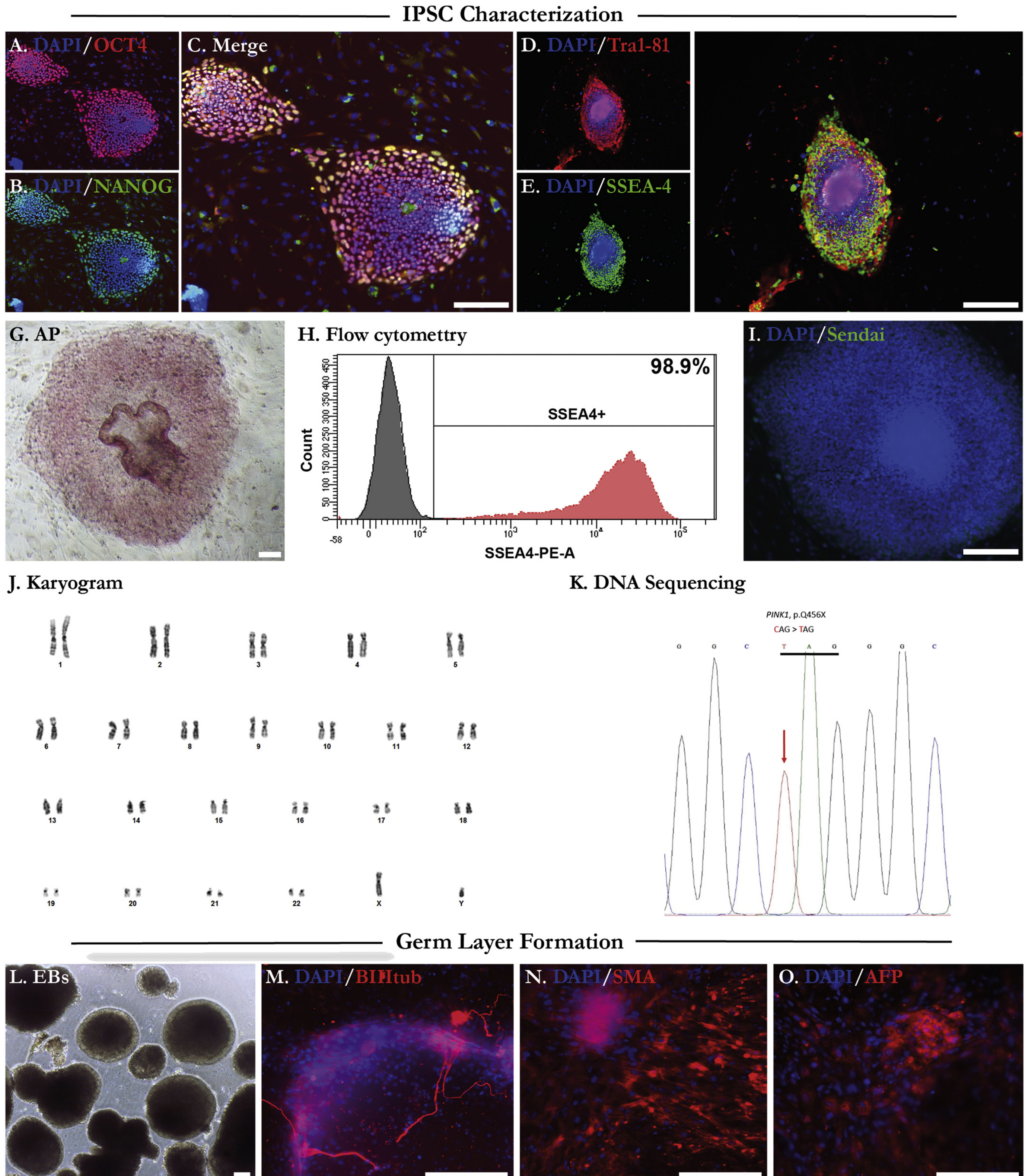

Fig. 1. Characterization of the iPSC line CSC-40F.

(Valente et al., 2001). Here we report the generation of a novel iPSC line, named CSC-40, from dermal fibroblasts obtained from a 59-year-old male PD patient with a homozygous p.Q456X mutation in *PINK1*.

Reprograming of patient fibroblasts was done using non-integration Sendai virus to deliver the reprograming factors OCT3/4, SOX2, c-MYC and KLF4 to the cells. Three clones (CSC-40F, CSC-40I and CSC-40G) were selected based on the morphology of the colonies, and characterized as previously described (Holmqvist et al., 2016). Here, we report the characterization of the clone CSC-40F.

The generated CSC-40F line stained positive for the common nuclear and cell surface pluripotency markers, OCT4, NANOG, TRA1-81 and SSEA4 (Fig. 1A–F). Flow cytometry analysis showed that >98% of the iPSCs were positive for SSEA4 (Fig. 1H; non-stained iPSCs are shown in grey). An alkaline phosphatase staining further confirmed the pluripotency of CSC-40F iPSC line (Fig. 1G). The CSC-40F line was clear of Sendai virus by passage 7 (Fig. 1I) and showed normal karyotyping (Fig. 1J). Genetic integrity was confirmed by fingerprint analysis of isolated DNA from both patient fibroblasts and CSC-40F iPSC line, while the presence of the homozygous p.Q456X mutation was detected by DNA sequencing (Fig. 1K). Finally, CSC-40F iPSC line was cultured as embryoid bodies (EBs) and then plated onto a 96-well plate to demonstrate its ability to differentiate into each of the three germ layers, in vitro. Immunocytochemistry revealed the expression of ectodermal marker beta-III-tubulin (BIIItub), the mesodermal marker smooth muscle actin (SMA), and the endodermal marker alpha-fetoprotein (AFP; Fig. 1L–O). Plasmocin was used during the reprogramming and early passages as prophylactic dose to prevent Mycoplasma (Table 1).

Materials and methods

Collection and reprogramming of patient's fibroblasts

Induced PSCs were generated from human dermal fibroblasts, harvested using a skin punch biopsy, from patient with a confirmed clinical diagnosis of PD. Patient fibroblasts were cultured and expanded in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin (v/v, all ThermoFisher Scientific) at 37 °C and 5% CO_2 for several passages before being cryopreserved and stored at −150 °C.

For reprogramming, fibroblasts were thawed and plated on a 0.1% gelatin-coated 12-well plate at a concentration of 75,000 per well. Two days after, the cells were transduced with OCT3/4, SOX2, c-MYC and KLF4 reprogramming factors using CytoTune™-iPS 2.0 Sendai reprogramming kit according to manufacturer's instructions (ThermoFisher Scientific). Fibroblast growth media was changed every other day for 7 days following transduction. At day 7, the fibroblasts were passaged using 0.05% trypsin-EDTA (Sigma-Aldrich) and plated onto irradiated mouse embryonic fibroblasts feeder cells (CF-1 MEF, GlobalStem) with WiCell media composed of advanced DMEM/F12 (Thermo Fisher Scientific), 20% Knock-Out Serum Replacement (v/v, ThermoFisher Scientific), 2 mM L-glutamine (Thermo Fisher Scientific), 1% non-essential amino acids (NEAA, v/v, Millipore), 0.1 mM β-mercaptoethanol (Sigma-Aldrich) and 20 ng/ml FGF2 (ThermoFisher Scientific). At day 28, single colonies were manually picked and re-seeded separately onto a fibroblast feeder cell-coated 12-well plate. Individual clones were passaged using dispase (ThermoFisher Scientific) weekly with media changed daily. Cells were then frozen in WiCell media and freezing media (20% DMSO + FBS 80%) 1:1 with 10 μM Rock inhibitor (Sigma-Aldrich) and stored at −150 °C.

Alkaline phosphatase activity

Alkaline phosphatase staining was completed using the Alkaline Phosphatase Staining Kit according to manufacturer's protocol (Stemgent, MA).

Karyotyping

The G-banding analysis was performed at 300–400 band resolution in average after 14 passages at the Department of Clinical Genetics and Pathology in Lund.

Mutation sequencing and fingerprint analysis

The mutation p.Q456X in *PINK1*, was confirmed by direct DNA sequencing (Macrogen Europe, Amsterdam, The Netherlands) following DNA isolation using a lysis buffer (100 mM Tris (pH 8.0), 200 mM NaCl, 5 mM EDTA, and 0.2% SDS in distilled and autoclaved water supplemented with 1.5 mg/ml Proteinase K). Primers used for amplification and directed sequencing of *PINK* around the mutation site are listed in the Table 2. To confirm the genetic integrity of the DNA between patient fibroblasts and iPSCs, DNA was isolated and sent to IdentiCell STR profiling service (Department of Molecular Medicine, Aarhus University Hospital, Denmark).

In vitro differentiation

For spontaneous differentiation, iPSCs were grown as EBs for 2 weeks on ultra low-attachment 24-well plates (Corning) with WiCell media. At the end of 2 weeks, EBs were dissociated and plated onto adherent 96-well plates (Greiner Bio-One) coated with 0.1% gelatin (Millipore) in DMEM supplemented with 10% fetal bovine serum and

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Not shown but available with author
Phenotype	Immunocytochemistry	Positive staining for pluripotency markers OCT4, NANOG, TRA1–81, SSEA4.	Fig. 1 panel A-F
	Flow cytometry	98.9% SSEA4	Fig. 1 panel H
	Alkaline phosphatase activity	Visible activity	Fig. 1 panel G
Genotype	Karyotype (G-banding) and resolution	46,XY, (300–400 bands resolution in average)	Fig. 1 panel J
Identity	STR analysis	10 sites analyzed, all matched with fibroblasts from the original donor	Available with author
Mutation analysis	Sequencing	homozygous p.Q456X mutation in *PINK1*	Fig. 1 panel K
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative.	Not shown but available with author
Differentiation potential	Embryoid body formation	Positive staining for smooth muscle actin, beta-III-tubulin and alpha-fetoprotein after spontaneous differentiation of embryoid bodies	Fig. 1 panel L-O
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

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:200	Millipore Cat# MAB4401, RRID:AB_2167852
	PE-conjugated mouse anti-human NANOG	1:200	BD Biosciences Cat# 560483, RRID:AB_1645522
	Mouse anti- TRA-1-81	1:200	Thermo Fisher Scientific Cat# 41–1100, RRID:AB_2533495
	PE-conjugated mouse anti-SSEA4	1:200	Thermo Fisher Scientific Cat# A14766, RRID:AB_2534281
Sendai	Chicken anti-Sendai virus	1:1000	Abcam Cat# ab33988, RRID:AB_777877
Differentiation Markers	Mouse anti-AFP	1:200	Sigma-Aldrich Cat# A8452, RRID:AB_258392
	Mouse anti-SMA	1:200	Sigma-Aldrich Cat# A2547, RRID:AB_476701
	Mouse anti-beta-III tubulin	1:200	Sigma-Aldrich Cat# T8660, RRID:AB_477590
Secondary antibodies	Donkey anti-mouse Alexa Fluor® 488	1:400	Molecular Probes Cat# A-21202, RRID:AB_141607
	Donkey anti-chicken Alexa Fluor® 488	1:400	Jackson ImmunoResearch Labs Cat# 703-545-155, RRID:AB_2340375
	Donkey anti-mouse Alexa Fluor® 555	1:400	Thermo Fisher Scientific Cat# A-31570, RRID:AB_2536180

Primers

	Target	Forward/Reverse primer (5′–3′)
Mutation sequencing	*PINK1*	TGGATCAGGTGATGTGCAGGA/ AGGATCTGTCACTGTGGCTCT

1% Penicillin-Streptomycin. iPSCs were cultured for 2 additional weeks with media changed every 2 days before being stained with antibodies against BIIItub, SMA, and AFP.

Immunocytochemistry

For immunocytochemical analysis, iPSCs were fixed with 4% PFA for 10 min at RT followed by standard immunocytochemical procedures. Blocking was done in 10% donkey serum in PBS with 0.1% Triton-X100 (Sigma-Aldrich) for 1 h at RT. Primary antibodies, listed in the Table 2, were then added to the cells and incubated overnight at 4 °C. Next the secondary antibodies, listed in the Table 2, were added and incubated for 1 h at RT in the dark. Additionally, nuclei were stained by DAPI (1:10,000; Life Technologies). All fluorescent photomicrographs were taken using an inverted epifluorescence microscope (LRI - Olympus IX-73). Scale bars are 200μm.

Mycoplasma detection

Absence of mycoplasma contamination was confirmed by the real-time PCR method at GATC Biotech AG (European Genome and Diagnostics Centre, Konstanz, Germany).

Acknowledgements

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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.2018.01.001.

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