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Lab resource: Stem Cell Line

Generation of an induced pluripotent stem cell (iPSC) line from a 42-year-old adult cerebral type X-linked adrenoleukodystrophy (X-ALD) patient

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A B S T R A C T

X-linked Adrenoleukodystrophy (X-ALD) is a neuro-metabolic disorder that is caused by malfunction of a peroxisomal transporter protein, adenosine ATP-binding cassette transporter superfamily D member 1 (ABCD1). We established an induced pluripotent stem cell (iPSC) line from a 42-year-old male X-ALD patient-derived dermal fibroblasts with Sendai virus-mediated reprogramming. Established iPSCs stably expanded, expressed genes of pluripotency, and maintained normal karyotype. *In vitro* differentiation assay revealed the characteristics of all three germ layers.

Resource table

Unique stem cell line identifier	KURNDi007-A
Alternative name(s) of stem cell line	acALD7
Institution	Department of Biotechnology, College of Life Science and Biotechnology, Korea University
Contact information of distributor	Dae-Sung Kim, sonnet10@korea.ac.kr Seungkwon You, bioseung@korea.ac.kr
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 42 Sex: Male Ethnicity if known: Asian (Korean)
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus (Cytotune® 2.0)
Genetic Modification	No
Type of Modification	N/A
Associated disease	X-linked adrenoleukodystrophy (X-ALD)
Gene/locus	ABCD1 gene/ Xq28
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	2017.06.14
Cell line repository/ bank	N/A
Ethical approval	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional Review Board Approval number: 4–2016-0194

Resource utility

This iPSC line will provide an *in vitro* system for future studies in pathophysiology and will contribute to the development of new therapies for X-ALD.

Resource details

X-linked adrenoleukodystrophy (X-ALD) is an inherited neuro-metabolic disorder caused by mutations in ATP-binding cassette transporter subfamily D member 1 (ABCD1) (Kemp et al., 2016). We generated an induced pluripotent stem cell (iPSC) line from a 42-year-old patient diagnosed with adult cerebral type-ALD (acALD) by implementing a commercial reprogramming kit (Cytotune®-iPS 2.0). These cells (KURNDi007-A) maintained the typical morphology of undifferentiated iPSCs, and were found positive for alkaline phosphatase (Fig. 1A). The majority of cells expressed various pluripotency markers

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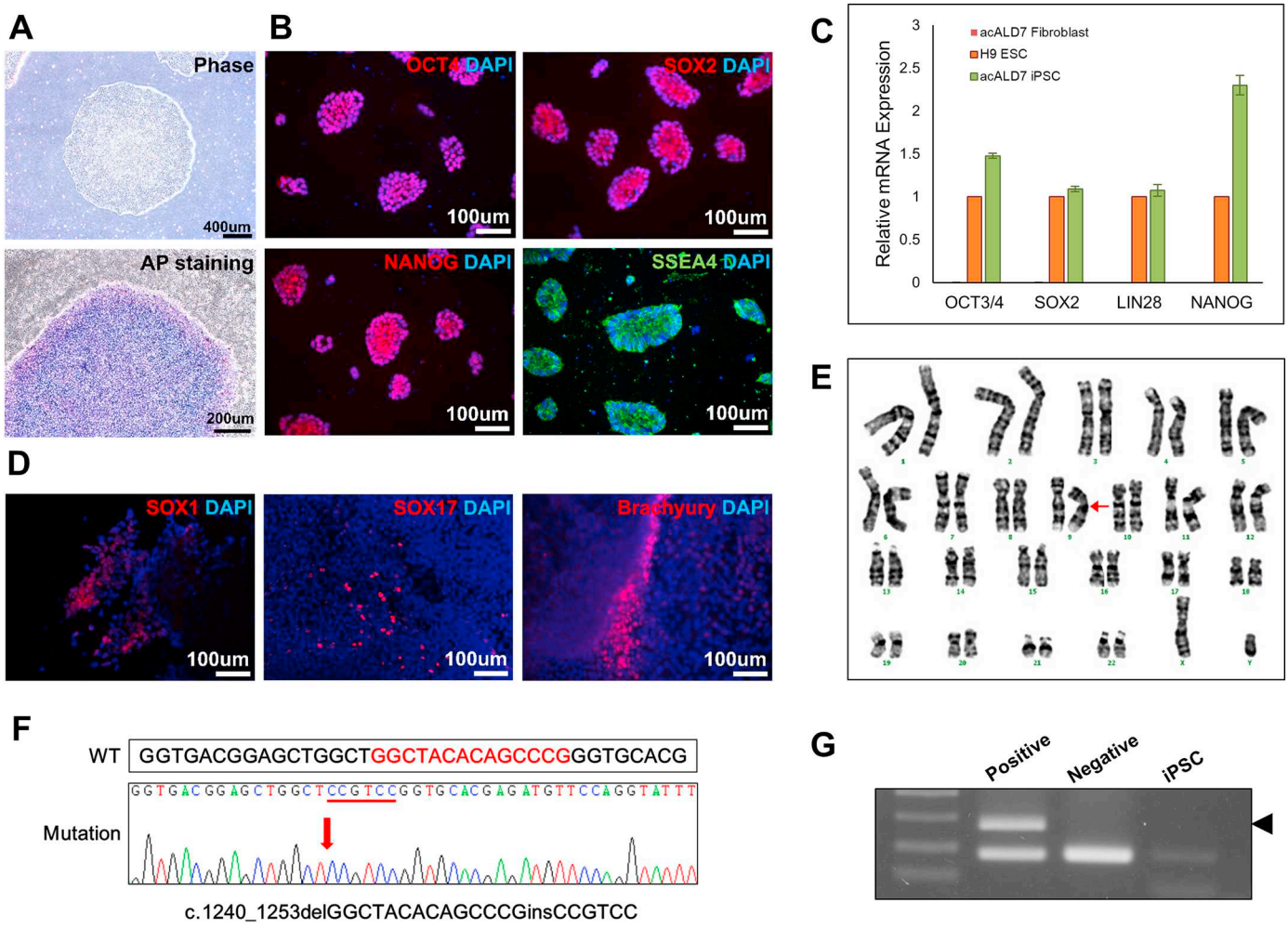

Fig. 1. Characterization of KURNDi007-A iPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Qualitative analysis	Positive staining for OCT4, NANOG, SOX2, SSEA4	Fig. 1 panel B
	Quantitative analysis	Expression of pluripotency markers equivalent or greater than H9 embryonic stem cells	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46, XY, inv.(9) (p12q13), Resolution 550	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	18 locus tested. 100% match	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Hemizygote mutation	Fig. 1 panel F
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Negative	Fig. 1 panel G
Differentiation potential	Spontaneous differentiation	SOX1, SOX17, Brachyury	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

including OCT4, SOX2, NANOG, and SSEA4 (Fig. 1B–C). Under the *in vitro* condition, the cells spontaneously differentiated into all three germ layers, as examined by immunofluorescence staining with markers like SOX1 (for ectoderm), SOX17 (for endoderm), and Brachyury (for mesoderm) (Fig. 1D). Karyotype of the expanded iPSCs displayed normal (46, XY) characteristics except for a small inversion detected at p12q13 locus (Fig. 1E, indicated by a red arrow). However, the same was observed in the donor's fibroblasts (Supplementary Fig. 1, indicated by a red arrow), indicating that this alteration was not created during reprogramming nor expansion. Genomic sequencing revealed that the iPSCs carries 14 bp deletion and 6 bp insertion in exon 4 of ABCD1 (c.1240_1253delGGCTACACAGCCCGinsCCGTCC (Fig. 1E), and STR analysis showed that the original fibroblasts share identical alleles with the newly created iPSCs (Supplementary Table 1). No mycoplasma contamination was detected (Table 1, Fig. 1G, indicated by an arrow head).

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT3/4	1:500	Santa Cruz Cat# SC-9081, RRID: AB_2167703
Pluripotency markers	Rabbit anti-SOX2	1:500	Millipore Cat# AB5603, RRID: AB_2286686
Pluripotency markers	Mouse anti-SSEA4	1:1000	Millipore Cat# MAB4304, RRID: AB_177629
Pluripotency markers	Rabbit anti-NANOG	1:500	Abcam Cat# AB21624, RRID: AB_446437
Differentiation markers	Goat anti-SOX1	1:200	R&D Cat# AF3369, RRID: AB_2239879
Differentiation markers	Goat anti-BRACHYURY	1:200	R&D Cat# AF2085, RRID: AB_2200235
Differentiation markers	Goat anti-SOX17	1:200	R&D Cat# AF1924, RRID: AB_355060
Secondary antibodies	Alexa Fluor®568 donkey anti-goat IgG (H + L)	1:1000	Thermo Fisher Scientific Cat# A11057, RRID: AB_2534104
Secondary antibodies	Alexa Fluor®568 donkey anti-rabbit IgG (H + L)	1:1000	Thermo Fisher Scientific Cat# A10042, RRID: AB_2534017
Secondary antibodies	Alexa Fluor®488 donkey anti-mouse IgG (H + L)	1:1000	Thermo Fisher Scientific Cat# A21202, RRID: AB_141607

Primers		
	Target	Forward/Reverse primer (5′-3′)
Targeted mutation analysis/sequencing	ABCD1	ATTGAGCGGATCATGTCGTC/ CATGGAGGTCCCTGAGTGAG
Pluripotency Marker (qPCR)	OCT3/4	CCTGAAGCAGAAGAGGATCACC/ AAAGCGGCAGATGGTCGTTTGG
Pluripotency Marker (qPCR)	SOX2	TGGACAGTTACGCGCACAT/ CGAGTAGGACATGCTGTAGGT
Pluripotency Marker (qPCR)	LIN28	TTGAGGAGCAGGCAGAGTGG/ TGCATTTGGACAGAGCATGG
Pluripotency Marker (qPCR)	NANOG	CTCCAACATCCTGAACCTCAGC/ CGTCACACCATTGCTATTCTTCG
Housekeeping gene (qPCR)	ACTB	CACCATTGGCAATGAGCGGTTC/ AGGTCCTTGCGGATGTCCACGT

Materials and methods

Cell culture

The dermal fibroblasts were obtained from a 42-year-old X-ALD patient at the Severance Biomedical Science Institute, Seoul, Korea under the guidelines of the Institutional Review Board (Approval No. 4-2013-0570). The fibroblasts were cultured in growth media (GM; DMEM supplemented with 15% FBS, 1% penicillin/streptomycin, 2 mM L-glutamine) at 37 °C in 5% CO_2.

Generation of iPSC

Fibroblasts were seeded on a 6-well plate at the density of 1×10^5 cells/well a day before reprogramming. CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) was used to reprogram the fibroblasts following manufacturer's instruction. After viral infection, the cells were cultured for another week and then transferred onto mitotically inactivated STO feeder cells (ATCC). Several iPSC-like colonies were observed in a week afterward. The colonies with the typical morphology of iPSCs were manually selected and passed onto newly prepared feeder cells from the third week after viral infection (counted as passage 1), and then subsequently expanded as a constructed cell line in media composed of DMEM/F12 supplemented with 20% knockout serum replacement, 1% penicillin/streptomycin, 1× non-essential amino acid, 1× beta-mercaptoethanol (Thermo Fisher Scientific), and 10 ng/ml basic fibroblast growth factor (PeproTech) at 37 °C in 5% CO_2. After 10 passages, iPSCs grown with feeder cells have been adapted to the feeder-free culture medium (StemMACS™ medium, Miltenyi Biotech) on the coating of Matrigel® (hESC-Qualified, Corning).

In vitro differentiation

iPSC colonies were mechanically detached and cultured as embryoid bodies (EBs) in ultra-low attachment 6 well plates (Corning) in the EB medium (composed of DMEM/F12, 20% KSR, 1× NEAA, and 0.1 mM beta-mercaptoethanol) supplemented with 5% FBS. At day 7, EBs were plated on Matrigel-coated coverslips and cultured for additional 2–3 weeks in the same medium. Then, EB colonies were subjected to immunostaining for germ layer marker expression, as described below.

Alkaline phosphatase staining

Alkaline phosphatase activity was detected using the Leukocyte Alkaline Phosphatase Kit (Sigma-Aldrich) according to a manual provided by the manufacturer.

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) in phosphate buffered saline (PBS) for 10 min, permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 5 min, and then blocked with 2% bovine serum albumin (Bovogen biologicals) in PBS for at least one hour at room temperature. Then, the cells were incubated with primary antibodies (Table 2) at 4 °C over-night. After thorough washing with PBS, the samples were exposed to the secondary antibodies for one hour at room temperature. The samples were mounted on slide glasses with VECTASHIELD® DAPI mounting medium (Vector Labs) and observed under the fluorescent microscope (IX73) (Olympus Corp.) with digital camera (DP73) (Olympus).

Sequencing analysis of the ABCD1 mutant alleles and Karyotyping

Genomic DNA was isolated from the iPSC lines using a G-spin® Total DNA Extraction Mini Kit (iNtRon Biotechnology). Mutation sequencing was performed using acALD7-specific primers (Table 2). Karyotyping using GTG banding was performed by CANCERROP Corp.

Mycoplasma contamination detection

The absence of mycoplasma contamination was confirmed using a e-

Myco™ Mycoplasma PCR Detection kit (iNtRON Biotechnology).

STR analysis

STR analysis was performed by Cosmo Genetech.

Acknowledgements

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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.2019.101425.

References

Kemp, S., Huffnagel, I.C., Linthorst, G.E., et al., 2016. Adrenoleukodystrophy – neuroendocrine pathogenesis and redefinition of natural history. Nat. Rev. Endocrinol 12, 606–615.