Stem Cell Research 16 (2016) 113–115

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem cell line

Lymphoblast-derived integration-free iPS cell line from a 65-year-old Alzheimer's disease patient expressing the TREM2 p.R47H variant

Friederike Schröter [a], Kristel Sleegers [b,c], Elise Cuyvers [b,c], Martina Bohndorf [a], Wasco Wruck [a], Christine Van Broeckhoven [b,c], James Adjaye [a,*]

[a] Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine-University Düsseldorf, Moorenstr, 5, 40225 Düsseldorf, Germany
[b] Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerp, Belgium
[c] Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Belgium

ARTICLE INFO

Article history:
Received 17 December 2015
Accepted 23 December 2015
Available online 28 December 2015

ABSTRACT

Human lymphoblast cells from a male patient diagnosed with Alzheimer's disease (AD) expressing the TREM2 p.R47H variant were used to generate integration-free induced pluripotent stem (iPS) cells employing episomal plasmids expressing OCT4, SOX2, NANOG, LIN28, c-MYC and L-MYC. The iPS cells retained the TREM2 mutation, and were defined as pluripotent based on (i) expression of pluripotent-associated markers, (ii) embryoid body-based differentiation into cell types representative of the three germ layers and (iii) the similarity between the transcriptomes of the iPS cell line and the human embryonic stem cell line H1 with a Pearson correlation of 0.966.

1. Resource table

Name of stem cell construct	pCXLE-hOCT3/4-shp53-F (ID 27077) pCSLE-hSK (ID 27078) pCXLE-hUL (ID 27080)
Institution	Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine-University VIB — Department of Molecular Genetics, Antwerp, Belgium
Person who characterized resource	Friederike Schröter
Contact person and email	James Adjaye james.adjaye@med.uni-duesseldorf.de
Date archived/stock date	4 June 2014
Origin	Human lymphoblast cells
Type of resource	Biological reagent: induced pluripotent stem cell, derived from human lymphoblast cells
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, NANOG, LIN28, c-MYC, L-MYC
Authentication	Identity and purity of cell line confirmed as shown in Fig. 1
Link to related literature (direct URL links and full references)	Cuyvers E, Bettens K, Philtjens S, Van Langenhove T, Gijselinck I, van der Zee J, Engelborghs S, Vandenbulcke M, Van Dongen J, Geerts N, Maes G, Mattheijssens M, Peeters K, Cras P, Vandenberghe R, De Deyn PP, Van Broeckhoven C, Cruts M, Sleegers K; BELNEU consortium, Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. *Neurobiol Aging* 2014 Mar.;35(3):726.e11-9
Information in public databases	Not yet

2. Resource details

Lymphoblast cells (AD2-2), derived from a 65-year-old AD patient expressing the TREM2 missense p.R47H mutation (Cuyvers et al., 2014), were reprogrammed employing oriP/EBNA-1-based episomal plasmids expressing OCT4, SOX2, KLF4, C-MYC, L-MYC, LIN28 and a p53 shRNA. After picking the clones, clone A of Lymph2-AD2-iPS cell line retained traces of EBNA1 (Fig. 1A) but was diluted out after several passages (*data not shown*). Clone B was negative for EBNA-1 and oriP (Fig. 1A), but both clones were positive for the pluripotency-associated genes *OCT4*, *SOX2*, *NANOG* and *TGDF1* (Fig. 1A). Pluripotency was confirmed by (i) expression of OCT4, SOX2, NANOG, TRA-1-60 and TRA-1-81 and SSEA4 (Fig. 1B) and (ii) embryoid body (EB)-based spontaneous differentiation into cell types representative of the three germ layers, namely ectoderm (Nestin, PAX6), mesoderm (SMA — smooth muscle actin) and endoderm (AFP — α-feto protein, SOX17) (Fig. 1C).

The DNA fingerprint of Lymph2-AD2-iPS cell line was identical to the parental lymphoblast line AD2-2 (Fig. 1D). Karyotype analysis was male (XY) and both lines exhibited a normal diploid chromosomal content (Fig. 1E). As depicted in the Dendrogram (Fig. 1F), the transcriptome of the parental lymphoblast cells differs from the pluripotent cell lines. The transcriptomes of the Lymph2-AD2-iPS cells and the embryonic stem

* Corresponding author.
E-mail address: James.Adjaye@med.uni-duesseldorf.de (J. Adjaye).

http://dx.doi.org/10.1016/j.scr.2015.12.017

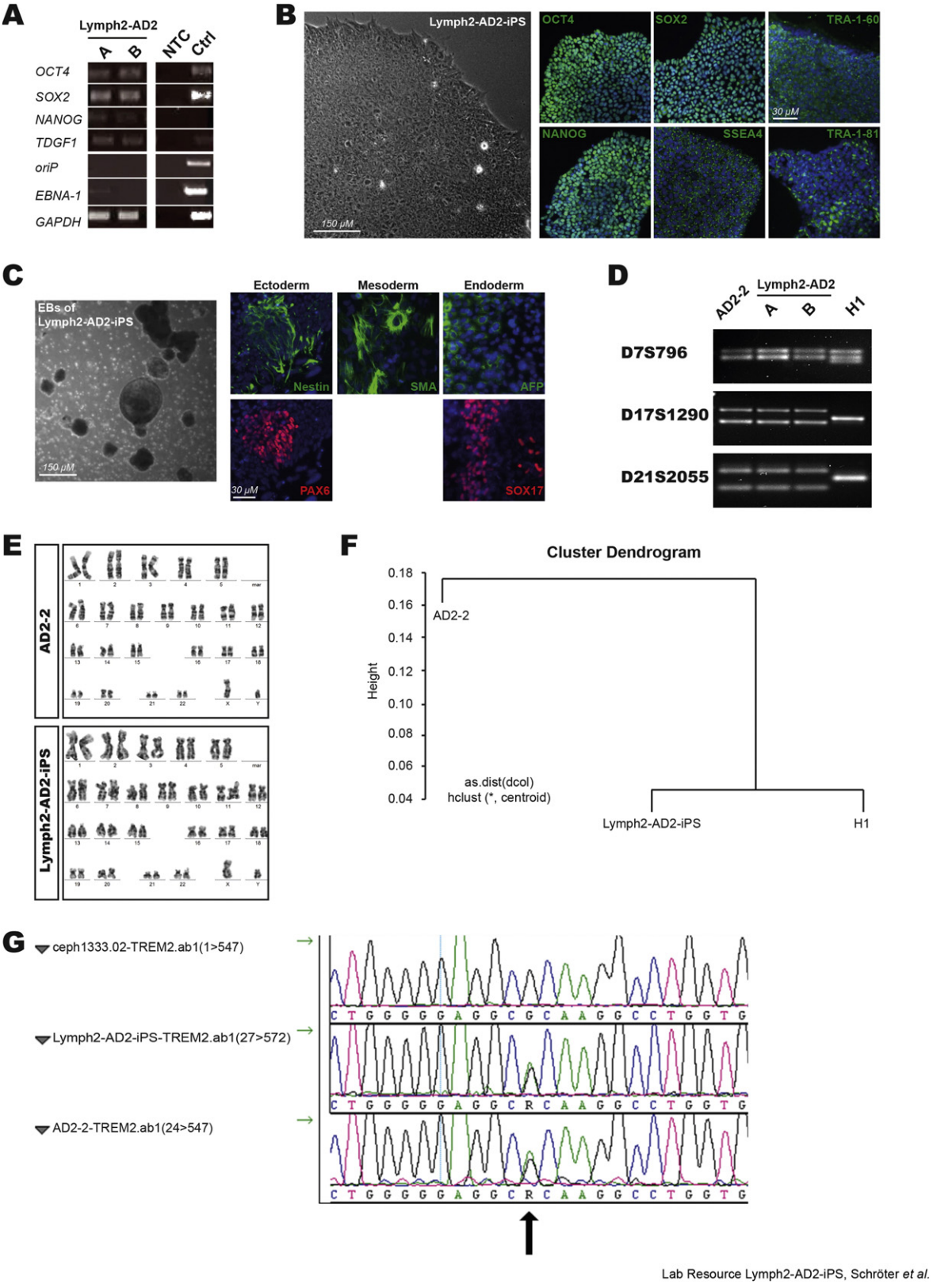

Fig. 1. Characterization of the Lymph2-AD2-iPS line. (A) Confirmation of mRNA expression of pluripotency-associated, oriP and EBNA-1 genes. NTC and control are the same as described in Schröter et al., (2016); NTC, non-template control; Ctrl, control line with oriP and EBNA-1 detection. (B) Immunofluorescence-based detection of human pluripotency-associated proteins OCT4, SOX2, NANOG and surface markers TRA-1-60, TRA-1-81 and SSEA4. Hoechst 33,258 was used for the nuclei staining. Scale bar: big — 150 µm, small — 30 µm. (C) Embryoid body (EB) formation was induced in Lymph2-AD2-iPS cells *in vitro* and analyzed by immunofluorescence-based detection of different germ layer marker: ectoderm — Nestin, PAX6; mesoderm — α-smooth muscle actin (SMA) and endoderm — α-Feto Protein (AFP), SOX17. Scale bar: EBs — 150 µm, germ layer — 30 µm. (D) Gel electrophoresis of DNA fingerprinting PCR products. Genomic DNA was isolated from parental lymphoblast cells AD2-2, Lymph2-AD2-iPS cells (separated into two clones, A and B) and embryonic stem cell line H1. DNA was amplified using PCR primers that flank different genomic regions (D7S796, D17S1290 and D21S2055). (E) Karyotyping analysis of parental lymphoblast AD2-2 and Lymph2-AD2-iPS line. Presence of male karyogram 46, XY. (F) Cluster Dendrogram of lymphoblast AD2-2, Lymph2-AD2-iPS line and embryonic stem cell line H1. (G) Sequence trace of control CEPH (first line), Lymph2-AD2-iPS (middle) and parental lymphoblast AD2-2 (last) DNA. The TREM2 missense mutation p.R47H results from a single base substitution (G > A) (black arrow).

cell line-H1, clustered together with a Pearson correlation of 0.966. The reprogramming process did not alter the TREM2 missense mutation as demonstrated in Fig. 1G.

3. Materials and methods

3.1. Ethic statements

The EBV-transformed lymphoblastoid cell line, AD2-2, used for the generation of the iPS cell line Lymph2-AD2, was generated from peripheral blood lymphocytes from a 65-year-old male donor diagnosed with Alzheimer's disease. The research protocol was approved by the Ethics Committee of the University Hospital Antwerp and the University of Antwerp, Belgium.

3.2. Cell culture

The lymphoblast cell line AD2-2 (Cuyvers et al., 2014) was cultured in RPMI1640 supplemented with 15% fetal bovine serum (Invitrogen™), 1% GlutaMAX (Invitrogen™), 1% Sodium pyruvate (Invitrogen™) and 1% Penicillin/Streptomycin (Invitrogen™) at 37 °C and 5% CO_2.

3.3. Derivation of the iPS cell line

Lymphoblast cells (AD2-2) were reprogrammed by nucleofection of oriP/EBNA-1-based episomal plasmids (expressing OCT4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA) (Okita et al., 2011) at the Biomedicum Stem Cell Center, University of Helsinki, Finland, (http://research.med.helsinki.fi/neuro/Otonkoski/core/default.html) as a service. The iPS cells were cultured under feeder-free conditions on Matrigel®-coated plates in E8 medium (Invitrogen™).

3.4. Polymerase chain reaction

RT-PCR to assess the expression levels of the transgene and endogenous stem cell markers were carried out by the Biomedicum Stem Cell Center, University of Helsinki, Finland.

3.5. Embryoid body formation

Embryoid body (EB) formation was carried out as described in (Matz and Adjaye, 2015). In brief, after culturing of the iPS cells in ultra-low attachment flask (Corning) in FDTA medium, the EBs were replated onto gelatin-coated plates, again in FDTA medium lacking bFGF and Dorsomorphin (Frank et al., 2012).

3.6. Immunocytochemistry

Immunocytochemistry was performed as described in (Schröter et al., 2016), except for the germ layers staining. The following antibodies were used: rabbit anti-Nestin (Sigma Aldrich; 1:1000), mouse anti-PAX6 (SYSY, 1:1000), mouse anti-SMA (Cell Signaling; 1:1000), rabbit anti-AFP (Sigma Aldrich; 1:500) and mouse anti-SOX17 (Novus Biological, 1:500). The fluorescent images obtained by an inverse fluorescence microscope LSM 700 (Carl Zeiss) and analyzed employing Adobe Photoshop software (Adobe, USA).

3.7. Karyotype analysis

Karyotype analysis was evaluated and performed at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf. Thirty and twelve metaphases were counted for the parental lymphoblast line AD2-2 and the iPS cell line Lymph2-AD2, respectively.

3.8. DNA fingerprinting

Genomic DNA was isolated according to the manufacturer protocol from the lymphoblast line AD2-2, the Lymph2-AD2-iPS line, separated in two independent lines A and B, and the human embryonic stem cell line (H1). The STR analysis was performed by PCR amplification with specific primers (Prigione et al., 2011; Wang and Adjaye, 2011).

3.9. Bidirectional Sanger sequencing

Genotyping of TREM2 p.R47H was performed on genomic DNA from the lymphoblast line AD2-2 and the Lymph2-AD2-iPS line by PCR amplification and Sanger sequencing of exon 2 as described in Cuyvers et al. (2014).

3.10. RNA-based microarray analysis

RNA-based microarray analysis was performed as described in Schröter et al. (2016). Microarray analysis was outsourced to the Genomics/Transcriptomic Laboratory of the BMFZ, Heinrich-Heine-University, Düsseldorf.

Acknowledgments

JA acknowledges support from the Medical Faculty, Heinrich-Heine-University, Düsseldorf. Research at the Antwerp site is funded in part by the Belgian Science Policy Office Interuniversity Attraction Poles program (BELSPO, www.belspo.be), the Flanders Impulse Program on Networks for Dementia Research (VIND) and the University of Antwerp Research Fund (http://www.uantwerpen.be/). JA, FS, KS and CVB are members of the EU project-AgedBrainSYSBIO. The AgedBrainSYSBIO project received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 305299.

References

Cuyvers, E., Bettens, K., Philtjens, S., van Langenhove, T., Gijselinck, I., van der Zee, J., Engelborghs, S., Vandenbulcke, M., van Dongen, J., Geerts, N., Maes, G., Mattheijssens, M., Peeters, K., Cras, P., Vandenberghe, R., Deyn, P.P., de, van Broeckhoven, C., Cruts, M., Sleegers, K., 2014. Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. Neurobiol. Aging 35 (3), 726, e11-9. http://dx.doi.org/10.1016/j.neurobiolaging.2013.09.009.

Frank, S., Zhang, M., Schöler, H.R., Greber, B., 2012. Small molecule-assisted, line-independent maintenance of human pluripotent stem cells in defined conditions. PLoS One 7 (7), e41958. http://dx.doi.org/10.1371/journal.pone.0041958.

Matz, P., Adjaye, J., 2015. Generation of iPSC line epiHUVEC from human umbilical vein endothelial cells. Stem Cell Res. 15 (3), 581–583. http://dx.doi.org/10.1016/j.scr.2015.10.004.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K.-i., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H., Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8 (5), 409–412. http://dx.doi.org/10.1038/nmeth.1591.

Prigione, A., Hossini, A.M., Lichtner, B., Serin, A., Fauler, B., Megges, M., Lurz, R., Lehrach, H., Makrantonaki, E., Zouboulis, C.C., Adjaye, J., 2011. Mitochondrial-associated cell death mechanisms are reset to an embryonic-like state in aged donor-derived iPS cells harboring chromosomal aberrations. PLoS One 6 (11), e27352. http://dx.doi.org/10.1371/journal.pone.0027352.

Schröter, F., Sleegers, K., Bohndorf, M., Wruck, W., van Broeckhoven, C., Adjaye, J., 2016. Lymphoblast-derived integration-free iPS cell line from a 69-year-old male. Stem Cell Res. 16 (1), 29–31. http://dx.doi.org/10.1016/j.scr.2015.11.016.

Wang, Y., Adjaye, J., 2011. A cyclic AMP analog, 8-Br-cAMP, enhances the induction of pluripotency in human fibroblast cells. Stem Cell Rev. 7 (2), 331–341. http://dx.doi.org/10.1007/s12015-010-9209-3.