Stem Cell Research 29 (2018) 170–173

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem Cell Line

Generation of the human induced pluripotent stem cell (hiPSC) line PSMi003-A from a patient affected by an autosomal recessive form of Long QT Syndrome type 1

Manuela Mura [a,b], Monia Ginevrino [c,d], Rita Zappatore [e], Federica Pisano [a,b,f], Marina Boni [e], Silvia Castelletti [g], Lia Crotti [g,h], Enza Maria Valente [c,d], Peter J. Schwartz [g], Massimiliano Gnecchi [a,b,f,i,*]

[a] Coronary Care Unit, IRCCS Policlinico San Matteo Foundation, Pavia, Italy.
[b] Laboratory of Experimental Cardiology for Cell and Molecular Therapy, IRCCS Policlinico San Matteo Foundation, Pavia, Italy.
[c] Department of Molecular Medicine, University of Pavia, Pavia, Italy.
[d] Neurogenetics Unit, IRCCS Santa Lucia Foundation, Rome, Italy
[e] Laboratory of Oncohaematological Cytogenetic and Molecular Diagnostics, Division of Haematology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy.
[f] Department of Molecular Medicine, Unit of Cardiology, University of Pavia, Pavia, Italy.
[g] IRCCS Auxologic Italian Institute, Center for Cardiac Arrhythmias of Genetic Origin, Laboratory of Cardiovascular Genetics, Milan, Italy.
[h] Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital IRCCS Auxologic Italian Institute, Milan, Italy.
[i] Department of Medicine, University of Cape Town, Cape Town, South Africa.

ARTICLE INFO

Article history:
Received 16 February 2018
Received in revised form 9 March 2018
Accepted 4 April 2018
Available online 06 April 2018

ABSTRACT

We generated human induced pluripotent stem cells (hiPSCs) from dermal fibroblasts of a 51 years old female patient homozygous for the mutation c.535 G>A p.G179S on the KCNQ1 gene, causing a severe form of autosomal recessive Long QT Syndrome type 1 (AR-LQT1), not associated with deafness. The hiPSCs, generated using four retroviruses each encoding for a reprogramming factor OCT4, SOX2, KLF4, cMYC, are pluripotent and can differentiate into spontaneously beating cardiomyocytes (hiPSC-CMs).

Resource table

Unique stem cell line identifier	PSMi003-A
Alternative name of stem cell line	HDF32-ARLQT-iPS
Institution	Fondazione IRCCS Policlinico San Matteo
Contact information of distributor	Massimiliano Gnecchi, m.gnecchi@unipv.it
Type of cell line	hiPSC
Origin	human
Additional origin info	Age: 51 Gender: female Ethnicity: Caucasian
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviruses encoding for the human cDNA of OCT4, SOX2, cMYC, KLF4
Genetic Modification	No
Type of Modification	N/A
Associated disease	Long QT Syndrome type 1 (OMIM #192500)

(*continued*)

Unique stem cell line identifier	PSMi003-A
Gene/locus	c.535 G>A mutation on KCNQ1 (NM_000218.2)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/Constitutive system	N/A
Date archived/stock date	Feb 28, 2013
Cell line repository/bank	No
Ethical approval	The study has been approved by the Ethics Committee of our Institution, Fondazione IRCCS Policlinico San Matteo, on the 29th of October 2010, protocol number 20100004354, proceeding P-20100003369. We obtained patient written informed consent for both skin biopsy procedure and conservation of biological samples.

* Corresponding author at: Fondazione IRCCS Policlinico San Matteo, Unità Coronarica, DEA Torre A, Viale Golgi 19, 27100 Pavia, Italy.
E-mail address: m.gnecchi@unipv.it. (M. Gnecchi).

https://doi.org/10.1016/j.scr.2018.04.003

Resource utility

It has been proven that iPSCs and iPSC-CMs can be efficiently used to model LQTS and test targeted therapies (Gnecchi et al., 2017; Mehta et al., 2017; Rocchetti et al., 2017). Accordingly, we anticipate that the PSMi003-A will be useful for: 1) modelling AR-LQT1; 2) testing novel therapies for the treatment of AR-LQT1.

Resource details

The PSMi003-A line was reprogrammed from dermal fibroblasts of a 51 years old woman affected by an autosomal recessive form of Long QT

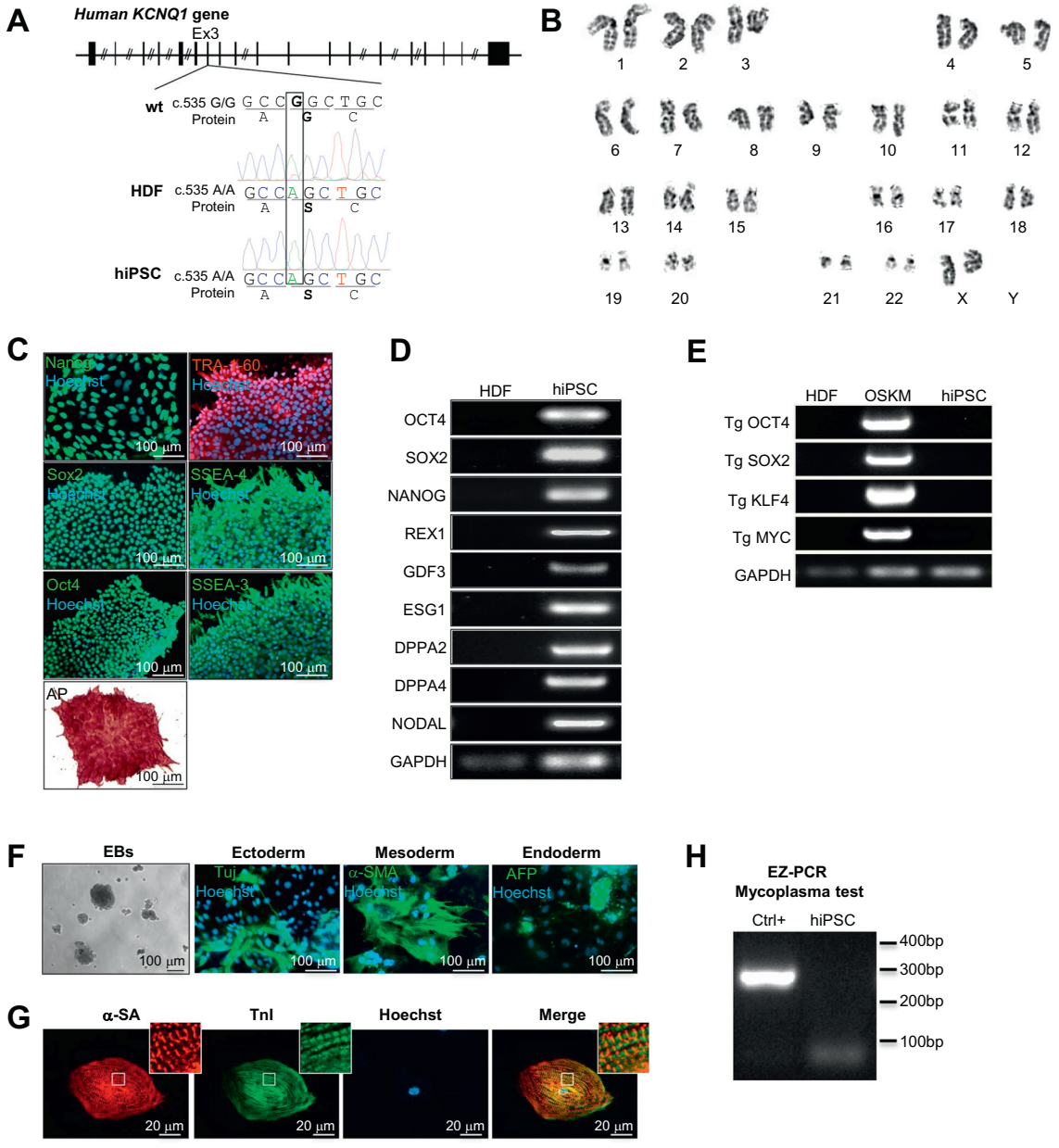

Fig. 1. Characterization of the PSMi003-A cell line. A. On top schematic representation of the KCNQ1 gene with introns (horizontal lines) and exons (vertical lines/boxes). In the lower panel, DNA sequencing results showing the presence of the mutation 535 G>A in homozygosis on Exon 3 of the KCNQ1 gene of patient-derived dermal fibroblasts (HDF) and PSMi003-A cell line (hiPSC) derived from the same HDF. The KCNQ1 coding sequence (CDS) used as a reference is the NCBI sequence NM_000218.2. B. Karyotype analysis of PSMi003-A (300 G-bandings) showing normal female karyotype (46, XX). C. Immunofluorescence staining showing uniform expression of the indicated markers of pluripotency in the PSMi003-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). The AP panel reports an alkaline phosphatase colorimetric staining. D. RT-PCR analysis showing expression of the indicated markers of pluripotency in PSMi003-A (hiPSC), compared with their parental fibroblasts (HDF). E. RT-PCR analysis showing no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), expression of Tg OCT4, SOX2, KLF4 and cMYC five days after transduction (OSKM) and silencing of the four Tg in PSMi003-A. F. Far left panel: floating embryoid bodies (EBs) formed after 7 days of PSMi003-A culture in suspension. Panels on the right: Immunofluorescence staining for markers of the 3 germ layers in iPSC-derived EBs: neuronal class tubulin beta III (Tuj) for ectoderm, smooth muscle actin (SMA) for mesoderm, and Alpha Fetoprotein (AFP) for endoderm. G. Co-immunofluorescence staining for the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA, red) and troponin I (TnI, green) in cardiomyocytes differentiated from the PSMi003-A. Nuclei were counterstained with Hoechst. The insets show areas of cross-striations. H. EZ-PCR test showing the absence of mycoplasma contamination in PSMi003-A. Ctrl+ is the positive PCR control provided by the kit.

Syndrome (AR-LQTS) type 1 (LQT1) without deafness. LQTS is an inherited disease characterized by the prolongation of cardiac repolarization, which is quantified as the duration of the QT interval on the surface electrocardiogram (ECG). This predisposes to ventricular tachycardia, often causing syncope or sudden cardiac death (SCD) (Schwartz et al., 1975). LQT1 is the commonest LQTS sub-type, accounting for ~40–50% of all cases. It is caused by mutations in the KCNQ1 gene, encoding for the α-subunit of the voltage-dependent potassium channel responsible for the delayed rectifier potassium current (I_{Ks}), one of the repolarization currents in the heart (Schwartz et al., 2012).

The enrolled patient was diagnosed with LQTS at age 10, when she was hospitalized after several syncopal episodes triggered by emotional and physical stresses. The ECG showed a markedly prolonged QTc (QT corrected for heart rate) of 664 ms. Given the severity of the clinical phenotype, she underwent left cardiac sympathetic denervation (LCSD) and beta-blocker therapy was started. At age 45, she received implantable cardioverter defibrillator (ICD) because of the presence of T-wave alternans at the ECG during the recovery phase of an exercise stress test. No appropriate ICD shocks or syncopal events occurred in the following years. Genetic screening revealed the presence a homozygous c.535 G/A mutation on the KCNQ1 gene, leading to the substitution of the glycine in position 179 with serine.

Fibroblasts were reprogrammed by retroviral infection of OCT4, SOX2, KLF4 and c-MYC. The obtained hiPSCs were maintained on feeders, retaining pluripotent features up to passage 50. Both patient's fibroblasts and hiPSCs present the disease-causing mutation on the KCNQ1 gene, as proved by DNA sequencing (Fig. 1A. The KCNQ1 coding sequence-CDS-used as a reference is the NCBI sequence NM_000218.2), and an identical DNA profile at seven polymorphic microsatellite loci, as shown by Short tandem Repeat (STR) analysis (submitted in archive with journal). Moreover, DNA karyotyping revealed normal female karyotype (46, XX) (Fig. 1B). The PSMi003-A uniformly expresses the human ES surface antigens Tumor Related Antigen-1-60 (TRA-1-60), Stage Specific Embryonic Antigen-3 and -4 (SSEA-3, SSEA-4) (Fig. 1C), and the pluripotent markers NANOG, OCT4, SOX2 (Fig. 1C and D), REX1, GDF3, ESG1, DPPA2, DPPA4 and NODAL (Fig. 1D). Likewise, it shows alkaline phosphatase (AP) activity (Fig. 1C). RT-PCR in Fig. 1E shows no expression of viral transgenes (Tg) in naïve fibroblasts (HDF), clear expression of Tg Oct4, Sox2, KLF4 and cMyc in fibroblasts five days after transduction (OSKM) and silencing of the four Tg in the PSMi003-A.

As expected, the PSMi003-A can spontaneously form embryoid bodies (EBs) able to differentiate into cells belonging to the three germ layers: endoderm, mesoderm and ectoderm (Fig. 1F). Most importantly, we have successfully differentiated PSMi003-A into cardiomyocytes displaying spontaneous beating activity and expressing the sarcomeric proteins alpha-actinin (α-SA) and troponin I (TnI) (Fig. 1G, insets show areas of cross-striation). We also verified the absence of mycoplasma contamination in our PSMi003-A (Fig. 1H).

Materials and methods

Generation and clonal expansion of hiPSCs

The detailed protocol is provided as Supplemental Methods.

Briefly, skin fibroblasts were reprogrammed using four retroviral vectors expressing OCT4, SOX2, KLF4 and cMYC. Clonal selection of fully reprogrammed cells was performed manually under sterile conditions and using an EVOS XL Core Imaging System (ThermoFisher), by picking individual clones morphologically similar to embryonic stem cells. Colonies were cut, harvested with a pipette, individually placed into a separate cell culture well and expanded (Table 1).

Mutation analysis

The presence of the KCNQ1-G179S mutation was confirmed by PCR and conventional sequencing of genomic DNA extracted from hiPSCs and their parental fibroblasts with QIAamp DNA Blood Mini kit (Qiagen). Genomic DNA was amplified with the GoTaq G2 DNA polymerase (Promega) (see Table 2 for primer sequences). The resulting amplicons were purified and sequenced (Lightrun service - GATC Biotech AG – Germany).

STR analysis

The PowerPlex® CS7 human identification kit (Promega) was used to co-amplify a set of seven variable short tandem repeat loci (LPL, F13B, FESFPS, F13A01, Penta_D, Penta_C, Penta_E) on genomic DNA from fibroblasts and hiPSCs in order to compare the genetic profile of the two cell lines. After PCR-amplification, fragments were run on a 3130xl capillary sequencer (Applied Biosystems) and then analyzed using GeneMarker software (SoftGenetics).

Table 1
Characterization and validation of PSMi003-A cell line.

Classification	Test	Result	Data
Morphology	Photography	Normal	Not shown but available upon request
Phenotype	Immunocytochemistry	Positive staining for the pluripotency markers OCT4, NANOG, SOX2, TRA-1-60, SSEA-3, SSEA-4	Fig. 1 panel C
	Alkaline phosphatase assay	Positive staining for the alkaline phosphatase	Fig. 1 panel C
	RT-PCR	Expression of the pluripotency markers OCT3/4, SOX2, NANOG, REX1, GDF3, ESG1, DPPA2, DPPA4, NODAL	Fig. 1 panel D
Genotype	Karyotype (300 G-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	Seven sites tested for iPSC, all sites matched with donor HDF STR profile	Online archive
Mutation analysis	Sequencing	Homozygous for the mutation c.535 G>A p.G179S on the KCNQ1 gene	Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Fig. 1 panel H
Differentiation potential	Embryoid body formation	The EBs expressed neuronal class tubulin beta III (Tuj) (ectoderm), smooth muscle actin (SMA) (mesoderm), and Alpha Fetoprotein (AFP) (endoderm).	Fig. 1 panel F
	Differentiation into cardiomyocytes	The iPSC-derived cardiomyocytes expressed the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA) and troponin I (TnI)	Fig. 1 panel G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not available
Genotype additional info	Blood group genotyping	Not performed	Not available
	HLA tissue typing	Not performed	Not available

Karyotyping

hiPSCs were blocked at metaphase by exposition to 10 μg/ml demecolcine solution for 3 h (Sigma Aldrich). Karyotyping was performed using 300 G-banding chromosome analysis, according with International System for Human Cytogenetic Nomenclature (ISCN) (Shaffer et al., 2013).

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti Nanog	1:200	Stemgent Cat# 09-0020, RRID: AB_2298294
	Mouse anti Oct3/4 (C-10)	1:500	SCBT Cat# sc-5279, RRID: AB_628051
	Mouse anti Sox2	1:500	R&D Systems Cat# MAB2018, RRID: AB_358009
	Mouse anti TRA-1-60	1:100	Stemgent Cat# 09-0010, RRID: AB_1512170
	Rat anti SSEA-3	1:100	Millipore Cat# MAB4303, RRID: AB_177628
	Mouse anti SSEA-4	1:100	Stemgent Cat# 09-0006, RRID: AB_1512169
Differentiation Markers (EBs)	Mouse anti neuronal class tubulin beta III (Tuj)	1:500	Covance Cat# MMS-435P, RRID: AB_2313773
	Mouse anti smooth muscle actin	1:1000	Millipore Cat# CBL171, RRID: AB_2223166
	Mouse anti alpha-fetoprotein	1:500	Millipore Cat# SCR030, RRID: AB_597591
Cardiac Markers	Rabbit anti Troponin I	1:250	Abcam Cat# ab52862, RRID: AB_869983
	Mouse anti alpha actinin	1:800	Sigma Aldrich Cat# A7811, RRID: AB_476766
Secondary antibodies	Alexa-Fluor® 488 Goat anti-rabbit IgG	1:500	ThermoFisher Cat# A11008, RRID:AB_143165
	Alexa-Fluor® 488 Goat anti-rat IgM	1:500	ThermoFisher Cat# A21212, RRID: AB_11180047
	Alexa-Fluor® 488 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11001, RRID: AB_2534069
	Alexa-Fluor® 546 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11003, RRID: AB_141370

Primers		
	Target	Forward/Reverse primer (5′–3′)
Targeted mutation analysis/sequencing	KCNQ1 Exon 3	Fw: 5′-gttcaaacaggttgcagggtctga-3′ Rev: 5′-ccaggtttccagaccaggaag-3′
Pluripotency Markers (RT-PCR)	OCT4	Fw: 5′-gtactcctcggtccctttcc-3′ Rev: 5′-caaaaaccctggcacaaact-3′
	SOX2	Fw: 5′-acaccaatcccatccacact-3′ Rev: 5′-tttttcgtcgcttggagact-3′
	NANOG	Fw: 5′-ttccttcctccatggatctg-3′ Rev: 5′-tctgctggaggctgaggtat-3′
	REX1	Fw: 5′-cagatcctaaacagctcgcagaat-3′ Rev: 5′-gcgtacgcaaattaaagtccaga-3′
	GDF3	Fw: 5′-cttatgctacgtaaaggagctggg-3′ Rev: 5′-gtgccaacccaggtcccggaagtt-3′
	ESG1	Fw: 5′-atatcccgccgtgggtgaaagttc-3′ Rev: 5′-actcagccatggactggagcatcc-3′
	DPPA4	Fw: 5′-ggagccgcctgccctggaaaattc-3′ Rev: 5′-tttttcctgatattctattcccat-3′
	DPPA2	Fw: 5′-ccgtccccgcaatctccttccatc-3′ Rev: 5′-atgatgccaacatggctcccggtg-3′
	NODAL	Fw: 5′-gggcaagaggcaccgtcgacatca-3′ Rev:5′-gggactcggtggggctggtaacgtttc-3′
House-Keeping Genes (RT-PCR)	GAPDH	Fw 5′-catgttccaatatgattccaccc-3′ Rev 5′-gggatctcgctcctggaagat-3′
Retroviral transgenes	OCT4 cDNA on pMXs-hOCT3/4	Fw: 5′-ccccagggccccatttttggtacc-3′
	SOX2 cDNA on pMXs-hSOX-2	Fw: 5′-ggcacccctggcatggctcttggctc-3′
	cMYC cDNA on pMXs-hcMYC	Fw: 5′-caacaaccgaaaatgcaccagccccag-3′
	KLF4 cDNA on pMXs-hKLF4	Fw: 5′-acgatcgtggccccggaaaaggacc-3′
	pMX viral vector	Rev: 5′-ccctttttctggagactaaataaa-3′

Immunocytochemistry

hiPSCs grown on glass coverslips were fixed for 15 min in 4% paraformaldehyde (Affimetrix USB), permeabilized in PBS containing 0.1% Triton X-100 (Sigma Aldrich) for 5 min, and blocked in 1% bovine serum albumin (BSA, Sigma Aldrich) for 1 h at room temperature (RT). Then they were incubated for 1 h at RT with the primary antibody (Table 2) diluted in blocking solution, washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (Table 2). Finally, the cells were stained with 1 μg/ml of Hoechst 33258 (Sigma Aldrich). Images were acquired using the Carl Zeiss fluorescence microscope Observer.Z1 equipped with the Apotome system and AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany).

AP colorimetric assay

AP was detected by using the Alkaline Phosphatase Staining kit (00-0009 Stemgent).

RT-PCR

Total RNA was purified using TRIzol (ThermoFisher Scientific). cDNA was synthesized using the Superscript II Reverse Transcriptase (ThermoFisher). RT-PCR was performed with the GoTaq G2 DNA polymerase (Promega) and primers in Table 2.

EB formation

hiPSCs were grown for 7 days in non-adherent conditions in a modified iPS medium deprived of bFGF and containing 20% FBS instead of KO-SR. Forming EBs were then transferred to gelatin-coated dishes to allow differentiation in adhesion for additional 7 days. Finally, the cells were processed for immunostaining of the three germ layers as described above.

Cardiac differentiation

Cardiac differentiation was induced using the PSC Cardiomyocyte Differentiation Kit (ThermoFisher).

Mycoplasma test

For the detection of mycoplasma in cell culture we used the EZ-PCR Mycoplasma Test Kit (Biological Industries).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.003.

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