Stem Cell Research 49 (2020) 102104

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell lines, UNIBSi012-A and UNIBSi013-A, from two patients with treatment-resistant depression

Federica Bono [a], Veronica Mutti [a], Giovanna Piovani [b], Alessandra Minelli [b,c], Jessica Mingardi [b], Adele Guglielmi [a], Chiara Fiorentini [a], Alessandro Barbon [b], Cristina Missale [a], Massimo Gennarelli [b,c,*]

[a] Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
[b] Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
[c] Genetic Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

A B S T R A C T

Novel and complementary experimental models are required for investigating the molecular mechanisms underlying the resistance to the available therapies of patients with major depression (Treatment-Resistant Depression, TRD) that occurs in at least one third of patients and need to be deeply investigated. Here, we have established a patient-specific disease model for TRD by reprogramming peripheral blood mononuclear cells (PBMCs) from two TRD patients into induced pluripotent stem cells (iPSCs), using non-integrating Sendai virus. These lines show the typical morphology of pluripotent cells, express pluripotency markers and display~~ed~~ in vitro differentiation potential toward cells of the three embryonic germ layers.

1. Resource Table

Unique stem cell lines identifier	UNIBSi012-A UNIBSi013-A
Alternative names of stem cell lines	P1MD40 (UNIBSi012-A) P2MD06 (UNIBSi013-A)
Institution	Department of Molecular and Translational Medicine, University of Brescia, 25,123 Brescia, Italy
Contact information of distributor	Massimo Gennarelli, massimo.gennarelli@unibs.it
Type of cell lines	iPSCs
Origin	Human
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
Multiline rationale	Non-isogenic cell lines obtained from patients with the same clinical condition
Gene modification	NO
Type of modification	N/A
Associated disease	Treatment-Resistant Depression (TRD)
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	Jun 2020
Cell line repository/bank	https://hpscreg.eu/cell-line/UNIBSi012-A https://hpscreg.eu/cell-line/UNIBSi013-A
Ethical approval	Before samples collection, all the patients involved in this study have previously signed the informed consent, in turn approved by local ethics committee (Ethics Committee of the Province of Verona N: 4997/09.11.01).

2. Resource utility

TRD is a clinical condition that affects about 30% of patients treated for major depression. Generation of human iPSCs (hiPSCs) offers a great opportunity to study viable patient-derived neurons with the goal of understanding the molecular mechanisms underlying TRD and identifying novel targets for antidepressant innovative therapies.

3. Resource details

Major depressive disorder (MDD) is the most common psychiatric

* Corresponding author at: Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy. Genetic Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy
E-mail address: massimo.gennarelli@unibs.it (M. Gennarelli).

https://doi.org/10.1016/j.scr.2020.102104
Received 3 September 2020; Received in revised form 18 November 2020; Accepted 27 November 2020
Available online 2 December 2020

disorder affecting several people worldwide (Hasin et al., 2018). Despite the availability of different classes of antidepressants, about 30% MDD patients do not achieve a complete remission of symptoms and they are classified as having treatment-resistant depression (TRD). TRD is a serious and dangerous situation causing suffering for patients and their families and pushing up costs for healthcare services (Thomas et al., 2013). Unfortunately, to date, the mechanisms underpinning TRD are poorly understood also due the absence of adequate "in vitro" and "in vivo" models for investigating its molecular and cellular basis. Taking advantage of hiPSCs technology, we developed a very promising tool that will allow to identify the molecular abnormalities underlying TRD in each individual patient and will help to identify novel and personalized targets for antidepressant therapies.

Here, hiPSCs were generated from two patients affected by TRD (Table 1). Peripheral blood mononuclear cells (PBMCs) were collected from whole blood samples and reprogrammed into hiPSCs by using the CytoTune-iPS 2.0 Sendai Reprogramming Kit, based on a non-integrating form of Sendai virus (SeV) carrying the Yamanaka's factors OCT4, SOX2, KLF4, and c-MYC in a feeder-free condition. At least three hiPSCs clones were generated from each patient; UNIBSi012-A and UNIBSi013-A clones, reported in this work, were randomly selected and characterized, as reported in Table 2.

As shown in Fig. 1A, hiPSCs lines exhibited an embryonic stem cell (ESC)-like morphology, characterized by a typical round shape morphology with small, tightly packed cells with a high nucleus/cytoplasm ratio and prominent nucleoli. Moreover, in immunofluorescence experiments, UNIBSi012-A and UNIBSi013-A expressed both the nuclear pluripotency markers OCT4 and SOX2 and the cell-specific surface antigens SSEA-4 and TRA-1–60 (Fig. 1B). UNIBSi012-A and UNIBSi013-A clones pluripotency was also tested by TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis, confirming the upregulation of endogenous pluripotency genes (Fig. 1C and Supplementary Fig. 1A). Moreover, after 10 passages in vitro, both hiPSCs lines were negative for KLF4, KOS and C-MYC transgenes as well as for SeV, as indicated by end point PCR analyses (Supplementary Fig. 1B).

The UNIBSi012-A and UNIBSi013-A potential three-germ-layer differentiation was assessed by the TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis. Each line showed a negative score for self-renewal gene expression and a positive score for the expression of genes involved in ectodermal, mesodermal, and endodermal formation (Fig. 1D-E and Supplementary Fig. 1A).

The genome integrity of UNIBSi012-A and UNIBSi013-A lines has been defined by using Q-band karyotyping showing that each hiPSCs line displayed a normal female karyotype (Fig. 1F). Moreover, the STR analysis proved the genetic match between the hiPSCs and the original PBMCs (Table 2, available with the authors). Both UNIBSi012-A and UNIBSi013-A clones were mycoplasma-free (Fig. 1G).

In conclusion, hiPSCs lines from two TRD patients, generated and fully characterized, may be a useful "in vitro" model for deepening the mechanisms responsible for antidepressants resistance in MDD pathology.

Table 2
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: TaqMan® Human Pluripotent Stem Cell Scorecard™ analysis	Positive score for self-renewal gene expression and a negative score for ectodermal, mesodermal, and endodermal gene expression.	Fig. 1, panel C
Genotype	Karyotype (G-banding) and resolution	Normal karyotype: 46, XX for all the two iPSCs lines (400–450 banding resolution)	Fig. 1, panel F
Identity	STR analysis: GenePrint® 10 System, PROMEGA	20 markers tested with 100% match	Available with the authors
Mutation analysis	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 G
Differentiation potential	Trilineage in vitro differentiation	TaqMan® hPSC Scorecard™ analysis: negative score for self-renewal gene expression and positive score for trilineage gene expression	Fig. 1, panel D and E
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

4. Materials and methods

4.1. Reprogramming of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples from two TRD patients using Ficoll-sodium diatrizoate centrifugation procedure (Ficoll-PaqueTM PLUS, GE Healthcare Life Science) and cultured in StemProR-34 (Gibco™, Termo Fisher Scientific) medium supplemented with cytokine cocktail StemSpan 100X (STEMCELL Technologies) for four days before transduction. hiPSCs were generated by using CytoTune iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), following the manufacturer's guideline protocol. Single colonies were manually picked 21 days post-transduction, seeded onto Matrigel-coated plates (Corning; cat. no: 354277) and cultured in StemFlex™ Medium (Gibco™, Termo Fisher Scientific; cat. no.: A3349401). The iPSCs were then amplified in StemFlex™ Medium on Matrigel coated plates at 5% CO2 and 37 °C. Cells were passaged using ReLeSR™ (Stemcell technologies; cat. no.: 05872) every 4–6 days (60–70% confluency) at a ratio of 1:5.

4.2. Karyotype analysis

Genomic stability was assessed by karyotyping performed at passages (p) 15 for both lines. Briefly, quite confluence hiPSCs were blocked at metaphase by Colcemid Solution (IrvineScientific), then detached with trypsin (Sigma Aldrich), swollen by exposure to hypotonic solution

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNIBSi012-A	UNIBSi012-A	*Female*	*45*	*Caucasian*	*N/A*	*TRD*
UNIBSi013-A	UNIBSi013-A	*Female*	*59*	*Caucasian*	*N/A*	*TRD*

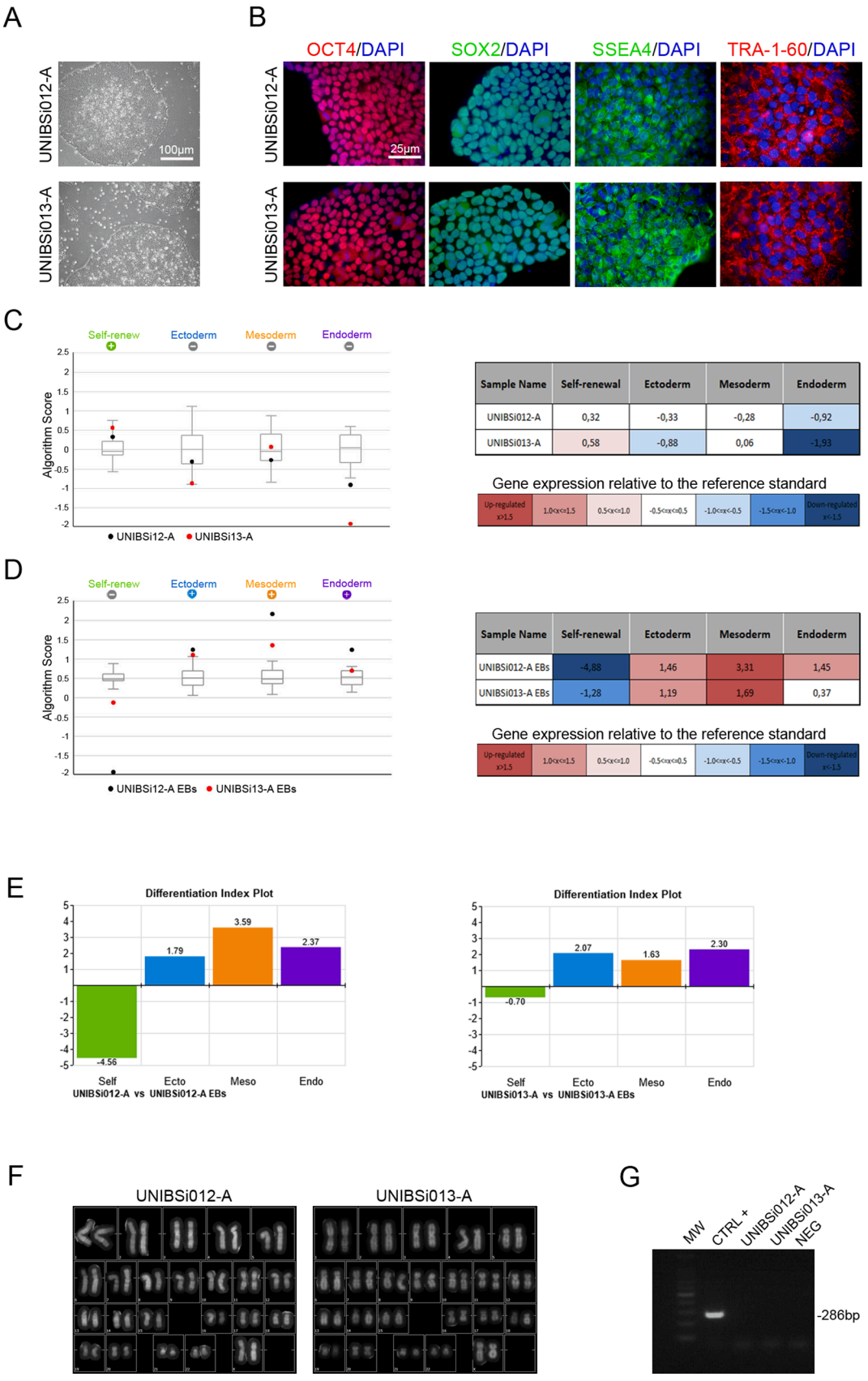

Fig. 1. A. UNIBSi012-A and UNIBSi013-A hiPSCs morphology B. Stemness markers staining by immunofluorescence: Oct4 (red), SOX2 (green), SSEA4 (green) and TRA-1-60 (red); nuclei staining with DAPI (blu). C. UNIBSi012-A and UNIBSi013-A hiPSCs score graph and the score table for pluripotency markers. D. UNIBSi012-A and UNIBSi013-A hiPSCs score graph and the score table for trilineage differentiation capability E. Differentiation Index Plot of each EB-derived hiPSCs vs the corresponding hiPSCs line. F. UNIBSi012-A and UNIBSi013-A hiPSCs Karyotype analysis. G. Mycoplasma detection.

and fixed with methanol/glacial acetic acid (3:1). Cytogenetic analysis was performed using Q-banding at 450 bands resolution, according to the International System for Human Cytogenetic Nomenclature (ISCN 2016). A minimum of 50 metaphase spreads were analyzed for each sample and karyotyped using a chromosome imaging analyzer system.

4.3. Immunofluorescence

hiPSCs phenotypic characterization was carried out using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Thermo Fischer Scientific). Briefly, hiPSCs were seeded and cultured on glass coverslips in 24-well plates. When they reached the 60–70% confluence, hiPSCs colonies were fixed, permeabilized, and blocked using the solutions supplied by kit. The primary antibodies (Abs; Table 3) incubation was performed over-night (O.N.) at 4 °C, followed by secondary Abs (Table 3) incubation for 1 h at room temperature (RT). Nuclei were counterstained with DAPI. Digital images of the immunofluorescence staining were captured with an Olympus IX51 microscope connected to an Olympus digital camera and analyzed using ImageJ software (NIH, Bethesda, MD, USA).

4.4. Mycoplasma detection

The absence of mycoplasma contamination was evaluated in the supernatant of confluent cell cultures by PCR analysis using the N-GARDE Mycoplasma PCR Reagent set (Euroclone).

4.5. STR analysis

DNAs from parental PBMCs and hiPSCs lines were extracted using the NucleoSpin® Tissue, Macherey-Nagel. Lines were authenticated by GenePrint® 10 System (PROMEGA) following the manufacturer's guideline.

4.6. In vitro embryoid body formation

Embryoid bodies (EBs) formation was performed as previously described (Bono et al., 2018). Briefly, cells were cultured in ultra-low adhesion culture plates with EB medium (DMEM/F12 supplemented with 20% FBS), with medium change performed every other day, for 14 days. Emergent EBs were then processed for RNA isolation.

4.7. Quantitative RT-PCR (qPCR) and Scorecard analysis

Total RNA was extracted from hiPSCs and EBs pellets using Tri Reagent (Sigma). Eight cDNA reactions were set up from 1 μg of total RNA per sample using a High-Capacity cDNA RT kit (Life Technologies) and following manufacturer's instructions. qPCR was performed on TaqMan® Human Pluripotent Stem Cell Scorecard™ Panel, 96-well Fast (Life Technologies) plates using Viia7 RUO software and a ViiA7 instrument (Applied Biosystems). Data were analyzed through TaqMan® hPSC Scorecard™ Analysis Software through Life Technologies website.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102104.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	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 (528 bp)	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG	
Vector detection	C-MYC transgene (532 bp)	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
Vector detection	KLF4 transgene (410 bp)	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
Vector detection	SeV transgene (181 bp)	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Housekeeping gene	GAPDH (230 bp)	AGGTCGGAGTCAACGGATTT/ ATCTCGCTCCTGGAAGATGG	

References

Bono, F., Savoia, P., Guglielmi, A., Gennarelli, M., Piovani, G., Sigala, S., Leo, D., Espinoza, S., Gainetdinov, R.R., Devoto, P., Spano, P., Missale, C., Fiorentini, C., 2018. Role of Dopamine D2/D3 Receptors in Development, Plasticity, and Neuroprotection in Human iPSC-Derived Midbrain Dopaminergic Neurons. Mol. Neurobiol. 55 (2), 1054–1067. https://doi.org/10.1007/s12035-016-0376-3.

Hasin, D.S., Sarvet, A.L., Meyers, J.L., Saha, T.D., Ruan, W.J., Stohl, M., Grant, B.F., 2018. Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. JAMA Psychiatr. 75 (4), 336. https://doi.org/10.1001/jamapsychiatry.2017.4602.

Thomas, L., Kessler, D., Campbell, J., Morrison, J., Peters, T.J., Williams, C., Lewis, G., Wiles, N., 2013. Prevalence of treatment-resistant depression in primary care: cross-sectional data. Br. J. Gen. Pract. 63 (617), e852–e858.