Lab resource: Stem Cell Line

Generation of an integration-free induced pluripotent stem cell line (CSC-43) from a patient with sporadic Parkinson's disease

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ARTICLE INFO

ABSTRACT

An induced pluripotent stem cell (iPSC) line was generated from a 36-year-old patient with sporadic Parkinson's disease (PD). Skin fibroblasts were reprogrammed using the non-integrating Sendai virus technology to deliver OCT3/4, SOX2, c-MYC and KLF4 factors. The generated cell line (CSC-43) exhibits expression of common pluripotency markers, *in vitro* differentiation into three germ layers and normal karyotype. This iPSC line can be used to study the mechanisms underlying the development of PD.

Resource table

Unique stem cell line identifier	ULUNDi005-A
Alternative name(s) of stem cell line	CSC-43 J
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: 31 Sex of patient: male 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

(continued)

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

This iPSC line can be used to explore the development of sporadic Parkinson's disease *in vitro*.

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Resource details

Dopaminergic neurons obtained from the differentiation of sporadic Parkinson's disease (PD) patient derived - induced pluripotent stem cells (iPSCs) have been shown to express a pathogenic phenotype, including morphological alterations and accumulation of autophagic vacuoles (Sánchez-Danés et al., 2012). Here we report the generation of an induced pluripotent stem cell line (CSC-43) from a patient with early-onset PD with no identified genetic cause, which can be used to understand the molecular basis of this neurodegenerative disease.

To generate CSC-43 iPSC line, skin fibroblasts collected by punch skin biopsy from a 36-year-old PD patient were reprogrammed using a non-integrating Sendai virus technology. Briefly, fibroblasts were seeded (75,000 cells/well) on a 12-well plate, two days before transduction. The CytoTune™-iPS 2.0 Sendai Reprogramming Kit was then used to deliver the four reprogramming factors, OCT3/4, SOX2, c-MYC and KLF4. At day 7 post-transduction, the cells were re-seeded onto mouse embryonic fibroblasts (MEF)-feeder layer and expanded until colonies presented an embryonic stem cell (ES)-like morphology. At day 28, 12 colonies were picked and expanded as individual clones for 7 days.
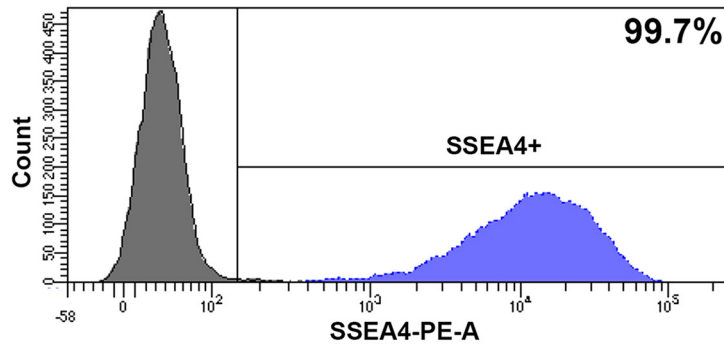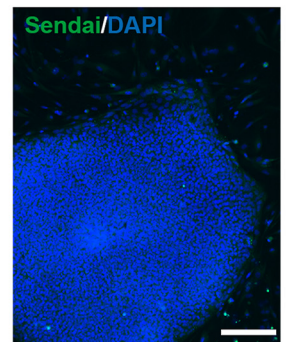
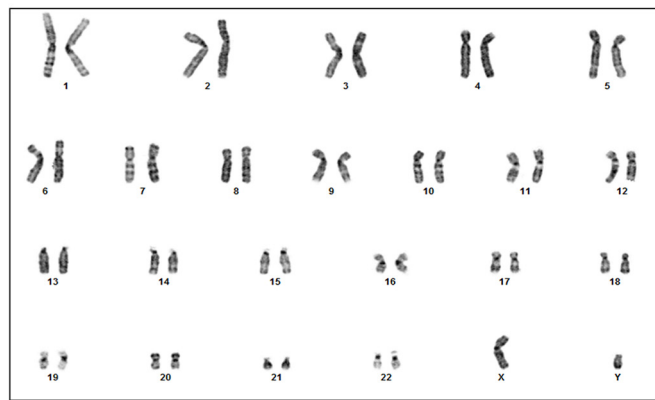### A. Pluripotency markers
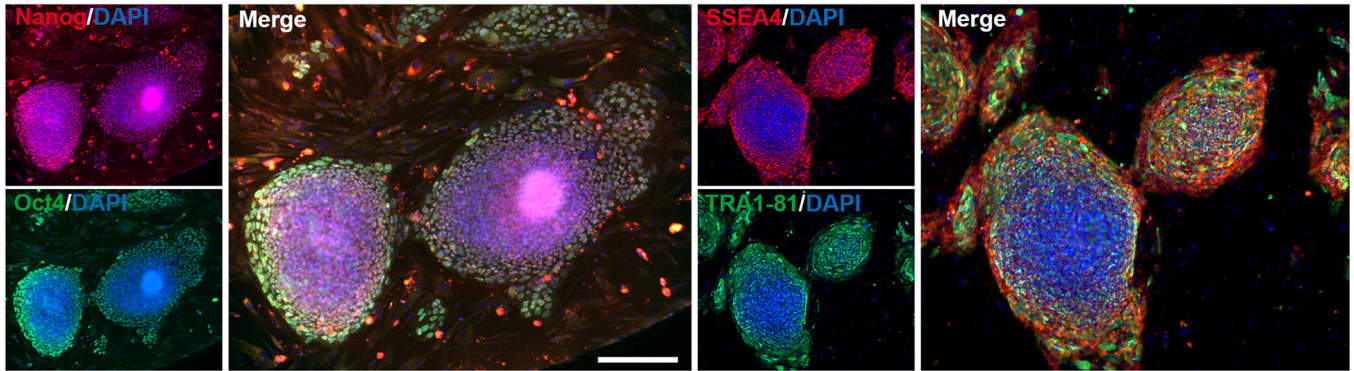
B. Alkaline phosphatase
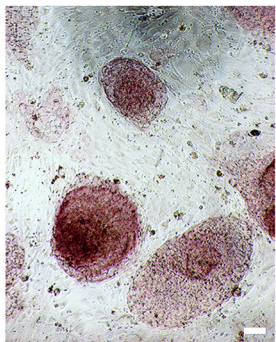
C. Flow cytometry

D. Sendai virus expression

E. Karyogram
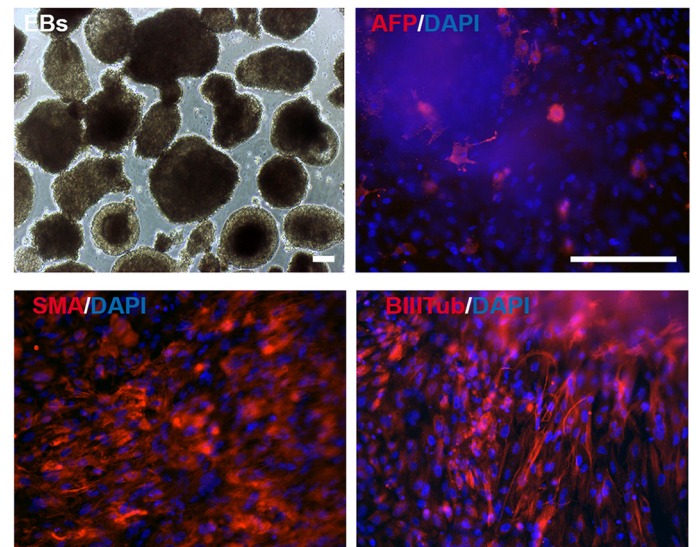
F. *In vitro* differentiation

Fig. 1. Characterization of the iPSC line CSC 43J.

Three clones (CSC-43D, CSC-43I and CSC-43J) were further selected for expansion and karyotype analysis. All clones were characterized using the methods we previously described (Holmqvist et al., 2016). We report, herein the characterization of clone CSC-43J.

The generated clone, CSC-43J, expressed the common nuclear and cell surface pluripotency markers, OCT4/Nanog and TRA1-81/SSEA4 (Fig. 1A) and showed alkaline phosphatase (ALP) activity (Fig.1B). Flow cytometry analysis showed >99% of the iPSCs positive for SSEA4 (Fig. 1C; non-stained iPSCs are shown in grey). Additional immunocytochemistry analysis revealed elimination of the Sendai virus at passage 8 (Fig. 1D). Fig. 1E depicts a normal male karyogram identified in CSC-43J clone. Embryoid bodies (EBs) generated from CSC-43J iPSC present three-germ layer differentiation capacity as shown by the in vitro expression of alpha-fetoprotein (AFP), an endodermal marker, smooth muscle actin (SMA), a mesodermal marker, and beta-III tubulin (BIIITub), an ectodermal marker (Fig. 1F). The identity of the generated iPSC line was confirmed by DNA fingerprint, showing genetic correspondence to parental fibroblasts. Mycoplasma infection was prevented by routine addition of plasmocin in cell culture media, at early passages.

Materials and methods

Fibroblast culture

Dermal fibroblasts were collected by punch skin biopsy from a patient diagnosed with PD, after obtaining informed consent. The fibroblasts were maintained in fibroblast growth medium, composed of DMEM media (ThermoFisher Scientific) with 10% fetal bovine serum and 1% Penicillin-Streptomycin and passaged with 0.05% trypsin.

iPSC generation and expansion

For reprogramming, 75,000 cells were seeded on a 12-well plate and maintained in fibroblast growth medium. Two days after (day 0), the cells were transduced using the three vector preparations (MOI = 5, 5, 3) included in the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific). On the following day and on every other day, the medium was replaced with fresh fibroblast growth medium. At day 7, the cells were re-seeded onto irradiated mouse embryonic fibroblasts (MEFs) feeder cells with fibroblast growth medium. On the day after and until colony picking, the cells were cultured in WiCell medium composed of 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) and 0.1 mM β-mercaptoethanol, supplemented

with 20 ng/ml FGF2 (ThermoFisher Scientific). On day 28, individual colonies were picked and re-seeded on a 24-well plate containing fresh MEFs. One week after, three clones were selected and further expanded on 6-well plates. The cells were passaged once a week and seeded on the appropriate cell culture surface for characterization assays at the indicated passage numbers (Table 1).

Immunocytochemistry

The iPSCs cultures were fixed with 4% paraformaldehyde for 15 min at room temperature (RT), permeabilized and blocked for 1 h at RT with PBS containing 10% donkey serum and 0.1% TritonX-100 (Sigma) and incubated overnight at +4 °C with the primary antibodies (Table 2) diluted in the blocking buffer. The secondary antibodies were thereafter added for 1 h at RT in the dark, followed by nuclei counterstain with DAPI (1:10,000 (Life Technologies)) and image acquisition on inverted epifluorescence microscope LRI - Olympus IX-73. Scale bars are 200μm.

Alkaline phosphatase activity

Alkaline phosphatase staining was performed using Alkaline Phosphatase Staining Kit (Stemgent, MA).

In vitro differentiation by embryoid body (EB) formation

Human iPSC were grown for 2 weeks as embryoid bodies (EBs) in low-attachment 24-well plates (Corning) in WiCell medium supplemented with 20 ng/ml FGF2. The EBs were then seeded on a 0.1% gelatin-coated 96-well plate (Greiner Bio-One) in DMEM media containing 10% fetal bovine serum and 1% Penicillin-Streptomycin for subsequent spontaneous differentiation, with media changes every 2–3 days. After 2 weeks, the cells were fixed and stained for three germ-layer markers as described in Table 2.

Karyotype analysis

The G-banding analysis was performed at 300–400 band resolution in average after 11 passages, at the Department of Clinical Genetics and Pathology in Lund.

DNA fingerprinting

Genomic DNA from fibroblasts and hiPSCs was extracted 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

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal	Not shown but available with the author
Phenotype	Immunocytochemistry	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	99.7% SSEA4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XY (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 the 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	Staining of 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-BIII 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

with 1.5 mg/ml Proteinase K. Fingerprinting analyses were outsourced to the IdentiCell STR profiling service (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).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.01.007.

References

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