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

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<tr>
<th>Unique stem cell lines identifier</th>
<th>Alternative names of stem cell lines</th>
<th>Institution</th>
<th>Contact information of distributor</th>
<th>Type of cell lines</th>
<th>Origin</th>
<th>Cell Source</th>
<th>Clonality</th>
<th>Method of reprogramming</th>
<th>Multiline rationale</th>
<th>Gene modification</th>
<th>Type of modification</th>
<th>Associated disease</th>
<th>Gene/locus</th>
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<td>Eric Metzler (<a href="mailto:eric.metzler@mdc-berlin.de">eric.metzler@mdc-berlin.de</a>)</td>
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<td>Human</td>
<td>Myoblasts (MDCi011-A, MDCi012-A, MDCi013-A) Peripheral Blood Mononuclear Cells (PBMCs) (MDCi011-B, MDCi012-B, MDCi013-B)</td>
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<td>Sendai-virus (OCT3/4, SOX2, KLF4, c-Myc)</td>
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<td></td>
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<td>MDCi012-A</td>
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Method of modification: N/A
Name of transgene or resistance: N/A
Inducible/constitutive system: N/A
Date archived/stock date: 01-02/2020
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...
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 modeling 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) (94.7%-99.1%) via flow cytometry. 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 mesodermal, ectodermal and endodermal origin on the characteristics of hiPSCs.

### Materials and methods

#### 4. Reprogramming

Myoblasts were isolated from muscle biopsy specimen and PBMCs from whole blood. Both were cultured in 21% O2, 5% CO2, 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, 5x10⁴ 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, 3x10⁵ 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% O2, 5% CO2, 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% O2. 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% Triton X-100 (10 min) followed by blocking with 1% BSA/PBS (1 h). Primary antibodies were diluted in 1% BSA/PBS and incubated overnight 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.

### Table 1

<table>
<thead>
<tr>
<th>iPSC line names</th>
<th>Abbreviation in figures</th>
<th>Gender</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Genotype of locus</th>
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</table>

For reprogramming, Sendai-virus delivery of 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) (94.7%-99.1%) via flow cytometry. 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.
Fig. 1. Characterization of the generated myoblast- (MDCi011-A, MDCi012-A, MDCi013-A) and PBMC-derived (MDCi011-B, MDCi012-B, MDCi013-B) hiPSCs.

A. Myoblast derived hiPSCs
B. PBMC derived hiPSCs

C. OCT3/4, NANOG, SOX2, TRA-1-60

D. Mesoderm, Ectoderm, Endoderm
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^scidIl2rg^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.2020.101987.

References


Table 3

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<th>Antibodies used for immunocytochemistry/flow-cytometry</th>
<th>Antibody</th>
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Antibody Dilution Company Cat # and RRID

Pluripotency Markers (Immunofluorescence)
- Rabbit anti-OCT4, Cat# ab19857, RRID: AB_445175
- Rabbit anti-SOX2, Cat# ab97959, RRID: AB_2341193
- Rabbit anti-NANOG, Cat# ab21624, RRID: AB_446437
- Mouse anti-TRA-1-60, Cat# ab16288, RRID: AB_779563

Secondary antibodies (Immunofluorescence)
- Alexa Fluor 568 donkey anti-rabbit, Cat# A10042, RRID: AB_2534017
- Alexa Fluor 488 goat anti-mouse, Cat# A10042, RRID: AB_2534069

Pluripotency Markers (Flow Cytometry)
- Anti-OCT3/4 APC, Cat# 130-117-709, RRID: AB_2784444
- Anti-NANOG PE, Cat# 149555, RRID: N/A
- Anti-TRA-1-60 Vio488, Cat# 130-106-872, RRID: AB_2654228
- Anti-SSEA4 VioBlue, Cat# 130-098-366, RRID: AB_2653521
- Anti-CD15 Vio770, Cat# 130-113-486, RRID: AB_2733201

Primers
- SeV (total), Forward/Reverse primer: GGATCACTAGGTATACGAGGC/ACCCAGCAAAGCTTTAGAGATAGTATC
- SeV-KOS, Forward/Reverse primer: ATGCACCGCTAGGATATGTGCG/ACCTTGCAATTGCTAGTGG
- SeV-KLF-4, Forward/Reverse primer: TTCCTCGATCTGGAGGAGGAGCC/AAATATCAGAACTTACGCAAA
- SeV-c-Myc, Forward/Reverse primer: TAACTGACTAGGCTTTGCTG/TCGAACATTGCTGGATAGTATC
- Hu18SRNA, Forward/Reverse primer: GTAACCCGTTGAACCCATT/CAATTCAGTGGTAGTGG

Antibodies used for immunocytochemistry/flow-cytometry

- Rabbit anti-OCT4, Dilution: 1:1000, Cat# ab19857, RRID: AB_445175
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