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Lab Resource: Single Cell Line

Generation and characterization of the human induced pluripotent stem cell (hiPSC) line NCUFi001-A from a patient carrying KCNQ1 G314S mutation

Luca Lavra [a,*,1], Fiorenza Magi [a,1], Alessandra Ulivieri [a], Alessandra Morgante [a], Marianna Paulis [b,c], Luca Sala [d], Matteo Pedrazzini [d], Patrizio Polisca [a,e], Marcella Rocchetti [f], Leonardo Calò [g], Salvatore Sciacchitano [a,h], Leila B. Salehi [a,i]

[a] Laboratory of Biomedical Research, Niccolò Cusano University Foundation, Via Don Carlo Gnocchi 3, 00166 Rome, Italy
[b] Humanitas Clinical and Research Center-IRCCS, Rozzano, 20089 Milan, Italy
[c] Institute of Genetic and Biomedical Research (IRGB)-UOS of Milan, National Research Council (CNR), 20138 Milan, Italy
[d] Center for Cardiac Arrhythmias of Genetic Origin and Laboratory of Cardiovascular Genetics, Istituto Auxologico Italiano IRCCS, Via Zucchi 18, Cusano Milanino, MI, Italy
[e] Department of Biomedicine and Prevention, University of Rome Tor Vergata, Viale Montpellier 1, 00133 Rome, Italy
[f] Department of Biotechnology and Biosciences, Università degli Studi di Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy
[g] Division of Cardiology, Policlinico Casilino, Via Casilina 1049, 00169 Rome, Italy
[h] Department of Clinical and Molecular Medicine, Sapienza University, Via di Grottarossa 1035/1039, 00189 Rome, Italy
[i] Medical Genetics Unit, Tor Vergata University Hospital, PTV, Viale Montpellier 1, 00133 Rome, Italy

A B S T R A C T

In this study we describe the generation and characterization of an human induced pluripotent stem cell (hiPSC) line from a long QT syndrome type 1 (LQT1) patient carrying the *KCNQ1* c.940 G > A (p.Gly314Ser) mutation. This patient-specific iPSC line has been obtained by using non-integrational Sendai reprogramming method, expresses pluripotency markers and has the capacity to differentiate into the three germ layers and into spontaneously beating cardiomyocytes (iPSC-CMs).

Resource Table:

Unique stem cell line identifier	NCUFi001-A
Alternative name(s) of stem cell line	N/A
Institution	Niccolò Cusano University Foundation
Contact information of distributor	Luca Lavra luca.lavra@fondazioneniccolocusano.it
Type of cell line	hiPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 17 years Sex: male Ethnicity: caucasian
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Associated disease	Long QT Syndrome type 1 (LQT1) (OMIM #192500)
Gene/locus	*KCNQ1* c.940 G > A, 11p15-5
Date archived/stock date	July 2019
Cell line repository/bank	No
Ethical approval	The Project "Caritmo" has been approved by the Ethics Committee ASL RM/B on 2011/01/13. Patient written informed consent was obtained for blood sampling and conservation of biological samples

1. Resource utility

Patient-specific iPSC-CMs provide a valuable resource to *in-vitro* explore long-QT syndrome disease and therapeutic interventions such as new drug screening and gene therapy (Lodrini et al., 2020). The new NCUFi001-A iPSC-CMs carrying the KCNQ1 G314S mutation will be a useful model for LQTS type 1 pathogenesis studies and targeted drug testing.

* Corresponding author.
E-mail address: luca.lavra@fondazioneniccolocusano.it (L. Lavra).
[1] These authors contributed equally to the study.

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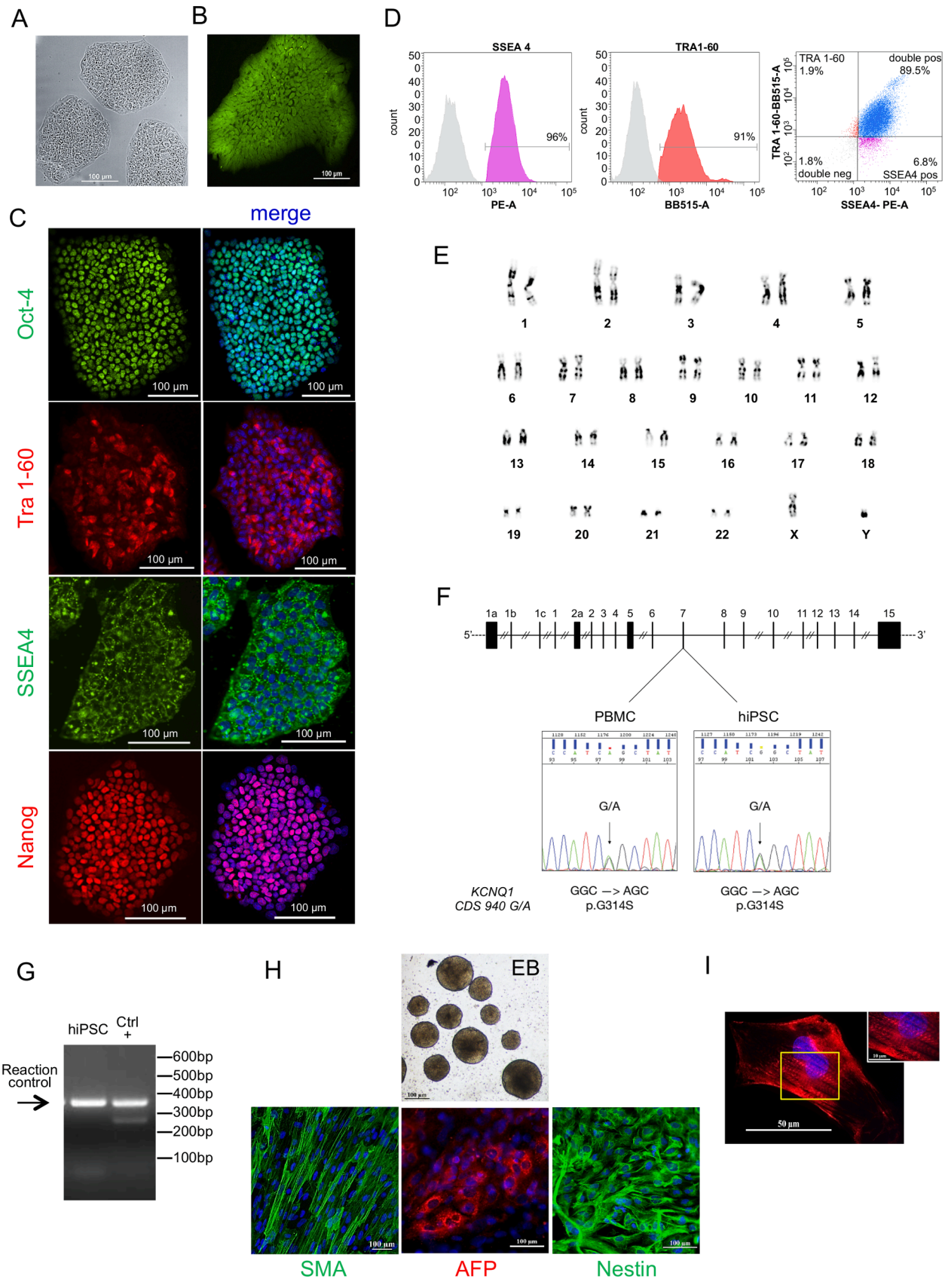

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Fig. 1. Characterization of the NCUFi001-A cell line. (A) Phase contrast image and (B) alkalin phosphatase staining of NCUFi001-A hiPSC colonies. (C) Immunofluorescence images of iPSCs positive for stem cell markers OCT4, TRA 1-60, SSEA4 and NANOG. Nuclei are labeled with Hoechst 33258 (blue). (D) Flow cytometry analysis of pluripotency protein markers SSEA4 and TRA-1-60. (E) Cytogenetic analysis of NCUFi001-A hiPSC showing normal male karyotype (46, XY). (F) Top: schematic representation of KCNQ1 gene with exons indicated as vertical lines or boxes. Bottom: DNA sequencing analysis showing the presence of heterozygous gene mutation c.940G>A in the exon 7 of KCNQ1 gene in both parental PBMC and NCUFi001-A hiPSC cells (NCBI Reference Sequence NM_000218.3). (G) EZ-PCR test showing the absence of mycoplasma contamination in NCUFi001-A hiPSC. A positive control provided by the kit is showed (Ctrl+). (H) Top: phase contrast image of floating EBs formed by NCUFi001-A hiPSC cultured in suspension. Bottom: immunofluorescence images of EBs positive for the three germ layers differentiation markers SMA (mesodermal), AFP (endodermal) and Nestin (ectodermal). (I) Immunofluorescence analysis of cardiac troponin T (cTnT) in NCUFi001-A hiPSC-derived cardiomyocytes.

2. Resource details

Long QT syndrome (LQTS) is an inherited primary arrhythmia syndrome that presents with recurrent syncope or, in rare cases, sudden cardiac death (SCD) secondary to malignant "Torsades de points" (TdP). It is an autosomal dominant inherited disease characterized by the prolongation of cardiac repolarization and by the elongation of the QT interval on the electrocardiogram (ECG). This repolarization defect predisposes to TdP, syncope, and SCD (Schwartz et al., 2012). Molecular genetic studies revealed that congenital LQTS is linked to mutations in genes encoding for cardiac ion channels subunits or adapter proteins that modify the channel functions. From the first study in 1991, where the single genetic locus 11p15.5 was associated with LQTS within a single family, today at least 17 types of genes have been found to be linked to 17 different types of syndromes (LQT1-17) (Skinner et al., 2019). Type 1 LQTS (LQTS1) is present in approximately 40–50% of all genotyped LQT patients and is caused by mutation in *KCNQ1* gene which encodes the α-subunit of the slow component of delayed rectifier K + current (I_{ks}) Voltage-Gated Potassium Channel Kv7.1 (Schwartz et al., 2012).

In this study, the NCUFi001-A cell line was generated by the reprogramming of PBMCs obtained from a 17-year-old man diagnosed with LQTS1. The patient carries the heterozygous mutation c.940 G > A on the *KCNQ1* gene leading to the substitution with serine of the glycine 314, localized in the signature sequence of KCNQ1 pore region. This mutation has been demonstrated to exert a dominant-negative effect in oocytes (Du et al., 2007; Li et al., 2009). The patient has a prolonged QTc (QT corrected for heart rate) and experienced cardiac symptoms.

PBMCs were reprogrammed using non-integrating Sendai-virus vectors encoding four Yamanaka factors (OCT4, SOX2, KLF4, and c-MYC). The obtained hiPSC cell line, maintained in feeder-free conditions, showed the typical ES-like morphology (Fig. 1A) and alkaline phosphatase (AP) activity (Fig. 1B). The pluripotency of the new hiPSC cell line was confirmed either by immunofluorescence staining for OCT4, SSEA4, Nanog, TRA-1-60, and FACS analysis for TRA-1-60 and SSEA4 (Fig. 1 C and D). DNA karyotyping revealed normal male karyotype (46, XY) (Fig. 1E). DNA sequencing analysis showed the presence of the *KCNQ1* c.940 G > A heterozygous mutation in both parental PBMCs and NCUFi001-A hiPSCs (Fig. 1F) and data from short tandem repeat (STR) analysis confirmed a complete match between NCUFi001-A hiPSC line and parental PBMC. The absence of mycoplasma contamination was demonstrated (Fig. 1G). To test the ability to differentiate into the three germ lineages, *in vitro* embryoid bodies (EBs) formation analysis was performed and immunofluorescence staining exhibited evidence for endodermal (AFP and SOX17), mesodermal (SMA and TnI) and ectodermal (Nestin and MAP2) markers expression (Fig. 1H and data not shown). Moreover, the NCUFi001-A hiPSC cell line successfully differentiated into spontaneously beating cardiomyocytes expressing the cardiac marker troponin T (cTnT) (Fig. 1I).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal human pluripotent stem cell morphology	Fig. 1 panel A
Phenotype	Qualitative analysis: Immunocytochemistry	Positive staining for expression of pluripotency markers: AP, OCT4, SSEA4, Nanog and TRA-1–60	Fig. 1 panel B and C
	Quantitative analysis: Flow cytometry	Assess % of positive cells for pluripotency markers: Tra 1–60: 91% SSEA-4: 96%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 350–400	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR)	DNA Profiling not performed	N/A
	STR analysis	AmpFlSTR® IdentifilerTM kit (Applied Biosystem). All 24 sites matched	Available in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous *KCNQ1* c.940 G > A	Fig. 1 panel F
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR Negative	Fig. 1 panel G
Differentiation potential	Embryoid body (EB) formation and induced cardiac differentiation	Proof of pluripotency (three germ layers differentiation): AFP, SMA, and Nestin. Proof of cardiac differentiation (NCUFi001-A iPSC-derived cardiomyocyte): cTnT	Fig. 1 panel H and I
List of recommended germ layer markers	Expression of these markers has been demonstrated at protein (IF) levels	Expression of endodermal (AFP, SOX17), mesodermal (SMA and TnTI) and ectodermal (Nestin, MAP2) proteins Expression of cardiac protein (cTnT)	IF with specific antibodies
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

3. Materials and Methods

3.1. iPSC Generation

Detailed protocols are reported in the Supplementary data section. PBMCs were reprogrammed to hiPSCs using CytoTune™-iPS 2.0

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4 (IgG)	1:500	Abcam Cat# ab19857	RRID: AB_445175
	Rabbit anti-Nanog (IgG)	1:100	Abcam Cat# ab21624	RRID: AB_446437
	Mouse anti-Tra-1-60 (IgM)	1:200	Abcam Cat# ab16288	RRID: AB_778563
	Mouse anti-SSEA4 (IgG)	1:500	Abcam Cat# ab16287	RRID: AB_778073
	anti SSEA4-PE Mouse IgG3	1:12.5	BD Biosciences Cat# 560,128	RRID: AB_1645533
	anti TRA1-60-BB515 mouse IgM, k	1:50	BD Biosciences Cat# 565,343	RRID: AB_2739196
	PE mouse IgG3, K Isotype	1:12.5	BD Biosciences Cat# 559,926	RID: AB_10050453
	BB515 mouse IgM, k isotype	1:50	BD Biosciences Cat#564680	RRID: AB_2869601
Differentiation Markers	Mouse anti-alpha-fetoprotein (AFP)	1:100	Millipore Cat#SCR030	RRID: AB_597591
	Rabbit anti SOX17	1:100	Millipore Cat# 09-038	RRID: AB_1587525
	Mouse anti smooth muscle actin (SMA)	1:100	Millipore Cat# CBL171	RRID: AB_2223166
	Mouse anti Troponin I (TnI)	1:200	Millipore Cat# MAB1691	RRID: AB_2256304
	Rabbit anti Nestin	1:100	Millipore Cat# ABD69	RRID: AB_2744681
	Mouse anti microtubule associated protein 2 (MAP2)	1:200	Millipore Cat# MAB3418	RRID: AB_94856
	Rabbit anti cardiac Troponin T (cTnT)	1:400	Abcam Cat# ab45932	RRID: AB_956386
Secondary Antibodies	Alexa Fluor 488 Donkey anti-Mouse IgG (H + L)	1:500	Thermo Fisher Cat# A-21202	RRID: AB_141607
	Alexa Fluor 488 Donkey anti-Rabbit IgG (H + L)	1:500	Thermo Fisher Cat# A-21206	RRID: AB_2535792
	Alexa Fluor 568 Donkey anti-Mouse IgG (H + L)	1:500	Thermo Fisher Cat# A10037	RRID: AB_2534013
	Alexa Fluor 568 Donkey anti-Rabbit IgG (H + L)	1:500	Thermo Fisher Cat# A10042	RRID: AB_2534017
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Targeted mutation analysis/ sequencing	KCNQ1 exon 7	312 bp	CAGGGTCTCTTGCCGGCCT/ TGGGTCTGCTCACAGGGAGG	

Sendai Reprogramming Kit (ThermoFisher) following the manufacturer instructions. hiPSCs were cultured on Vitronectin (VTN-N)-coated plates (0.5 μg/cm^2), in Essential-8 Medium and maintained at 37 °C in humidified atmosphere containing 5% CO_2.

3.2. Mutation analysis

DNA extraction was performed by using Wizard Genomic DNA Purification Kit (Promega). The exon-7 of *KCNQ1* gene was amplified by PCR using home-designed primers with annealing temperature of 60 °C (GoTaq® Hot Start Polymerase Promega). Amplicons were sequenced using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with a 3500 Genetic Analyzer (Applied Biosystems) (See Table 1).

3.3. STR analysis

STR analysis was conducted by Eurofins Genoma Group Srl using the AmpFlSTR® IdentifilerTM kit (Applied Biosystem). The 24 markers were sequenced on ABI PRISM 310 Genetic Analyzer and analyzed using the Genotyper software (Applied Biosystems).

3.4. Immunocytochemistry

hiPSCs, grown on VTN-N-coated coverslips, were fixed with 4% paraformaldehyde (PFA) solution (Sigma-Aldrich) at room temperature (RT) for 15 min, washed three times with PBS, permeabilized with 0.25% Triton X-100 (Sigma-Aldrich) for 10 min at RT and blocked in PBS containing 3% BSA (Sigma-Aldrich) for 1 h at RT. Then they were incubated O.N. at 4 °C with the primary antibody (See Table 2) diluted in blocking solution, washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (See Table 2). Nuclei were stained with 1 μg/ml of Hoechst 33258 (Sigma-Aldrich). Images were captured using Nikon Eclipse Ti fluorescence microscope equipped with the Neo 5.5 sCMOS camera (Andor Technology) and NIS-Elements software (Nikon) (See Table 1).

3.5. Flow cytometry analysis

hiPSCs were harvested with 0.25% trypsin-EDTA (EuroClone) and resuspended in PBS at 1×10^6 cells/ml. Cells were incubated for 7 min at 37 °C with Fixable Viability Dye 510 (BD Bioscience). Samples were then incubated for 30 min at 4 °C with direct-labeled SSEA4, TRA1-60 and isotypes control antibodies (BD Bioscience) diluted in staining buffer (PBS/FBS 1%/EDTA 2 mM) (See Table 2). After washing, cells were analyzed using BD FACS Melody flow cytometer and BD FACSDiva software (See Table 1).

3.6. Karyotyping

hiPSCs grown on coverslip were treated with 0.1 mg/ml KaryoMAX® Colcemid™ Solution (ThermoFisher) for 2 h at 37 °C. After hypotonic treatment with 0.075 M KCl and fixation in methanol:acetic acid (3:1 v/v), slides were air dried and mounted in Eukitt (Fluka). Chromosome counts and karyotype analyses were performed on metaphases stained with Vectashield mounting medium with DAPI (Vector Laboratories) for Q-banding. Images were captured using Olympus BX61 Research Microscope equipped with a cooled CCD camera and analyzed with Applied Imaging Software CytoVision (CytoVision Master System with mouse karyotyping). At least 20 karyotypes were analyzed (See Table 1).

3.7. EB formation

hiPSCs were seeded into ultra-low attachment plates (Corning Inc.) for 7 days in Essential 8 medium and then transferred on VTN-N-coated coverslip in Essential 6 medium (ThermoFisher) for additional 7 days.

Differentiated EBs were immunostained using 2 markers per germ layer (See Table 1and Table 2).

3.8. Cardiac differentiation

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

3.9. Mycoplasma test

The EZ-PCR Mycoplasma Detection Kit (Biological Industries) was used to prove the absence of mycoplasma contamination in cell culture (See Table 1).

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.2021.102418.

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