Stem Cell Research 49 (2020) 102051

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of iPSC lines from peripheral blood mononuclear cells of identical twins both suffering from type 2 diabetes mellitus and one of them additionally diagnosed with atherosclerosis

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A B S T R A C T

Here we describe the generation of induced pluripotent stem cell (iPSC) lines from peripheral blood samples of identical twin sisters with type 2 diabetes mellitus (DM2). Two clonal lines from each patient (HU-DM2-A-1, HU-DM2-A-2 and HU-DM2-B-1, HU-DM2-B-2) were established via Sendai viral reprograming of peripheral blood mononuclear cells, and characterized to confirm pluripotency and genetic integrity. The established iPSC lines can help to investigate DM2 related cellular phenotypes and provide a model system for drug testing.

1. Resource Table

Unique stem cell lines identifier	*RCNSi001-A* *RCNSi001-B* *RCNSi002-A* *RCNSi002-B*
Alternative names of stem cell lines	*HU-DM2-A-1* *HU-DM2-A-2* *HU-DM2-B-1* *HU-DM2-B-2*
Institution	1. Research Center for Natural Sciences, Institute of Enzymology, Eötvös Loránd Research Network
Contact information of distributor	Ágota Apáti, apati.agota@ttk.mta.hu
Type of cell lines	*iPSC lines*
Origin	*Human*
Cell Source	*PBMCs*
Clonality	*Clonal*
Method of reprogramming	*Sendai viral*
Multiline rationale	*isogenic clones of DM2 patients and control and patient (atherosclerosis) pairs*
Gene modification	*NO*
Type of modification	*NA*
Associated disease	*Diabetes; atherosclerosis*
Gene/locus	*NA*
Method of modification	*NA*
Name of transgene or resistance	*NA*
Inducible/constitutive system	*NA*
Date archived/stock date	*June 29, 2018.*
Cell line repository/bank	*NA*
Ethical approval	*Health Care Research Council, Human Reproduction Committee in Hungary (in Hungarian: Egészségügyi Tudományos Tanács, Humán Reprodukciós Bizottság (ETT HRB)Approval number: 42592-2/2016-EHR*

2. Resource utility

DM2 is regarded as metabolic disease resulting from insulin resistance of peripheral tissues and inadequate insulin-secreting pancreatic cell function. It is one of the largest global health burdens having frequent complications related to chronic hyperglycemia (Harding et al, 2019.). Generation of disease specific iPSC lines offers a platform to improve therapeutic options.

3. Resource details

Even though diabetes is one of the most studied diseases all over the world, treatment opportunities for diabetic patients remain limited. iPSC technology provides a new opportunity for investigation and treatment of the disease. Along with the investigation of iPSC-derived

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https://doi.org/10.1016/j.scr.2020.102051
Received 2 August 2020; Received in revised form 5 October 2020; Accepted 11 October 2020
Available online 15 October 2020

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
RCNSi001-A	HU-DM2-A-1	Female	66	Caucasian	NA	DM2
RCNSi001-B	HU-DM2-A-2	Female	66	Caucasian	NA	DM2
RCNSi002-A	HU-DM2-B-1	Female	66	Caucasian	NA	DM2, atherosclerosis
RCNSi002-B	HU-DM2-B-2	Female	66	Caucasian	NA	DM2, atherosclerosis

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Olympus CKX 41	Normal iPSCs formation	Fig. 1 panel C
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining of pluripotency markers: Oct4, Nanog	Fig. 1 panel D
	Quantitative analysis Flow cytometry RT-qPCR	Cell surface markers: SSEA-4: > 90% all of cell lines RT-qPCR: Nanog, Brachyury, AFP, PAX6	Fig. 1 panel E, G
Genotype	Karyotype (G-banding) and resolution	46XX for all clones Resolution: 450–500 bands per haploid chromosome set	Fig. 1 panel A
Identity	STR analysis	DNA Profiling performed	Supplementary file STR analysis.
		17 sites tested, all matching between PBMC and iPSC lines.	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	NA	
	Southern Blot OR WGS	NA	
Microbiology and virology	Mycoplasma	Mycoplasma testing /Negative	Supplementary file mycoplasma test.
Differentiation potential	Embryoid body formation (Spontaneous differentiation)	Immunocytochemistry of AFP, SMA and B-III-TUBULIN as germ layer markers. mRNA expression of AFP, BRY and PAX6 as germ layer markers.	Fig. 1 panel F, G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	
	HLA tissue typing	NA	

insulin producing beta cells (Gaertner et al., 2019.) there is an increasing number of studies focusing other affected-derived cell types such as cardiac or mesenchymal cells derived from diabetic patients' samples (Granéli et al., 2019., Balhara et al., 2015.). Generation of well characterized iPSC lines from patients diagnosed with diabetes could help to understand the involvement of different cell types in the pathology of diabetes. Here we describe four iPSC clones generated from identical twin patients diagnosed with idiopathic DM2, in whom the gene loci for diabetes are unknown (Tables 1 and 2).

Peripheral blood samples of the 66-year-old twin sisters were taken at the Heart and Vascular Center, Semmelweis University. One of the two patients had developed atherosclerosis as well, possibly due to her smoking habits. Mononuclear cell fractions of the samples were reprogramed via Sendai viral transduction of the four "classical" Yamanaka factors (Oct3/4, Sox2, klf4, cMyc). Two clones of each patient specific iPSC-s were selected and characterized. All four clones displayed normal diploid 46, XX karyotype (Fig. 1.A) according to standard G-banding and their identity was verified by STR analysis. After passage 12 real time quantitative PCR analysis (RT-PCR) using Sendai virus specific primers revealed clearance of viral particles (Fig. 1.B). According to our lab routine we used the 62F iPCS line generated from fibroblast sample of a healthy volunteer by Sendai virus (Vőfély et al. 2018.) as negative control for SeV assessment. Mycoplasma tests were negative (see Supplementary file).

Pluripotency was confirmed by assessment of morphology (Fig. 1.C), immunofluorescent staining of endogenous pluripotency transcription factors Oct4 and Nanog (Fig. 1.D), as well as flow cytometry analysis of surface marker SSEA4 (Fig. 1.E). In vitro spontaneous differentiation potential was examined via embryoid body (EB) formation. Derivatives of the three germ layers were evinced by immunofluorescent staining after 6 days of differentiation in free-floating EB culture and additional 12 days on gelatine coated chambered cover glasses. Expression of alpha-fetoprotein (AFP) as endoderm, smooth muscle actin (SMA) as mesoderm and beta-III tubulin (TUB) as ectoderm markers were detected by confocal microscopy (Fig. 1.F). Besides RNA expression levels of pluripotency marker Nanog, endoderm marker AFP, mesoderm marker brachyury (BRY) and ectoderm marker Paired Box 6 (PAX6) were compared in iPS and 6 + 6 days differentiated EB cultures by RT-PCR. In pooled RNA samples of 8 parallel differentiations we found considerable downregulation of pluripotency-, and upregulation of differentiation markers similar to HUES9 human embryonic cell line that we used as a positive control (Erdei et al., 2014.) (Fig. 1.G).

The characterization and quality control of the established cell lines verified the pluripotency and the genomic integrity of the iPSC clones.

4. Materials and methods

4.1. Reprogramming of peripheral blood mononuclear cells (PBMC)

PBMCs were prepared according to manufacturer's instruction (BD Vacutainer CPT). iPSC lines were generated from PBMC by Sendai virus (Thermo Fisher Scientific) based reprogramming and manually selected clones were maintained on mitomycin-C (Sigma) treated mouse embryonic fibroblast feeder cells (Millipore) until passage 10. Clones were transferred onto Matrigel (Corning) coated plates in mTeSR medium (Stemcell Technologies) and medium was changed every day. Cells were passaged with Accutase (Thermofisher Scientific) when reached confluence and replated in mTeSR1 containing 10uM Y27632-2HCl (Selleckchem).

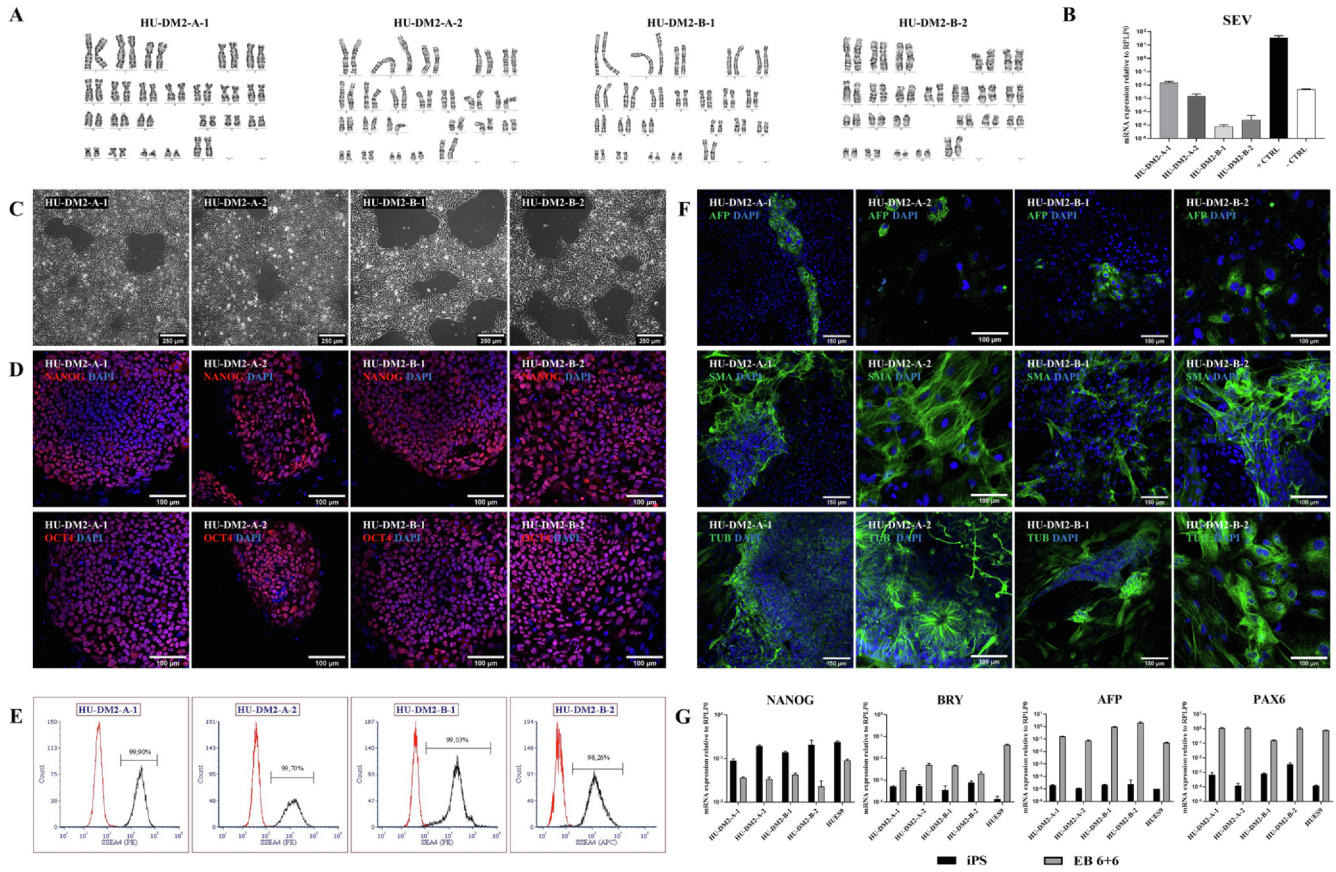

Fig. 1. Characterization of induced pluripotent stem cell lines (HU-DM2-A-1, HU-DM2-A-2, HU-DM2-B-1 and HU-DM2-B-2). A. Karyotype analysis of iPSC clones B. Sendai virus clearance by RT-PCR C. Immunofluorescence staining of pluripotency markers (NANOG and OCT4). D. Cell surface expression of SSEA4 by flow cytometry E. Immunofluorescence staining of germ layer markers (AFP as endoderm, SMA as mesoderm and beta-III tubulin (TUB) as ectoderm markers). F. Expression of pluripotency and differentiation markers by RT-PCR.

4.2. Karyotype and STR analyses

Analyses were performed by UD-GENOMED Medical Genomic Technologies Ltd. (Hungary). Chromosomes were classified using the standard G-banding technique between passages p14-25. STR analysis was performed by GenePrint® 10 System (Promega).

4.3. Mycoplasma detection

Mycoplasma detection was performed with MyxoAlert™ Mycoplasma Detection Kit (Lonza) according to the manufacturer's instructions.

4.4. In vitro spontaneous differentiation

For in vitro spontaneous differentiation experiments, iPSCs were detached with collagenase (Thermofisher Scientific) and transferred to ultra-low attachment plates (Nalgene Nunc International) to allow embryoid body (EB) formation. EB medium (KO-DMEM supplemented with 20% FBS, 1 mM L-GLU, 1% non-essential amino acids, and 0,1 mM ß-mercaptoethnol (Thermofisher Scientific)) was changed in every 2 days for 6 days. Then EBs were transmitted onto 0.1% gelatine (Merck) coated eight-well Nunc Lab-Tek II Chambered Coverglasses (Nalgene Nunc International) for subsequent immunocytochemical staining, or 24 well tissue culture plates (Avantor) for subsequent RT-PCR analysis, and allowed to attach and differentiate spontaneously for another 12 or 6 days in DMEM supplemented with 10% FBS.

4.5. RT-PCR analysis

Total RNA was isolated from iPSCs using TriFast reagent (Peqlab Ltd.) following the manufacturer's instructions. cDNA samples were prepared from 1 µg total RNA using the Promega Reverse Transcription System Kit (Promega) as specified by the manufacturer. For real-time quantitative PCR (RT-PCR) Pre- Developed TaqMan® assays were purchased (Thermo Fisher Scientific). RPLP0 ribosomal protein was used as endogenous control. RT-PCR analyses were carried out in StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific). Relative mRNA levels were calculated by the $2^{-\Delta Ct}$ method.

4.6. Immunocytochemical staining

For immunofluorescence staining, iPSCs clones and their spontaneously differentiated derivates cells were plated on eight-well chambers as described previously (Erdei et al., 2014.). Briefly; cells were fixed on the indicated days with 4% paraformaldehyde (Thermo Fisher Scientific) in Dulbecco's modified PBS (DPBS) (Sigma) for 15 min at room temperature. After washing with DPBS, the samples were blocked for 1 h at room temperature in DPBS containing 2 mg/ml bovine serum albumin (BSA-Sigma), 1% gelatine from cold water fish skin (Sigma), 0.1% Triton-× 100 (Sigma) with or without 5% goat serum (Sigma) depending on the primary antibodies. The samples were then incubated for 1 h at room temperature or overnight at 4 °C with the primary antibodies (Table 3.). After washing with DPBS, the cells were incubated for 1 h at room temperature with Alexa Fluor 488-conjugated IgG secondary antibodies (Thermo Fisher Scientific). The nuclei were stained with DAPI (Thermo Fisher Scientific).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-Oct3/4	1:50	Santa Cruz Biotechnology Cat# sc-5279; RRID:AB_628051
Pluripotency marker	Goat anti-Nanog	1:100	R and D Systems Cat# AF1997; RRID:AB_355097
Endoderm marker	Mouse anti-AFP	1:500	Sigma-Aldrich Cat# A8452; RRID:AB_258392
Mesoderm marker	Mouse anti-SMA	1:500	Abcam Cat# ab7817; RRID:AB_262054
Ectoderm marker	Mouse anti-ß-III-Tubulin	1:2000	R and D Systems Cat# MAB1195; RRID:AB_357520
Secondary antibody	Goat-Anti-Mouse (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-11029; RRID:AB_2534088
Secondary antibody	Donkey-Anti-Goat (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-11055; RRID:AB_2534102
Primers			
Sendai viral vector	Sendai-SevFam	Mr04269880_mr (Applied Biosystems)	
Pluripotency Markers (qPCR)	NANOG	Hs02387400_g1 (Thermo Fisher Scientific)	
Pluripotency Markers (qPCR)	OCT4	Hs00999632_g1 (Thermo Fisher Scientific)	
Endoderm differentiation marker (qPCR)	AFP	Hs00173490_m1 (Thermo Fisher Scientific)	
Mesoderm differentiation markers (qPCR)	T-BRY	Hs00610080_m1 (Thermo Fisher Scientific)	
Ectoderm differentiation markers (qPCR)	PAX6	Hs00240871_m1 (Thermo Fisher Scientific)	
House-Keeping Gene (qPCR)	RLP0	Hs99999902_m1 (Thermo Fisher Scientific)	

Acknowledgements

The authors thank Beáta Haraszti, Zsuzsa Erdei and László Homolya for advice and technical assistance. 62F iPS cell line and HUES9 embryonic cell line were kindly provided by Fred Gage (Salk Institute) and Douglas Melton (HHMI) respectively.

Funding

This study was funded by the National Brain Research Program (NAP) of Hungary (grant numbers: 2017-1.2.1-NKP-2017-00002 to ÁA, LH, and JMR), National Research, Development and Innovation Office (OTKA- K128369 to AÁ and FG) and PRIME Consortium (H2020 Programme): Prevention and Remediation of Insulin Multimorbidity in Europe. Grant agreement ID: 847879.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102051.

References

Balhara, B., Burkart, A., Topcu, V., Lee, Y.K., Cowan, C., Kahn, C.R., Patti, M.E., 2015. Severe insulin resistance alters metabolism in mesenchymal progenitor cells. Endocrinology 156 (6), 2039–2048.

Erdei, Z., Lőrincz, R., Szebényi, K., Péntek, A., Varga, N., Likó, I., Várady, G., Szakács, G., Orbán, T.I., Sarkadi, B., Apáti, A., 2014. Expression pattern of the human ABC transporters in pluripotent embryonic stem cells and in their derivatives. Cytometry B Clin. Cytom. 86 (5), 299–310.

Gaertner, B., Carrano, A.C., Sander, M., 2019. Human stem cell models: lessons for pancreatic development and disease. Genes Dev. 33 (21-22), 1475–1490.

Granéli, C., Hicks, R., Brolén, G., Synnergren, J., Sartipy, P., 2019. Diabetic cardiomyopathy modelling using induced pluripotent stem cell derived cardiomyocytes: recent advances and emerging models. Stem Cell Rev. Rep. 15 (1), 13–22.

Harding, J.L., Pavkov, M.E., Magliano, D.J., Shaw, J.E., Gregg, E.W., 2019. Global trends in diabetes complications: a review of current evidence. Diabetologia 62 (1), 3–16.

Vőfély, G., Berecz, T., Szabó, E., Szebényi, K., Hathy, E., Orbán, T.I., Sarkadi, B., Homolya, L., Marchetto, M.C., Réthelyi, J.M., Apáti, Á., 2018. Characterization of calcium signals in human induced pluripotent stem cell-derived dentate gyrus neuronal progenitors and mature neurons, stably expressing an advanced calcium indicator protein. Mol. Cellular Neurosci. 88, 222–230.