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Lab Resource: Single Cell Line

Generation and cardiac differentiation of a human induced pluripotent stem cell line UALGi002-A from a female 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 abnormal number and prominence of trabeculations of the left ventricle of the heart. Although LVNC has been associated with mutations in several genes encoding for transcriptional regulators, ion channels, sarcomeric and mitochondrial proteins, approximately 60% of LVNC patients do not present these genetic alterations. Here, we describe an induced pluripotent stem cell (hiPSC) line (UALGi002-A) originated from a LVNC female patient (LVNC-hiPSC) who does not present any previously known mutations associated to LVNC. The LVNC-hiPSC exhibited full pluripotency and differentiation potential and retained a normal karyotype after reprogramming. Moreover, the LVNC-hiPSC differentiated into contracting cardiomyocytes. This cellular model will be useful to study the molecular, genetic and functional aspects of LVNC *in vitro*.

1. Resource Table

Unique stem cell line identifier	UALGi002-A
Alternative name(s) of stem cell line	*VITAL8*
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: 51* *Sex: Female* *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	*June 2019*
Cell line repository/bank	https://hpscreg.eu/cell-line/UALGi002-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. The UALGi002-A cell line, derived from a female patient with LVNC offers a useful tool for molecular, cellular and physiological analysis to study mechanisms involved in this cardiomyopathy.

3. Resource details

Mononucleated cells were collected from 4 mL of peripheral blood sample from a 51-year-old female. The patient has a history of palpitations and was clinically diagnosed with left ventricular trabeculations located in the middle and apical segments, with a diastolic ratio of >2.3,

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compatible with Left Ventricular Noncompaction Cardiomyopathy (LVNC). A genetic study of this patient showed no clinically relevant mutations in LDB3, TAZ, LMNA/C, MYH7, MYBPC3, TNNT2, ACTC1, TPM1, CSRP3, TCAP, SGCD, and PLN genes that have been previously associated with LVNC. LVNC is characterized by the presence of vast trabeculations likely due to endomyocardial morphogenesis arrest during embryogenesis. Clinical manifestations and their onset are variable, and may include heart failure, thromboembolism, ventricular arrhythmias, and ultimately sudden cardiac death (Finsterer et al., 2017). The human induced pluripotent stem cell (hiPSC) line UALGi002-A was generated 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 hiPSC lines were established and further characterized (Table 1), formed colonies with a standard stem-like morphology visible by phase contrast (Fig. 1A) and were positive for Alkaline Phosphatase (AP) activity (Fig. 1B). The expression of pluripotency markers was demonstrated by immunofluorescence staining of NANOG, SOX2, OCT4 and SSEA4 proteins (Fig. 1C), as well as by detection of NANOG expressing hiPSCs by flow cytometry (Fig. 1D), and by detection of NANOG, OCT4, SOX2 and REX1 transcripts expression by RT-PCR (Supplementary Fig. S1A). Viral clearance was confirmed at passage 11 (Fig. 1E).

Genomic integrity was assessed by karyotype analysis, showing that UALGi002-A, at passage 22, presented a normal diploid (46, XX) chromosomal content (Fig. 1F). The capacity of hiPSCs to differentiate into three germ layers was confirmed by embryoid body (EB) differentiation assay, and by the detection of ectoderm (TUJ1), mesoderm (α-SMA) and endoderm (AFP) markers expression (Fig. 1G). In addition, hiPSC were differentiated into contracting cardiomyocytes as previously described (Lian et al., 2013), and expressed the sarcomeric protein α-actinin (Figure H). DNA fingerprinting was used to prove the genetic identity between hIPSCs and parental mononucleated blood cells (archived with journal). Mycoplasma was regularly tested negative throughout cell culture indicating that UALGi002-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

The collection of blood samples was performed has we previously reported (Calado et al., 2021). In brief, 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), 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, reprogrammed cells were transferred onto a 6-well plate covered with irradiated mouse embryonic fibroblasts (Gibco) and cultured in hiPSC medium (Knockout-DMEM, KSR 20%, Glutamax 2 mM, non-essential amino acids 0.1 mM, β-mercaptoethanol 0.23 mM, bFGF 10 ng/ml, penicillin/streptomycin, Gibco). Individual colonies with stem-like morphology were manually picked and expanded for 14 to 21 days post-transduction. hiPSC were adapted to feeder-free cell culture on plates coated with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco) using mTeSR1 medium (StemCell Technologies). Passages were performed using ReLeSR (StemCell Technologies), using a split ratio of 1:5 and cells were maintained at 37 °C in humidified atmosphere containing 5% CO_2.

4.2. Immunocytochemistry

Cells were grown 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 with PBS and placed in blocking solution (2% bovine serum albumin in 0.2% Triton-X100/PBS) for 1 h, at room temperature. Next, 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).

4.3. In vitro differentiation assay

Three germ layers differentiation *in vitro* was performed by EB formation. hiPSC colonies were lifted manually and cultured in non-adherent conditions in mTeSR1 medium, containing 0.4% of polyvinyl alcohol, for 48 h. Next, EBs were seeded on glass coverslips coated with 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). Directed cardiomyocyte differentiation was performed following the GiWi protocol, as described by Lian and co-workers (Lian et al., 2013). The coverslips were fixed

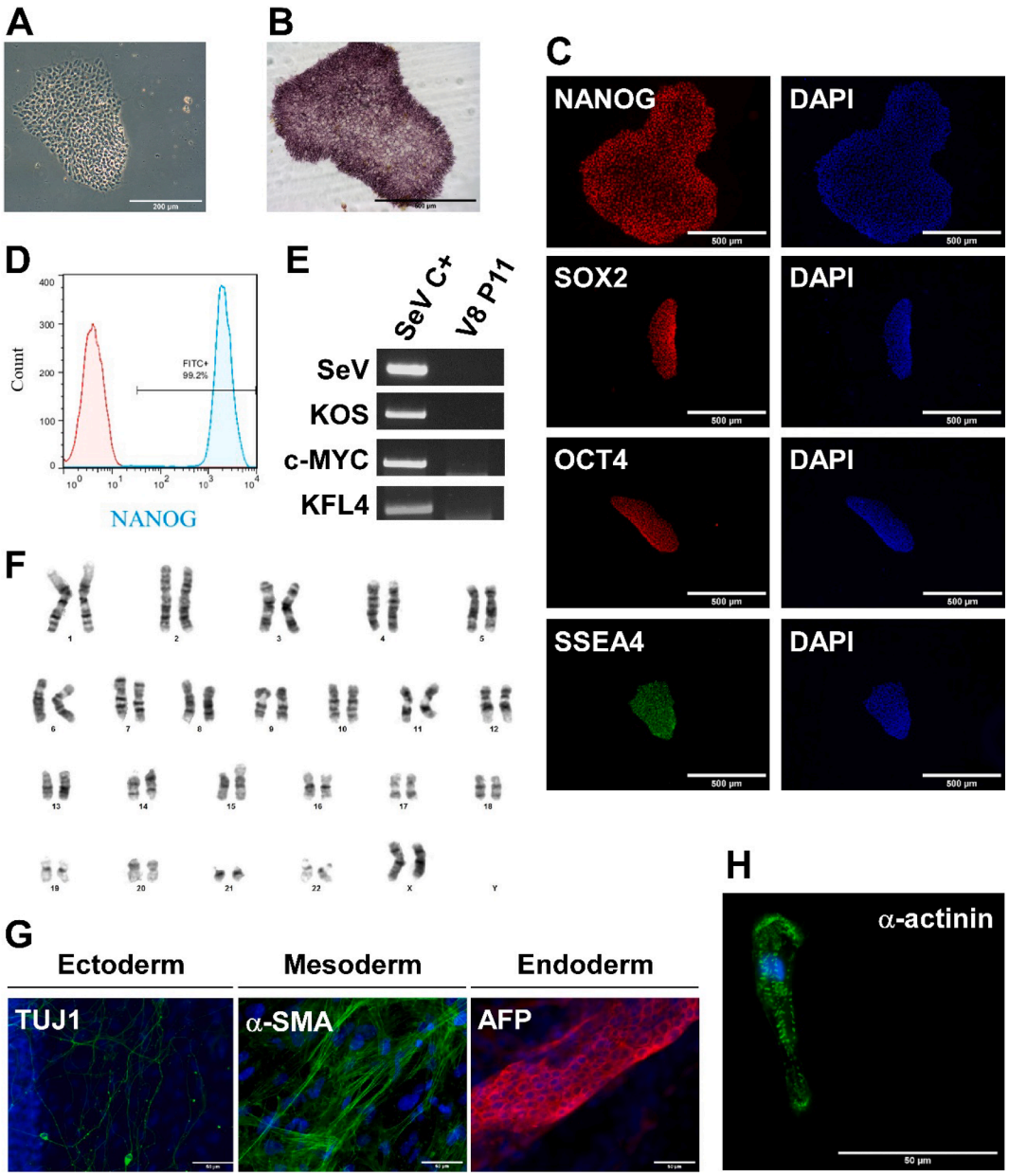

Fig. 1. Characterisation of the induced pluripotent stem cell line (UALGi002-A) from a patient with LVNC. (A) Phase contrast micrograph of UALGi002-A colony cultured in feeder-free conditions. (B) Alkaline phosphatase positive staining. (C) Immunofluorescence for pluripotency markers NANOG, SOX2, OCT4 and SSEA4. Nuclei were counterstained with DAPI (blue). (D) Flow cytometry of nuclear NANOG pluripotency marker. (E) RT-PCR analysis of Sendai virus and reprogramming transgenes at day 7 after transduction (SeV C+) and at passage 11 in UALGi002-A line (V8 P11). (F) Representative metaphase showing normal diploid 46, XY karyotype. (G) Immunocytochemistry for ectodermal (TUJ1), mesodermal (α-SMA) and endodermal (AFP) markers. Nuclei were counterstained with DAPI (blue). (H) Detection by immunofluorescence of the sarcomeric protein α-actinin (green) and staining of the nuclei by DAPI (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with 4% paraformaldehyde (PFA) for 15 min and analyzed by immunofluorescence (Table 2) on Axioimager Z2/Apotome fluorescence microscope (Carl Zeiss).

4.4. Flow cytometry

hiPSCs 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).

4.6. RT-PCR for detection of viral clearance

Total RNA was isolated from cultured hiPSC 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# 560073	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 GC	
			TGC 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.

4.7. Karyotype analyses

Genome integrity of the hiPSC was evaluated by G-banding at 400–550 band resolution, with a minimum of 20 metaphase spreads analyzed (Genomed, Lisbon, Portugal).

4.8. Fingerprinting

Genomic DNA from PBMC and hiPSC 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.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Braganca reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Fundação para a Ciência e Tecnologia (FCT).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102462.

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