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Lab resource: Stem Cell Line

Establishment and characterization of induced pluripotent stem cell (iPSCs) line UNIBSi014-A from a healthy female donor

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A B S T R A C T

Peripheral blood mononuclear cells (PBMCs) derived from a healthy 40-year-old female were successfully transformed into induced pluripotent stem cells (iPSCs) by using the integration-free CytoTune-iPS Sendai Reprogramming method. The resulting iPSCs line exhibits a normal karyotype, expresses stemness markers and displays the differentiation capacity into the three germ layers. This human iPSCs line can be used as healthy control in disease modelling studies.

1. Resource table

Unique stem cell line identifier	UNIBSi014-A
Alternative name(s) of stem cell line	C1MD14
Institution	Department of Molecular and Translational Medicine, University of Brescia, 25,123 Brescia, Italy
Contact information of distributor	Chiara Fiorentini, chiara.fiorentini@unibs.it
Type of cell line	iPSCs
Origin	Human
Additional origin info	Age: 40 Sex: Female Ethnicity: Caucasian
Cell Source	PBMCs
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific), expressing the four Yamanaka factors Oct4, Sox2, Klf4, and c-Myc
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Healthy control
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	Dec 2020
Cell line repository/bank	https://hpscreg.eu/cell-line/UNIBSi014-A
Ethical approval	Before samples collection, the healthy control has previously signed the informed consent, in turn approved by local ethics committee (CEIO IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli N.50/2008).

2. Resource utility

The UNIBSi014-A iPSCs line was generated from a healthy woman enrolled as control subject in a treatment-resistant depression (TRD) study (Minelli et al., 2015). Moreover, iPSCs-derived neuronal and non-neuronal cells could be a useful tool for examine the properties of any human protein in its physiological environment (Bono et al., 2018, 2019).

3. Resource details

UNIBSi014-A human iPSCs (hiPSCs) line was obtained by reprogramming PBMCs of a 40-year-old healthy woman (Table 1). The donor represents a control subject enrolled for a project focused on the molecular abnormalities underlying TRD in order to identify novel and personalized targets for antidepressant therapies. Two hiPSCs lines

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal morphology	Fig. 1, panel A
Phenotype	Qualitative analysis of immunofluorescence staining	Positive immunostaining of pluripotency markers: OCT4; SOX2; SSEA4; TRA-1–60.	Fig. 1, panel B
	Quantitative analysis (RT-qPCR)	Positive for the expression of three pluripotency markers, assessed by qPCR (OCT4, SOX2, NANOG).	Fig. 1, panel C
Genotype	Karyotype (Q-banding) and resolution	Normal karyotype: 46, XX (400–450 banding resolution)	Fig. 1, panel E
Identity	STR analysis: GenePrint® 10 System, PROMEGA	20 markers tested with 100% match	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR analysis: Negative	Fig. 1, panel F
Differentiation potential	Trilineage in vitro differentiation	Expression of markers specific for each germ layer was detected by qPCR upon directed differentiation, as follows: - for the mesoderm layer: MSX1, RUNX1, ACTA2. - for the endoderm layer: SOX17, GATA4, GATA6. -for ectoderm layer: PAX6, TUBB3.	Fig. 1, panel D
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

derived from female TRD patients (UNIBSi012-A and UNIBSi013-A) have been previously obtained and characterized, and published earlier (Bono et al., 2020). The donor has been screened for DSM-IV Axis I disorders diagnoses using the Mini-International Neuropsychiatric Interview (M.I.N.I.) and she is without history of drug or alcohol abuse or dependence and without a personal or first-degree family history of psychiatric disorders.

PBMCs were isolated from peripheral blood samples using Ficoll-Paque (GE Healthcare Life Sciences) and SepMate tubes (StemCell Tech), according to the manufacturer's protocols, and cryopreserved in liquid nitrogen. The generation of the hiPSC line UNIBSi014-A was carried out using non-integrative Sendai virus containing the human reprogramming factors, Oct4, Sox2, C-Myc and Klf4 in a feeder-free condition. After 30 days, multiple hiPSCs lines were generated as colonies with embryonic stem cells (ESC) morphology, and one line, UNIBSi014-A (Fig. 1, panel A) was further expanded and characterized, as reported in Table 1. Cell line authentication was investigated by performing short tandem repeat (STR) analyses showing an identical DNA profile at 20 polymorphic loci with the parental PBMCs (data available upon request).

UNIBSi014-A hiPSCs pluripotency was first assessed by immunocytochemistry using the Fluorescent Human ES/iPS cell Characterization Kit (Millipore, Billerica, MA, USA), following manufacturer's directions. We found that UNIBSi014-A hiPSCs clone uniformly expressed the nuclear stem cell markers OCT4 and SOX2 (Fig. 1, panel B), as well as the surface markers (TRA)-1-60 and SSEA-4 (Fig. 1, panel B). Pluripotency was further assessed by using quantitative real-time PCR (qPCR), showing that in the newly generated hiPSCs clone the expression of endogenous OCT4, SOX2, and Nanog was comparable to that a commercial certificated control hiPSCs line (CTRL-hiPSCs, Gibco® Episomal hiPSCs Line, Cat#A18945) and significantly higher than those measured in the parental PBMCs, (Fig. 1, panel C). Moreover, loss of exogenous expression of the of KLF4, KOS and C-MYC reprogramming factors as well as of SeV was also confirmed by end point PCR analyses (Supplementary Fig. 1). The UNIBSi014-A hiPSCs line was able to form embryoid bodies (EBs) and spontaneously differentiate into cells of the three germ layers, as confirmed by qPCR analysis for mesodermal (MSX1, RUNX1 and ACTA2), endodermal (SOX17, GATA4 and GATA6) and ectodermal (PAX6 and TUBB3) markers (Fig. 1, panel D). The genome integrity of UNIBSi014-A line has been defined by using Q-band karyotyping showing that this hiPSCs line displayed a normal female karyotype (Fig. 1, panel E). Additionally, in the UNIBSi014-A hiPSCs clone no trace of mycoplasma has been detected (Fig. 1, panel F).

4. Materials and methods

4.1. Reprogramming of peripheral blood mononuclear cells (PBMCs)

Ficoll-sodium diatrizoate centrifugation procedure (Ficoll-PaqueTM PLUS, GE Healthcare Life Science) was used to isolate peripheral blood mononuclear cells (PBMCs) from whole blood samples. Before transduction procedures, PBMCs were cultured for 4 days in StemProR-34 (Gibco™, Termo Fisher Scientific) medium supplemented with cytokine cocktail StemSpan 100X (STEMCELL Technologies). Then, hiPSCs were generated by using CytoTune iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), following the manufacturer's guideline protocol. Emerging single colonies were manually picked 20 days post-transduction, seeded on Matrigel coated plates and cultured at 37 °C with 5% CO2. Cells were daily refreshed with the StemFlex™ Medium (Gibco™, Termo Fisher Scientific; cat. no.: A3349401). Every 4–6 days (60–70% confluence), cells were passaged at a ratio of 1:5 using ReLeSR™ (Stemcell technologies, cat. no.: 05872).

4.2. Karyotype analysis

UNIBSi014-A hiPSCs karyotyping was performed at passages (p) 15. Quite confluence hiPSCs were blocked at metaphase by Colcemid Solution (IrvineScientific). Cells were then detached with trypsin (Sigma Aldrich), swollen by exposure to hypotonic solution and fixed with methanol/glacial acetic acid (3:1). Analyses was performed by using Q-banding at 450 bands resolution, according to the International System for Human Cytogenetic Nomenclature (ISCN 2016). At least 50 metaphase plates were analyzed and karyotyped using a chromosome imaging analyzer software (Chromowin software, Tesi Imaging).

4.3. Immunofluorescence

For immunofluorescence analyses, the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Thermo Fischer Scientific) was used. hiPSCs (p15), seeded on glass coverslips in 24-well plates, were cultured until reaching the 60–70% confluence. Cells were then fixed, permeabilized and blocked, according to the manufacturer's protocol

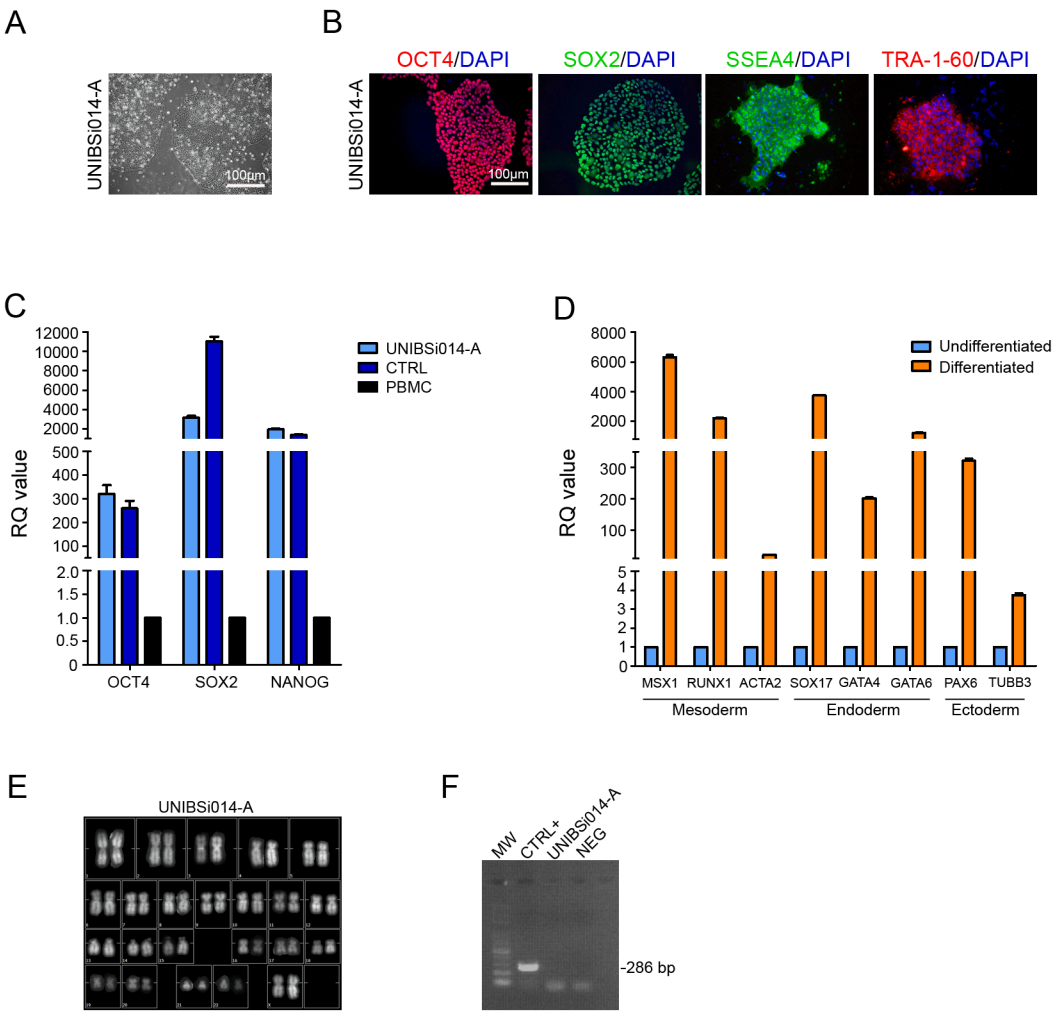

Fig. 1. A. hiPSCs morphology B. Stemness markers staining by immunofluorescence: OCT4 (red), SOX2 (green), SSEA4 (green), TRA-1–60 (red) and nuclei staining with DAPI; C. Pluripotency markers analysis by qPCR; D. Expression analysis of three germ layers markers by qPCR; E. Karyotype analyses; F. Mycoplasma detection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and incubated with primary antibodies (Abs; Table 2) over-night (O.N.) at 4 °C. Incubation with the appropriate secondary Abs (Table 2) was performed for 1 h at room temperature (RT). Nuclei were counterstained with DAPI. Digital images were captured with an Olympus IX51 microscope connected to an Olympus digital camera. Analyses was performed by using the ImageJ software (NIH, Bethesda, MD, USA).

4.4. Mycoplasma detection

Supernatants from UNIBSi014-A hiPSCs line were collected and analyzed by using the N-GARDE Mycoplasma PCR Reagent set (Euroclone), according to the manufacturer's instructions.

4.5. STR analysis

NucleoSpin® Tissue, Macherey-Nagel was used to extract genomic DNAs from both hiPSCs (p15), and the parental PBMCs. The subsequent analyses were performed by using the GenePrint® 10 System (PROMEGA), following the manufacturer's guideline.

4.6. In vitro embryoid body formation

To obtain embryoid bodies (EBs), cells were plated on ultra-low adhesion culture plates and cultured with EB medium (DMEM/F12 supplemented with 20% FBS) for 14 days. Medium was refreshed every other day. At day 14, EBs were processed for RNA isolation and trilineage differentiation potential assessed by qPCR.

4.7. Gene expression analyses

Total RNA was isolated from hiPSCs (p15) and EBs by using the Tri Reagent (Sigma). cDNA was synthetized using a High-Capacity cDNA RT kit (Life Technologies), from 1 μg of total RNA, following the manufacturer's instructions. Quantitative PCR (qPCR) was performed using Sybr®Green Master Mix (Bio-Rad) and 20 ng of cDNA, in triplicate for each gene, and run on ViiA7 instrument (Applied Biosystems). The following conditions were used: 95 °C for 10 min for 1 cycle, 95 °C for 15 s and 60 °C for 1 min for 40 cycles. The relative quantification (RQ value) of target genes was calculated by the 2^− ΔΔCt method, using GAPDH as housekeeping gene. Primers sequences are listed in Table 2.

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:200	Thermo Fisher Scientific Cat# A24867, RRID: AB_2650999
Pluripotency Markers	Rat anti-SOX2	1:100	Thermo Fisher Scientific Cat# A24759, RRID: AB_2651000
Pluripotency Markers	Mouse anti-SSEA4 (IgG3)	1:200	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Pluripotency Markers	Mouse anti-TRA-1–60 (IgM)	1:100	Thermo Fisher Scientific Cat# A24868, RRID: AB_2651002
Secondary antibody	Alexa Fluor 555 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A24869, RRID: AB_2651006
Secondary antibody	Alexa Fluor 488 donkey anti-rat	1:250	Thermo Fisher Scientific Cat# A24876, RRID: AB_2651007
Secondary antibody	Alexa Fluor 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877, RRID: AB_2651008
Secondary antibody	Alexa Fluor 555 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A24871, RRID: AB_2651009
Primers			
Transgenes Primers for endpoint PCR	Target	Forward/Reverse primer (5′-3′)	
Vector detection	KOS transgene	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG	
Vector detection	C-MYC transgene	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
Vector detection	KLF4 transgene	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
Vector detection	SeV transgene	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Pluripotency Primers for RT-qPCR with SYBR Green chemistry	Target	Forward/Reverse primer (5′-3′)	
Pluripotency Markers	OCT4	GACAGGGGGAGGGGAGGAGCTAGG/ CTTCCCTCCAACCAGTTGCCCCAAAC	
Pluripotency Markers	SOX2	GGGAAATGGGAGGGGTGCAAAAGAGG/ TTGCGTGAGTGTGGATGGGATTGGTG	
Pluripotency Markers	NANOG	TGCAAGAACTCTCCAACATCCT/ ATTGCTATTCTTCGGCCAGTT	
Trilineage Primers for RT-qPCR with SYBR Green chemistry	Target	Forward/Reverse primer (5′-3′)	
Endoderm marker	SOX17	CTCTGCCTCCTCCACGAA/ CAGAATCCAGACCTGCACAA	
Endoderm marker	GATA4	CTAGACCGTGGGTTTTGCAT/ TGGGTTAAGTGCCCCTGTAG	
Endoderm marker	GATA6	ACCACCTTATGGCGCAGAAA/ ATAGCAAGTGGTCTGGGCAC	
Ectoderm marker	PAX6	GTCCATCTTTGCTTGGGAAA/ TAGCCAGGTTGCGAAGAACT	
Ectoderm marker	TUBB3	GCTCAGGGGCCTTTGGACATCTCTT/ TTTTCACACTCCTTCCGCACCACATC	
Mesoderm marker	MSX1	CGAGAGGACCCCGTGGATGCAGAG/ GGCGGCCATCTTCAGCTTCTCCAG	
Mesoderm marker	RUNX1	CCCTAGGGGATGTTCCAGAT/ TGAAGCTTTTCCCTCTTCCA	
Mesoderm marker	ACTA2	CCTATCCCCGGGACTAAGAC/ AGGCAGTGCTGTCCTCTTCT	
Housekeeping gene	GAPDH	AGGTCGGAGTCAACGGATTT/ ATCTCGCTCCTGGAAGATGG	

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102216.

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