Stem Cell Research 44 (2020) 101745

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of three iPSC lines from two patients with heterozygous *FOXF1* mutations associated to Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins

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A B S T R A C T

Diagnosing Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins (ACD/MPV) based on a genetic alteration in the *FOXF1* gene, is complicated by the poor understanding of the causal relation between *FOXF1* variants and the ACD/MPV phenotype. Here, we report the generation of human iPSC lines from two ACD/MPV patients, each carrying a different heterozygous *FOXF1* mutation, which enables disease modeling for further research on the effect of *FOXF1* variants *in vitro*. The iPSC lines were generated from skin fibroblasts using the non-integrating Sendai virus. The lines expressed pluripotency genes, retained the heterozygous mutation and were capable of trilineage differentiation.

Resource Table

Unique stem cell lines identifier	EMC127i-A EMC127i-B EMC128i-A
Alternative names of stem cell lines	EMC127i-A: ACD871C4 EMC127i-B: ACD871C8 EMC128i-A: ACD874C9
Institution	Erasmus University Medical Center Rotterdam, The Netherlands
Contact information of distributor	Robbert Rottier; r.rottier@erasmusmc.nl
Type of cell lines	iPSC
Origin	Human
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune-iPS 2.0 Sendai reprogramming
Multiline rationale	Two isogenic iPSC clones from ACD/MPV patient 1 and one iPSC clone from ACD/MPV patient 2.
Gene modification	Yes
Type of modification	Congenital, *de novo*
Associated disease	Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins (ACD/MPV)
Gene/locus	Patient 1: FOXF1; 16q24.1; Chr16(GRCh37): g.86544341C>G (c.166C>G) Patient 2: FOXF1; 16q24.1; Chr16(GRCh37): g.86544428T>A (c.253T>A)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	EMC127i-A: 2019-5-1 EMC127i-B: 2019-4-25 EMC128i-A: 2019-5-28
Cell line repository/bank	https://hpscreg.eu/cell-line/EMCi127-A https://hpscreg.eu/cell-line/EMCi127-B https://hpscreg.eu/cell-line/EMCi128-A
Ethical approval	Medical Ethics Committee Erasmus MC Rotterdam, The Netherlands. Approval number: MEC-2017-302

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https://doi.org/10.1016/j.scr.2020.101745
Received 11 December 2019; Received in revised form 6 February 2020; Accepted 20 February 2020
Available online 04 March 2020

1. Resource utility

The ACD/MPV patient specific iPSC lines are useful for disease modeling to investigate the pathogenesis of ACD/MPV. The iPSC lines will help to elucidate the effect of specific *FOXF1* mutations on the function of different cell types that are important during lung development.

2. Resource details

Diagnosing congenital lung disorder Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins (ACD/MPV) based on a genetic alteration in the *FOXF1* gene, is complicated by the poor understanding of the causal relation between *FOXF1* variants and the ACD/MPV phenotype. Although several studies revealed a strong association with mutations and copy number variations in the *FOXF1* gene, an invasive lung biopsy is still necessary to confirm ACD/MPV (Stankiewicz et al., 2009; Slot et al., 2018). Studies in mice confirmed that FOXF1 is important in early lung development. However, rodent knock-down models do not display all ACD/MPV features that are observed in human patients (Mahlapuu et al., 2001). Unfortunately, there is limited accessibility of patient samples, which complicates further research on ACD/MPV and FOXF1 function in human. Here, we report the generation of human iPSC lines from two ACD/MPV patients, each carrying a different heterozygous *FOXF1* mutation, which is a major contribution to the research field by enabling the investigation of FOXF1 function *in vitro*.

The iPSC lines were generated at the Erasmus MC iPS Core Facility from patient derived skin fibroblasts. From patient 1, who carried *FOXF1* mutation c.166C>G, we generated two isogenic iPSC clones. From patient 2, who carried *FOXF1* mutation c.253T>A (Sen et al., 2013), we generated one iPSC clone (Table 1:). All lines showed iPSC morphology and expression of pluripotent markers NANOG, OCT4 and SSEA4 (Fig. 1A). In addition, quantitative RT-PCR revealed increased expression of NANOG and OCT3/4 compared to patients' skin fibroblasts, and similar expression as control line HuES9 (Cowan et al., 2004) (Fig. 1B). Sanger sequencing confirmed the presence of the heterozygous mutations (Fig. 1C) and SNP arrays confirmed the absence of major copy number variations other than balanced translocations (Fig. 1D). Further, the numbers of SNP counts verified the identity of the iPSC lines (Suppl. Fig. 1B). All clones were mycoplasma free (Suppl. Fig. 1C) and able to differentiate into the three germ layers as shown by expression of trilineage markers (Fig. 1E) (Table 2:).

3. Materials and methods

3.1. Ethical approval

iPSC lines were generated from anonymized fibroblasts that were previously isolated from skin tissues of ACD/MPV patients. The research proposal was approved by the Daily Board of the Medical Ethics Committee (METC) Erasmus University Medical Center Rotterdam, The Netherlands.

Table 1.
Summary of lines.

iPSC line names	Gender	Age	Ethnicity	Genotype of locus	
ACD871C4	Male	Newborn	Caucasian	c.166C>G	ACD/MPV
ACD871C8	Male	Newborn	Caucasian	c.166C>G	ACD/MPV
ACD874C9	Male	Newborn	Caucasian	c.253T>A	ACD/MPV

3.2. Generation and culture of human iPSC lines

Skin fibroblasts were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) according to the manufacturer's instructions. After reprogramming, single colonies were collected and maintained on Matrigel (Corning) plates in mTeSR™1 (STEMCELL Technologies) at 37 °C with 5% CO2. Every four days (at 80–90% confluency), the cells were passaged in an 1:6 ratio. The first five passages were done by means of mechanical passaging, all following passages were done using ReLeSR™ (Stem Cell Technologies). The absence of Sendai virus was confirmed by quantitative RT-PCR (Suppl. Fig. 1A) at passage 7 (ACD871C4-C8) and passage 9 (ACD874C9). As negative and positive controls, RNA of non-transduced skin fibroblasts and skin fibroblasts 7 days after transduction were used.

3.3. Immunofluorescence staining

iPS cells were cultured on Geltrex (ThermoFisher Scientific) coated 4-well chamber slides (Sarstedt) and fixed for 15 min with 4% PFA at room temperature. Thereafter, cells were permeabilized with 0.1% Triton-X100 for 10 min and blocked with 1% BSA/0.05% Tween 20/PBS for 30 minTable 1: at room temperature. The cells were incubated overnight at 4 °C with primary antibodies (Table 3) diluted in blocking buffer. The next day, the cells were washed and incubated with fluorophore-tagged secondary antibodies (Table 3) for 1 h at room temperature. Finally, the cells were stained with DAPI and imaged with a Leica SP5 confocal microscope.

3.4. Quantitative gene expression analysis

RNA was isolated from iPS cells, skin fibroblasts and HuES9 cells using the ReliaPrep™ RNA Cell Miniprep System (Promega) and cDNA was prepared from mRNA using SuperScript™ II Reverse Transcriptase kit (Invitrogen). qPCR was performed with the CFX96 C1000 Thermal Cycler (Bio-rad) using SYBR Green premix (Bio-rad) and the primers listed in Table 3. mRNA expression was normalized to GAPDH.

3.5. Genotyping of the human iPSC lines

Genomic DNA was isolated from iPS cells (passage 12 for ACD871C4-C8 and passage 16 for ACD874C9) and fibroblasts (passage 7) using the QIAamp DNA Mini Kit (Qiagen) and tested for copy number variations with GSAMD24 v1 Illumina Infinium SNP array 700k (Illumina). Data was analyzed with GenomeStudio software (IIllumina) and visualized using Nexus Copy Number 9.0 (BioDiscovery). To confirm iPSC identities, we compared the number of SNPs found in iPSC lines and patient fibroblasts with R software. To assure the presence of the heterozygous *FOXF1* mutations, the regions of interest were amplified with PCR using M13 tailed primers (Table 3), followed by Sanger sequencing with the 3730xl DNA Analyzer (Applied Biosystems, ThermoFisher Scientific). The PCR was performed with the Biometra TAdvanced Thermocycler (Westburg) and consisted of 35 cycles of the following steps: 30 s at 94 °C, 30 s at 60 °C and 90 s at 72 °C.

3.6. Trilineage differentiation

In vitro trilineage differentiation was induced with the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) according to manufacturer's instructions. In brief, cells were plated in a single cell suspension on Geltrex coated 4-well chamber slides (Sarstedt). The cells were daily fed with either ectoderm, mesoderm or endoderm specific medium. After five (mesoderm and endoderm) or seven (ectoderm) days, the cells were fixed with 4% PFA and used for immunofluorescence staining.

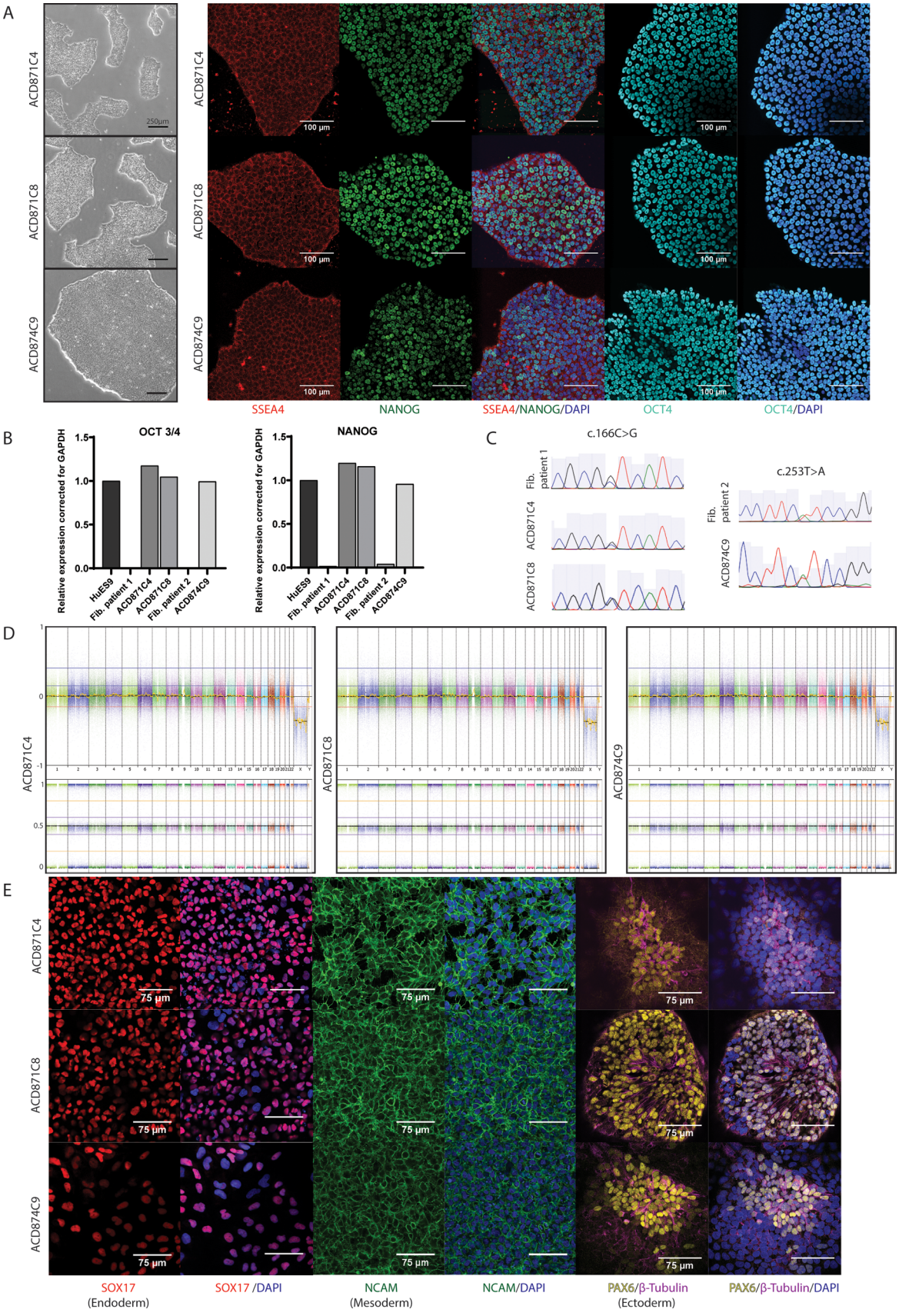
A
ACD871C4
ACD871C8
ACD874C9
250µm
100 µm
SSEA4
NANOG
SSEA4/NANOG/DAPI
OCT4
OCT4/DAPI
B
OCT 3/4
NANOG
Relative expression corrected for GAPDH
1.5
1.0
0.5
0.0
HuES9
Fib. patient 1
ACD871C4
ACD871C8
Fib. patient 2
ACD874C9
C
c.166C>G
c.253T>A
Fib. patient 1
Fib. patient 2
ACD871C4
ACD871C8
ACD874C9
D
ACD871C4
ACD871C8
ACD874C9
E
75 µm
SOX17
(Endoderm)
SOX17 /DAPI
NCAM
(Mesoderm)
NCAM/DAPI
PAX6/β-Tubulin
(Ectoderm)
PAX6/β-Tubulin/DAPI

Fig. 1. *

Table 2.
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1A
Phenotype	Qualitative analysis by immunohistochemistry	Expression of SSEA4, NANOG and OCT4	Fig. 1A
	Quantitative analysis by RT-qPCR	Expression of NANOG and OCT3/4	Fig. 1B
Genotype	GSAMD24 v1 Illumina Infinium SNP array 700k	Resolution 50kb: No major copy number variations or allelic changes	Fig. 1D
Identity	GSAMD24 v1 Illumina Infinium SNP array 700k	100.00% identical SNPs between fibroblasts and iPSCs	Supplementary fig. 1B
Mutation analysis	Sanger Sequencing	ACD871C4: Chr16(GRCh37): g.86544341C>G (c.166C>G) ACD871C8: Chr16(GRCh37): g.86544341C>G (c.166C>G) ACD874C9: Chr16(GRCh37): g.86544428T>A (c.253T>A)	Fig. 1C
Microbiology and virology	Mycoplasma testing by luminescence	Negative	Supplementary fig.1C
Differentiation potential	In vitro trilineage differentiation	Expression of specific germ layer markers SOX17 (endoderm), NCAM (mesoderm) and β-Tubulin (ectoderm)	Fig. 1E

Table 3
Reagents details.

Antibodies used for immunocytochemistry	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-SSEA4	1:75	Abcam, ab16287, RRID: AB_778073
	Rabbit anti- NANOG	1:75	Abcam, ab21624, RRID: AB_446437
	Rabbit anti-OCT4	1:250	Abcam, ab19857, RRID: AB_445175
Differentiation markers	Goat anti-SOX17	1:100	R&D Systems, AF1924, RRID: AB_355060
	Goat anti-NCAM	1:100	R&D Systems, AF2408, RRID: AB_442152
	Mouse anti-β-Tubulin	1:1000	Sigma-Aldrich, T8660, RRID: AB_477590
	Rabbit anti-PAX6	1:250	Biolegend, 901301, RRID: AB_2565003
Secondary antibodies	Goat anti-Mouse IgG (H + L) Alexa Fluor 546	1:500	ThermoFisher Scientific, A-11003, RRID:AB_2534071
	Goat anti-Rabbit IgG (H + L) Alexa Fluor 488	1:500	ThermoFisher Scientific, A-11008, RRID:AB_143165
	Donkey anti-Goat IgG (H + L) Alexa Fluor 488	1:500	ThermoFisher Scientific, A-11055, RRID: AB_2534102
	DyLight 594 Goat anti-Mouse IgG +IgM (H + L)	1:500	Jackson, 115-515-044, RRID: AB_2338823
	Donkey anti-Rabbit IgG (H + L) Alexa Fluor 488	1:500	Jackson, 711-545-152, RRID: AB_2313584
Primers			
	Target	Forward/Reverse primer (5′−3′)	
Pluripotency Markers (qPCR)	NANOG	CAGCCCCGATTCTTCCACCAGTCCC/ CGGAAGATT CCCAGTCGGGTTCACC	
	OCT3/4	AGCCACATCGCTCAGACAC/ GCCCAATACGACCAAATCC	
Absence of Sendai virus	SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAG TTTAAGAGATATGTATC	
House-Keeping Gene (qPCR)	GAPDH	CCTTCATTGACCTCAACTAC/ GGAAGGCCATGCCAGTGAGC	
Targeted mutation analysis (Sanger sequencing)	FOXF1 ROI (GRch37/Hg19 Chr16: 86,544,274–86,544,511; c.99–c.330; 237 bp)	GGCGGCGCGGCCATGGACC/ GCCCTTGGGTAGCTTGATG	

3.7. Mycoplasma detection

Cell cultures were tested for mycoplasma contamination with the MycoAlert™ Mycoplasma Detection Kit (Lonza), according to manufacturer's 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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101745.

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