Stem Cell Research 58 (2022) 102617

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

Establishment of a patient-specific induced pluripotent stem cell line DHMi004-A from a male Holt-Oram syndrome patient with verified *TBX5* mutation

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A B S T R A C T

The Holt-Oram syndrome (HOS) is a rare autosomal dominant disorder, mostly based on mutations in the *TBX5* gene. Patients show malformation of at least one upper limb along with congenital heart defects. The established induced pluripotent stem cell (iPSC) line was generated from a patient displaying pronounced and typical features of HOS and carrying a single-nucleotide change c.920_C>A leading to an amino acid change from proline to threonine at amino acid position 85, which appeared *de novo*. Adipose fibroblasts from the patient were reprogrammed using Sendai virus. Pluripotency of the iPSCs was fully demonstrated.

Resource table

Unique stem cell line identifier	DHMi004-A https://hpscreg.eu/cell-line/DHMi004-A
Alternative name(s) of stem cell line	HOS_1460
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: 15 months Sex: male Ethnicity if known: Caucasian
Cell Source	Adipose fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune-iPS™-iPS 2.0 Sendai Reprogramming (Invitrogen, ThermoFisher Scientific)
Genetic Modification	YES
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Sendai-footprinting, RT-PCR (passage 7)
Associated disease	Holt-Oram Syndrome

(*continued on next column*)

Resource table (*continued*)

Gene/locus	*TBX5* gene, Chromosome 12, NC_000012.12 (114353911..114408708, complete)
Date archived/stock date	October 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/DHMi004-A
Ethical approval	Ethical committee of the Medical Faculty of the Technical University of Munich 5943/13

1. Resource utility

The iPSC line was generated from a 15-month-old male patient who displayed pronounced and typical features of HOS, carrying a heterozygous *TBX5* mutation (Dreßen et al., 2016). This patient-specific HOS iPSC line allows the investigation of pathogenic mechanisms caused by TBX5 in cardiogenesis.

2. Resource details

The cell line described here was generated from adipose fibroblasts using the non-integrative Sendai virus (Table Resource table). Clones were manually picked and characterized. The iPSC colonies showed flat,

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https://doi.org/10.1016/j.scr.2021.102617
Received 20 October 2021; Received in revised form 1 December 2021; Accepted 5 December 2021
Available online 6 December 2021

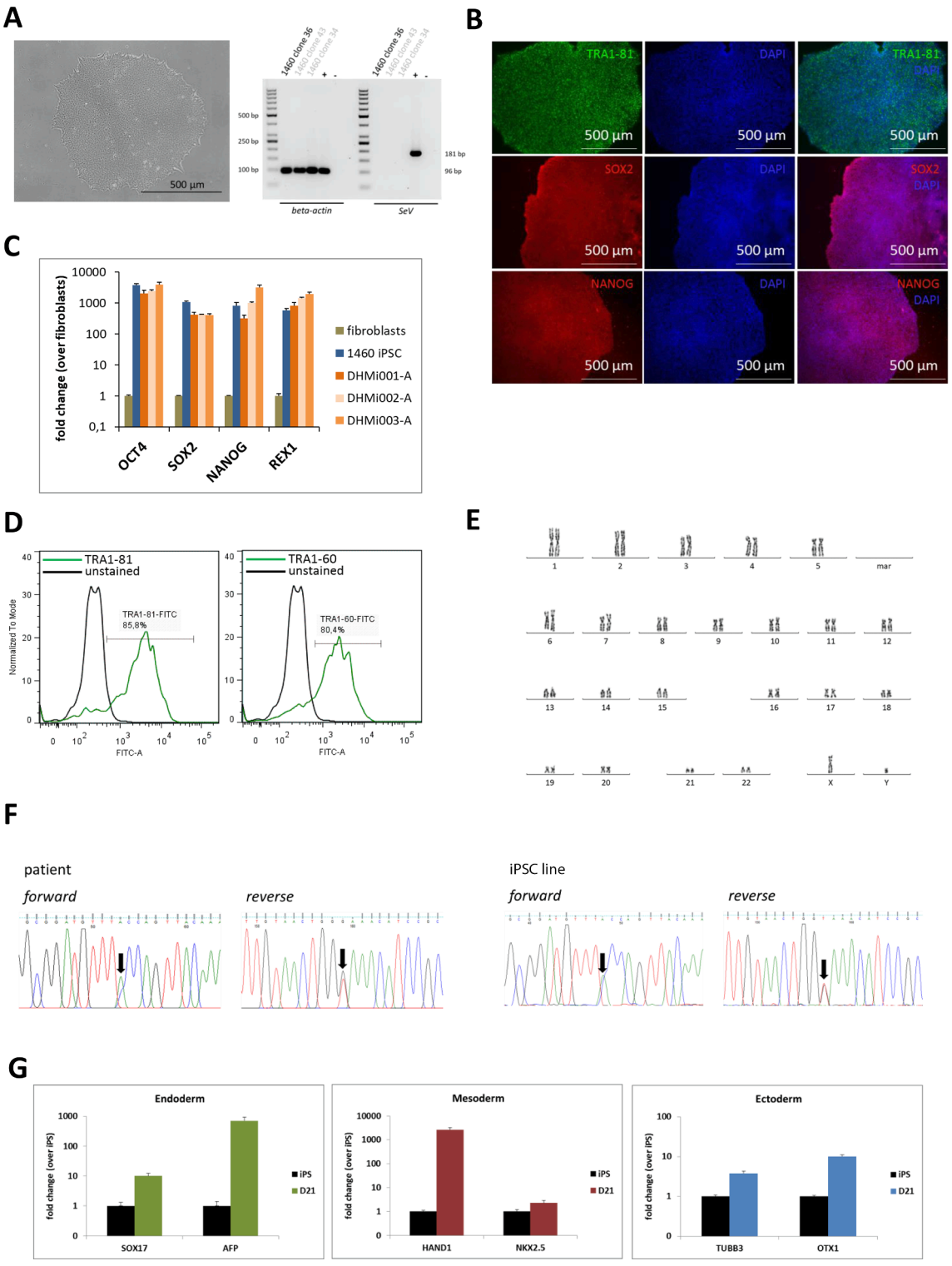

Fig. 1. Characterization of the iPSC line DHMi004-A from a male Holt-Oram Syndrome (HOS) patient carrying a heterozygous de novo TBX5 mutation.

colony-like growth with defined margins, without differentiating areas (Fig. 1A, left panel). At passage 7 (p7) after reprogramming, the Sendai virus was no longer detectable (Fig. 1A, right panel). Immunofluorescence staining showed the iPSC line positive for the pluripotency markers TRA-1-81, SOX2 and NANOG (Fig. 1B). Gene expression of the pluripotency markers *OCT4*, *SOX2*, *NANOG*, and *REX1* was detected in the iPSCs (Fig. 1C). Fluorescence-activated cell sorting revealed the iPSCs positive for TRA-1-60 and TRA-1-81 (Fig. 1D). STR analysis was performed in both DNA isolated from the patient and the iPSC line, clearly confirming the identity of the cell line (submitted in archive with journal). Karyogram of a representative clone from the iPSCs showed normal karyotype (Fig. 1E). The identified *TBX5* mutation of the patient (Dreßen et al., 2016) was verified in the patient-specific iPSC line (Fig. 1F). 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 *TUBB3*, *OTX1*, Fig. 1G). The cell line tested negative for mycoplasma (Supplementary data) (Table 1).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	normal	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry, RT-PCR)	Positive staining for TRA1-81, SOX2 and NANOG Expression of Pluripotency markers: *OCT4*, *SOX2*, *NANOG*, *REX1*	Fig. 1B Fig. 1C
	Quantitative analysis (Flow cytometry)	80,4% of positive cells for cell surface markers Tra 1–60 85,8% of positive cells for cell surface marker Tra 1-81	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	*46, XY* Resolution 600 dpi	Fig. 1E
Identity	Microsatellite PCR (mPCR) STR analysis	N/A Tested 16 sites (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818, FGA), all matched	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation DNA c.920_C>A on the TBX5 gene	Fig. 1F
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Supplementary
Differentiation potential	Embryoid body formation	Expression of genes in embryoid bodies: *SOX17* and *AFP* (endoderm), *HAND1* and *NKX2.5* (mesoderm), *TUBB3* and *OTX1* (ectoderm)	Fig. 1G
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR), at least 2 markers need to be shown per germ layer	Endoderm: *SOX17, AFP* Mesoderm: *HAND1, NKX2.5* Ectoderm: *OTX1, TUBB3*	qRT-PCR with *b-actin* as reference gene, Fig. 1G
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

3. Materials and methods

3.1. Reprogramming and culture

Adipose tissue taken during surgery of the donor was used to obtain adipose fibroblasts. Patient-derived fibroblasts were cultured in high glucose DMEM (Gibco), containing 10% fetal-calf-serum (ThermoFisher Scientific), 1% sodium-pyruvate (Gibco) and 1% antibiotics (PanReac AppliChem). Fibroblasts were reprogrammed using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (MAN0009378, 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

iPSC colonies (p20) were cultured on Matrigel-coated chamber slides. Cells were fixed for 10 min at −20 °C with methanol (TRA1-81) or acetone (SOX2 and NANOG). Cells were washed (1x PBS) and permeabilized with PBS/0.1% Triton-X-100 (1x PBS-T) for 10 min at room temperature (RT). After blocking with 5% goat serum (30 min, RT), cells were washed three times with PBS-T and incubated with mouse anti-TRA-1–81 (Merck Millipore) or rabbit anti-SOX2 (Santa Cruz). For staining with rabbit anti-NANOG (abcam), cells were blocked with 20% goat serum (30 min, RT). Primary antibodies were diluted in PBS-T containing 1.5% goat serum and were incubated overnight at 4 °C. Cells were washed three times with PBS-T and incubated with the appropriate secondary antibodies diluted in PBS-T containing 1.5% goat serum for 1 h in the dark at 4 °C. After washing the cells three times with PBS, cells were washed with *aq. bidest.* Slides were air dried and embedded with mounting medium with DAPI (abcam). Immunostaining 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 RT for 5 min unless otherwise described. Primary and secondary antibodies used are given in Table 2.

3.3. In vitro differentiation potential

Spontaneous differentiation of iPSCs (p20) into all three germ layers was induced by embryoid body formation (Moretti et al., 2010)(). Expression of endodermal (*SOX17*, *AFP*), mesodermal (*HAND1*, *NKX2.5*) and ectodermal markers (*TUBB3*, *OTX1*) was measured on day 21 as fold gene expression compared to iPSCs. RNA extraction, cDNA production and RT-PCR were performed as described. Used primers are listed in Table 2. *β-actin* was used as house-keeping gene.

3.4. Targeting mutation, sequencing

Genomic DNA of the iPSCs (p10) was purified using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's recommendation. Amplification of the genomic *TBX5* sequence and sequencing was done as previously described (Dreßen et al., 2016)() with the primers given in Table 2.

3.5. Karyotype analysis

Treatment of iPSC culture (p40) and karyotype analysis was done according to standard conditions (Bangs and Donlon, 2005) , with the following modifications. iPSCs were incubated with Colcemid (40 ng/ml) for 2 h. Cells were detached using 0.5 mM PBS/EDTA (Gibco). Twenty well-spread metaphases were analysed and karyotyped.

3.6. Mycoplasma detection

Supernatant of dense iPSC culture (p35) was collected after 24 h and Mycoplasma Detection Kit Venor®GeM Classic (MB Minerva Biolabs) was used according to the manufacturer's instructions.

Declaration of Competing Interest

The authors declare that they have no known competing financial

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers (ICC)	Anti-TRA1-81 mouse monoclonal IgM antibody; Anti-SOX2 rabbit polyclonal IgG antibody; Anti-Nanog rabbit polyclonal IgG antibody	1:75, 1:75, 1:250	Merck Millipore, Cat# MAB4381; Abcam, Cat# ab106465; Abcam, Cat# ab137385	AB_177638, AB_10858563, AB_2814892
Secondary antibodies (ICC)	Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody; Goat Anti-Rabbit IgG H&L (Alexa Fluor 555) antibody	1:500, 1:500	Abcam, Cat# ab150113; Abcam,Cat# ab150078	AB_2576208AB_2722519
Pluripotency Markers (FACS)	Anti-TRA1-81 mouse monoclonal IgM antibody; Anti TRA1-60 mouse anti-Human	1:1001:5	Merck Millipore, Cat# MAB4381BD; Bioscience, Cat#ab560876	AB_177638AB_10564094
Secondary antibodies (FACS)	Goat Anti-Mouse IgM (Alexa Fluor® 488)	1:2000	Abcam, Cat#150121	AB_2801490

	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Sendai-Footprinting	*SeV*	181 bp	5‘-GGATCACTAGGTGATATCGAGC-3‘ 5‘-ACCAGACAAGAGTTTAAGAGATATGTATC-3‘
	ACTB (β-actin)	96 bp	5‘-CCAACCGCGAGAAGATGA-3′ 5‘-CCAGAGGCGTACAGGGATAG-3‘
Pluripotency Markers (qRT-PCR)	*4-Oct*	148 bp	5‘-GGGATGGCGTACTGTGGG-3‘ 5‘-GCACCAGGGGTGACGGTG-3‘
	SOX2	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	5́-GTGCCAAGCTCAGGGTGTAG-3́ 5́-CAGCCTCAAGTTGTTCCTCTG-3́
Mesoderm	*HAND1*	72 bp	5’-AACTCAAGAAGGCGGATGG-3’ 5’-GGAGGAAAACCTTCGTGCT-3’
	NKX2.5	102 bp	5‘-TTCTATCCACGTGCCTACAGC-3́ 5́-CTGTCTTCTCCAGCTCCACC-3́
Ectoderm	*TUBB3*	84 bp	5’-GCAACTACGTGGGCGACT-3’ 5’-CGAGGCACGTACTTGTGAGA-3’
	OTX1	125 bp	5’-GATCAACCTGCCGGAGTCTA-3’ 5’-CGCACTGGAGAGGACTTCTT-3’
House-keeping gene (qRT-PCR)	*ACTB (b-actin)*	96 bp	5‘-CCAACCGCGAGAAGATGA-3‘ 5‘-CCAGAGGCGTACAGGGATAG-3‘
Genotyping	*TBX5*	579 bp	5́-GTTACCTCCTCCCTTCGCCTTT-3́ 5́-CAACCTGTGGGTGCAGCAATAG-3́
Targeted mutation analysis/sequencing	*TBX5*	579 bp	5́-GTTACCTCCTCCCTTCGCCTTT-3́ 5́-CAACCTGTGGGTGCAGCAATAG-3́

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102617.

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