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Lab Resource: Multiple Cell Lines

Generation of the Human iPSC Line from Spontaneous Late-Onset Alzheimer's Disease Patient with ApoE3/3 Genotype and Sex-, Age-, and ApoE-Matched Healthy Control

Sona Kadakova [a], Jan Raska [a,b], Hana Hribkova [a], Veronika Fedorova [a], Jiri Sedmik [a], Klara Plesingrova [a], Katerina Sheardova [b], Dasa Bohaciakova [a,b,*]

[a] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[b] International Clinical Research Center (ICRC), St. Anne's University Hospital, Masaryk University, Brno, Czech Republic

A B S T R A C T

Human induced pluripotent stem cell (iPSC) lines were generated from peripheral blood mononuclear cells (PBMCs) isolated from a patient diagnosed with spontaneous late-onset Alzheimer's disease (AD) carrying ApoE3/3 gene and one age-, sex-, and ApoE-matched healthy control. Reprogramming was done using a commercially available Epi5 Reprogramming Kit containing *OCT4, SOX2, KLF4, LIN28, and L-MYC* as reprogramming factors. The pluripotency of the iPSC lines was verified by the expression of pluripotency markers and by their capacity to differentiate into all three embryonic germ layers *in vitro*. These newly established iPSC lines offer a valuable platform for *in vitro* modeling of AD.

Resource Table:

Unique stem cell lines identifier	*MUNIi020-A*; *MUNIi021-A*
Alternative name(s) of stem cell lines	*sHC5 (MUNIi020-A)*; *sAD5 (MUNIi021-A)*
Institution	*Masaryk University, Brno, Czech Republic*
Contact information of distributor	*Dáša Bohačiaková; bohaciakova@med.muni.cz*
Type of cell lines	*iPSC*
Origin	*human*
Additional origin info	*sHC5 (MUNIi020-A)Age: 66Sex: F*; *sAD5 (MUNIi021-A)Age: 79Sex: F*
Cell Source	*Peripheral blood mononuclear cells*
Clonality	*Clonal*
Method of reprogramming	*Epi5™ Episomal iPSC Reprogramming Kit (episomal vectors with the oriP/EBNA-1 backbone containing OCT4, SOX2, KLF4, LIN28, and L-MYC)*
Genetic Modification	*NO*
Type of Genetic Modification	*NO*
Evidence of the reprogramming transgene loss	*PCR*
Associated disease	*Alzheimer's disease*
Gene/locus	*N/A*
Date archived/stock date	*March 2023 – September 2023*
Cell line repository/bank	https://hpscreg.eu/cell-line/MUNIi020-A; https://hpscreg.eu/cell-line/MUNIi021-A
Ethical approval	*Human blood samples were obtained with the approval of the local ethical committee (FNUSA-ICRC, Brno; approval nr. 03G/2019), and the donor gave written informed consent. IPSC lines were reprogrammed with the approval of the local ethical committee (Masaryk University, Faculty of Science, Brno, Czech Republic; approval nr. EKV-2018–051)*

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1. Resource utility

iPSC lines generated from late-onset Alzheimer's patient and matched control were created as an *in vitro* model for the AD studies. These lines can differentiate into 2D/3D neuronal models and other relevant cell types, allowing the study of AD-related phenotype and pathology using standard protocols.

2. Resource details

Peripheral blood mononuclear cells (PBMCs), shown in Fig. 1A, were isolated from the whole peripheral blood of patients enrolled in the Czech Brain Aging Study (Sheardova et al., 2019). PBMCs were subsequently reprogrammed into induced pluripotent stem cells (iPSCs) by the commercially available Epi5™ Episomal iPSC Reprogramming Kit

* Corresponding author.
E-mail address: bohaciakova@med.muni.cz (D. Bohaciakova).

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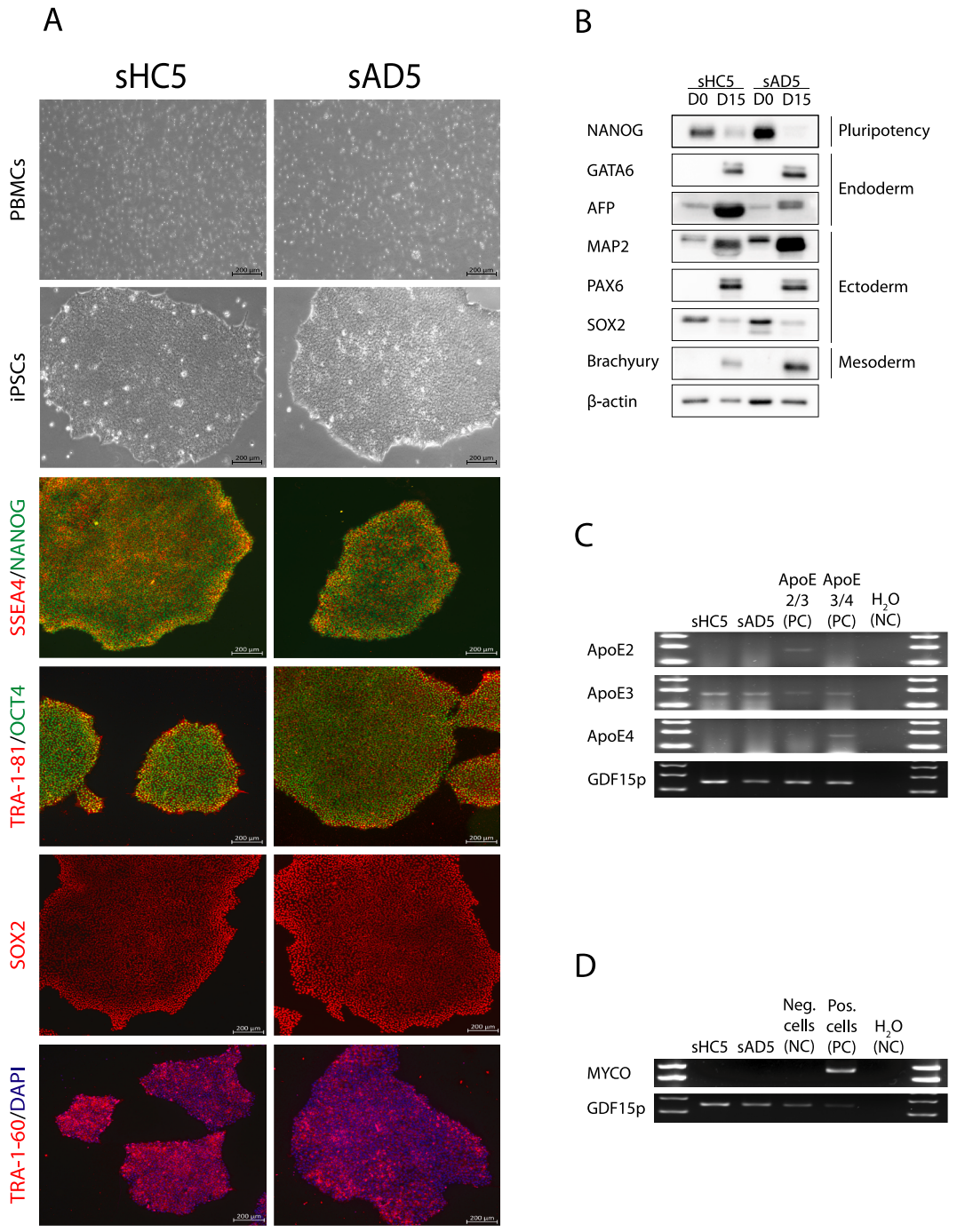

Fig. 1. Characterization and validation of sHC5/sAD5 iPSC lines.

(Thermo Fisher Scientific) using transcription factors *OCT4*, *SOX2*, *LIN28*, *L-MYC*, and *KLF4* and cultivated in feeder-free conditions on Matrigel® (Corning) coated plates with mTeSR™1 medium (STEMCELL Technologies). Newly derived iPSCs were grown as compact colonies with smooth edges (Fig. 1A). Their pluripotency was further confirmed by immunofluorescence, showing the expression of cell surface markers TRA-1–81, TRA-1–60, and SSEA4, and nuclear markers OCT4, NANOG, and SOX2 (Fig. 1A). Additionally, the protein expression of NANOG and SOX2 was validated by Western blot analysis (Fig. 1B). The differentiation potential of iPSC lines was demonstrated *in vitro* by Embryoid bodies formation assay. Cells were allowed to undergo spontaneous differentiation for a duration of 15 days on a non-adhesive culture plate. Markers associated with ectoderm (SOX2, PAX6, MAP2), endoderm (GATA6, AFP), and mesoderm (BRACHYURY) were successfully detected by Western blot analysis (Fig. 1B). The ApoE genotype, a known risk factor for Alzheimer's disease, was determined using PCR analysis (Fig. 1C). The results confirmed that the sHC5/sAD5 iPSC lines had an ApoE3/3 genotype. GDF15p was used as a loading control, and positive control (PC) for ApoE2, ApoE3, and ApoE4 genotype, and negative control (NC) for PCR reaction (H_2O) were also included in this analysis. Lastly, iPSCs were proved to be negative for mycoplasma contamination by PCR analysis (Fig. 1D). Here, the analysis also included positive control – cells positive for mycoplasma (PC), negative control – cells negative for mycoplasma (NC), and negative control for PCR reaction (H_2O).

Additionally, cell lines were tested for several other parameters.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	OCT4, NANOG, SOX2, SSEA4, TRA-1–81, TRA-1–60	Fig. 1 panel A
	Quantitative analysis Immunocytochemistry	sHC5: OCT4 100 %; NANOG 100 %; SOX2 100 % sAD5: OCT4 100 %; NANOG 100 %; SOX2 100 %	–
Genotype	Karyotype (G-banding) and resolution	sHC5: 46, XX sAD5: 46, XX Resolution 550	Supplementary Fig. 1 panel B
Identity	STR analysis	18 sites tested, all matched	Supplementary information
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	–
	Southern Blot OR WGS	N/A	–
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR Negative	Fig. 1 panel D
Differentiation potential	Embryoid body formation assay (Western blot)	sHC5: Ectoderm – PAX6; MAP2 expressed Endoderm – GATA6; AFP expressed Mesoderm – BRACHYURY expressed sAD5: Ectoderm – PAX6; MAP2 expressed Endoderm – GATA6; AFP expressed Mesoderm – BRACHYURY expressed	Fig. 1 panel B
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	–
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	–
	HLA tissue typing	N/A	–

Specifically, the presence or absence of reprogramming vectors in cells was tested by the PCR analysis. Results show the absence of the Epstein-Barr virus nuclear antigen 1 (EBNA1) gene and the oriP element in both cell lines (Supplementary Figure S1A). A karyotype of cell lines was also evaluated by standard G-banding methodology, showing that both cell lines have a normal karyotype (Supplementary Figure S1B). Finally, to confirm the exact identity of the parental PBMCs and prevent any possibility of cross-contamination, the STR analysis was done by Generi Biotech (Hradec Králové, Czech Republic). Results confirmed that reprogrammed cell lines were identical to control parental PBMCs with a conformity level of 100 % in both cases (sHC5/sAD5). In summary, two new iPSC lines were successfully generated and represent a resource for *in vitro* investigations into Alzheimer's disease using stem cell-based models Table 1.

3. Materials and methods

3.1. Cell culture and reprogramming

Peripheral blood mononuclear cells (PBMCs) were obtained from whole blood through gradient centrifugation using Histopaque ®-1077 (Merck). Cells were cultured in Gibco™ StemPro™-34 SFM media supplemented with 2 mM Glutamax (all Thermo Fisher Scientific) 1X Zellshield™ (Minerva Biolabs), 100 ng/mL SCF and Flt-3, 20 ng/mL IL-3 and 10 ng/mL IL-6 (all Peprotech). After three days of cultivation, reprogramming was done using the Epi5™ Episomal iPSCs Reprogramming Kit by electroporation using a Neon transfection system (Thermo Fisher Scientific) based on the manufacturer's recommendation (Pulse voltage: 1,600 V; Pulse width 10 ms; Pulse number: 3). Reprogrammed iPSCs were cultured under feeder-free conditions on Matrigel®-coated plates in mTeSR™1 medium supplemented with 1X Zellshield™. These cells were kept on laboratory plastic (TPP) in a humidified incubator set at 37 °C with 5 % CO_2. Cells were mechanically passaged every 6 – 7 days to fresh mTeSR™1 medium.

3.2. Embryoid bodies formation assay

iPSCs cells were collected and plated into a non-adhesive V-shaped 96-well plate (coated with poly-2-hydroxyethyl methacrylate, Merck). Cells were plated at the concentration of 9 000 – 18 000 cells per well. Plates with plated cells were spun down at 200 rpm for 6 min and cultivated in the mTeSR™1 medium supplemented with 1X Zellshield™ and 20 μM Rock inhibitor (Y-27632; Selleckchem) for 1 day (D0). After 24 h (D1), embryoid bodies were cultured in the differentiation medium consisting of DMEM/F12 supplemented by 20 % KnockOut™ Serum Replacement, 3 % Embryonic Stem Cell Fetal Bovine Serum Qualified, 1 % GlutaMAX, 1 % non-essential amino acids (all from Thermo Fisher Scientific), 1X ZellShield™, 100 μM β-mercaptoethanol (Merck). Embryoid bodies were harvested after 15 days of cultivation.

3.3. PCR analysis of vector presence, mycoplasma contamination, and ApoE genotype

For the DNA isolation, a DNeasy Blood & Tissue Kit (Qiagen) was used. PCR was conducted using Taq DNA polymerase (Top-Bio). To detect EBNA and OriP, we followed the primer sequences and conditions recommended by the manufacturer of the reprogramming kit. Mycoplasma detection was carried out as previously outlined in (Dreolini et al., 2020). For the analysis of ApoE polymorphism, we utilized the allele-specific PCR method as described in (Pantelidis et al., 2003). GDF15p was chosen as a loading control.

3.4. Western blot and immunocytochemistry

Western blot and immunocytochemistry were performed as described previously (Fedorova et al., 2019). Microphotographs were taken on an Axio Observer Z1 microscope (Zeiss). All used antibodies are listed in Table 2.

3.5. STR analysis

STR analysis was done commercially by Generi Biotech (Hradec Králové, Czech Republic).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/western blot				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Rabbit anti- OCT3/4	1:250	Santa Cruz Cat# 9081	RRID: AB_2167703
Pluripotency Markers	Mouse anti-SSEA4	1:300	Thermo Fisher Cat# MA1-021	RRID: AB_2536687
Pluripotency Markers	Mouse anti-SOX2	1:400	Cell Signaling Technology Cat# 4900S	RRID: AB_10560516
Pluripotency Markers	Rabbit anti-NANOG	1:300	Thermo Fisher Cat# PA1-097	RRID: AB_2539867
Pluripotency Markers	Mouse anti-TRA-1–81	1:300	Thermo Fisher Cat# MA1-024	RRID: AB_2536706
Secondary Antibodies	CF488A Donkey Anti-Rabbit IgG	1:500	Thermo Fisher Cat# A21206	RRID: AB_2535792
Secondary Antibodies	CF594A Donkey Anti-mouse IgG	1:500	Thermo Fisher Cat# A21203	RRID: AB_141633
Differentiation Markers	Rabbit anti-NANOG	1:1000	Cell Signaling Technology Cat# 3580S	RRID: AB_2150399
Differentiation Markers	Rabbit anti-GATA6	1:500	Cell Signaling Technology Cat# 5851 T	RRID: AB_10705521
Differentiation Markers	Mouse anti-AFP	1:500	Sigma-Aldrich Cat# A8452	RRID: AB_258392
Differentiation Markers	Rabbit anti-PAX6	1:1000	Cell Signaling Technology Cat# 60,433	RRID: AB_2797599
Differentiation Markers	Mouse anti-SOX2	1:500	Cell Signaling Cat# 4900	RRID: AB_10560516
Differentiation Markers	Rabbit anti-Brachyury	1:1000	Cell Signaling Technology Cat# 81,694	RRID: AB_2799983
Differentiation Markers	Mouse anti-β-actin	1:10000	Cell Signaling Technology Cat# 3700	RRID: AB_2242334
Secondary Antibodies	Anti-rabbit IgG, HRP-linked Antibody	1:3000	Cell Signaling Technology Cat# 7074	RRID: AB_2099233
Secondary Antibodies	Anti-mouse IgG, HRP-linked Antibody	1:3000	Cell Signaling Technology Cat# 7076	RRID:AB_330924
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Epi5 vectors (PCR)	Epstein-Barr virus oriP	544 bp	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC	
Epi5 vectors (PCR)	EBNA1	666 bp	ATCGTCAAAGCTGCACACAG/CCCAGGAGTCCCAGTAGTCA	
Loading control (PCR)	GDF15p	242 bp	ACACATCAAGGTTGCCCTTC/GGGCCTCAGTATCCTCTTCC	
Mycoplasma contamination (PCR)	Mycoplasma	500 bp	GGCGAATGGGTGAGTAACACG/CGGATAACGCTTGCGACCTAT	
Apolipoprotein E genotyping (PCR)	ApoE2	173 bp	CGGACATGGAGGACGTGT/CTGGTACACTGCCAGGCA	
Apolipoprotein E genotyping (PCR)	ApoE3	173 bp	CGGACATGGAGGACGTGT/CTGGTACACTGCCAGGCG	
Apolipoprotein E genotyping (PCR)	ApoE4	173 bp	CTGGTACACTGCCAGGCG/CGGACATGGAGGACGTGC	

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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.2023.103273.

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