Stem Cell Research 48 (2020) 101998

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 derived from myoblasts (MDCi014-A) and from peripheral blood mononuclear cells (MDCi014-B) from the same donor

Eric Metzler[a,d], Narasimha Telugu[d], Sebastian Diecke[b,c,d], Simone Spuler[a,d,e], Helena Escobar[a,d,*]

[a] Muscle Research Unit, Experimental and Clinical Research Center, A Joint Cooperation of Charité, Universitätsmedizin Berlin and the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[b] Berlin Institute of Health (BIH), Berlin, Germany
[c] DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany
[d] Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[e] Charité, Universitätsmedizin Berlin, Germany

A B S T R A C T

We describe the generation and characterization of two human induced pluripotent stem cell (hiPSCs) lines reprogrammed from myoblasts and from peripheral blood mononuclear cells (PBMCs) from the same donor. The donor was free of neuromuscular disorders, male and 18 years of age. For reprogramming we used Sendai-virus delivery of the four Yamanaka factors. The pluripotent identity of the hiPSC lines was confirmed by the expression of pluripotency markers and their capacity to differentiate into all three germ layers. These hiPSCs constitute a tool to study tissue of origin specific differences in the identity of hiPSCs.

1. Resource Table

Unique stem cell lines identifier	MDCi014-A MDCi014-B
Alternative names of stem cell lines	N/A
Institution	Max Delbrück Center for Molecular Medicine, Berlin, Germany
Contact information of distributor	Eric Metzler (eric.metzler@mdc-berlin.de) Helena Escobar (helena.escobar@mdc-berlin.de) Simone Spuler (simone.spuler@charite.de)
Type of cell lines	iPSCs
Origin	Human
Cell Source	Myoblasts (MDCi014-A) Peripheral Blood Mononuclear Cells (PBMCs) (MDCi014-B)
Clonality	Mixed
Method of reprogramming	Sendai-virus (OCT3/4, SOX2, KLF4, c-Myc)
Multiline rationale	hiPSCs generated from myoblasts and PBMCs from the same donor
Gene modification	NO
Type of modification	N/A
Associated disease	No disease reported
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	01/2020 07/2020
Cell line repository/bank	https://hpscreg.eu/cell-line/MDCi014-A https://hpscreg.eu/cell-line/MDCi014-B
Ethical approval	Ethics Committee of Charité Universitätsmedizin Berlin EA2/175/17

2. Resource utility

Several studies have shown that the cell type of origin can influence the epigenetic profile and the differentiation capacity of hiPSC into different tissues. The question of what tissue source is optimal to generate hiPSC for specific downstream applications is highly relevant and yet to be categorically answered.

3. Resource details

The tissue of origin of iPSCs has been shown to influence their epigenetic profile (Kim et al., 2011) and differentiation capacity into different tissue cell types (Bar-Nur et al., 2011; Sanchez-Freire et al., 2014). Understanding these tissue-of-origin-specific differences is fundamental to enhance the application of hiPSCs for e.g. disease modelling or cell therapies. In this study, we describe the generation of two hiPSC lines generated from a donor free of neuromuscular disorders, male and 18 years of age (see Table 1). One hiPSC line was

* Corresponding author.

https://doi.org/10.1016/j.scr.2020.101998
Received 22 July 2020; Received in revised form 1 September 2020; Accepted 10 September 2020
Available online 17 September 2020

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
MDCi014-A	MDCi014-A	male	18	Caucasian	N/A	N/A
MDCi014-B	MDCi014-B	male	18	Caucasian	N/A	N/A

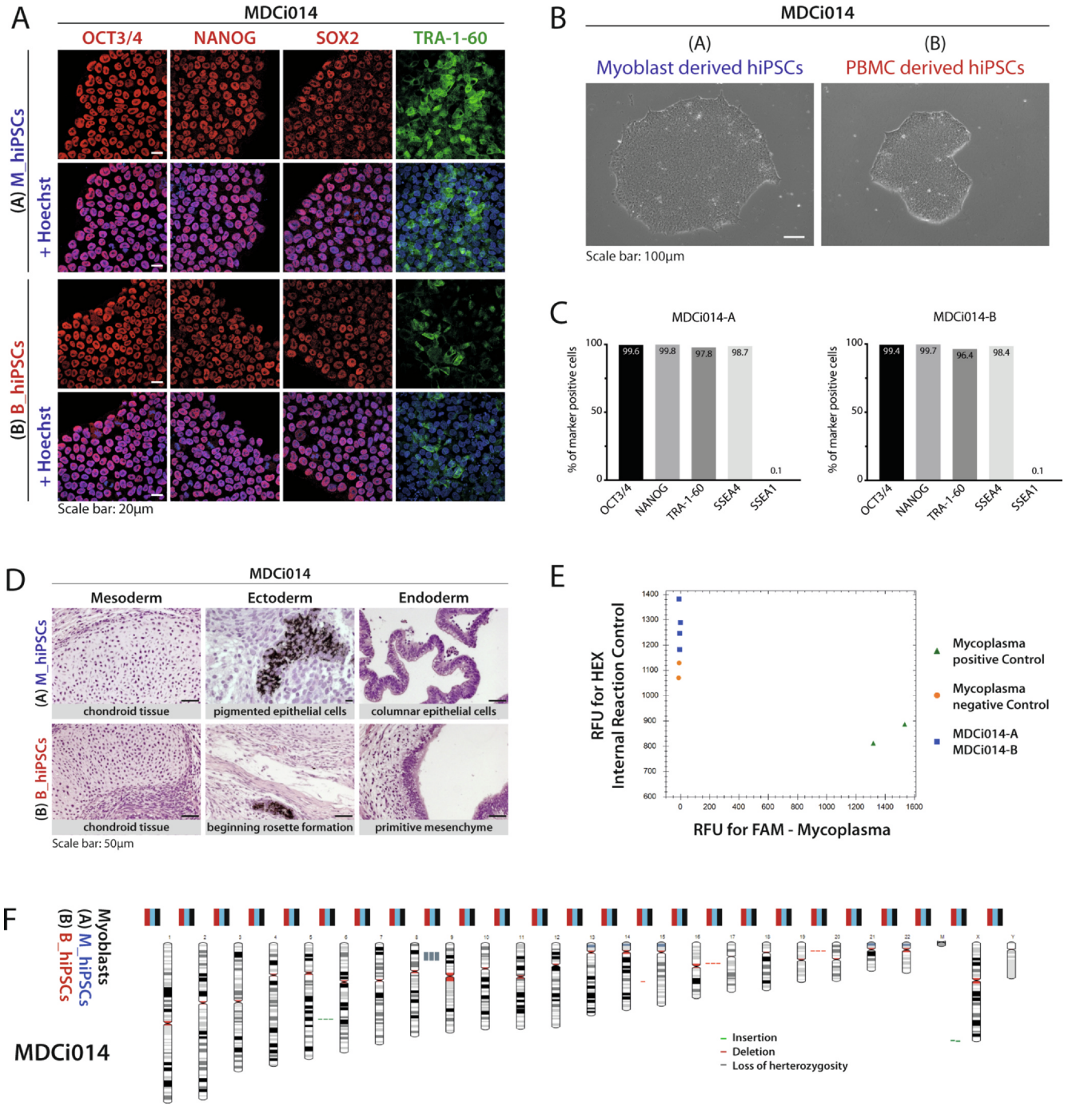

Fig. 1. Characterization of the generated myoblast- (MDCi014-A) and PBMC-derived (MDCi014-B) hiPSCs.

reprogrammed from myoblasts and one from peripheral blood mononuclear cells (PBMCs).

Myoblasts and PBMCs were reprogrammed using Sendai-virus delivery of the 4 Yamanaka factors, OCT3/4, SOX2, KLF4 and c-Myc. Each hiPSC line was derived from a single colony that was isolated and expanded after reprogramming. Both hiPSC lines were tested negative for the presence of remaining Sendai-virus particles by RT-PCR in passages 13–15 (Suppl. Fig. S1). The master cell bank was generated for all lines in passage 15–17 and was proven to be negative for any mycoplasma contamination by RT-qPCR (Fig. 1E). Both hiPSC lines, cultured in mTeSR™1 medium on Matrigel-coated plates, show well-defined colony borders and packed colony morphology with no signs of spontaneous

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis (Immunofluorescence Microscopy)	OCT3/4 SOX2 NANOG TRA-1-60	Fig. 1 panel A
	Quantitative analysis (Flow cytometry)	MDCi014-A: OCT3/4: 99.6% NANOG: 99.8% TRA-1-60: 97.8% SSEA-4: 98.7% SSEA-1: 0.1% MDCi014-B: OCT3/4: 99.4% NANOG: 99.7% TRA-1-60: 96.4% SSEA-4: 98.4% SSEA-1: 0.1%	Fig. 1 panel C Raw data available at hPSCreg
Genotype	Karyotype (Single Nucleotide Polymorphism Analysis (SNPs))	46, XX No numerical aberrations No large deletions/insertions Total number of markers: 962,215	Fig. 1 panel F
Identity	STR analysis	Identity of myoblast- and PBMC-derived hiPSCs confirmed comparing the hiPSCs against the primary myoblasts of each donor	
		16 STR-sites analyzed, all matching	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, all negative	Fig. 1 panel E
Differentiation potential	Teratoma formation	Formation of all three germ layers confirmed by histopathological analysis for all samples	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

differentiation (Fig. 1B). Immunofluorescence analysis showed the expression of markers for undifferentiated pluripotent stem cells as octamer-binding transcription factor 3/4 (OCT3/4), sex determining region Y-box 2 (SOX2), NANOG and tumour rejection antigen (TRA-1–60) (Fig. 1A). Additionally, the purity of the pluripotent cell populations was confirmed by quantifying the percentage of cells positive for OCT3/4 (99.6%/99.4%), NANOG (99.8%/99.7%), TRA-1–60 (97.8%/96.4%) and stage specific embryonic antigen 4 (SSEA4) (98.7%/98.4%) via flow cytometry. Additionally, the differentiation marker stage specific embryonic antigen 1 (SSEA1) was expressed in 0.1% of the cells in both hiPSC lines, thus confirming pluripotent cell states (Fig. 1C, Table 2, raw data available at hPSCreg). The three-germ layer differentiation capacity was tested via teratoma formation assay. Histopathological examination of the teratoma tissues confirmed the generation of tissues of mesodermal, ectodermal and endodermal origin for both hiPSC lines (Fig. 1D). Single nucleotide polymorphism (SNP)- analysis confirmed typical karyotypes without numerical chromosomal abnormalities for M_hiPSCs and B_hiPSCs and the corresponding myoblasts of origin. Regions that show differences to the human reference genome like insertions (green), deletions (red) or LOH (loss of heterozygosity) are depicted on the left side of each chromosome as colored bars with no areas of insertions or deletions above 1.2 MB. Most abnormalities are already present in the parental cell populations and minor abnormalities may be attributed to the comparison to the standard human genome (Fig. 1F). Short tandem repeat (STR)- analysis of 16 genomic loci showed identical DNA profiles between the generated hiPSC lines and the corresponding donor-derived myoblast sample (available with journal).

In conclusion we generated and fully characterised two hiPSC lines reprogrammed from myoblasts and PBMCs of the same donor. Those lines can be used to investigate the influence of tissues of origin on the characteristics of hiPSCs.

4. Materials and methods

4.1. Reprogramming

Myoblasts were isolated from muscle biopsy specimen and PBMCs from whole blood. Both were cultured in 21% O_2, 5% CO_2, 37 °C, 95% rH. For reprogramming, Sendai-virus delivery of OCT3/4, SOX2, KLF4 and c-Myc (CytoTune™-iPS 2.0 Sendai Reprogramming Kit, Invitrogen, Fusaki et al., 2009) was used. Briefly, $5x10^4$ myoblasts were seeded on Matrigel (concentration according to the dilution factor as specified for each lot number) in Skeletal Muscle Cell Growth Medium (SMCGM, ProVitro) + Polybrene (10 µg/ml) + Sendai virus mix. After 24 h 50% fresh SMCGM was added. The next day medium was exchanged for fresh SMCGM + Sodium Butyrate (200 µM) + Ascorbic acid (64 µg/µl) and exchanged every other day. Accordingly, $3x10^5$ PBMCs were infected with the Sendai-virus mix in PBMC medium (StemPro™-34 SFM medium + supplement, L-Glutamine (2 mM), SCF (100 ng/ml), FLT-3 (100 ng/ml), IL-3 (20 ng/ml), IL-6 (20 ng/ml), Epo (2U/ml)) + Polybrene. Sendai-virus was removed by centrifugation on the next day and 50% medium was exchanged every other day using fresh PBMC medium + Sodium Butyrate + Ascorbic acid. When hiPSC colonies showed well-defined borders medium was changed to mTeSR™1 (Stemcell Technologies) and cells were transferred to 5% O_2, 5% CO_2, 37 °C, 95% rH.

4.2. Test for the absence of Sendai virus

Absence of Sendai-virus particles was analysed by RT-PCR (Hildebrand et al., 2016) using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific) together with the primers listed in Table 3 following this program: 95 °C 5 min, 35 cycles: 95 °C 30 sec, 55 °C 30 sec, 72 °C 30 sec and finally 72 °C for 10 min. PCR products were

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers (Immunofluorescence)	Rabbit anti-OCT4	1:1000	Abcam Cat# ab19857, RRID: AB_445175
	Rabbit anti-SOX2	1:300	Abcam Cat# ab97959, RRID: AB_2341193
	Rabbit anti-NANOG	1:100	Abcam Cat# ab21624, RRID: AB_446437
	Mouse anti-TRA-1–60	1:500	Abcam Cat# ab16288, RRID: AB_778563
Secondary antibodies (Immunofluorescence)	Alexa Fluor 568 donkey anti-rabbit	1:1000	Thermo Fisher Cat# A10042, RRID: AB_2534017
	Alexa Fluor 488 goat anti-mouse	1:1000	Thermo Fisher Cat# A10042, RRID: AB_2534069
Pluripotency Markers (Flow Cytometry)	Anti-OCT3/4 APC	1:50	Miltenyi Biotec Cat# 130–117-709, RRID: AB_2784444
	Anti-NANOG PE	1:100	Cell Signaling Cat# 14955S, RRID: N/A
	Anti-TRA-1–60 Vio488	1:600	Miltenyi Biotec Cat# 130–106-872, RRID: AB_2654228
	Anti-SSEA4 VioBlue	1:20	Miltenyi Biotec Cat# 130–098-366, RRID: AB_2653521
	Anti-CD15 Vio770	1:100	Miltenyi Biotec Cat# 130–113-486, RRID: AB_2733201
Primers			
	Target	Forward/Reverse primer (5′–3′)	
Sendai-virus (PCR)	SeV (total)	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
	SeV-KOS	ATGCACCGCTACGAGTGAGCGC/ACCTTGACAATCCTGATGTGG	
	SeV-KLF-4	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA	
	SeV-c-Myc	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
House-keeping gene (PCR)	Hu18SRNA	GTAACCCGTTGAACCCCATT/CCATCCAATCGGTAGTAGCG	

analysed using a 2% agarose gel.

4.3. hiPSC culture

hiPSCs were cultured in mTeSR™1 medium (Stemcell Technologies) on Matrigel-coated 6-well plates at 5% O_2. Cells were passaged routinely at a ratio of 1:10 every 3 days using 0.5 mM PBS/EDTA. Mycoplasma was tested using the Venor® GeM qOneStep kit (Minerva Biolabs).

4.4. Immunofluorescence

hiPSCs grown on Matrigel-coated 8-well IbiTreat slides (Ibidi) were fixed with 3.7% Formaldehyde (10 min). Except TRA-1-60 staining, all cells were permeabilized with 0.2% Trition X-100 (10 min) followed by blocking with 1% BSA/PBS (1 h). Primary antibodies were diluted in 1% BSA/PBS and incubated over night at 4 °C followed by secondary antibodies for 1 h in PBS at RT (Table 3). Confocal immunofluorescence imaging was performed using the Laser Scan Microscope LSM 700 (Carl Zeiss).

4.5. Flow cytometry

Single cells were labelled with conjugated antibodies (Table 3). Surface marker (SSEA1, SSEA4 and TRA1-60) staining was performed in unfixed cells by incubation with antibodies diluted in 0.5% BSA/PBS (10 min, 4 °C, dark). Intracellular marker (OCT3/4, NANOG) were stained by fixation/permeabilization solution (Miltenyi Biotec, 130-093-142) (30 min, 4 °C, dark) followed by antibody incubation in permeabilization buffer (Miltenyi Biotec, 130-093-142) (30 min, 4 °C, dark). Analysis was done using the MACSQuant® AnalyzerVYB and FlowJo v10.4.

4.6. Teratoma formation assay

$2.5*10^6$ hiPSCs in 100 µl PBS/Matrigel (1:1) were injected subcutaneously into the flank of immunodeficient NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/JigTac mice (Taconic Biosciences). Animals were sacrificed when tumour reached more than 1 cm^3 or 8 weeks after transplantation.

4.7. Single nucleotide polymorphism (SNP)- Karyotype

hiPSCs where karyotyped using the OMNI-EXPRESS-8v1.6 chip (Illumina). Karyostudio 1.3 software was used based on the information of GRCh36/hg18 dataset.

4.8. Short tandem repeat (STR)- analysis

hiPSCs identity was confirmed using myoblast samples of the corresponding donors. For analysis, the AmpFLSTR™ NGM SElect™ PCR Amplification Kit (ThermoFisher Scientific) was used.

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.

Acknowledgements

We thank the donors of the primary cell types. We also thank Norman Krüger and Sandra Schommer for excellent technical assistance. This work was financially supported by the German Research Foundation (DFG SP1152), the Berlin Institute of Health (BIH), the Charité Medical Faculty and the Foundation Gisela Krebs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.101998.

References

Bar-Nur, O., Russ, H.A., Efrat, S., Benvenisty, N., 2011. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Stem Cell 9, 17–23. https://doi.org/10.1016/j.stem.2011.06.007.

Fusaki, N., Ban, H., Nishiyama, A., Saeki, K., Hasegawa, M., 2009. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci. 85, 348–362. https://doi.org/10.2183/pjab.85.348.

Hildebrand, L., Rossbach, B., Kühnen, P., Gossen, M., Kurtz, A., Reinke, P., Seemann, P., Stachelscheid, H., 2016. Generation of integration free induced pluripotent stem cells from fibrodysplasia ossificans progressiva (FOP) patients from urine samples. Stem Cell Res. 16, 54–58. https://doi.org/10.1016/j.scr.2015.11.017.

Kim, K., Zhao, R., Doi, A., Ng, K., Unternaehrer, J., Hongguang, H., Loh, Y., Aryee, M.J.,

Lensch, M.W., Li, H., Collins, J.J., Feinberg, A.P., Daley, G.Q., 2011. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells Kitai. Nat. Biotechnol. 29, 1117–1119. https://doi.org/10.1038/nbt.2052.Donor.

Sanchez-Freire, V., Lee, A.S., Hu, S., Abilez, O.J., Liang, P., Lan, F., Huber, B.C., Ong, S.G., Hong, W.X., Huang, M., Wu, J.C., 2014. Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells. J. Am. Coll. Cardiol. 64, 436–448. https://doi.org/10.1016/j.jacc.2014.04.056.