Stem Cell Research 30 (2018) 206–209

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of an induced pluripotent stem cell line from a patient with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP): HIHCNi003-A

Stefanie Nicole Hayer[a,b], Yvonne Schelling[b], Philip Hoeflinger[a,b], Stefan Hauser[a,b], Ludger Schöls[a,b,*]

[a] Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research & Center of Neurology, University of Tübingen, Tübingen, Germany
[b] German Research Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Tübingen, Germany

A B S T R A C T

An induced pluripotent stem cell line, HIHCNi003-A (iPSC-ALSP), was created from a skin biopsy of a patient with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) caused by a heterozygous c.2512G > C, p.Val838Leu mutation in the *CSF1R* gene. Skin fibroblasts were reprogrammed using episomal plasmids carrying *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, and *hLIN28*. The iPSC-ALSP line exhibits chromosomal stability with conservation of the *CSF1R* mutation, expresses pluripotency markers and differentiates into endo-, meso-, and ectodermal cells *in vitro*.

Resource table

Unique stem cell line identifier	HIHCNi003-A
Alternative name(s) of stem cell line	iPSC-ALSP
Institution	Hertie-Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
Contact information of distributor	Ludger Schöls; ludger.schoels@uni-tuebingen.de
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 55 Sex: male Ethnicity: German
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrating episomal plasmids
Genetic modification	No
Type of modification	N/A
Associated disease	Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, OMIM #221820
Gene/locus	*CSF1R* (GenBank: NM_005211.3), c.2512G > C, p.Val838Leu/ 5q32
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	July 2017
Cell line repository/ bank	N/A
Ethical approval	Ethics committee of the University Hospital Tübingen, approval number 598/2011

Resource utility

Generation of ALSP patient-derived iPSCs allows the investigation of *CSF1R* mutations in a human cell model. As the pathophysiology of the neurodegenerative disorder ALSP is still largely unknown, iPSC-ALSP is an important tool to generate and study different cell types

* Corresponding author at: Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research & Center of Neurology, University of Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany.
E-mail address: ludger.schoels@uni-tuebingen.de (L. Schöls).

https://doi.org/10.1016/j.scr.2018.06.011
Received 23 April 2018; Received in revised form 17 May 2018; Accepted 19 June 2018
Available online 20 June 2018

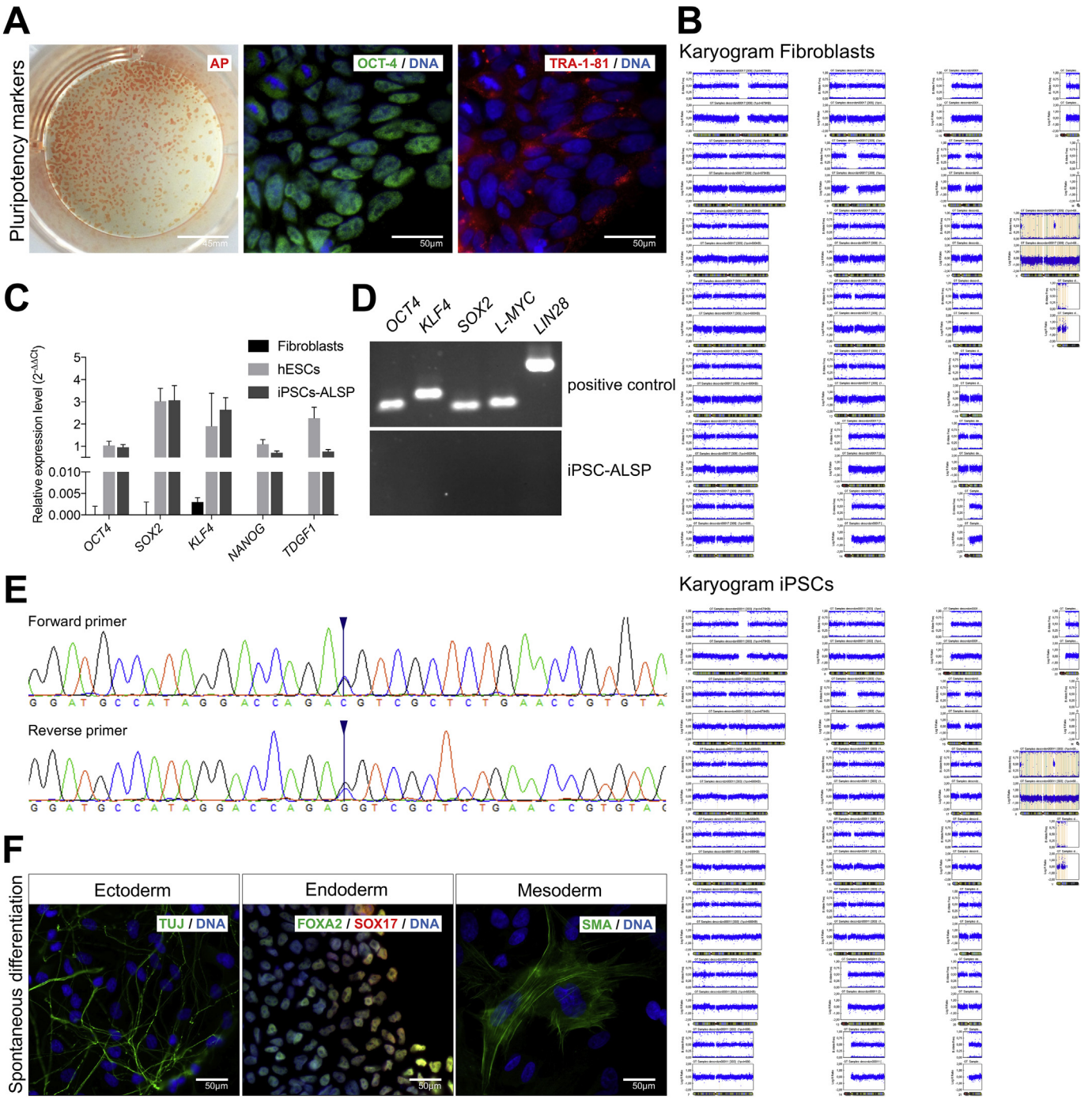

Fig. 1. Characterization of iPSC-ALSP.

potentially involved in the disease, e.g. neurons and microglia.

Resource details

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare, adult-onset neurodegenerative disorder characterized by cognitive decline, personality changes, and motor impairment (Konno et al., 2017). The onset of symptoms is typically in the fourth or fifth decade followed by a rapid progression, resulting in death within approximately seven years (Konno et al., 2017). The disease is caused by autosomal-dominant mutations in the *colony-stimulating factor 1 receptor* (*CSF1R*) gene (Rademakers et al., 2012).

To study the pathophysiology of ALSP, the iPSC line HIHCNi003-A (iPSC-ALSP) was generated by delivery of episomal plasmids encoding human *OCT4*, *SOX2*, *KLF4*, *L-MYC* and *LIN28* (Okita et al., 2011) into skin fibroblasts from a 55-year-old patient with a ALSP. The patient developed word-finding difficulties at the age of 51, followed by rapidly progressing dementia with apathy, poverty of speech, and pronounced apraxia.

To characterise and validate the iPSC-line, genomic integrity was analysed by comparative SNP analysis of fibroblasts and the generated iPSCs; the analysis showed a normal karyotype (46, XY) without chromosomal aberrations at a resolution of 350kbp (Fig. 1B, Table 1). Sequencing of the *CSF1R* gene confirmed the heterozygous mutation in the iPSCs (Fig. 1E, Table 1). To exclude genomic integration of the episomal plasmids that were used for reprogramming of the fibroblasts,

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	not shown but available with author
Phenotype	Immunocytochemisty	Positive staining for AP, OCT4 and TRA-1-81	Fig. 1 panel A
	qRT-PCR	Expression of pluripotency markers *OCT4*, *SOX2*, *KLF4*, *NANOG*, *TDGF1*	Fig. 1 panel C
Genotype	Karyotype	46, XY No larger chromosomal aberrations or copy number variations (resolution 350 kbp)	Fig. 1 panel B
Identity	Whole genome SNP genotyping	Fibroblast-iPSC genotypic identicalness confirmed	Fig. 1 panel B
Mutation analysis	Sequencing	Heterozygous *CSF1R* mutation	Fig. 1 panel E
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 1
Differentiation potential	Spontaneous differentiation	Expression of germ layer markers: Ectoderm – TUJ Endoderm – FOXA2, SOX17 Mesoderm – SMA	Fig. 1 panel F
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

standard PCR with specific primers for *OCT4*, *SOX2*, *KLF4*, *L-MYC* and *LIN28* (Table 2) was performed; there was no amplification of either gene in the iPSCs (Fig. 1D). The expression of pluripotency markers was assessed on protein and RNA level. Alkaline phosphatase (AP) and immunocytochemical stainings of OCT4 and TRA-1-81 revealed a robust Fast Red (AP) and fluorescence signal, respectively (Fig. 1A, Table 1). Expression of the pluripotency genes *OCT4*, *SOX2*, *KLF4*, *NANOG*, and *TDGF1* was upregulated in comparison to fibroblasts and similar to that of the human embryonic stem cell (hESC)-line I3 as assessed by qRT-PCR (Fig. 1C, Table 1). The potential of the generated iPSCs to differentiate into cells of all three germ layers was investigated by spontaneous differentiation. iPSCs were able to differentiate into neurons expressing β-III-tubulin, early endodermal cells positive for FOXA2 and SOX17, and muscle cells positive for α-smooth muscle actin (SMA) (Fig. 1F, Table 1).

Materials and methods

Reprogramming

Patient fibroblasts carrying the heterozygous mutation c.2512G > C, p.Val838Leu in *CSF1R* were derived from a skin biopsy and reprogrammed by nucleofection of 1×10^5 cells with 1 µg of each plasmid (*hOCT3/4*, *hSOX2*, *hKLF4*, *hL-MYC* and *hLIN28* (Okita et al., 2011)) using the Nucleofector 2D system (Lonza). After a period of 2 days in fibroblast medium containing 2 ng/ml FGF-2 (Peprotech), cells were transferred to Essential 8 (E8) medium with 100 µM sodium butyrate (Sigma-Aldrich). 3–4 weeks after reprogramming, iPSC colonies were picked. At passage 5, cells were analysed as described or frozen in E8 with 40% KnockOut Serum Replacement (KOSR, Life technologies), 10% DMSO (Sigma-Aldrich) and 1 µM Y-27632 (Abcam Biochemicals). Mycoplasma negativity was confirmed by PCR using a PCR Mycoplasma Test Kit (AppliChem).

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Goat anti-OCT4	1:100	Santa Cruz, AB_653551
	Mouse anti-TRA-1-81	1:500	Millipore, AB_177638
Differentiation markers	Mouse anti-SMA	1:100	Dako, AB_2223500
	Mouse anti-SOX17	1:250	R&D Systems, AB_355060
	Rabbit anti-FOX-A2	1:300	Millipore, AB_390153
	Mouse anti-TUJ	1:1000	Sigma Aldrich, AB_477590
Secondary antibodies	Alexa Fluor 488 Donkey anti-Goat IgG	1:300	Life Technologies
	Alexa Fluor 488 Goat anti-Rabbit IgG	1:300	Life Technologies
	Alexa Fluor 488 Goat anti-Mouse IgG	1:300	Life Technologies
	Alexa Fluor 568 Goat anti-Mouse IgG	1:300	Life Technologies

Primers

	Target	Forward/Reverse primer (5′-3′)
Episomal plasmids	*OCT3/4* plasmid	CATTCAAACTGAGGTAAGGG TAGCGTAAAAGGAGCAACATAG
	KLF4 plasmid	CCACCTCGCCTTACACATGAAG TAGCGTAAAAGGAGCAACATAG
	L-MYC plasmid	GGCTGAGAAGAGGATGGCTAC TTTGTTTGACAGGAGCGACAAT
	SOX-2 plasmid	TTCACATGTCCCAGCACTACCAG TTTGTTTGACAGGAGCGACAAT
Pluripotency markers	*OCT4*	GGAAGGTATTCAGCCAAACG CTCCAGGTTGCCTCTCACTC
	NANOG	CAAAGGCAAACAACCCACTT TGCGTCACACCATTGCTATT
	SOX2	AGCTCGCAGACCTACATGAA CCGGGGAGATACATGCTGAT
	KLF4	CCCCAAGATCAAGCAGGAGG GGGCAGGAAGGATGGGTAAT
	TDGF1	GGTCTGTGCCCCATGACA AGTTCTGGAGTCCTGGAAGC
Housekeeping gene	*GAPDH*	TCACCAGGGCTGCTTTTAAC GACAAGCTTCCCGTTCTCAG
Mutation sequencing	*CSF1R*	AACAGCTTTGTCCACCAGTG AGAGTCGGGGCCCAAAATAA

Genomic integrity

Whole-genome SNP genotyping was performed by Life & Brain GENOMICS with 2 µg iPSC- and fibroblast-DNA isolated using the DNeasy Blood & Tissue Kit (Qiagen). The analysis was conducted using Infinium OmniExpressExome-8-BeadChip (Illumina) and GenomeStudio V2.0.3 (Illumina) for evaluation. Copy number analysis was performed via the cnvPartition plugin (Illumina). Early mosaicism states were evaluated by manual review on B allele frequency plots on chromosomal level.

To confirm the *CSF1R* mutation, the sequence was analysed using the 3130xl Genetic Analyzer (Applied Biosystems) and primers flanking the *CSF1R* gene (Table 2), followed by visualization with Staden 2.0.0b10 software (Staden Sourceforge).

Non-integration of the three episomal plasmids was confirmed with plasmid specific primers (Table 2) via PCR using GoTaq G2 DNA Polymerase (Promega).

Pluripotency

Alkaline phosphatase (AP) staining was done with iPSCs cultivated on 12-well plates and fixed in 4% paraformaldehyde (PFA) for 1 min. After washing with PBS, staining solution (20 µl Naphthol AS-MX phosphate alkaline solution (Sigma-Aldrich) and 500 µl Fast Red (1 mg/ml, Sigma Aldrich)) was added over night.

Cells were immunostained for pluripotency markers using a standard staining protocol after cultivation on coverslips and fixation in 4% PFA for 15 min. Primary and secondary antibodies are listed in Table 2. After embedding in Pro-Long Diamond Antifade Mountant, photomicrographs were taken with the Axio Imager Z1 (Zeiss).

For real-time quantification of pluripotency markers, RNA was isolated with the High Pure RNA Isolation Kit (Roche), reverse transcribed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche) and quantified on the Light Cycler 480 using SYBR Green I Master (Roche) with specific primers (Table 2). Runs were performed as triplicates and CT-values were normalized using the $2^{-\Delta\Delta Ct}$ method; the hESCs line H9 was used as reference and *GAPDH* as housekeeping gene.

For embryoid body (EB) formation, iPSCs were cultivated in EB medium consisting of 80% DMEM/F12 (Life technologies), 20% KOSR, 1× NEAA, 1× Pen/Strep, 2 mM l-Glutamine and 0.1 mM 2-Mercaptoethanol on AggreWell 800 plates (Stemcell Technologies) with medium changed on day 2. EBs were collected on day 4, plated on 0.1% gelatine (Sigma-Aldrich)-coated coverslips, cultivated for additional 2–3 weeks with medium changed every other day, and analysed by immunostaining using specific antibodies (Table 2).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.06.011.

Acknowledgments

We thank Prof. Dr. Schöler from the Max Planck Institute for Molecular Biomedicine for kindly providing the cDNA of the human embryonic stem cell lines I3 and H9.

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