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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line (CSC-46) from a patient with Parkinson's disease carrying a novel p.R301C mutation in the GBA gene

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A B S T R A C T

Mutations in the glucocerebrosidase (GBA) gene have been associated with the development of Parkinson's disease (PD). An induced pluripotent stem cell (iPSC) line was generated from a 60-year old patient diagnosed with PD and carrying a new mutation variant p.R301C in *GBA*. Using non-integrating Sendai virus-based technology, we utilized OCT3/4, SOX2, c-MYC and KLF4 transcription factors to reprogram skin fibroblasts into iPSCs. The generated iPSC line retained the mutation, displayed expression of common pluripotency markers, differentiated into the three germ layers, and exhibited normal karyotype. The iPSC line can be further used for studying PD pathogenesis.

Resource table

Unique stem cell line identifier	ULUNDi007-A
Alternative name(s) of stem cell line	CSC-46 L
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	iPSCs
Origin	Human
Additional origin info	Age of patient: 60 Sex of patient: Male
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	N/A
Associated disease	Parkinson's disease
Gene/locus	*GBA* (MIM # 606463) on chromosome 1q22
Method of modification	N/A N/A
Name of transgene or resistance	
Inducible/constitutive system	N/A
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: 202100–3211 (delivered by Swedish work environment Arbetsmiljöverket).

Resource utility

GBA mutations are associated with the development of Parkinson's disease (PD). The CSC-46 iPSC line was generated from a PD patient carrying a new mutation variant p.R301C in the GBA gene. This iPSC line can be used to explore the association between PD and *GBA* mutation in disease modeling studies.

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Resource details

GBA encodes the enzyme glucocerebrosidase (GBA), a lysosomal hydrolase that digests glycolipids and which is deficient in Gaucher's disease, a recessive lysosomal storage disorder. Mutations in *GBA* are linked to an increased risk of developing PD (Sidransky and Lopez, 2012) and PD patients with identified heterozygous *GBA* mutations present with early-onset motor symptoms together with cognitive impairment (Sidransky and Lopez, 2012; Mata et al., 2016). Several mutations have been identified in *GBA*. For example, p.N370S and p.L444P are most frequently described in genetic screening studies (Asselta et al., 2014). Here we report the generation of an iPSC line (clone CSC-46 L) from a PD patient carrying a new mutation variant p.R301C in *GBA*. This line can be used to decipher molecular mechanisms underlying p.R301C *GBA* associated PD.

To generate the CSC-46 iPSC line, skin fibroblasts were collected by punch skin biopsy from a 60-year old male PD patient and reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Briefly, fibroblasts were seeded on a 12-well plate (75.000 cells/well) and two days later four separate vectors carrying genes OCT-3/4, KLF-4, SOX-2 and c-MYC were delivered to the cells to induce pluripotency. At day 7 post-transduction, the cells were re-seeded onto mouse embryonic fibroblasts (MEF)-feeder layer and expanded until an embryonic stem cell (ES)-like morphology was observed. At day 28, several colonies were collected and expanded as single clones for 7 days. Three clones (CSC-46F, CSC-46 K and CSC-46 L) were selected for further expansion and karyotype analysis. Here, we present the detailed characterization of clone CSC-46 L (Table 1) using the methods we previously described (Holmqvist et al., 2016).

The generated iPSC line CSC-46 L displayed typical colony shape and morphology when grown on mouse fibroblast feeders (Fig. 1A). The colonies expressed the common nuclear and cell surface pluripotency markers, Oct4, Nanog, TRA1–81 and SSEA4 (Fig. 1B) as well as alkaline phosphatase (ALP) activity (Fig. 1C). In addition flow cytometry confirmed that > 92% of the iPSCs were positive for the pluripotent cell marker SSEA4 (Fig. 1D). The elimination of the Sendai virus was demonstrated with immunohistochemistry analysis at passage 7 (Fig. 1E). The generated iPSC line had normal karyotype (Fig. 1F). The presence of the p.R301C mutation in GBA gene was confirmed by DNA sequence analysis (Fig. 1G). Immunocytochemistry for endodermal marker alpha-fetoprotein (AFP), the mesodermal marker smooth muscle actin (SMA), and the ectodermal marker beta-III-tubulin revealed formation of all three germ layers from the embryoid bodies (EBs) generated from the CSC-46 L line (Fig. 1H). DNA fingerprinting showed genetic equivalency to parental fibroblasts, thus confirming the identity of the generated iPSC line. Finally, standardised qPCR test showed absence of mycoplasma contamination in the generated iPSC line.

Materials and methods

Fibroblast culture

Human dermal fibroblasts were obtained by punch skin biopsy from a PD patient carrying *GBA* mutation after written informed consent. Fibroblasts were cultured and expanded in culture medium containing DMEM media (ThermoFisher Scientific), 10% fetal bovine serum and 1% Penicillin-Streptomycin. Cells were passaged with 0.05% trypsin (Invitrogen).

iPSC generation and expansion

For reprogramming, fibroblasts were transduced using the three vector preparations (MOI = 5, 5, 3) included in the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). The medium was replaced daily for 7 days, after which the cells were re-seeded onto irradiated mouse embryonic fibroblasts (MEF) feeder cells. From the day 8 and until day 28, the cells were cultured in WiCell medium composed of advanced DMEM/F12 (Thermo Fisher Scientific), 20% Knock-Out Serum Replacement (v/v, Thermo Fisher Scientific), 2 mM L-glutamine (Thermo Fisher Scientific), 1% non-essential amino acids (NEAA, v/v, (v/v, Thermo Fisher Scientific) and 0.1 mM β-mercaptoethanol (Sigma-Aldrich), supplemented with 20 ng/mL FGF2 (Thermo Fisher Scientific). On day 28, several single colonies were picked and re-seeded to fresh MEFs, on a 24-well plate. Seven days later, three individual clones were randomly selected and re-plated on a 6-well plate for further expansion. Cells were passaged weekly and re-plated on appropriate cell culture surface for characterization assays (Table 1).

Immunocytochemistry

The cells were fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature (RT) followed by blocking and permeabilization for 1 h at RT with PBS containing 10% donkey serum and 0.1% TritonX-10 (Sigma). Then the cells were incubated overnight at +4 °C with the primary antibodies (Table 2) diluted in the blocking buffer followed by incubation with secondary antibodies in the dark for 1 h at RT. DAPI (1:10000) was used for nuclei counterstaining. Image acquisition were performed on inverted epifluorescence microscope LRI – Olympus IX-73.

Alkaline phosphatase activity

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

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal morphology	Fig. 1 panel A
Phenotype	Immunocytochemistry	Positive staining for pluripotency markers: Oct4, Nanog, TRA1–81 and SSEA4	Fig. 1 panel B
	Alkaline phosphatase activity	Visible activity	Fig. 1 panel C
	Flow cytometry	92.8% SSEA4	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	Normal karyotype 46,XY (300–400 bands resolution in average)	Fig. 1 panel F
Identity	STR analysis	matched with parental fibroblasts	STR analysis
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous p.R301C mutation in *GBA*	Fig. 1 panel G
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative.	Not shown but available with author
Differentiation potential	Embryoid body formation	Spontaneous EB formation and positive staining for smooth muscle actin (SMA), beta-III-tubulin (BIIITub) and α-fetoprotein (AFP)	Fig. 1 panel H
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

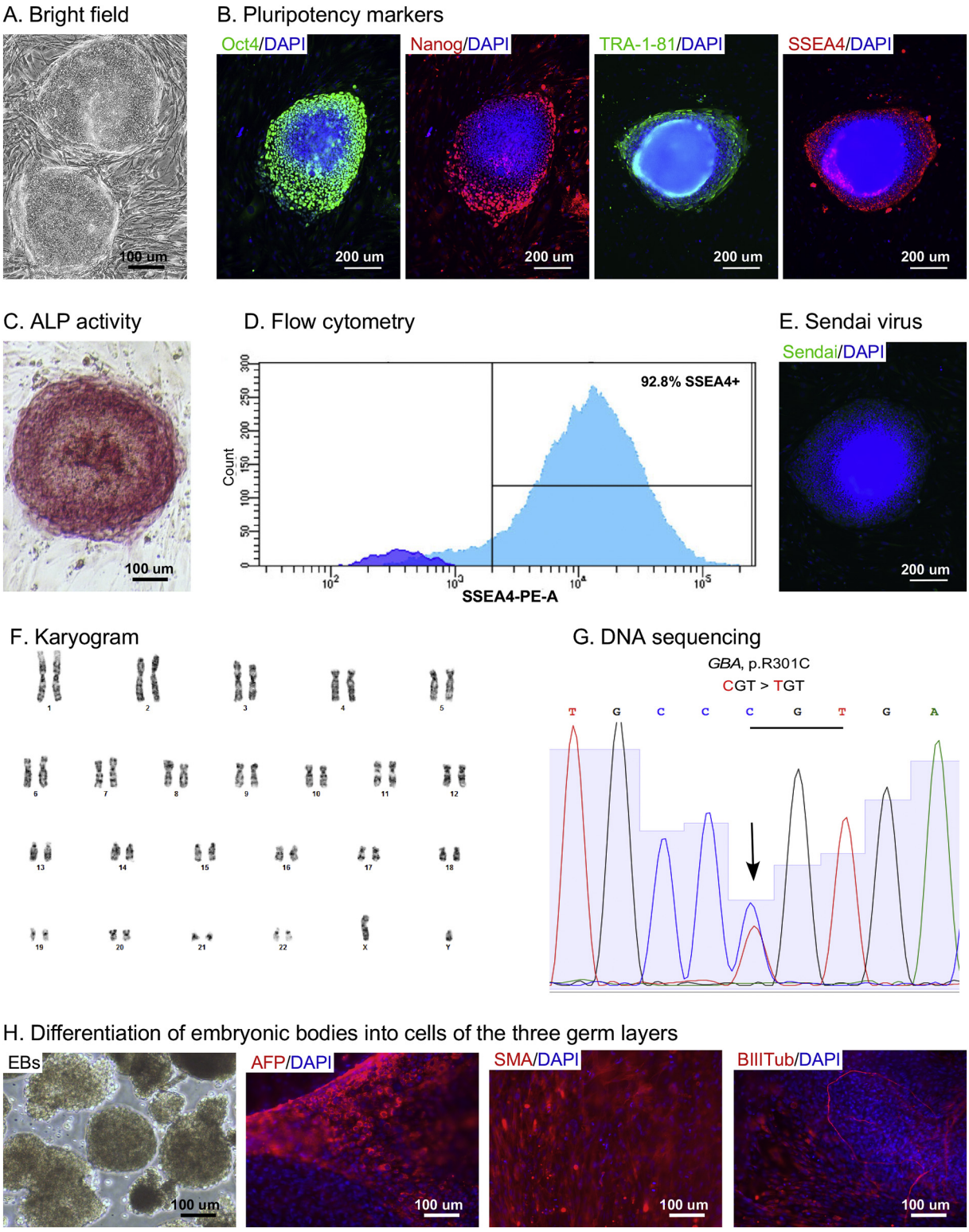

Fig. 1. Characterization of the iPSC line CSC 46L.

In vitro differentiation by embryoid body (EB) formation

iPSCs were plated on low-attachment 24-well plates and grown as embryoid bodies (EBs) for 2 weeks in WiCell medium supplemented with 20 ng/mL FGF2. For subsequent spontaneous differentiation, EBs were seeded on a 0.1% gelatin-coated 96-well plate in DMEM media containing 10% fetal bovine serum and 1% Penicillin-Streptomycin. Media was changed every 2–3 days. After 2 weeks, the cells were fixed and stained for markers against three germ layers (Table 2).

Karyotype analysis

The G-banding analysis was performed at 300–400 band resolution in a clinical diagnostic setting after 11 passages.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	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
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′)
Targeted mutation sequencing	*GBA*	TGGTCCACTTTCTTGGCCG/AGGGGAATGGTGCTCTAGGA

Mutation sequencing

Genomic DNA was extracted from fibroblasts and iPSCs with the use of conventional lysis buffer composed of 100 mM Tris (pH 8.0), 200 mM NaCl, 5 mM EDTA, 1.5 mg/mL Proteinase K, and 0.2% SDS in distilled autoclaved water. Direct DNA sequencing (Macrogen Europe, Amsterdam, The Netherlands) confirmed the presence of the p.R301C mutation in *GBA*. Primers used for amplification and directed sequencing of GBA around the mutation sites are listed in Table 2.

DNA fingerprinting

DNA fingerprinting analysis was performed by the IdentiCell STR profiling service (Department of Molecular Medicine, Aarhus University Hospital, Skejby, Denmark).

Mycoplasma detection

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

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