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Lab Resource: Multiple Cell Lines

Generation of three age and gender matched pairs of human induced pluripotent stem cells derived from myoblasts (MDCi011-A, MDCi012-A, MDCi013-A) and from peripheral blood mononuclear cells (MDCi011-B, MDCi012-B, MDCi013-B) from the same donor

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ABSTRACT

We describe the generation and characterization of three pairs of human induced pluripotent stem cell (hiPSC) lines reprogrammed from myoblasts and from peripheral blood mononuclear cells (PBMCs) of the same donor. All donors were free of neuromuscular disorders, female and between 47 and 50 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	MDCi011-A MDCi011-B MDCi012-A MDCi012-B MDCi013-A MDCi013-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 (MDCi011-A, MDCi012-A, MDCi013-A) Peripheral Blood Mononuclear Cells (PBMCs) (MDCi011-B, MDCi012-B, MDCi013-B)
Clonality	Mixed
Method of reprogramming	Sendai-virus (OCT3/4, SOX2, KLF4, c-Myc)
Multiline rationale	3 pairs of gender- and age-matched donors (45–49, female) with one clone generated from myoblasts and the other clone generated from PBMCs
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	01-02/2020
Cell line repository/bank	https://hpscreg.eu/cell-line/MDCi011-A https://hpscreg.eu/cell-line/MDCi011-B https://hpscreg.eu/cell-line/MDCi012-A https://hpscreg.eu/cell-line/MDCi012-B https://hpscreg.eu/cell-line/MDCi013-A https://hpscreg.eu/cell-line/MDCi013-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 hiPSCs into different tissues. The question of what tissue source is optimal to generate hiPSCs for specific downstream applications is highly relevant and yet to be categorically answered.

3. Resource details

The tissue of origin of hiPSCs has been shown to influence their epigenetic profile (Kim et al., 2011) and differentiation capacity into

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
MDCi011-A	MDCi011-A	Female	47	Caucasian	N/A	N/A
MDCi011-B	MDCi011-B	Female	47	Caucasian	N/A	N/A
MDCi012-A	MDCi012-A	Female	50	Caucasian	N/A	N/A
MDCi012-B	MDCi012-B	Female	50	Caucasian	N/A	N/A
MDCi013-A	MDCi013-A	Female	47	Caucasian	N/A	N/A
MDCi013-B	MDCi013-B	Female	47	Caucasian	N/A	N/A

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 six hiPSC lines from three age- and gender-matched healthy donors (female, age 47–50). For each donor, we generated one hiPSC line from myoblasts and one from peripheral blood mononuclear cells (PBMCs) (see Table 1).

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. All hiPSC lines were tested negative for the presence of remaining Sendai virus particles by RT-PCR in passages 13–15 (Suppl. Fig. S1B). 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 (Suppl. Fig. S1C). All 6 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 differentiation (Fig. 1A). 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. 1C). Additionally, the purity of the pluripotent cell populations was confirmed by quantifying the percentage of cells positive for OCT3/4 (99.4%-99.7%), NANOG (99.7%-99.8%), TRA-1–60 (96.2%-97.7%) and stage specific embryonic antigen 4 (SSEA4) (94.7%-99.1%) via flow cytometry. Additionally, the differentiation marker stage specific embryonic antigen 1 (SSEA1) was expressed in $<$ 0.2% of the cells in all hiPSC lines, thus confirming pluripotent cell states (Fig. 1B, 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 all 6 hiPSC lines (Fig. 1D). Single nucleotide polymorphism (SNP)-analysis confirmed typical karyotypes without numerical chromosomal abnormalities and only minor insertions or deletions below 1 Mb, with all abnormalities already present in the parental cell populations (Suppl. Fig. S1A). Short tandem repeat (STR)- analysis of 16 genomic loci showed identical DNA profiles between the generated hiPSC lines and the corresponding donor-derived myoblast samples (available with journal).

In conclusion we generated and fully characterised three pairs of 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 in Skeletal Muscle Cell Growth Medium (SMCGM, Pro-Vitro) + 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 30sec , 55 °C 30sec, 72 °C 30sec and finally 72 °C for 10 min. PCR products were 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.

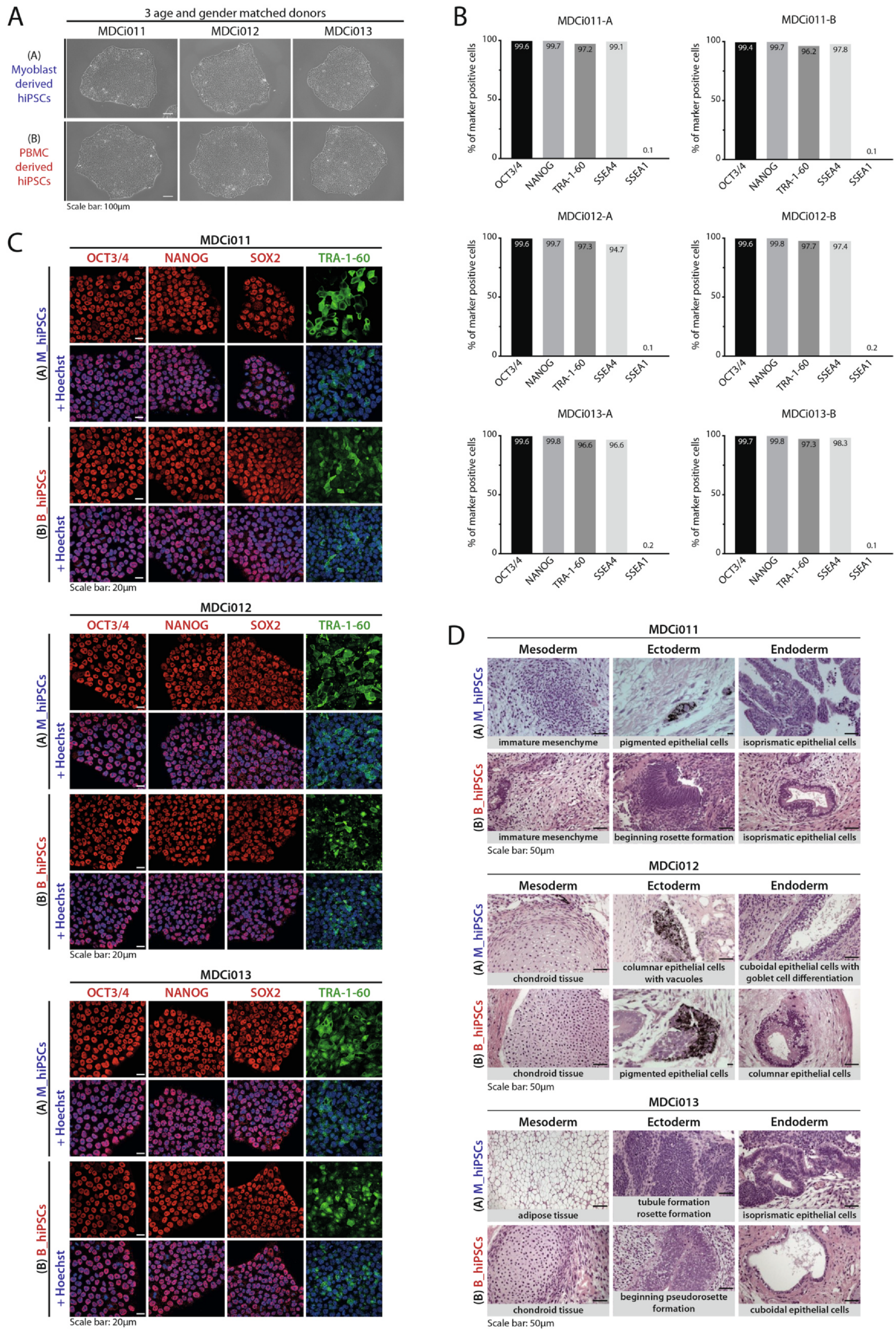

Fig. 1. Characterization of the generated myoblast- (MDCi011-A, MDCi012-A, MDCi013-A) and PBMC-derived (MDCi011-B, MDCi012-B, MDCi013-B) hiPSCs.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunofluorescence Microscopy)	OCT3/4 SOX2 NANOG TRA-1-60	Fig. 1 panel C
	Quantitative analysis (Flow cytometry)	MDCi011-A: OCT3/4: 99.6% NANOG: 99.7% TRA-1-60: 97.2% SSEA-4: 99.1% SSEA-1: 0% MDCi011-B: OCT3/4: 99.4% NANOG: 99.7% TRA-1-60: 96.2% SSEA-4: 97.8% SSEA-1: 0.1% MDCi012-A: OCT3/4: 99.6% NANOG: 99.7% TRA-1-60: 97.3% SSEA-4: 94.7% SSEA-1: 0.1% MDCi012-B: OCT3/4: 99.6% NANOG: 99.8% TRA-1-60: 97.7% SSEA-4: 97.4% SSEA-1: 0.2% MDCi013-A: OCT3/4: 99.6% NANOG: 99.8% TRA-1-60: 96.6% SSEA-4: 97.6% SSEA-1: 0.2% MDCi013-B: OCT3/4: 99.7% NANOG: 99.8% TRA-1-60: 97.3% SSEA-4: 98.3% SSEA-1: 0.1%	Fig. 1 panel B 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	Supplementary Fig. 1 panel A
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 Southern Blot OR WGS	N/A N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, all negative	Supplementary Fig. 1 panel C
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 HLA tissue typing	N/A N/A	

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.

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

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.2020.101987.

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