Stem Cell Research 53 (2021) 102302

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (UALGi001-A) from a patient with Left-Ventricular Noncompaction Cardiomyopathy

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A B S T R A C T

Left Ventricular Noncompaction Cardiomyopathy (LVNC) is characterized by excessive trabeculation of the left ventricle. To date, mutations in more than 40 genes have been associated with LVNC, however the exact mechanisms underlying the disease remain unknown. Here, we describe an induced pluripotent stem cell (iPSC) line (UALGi001-A) from a LVNC patient (LVNC-iPSC) that does not present mutations in the genes most commonly associated with the disease (van Waning et al., 2019). The LVNC-iPSC exhibited full pluripotency and differentiation potential, and retained a normal karyotype after reprogramming. This in vitro cellular model will be useful to study the molecular, genetic and functional aspects of LVNC.

1. Resource Table:

Unique stem cell line identifier	UALGi001-A
Alternative name(s) of stem cell line	*VITAL16*
Institution	*Universidade do Algarve, Faro, Portugal Algarve Biomedical Center Research Institute (ABC-RI), Faro, Portugal*
Contact information of distributor	jebraganca@ualg.pt
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 75* *Sex: Male* *Ethnicity: Caucasian/Portuguese*
Cell Source	*Peripheral blood mononuclear cells*
Clonality	*Clonal*
Method of reprogramming	*Non-integrating, Sendai Virus expressing hOCT3/4, hSOX2, hKLF4 and hC-MYC*
Genetic Modification	*NO*
Type of Modification	*N/A*
Associated disease	*Left Ventricular Non-Compaction Cardiomyopathy (LVNC)*
Gene/locus	*N/A*
Date archived/stock date	*July 2020*
Cell line repository/bank	https://hpscreg.eu/cell-line/UALGi001-A
Unique stem cell line identifier	UALGi001-A
Ethical approval	Approval ID: 78/19 by Ethics Committee for Health, Centro Hospital e Universitário do Algarve, Faro, Portugal

2. Resource utility

Left Ventricular Noncompaction Cardiomyopathy (LVNC) is a heart disorder characterized by endomyocardial noncompaction. UALGi001-A cell line, derived from a patient with LVNC offers a useful tool to study molecular and cellular mechanisms involved in this cardiomyopathy.

3. Resource details

Mononucleated cells were collected from 4 mL of peripheral blood sample from 75-year-old male. The patient was clinically diagnosed with left ventricular trabeculations located in the middle and apical segments and deep recesses, compatible with Left Ventricular Non-Compaction Cardiomyopathy (LVNC). LVNC is the third most prevalent heart muscle disease, accounting for almost 10% of all cardiomyopathies and it is

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https://doi.org/10.1016/j.scr.2021.102302
Received 2 March 2021; Received in revised form 8 March 2021; Accepted 13 March 2021
Available online 19 March 2021

characterized by the presence of a vast trabeculations likely due to endomyocardial morphogenesis arrest during embryogenesis (van Waning et al., 2019). LVNC clinical manifestations include heart failure, thromboembolism, ventricular arrhythmias, and ultimately sudden cardiac death (Finsterer et al., 2017). The human induced pluripotent stem cell (iPSC) line UALGi001-A was generated by using the CytoTune® iPSC-Reprogramming kit (Thermo Fisher Scientific, Invitrogen), encoding for the reprogramming factors hOCT3/4, hc-MYC, hKLF4, and hSOX2 (Takahashi and Yamanaka, 2006), according to manufacturer's instructions. Clonal iPSC lines were established and further characterized (Table 1), formed colonies with a standard stem-like morphology visible by phase contrast, and were positive for Alkaline Phosphatase (AP) activity (Fig. 1A-B). The expression of pluripotency markers was demonstrated by immunofluorescence staining of SOX2, NANOG, OCT4 and SSEA-4 (Fig. 1 C), as well as flow cytometry (Fig. 1D). Viral clearance was confirmed at passage 14 (Supplementary Fig. S1A).

Genomic integrity was assessed by karyotype analysis, showing that UALGi001-A, at passage 21, presented a normal diploid (46, XY) chromosomal content (Fig. 1E). The capacity of iPSCs to differentiate into three germ layers was confirmed by embryoid body (EB) differentiation assay, and expression of ectoderm (TUJ1), endoderm (AFP) and mesoderm (α-SMA) markers (Fig. 1F). DNA fingerprinting was used to prove the genetic identity to parental mononucleated blood cells (archived with journal). Mycoplasma was regularly tested negative throughout cell culture indicating that UALGi001-A line is mycoplasma-free (Supplementary Fig. S1B).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	*Normal*	*Fig. 1A*
Phenotype	Qualitative analysis *(Immunocytochemistry)*	*Positive pluripotency markers: Alkaline Phosphatase, Oct3/4, Nanog, Sox2, and SSEA-4*	*Fig. 1B and C*
	Quantitative analysis *(Flow cytometry)*	*Percentage of NANOG positive cells: 95%; SSEA-4 positive cells: 93,3%*	*Fig. 1D*
Genotype	Karyotype (G-banding) and resolution	*46XY, Resolution 400–500*	*Fig. 1E*
Identity	Microsatellite PCR (mPCR)	*Not performed*	*N/A*
	STR analysis	*16 loci analyzed, all matched*	*Supplementary table 1*
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	*Mycoplasma testing by RT-PCR: Negative*	*Supplementary Fig. 1*
Differentiation potential	e.g. Embryoid body formation	Positive staining for α-fetoprotein (AFP), βIII-tubulin (TUBB3), α-smooth muscle actin (SMA).	*Fig. 1F*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

4. Materials and methods

4.1. Reprogramming of PBMCs

PBMCs were isolated using the Vacutainer® CPT™ tubes (BD Biosciences). PBMCs were cultured (1.0×10^6 cells) in PBMCs Medium (StemPro-34 SFM medium; 1% GlutaMAX; 1% Pen/Strep, 100 ng/mL SCF, 100 ng/mL FLT-3; 20 ng/mL IL-3, 20 ng/mL 20 ng/mL IL-6; 25 ng/mL GM-CSF), for one week before transduction with CytoTune®-iPS 2.0 Sendai Reprogramming Kit. Briefly, 0.25×10^5 cells were transduced using MOI of 5–5-3 (hKOS, hc-MYC, hKLF4, respectively). After 24 h of incubation, cells were collected, centrifuged, and seeded in a 24-well plate containing PBMCs Medium. Two days later, cells were transferred onto a 6-well plate coated with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco) in ReproTeSR (STEMCELL Technologies), supplemented with 1% Pen/Strep. Individual colonies with stem-like morphology were manually isolated and expanded 14 to 21 days post-transduction. iPSC clones were cultured and expanded on 6-well plates coated with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix, using a slip ratio of 1:5 and were maintained at 37 °C in humidified atmosphere containing 5% CO_2.

4.2. In vitro differentiation assay

In vitro differentiation was performed by EB formation. iPSC colonies were lifted manually and cultured in non-adherent conditions in mTeSR1 medium, containing 0.4% of polyvinyl alcohol, for 48 h. Thereafter, the EBs were seeded on glass coverslips coated Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix and cultured for 3 weeks in differentiation medium (DMEM, 10% FBS, 1% Pen/Strep, 1% GlutaMAX, 1% MEM-NEAA). The coverslips were fixed with 4% paraformaldehyde (PFA) for 15 min and analyzed by immunofluorescence (Table 2) on Axioimager Z2/Apotome fluorescence microscope (Carl Zeiss).

4.3. Immunocytochemistry

Cells were allowed to grow in glass coverslips coated with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix and washed in ice-cold PBS before fixation in 4% PFA, for 15 min. Fixed cells were washed twice in PBS and placed in blocking solution (2% bovine serum albumin in 0.2% Triton-X100/PBS) for 1 h, at room temperature. Cells were incubated for 1 h at room temperature with the primary antibody (Table 2). After incubation, samples were washed 3 times with 0.2% Triton X100/PBS, and incubated with the secondary antibodies for 1 h, at room temperature (Table 2). After 3 washes, sections were mounted with Fluoromount G mounting medium (Thermo Fisher Scientific) containing 4,6-diamidino-2-phenylindole (DAPI) and analyzed on a Axioimager Z2 fluorescence microscope (Carl Zeiss)

4.4. Flow cytometry

iPSCs were dissociated using TrypLE Select (Gibco) for 3 min at RT, centrifuged at 300 g for 5 min and 100,000 cells resuspended in 200 μl of ice-cold 0.5 % PFA in PBS, for 20 min. Fixed cells were washed twice in PBS/0.5% BSA/0.1% Triton X100 and incubated with the primary antibody for 1 h at 4 °C (Table 2). After incubation, samples were washed 3 times with PBS/0.5% BSA/0.1% Triton X100, and incubated with the secondary antibodies for 1 h, at 4 °C (Table 2), protected from light. The cells were analyzed using a FACScalibur cell analyzer (BD Biosciences) and data was analyzed by CytExpert 2.0 software.

4.5. Alkaline phosphatase activity

Alkaline phosphatase staining was carried out using Alkaline Phosphatase Staining Kit II (Stemgent, MA).

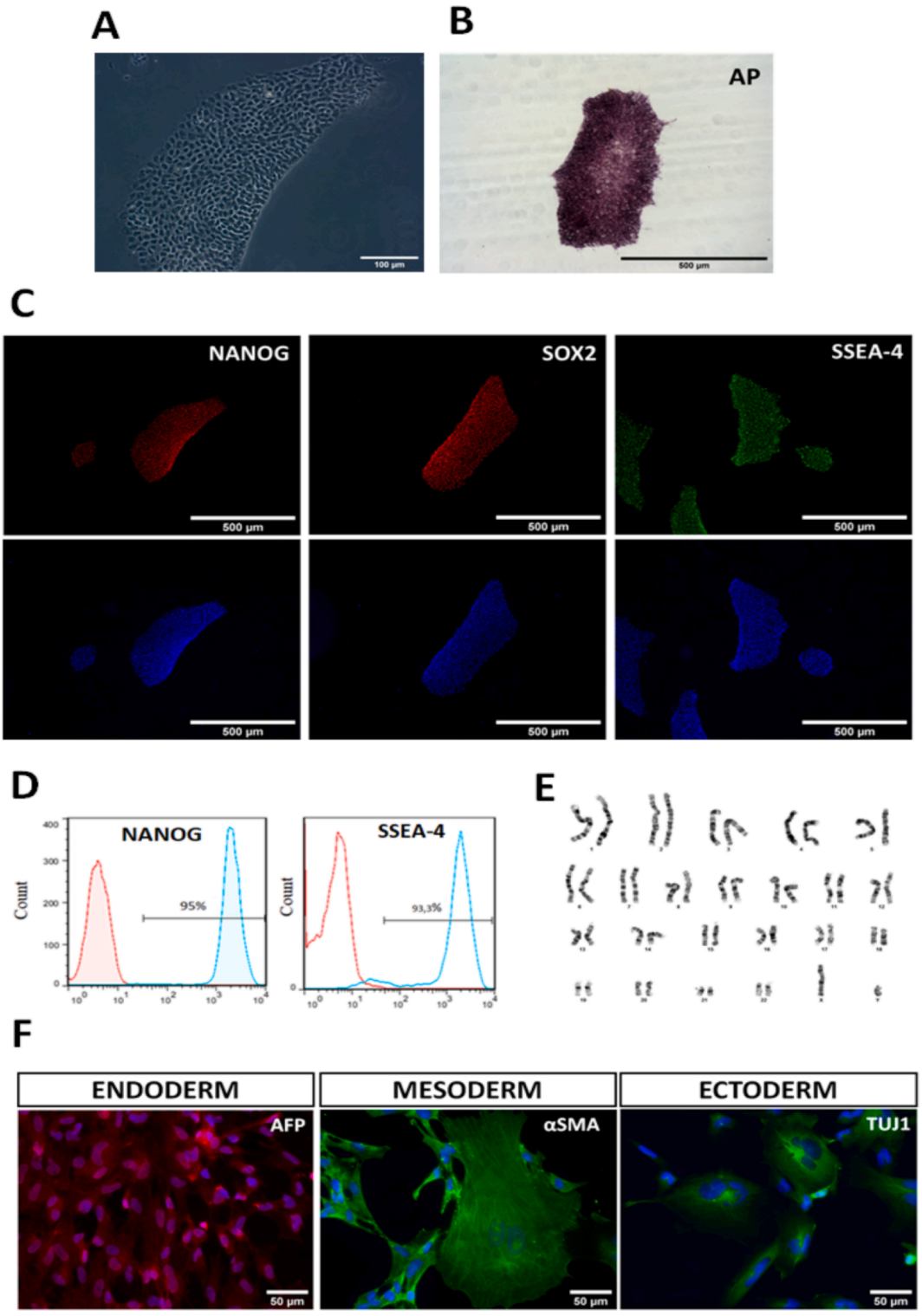

Fig. 1. Characterisation of the induced pluripotent stem cell line (UALGi001-A) from a patient with LVNC. (A) Phase contrast micrograph of UALGi001-A colony cultured in feeder-free conditions. (B) Alkaline phosphatase positive staining. CC) Flow cytometry of nuclear (NANOG) and surface (SSEA-4) pluripotency markers. (D) Immunofluorescence for pluripotency markers OCT4, SOX2, NANOG, and SSEA-4. Nuclei were counterstained with DAPI (blue). (E) Representative metaphase showing normal diploid 46, XY karyotype. (F) Immunocytochemistry for ectodermal (TUJ1), endodermal (AFP) and mesodermal (αSMA) markers. Nuclei were counterstained with DAPI (blue).

4.6. Karyotype analyses

Genome integrity of the iPSC was evaluated by G-banding at 400–550 band resolution, with a minimum of 30 metaphase spreads analyzed (Genomed, Lisbon, Portugal).

4.7. RT-PCR for detection of viral clearance

Total RNA was isolated from cultured iPSC with RNeasy Mini Kit (Qiagen). 1 µg of total RNA was used as template to obtain cDNA, using NZY First-Strand cDNA Synthesis Kit (nzytech). Viral clearance was

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers (immunofluorescence and flow cytometry)	*Rabbit anti-OCT4*	*1:400*	Cell Signaling Technology Cat# 2840	RRID: AB_2167691
	Rabbit anti-NANOG	*1:400*	Cell Signaling Technology Cat# 4903	RRID: AB_10559205
	Rabbit anti-SOX2	*1:400*	Cell Signaling Technology Cat# 3579	RRID: AB_2195767
	Mouse anti-SSEA-4	*1:200*	BD Biosciences Cat# 560,073	RRID: AB_1645601
Differentiation Markers	*Mouse anti-TUBB3*	*1:500*	BioLegend Cat#801213	RRID: AB_2728521
	Mouse anti-ASM	*1:500*	Sigma-Aldrich Cat#A5228	RRID: AB_262054
	Rabbit anti-AFP	*1:100*	Dako Cat# A0008	RRID: AB_2650473
Secondary antibodies for immunofluorescence	*Donkey anti-Mouse 488*	1:500	Molecular Probes Cat# A-21202	RRID: AB_141607
	Donkey anti-Rabbit 594	1:500	Molecular Probes Cat# A-21207	RRID: AB_141637
Secondary antibodies for flow cytometry	*Donkey anti-Rabbit 488*	1:200	Molecular Probes Cat# A-21206	RRID: AB_2535792
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids (RT-PCR)	*SeV Plasmid*	*181 bp*	GGATCACTAGGTGATATCGAGC/	
	hKLF4 Plasmid	*410 bp*	ACCAGACAAGAGTTTAAGAGATATGTATC	
	hKOS Plasmid	*528 bp*	TTCCTGCATGCCAGAGGAGCCC/	
	hC-MYC Plasmid	*532 bp*	AATGTATCGAAGGTGCTCAA	
			ATGCACCGCTACGACGTGAGCGC/	
			ACCTTGACAATCCTGATGTGG	
			TAACTGACTAGCAGGCTTGTCG/	
			TCCACATACAGTCCTGGATGATGATG	
Mycoplasma (PCR)	*M. arginine*	*500 bp*	*Forward primers:*	
	M. boris		CGC CTG AGT AGT ACG TTC GC	
	M. fermentans		CGC CTG AGT AGT ACG TAC GC	
	M. hominis		TGC CTG AGT AGT ACA TTC GC	
	M. hyorhinis		CGC CTG GGT AGT ACA TTC GC	
	M. orale		CGC CTG AGT AGT ATG CTC GCTGC CTG GGT AGT ACA TTC GC	
			Reverse primers:	
			GCG GTG TGT ACA AGA CCC GA	
			GCG GTG TGT ACA AAA CCC GA	
			GCG GTG TGT ACA AAC CCC GA	

analyzed using the primers described in Table 2. RT-PCR reaction was performed using DreamTaq DNA Polymerase (Thermo Scientific) and PCR products were visualized on a 2% agarose gel.

4.8. Fingerprinting

Genomic DNA from PBMC and iPSC was extracted using QIAamp DNA Blood mini kit (Qiagen). Fingerprinting analyses was performed using Promega's PowerPlex 16 kit and analyzed on ABI PRISM 3100 using GeneMapper (Thermo Fisher) by STABVida, Lisbon, Portugal.

4.9. Mycoplasma detection

The presence of mycoplasma was tested regularly by PCR (Uphoff and Drexler, 2001) using the Primers listed in Table 2.

Funding

This work was supported by Fundação para a Ciência e Tecnologia (FCT) and the Comissão de Coordenação e Desenvolvimento Regional do Algarve (CCDR Algarve) for the project ALG-01-0145-FEDER-28044 "VITAL", PTDC/BTM-TEC/28044/2017. We acknowledge the Light Microscopy Unit of CBMR-UAlg. The Microscopy Unit was partially supported by national portuguese funding FCT: PPBI-POCI-01-0145-FEDER-022122.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102302.

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