Stem Cell Research 29 (2018) 250–253

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Blood-derived integration-free iPS cell line UKBi011-A from a diagnosed male Alzheimer's disease patient with APOE ε4/ε4 genotype

Michael Peitz[a,b], Tamara Bechler[a], Catrin Cornelia Thiele[a,b], Monika Veltel[a], Melanie Bloschies[a], Klaus Fliessbach[b,c], Alfredo Ramirez[c,d,e], Oliver Brüstle[a,*]

[a] Institute of Reconstructive Neurobiology, Life & Brain Center, University of Bonn Medical Faculty, Bonn 53127, Germany,
[b] DZNE German Center for Neurodegenerative Diseases (DZNE), Bonn 53175, Germany,
[c] Department of Neurodegenerative Diseases and Geriatric Psychiatry, University Hospital Bonn, Germany,
[d] Department of Psychiatry and Psychotherapy, University of Cologne, Cologne, Germany,
[e] Department of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany.

ABSTRACT

Alzheimer's disease (AD) is most the frequent neurodegenerative disease, and the APOE ε4 allele is the most prominent risk factor for late-onset AD. Here, we present an iPSC line generated from peripheral blood cells of a male AD patient employing Sendai virus vectors encoding the transcription factors OCT4, SOX2, KLF4 and c-MYC. The characterized iPSC line expresses typical human pluripotency markers and shows differentiation into all three germ layers, complete reprogramming vector clearance, a normal SNP genotype and maintenance of the APOE ε4/ε4 allele.

Resource table

Unique stem cell line identifier	UKBi011-A
Alternative name(s) of stem cell line	iLB-AD-169bm-s24
Institution	University of Bonn
Contact information of distributor	Oliver Brüstle, r.neuro@uni-bonn.de
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 80 Sex: male Ethnicity: Caucasian, German
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	CytoTune-iPS 2.0 Sendai Reprogramming Kit
Genetic modification	No
Type of modification	N/A
Associated disease	Alzheimer's disease
Gene/locus	APOE with ε4/ε4 alleles
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	June 2017
Cell line repository/ bank	EBiSC; https://cells.ebisc.org/UKBi011-A
Ethical approval	The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn (approval number 275/08), and informed consent was obtained from the patient.

Resource utility

Since this APOE ε4/ε4 iPSC line is derived from a diagnosed AD patient, it is expected to serve as a valid in vitro research tool to study the contribution of the APOE ε4 risk allele to the development of AD pathology.

* Corresponding author.
E-mail address: brustle@uni-bonn.de (O. Brüstle).

https://doi.org/10.1016/j.scr.2018.04.011
Received 29 March 2018; Received in revised form 19 April 2018; Accepted 22 April 2018
Available online 23 April 2018

Resource details

Alzheimer's disease (AD) is most the frequent cause of neurodegeneration, and the APOE ε4 allele is the most prominent risk factor for sporadic late-onset AD (Yu et al., 2014) with two ε4 alleles conferring an approximately twelve-fold increased risk of developing the disease compared to APOE ε3/ε3 carriers (Michaelson, 2014). In this study, we report the generation of an iPSC line from a male diagnosed AD patient

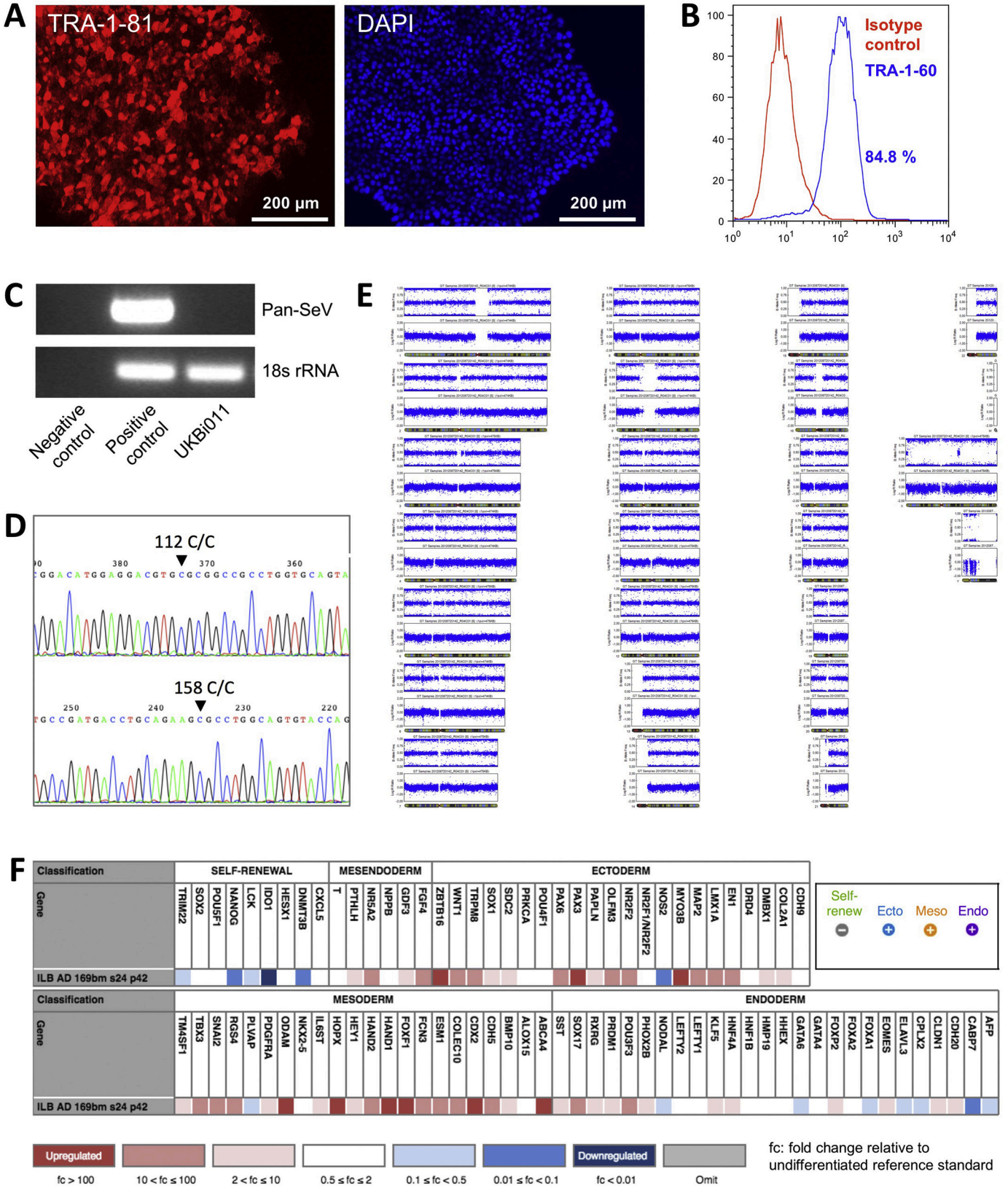

Fig. 1. Characterization of UKBi011-A.

with an APOE ε4/ε4 genotype. Human peripheral blood mononuclear cells (PBMCs) from this patient were cultivated under conditions that foster the induction and enrichment of erythroblasts (van den Akker et al., 2010) and subsequently infected with Sendai virus vectors of the non-integrative CytoTune iPS 2.0 Reprogramming Kit which express the transcription factors OCT4, SOX2, KLF4, and c-MYC. Twenty days after infection, several primary colonies were picked and expanded as clonal lines. The iPSC clone UKBi011-A was selected for in-depth characterization. This clonal line showed robust expression of the human pluripotency markers TRA-1-81 and TRA-1-60 as demonstrated by immunofluorescence (Fig. 1A) or FACS analysis (Fig. 1B). Further QC tests yielded the following results: i) An endpoint PCR analysis confirmed the loss of Sendai virus vectors (Fig. 1C); ii) mycoplasma testing using a qPCR-based detection kit was negative (Supplementary Fig. 1); iii) a short tandem repeat (STR) analysis of 21 different genomic loci confirmed genetic matching of this iPSC line with the blood cells of the donor (archived at SCR journal); iv) the presence of two APOE ε4 alleles was verified via Sanger sequencing (Fig. 1D). Furthermore, the molecular karyotype of UKBi011-A showed no detectable abnormalities in a genome-wide SNP analysis (Fig. 1E). In order to confirm the pluripotent potential of UKBi011-A, the line was differentiated according to three separate differentiation protocols that promote the induction of ectodermal, mesodermal or endodermal lineages. RNA from all three differentiation cultures was pooled in an equal ratio and analyzed with a TaqMan-based hPSC Scorecard panel. With this assay we could confirm the upregulation of several germ layer-specific markers including PAX6 and MAP2 (ectoderm), HAND1 and FOXF1 (mesoderm) as well as SOX17 and POU3F3 (endoderm) (Fig. 1E, Table 1).

Materials and methods

Reprogramming of peripheral blood cells

PBMCs were stimulated to yield erythroblast cultures (van den Akker et al., 2010) by cultivating for 7 days in erythroblast medium (EM1) consisting of StemSpan SFEM (Stem Cell Technologies) supplemented with 2 U/mL EPO, 100 ng/mL SCF, 40 ng/mL IGF-1, 10 ng/mL IL-3 (all R&D systems), 1 μM dexamethasone (Sigma), and 1:100 chemically defined lipid concentrate (Life Technologies). On day 7 erythroblasts were purified by Percoll density gradient centrifugation. Cultures were further expanded in EM1 without IL-3. On day 10, 3×10^5 erythroblasts were infected with viral particles of the CytoTune-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher) for 24 h. Three days post-transduction, cells were seeded onto Geltrex-coated dishes and moved to hypoxic conditions (5% O_2). From day four onwards cells were cultured in non-supplemented StemSpan Medium. On days 7 and 8 medium was gradually, and from day 9 onwards completely changed to E7 medium. Colonies were picked on day 20 and expanded in mTeSR1 (Stem Cell Technologies) or StemMACS iPS-Brew XF (Miltenyi) on Geltrex (ThermoFisher) in standard atmosphere.

ScoreCard analysis

UKBi011-A iPSCs were subjected to three individual differentiation protocols for 5 days according to the following procedures: endoderm and mesoderm was induced with Stem Diff Definitive Endoderm Kit and the Stem Diff Mesoderm Induction Medium (both StemCell Technologies), respectively, and ectoderm was induced with neural induction medium (DMEM/F12 and Neurobasal medium, mixed in 1:1 ratio, supplemented with 1:200 N2, 1:2000 B27, 15 μM SB431542 and 50 nM LDN193189). From each culture total RNA was extracted and mixed in a 1:1:1 ratio. From this mix 1 μg RNA was used for reverse transcription. cDNA was analyzed with the TaqMan hPSC Scorecard 384 well panel (ThermoFisher) according to manufacturer's instructions.

Sendai virus detection

Total RNA was extracted and 1 μg RNA was used for RT-PCR analysis using the following PCR conditions: 1 min at 95 °C, 30s at 95 °C, 30s at 60 °C, 1 min at 72 °C (40 cycles), 5 min at 72 °C (primers listed in Table 2).

SNP analysis

SNP genotyping was performed at the Institute of Human Genetics at the University of Bonn using an Infinium PsychArray-24 v1.1 BeadChip (Illumina). Data was analyzed using GenomeStudio (Illumina).

APOE genotyping

Purified PCR products of genotyping primers listed in Table 2 were sequenced by Microsynth Seqlab.

Mycoplasma detection

Genomic DNA was extracted and analyzed with the qPCR kit Venor GeM qEP (Minerva Biolabs).

Table 1
Characterization and validation summary.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal morphology	Not shown but available from authors upon request.
Phenotype	Immunocytochemisty	Expression of pluripotency marker TRA-1-81	Fig. 1 panel A
	Flow cytometry	84.8% of all cells express the pluripotency marker TRA-1-60	Fig. 1 panel B
Genotype	Molecular karyotyping with SNP array	Normal karyotype, resolution: 1–2 kb	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR)	Not performed	N/A
	STR analysis	21 sites tested: complete match with donor's blood	Submitted to SCR journal for archiving
Mutation analysis (IF APPLICABLE)	Sequencing of APOE gene	Confirmation of APOE ε4/ε4 variant	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 1
Differentiation potential	TaqMan hPSC Scorecard analysis upon directed three germlayer differentiation	Passed	Fig. 1 panel F
Donor screening (OPTIONAL)	HIV 1 + 2, Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency Marker	Mouse anti-TRA-1-81	1:300	Millipore Cat# MAB4381, RRID: AB_177638
Pluripotency Marker	Mouse anti-Oct-3/4	1:400	Santa Cruz Biotechnology Cat# sc-514,295, RRID: AB_2721911
Pluripotency Marker (Flow cytometry)	Mouse anti-TRA-1-60	1:1000	Millipore Cat# MAB4360, RRID: AB_2119183
Secondary antibody	Goat anti-Mouse IgG (H + L)	1:1000	ThermoFisher Scientific Cat# A-21422, RRID: AB_2535844
Secondary antibody	Goat anti-Mouse IgG (H + L)	1:1000	ThermoFisher Scientific Cat# A-11001, RRID: AB_2534069
Secondary antibody (Flow cytometry)	Goat anti-Mouse IgM Heavy Chain	1:1500	ThermoFisher Scientific Cat# A-21042, RRID: AB_2535711

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pan-SeV	Sendai virus backbone	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
18s	18s ribosomal RNA	TTCCTTGGACCGGCGCAAG/GCCGCATCGCCGGTCGG
Genotyping	APOE	ACTGACCCCGGTGGCGGAGGA/CAGGCGTATCTGCTGGGCCTGCTC

Immunocytochemisty

Cells were fixed with 4% paraformaldehyde at RT for 10 min, washed with PBS, blocked with 10% FBS in PBS with 0.1% TritonX-100 for 1 h. Primary antibody was incubated overnight at 4 °C, followed by secondary antibody incubation for 1 h at RT. Antibody list: Table 2.

Flow cytometry

10^6 cells were stained with the primary antibody in PBS for 15 min on ice followed by secondary antibody incubation for 30 min (4 °C). Cells were analyzed with a FACS Calibur (BD Biosciences). Antibody list: Table 2.

STR analysis

STR analysis was performed by Eurofins Medigenomix Forensik GmbH by using a PowerPlex 21 PCR kit (Promega).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.011.

Conflict of interest

The authors declare no conflict of interest.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.011.

Acknowledgements

The generation of the line was partly supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement number 115582 (EBiSC), the resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution; and partly supported the ADAPTED consortium, which has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115975. This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations.

The following is the supplementary data related to this article.

References

van den Akker, E., Satchwell, T.J., Pellegrin, S., Daniels, G., Toye, A.M., 2010. The majority of the in vitro erythroid expansion potential resides in CD34 - cells, outweighing the contribution of CD34 + cells and significantly increasing the erythroblast yield from peripheral blood samples. Haematologica 95, 1594–1598. http://dx.doi.org/10.3324/haematol.2009.019828.

Michaelson, D.M., 2014. APOE ε4: the most prevalent yet understudied risk factor for Alzheimer's disease. Alzheimers Dement. 10, 861–868. http://dx.doi.org/10.1016/j.jalz.2014.06.015.

Yu, J.-T., Tan, L., Hardy, J., 2014. Apolipoprotein E in Alzheimer's disease: an update. Annu. Rev. Neurosci. 37, 79–100. http://dx.doi.org/10.1146/annurev-neuro-071013-014300.