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Generation of human induced pluripotent stem cell lines (iPSC) from adipose-derived mesenchymal stromal cells from two patients with systemic sclerosis

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ABSTRACT

Systemic sclerosis (SSc) is a rare and complex connective tissue disease associated with high morbidity and mortality. SSc is characterized by ischemic vasculopathy, cutaneous and visceral fibrosis and a dysimmune state (Denton and Khanna, 2017; Volkmann et al., 2023; Barnes and Mayes, 2012). We have derived induced pluripotent stem cell (iPSC) lines from two SSc patients aged 38 and 67 years with severe vascular phenotype. These iPSC lines expressed pluripotent markers, exhibited normal and stable genome, and differentiated into trilineage embryonic layers in teratoma formation assays. These SSc-specific iPSC lines can be differentiated into endothelial cells, providing a valuable model to elucidate vascular dysfunction and develop personalized therapeutic approaches.

1. Resource table

Alternative name(s) of stem cell lines	REGi011-A,REGi012-A
Institution	Safe IPS facility
Contact information of distributor	Mélanie VELIER melanie.velier@ap-hm.fr
Type of cell lines	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age: 38 and 67 Sex: Female
Cell Source	Adipose-derived mesenchymal stromal/stem cells
Clonality	Pool
Method of reprogramming	Sendai
Genetic Modification	YES
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RNA seq, Phosphatase alkaline, Cytometry

(continued on next column)

(continued)

Alternative name(s) of stem cell lines	REGi011-A,REGi012-A
Associated disease	Systemic sclerosis
Gene/locus	N/A
Date archived/stock date	31/07/2024
Cell line repository/bank	hPSCReg (https://hpscereg.eu/user/dashboard)
Ethical approval	CPP Sud Mediterranee V (ref 14.051) N° 2014-003023-22.

2. Resource utility

Vascular alterations represent an early and critical event in SSc. However, direct access to endothelial cells from SSc patients remains challenging (Denton and Khanna, 2017; Fioretto et al., 2023). The generation of iPSC from SSc patients offers an alternative to overcome

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this limitation, providing a valuable and renewable model for investigating the mechanisms driving microvascular dysfunction in SS.

3. Resource details

The experimental approach for the development of a cellular model of systemic sclerosis with induced pluripotent stem cells (iPSC) is outlined in Fig. 1A. Adipose-derived mesenchymal stromal cells (ASC) were reprogrammed using the Sendai virus gene delivery system to express

four key reprogramming factors—OCT4, SOX2, SSEA4 and NANO-G—without genomic integration. The pluripotency of both cell lines was demonstrated by subcutaneous injection and the subsequent formation of teratoma. Histologic analysis of these teratoma, stained with May-Grünwald Giemsa, confirmed the presence of all three germ layers (Fig. 1B). In addition, the pluripotency of iPSC lines M018 and M027 was confirmed by enzyme staining for alkaline phosphatase (Fig. 1C), and corroborated by flow cytometry analysis of the undifferentiated human iPSC markers, which revealed the expression of OCT4, SSEA4,

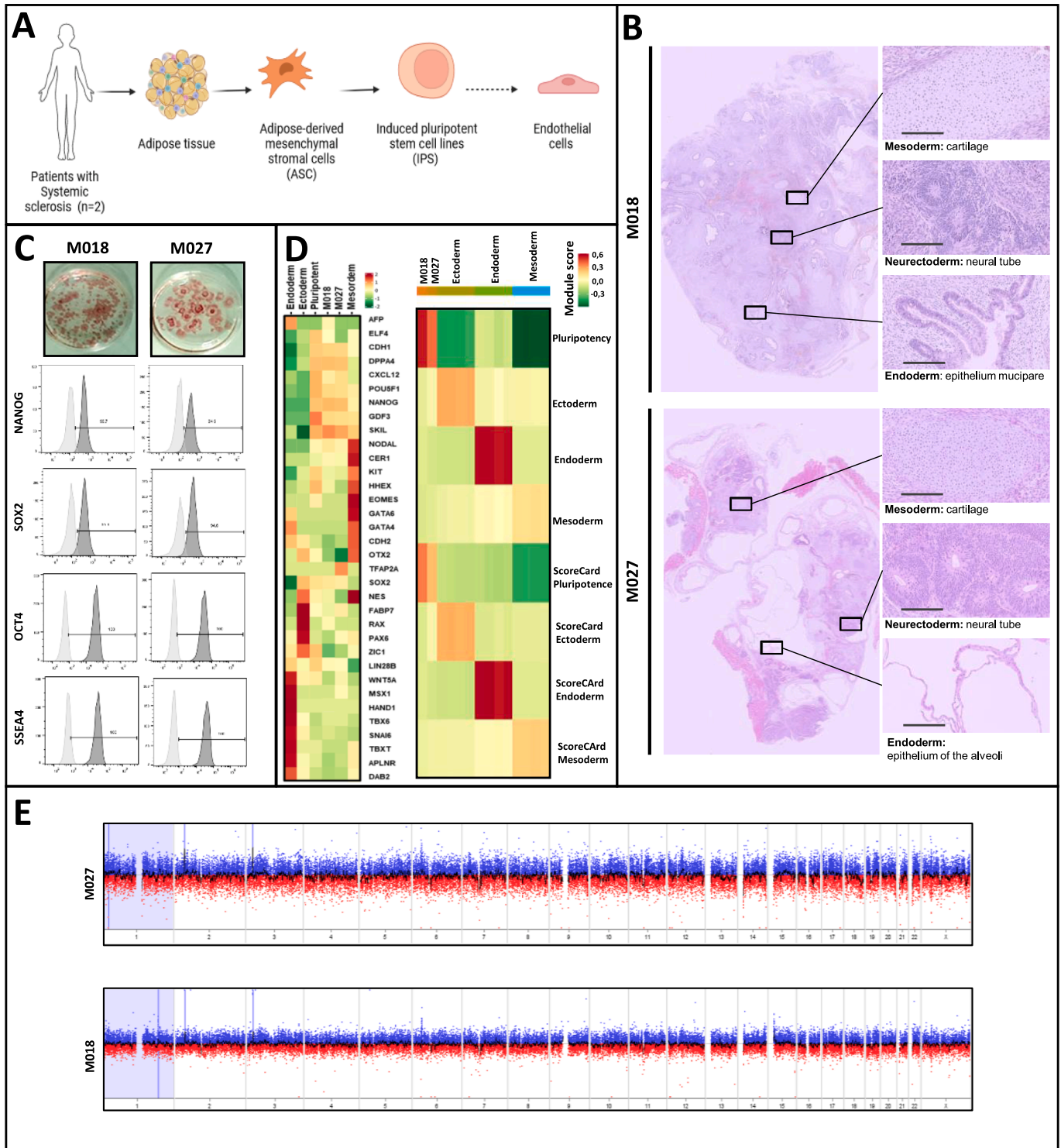


Fig. 1.

SOX2, and NANOG (Fig. 1C). The results of the transcriptome analysis of the two iPSC lines are shown in Fig. 1D: a heatmap shows the supervised clustering of genes involved in pluripotency (left panel), and RNA sequencing data of the iPSC used in the ScoreCard pluripotency assessment and Sequin platform (<https://sequin.ncats.io/app/>), which confirmed the pluripotent nature of the cells (right panel). Finally, the genomic stability of iPSC M018 and M027 was analyzed by CGH array (Fig. 1E), and no chromosomal abnormalities were detected. A summary of the characterization and validation of iPSC lines M018 and M027 can be found in Table 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Visual record of the lines is normal	Supplementary Fig. 1
Phenotype	Immunocytochemistry,	Assess staining/expression of undifferentiated human iPSC markers: Oct3/4, Nanog, Sox2, Tra 1–60, SSEA-4, SSEA-1, Tra-1–80.	Data not shown
	Quantitative analysis Flow cytometry	Assess % of positive cells or transcripts for antigen & cell surface markers e.g. Oct3/4: 97 %Tra 1–60: 98 %SSEA-4: 99 %SSEA-1: 6 %	Fig. 1 panel C
Genotype	CGH array	E.g. 46XX, Resolution 450–500	Fig. 1 panel E
Identity		DNA Profiling e.g. Performed/not performed	Supplementary Table1
	STR analysis	12 STR ATCC	e.g. submitted in archive with journal
Microbiology and virology Differentiation potential	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary-Table2
	Teratoma formation	Describe expression of genes in embryoid bodies: e.g. muscle actin, β -tubulin and α -feto protein. OR proof of three germ layers formation	Fig. 1 panel B
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer		<i>not shown but available with author</i>
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	<i>not shown but available with author</i>

4. Materials and methods

4.1. Reprogramming ASC to iPSC

ASC were isolated from two SSC patients using surgical residues obtained from routine lipotransfer procedures with informed consent provided for the scientific use of these samples. The collected lipooaspirate was washed and enzymatically digested at 37 °C for 45 min. The resulting cell suspension was then plated into a T75 flask to isolate ASC (adherent cells). The ASC were then reprogrammed using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, cat#A34546) containing Klf4, Oct3/4, Sox2, and c-Myc vectors, according to the manufacturer's protocol. The morphology of the IPS is presented in **Supplementary Fig. 1**.

4.2. Alkaline phosphatase activity

The cells were stained with Vector® Red AP substrate (Vector Laboratories kit #SK-5100) following the manufacturer's instructions. The culture media was removed before incubation of the cells with the substrate working solution for 20–30 min in the dark. The images are presented in **Fig. 1C**.

4.3. iPSC culture and genomic DNA extraction

The iPSC were cultured on hPSC-qualified Matrigel substrate as extracellular matrix (Fisher Scientific, cat#354277, lot number: 5194015), with a recommended dilution factor of 1:83. Cells were maintained in Essential 8™ culture media (Thermo Fisher Scientific, cat#A15169-01) following the manufacturer's instructions. Cultures were incubated at 37 °C under conditions of 5 % O₂ and 5 % CO₂. For cell dissociation, Versene solution (Thermo Fisher Scientific; cat#15040066) was used.

4.4. Flow cytometry analysis

For flow cytometry analysis, a single-cell suspension (at P12) for each cell line was fixed in a 4 % paraformaldehyde solution in phosphate-buffered saline (PBS). Following fixation, the cells were permeabilized and labelled according to the established protocols of the BD Stemflow Human and Mouse Pluripotent Stem Cell Analysis Kit (catalog no. 560477) and the BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit (catalog no. 560589). The staining of specific undifferentiated human iPSC markers was performed using dedicated antibodies. In the flow cytometry results, the white peaks correspond to IgG staining, while the black peaks represent specific markers, confirming the pluripotent status of the cells. Cell analysis was conducted using a Becton Dickinson CANTO II instrument, and the acquired data were processed using FlowJo software. The results are presented in **Fig. 1C** and the antibodies used are listed in **Table 2**.

4.5. RNA sequencing and supervised hierarchical clustering

Total RNA extraction was performed on 2 iPSC lines using the RNeasy kit (#74104, Qiagen) with on-column DNase digestion with the RNase-Free DNase Set (#79254, Qiagen) following the original manufacturer's protocol. RNA integrity was assessed using the RNA Screen Tape (Agilent 4150 TapeStation system). Bulk mRNA sequencing in paired-end mode (150 bp) was performed on BGISEQ sequencing platform by BGI Genomics. Filtered clean reads were aligned to the genome version reference sequence: homo_sapiens_9606.NCBI.GCF_000001405.40_GRCh38.p14.v2201, by HISAT, and then mapped to the reference genes using Bowtie2. Gene expression quantification was performed using RSEM software (v1.3.1) and heatmaps illustrating gene expression clustering across different samples were generated using pheatmap (v1.0.8). DESeq2 (v1.4.5) was used for differential gene

Table 2
Reagents details Characterization and validation.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers Flow cytometry	Pluripotency Markers Flow cytometry	1:200	Cell Signaling Technology Cat# 4903	RRID: AB_10559205
Pluripotency Markers Flow cytometry	Oct-4A Rabbit mAb (Clone C30A3) IgG	1:200	Cell Signaling Technology Cat# 2840	RRID: AB_2167691
Pluripotency Markers Flow cytometry	Sox2 XP® Rabbit mAb (Clone D6D9) IgG	1:200	Cell Signaling Technology Cat# 3579	RRID: AB_2195767
Pluripotency Markers Flow cytometry	SSEA4 Mouse mAb (Clone MC813) IgG3	1:200	Cell Signaling Technology Cat# 4755	RRID: AB_1264259
Pluripotency Markers Flow cytometry	TRA-1-60(S) Mouse mAb (Clone TRA-1-60(S)) IgM	1:200	Cell Signaling Technology Cat# 4746	RRID: AB_2119059
Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	
Sendai Virus detection	Sendai virus detection	181 bp	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAAGAGATATGTATC	
Sendai Virus detection	c-Myc detection	532 bp	F: TAACTGACTAGCAGGCTTGTCC R: TCCACATACAGTCCTGGATGATGATG	
Sendai Virus detection	Cluster KOS detection	528 bp	F: ATGCACCGCTACGACGTGAGCGC R: ACCTTGACAATCCTGATGTGG	

detection. A revised list of pluripotency genes was employed to generate the heatmap for supervised hierarchical clustering (Fig. 1D).

4.6. CGH array

Genomic DNA extracted from the iPSC line at passage 10 and was analyzed by ChromoStem facility of Montpellier using the chromosomal microarray was performed using SurePrint G3 Human CGH microarray Kit, 8 × 60 K with overall median probe 41 Kb genome-wide median probe spacing. Experiments were conducted according manufacturer's protocol. Commercial reference DNA (male and female) provided by Agilent Technologies were used for the analysis. Results are presented in Fig. 1E.

4.7. Sendai clearance

To confirm the absence of the Sendai virus, RNA was extracted from cells using the RNeasy kit (QIAGEN) according to the manufacturer's instructions. After 10 passages, of iPSC, extracted RNA was used as a matrix for RT-qPCR with 5x One-Step RT-PCR Mastermix (BioLabs). RT-PCR was conducted using primers provided with the Cytotune kit to detect the SeV backbone and OSKM genes under the following conditions: denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s for a total of 30–35 cycles. Results are presented in Supplementary Table 3.

4.8. Teratoma induction and histological analysis

The evaluation of differentiation potential was accomplished through in vivo teratoma formation. Clusters containing approximately 3 × 10⁶ iPSC cells were introduced into anesthetized NOD SCID gamma

(NOD.CgPrkdcscidll2rg tm1Wjl/SzJ) mice. Following a latency period of 4–8 weeks, a 100 % efficiency in teratoma formation was observed. These teratoma were subsequently fixed, embedded in paraffin blocks, subjected to Hematoxylin-Eosin-Saffron staining, and subsequently analysed by a pathologist. This analysis aimed to identify structures corresponding to the three primary embryonic germ layers (Fig. 1B).

4.9. Short tandem repeat analysis (STR)

Cell lines were authenticated using analysis performed on 17 STR. Genomic DNA from each iPSC was sent to ATCC (ATCC Manassas, Virginia 20110 USA).

4.10. Mycoplasma detection

To detect mycoplasma, the MycoAlert® Detection Kit (Lonza) was used according to manufacturer's instructions. Cells culture was done without any antibiotic affecting mycoplasma growth. Results are presented in Supplementary Table 2.

CRediT authorship contribution statement

Mélanie Velier: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Romain Appay:** Methodology, Data curation. **Robin Arcani:** Conceptualization. **Romain Desprat:** Methodology, Data curation. **Stéphanie Simoncini:** Conceptualization. **Anouck Zavarro:** Conceptualization. **Danièle Noel:** Writing – review & editing. **Lenha Bendaass:** Conceptualization. **Christian Jorgensen:** Supervision. **Quentin Gomes De Pinho:** Conceptualization. **Audrey Benyamine:** Conceptualization. **Brigitte Granel:** Writing – review & editing, Conceptualization. **Aurélie Dumas:** Conceptualization. **Francoise Dignat George:** Supervision. **Florence Sabatier:** Validation, Supervision, Conceptualization. **Jérémy Magalon:** Validation, Supervision, Conceptualization.

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.2024.103624>.

Data availability

Data will be made available on request.

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