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Lab Resource: Single Cell Line

Establishment of an induced pluripotent stem cell line DHMi005-A from a healthy male proband

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A B S T R A C T

We generated an induced pluripotent stem cell (iPSC) line from a healthy male 29-year-old proband. Adipose fibroblasts (AFs) were reprogrammed using Sendai virus. Generated iPSCs showed typical stem cell morphology. From passage 9 on, iPSCs were free of virus. Pluripotency in the iPSCs was verified and spontaneous differentiation showed expression of all three germ layers. Karyotyping indicated no anomalies for the generated iPSCs. Many patient-specific iPSCs are generated from subcutaneous fat fibroblasts obtained during surgical procedure. The described control iPSC line was generated equally and therefore serves as an ideal control for adipose-fibroblast-based patient-specific iPSC lines in disease modeling.

Resource Table

Unique stem cell line identifier	DHMi005-A https://hpscreg.eu/user/cellline/edit/DHMi005-A
Alternative name(s) of stem cell line	Control_L
Institution	Department of Cardiovascular Surgery, Institute Insure, German Heart Center Munich
Contact information of distributor	Dr. rer. nat. Martina Dreßen, dressen@dhm.mhn.de
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 29 years Sex: male Ethnicity: Caucasian
Cell Source	Adipose fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune-iPS™-iPS 2.0 Sendai Reprogramming (Invitrogen, ThermoFisher Scientific)
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Sendai-footprinting, RT-PCR (passage 9)
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	December 2021
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/DHMi005-A
Ethical approval	Ethical committee of the Medical Faculty of the Technical University of Munich 5943/13

1. Resource utility

The extent of initial cell source influencing iPSC reprogramming and subsequent differentiation is uncertain. Therefore, it is advantageous to use control iPSCs with the same origin as the patient's iPSCs. The generated adipose-fibroblast-based iPSCs serve as ideal control for disease modeling since many patient-specific iPSCs are generated from adipose fibroblasts.

2. Resource details

The established cell line described here was generated from

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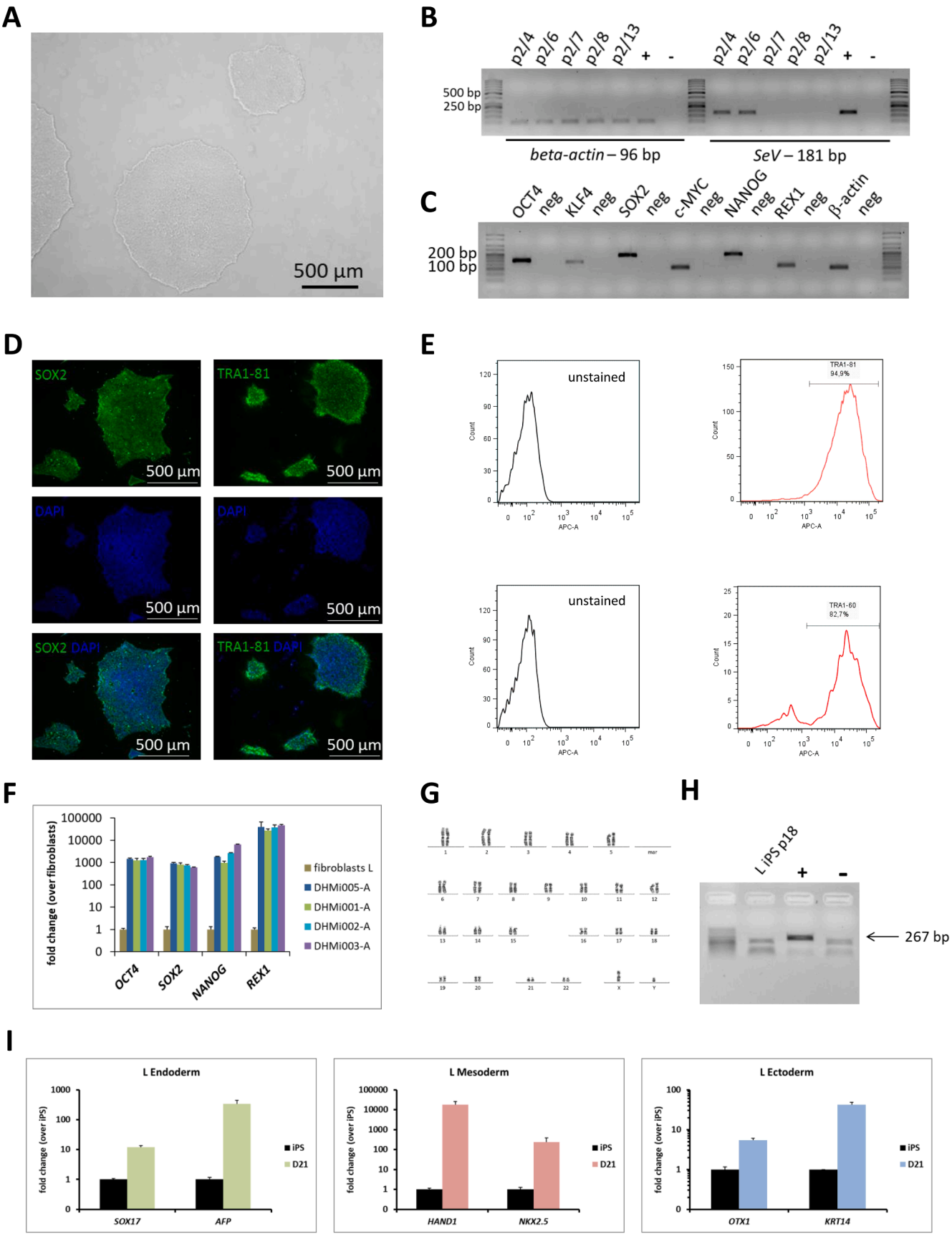

Fig. 1. Characterization of the iPSC control line DHMi005-A from a male healthy proband generated from adipose fibroblasts.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive staining for SOX2, TRA1-81	Fig. 1D
	Quantitative analysis (Flow cytometry, RT-qPCR)	94,90% cells positive for cell surface marker TRA1-81 and 82,7% cells positive for cell surface marker TRA1-60 Transcript expression of pluripotency markers *OCT4*, *SOX2, NANOG*, *REX1*	Fig. 1E Fig. 1C, F
Genotype	Karyotype (G-banding) and resolution	46XY	Fig. 1G
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	Tested 16 sites (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818, FGA), all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR	Fig. 1H
Differentiation potential	Embryoid body formation	*SOX17* and *AFP* (endoderm), *HAND1* and *NKX2.5* (mesoderm), *OTX1* and *KRT14* (ectoderm)	Fig. 1I
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) with β-*actin* as reference gene	Endoderm: *SOX17*, *AFP*; Ectoderm: *OTX1*, *KRT14*; Mesoderm: *HAND1*, *NKX2.5*	Fig. 1I qRT-PCR with reference gene β-*actin*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping		not shown but available with author
	HLA tissue typing		not shown but available with author

fibroblasts cultured from subcutaneous fat (AFs) using the non-integrative Sendai virus (Resource Table). Clones were manually picked and characterized. The iPSCs showed typical stem cell morphology without differentiating areas (Fig. 1A). At passage 9 (p9) after reprogramming, the Sendai virus was absent (Fig. 1B). Expression of endogenous reprogramming factors and pluripotency markers (Fig. 1C) was validated. Pluripotency was verified by immunocytochemistry for SOX2 and TRA1-81 (Fig. 1D) and by flow cytometry for TRA1-81 and TRA1-60 (Fig. 1E). Expression levels of the pluripotency markers *OCT4*, *SOX2*, *NANOG*, and *REX1* confirmed pluripotency in the generated iPSCs, in line with other iPSCs and in contrast to the patient-specific fibroblasts (Fig. 1F). STR analysis was performed in DNA samples isolated from the patient's fibroblasts and the iPSCs. This analysis clearly confirmed the identity of the cell line (submitted in archive with journal). Twelve well-spread metaphases were analysed and karyotyped. Representative normal karyogram of iPSCs is shown in Fig. 1G. The cell line tested negative for mycoplasma (Fig. 1H). Spontaneous differentiation induced by embryoid body formation (Moretti et al., 2010) generated cells of all three germ layers (endoderm *SOX17*, *AFP*; mesoderm *HAND1*, *NKX2.5*; and ectoderm *OTX1*, *KRT14*, Fig. 1I).

3. Materials and methods

3.1. Reprogramming and iPSC culture

Proband's adipose fibroblasts were cultured in high glucose DMEM (Gibco), containing 10% fetal-calf-serum (ThermoFisher Scientific), 1% sodium-pyruvate (Gibco) and 1% antibiotics (PanReac AppliChem). Reprogramming was done using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (A16517, Invitrogen, ThermoFisher Scientific). Outgrowing iPSC colonies were manually picked and cultured in TeSR™E8™ (StemCell Technologies) on Matrigel-coated plates (8.7 μg/cm^2, Corning) at 37 °C and 5% CO_2. iPSCs were passaged every 4–6 days at a 1:6 ratio using ReLeSR (StemCell Technologies) supplemented with ROCK inhibitor (10 μM, StemCell Technologies).

3.2. Pluripotency marker expression

iPSCs (p21) were cultured on Matrigel-coated chamber slides for 2 days. Cells were fixed for 10 min at −20 °C with acetone (SOX2) or methanol (TRA1-81). After washing (1x PBS) and permeabilization with PBS/0.1% Triton-X-100 (1x PBS-T, 10 min), cells were blocked with 1x PBS-T containing 5% goat serum for 30 min. After washing three times with 1x PBS-T, cells were incubated with the primary antibodies (Anti-SOX2 rabbit polyclonal IgG antibody, Anti-TRA1-81 mouse monoclonal IgM antibody) diluted in 1x PBS-T containing 1.5% goat serum overnight at 4 °C. Cells were washed three times with 1x PBS-T and incubated with the appropriate secondary antibodies diluted in 1x PBS-T containing 1.5% goat serum for 1 h in the dark at 4 °C. After three washing steps with PBS and one washing with *aq. bidest.*, slides were air dried and embedded with mounting medium containing DAPI (abcam). Images were taken with an Axiovert 200 M (Zeiss) using the Carl Zeiss™ Axio Vision Rel. 4.8.2. Software (Zeiss). All steps were performed at room temperature for 5 min unless otherwise described. Primary and secondary antibodies are indicated in Table 2.

3.3. Short tandem repeat (STR)

Genomic DNA of iPSCs (p28) and proband's fibroblasts (p3) was sent to Eurofins Genomics (Ebersberg, Germany). Genetic characteristics were determined by PCR-single-locus-technology. Sixteen independent markers, which are given in Table 1 were investigated.

3.4. In vitro differentiation potential

Expression of endodermal (*SOX17*, *AFP*), mesodermal (*HAND1*,

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers (ICC)	Anti-SOX2 rabbit polyclonal IgG antibody, Anti-TRA1-81 mouse monoclonal IgM antibody	1:75 1:75	Abcam Cat# ab137385, Merck Millipore Cat# MAB4381	AB_2814892 AB_177638
Secondary antibodies (ICC)	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody, Goat Anti-Mouse IgM H&L (Alexa Fluor® 488) antibody	1:500 1:500	Abcam Cat# ab150077, Abcam Cat# ab150121	AB_2630356 AB_2801490
Pluripotency Markers (FACS)	Anti-TRA1-81 mouse monoclonal IgM antibody,	1:100	Merck Millipore Cat# MAB4381	AB_177638
	Alexa Fluor® 647 Mouse anti-Human TRA-1–60 Antigen	1:5	BD Biosciences Cat# 560850	AB_10565983
Secondary antibodies (FACS)	Goat Anti-Mouse IgM mu chain (Alexa Fluor® 647) antibody	1:2000	Abcam Cat# ab150123	AB_2893175
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai-Footprinting (qRT-PCR)	*SeV*	181 bp	5′-GGATCACTAGGTGATATCGAGC-3′ 5′-ACCAGACAAGAGTTTAAGAGATATGTATC-3′	
	ACTB (β-actin)	96 bp	5′-CCAACCGCGAGAAGATGA-3′ 5′-CCAGAGGCGTACAGGGATAG-3′	
Pluripotency Markers (qRT-PCR)	*OCT4* (endogenous)	148 bp	5′-GGGATGGCGTACTGTGGG-3′ 5′-GCACCAGGGGTGACGGTG-3′	
	SOX2 (endogenous)	191 bp	5′-AGCAGACTTCACATGTCCCAG-3′ 5′-ACCGGGTTTTCTCCATGCTGT-3′	
	NANOG	193 bp	5′-TGCTTTGAAGCATCCGACTGT-3′ 5′-GGTTGTTTGCCTTTGGGACTG −3′	
	REX1	105 bp	5′-AGTAGTGCTCACAGTCCAGCAG-3′ 5′-TGTGCCCTTCTTGAAGGTTT-3′	
Germ layer				
Endoderm	*SOX17*	81 bp	5′-ACGCCGAGTTGAGCAAGA-3′ 5′-TCTGCCTCCTCCACGAAG-3′	
	AFP	90 bp	5′-GTGCCAAGCTCAGGGTGTAG-3́ 5′-CAGCCTCAAGTTGTTCCTCTG-3	
Mesoderm	*HAND1*	72 bp	5′-AACTCAAGAAGGCGGATGG-3′ 5′-GGAGGAAAACCTTCGTGCT-3′	
	NKX2.5	102 bp	5′-TTCTATCCACGTGCCTACAGC-3́ 5́-CTGTCTTCTCCAGCTCCACC-3	
Ectoderm	*OTX1*	125 bp	5′-GATCAACCTGCCGGAGTCTA-3′ 5′-CGCACTGGAGAGGACTTCTT-3′	
	KRT14	85 bp	5′-CACCTCTCCTCCTCCCAGTT-3′ 5′-ATGACCTTGGTGCGGATTT-3′	
House-keeping gene (qRT-PCR)	*ACTB (β-actin)*	96 bp	5′-CCAACCGCGAGAAGATGA-3′ 5′-CCAGAGGCGTACAGGGATAG-3′	
Endogenous reprogramming factors (qRT-PCR)	*KLF4* (endogenous)	133 bp	5′-TCTTCGTGCACCCACTTGGG-3′ 5′-CTGCTCAGCACTTCCTCAAG-3′	
	c-MYC (endogenous)	101 bp	5′-CACCAGCAGCGACTCTGA-3′ 5′-GATCCAGACTCTGACCTTTTGC-3′	

NKX2.5), and ectodermal (*OTX1, KRT14*) markers was measured in iPSCs and on day 21 after induction of embryoid body formation (Moretti et al., 2010). Differentiation potential was calculated as fold gene expression on day 21 compared to iPSCs with β-*actin* as house-keeping gene. RNA extraction, cDNA production and RT-PCR was performed as described in the Supplementary Material. Used primers are listed in Table 2.

3.5. Karyotype analysis

Treatment of iPSC culture (p22) and karyotype analysis was done according to standard conditions (Bangs and Donlon, 2005), with the following specified slight modifications. iPSCs were incubated with Colcemid (40 ng/mL) for 2 h. Cells were detached using 0.5 mM PBS/EDTA (Gibco).

3.6. Mycoplasma detection

Supernatant of dense iPSC culture (p18) was collected after 24 h and Mycoplasma Detection Kit Venor®GeM Classic (MB Minerva Biolabs) was used according to the manufactureŕs instructions.

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.

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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.2022.102662.

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