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Lab Resource: Stem Cell Line

Establishment of induced pluripotent stem cell (iPSC) line from a 63-year old patient with late onset Alzheimer's disease (LOAD)

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ABSTRACT

Peripheral blood mononuclear cells (PBMCs) were collected from a clinically characterised 63-year old woman with late onset Alzheimer's disease (LOAD). The PMBCs were reprogrammed with the human OSKM transcription factors using the Sendai-virus delivery system. The transgene-free iPSC showed pluripotency verified by immunocytochemistry for pluripotency markers and differentiated spontaneously towards the 3 germ layers in vitro. Furthermore, the iPSC line showed normal karyotype. Our model might offer a good platform to further study the pathomechanism of sporadic AD, to identify early biomarkers and also for drug testing and gene therapy studies.

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Resource table

Name of Stem Cell line	BIOT-0726-LOAD
Institution	BioTalentum Ltd.
Person who created resource	András Dinnyés
Contact person and email	andras.dinnyes@biotalentum.hu
Date archived/stock date	April 2016
Origin	Peripheral blood mononuclear cells (PBMCs)
Type of resource	Induced pluripotent stem cell (iPS); derived from a late onset Alzheimer disease (LOAD) patient
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	hOCT4, hSOX2, hC-MYC, hKLF4 (CytoTune™-iPS 2.0 Sendai Reprogramming Kit - Invitrogen, Thermo Fisher Scientific Inc.)
Authentication	Identity and purity of the cell line was confirmed by the following analyses: SeV specific polymerase chain reaction (PCR), karyotyping, expression analysis of pluripotency markers and in vitro differentiation potential
Link to related literature	N/A
Information in public databases	N/A
Ethics	Patient informed consent obtained/Ethics Review Board-competent authority approval obtained

Resource details

Blood samples were donated by a 63-year old female patient with clinically characterised late onset Alzheimer's disease (LOAD) by the Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest (Hungary).

To generate the BIOT-0726-LOAD iPSC line (Fig. 1A) the four "Yamanaka reprogramming factors" *OCT3/4*, *SOX2*, *KLF4*, and *C-MYC* were delivered into PBMCs using the integration-free Sendai virus gene-delivery method (Yang et al. 2008; Fusaki et al. 2009). The iPSC-like colonies were picked after 20–27 days post-transduction. Beginning from passage 5 of the iPSCs the absence/presence of Sendai virus vector was analysed by RT-PCR using Sendai virus vector (SeV) - specific primers (Table 1). After 7 passages, the elimination of the reprogramming vector was confirmed in BIOT-0726-LOAD iPSC line which was selected for further analysis (Fig. 1B).

The karyotype of the BIOT-0726-LOAD iPSC line was determined with Giemsa-banding, proving normal diploid 46, XX karyotype, without any detectable abnormalities (Fig. 1B).

Expression of pluripotency markers was examined by immunocytochemistry staining, using antibodies against human OCT3/4, E-CADHERIN, and NANOG (Fig. 1A). The in vitro spontaneous differentiation potential towards the three germ layer of the BIOT-0726-LOAD iPSC line was demonstrated by the expression of ectodermal (βIII-TUBULIN), mesodermal (BRACHYURY) and endodermal (GATA4) markers (Fig. 1A) (Itskovitz-Eldor et al. 2000; Carpenter et al. 2003).

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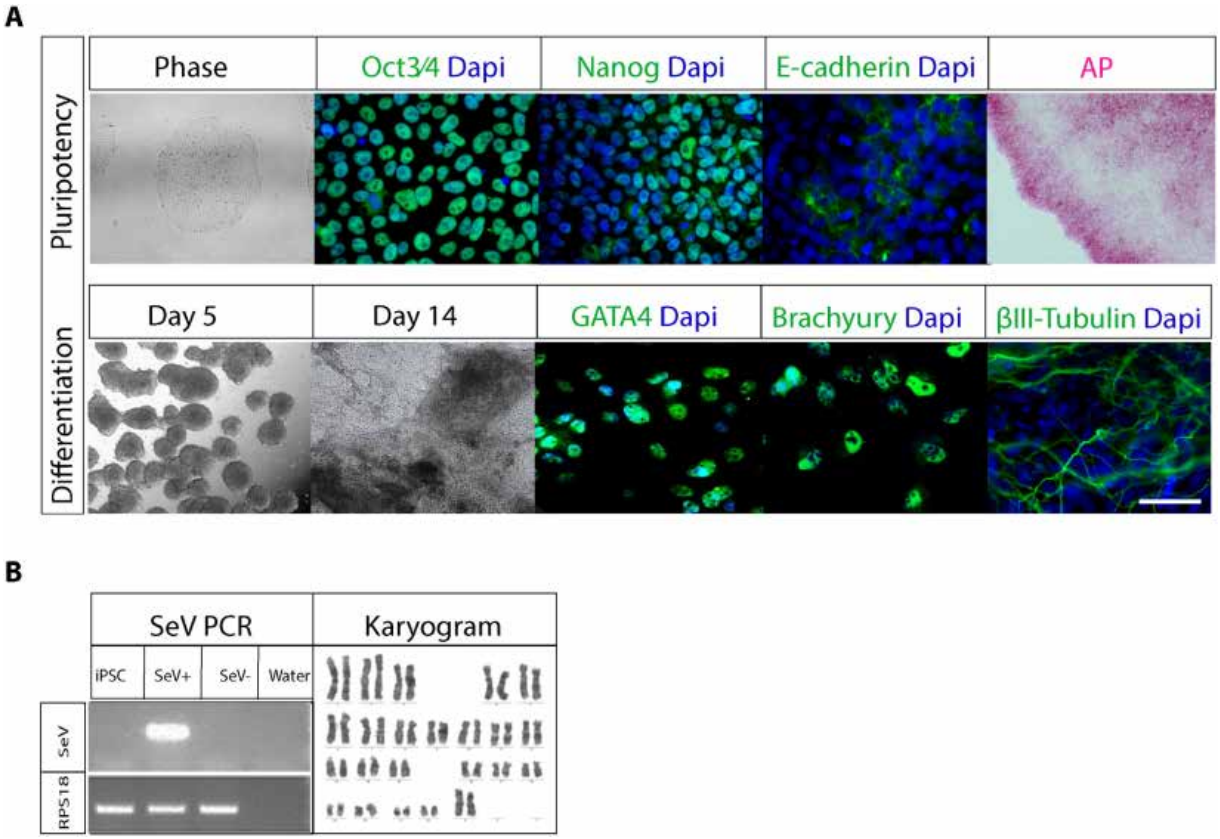

Fig. 1. Characterisation of BIOT-7183-PSEN1 hiPSC line A) Above: Morphology of generated hiPSCs (4×) and representative immunofluorescent micrographs of iPSCs positive for stem cells markers OCT3/4, NANOG and E-CADHERIN (in green). Alkaline phosphatase (AP) activity was also detected in iPSCs. Below: Spontaneously formed EBs in suspension culture (Day 5) and after attachment and further differentiation (Day 14). Immunostainings for endodermal (GATA4), mesodermal (BRACHYURY) and ectodermal (βIII-TUBULIN) germ layers, markers (in green). Nucleus is labelled with DAPI (in blue). B) RT-PCR verification of the absence of Sendai-virus from the reprogrammed cells and karyogram showing normal 46 chromosomes (XX).

Materials and methods

Reprogramming of peripheral blood mononuclear cells (PBMC)

PBMCs were isolated within 2 h post-collection using the Vacutainer® CPT™ Cell Preparation Tube with Sodium Heparin, (BD Biosciences) and separated by centrifugation (1800 rcf, 30 min, room temperature (RT). The freshly isolated PBMCs were seeded (0.5×10^6 cells) in Expansion Medium (EM): QBSF-60 medium (Quality Biological) supplemented with 50 μg/ml Ascorbic acid, 1% Penicillin/Streptomycin, 50 ng/ml SCF (R&D systems), 10 ng/ml IL-3 (R&D systems), 2 U/ml EPO (R&D systems), 40 ng/ml IGF-1 (R&D systems) and 1 μM Dexamethasone. Nine days later (Day 0) the cells were transduced by Sendai-virus delivery using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), following the manufacturer's instruction. Briefly, the cells were transduced on 1w/24wp in 1 ml EM supplemented with 4 μg polybrene. The calculated volumes of each of the three CytoTune™ 2.0 Sendai-virus (SeV) was added to the cells using MOI of 5-5-3 (KOS MOI = 5, hc-Myc MOI = 5, hKlf4 MOI = 3). The cell containing plate was centrifuged (900 rcf, 90 min, RT) and incubated for 24 h. The following day the cells were collected, centrifuged (300 rcf, 5 min, RT) and seeded in fresh EM (Day 1). Two days later (Day 3) the cells were passed onto a 6 cm dish covered with 0.1% gelatine and 0.65×10^6 Mitomycin C treated-MEF in QBSF-60 medium (Quality Biological) supplemented with 50 μg/ml Ascorbic acid. Seven days post-transduction (Day 7) the medium was replaced with HESC medium: DMEM/F12, 20% Knockout Serum Replacement, 1% MEM Non-Essential Amino Acid Solution, 0.1 mM β-mercaptoethanol, 0.5% Pen/Strep, 10 ng/ml bFGF and supplemented with 50 μg/ml Ascorbic acid. The colonies with an ES-like appearance were manually isolated based on morphology between Day 21 to Day 27 post-transduction and cultured as iPSCs thereafter. Human iPSC cultures were maintained on plates coated with Matrigel (BD Biosciences) in mTESR-1 medium (Stem Cell Technologies) following the manufacturer's instruction (Fig. 1A). All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2.

Table 1
Primers used in the study.

Gene symbol	Fwd and Rvs primers (5′-3′)	PCR product (bp)
SeV	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	181
RPS18S	TTAAGGGTGTGGGCCGAAGA GGGATCTTGTACTGGCGTGGA	142

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured cells with RNeasy Micro Kit (Qiagen) and reverse transcribed corresponding to SuperScript III Reverse Transcriptase protocol (Thermo Fisher Scientific). The RT-PCR reaction was performed using GoTaq Hot Start Green Master Mix (Promega). To confirm the transgene-free status of the iPSC lines, SeV specific primers were used described in CytoTune®-iPS 2.0 Sendai Reprogramming Kit protocol (Thermo Fisher Scientific; Table 1).

In vitro spontaneous differentiation

The iPSCs were harvested according to the mTeSR-1 kit protocol (Stem Cell Technologies) and the cell clumps were plated on low attachment dishes in mTeSR medium. Five days later the embryoid

Table 2
Antibodies used for immunocytochemistry.

	Antibody	Dilution	Company (Cat #)
Pluripotency	Mouse anti-E-CADHERIN	1:1000	Thermo Fisher Scientific (13-1700)
	Mouse anti-OCT4	1:50	Santa Cruz Biotechnologies (sc-5279)
	Goat anti-hNANOG	1:50	R&D (AF1997)
In vitro differentiation	Rabbit anti-β-III-TUBULIN	1:2000	Covance (PRB-435P)
	Rabbit anti-BRACHYURY T	1:50	Santa Cruz Biotechnologies (sc-20109)
	Mouse anti-GATA4	1:50	Santa Cruz Biotechnologies (sc-25310)
Secondary antibodies	Alexa Fluor 488 donkey anti-goat IgG	1:2000	Thermo Fisher Scientific. (A-11055)
	Alexa Fluor 488 donkey anti-mouse IgG	1:2000	Thermo Fisher Scientific (A-21202)
	Alexa Fluor 488 donkey anti-rabbit IgG	1:2000	Thermo Fisher Scientific (A-21206)
	Alexa Fluor 488 donkey anti-mouse IgM	1:2000	Thermo Fisher Scientific (A-21042)

bodies (EBs) that had formed were plated on 24-well plates containing cover slips (3–4 EBs/well), covered with 0.1% gelatin in differentiation medium: DMEM, 20% FBS, 1% MEM Non-Essential Amino Acid Solution, 0.1 mM β-mercaptoethanol, 1% Pen/Strep. The medium was changed in every second day hereafter. The cells were fixed on day 14 of differentiation with 4% PFA for ICC staining specific for the 3 germ layers.

Immunocytochemistry (ICC)

The expression of specific pluripotency and germ layer markers were analysed using conventional ICC staining. Cells were fixed in 4% PFA (20 min, RT), permeabilized with 0.1% Triton X-100 (5 min, RT) and blocked in 1% bovine serum albumin (1 h, RT). The cells were incubated with primary antibodies (overnight at 4 °C) and visualized with secondary antibodies. For nuclei counterstaining 0.2 μg/ml DAPI (20 min, RT) was used. The antibodies and applied dilutions are listed in Table 2. The cells were observed under fluorescent microscope equipped with 3D imaging module, (Axio Imager system with ApoTome; Carl Zeiss) controlled by AxioVision 4.8.1 microscope software (Carl Zeiss).

Alkaline phosphatase (AP) staining

For detecting Alkaline phosphatase activity the 4% PFA fixed cells (20 min, RT) were stained AP solution (29.7 mM TRIS base; 6 mM maleic acid, pH 8.5–9.0; with 0.08% $MgCl_2$, 10.8 mM Naphtol-As MX Phosphate; 23 μM Fast Red TR Salt; all from Sigma Aldrich) for 30 min (RT). Samples were observed with Axio Imager system (Carl Zeiss).

Karyotyping

Cells were treated with KaryoMAX® Colcemid™ Solution (Thermo Fisher Scientific) and processed with standard methods. Standard Giemsa-banded karyotype was performed by Istenhegyi GeneDiagnostic Center, Budapest (Hungary) and a minimum of 20 metaphases were analysed. The chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN).

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References

Carpenter, M.K., Rosler, E., Rao, M.S., 2003. Characterization and differentiation of human embryonic stem cells. Cloning Stem Cells 5 (1), 79–88.
Fusaki, N., Ban, H., Nishiyama, A., Saeki, K., Hasegawa, M., 2009. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85 (8), 348–362.
Itskovitz-Eldor, J., Schuldiner, M., Karsenti, D., Eden, A., Yanuka, O., Amit, M., Soreq, H., Benvenisty, N., 2000. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol. Med. 6 (2), 88–95.
Yang, W., Mills, J.A., Sullivan, S., Liu, Y., French, D.L., Gadue, P., 2008. iPSC Reprogramming From Human Peripheral Blood Using Sendai Virus Mediated Gene Transfer. StemBook, Cambridge (MA).