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Lab Resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line (CSC-42) from a patient with sporadic form of Parkinson's disease

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ABSTRACT

Skin fibroblasts were collected from a 44-year-old patient with sporadic case of Parkinson's disease (PD). The non-integrating Sendai virus vector encoding OCT3/4, SOX2, c-MYC and KLF4 was used to reprogram fibroblasts into induced pluripotent stem cells (iPSCs). Generated iPSCs had normal karyotypes, expressed common stem cell markers, and were capable of differentiating into all three germ layers. Generated line could be used for PD modeling to understand the mechanisms that influence the disorder.

Resource table

Unique stem cell line identifier	ULUNDi004-A
Alternative name(s) of stem cell line	CSC-42L
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: 38 Sex of patient: female Ethnicity: N/A
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus mediated delivery of OCT3/4, SOX2, c-MYC and KLF4
Genetic Modification	No modification
Type of Modification	No modification
Associated disease	Parkinson's disease
Gene/locus	N/A
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 sporadic Parkinson's disease (PD) is the second most frequent degenerative disorder of the human nervous system (Braak and Del Tredici, 2009). Generated induced pluripotent stem cell (iPSC) line can be utilized for in vitro disease modeling to study the mechanisms underlying Parkinson's disease (PD).

Resource details

Fibroblasts from a 44-year-old female patient with sporadic form of PD were reprogrammed through introduction of OCT3/4, SOX2, c-MYC and KLF4 factors. Delivery of the pluripotency-inducing factors was

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achieved using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. At day 28, colonies were manually picked and expanded clonally. Three clones (CSC-42I, CSC-42K, CSC-42L) were further selected based on the morphology of the colonies, for characterization using the methods we previously described in Holmqvist et al., 2016. Here, we present the characterization of clone CSC-42L. Pluripotency of generated cells was confirmed by immunocytochemistry using staining for OCT4, NANOG, TRA1–81 and SSEA4 markers (Fig. 1A) as well as by demonstration of alkaline phosphatase (ALP) activity in cultured cells (Fig. 1B). Flow cytometry analysis revealed that >99% of the iPSCs were positive for SSEA4 (Fig. 1C; non-stained iPSCs are shown in grey). The clearance of the virus was confirmed by staining against Sendai after 7 passages (Fig. 1D). G-banding analysis showed a normal karyotype (Fig. 1E). The genetic fingerprinting showed genetic identity of CSC-42 L line to parental fibroblasts. In addition, CSC-42 L iPSCs cultured on non-adherent surfaces were able to form embryoid bodies (EBs) and subsequently differentiate into all three embryonic germ layers, in vitro, as detected by the presence of lineage specific markers such as ecto dermal

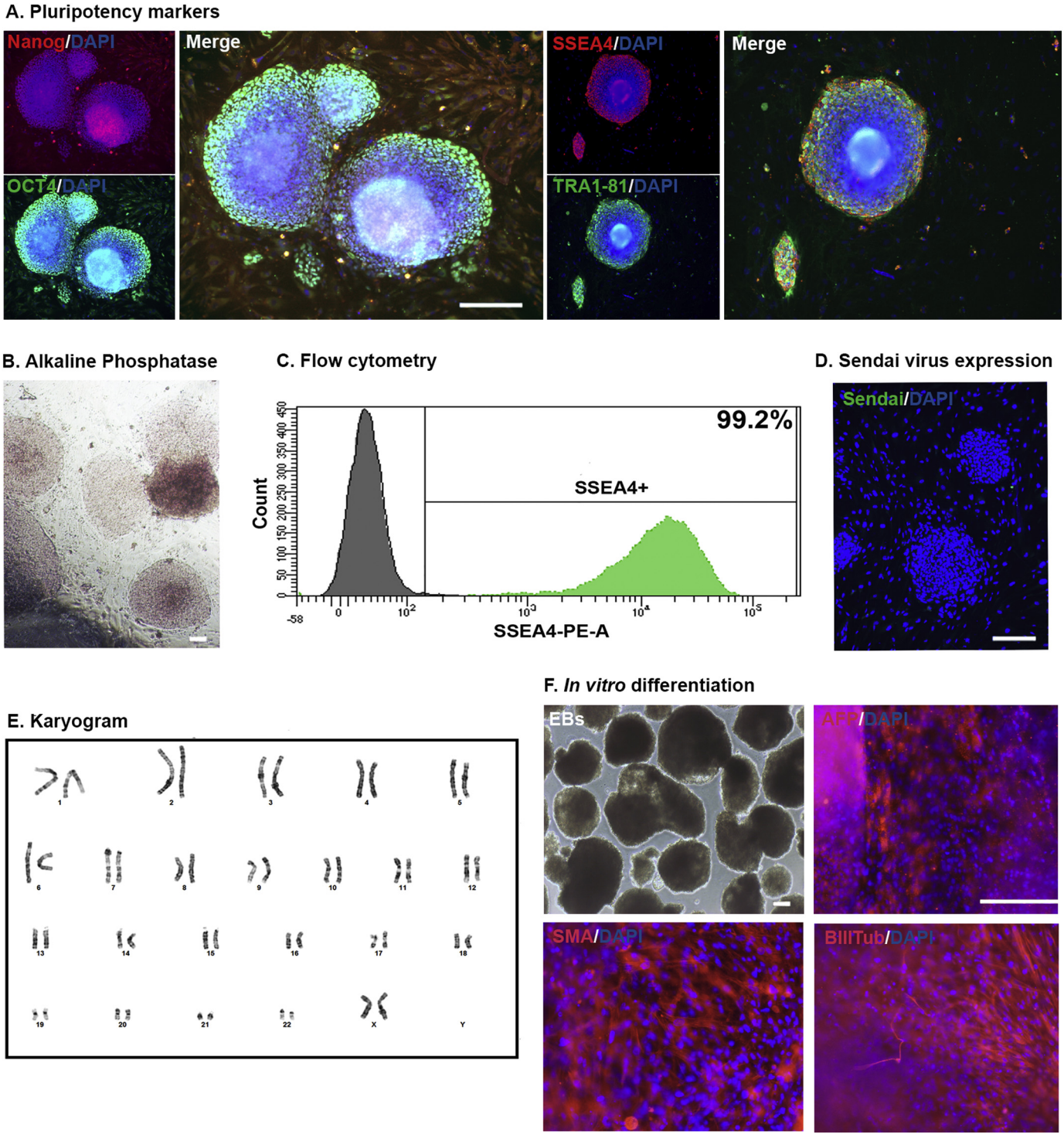

Fig. 1. Characterisation of the iPSC line CSC-42.

marker beta-III-tubulin (BIIITub), the mesodermal marker smooth muscle actin (SMA) and the endodermal marker alpha-fetoprotein (AFP) (Fig. 1F). Mycoplasma infection was prevented by routine addition of plasmocin in cell culture media.

Materials and methods

Fibroblast culture

The skin biopsy sample was collected from a 44-year-old patient with a confirmed sporadic form of PD, after obtaining informed consent. After collection, fibroblasts were cultured in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum and 1% Penicillin-Streptomycin and passaged with 0.05% trypsin.

iPSC generation

Human fibroblasts were reprogrammed to iPSCs through introduction of OCT3/4, SOX2, c-MYC and KLF4. For this purpose, cells were plated on a 12-well plate at a density of 75,000 cells per well. After two days of incubation cells were transduced with Sendai virus containing four reprogramming factors mentioned above using CytoTune™-iPS 2.0 Sendai reprogramming kit (ThermoFisher Scientific). After transduction, cell media was changed every second day. At day 7, the cells were collected and re-plated onto irradiated mouse embryonic fibroblasts (MEF) feeder cells in DMEM media containing 10% fetal bovine serum and 1% Penicillin-Streptomycin. On the day after and until the day 28, the cells were cultured in WiCell medium containing advanced DMEM/F12 (ThermoFisher Scientific), 20% Knock-Out Serum Replacement (v/v, ThermoFisher Scientific), 2 mM l-glutamine (ThermoFisher Scientific), 1% non-essential amino acids (NEAA, v/v, Millipore), 0.1 mM β-mercaptoethanol (Sigma-Aldrich), and 20 ng/ml FGF2 supplemented daily (ThermoFisher Scientific). On day 28, single colonies were picked and seeded on a MEF-coated 24-well plate. A week after, three separate clones were randomly selected for further expansion. Once a week cells were cryopreserved or re-plated on the appropriate cell culture surface for further analysis.

Immunocytochemistry

For immunocytochemistry, iPSC cells were fixed with 4% paraformaldehyde (PFA), permeabilized and blocked with solution containing 0.1% TritonX-10 (Sigma) and 10% donkey serum in PBS for 1 h at room temperature (RT). Then, the cells were incubated overnight at +4 °C with the primary antibodies (Table 2) diluted in the blocking solution. Next day, the cells were washed with PBS and incubated for 1 h at RT in the dark with secondary antibodies diluted in PBS. DAPI (Life Technologies) was used to counterstain nuclei (1:10,000). The staining was visualized using a fluorescence microscope LRI - Olympus IX-73. Scale bars are 200 μm (Table 1).

Alkaline phosphatase activity

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

Embryoid body (EB) formation

For in vitro embryoid body formation, iPSCs were plated on low-attachment 24-well plates in WiCell medium supplemented with 20 ng/ml FGF2 allowing them to grow for 2 weeks as EBs. After that, EBs were collected, dissociated and plated onto 96-well plate in DMEM media containing 10% fetal bovine serum and 1% Penicillin-Streptomycin for 2 more weeks. Then the cells were harvested, fixed and stained for markers against three germ layers (Table 2).

Karyotype analysis

Karyotype analysis was performed at 300–400 band resolution in average after 10 passages using G-banding technique at the Department of Clinical Genetics and Pathology in Lund.

DNA fingerprinting

Genomic DNA was extracted from fibroblasts and iPSCs using conventional lysis buffer composed of 100 mM Tris (pH 8.0), 200 mM NaCl, 5 mM EDTA and 0.2% SDS in distilled autoclaved water supplemented with 1.5 mg/ml Proteinase K. DNA fingerprinting was performed by IdentiCell STR profiling service at the Department of Molecular Medicine, Aarhus University Hospital, Skejby, Denmark.

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).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal	Not shown but available with author
Phenotype	Immunocytochemisty	Positive staining for pluripotency markers: OCT4, NANOG, TRA1-81 and SSEA4	Fig. 1 panel A
	Alkaline phosphatase activity	Visible activity	Fig. 1 panel B
	Flow cytometry	99.2% SSEA4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XX (300–400 bands resolution in average)	Fig. 1 panel E
Identity	STR analysis	10 sites analyzed, all matched with parent fibroblast cell line	Available with author
Mutation analysis (IF APPLICABLE)	N/A	N/A	N/A
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 F
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

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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.2018.01.002.

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