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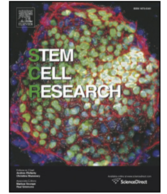
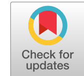


Lab Resource: Stem Cell Line

# Induced pluripotent stem cells line (UNIPDi003-A) from a patient affected by EEC syndrome carrying the R279H mutation in TP63 gene



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## ABSTRACT

Oral mucosa epithelial stem cells from a patient affected by Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) syndrome carrying the R279H mutation in the TP63 gene were reprogrammed into human induced pluripotent stem cells (hiPSCs) with episomal vectors. The generated UNIPDi003-A-hPSC line retained the mutation of the parental cells and showed a normal karyotype upon long term culture. Analysis of residual transgenes expression showed that the episomal vectors were eliminated from the cell line. UNIPDi003-A-hiPSCs expressed the undifferentiated state marker alkaline phosphatase along with a panel of pluripotency markers, and formed embryoid bodies capable of expressing markers belonging to all the three germ layers.

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Resource table

| | |
|---|---|
| Unique stem cell line identifier | UNIPDi003-A-hiPSC |
| Alternative name(s) of stem cell line | EEC-A-hiPSCs |
| Institution | Department of Molecular Medicine, University of Padova, Italy |
| Contact information of distributor | Marta Trevisan; marta.trevisan@unipd.it |
| Type of cell line | iPSC |
| Origin | human |
| Additional origin info | Age: N/A<br>Sex: female<br>Ethnicity if known: Caucasian |
| Cell source | human oral mucosa epithelial stem cells (hOMESCs) |
| Clonality | Clonal |
| Method of reprogramming | Transgene free |
| Genetic modification | NO |
| Type of modification | No modification |
| Associated disease | Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) syndrome |
| Gene/locus | TA-p63α; AF075430: c.953G>A; p.R279H |
| 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. |

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## Resource utility

Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome is a genetic disorder caused by mutations in the TP63 genes and R279H 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 dominantly inherited disease caused by several mutations in TP63 gene (van Bokhoven et al., 2001). Among these, R279H is one of the most severe, causing a loss of limbal stem cells in the cornea and, as consequence, patients progressive and irreversible degeneration of visual capabilities (Ianakiev et al., 2000). Upon signature of an informed consent, human oral mucosa epithelial stem cells (hOMESCs) were isolated from a fresh oral biopsy obtained from a female patient carrying the R279H mutation in the TP63 gene at the Fondazione Banca degli Occhi del Veneto (Venice, Italy). hOMESCs were expanded and used to generate hiPSCs by nucleofection of episomal vectors (Okita et al., 2011) using the 4D Nucleofector Device. At day 15 post-transfection (p.t.) human embryonic stem (ES) cell-like colonies started to emerge and were manually picked after 5 more days in culture. The UNIPDi003-A-human induced pluripotent stem cell (hiPSC) line retained the parental cells R279H mutation, as demonstrated by Sanger sequencing (Fig. 1A). The absence of residual expression of the episomal transgenes (OCT4, SOX2, KLF4, l-MYC and

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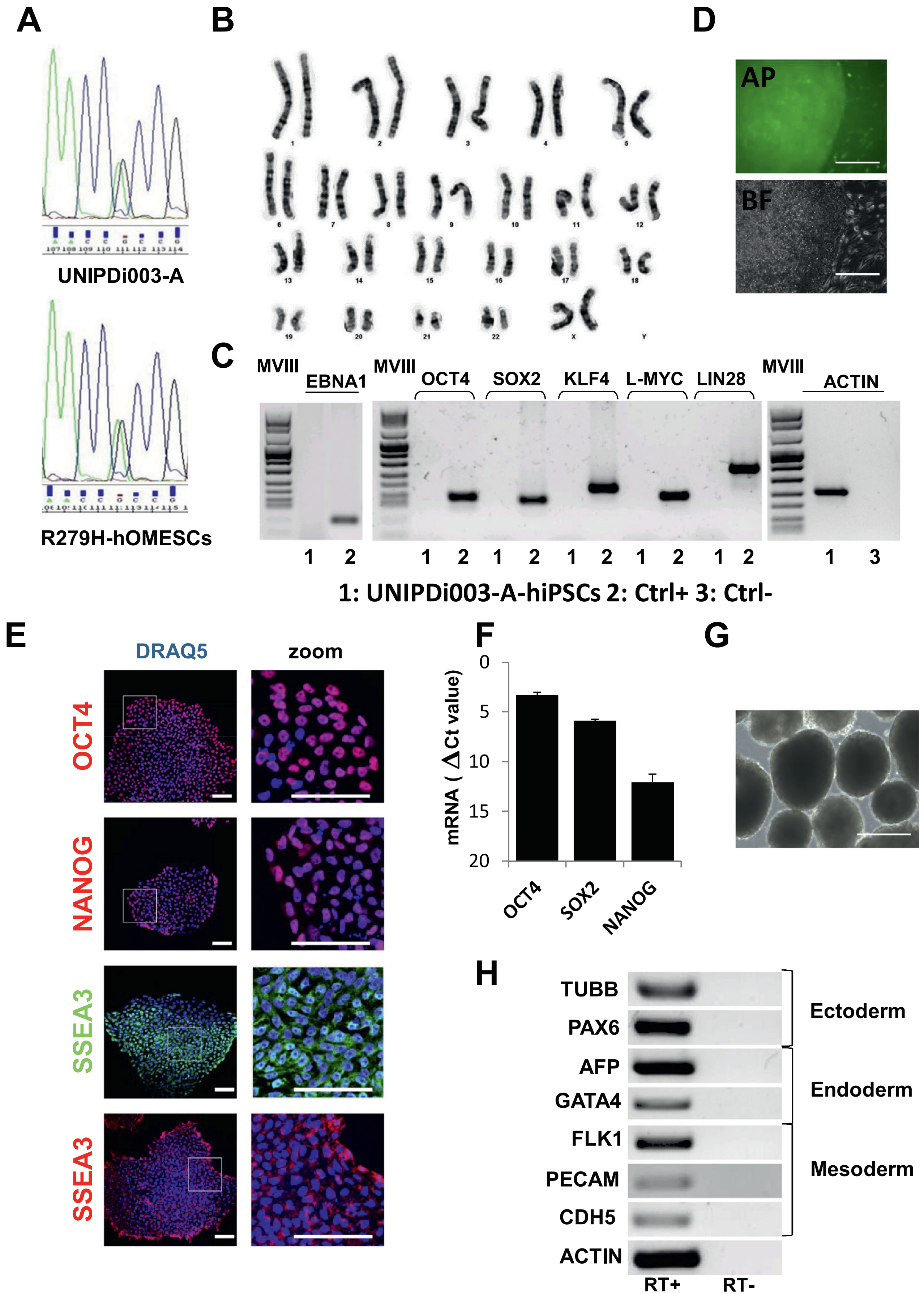


**Fig. 1.** Characterization of UNIPDi003-A-hiPSC line.

LIN28) in UNIPDi003-A-hiPSCs was confirmed by RT-PCR at passage 20 (Fig. 1C). Moreover, no EBNA1 sequences could be detected in the cell line by PCR (Fig. 1C). UNIPDi003-A-hiPSCs maintained a normal karyotype in long term culturing (passage 68, Fig. 1B) and expressed the undifferentiated state marker alkaline phosphatase (AP; Fig. 1D; BF: bright field; scale bar = 100 μm). UNIPDi003-A-hiPSC line expressed several pluripotency markers, as assessed by indirect immunofluorescence (OCT4, SSEA3, SSEA and Nanog; Fig. 1E; scale bars = 100 μm), and by qRT-PCR (OCT4, NANOG and SOX2; Fig. 1F). UNIPDi003-A-hiPSCs formed embryoid bodies (EBs) in vitro (Fig. 1G; scale bar = 200 μm), confirming the potential to give rise to the derivatives of the three germ layers, as showed by the expression by RT-PCR of ectodermal (TUBB and PAX6), endodermal (AFP and GATA4) and mesodermal (FLK1, PECAM and CDH5) markers (Fig. 1H).

## Materials and methods

### *Cell cultures*

R279H-hOMESCs were isolated from a fresh oral mucosa biopsy of a patient affected by EEC syndrome. Cells were grown as previously described (Barbaro et al., 2016). UNIPDi003-A-hiPSC were grown on irradiated mouse embryonic fibroblasts (MEFs) in human Embryonic Stem (hES) cell medium (Table 1).

### *Reprogramming of R279H-hOMESCs by episomal vectors nucleofection*

R279H-hOMESCs ($10^6$ cells) were re-suspended in 100 μl of P3 solution (Lonza, Switzerland) with 1 μg of each of the episomal plasmids pCXLE-hOCT3/4, pCXLE-hSK and pCXLE-hUL (Addgene plasmids: #27077, #27078, #27080). The solution was nucleofected with the 4D Nucleofector device (Lonza) as described in (Barbaro et al., 2016). Transfected cells were then seeded on dishes previously coated with MEFs in Keratinocyte growth medium (KGM). The medium was changed every other day for one week gradually switching from KGM to hES medium. From day 7 on, hES medium was changed on a daily basis.

### *Sequencing analysis*

TP63 gene exon 7 was amplified from DNA isolated from EEC-A-hiPSCs and R279H-hOMESCs by PCR (Table 2) and used for Sanger sequencing reaction.

### *Karyotype analysis*

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

### *Residual presence of episomal plasmids and transgene expression analysis by PCR and RT-PCR*

DNA isolated from UNIPDi003-A-hiPSCs was used for the analysis of the presence of residual EBNA1 sequences by PCR as previously described (Okita et al., 2011). For residual transgenes expression analysis, total RNA was isolated from UNIPDi003-A-hiPSCs (passage 20) and reverse transcribed. cDNAs were used to amplify the transgenes of interest (OCT4, SOX2, KLF4, l-MYC and LIN28; Table 2) by RT-PCR (Okita et al., 2011).

### *Alkaline phosphatase live staining assay*

The UNIPDi003-A-hiPSC line was incubated for 30 min at RT with 1× AP Live Stain Solution (Thermo Fisher Scientific, USA). Live cells were observed under a Leica DFC420 inverted epifluorescence microscope (Leica, Germany).

### *Pluripotency marker expression analysis by qRT-PCR*

cDNA previously reverse transcribed from UNIPDi003-A-hiPSCs was used to amplify by qRT-PCR pluripotency-associated genes (i.e. OCT4, NANOG and SOX2; Table 2).

### *Pluripotency marker expression analysis by immunofluorescence*

UNIPDi003-A-hiPSCs growing on MEFs were fixed in 4% paraformaldehyde (Sigma Aldrich, USA) for 20 min at RT and treated with PBS/0.1% Triton X-100 (Sigma Aldrich) for 15 min at RT. Cells were subsequently incubated at 4 °C overnight with 4% BSA/PBS (Sigma Aldrich). The next day, primary antibodies (Table 2) were added and incubated for 1 h at RT and cells were incubated for 1 h at RT with secondary antibodies (Table 2). Finally, cells were stained with DRAQ5 5 μM (Thermo Fisher Scientific) for 10 min at RT.

### *Embryoid bodies test*

The UNIPDi003-A-hiPSC line growing on MEFs was forced to grow for 7 days in suspension in hES medium depleted of bFGF. Cells were then seeded on 0.1% gelatine-coated plates (Merck Millipore, USA) in DMEM medium supplemented with 10% FBS (Gibco, Thermo Fisher

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | *Normal* | Fig. 1D |
| Phenotype | Qualitative analysis | *Expression of pluripotency markers: OCT4, SSEA3, SSEA4, Nanog* | Fig. 1, panel E |
| | Quantitative analysis (qPCR) | *OCT4, NANOG and SOX2* | Fig. 1, panel F |
| | Immunocytochemistry counting | *Oct4: 99%; Nanog: 98%; SSEA4: 96%; SSEA3: 99%* | |
| Genotype | Karyotype (G-banding) and resolution | *46XX, Resolution 450–500* | Fig. 1, panel B |
| Identity | Microsatellite PCR (mPCR) OR | *DNA Profiling: N/A* | N/A |
| | STR analysis | *15 sites tested, all matched* | Fig. S2; submitted in archive with journal |
| Mutation analysis (if applicable) | Sequencing | *Heterozygous, R279H* | Fig. 1, panel A |
| | Southern Blot OR WGS | *N/A* | |
| Microbiology and virology | Mycoplasma | *Mycoplasma testing by PCR: Negative* | Fig. S1 |
| Differentiation potential | Embryoid body formation | *RT-PCR of ectodermal (TUBB and PAX6), endodermal (AFP and GATA4) and mesodermal (FLK1, PECAM and CDH5) markers* | Fig. 1, panels G and H |
| 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* | |

**Table 2**
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

| | Antibody | Dilution | Company Cat # and RRID |
|---|---|---|---|
| Pluripotency markers | - Goat anti-OCT3/4 [N-9] | 1:200 | - Santa Cruz Cat#: sc-8628, RRID:AB_653551 |
| | - Rat anti-SSEA3 [MC631] | 1:50 | - Abcam Cat#: ab16286, RRID:AB_882700 |
| | - 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 |
| Differentiation Markers | N/A | N/A | N/A |
| Secondary antibodies | - Goat anti-Rat IgG H&L (FITC) | 1:250 | - Abcam Cat#: ab6840, RRID:AB_955326 |
| | - 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 |

Primers

| | Target | Forward/reverse primer (5′–3′) |
|---|---|---|
| Episomal plasmids | OCT4 plasmid | CAT TCA AAC TGA GGT AAG GG<br>TAG CGT AAA AGG AGC AAC ATA G |
| | KLF4 plasmid | CCA CCT CGC CTT ACA CAT GAA GA<br>TAG CGT AAA AGG AGC AAC ATA G |
| | SOX2 plasmid | TTC ACA TGT CCC AGC ACT ACC AGA<br>TTT GTT TGA CAG GAG CGA CAA T |
| | l-MYC plasmid | GGC TGA GAA GAG GAT GGC TAC<br>TTT GTT TGA CAG GAG CGA CAA T |
| | LIN28 plasmid | AGC CAT ATG GTA GCC TCA TGT CCG C<br>TAG CGT AAA AGG AGC AAC ATA G |
| | EBNA1 | ATC AGG GCC AAG ACA TAG AGA TG<br>GCC AAT GCA ACT TGG ACG TT |
| Pluripotency markers (qPCR) | OCT4 | GGTGCCTGCCCTTCTAGGAATGGG GGA<br>CAAAAACCCTGGCACAAACTC<br>Probe: CTAGGGAAAGAAAACC |
| | SOX2 | GAGAAGTTTGAGCCCCAGG<br>AGAGGCAAACTGGAATCAGG<br>Probe: ATCATCGGCGGCGGCA |
| | NANOG | GGTCTCGATCTCCTGACCTTGT<br>GCCTGTAAATCCCAGCTGTTAGG<br>Probe: TCCACCCGCCTCGG |
| House-keeping genes (qPCR) | ACTIN | CGGGACCTGACTGACTACCTC<br>CCATCTCTTGCTCGAAGTCCAG<br>Probe: TCCTTAATGTCACGCACGATT TCCCGCT |
| Differentiation markers (RT-PCR) | TUBB | CAGATGTTCGATGCCAAGAA<br>TGCTGTTCTTGCTCTGGATG |
| | PAX6 | TCTAATCGAAGGGCCAAATG<br>TGTGAGGGCTGTGTCTGTTC |
| | AFP | AGCTTGGTGGTGGATGAAAC<br>CCCTCTTCAGCAAAGCAGAC |
| | GATA4 | CTAGACCGTGGGTTTTGCAT<br>TGGGTTAAGTGCCCCTGTAG |
| | FLK1 | AGTGATCGGAAATGACACTGGA<br>GCACAAAGTGACACGTTGAGAT |
| | PECAM | CCCAGCCCAGGATTTCTTAT<br>ACCGCAGGATCATTTGAGTT |
| | CDH5 | CAGCCCAAAGTGTGTGAGAA<br>TGTGATGTTGGCCCGTGTTAT |
| Genotyping | TP63 exon 7 | GGGAAGAACTGAGAAGGAACAAC<br>CAGCCACGATTTCACTTTG |
| Targeted mutation analysis/sequencing | N/A | |

Scientific). After 7 days cells were harvested and total RNA was isolated and reverse transcribed. cDNAs were used as template to amplify genes (Table 2) belonging to the three germ layers ectoderm (TUBB, PAX6), endoderm (AFP, GATA4) and mesoderm (FLK1, PECAM, CDH5).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.02.008.

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