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Lab Resource: Single Cell Line

Generation of DMBi002-A human induced pluripotent stem cell line from patient with Spinal muscular atrophy type 3

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A B S T R A C T

Spinal Muscular Atrophy (SMA) is a genetic neuromuscular disease caused by mutations in*SMN1* gene encoding survival motor neuron (SMN) protein. Lack of this protein leads to progressive loss of motor neurons and therefore to gradual loss of signal transmission between motor neurons and skeletal muscle cells. As a consequence, patients develop muscle atrophy and lose the ability to move independently, what is also related to problems with breathing and swallowing. Here, we describe the generation of human induced pluripotent stem cells (hiPSC) from peripheral blood mononuclear cells (PBMC) of adult SMA type 3 patient with a use of Sendai virus vectors.

1. Resource table

Unique stem cell line identifier	**DMBi002-A**https://hpscreg.eu/cell-line/DMBi002-A
Alternative name(s) of stem cell line	*SMA6.26*
Institution	*Jagiellonian University in Krakow, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology*
Contact information of distributor	*Prof. Józef Dulak, PhD, DSc.; jozef.dulak@uj.edu.pl*
Type of cell line	*hiPSC*
Origin	*human*
Additional origin info requiredfor human ESC or iPSC	*Age: 32* *Sex: Male* *Ethnicity if known: Caucasian*
Cell Source	*Peripheral blood mononuclear cells*
Clonality	*Clonal*
Associated disease	*Spinal muscular atrophy (SMA)*
Gene/locus	*SMN1*
Date archived/stock date	*21.07.2021*
Cell line repository/bank	hPSCreg, https://hpscreg.eu/cell-line/DMBi002-A
Ethical approval	*Warsaw Medical University Bioethical Committee, approval No. KB/120/2020 Agreement of the Ministry of the Environment for the use of GMO/GMM (decision 41/2016)*

2. Resource utility

This hiPSC cell line was generated in order to study the mechanisms of neuromuscular junction disruption in patients with spinal muscular atrophy (SMA) type 3. These cells will be differentiated into skeletal muscle cells and motor neurons and will be used for omics analyses and drug testing.

3. Resource details

Spinal Muscular Atrophy (SMA) is a genetic neuromuscular disorder caused by mutations in SMN1 gene encoding survival motor neuron (SMN) protein. Lack of this protein leads to progressive loss of motor neurons and therefore to gradual loss of signal transmission between motor neurons and skeletal muscle cells. As a consequence, patients develop muscle atrophy and lose the ability to move independently, what is also related to problems with breathing and swallowing. It is claimed, that the copy number of similar gene SMN2, which also encodes SMN can be a predictor of disease severity, as patients with milder types of SMA – SMA2 and SMA3 usually have more SMN2 copy numbers (Wirth et al., 2006; Tiziano et al., 2007; Elsheikh et al., 2009). Due to the single change of nucleotides in exon 7 of SMN2 gene the sequence of exonic splice enhancer is modified and exon 7 is excluded from majority of SMN transcripts as a result of alternative splicing. Therefore, only small amount (~10–20%) of functional protein can be produced by SMN2 gene.

In this study, hiPSC were generated from PBMC isolated from 32-years old male donor upon obtaining an informed consent. Reprogramming of

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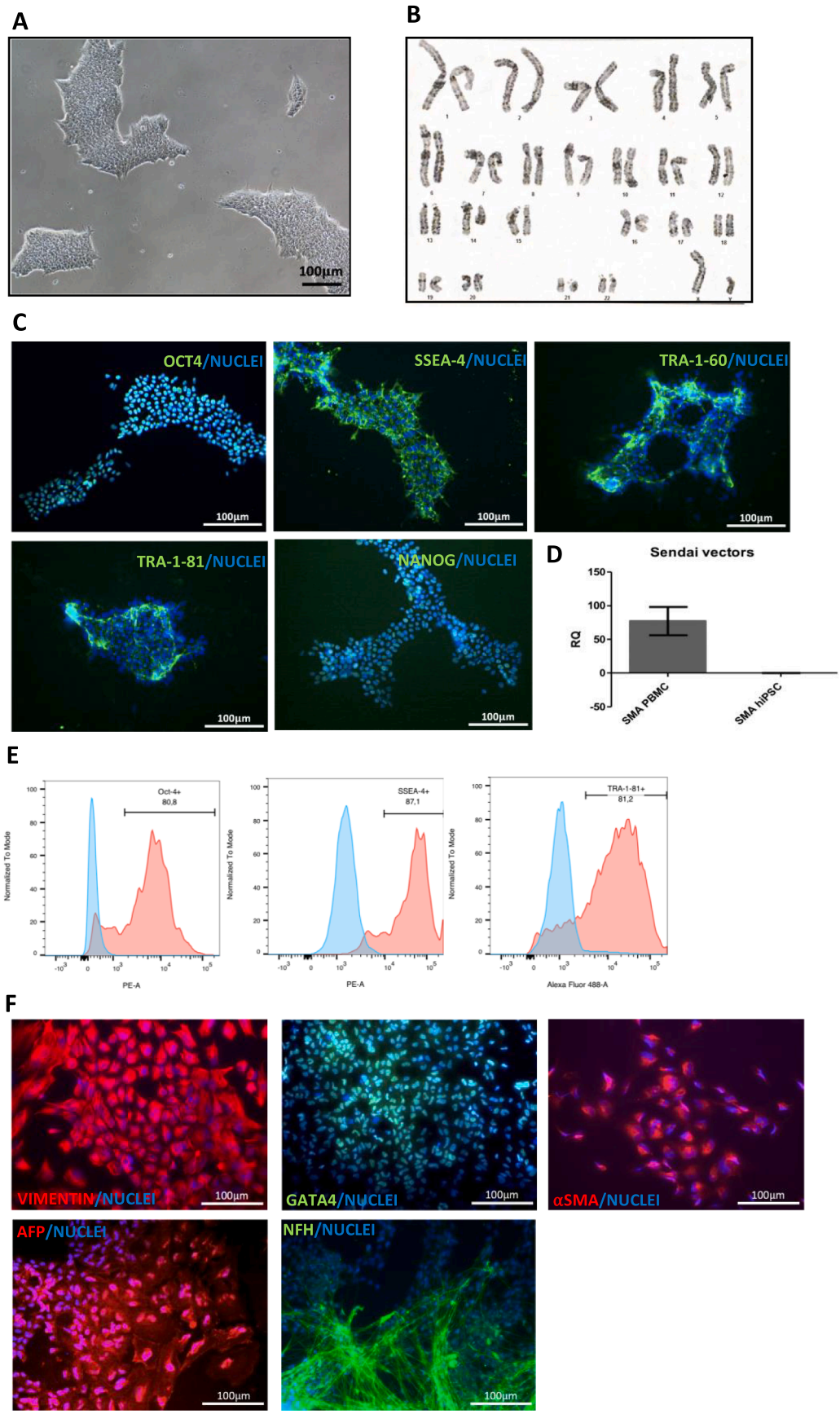

Fig. 1. Characterization of DMBi002-A hiPSC cell line. A. Picture of hiPSC colonies showing normal cell morphology; B. Karyotype showing lack of abnormalities in ploidy; C. Pictures of hiPSC stained for makers of pluripotency; D. qPCR results confirming lack of Sendai virus vectors in generated hiPSC; E. Quantitative flow cytometry analysis of pluripotency markers-positive hiPSC; F. Pictures of hiPSC-derived embryoid bodies stained for markers of all three germ layers.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field *[mandatory]*	*normal*	Fig. 1 *panel A*
Phenotype	Qualitative analysis *(Immunocytochemistry) [mandatory]*	*hiPSCs are positive for all tested markers: OCT4, SSEA-4, TRA-1–60, TRA-1–81 and NANOG*	Fig. 1 *panel C*
	Quantitative analysis *(flow cytometry analysis)[mandatory]*	*Flow cytometry analysis revealed that in generated hiPSC line more than 70% cells are positive for OCT4 (80,8%), SSEA-4 (87,1%), and TRA-1–81 (81,2%)*	Fig. 1 *panel E*
Genotype	Karyotype (G-banding) and resolution *[mandatory]*	*46XY, Resolution 450–500*	Fig. 1 *panel B*
Identity	Microsatellite PCR (mPCR) OR	*Not performed*	*N/A*
	STR analysis *[mandatory]*	*10 sites tested, all matched*	*Supplementary File 2*
Mutation analysis (IF APPLICABLE)	Sequencing	*No copies of SMN1 exon7, 1 copy of SMN1 exon 8, 4 copies of SMN2 exon 7, 3 copies of SMN2 exon 8*	*genetic analysis performed for diagnostic purpose, with authors*
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma *[mandatory]*	*Mycoplasma testing by* PCR. *Negative*	*Supplementary File 1*
Differentiation potential	Embryoid body formation*[mandatory]*	*Embryoid bodies subjected to spontaneous differentiation formed three germ layers as confirmed by staining for GATA4, vimentin, α-SMA, AFP and NFH (neurofilament heavy chain)*	Fig. 1 *panel F*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	N/A
	HLA tissue typing	*N/A*	N/A

PBMC was performed using commercially available non-integrating Sendai vectors (CytoTune-iPS 2.0). Obtained DMBi002-A line showed typical morphology of normal undifferentiated hiPSC (Fig. 1A). Subsequently, it was assessed for the expression of pluripotency markers on protein level as well as for the potential to spontaneously differentiate via embryoid bodies into cells originating from all three germ layers. Immunocytochemical analyses confirmed presence of pluripotency markers (OCT4, SSEA-4, TRA-1-60, TRA-1-81 and NANOG) in DMBi002-A line (Fig. 1C) as well as markers of all three germ layers-derived cells (GATA-4, α-SMA, AFP, NFH and vimentin) upon its spontaneous differentiation (Fig. 1F). Quantitative flow cytometry analysis demonstrated 80,8 % of OCT4-positive, 87,1% of SSEA-4-positive and 81,2% of TRA-1–81-positive cells within DMBi002-A colonies (Fig. 1E). The Sendai vectors were not detected in generated line (Fig. 1D). Additionally, karyotyping did not reveal any changes in ploidy (Fig. 1B). Presence of mutation was confirmed by medical genetic examination commissioned by the patient's physicians during diagnostics (Table 1). *Mycoplasma testing revealed no contamination of DMBi002-A line (Supplementary File 1).*

4. Materials and methods

PBMC isolation: PBMC were isolated with a use of BD Vacutainer® cellular preparation Tubes™ according to the manufacturer's instruction.

PBMC reprogramming: 200.000 PBMC were transduced with Sendai vectors (MOI: hKlf4 = 3, hc-Myc = 5 , KOS = 5) in PBMC medium accordingly to the manufacturer's instruction (CytoTune-iPS 2.0). After 24 h the medium was refreshed and cells were cultured for next 48 h. Three days after transduction PBMC were seeded into Geltrex™-coated wells and adapted to Essential 8™ (E8) medium on day 7. Henceforth, the E8 medium was changed daily and around the 15th day hiPSC colonies were collected to separate wells.

*hiPSC culture: hiPSC were cultured on Geltrex™-coated plates in E8 medium refreshed daily. Passaging was performed when the cells reached 70% confluency using 0.5 mM EDTA. ROCK inhibitor (*10 μ*M, Abcam) was added to the cell culture for 24 h after each passage.*

Embryoid bodies formation and differentiation: 3000 hiPSC were seeded into non-adherent U-shaped 96-well plate in Essential 6 medium. After three days, created embryoid bodies were transferred into Geltrex™-coated wells and subjected to spontaneous differentiation for two weeks.

Immunofluorescent staining: Prior to staining, cells were fixed with 4% paraformaldehyde for 15 min. Subsequently, they were washed with PBS and permeabilized in 0,1% Triton X-100 for 15 min. Afterwards cells were washed with PBS and blocked in 3% bovine serum albumin for 1 h at room temperature (RT). Primary antibodies were diluted in blocking solution accordingly to information in Table 2 *and incubated overnight in 4 °C. Next day, cells were washed in PBS and secondary antibodies (*Table 2*) were added for 1 h (RT). Nuclei were stained with 0.2 ug/mL DAPI. For flow cytometry analysis primary antibodies were incubated for 30* min *and secondary antibodies for 20* min *(both at 4 °C). Analysis was performed using LSR Fortessa cytometer (BD) and FlowJo software .*

RNA isolation, reverse transcription and qPCR: RNA was isolated using GTC extraction method. 300 μg *of RNA (concentration and purity determined by NanoDrop 1000) was transcribed to cDNA with a use of Revert Aid Reverse Transcriptase according to manufacturer's protocol. Reaction mixture contained* 3 μL *of cDNA (diluted 5x after reverse transcription),* 0.75 μL *of each primer (listed in* Table 2.*),* 7.5 μL *of SybrGreen Mix (Sigma-Aldrich) and* 3 μL *of water and was performed on StepOnePlus™ Real-time PCR System.*

STR: STR analysis was performed in Human Genome Variation Research Group (Małopolska Centre of Biotechnology, Jagiellonian University, Poland) using DNA isolated (using Genomic Mini kit, A&A Biotechnology) from DMBi002-A line and parental PBMC used for reprogramming.

Kariotype: Karyotype analysis was performed using G-banding method by Kariogen laboratory (Krakow, Poland).

Mycoplasma detection: Mycoplasma testing was performed by PCR using primers listed in(Table 2) *and KAPA2G Fast Genotyping Mix (Merck). Reaction was run on Applied Biosystems™ ProFlex™ PCR System (accordingly to reaction set-up provided in KAPA mix manufacturer's instruction).*

Cell culture was performed under standard conditions (37C, 5% CO_2, 20% O_2). All experiments were performed on hiPSC at passage between 15 and 20.

Unless stated otherwise all reagents were purchased from Thermo Fisher Scientific.

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Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	*Goat anti-OCT3/4*	*1:200*	*Santa Cruz Biotechnology Cat# 8628*	*RRID:AB_653551*
Pluripotency Markers	*PE anti-OCT3/4*	*1:500*	*BioLegend #653703*	*RRID:AB_2562017*
Pluripotency Markers	*Mouse anti-SSEA4*	*1:200*	*Santa Cruz Biotechnology Cat# sc-21704*	*RRID:AB_628289*
Pluripotency Markers	*Mouse anti-TRA-1-60*	*1:200*	*Sigma Aldrich # MAB4360*	*RRID:AB_2119183*
Pluripotency Markers	*Mouse anti-TRA-1-81*	*1:200*	*Sigma Aldrich # MAB4381*	*RRID:AB_177638*
Pluripotency Markers	*Mouse anti-NANOG*	*1:200*	*Santa Cruz Biotechnology Cat# sc-293121*	*RRID:AB_2665475*
Differentiation Markers	*Mouse anti-GATA4*	*1:200*	*Santa Cruz Biotechnology Cat# sc-25310*	*RRID:AB_627667*
Differentiation Markers	*Rabbit anti- αSMA*	*1:200*	*Abcam Cat# ab5694*	*RRID:AB_2223021*
Differentiation Markers	*Rabbit anti-vimentin*	*1:200*	*Abcam Cat# ab92547*	*RRID:AB_10562134*
Differentiation Markers	*Rabbit anti-NFH*	*1:400*	*Abcam Cat # ab8135*	*RRID:AB_306298*
Differentiation Markers	*Goat anti-AFP*	*1:200*	*Santa Cruz Biotechnology Cat# 8108*	*RRID:AB_633815*
Secondary antibodies	*AF488 Goat Anti-Mouse IgG*	*1:400*	*ThermoFisher Scientific #A11001*	*RRID:AB_2534069*
Secondary antibodies	*AF488 Rabbit Anti-Goat IgG*	*1:400*	*ThermoFisher Scientific #A11078*	*RRID: AB_2534122*
Secondary antibodies	*AF568 Goat Anti-Rabbit IgG*	*1:400*	*ThermoFisher Scientific #A11036*	*RRID: AB_10563566*
Secondary antibodies	*AF488 Donkey Anti-Rabbit IgG*	*1:400*	*ThermoFisher Scientific #A21206*	*RRID:AB_2535792*
Secondary antibodies	*AF568 Donkey Anti-Goat IgG*	*1:400*	*Thermo Fisher Scientific #A11057*	*RRID:AB_142581*
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Sendai vectors (qPCR)	*Sendai vectors*	*181 bp*	*GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC*	
House-Keeping Genes (qPCR)	*EEF2*	*214 bp*	*TCAGCACACTGGCATAGAGG/GACATCACCAAGGGTGTGCA*	
Mycoplasma testing	*Mycoplasma* spp.	*715 bp*	*ACTCCTACGGGAGGCAGCAGTA/ACTCCTACGGGAGGCAGCAGTA*	

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102563.

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