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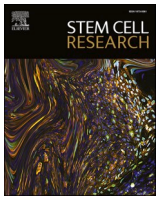

Generation of two induced pluripotent stem cell lines from psoriatic patient with cardiovascular comorbidity

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A B S T R A C T

Psoriasis (Ps) is a chronic, inflammatory skin disease characterized by thickened, red and scaly plaques. Systemic inflammation associated with psoriasis results in an increased risk of death due to the development of psoriasis-associated comorbidities such as cardiovascular disease (CVD) and metabolic syndrome. Although the cardiometabolic features in psoriasis are clinically well described, the underlying molecular mechanisms linking these comorbidities remain poorly understood. Generation of induced pluripotent stem cells (hiPSCs) from peripheral blood mononuclear cells (PBMCs) and skin fibroblasts (SFs) of psoriatic patients provides a novel approach to investigate the pathway by which cutaneous inflammation promotes CV complications in this disorder.

1. Resource table

Unique stem cell lines identifier	1. DMBi007-A https://hpscreg.eu/cell-line/DMBi007-A DMB008-A https://hpscreg.eu/cell-line/DMBi008-A
Alternative name(s) of stem cell lines	2. 30.8 (DMBi007-A) 300.25 (DMBi008-A)
Institution	Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
Contact information of distributor	Monika Biniecka, PhD, DSc.; monika.biniecka@uj.edu.pl
Type of cell lines	hiPSCs
Origin	human
Additional origin info required for human ESCs or iPSCs	Age: 72 Sex: Female Ethnicity if known: Caucasian
Cell Source	1. Peripheral blood mononuclear cells (PBMCs)Skin fibroblasts (SFs)
Clonality	Clonal
Method of reprogramming	Sendai virus encoding transgenes hOct3/4, hSox2, hKlf4, and hc-Myc

(continued on next column)

(continued)

Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Psoriasis
Gene/locus	N/A
Date archived/stock date	DMBi007-A, October 2022; DMBi008-A, January 2023
Cell line repository/bank	hPSCreg: https://hpscreg.eu/cell-line/DMBi007-A https://hpscreg.eu/cell-line/DMBi008-A
Ethical approval	Wrocław Medical University Bioethical Committee approval no. KB-110/2020

2. Resource utility

hiPSC lines obtained from PBMCs and SFs of psoriatic patient having associated cardiometabolic complications provide a platform for the derivation of cardiac lineage differentiated cells, like cardiomyocytes

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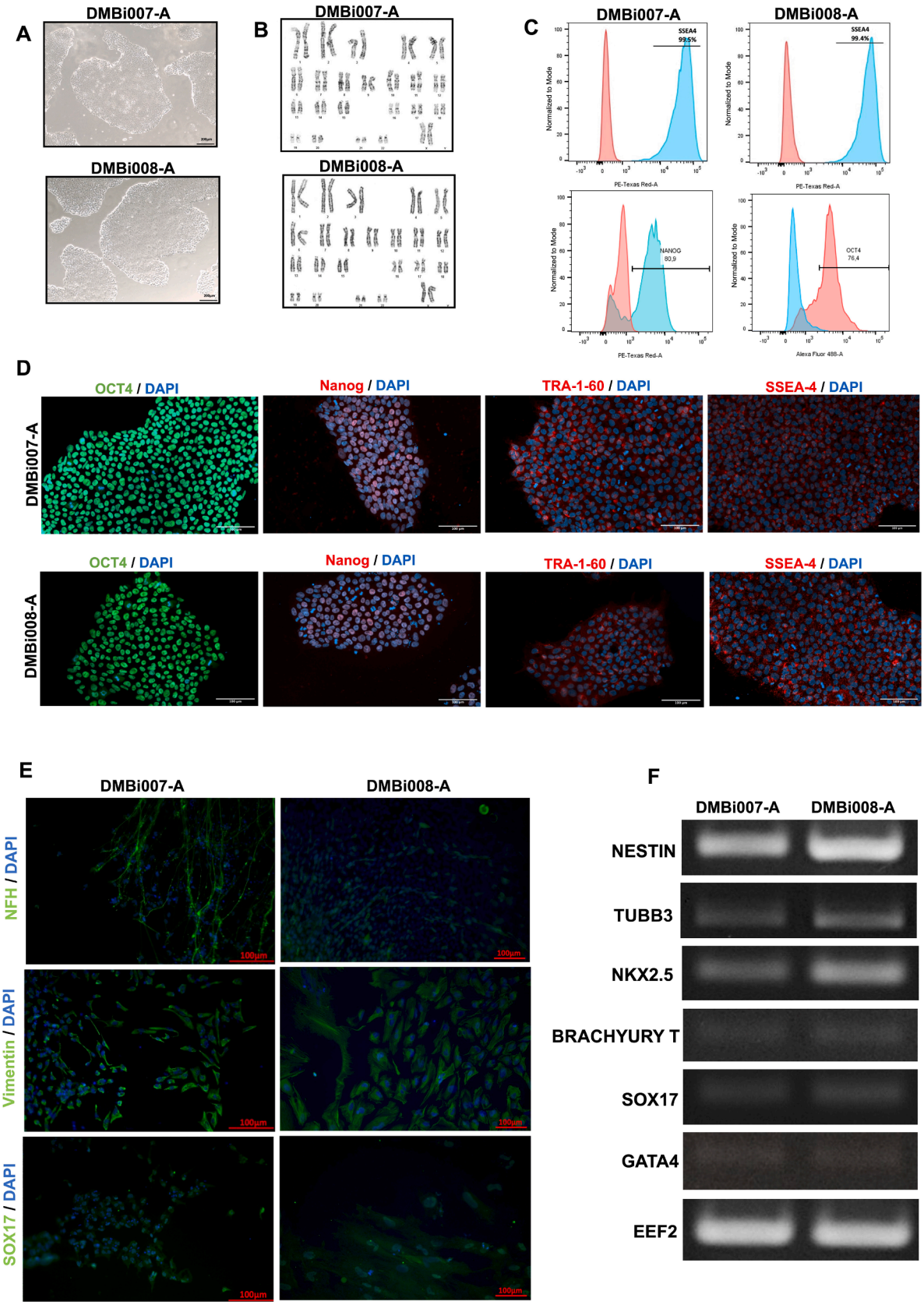
A
DMBi007-A
DMBi008-A
B
DMBi007-A
DMBi008-A
C
DMBi007-A
DMBi008-A
SSEA4
99.5%
99.4%
Normalized to Mode
PE-Texas Red-A
NANOG
80.9
OCT4
76.4
Alexa Fluor 488-A
D
OCT4 / DAPI
Nanog / DAPI
TRA-1-60 / DAPI
SSEA-4 / DAPI
DMBi007-A
DMBi008-A
E
DMBi007-A
DMBi008-A
NFH / DAPI
Vimentin / DAPI
SOX17 / DAPI
100µm
F
DMBi007-A
DMBi008-A
NESTIN
TUBB3
NKX2.5
BRACHYURY T
SOX17
GATA4
EEF2

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	normal	Fig. 1 panel A
Phenotype	Qualitative analysis *(Immunocytochemistry)*	DMBi007-A and DMBi008-A lines expressed the pluripotency markers: OCT4, NANOG, TRA-1–60 and SSEA-4	Fig. 1 panel D
	Quantitative analysis *(Flow cytometry analysis)*	DMBi007-A line expressed SSEA-4 (99.5 %) and NANOG (80.9 %) DMBi008-A line expressed SSEA-4 (99.4 %) and OCT4 (76.4 %)	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	Both lines 46XX, Resolution 450–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	10 sites tested, all matched	Supplementary file 2
Mutation analysis *(IF APPLICABLE)*	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	PCR-based Negative	Supplementary file 1B
Differentiation potential	Embryoid body formation	DMBi007-A and DMBi008-A lines differentiated into the three germ layers including ectoderm (NFH, NESTIN, TUBB3), mesoderm (vimentin, NKX 2.5, BRACHYURY T), and endoderm (SOX17, GATA4)	IF: Fig. 1 panel E RT-PCR: 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

and endothelial cells. This will constitute an *in vitro* model to investigate heart and endothelial dysfunctions in psoriasis, Ps-oriented drug discovery, and toxicology.

3. Resource details

Psoriasis is an immune-mediated inflammatory skin condition characterized by the spontaneous development of skin lesions manifesting as red plaques with a silvery-white scale (Griffiths and Barker, 2007, Nestle et al., 2009), resulting from a combination of genetic, immunological, and environmental factors (Menter and Griffiths, 2015). Psoriatic immune response enhances activation of plasmacytoid dendritic cells, myeloid cells and T cells, leading to up-regulated secretion of type I interferons, TNF-α, and interleukins (ILs) IL-23, IL-17, and IL-6, which are linked to vascular inflammation and development of atherosclerosis. Indeed, it is well established that psoriatic patients, especially those with >10 % body surface area affected, or a long duration of the disease are at increased risk for CV complications including myocardial infarction, stroke, and cardiovascular death. Furthermore, psoriasis is associated with a greater prevalence of CV risk factors, such as hypertension, diabetes mellitus, dyslipidemia, obesity, and the metabolic syndrome (Yamazaki, 2021).

Previously, PBMCs from psoriatic patients were reprogrammed to obtain hiPSCs (Vallejo-Diez et al., 2020). In our study, we have successfully generated and characterized two hiPSC lines from PBMCs and SFs obtained from psoriatic donor. PBMC-derived hiPSCs and SF-derived hiPSCs can be subsequently differentiated into a broad range of patient-specific cell types, like cardiomyocytes (CMs), endothelial cells (ECs), and cardiac fibroblasts (CFs). Therefore, using Ps-specific hiPSC-CMs/ECs/CFs will provide a unique opportunity to investigate the relationship between psoriasis and cardiovascular complications at the preclinical level.

We utilized commercially available Sendai virus containing Yamanaka transcription factors (CytoTune-iPS 2.0) to generate two hiPSC lines (DMBi007-A and DMBi008-A) from one donor – 72 years old psoriatic female with high Psoriasis Area and Severity Index (PASI = 25) (approval by Wrocław Medical University Bioethical Committee, no. KB 110/2020). Importantly, this patient developed psoriasis-associated comorbidities, including atrial fibrillation, hypertension, and hypercholesterolemia. DMBi007-A line was generated from PBMCs, and DMBi008-A line was generated from SFs. DMBi007-A and DMBi008-A lines demonstrated typical colony-like morphology of hiPSCs (Fig. 1A) and normal chromosome count (Fig. 1B, Table 1). Furthermore, both lines were confirmed to be free from Sendai virus genome (passage numbers 5–10) (Supplementary file 1A) and showed no presence of mycoplasma contamination (Table 1, Supplementary file 1B). The pluripotent-like state of generated hiPSC lines was confirmed by quantitative flow cytometry, where specific expression of SSEA-4, NANOG, and OCT4 was more than 75 % of positive cells within DMBi007-A and DMBi008-A colonies (Fig. 1C, Table 1) and by immunofluorescent analysis of OCT4, NANOG, TRA-1–60, and SSEA-4 (Fig. 1D, Table 1). Next, spontaneous differentiation *via* embryoid bodies (EBs) was examined to prove the differentiation potential of hiPSCs into cells originating from three germ layers. The expression of ectoderm markers (NFH, NESTIN, TUBB3), mesoderm markers (Vimentin, NKX2.5, BRACHYURY T), and endoderm markers (SOX17, GATA4) in the differentiated cells was shown by immunocytochemistry (Fig. 1E, Table 1) and RT-PCR (Fig. 1F, Table 1). Authentication of the generated hiPSC lines was verified using short tandem repeat (STR) analysis and found identical to their parental PBMCs and SF (Supplementary file 2). All data for the characterization of hiPSC lines are summarized in Tables 1 and 2.

4. Materials and methods

4.1. PBMCs isolation and culture

PBMCs were isolated using BD Vacutainer mononuclear cell preparation tubes and cultured in StemPro-34 SFM medium with L-Glutamine (2 mM),

SCF (100 ng/mL), FLT-3 (100 ng/mL), IL-3 (20 ng/mL) and IL-6 (20 ng/mL) for 4 days.

4.2. Skin fibroblasts isolation and culture

Skin biopsies were digested with the Whole Skin Dissociation Kit (Miltenyi Biotech), then processed with gentleMACS™ Dissociator (Miltenyi Biotech). Released cells were cultured with fresh DMEM medium containing 10 % FBS, penicillin, streptomycin, and amphotericin B.

4.3. Reprogramming

PBMCs (150.000) and skin fibroblasts (100.000) were transduced with Sendai Virus vectors (CytoTune-iPS 2.0 Sendai Reprogramming

Table 2
Reagents details.

	ANTIBODIES USED FOR IMMUNOCYTOCHEMISTRY / FLOW-CYTOMETRY			
	Antibody	**Dilution**	**Company Cat#**	**RRID**
Pluripotency marker	Goat anti-OCT4	1:200	Santa Cruz Biotechnology Cat# sc-8628	RRID: AB_653551
Pluripotency marker	Mouse anti-NANOG	1:200	Santa Cruz Biotechnology Cat# sc-293121	RRID: AB_2665475
Pluripotency marker	Mouse anti-TRA-1–60	1:400	Thermo Fisher Scientific Cat# MA1023	RRID: AB_2536699
Pluripotency marker	Mouse anti-SSEA4	1:200	Sigma-Aldrich, #MAB4304	RRID: AB_177629
Differentiation marker	Rabbit anti-NFH	1:200	Abcam Cat# ab8135	RRID: AB_306298
Differentiation marker	Rabbit anti-vimentin	1:200	Abcam Cat# ab92547	RRID: AB_10562134
Differentiation marker	Mouse anti-SOX17	1:50	R&D Systems Cat# MAB1924	RRID: AB_2195646
Secondary antibody	AF488 Rabbit Anti-Goat IgG	1:400	Thermo Fisher Scientific Cat# A-11078	RRID: AB_2534122
Secondary antibody	AF488 Goat Anti-Rabbit IgG	1:400	Thermo Fisher Scientific Cat# A-11034	RRID: AB_2576217
Secondary antibody	AF488 Goat Anti-Mouse IgG	1:400	Thermo Fisher Scientific Cat# A-11001	RRID: AB_2534069
Secondary antibody	AF568 Goat Anti-Mouse IgG	1:400	Thermo Fisher Scientific Cat# A-11004	RRID: AB_2534072

PRIMERS			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Ectodermal derivative	*Nestin*	206 bp	GTAGCTCCCAGAGAGGGGAA/ TCTAGAGGGCCAGGGACTT
Ectodermal derivative	*TUBB3*	397 bp	TCTTCCCTCGTGGAGACAGG/ TCGAGGCACGTACTTGTG
Mesodermal derivative	*NKX2.5*	113 bp	CAACATGACCCTGAGTCCCC/ TAATCGCCGCCACAAACTCT
Mesodermal derivative	*Brachyury T*	148 bp	GGGTACTCCCAATCCTATTCTGAC/ CTGACTGGAGCTGGTAGGT
Endodermal derivative	*SOX17*	140 bp	TCATAAGCTTGACCTGCCCC/ TTAAACCCAGCGATGCTTGC
Endodermal derivative	*GATA4*	238 bp	TGTGCAGAGTTTGCCTCACA/ AGAACGAATGCCGAGTCCTG
House-keeping gene	*EEF2*	173 bp	GCAAGTTCAGCAAGTCAGCC/ CTTGTCCTCGCTGTCCAGTT
Mycoplasma testing	*Mycoplasma* spp.	715 bp	ACTCCTACGGGAGGCAGCAGTA/ TGCACCATCTGTCACTCTGTTAACCTC
Sendai Vectors	*Sendai vectors*	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC

Kit). After 24 h the medium was replaced, and cells were kept in culture. Three days (PBMCs)/seven days (SFs) after transduction, cells were seeded onto Geltrex™-coated wells. From day 7, the cells were cultured in Essential 8™ (E8) medium. Medium was replaced every day and the cells were observed for the formation of mature colonies resembling human embryonic stem cells. Then the colonies were picked and transferred into another well-plate.

4.4. hiPSCs culture

hiPSCs were cultured on Geltrex™-coated plates in E8 medium with daily medium replacement. Cells were passaged at confluency 80–90 % using EDTA (0.5 mM) and cultured in E8 with the addition of 10 μM Y-27632 (Rho kinase inhibitor, Abcam) for 24 h. Cells were cultured at 37 °C in 5 % CO_2.

4.5. Germ layer differentiation

20.000 of hiPSCs were seeded into a non-adherent U-shaped 96-well plate in Essential 6 medium (E6) supplemented with 10 μM Y-27632. On the next day, the media was replaced with fresh E6, and cells were cultured for 7 days to form EBs which were transferred on Geltrex™-coated wells and observed for spontaneous differentiation of the cells.

4.6. Immunocytochemistry

hiPSCs and EBs were fixed with 4 % PFA, permeabilized with 0.1 % Triton X-100, and blocked with 3 % bovine serum albumin (BSA) (1 h/RT). Cells were treated with primary antibodies diluted in BSA (as specified in Table 2) and incubated overnight at 4 °C.

Next, cells were washed with PBS, incubated with secondary antibodies (1 h/RT) (Table 2), and counterstained with 0.2 μg/mL DAPI (Sigma-Aldrich). Cells were imaged and analyzed in Axio Observer (Zeiss).

4.7. Flow cytometry

hiPSCs were fixed with 4 % PFA and permeabilized with 0.1 % Triton X-100, followed by staining with primary antibodies (1 h/RT) and secondary antibodies (30 min/RT) (Table 2). Cells were counterstained with 0.2 μg/ml DAPI and analyzed with LSRFortessa cytometer and FlowJo software.

4.8. PCR

RNA was isolated (Qiagen) and transcribed with RevertAid Reverse Transcriptase. 50 ng cDNA was used for each PCR reaction with KAPA2G Fast Genotyping Mix (Merck). PCR reaction was done on Applied Biosystems ProFlex thermocycler using specific primers (Table 2).

4.9. STR analysis

DNA was isolated from hiPSC lines, parental PBMCs, and parental skin fibroblasts. STR was performed at Malopolska Centre of Biotechnology, Kraków, Poland.

4.10. Karyotyping

Karyotyping was performed on hiPSC lines using the G-banding method (GTG-450, 15 mitoses/sample) (Kariogen, Poland). Karyotype analyses were performed on iPSC lines at passage numbers 10–15.

4.11. Mycoplasma detection

DNA was polymerized with KAPA2G Fast Genotyping Mix (Merck), and PCR was carried out with specific primers (Table 2). Products were electrophoresed on

2 % agarose gel.

All reagents were purchased from ThermoFisher Scientific, unless otherwise specified.

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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.2023.103251.

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