Stem Cell Research 18 (2017) 26–28

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation and characterization of human iPSC line MML-6838-Cl2 from mobilized peripheral blood derived megakaryoblasts

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ARTICLE INFO

Article history:
Received 6 November 2016
Accepted 5 December 2016
Available online 10 December 2016

ABSTRACT

Mobilized peripheral blood (MPB) CD34+ cells were cultured to CD41+/CD34+ megakaryoblasts. Cells were sorted to obtain a pure megakaryoblast population that was reprogramed by a hOKSM self-silencing polycistronic vector using lentiviral delivery. The generated induced pluripotent stem cell (iPSC) lines were tested for silencing of the reprogramming construct by flow cytometry. Pluripotency of MML-6838-Cl2 iPSC line was confirmed by expression of associated markers and by in vivo spontaneous differentiation towards the 3 germ layers. The genomic integrity of iPSC line was shown by karyotyping. The MML-6838-Cl2 iPSC is, to our knowledge, the first to be generated from megakaryoblasts.

Resource table.

Name of Stem Cell line	MML-6838-Cl2
Institution	Sanquin blood bank, Amsterdam, The Netherlands
Person who created resource	Marten Hansen
Contact person and email	e.vandenakker@sanquin.nl
Date archived/stock date	January, 2014
Origin	Human megakaryoblasts
Type of resource	Induced pluripotent stem cell (iPSC); derived from mobilized peripheral blood donation.
Sub-type	Induced Pluripotent Stem Cells (iPSC)
Key transcription factors	OCT4, SOX2, KLF4, C-MYC
Authentication	Purity of iPSC was confirmed by: Flow cytometry, ALP staining, karyotyping and in vivo spontaneous differentiation potential.
Link to related literature	N/A
Information in public databases	N/A
Ethics	Informed consent was given in accordance with the Declaration of Helsinki and Dutch national and Sanquin internal ethic boards.

1. Resource details

MPB CD34+ cells of a male donor were isolated and differentiated to the megakaryocytic lineage as described before (Heideveld et al., 2015) and FACS sorted to obtain pure $CD34^+/CD41^+$ megakaryoblasts. Sorted Megakaryoblasts were transduced with the self-inactivating pRRL.PPT.SF.hOKSMco.GFP.preFRT lentiviral vector (Warlich et al., 2011; Voelkel et al., 2010). Reprogramming was performed on an irradiated mouse embryonic fibroblast (iMEF) feeder layer. The iPSC-like colonies were individually picked 14–20 days post-transduction and based on morphology criteria MML-6838-CL2 iPSC was chosen for further examination (Fig. 1A).

Background green fluorescence was detected in the lentiviral reprogrammed MML-6838-CL2 iPSC line and a control episomal (non-GFP) reprogrammed iPSC, indicating silencing (Fig. 1B). Pluripotency was confirmed by detecting expression of SOX2, OCT4, SSEA4, TRA1-61 and TRA1-80 pluripotency-associated markers by flow cytometry and alkaline phosphatase staining (Fig. 1C, D).

The spontaneous differentiation potential of MML-6838-CL2 iPSC line was shown in vivo by analyzing teratomas formed in NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG). Three mice were injected and 2/3

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http://dx.doi.org/10.1016/j.scr.2016.12.004

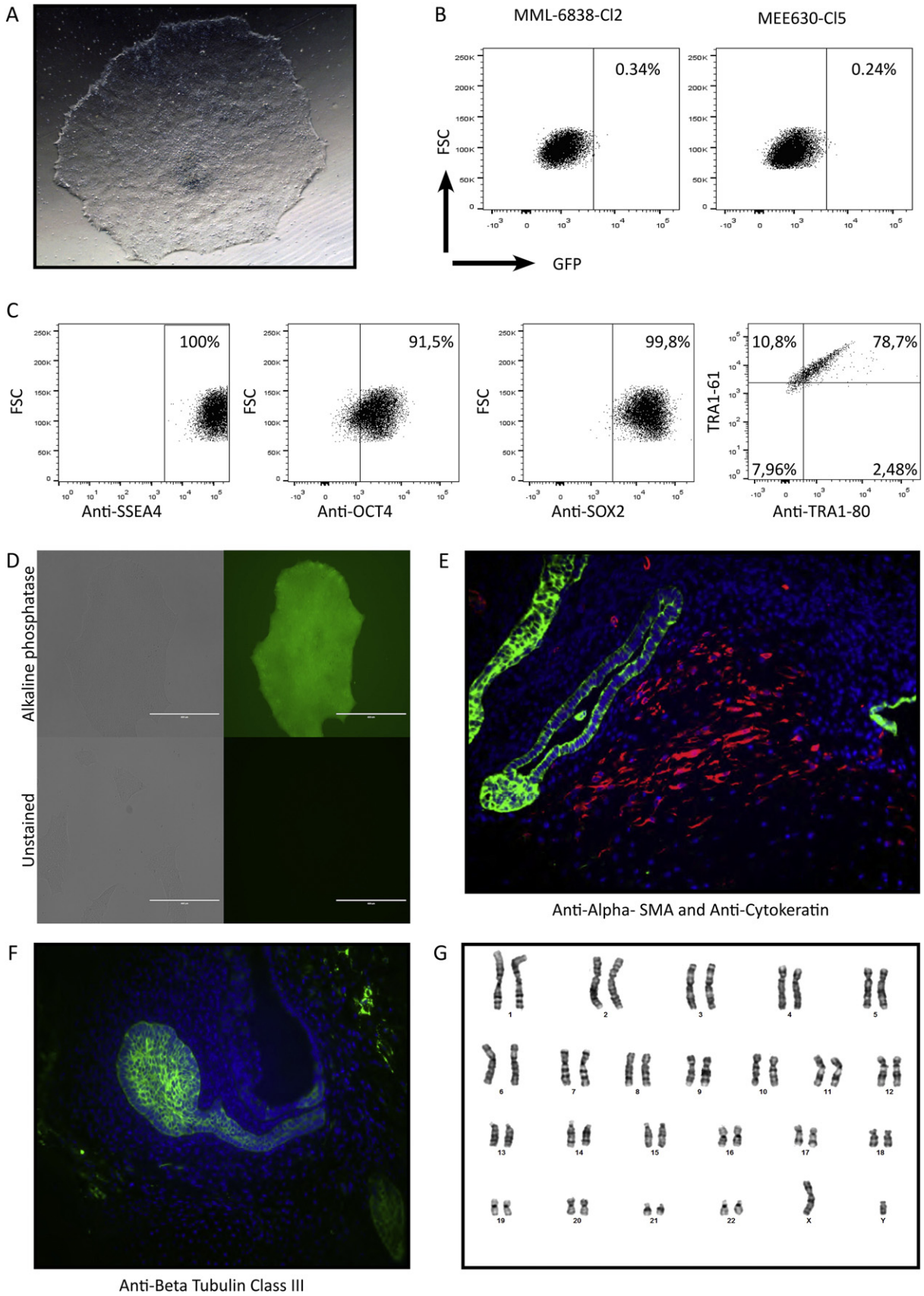

Fig. 1. Characterization of MML-6838-Cl2 iPSC line. A) Morphology of MML-6838-Cl2 iPSC line, 4× enlargement. B) Flow cytometry analysis of MML-6838-Cl2 iPSC to show the silencing of the reprogramming cassette measured by flow cytometry as the absence of green fluorescent protein (GFP; left); an episomal reprogramed iPSC was used as negative control (right). C) Expression of pluripotency associated markers SSEA4, OCT4, SOX2, TRA1-61 and TRA1-80 by flow cytometry all isotype controlled. D) Alkaline phosphatase staining (top) and unstained control (bottom), showing bright field (left) and green fluorescence channel (right). E) Teratomas stained for mesoderm (Alpha-SMA, red), endoderm (cytokeratin, green) and F) ectoderm (Beta Tubulin Class III, green) and dapi (blue). G) Representative G-banded karyogram of MML-6838-Cl2.

formed teratomas. The commitment of the iPSC line to all three germ layers was confirmed by immunohistochemistry staining in which endodermal (PAN-CYTOKERATIN), mesodermal (α-SMA) and ectodermal (βIII-TUBULIN) markers were detected (Fig. 1E, F).

The Genomic integrity of MML-6838-CL2 iPSC line was determined by Giemsa-banding, proving normal diploid 46XY karyotype, without any detectable abnormalities (Fig. 1G).

2. Materials and methods

2.1. Experimental procedures

The chemicals were purchased from Sigma-Aldrich (Munich, Germany) and all the culture reagents from Thermo Fisher Scientific (Waltham, Massachusetts, USA), unless otherwise specified.

2.2. Cell culture

All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2. The iPSCs were cultured on Matrigel (BD Biosciences, Breda, The Netherlands) in essential 8 medium (E8) following the manufacturer's instructions.

2.3. Lentiviral production

Reprogramming virus was produced in 293 T cells in Gibco DMEM, 1% Pen/Strep, 2 mM/ml L-Glut, 10% fetal calve serum. In Brief, cells were seeded and 24 h later transfected with the pRRL.PPT.SF.hOKSMco.GFP.preFRT lentiviral plasmid and lentiviral packaging constructs: pVSV, pRSV-REV and pMDI using calcium phosphate transfection. Lentivirus particle containing supernatant was collected 48 and 72 h after transfection then pooled. Cell debris was cleared by centrifugation at 3000g, 5 min and the supernatant was filtered using a 0,45 μm filter. Lentiviral particles were immobilized by PEG (final concentration of 5%) and virus-PEG precipitates were spun down at 2000 g, 20 min, 4 °C and snap-frozen in liquid nitrogen and stored at −80 °C until use.

2.4. Isolation and culture of megakaryoblast from MPB and reprogramming

CD34+ hematopoietic stem and progenitor cells were isolated from MPB using CD34 Microbeads (Miltenyi Biotec, Leiden, The Netherlands) magnetic-activated cell sorting according to manufacturer instructions. CD34+ cells were cultured in IMDM (Biochrome, Merck, Berlin, Germany) supplemented with growth factors: 50 ng/ml stem cell factor (SCF), 50 ng/ml TPO, 1 ng/ml IL-3 and 20 ng/ml IL-6. 4 days later the growth factor mix was changed to 50 ng/ml TPO and 10 ng/ml IL-1β as described previously by Heideveld et al. (all from PeproTech, Pittsburgh, USA). Megakaryocyte cultures were sorted on $CD34^+/CD41^+$ positivity to obtain a pure megakaryoblasts population (ARIA III, BD Bioscience). $1.5.10^6$ megakaryoblasts were transduced by the hOKSM-carrying lentivirus and further cultured for one day in IMDM (Biochrome, Merk) supplemented with 50 ng/ml TPO, 10 ng/ml IL-1β, 10μg/ml polybrene and 2 mM valproic acid (VPA). Cells were seeded on an iMEF (GlobalStem, Rockville, USA) layer with VPA, 50 ng/ml TPO and IL-1β for 7 days. Day 10 post-transduction the medium was changed to E8 and iPSCs-like colonies were individually isolated between day 14–20 post-transduction. iPSC were maintained on Matrigel (BD Biosciences) in E8 medium following the manufactures instructions.

2.5. Karyotyping

Cells were treated with Demecolcine solution (10 μg/ml in HBSS), and processed with standard methods. Giemsa-banded karyotyping was performed and a minimum of 10 metaphases were analyzed. The chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN), (2016).

2.6. Teratoma formation

Cells were harvested using TripLE Select or trypsin. $1–3 \times 10^6$ human IPSC and $1–2.10^6$ OP9 cells were mixed together with Matrigel (BD bioscience) prior subcutaneous injection into 8–9 weeks old NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ). The teratoma formation ability of iPSCs was recorded and upon reaching 15 mm diameter teratomas were excised and further processed for histopathology analyses. Teratomas were stained for pan-Cytokeratin, α-SMA and βIII-Tubulin all from eBioscience (San Diego, California, USA).

2.7. Alkaline phosphatase staining

Alkaline phosphatase (AP) staining was performed by alkaline phosphatase live stain (Thermo Fisher Scientific). In short, the cultured iPSCs were washed with DMEM/F-12 prior staining. E8 was added on plates with AP live stain (500×) and incubated for 30 min, then washed 2× with DMEM/F-12 and visualized on EVOS-FL.

2.8. Flow cytometry

IPSC single cell suspensions were made using TrypLE Select, washed in PBS then re-suspended in PBS-BSA (0.1%). Anti-TRA1-80-FITC (1:100; Stem cell technologies) or anti-TRA1-61-APC (1:100; Millipore, Amsterdam, The Netherlands) were incubated with iPSC for 30 min at 4 °C. Intracellular staining for OCT4, SOX2, SSEA4 were performed according to manufacturer instructions (R&D Systems, Abingdon, United Kingdom). Cells were washed and measured on an LSR-II (BD Bioscience) and analyzed using Flowjo software (Flowjo, Ashland, USA).

References

Heideveld, E., et al., 2015. CD14+ cells from peripheral blood positively regulate hematopoietic stem and progenitor cell survival resulting in increased erythroid yield. Haematologica 100 (11), 1396–1406.

ISCN, 2016. In: McGowan-Jordan, J., Simons, A., Schmid, M. (Eds.), An International System for Human Cytogenomic Nomenclature (2016). S. Karger AG, Basel, Switzerland.

Voelkel, C., et al., 2010. Protein transduction from retroviral Gag precursors. Proc. Natl. Acad. Sci. U. S. A. 107 (17), 7805–7810.

Warlich, E., et al., 2011. Lentiviral vector design and imaging approaches to visualize the early stages of cellular reprogramming. Mol. Ther. 19 (4), 782–789.