Stem Cell Research 28 (2018) 149–152

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of a transgene-free induced pluripotent stem cells line (UNIPDi002-A) from oral mucosa epithelial stem cells carrying the R304Q mutation in TP63 gene

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ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form 6 February 2018
Accepted 11 February 2018
Available online 16 February 2018

ABSTRACT

Transgene free UNIPDi002-A-human induced pluripotent stem cell (hiPSC) line was generated by Sendai Virus Vectors reprogramming from human oral mucosal epithelial stem cells (hOMESCs) of a patient affected by ectrodactyly-ectodermal dysplasia-clefting (EEC)-syndrome, carrying a mutation in exon 8 of the TP63 gene (R304Q). The UNIPDi002-A-hiPSC line retained the mutation of the parental R304Q-hOMESCs and displayed a normal karyotype. No residual expression of transgenes nor Sendai virus vector sequences were detected in the line at passage 8. UNIPDi002-A-hiPSC expressed a panel of pluripotency-associated markers and could form embryoid bodies expressing markers belonging to the three germ layers ectoderm, endoderm and mesoderm.

Resource Table

Unique stem cell line identifier	UNIPDi002-A-hiPSC
Alternative name(s) of stem cell line	304D- hiPSC
Institution	Department of Molecular Medicine, University of Padova, Padua, Italy
Contact information of distributor	Marta Trevisan, marta.trevisan@unipd.it
Type of cell line	iPSC
Origin	human
Additional origin info	Age:18 Sex: female Ethnicity if known: caucasian
Cell source	human oral mucosa epithelial stem cells (hOMESCs)
Clonality	Clonal
Method of reprogramming	Transgene free, Sendai virus vectors
Genetic modification	NO
Type of modification	N/A
Associated disease	Ectrodactyly-ectodermal dysplasia-clefting syndrome (EEC)
Gene/locus	TA-p63α: AF075430: c.1028G > A; p.R304Q
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	Patient informed consent obtained and approved by the Venetian Ethical Committee for Clinical Research Studies (Prot. 2009/77661, November 19, 2009).

Resource utility

Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome is a rare hereditary disorder caused by mutations in the TP63 genes and R304Q is one of the most severe. The generated hiPSC line offers a useful resource to investigate pathogenic mechanisms in EEC syndrome, as well as a source of cells for future cell-based therapies.

Resource details

Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome (OMIM #*604292*) is a rare autosomal dominant disorder caused by mutations in the TP63 gene (Celli et al., 1999). Among mutations affecting the DNA binding site of TP63, R304Q (exon 8) is one of the most severe (Ianakiev et al., 2000). Human oral mucosa epithelial stem cells (hOMESCs) were isolated, after an informed consent was signed, from

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https://doi.org/10.1016/j.scr.2018.02.006

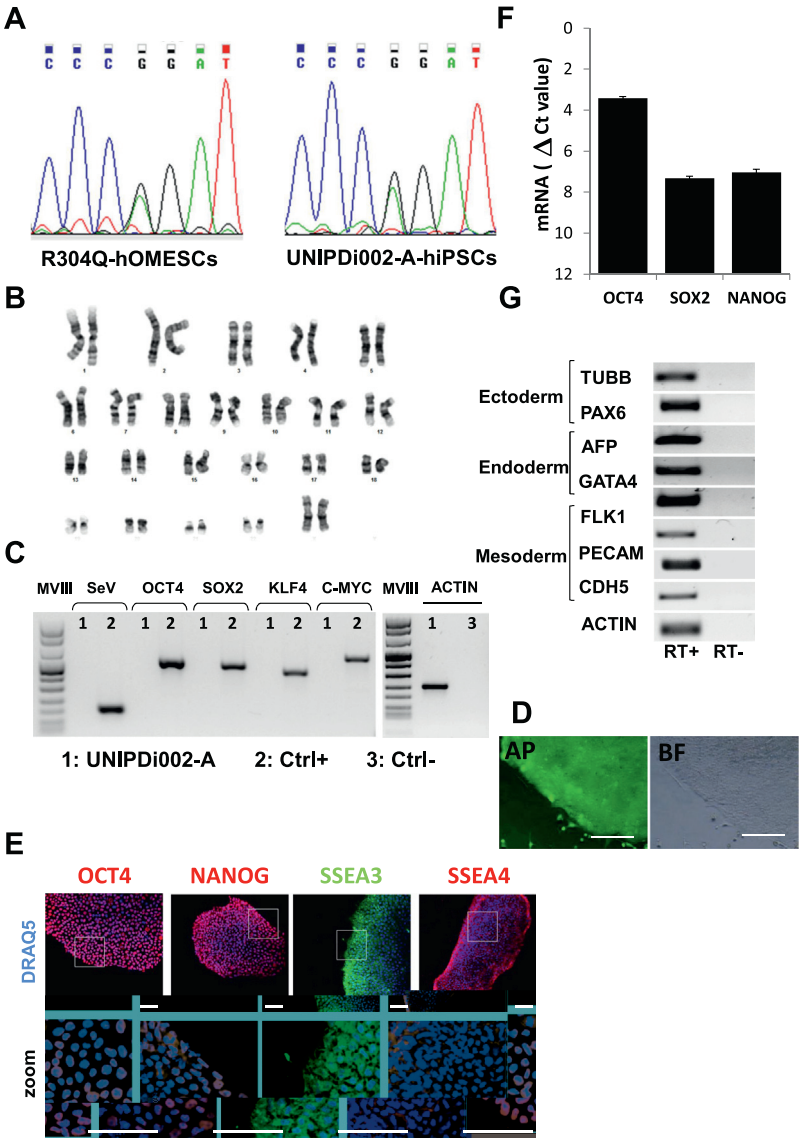

Fig. 1. Characterization of UNIPDi002-A-hiPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel D
Phenotype	Qualitative analysis (Immunocytochemistry)	Staining of pluripotency markers: Oct4, Nanog,SSEA3, SSEA-4	Fig. 1 panel E
	Quantitative analysis (RT-qPCR)	OCT4, NANOG, SOX2	Fig. 1 panel F
	Immunocytochemistry counting	Oct4: 100%, Nano: 99%; SSEA3: 97%; SSEA4: 95%	
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel B
Identity		DNA Profiling: N/A	N/A
	STR analysis	15 sites tested, all matched	Fig. 2/Supplementary; submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	heterozygous Arg304Gln	Fig. 1 panel A
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Fig. 1/supplementary
Differentiation potential	Embryoid body formation	Ectoderm (TUBB and PAX6), mesoderm (FLK1, PECAM and CDH5) and endoderm (AFP and GATA4)	Fig. 1 panel G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

a 18 years old female carrying the R304Q mutation in exon 8 of the TP63 gene. Cells were expanded and characterized at the Fondazione Banca degli Occhi del Veneto, Venice, Italy as previously described (Barbaro et al., 2016). R304Q-hOMESCs were grown on a feeder layer of irradiated 3 T3-J2 in keratinocyte growth medium (KGM) and, at passage 3, were subsequently transduced with Sendai virus (SeV) vectors expressing the four Yamanaka factors (i.e. OCT4, SOX2, KLF4, c-MYC) at a multiplicity of infection (MOI) of 3. iPSCs-like colonies started to appear as early as day 18 post transduction (p.t.). Cell line UNIPDi002-A-hiPSC was manually picked 7 days later and transferred to a plate coated with mouse embryonic fibroblasts (MEFs) feeder layer for expansion and characterization. UNIPDi002-A-hiPSC retained the parental R304Q mutation, as confirmed by Sanger sequencing analysis of exon 8 of the TP63 gene (Fig. 1A). The cell line displayed a normal karyotype (46XX) upon long term culture (passage 35) (Fig. 1B) and did neither show any residual presence of SeV sequences, nor expression of any of the transgenes as early as at passage 8, as demonstrated by RT-PCR analysis (Fig. 1C). UNIPDi002-A-hiPSCs expressed the undifferentiated state marker alkaline phosphatase (AP, Fig. 1D, BF = bright field, scale bar = 100 μm), along with a panel of key pluripotency markers, as assessed by either indirect immunofluorescence (IIF) analysis (Fig. 1E: OCT4, Nanog, SSEA3, SSEA4; scale bars = 100 μm), or qRT-PCR (Fig. 1F: OCT4, NANOG and SOX2), confirming the pluripotency of the line. Moreover, UNIPDi002-A-hiPSCs formed embryoid bodies (EBs) in vitro and, upon random differentiation in adhesion, it expressed markers belonging to the three germ layers, ectoderm (TUBB and PAX6), endoderm (AFP and GATA4) and mesoderm (FLK1, PECAM and CDH5), as assessed by RT-PCR (Fig. 1G). (See Table 1.)

Materials and methods cell cultures

R304Q-hOMESCs were isolated from a fresh oral mucosa biopsy obtained from a patient affected by EEC syndrome carrying the heterozygous mutation R304Q in *TP63 gene*. Cells were grown as previously described (Barbaro et al., 2016) in keratinocytes growth medium (KGM). UNIPDi002-A-hiPSC line was grown on irradiated MEFs (MTI-GlobalStem, Thermo Fisher Scientific, USA), in human Embryonic Stem (hES) cell medium as previously described (Trevisan et al., 2017).

Sendai virus vector reprogramming

R304Q-hOMESCs ($5 * 10^5$) were transduced with SeV vectors (Cytotune®-iPS Sendai Reprogramming Kit, Life Technologies, Thermo Fisher Scientific) at a MOI of 3 and the medium was changed every other day. On day 7, cells were detached and plated onto a 10 cm dish previously coated with MEFs. From the day after for 48 h, the medium was composed by a mixture in a ratio 1:1 of KGM and hES medium before switching to hES medium. Cells were then fed every other day for a week before switching to a daily feeding.

Mutation analysis of the TP63 gene

Genomic DNA was isolated from hOMESCs-R304Q and UNIPDi002-A-hiPSCs and underwent PCR amplification of exon 8 of TP63 (Table 2) and Sanger sequencing. Karyotype analysis.

Karyotype analysis was performed by Cell Guidance system (Cambridge, CB22 3AT, UK).

Residual transgene expression analysis

Total RNA was isolated from UNIPDi002-A-hiPSCs at passage 8, using the RNAeasy mini kit (Qiagen, Germany), and reverse transcribed. cDNA were used to amplify by RT-PCR SeV genome and the four transgenes (Table 2). As positive controls, transduced hOMESCs-R304Q at day 7 p.t. were processed in parallel.

Alkaline phosphatase live staining assay

The UNIPDi002-A-hiPSC line was incubated for 30 min at RT with 1× AP Live Stain Solution (Thermo Fisher Scientific) and observed under a Leica DFC420 inverted epifluorescence microscope (Leica, Germany).

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	- Goat anti-OCT3/4 [N-9]	1:200	- Santa Cruz Cat#: sc-8628, RRID:AB_653551
	- Mouse anti-SSEA4 [MC813]	1:50	- Abcam Cat#: ab16287, RRID:AB_778073
	- Mouse anti--NANOG [NNG-811]	1:50	- Abcam Cat#:ab62734, RRID:AB_956161
	- Rat anti-SSEA3 [MC631]	1:50	- Abcam Cat#: ab16286, RRID:AB_882700
Differentiation markers	N/A	N/A	N/A
Secondary antibodies	- Donkey anti-goat IgG-R	1:250	- Santa Cruz Cat# sc-2094, RRID:AB_641161
	- Goat anti-Mouse IgG	1:500	- Millipore Cat# AP124R, RRID:AB_90563
	- Goat anti-Rat IgG H&L (FITC)	1:250	- Abcam Cat#: ab6840, RRID:AB_955326

Primers

	Target	Forward/Reverse primer (5′-3′)
Sendai virus vectors transgenes	SeV	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC
	OCT4 (transgene)	CCC GAAAGAGAAAGCGAACCAG AATGTATCGAAGGTGCTCAA
	SOX2 (transgene)	ATGCACCGCTACGACGTGAGCGC AATGTATCGAAGGTGCTCAA
	KLF4 (transgene)	TTCCTGCATGCCAGAGGAGCCC AAT GTATCG AAG GTG CTC AA
	cMYC (transgene)	TAA CTG ACT AGC AGG CTT GTC G TCC ACA TAC AGT CCT GGA TGA TGA TG
Episomal plasmids (qPCR)	N/A	
Pluripotency markers (qPCR)	OCT4	GGTGCCTGCCCTTCTAGGAATGGGGGA CAAAAACCCTGGCACAAACTC Probe: CTAGGGAAAGAAAACC
	SOX2	GAGAAGTTTGAGCCCCAGG AGAGGCAAACTGGAATCAGG Probe: ATCATCGGCGGCGGCA
	NANOG	GGTCTCGATCTCCTGACCTTGT GCCTGTAAATCCCAGCTGTTAGG Probe: TCCACCCGCCTCGG
House-keeping genes (qPCR)	ACTIN	CGGGACCTGACTGACTACCTC CCATCTCTTGCTCGAAGTCCAG Probe: TCCTTAATGTCACGCACGATTTCCCGCT
Differentiation markers (RT-PCR)	TUBB	CAGATGTTCGATGCCAAGAA TGCTGTTCTTGCTCTGGATG
	PAX6	TCTAATCGAAGGGCCAAATG TGTGAGGGCTGTGTCTGTTC
	AFP	AGCTTGGTGGTGGATGAAAC CCCTCTTCAGCAAAGCAGAC
	GATA4	CTAGACCGTGGGTTTTGCAT TGGGTTAAGTGCCCCTGTAG
	FLK1	AGTGATCGGAAATGACACTGGA GCACAAAGTGACACGTTGAGAT
	PECAM	CCCAGCCCAGGATTTCTTAT ACCGCAGGATCATTTGAGTT
	CDH5	CAGCCCAAAGTGTGTGAGAA TGTGATGTTGGCCGTGTTAT
Genotyping	EXON 8 TP63	GGTAGATCTTCAGGGGACTTTC TTCTCACTGGCTCTGAGGG
Targeted mutation analysis/sequencing	N/A	

Pluripotency marker expression analysis by qRT-PCR

UNIPDi002-A-hiPSCs were harvested by Accutase (Stemcell Technologies) treatment and total RNA was isolated as described above. Upon reverse transcription, cDNA were used as template to amplify by qRT-PCR a panel of pluripotency associated genes (Table 2).

Pluripotency marker expression analysis by immunofluorescence

UNIPDi002-A-hiPSCs growing on MEFs in hES medium were fixed in 4% paraformaldehyde (Sigma Aldrich, USA) for 20 min at RT, permeabilized in PBS/0.1% Triton X-100 (Sigma Aldrich) for 15 min at RT and saturated with 4% BSA/PBS (Sigma Aldrich) at 4 °C overnight. The following day, primary antibodies (Table 2) were added and incubated for 1 h at RT, after which secondary antibodies (Table 2) were added for 1 h in the dark at 37 °C. Cells were then stained with *DRAQ5 5* μM, (Thermo Fisher Scientific) for 5 min at RT.

Embryoid bodies test

The UNIPDi002-A-hiPSC line was forced to grow for 7 days in suspension in hES medium not supplemented with b-FGF. After one week, cells were seeded in plates previously coated with 0.1% porcine gelatine (Merck Millipore) in DMEM medium supplemented with 10% FBS (both from Gibco, Thermo Fisher Scientific). After 7 days total RNA was isolated and reverse transcribed, and cDNAs were used to amplify markers belonging to the three germ layers (Table 2).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.02.006.

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