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Human induced pluripotent stem cells generated from STING-associated vasculopathy with onset in infancy (SAVI) patients with a heterozygous mutation in the *STING* gene

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Abstract

We have successfully created induced pluripotent stem cells (iPSC) from patients carrying a heterozygous mutation in the gene encoding STING. The gain-of-function mutation leads to constitutive activation of STING which leads to the development of the disease STING-associated vasculopathy with onset in infancy (SAVI). The iPSC lines derived from the SAVI patitents are shown to be morphologically and phenotypically normal and have the potential to self renew and differentiate into the three germ layers. These iPSC provide a powerful tools to investigate the role of STING in the regulation of immune responses and vascular renegeration.

1. Resource Table

Unique stem cell lines identifier	NIHTVBi024-A
	NIHTVBi025-A

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[1]These authors contributed equally to this work.

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.2022.102974.

	NIHTVBi026-A
	NIHTVBi027-A
Alternative names of stem cell lines	182–5 (NIHTVBi024-A)
	189–2 (NIHTVBi025-A)
	199–2 (NIHTVBi026-A)
	1131 (NIHTVBi027-A)
Institution	National Heart, Lung, and Blood Institute (NHLBI),
	National Institutes of Health (NIH), Bethesda,
	Maryland, USA
Contact information of distributor	Manfred Boehm; boehmm@nhlbi.nih.gov
Type of cell lines	iPSC
Origin	Human
Cell Source	Dermal fibroblasts and peripheral blood mononuclear cells
Clonality	Clonal cell lines
Method of reprogramming	Sendai-virus vectors containing the transcription factors Oct-4, Klf4, Sox2 and c-MYC
Multiline rationale	Lines derived from four individual
Gene modification	Yes
Type of modification	Hereditary
Associated disease	STING-associated vasculopathy with onset in infancy (SAVI)
Gene/locus	*STING1*
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	July 1, 2016
Cell line repository/bank	https://hpscreg.eu/cell-line/NIHTVBi024-A
	https://hpscreg.eu/cell-line/NIHTVBi025-A
	https://hpscreg.eu/cell-line/NIHTVBi026-A
	https://hpscreg.eu/cell-line/NIHTVBi027-A
Ethical approval	NCT02974595

1.1. Resource utility

Human induced pluripotent stem cells (hiPSC) carrying a heterozygous mutation in *STING1* gene exhibit the potential of selfrenew and differentiating into variety of cell types including vascular cells and immune cells. The derivatives sustaining the mutation could be a powerful platform allowing for the investigation of the molecular mechanisms and identification of potential therapeutic targets in patients.

2. Resource details

*S*TING-associated vasculopathy with onset in infancy (SAVI) is a rare monogenic autoinflammatory interferonopathy with a gain-of-function mutation in the stimulator of interferon response cGAMP interactor 1 (*STING1*) gene (Liu et al., 2014). This results in a constituently activated stimulator of interferon genes (STING) protein which is downstream

to cyclic GMP-AMP synthase (cGAS), an innate cytosolic nucleic acid sensor (Liu et al., 2014, Zhang et al., 2022). The STING mutation is associated with high levels of type 1 interferon along with a spectrum of clinical features featuring fevers, a peripheral vasculopathy presenting as a violaceous scaling lesions that can progress to acral ulceration, necrosis, and autoamputation, and interstitial lung disease (including pulmonary fibrosis) with respiratory failure the most common cause of death (Liu et al., 2014). Patients can present as early as 6–8 weeks following birth (Liu et al, 2014). Specific treatments include the use of JAK-STAT 1/2 inhibitors which has shown to have partial benefits (Sanchez et al., 2018). While the cGAS-STING pathway has previously been explored (Zhang et al., 2022), it is unclear whether this exact pathway is the mechanistic driver of the changes seen in different cell types with constitutively activated STING.

We initially identified four patients carrying the heterozygous mutation in the *STING1* gene by Sanger sequencing after which they were enrolled into our NHLBI clinical protocols for further investigation. Information regarding clinical features of these patients were obtained using the standard clinical questionnaire (Table 1). Skin punch biopsy from all patients and peripheral blood sample from patient 199 were collected at the NIH Clinical Center. Using a Sendai-OKSM delivery system expressing four transcription factors (OCT4, SOX2, KLF4, and CMYC), iPSC lines were generated from skin fibroblasts (185–2, 198–2, and 1131) or peripheral blood mononuclear cells (PBMNC) (199–2) from the patients. Typical iPSC morphology was observed for all lines, and the generated iPSCs expressed the pluripotency markers of OCT4, NANOG, TRA-160, SSEA4, and SOX2 as shown by immunofluorescent staining (Fig. 1A), by FACS (Fig. 1B), and/or real-time (RT)-qPCR (Fig. 1C). The iPSC lines were free of Sendai virus as confirmed by RT-PCR at the 15th passage (Supplementary File1). Genotyping of the generated iPSC lines confirmed the heterozygous mutation in the *STING1* gene was the same as their parental lines (Fig. 1D). To test the differentiation potential of the iPSC lines, we performed a monolayer differentiation assay to drive the cells towards the three germ layers *in vitro.* We showed that the expression of marker genes for the mesoderm (*HAND1*), endoderm (*SOX17*), and ectoderm (*PAX6*) germ layers by RT-qPCR confirmed that all four iPSC lines were able to differentiate into mesoderm, endoderm, and ectoderm cells (Fig. 1C). Short tandem repeat (STR) profiles indicated that the iPSC lines matched with its correspodning somatic cells completely in 15 amplified STR loci. All cultures were routinely tested for Mycoplasma contamination and were found to be Mycoplasma free (Supplementary File 2). All four iPSC lines demonstrated chromosomal stability and a normal karyotype with G-banding (Fig. 1E). Overall, the four patient derived iPSC lines demonstrated the pluripotent potential for self-renew and differentiation, suggesting the successful generation of iPSCs from the SAVI patients.

3. Materials and methods

3.1. Subjects and derivation of fibroblasts and peripheral blood mononuclear cells (PBMNC)

The fibroblasts were derived from skin punch biopsy samples obtained from three of the SAVI patients with the heterozygous *STING1* gene mutation and PBMNC was used for the remaining patient (199–2). Fibroblasts were propagated as previously described (Chen et al.,

2019). PBMNC were obtained from the remaining patient which were isolated from whole blood using density gradient centrifugation via Ficoll-Paque according to the manufacturer's direction. This study was approved by the NHLBI's institutional review board, and samples were collected after obtaining informed written consent.

3.2. Generation and culture of human iPSC from fibroblasts or PBMNC

Fibroblasts or PBMNC from the SAVI patients were reprogrammed and expanded in a typical human embroynic stem cell/iPSC culture condition as previously described (Chen et al., 2019) and Supplementary File 3.

3.3. Immunofluorescent staining and flow cytometry analysis (FACS)

iPSC colonies (passage 12) were fixed with 4% paraformaldehyde and stained for NANOG, SOX2, SSEA4, and TRA-1–60 (Table 3) following the previous protocol (Chen et al., 2019).

FACS data acquisition and analysis was performed on the iPSCs (passage 12) for the designed antibodies (Table 3) as previously described (Chen et al., 2019).

3.4. Monolayer differentiation assay

The *in vitro* assessment of iPSC differentiation ability at the 12th passage was performed as per the previous protocol with harvesting of cells for analysis at day 7 (Chen et al., 2019).

3.5. Gene expression analysis by RT-PCR

Total RNA isolation, cDNA synthesis, and PCR using the primers indicated in Table 2 was performed as previously described (Chen et al., 2019) with the iPSC obtained at passage 5 serving as the positive control (*Pos*) (Supplementary File 1).

After 15 passages, iPSCs were tested for Sendai virus (SeV) residues as outlined before (Alonso-Barroso et al., 2017).

Endogenous mRNA expression levels of *OCT4, NANOG*, *SOX2*, *SOX17, PAX6,* and *HAND1* were determined in iPSCs and in differentiating cells at day 7 (*D7*) (Fig. 1C) via RT-qPCR as previously described (Chen et al., 2019) using the primers catalogued in Table 3.

3.6. Karyotyping assay

The iPSC karyotypes were evaluated (182–5 at passage 15, 198–2 at passage 34, 199–2 at passage 6, and 1131 at passage 30) by the WiCell Research Institute using G-banding metaphase karyotype analysis.

3.7. DNA sequencing and STR

DNA sequencing and STR analysis was derived from previous methods (Chen et al., 2019). PCR was performed using specific primers listed in Table 3 to amplify the corresponding deletion positions in the *STING1* gene.

3.8. Mycoplasma detection

The supernatant from the iPSCs underwent mycoplasma testing as per the protocol detailed in Chen et al., 2019 using the MycoAlert™ Mycoplasma Detection Kit (Lonza, LT27–224) (Supplementary File 2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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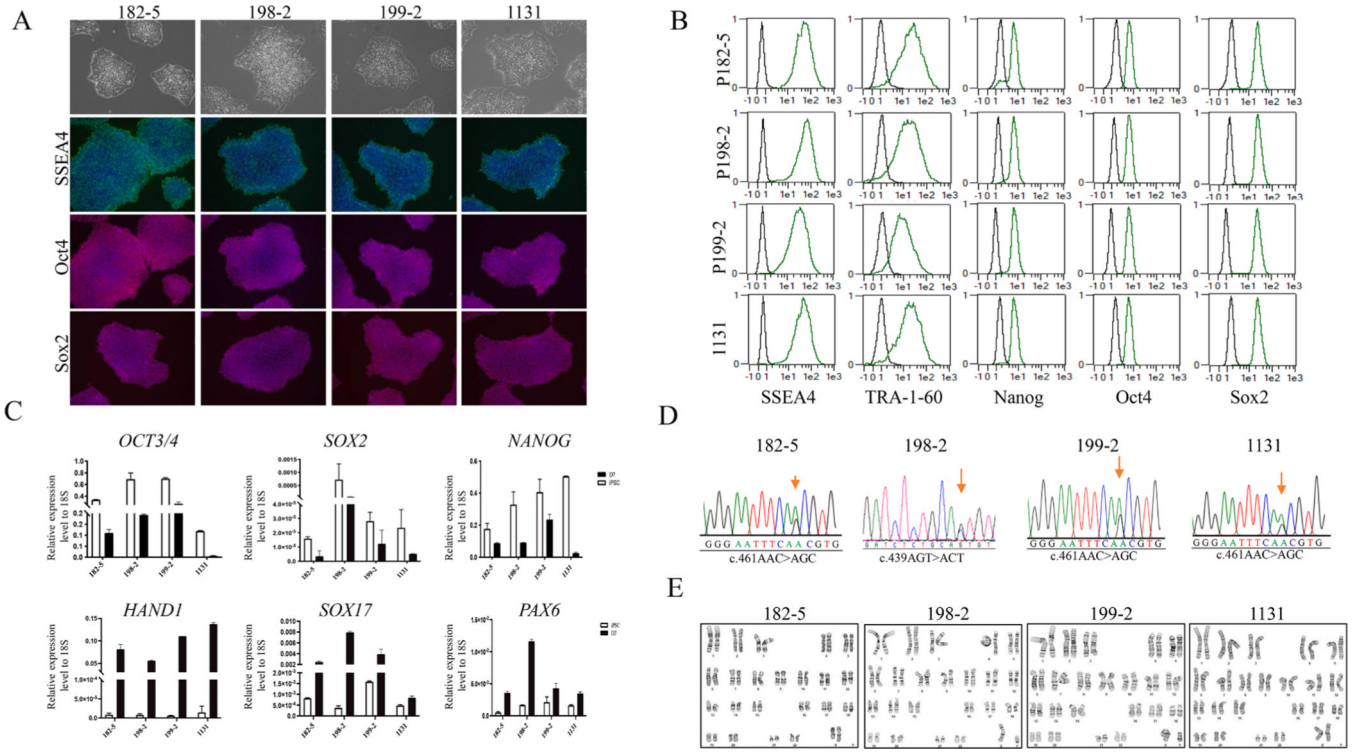

Fig. 1.

Generation and characterization of human induced pluripotent stem cells (iPSCs) from four SAVI patients carried a heterozygous mutation in the *STING* gene.

Table 1

Summary of patients with a SAVI disease.

iPSC line names	Abbreviation in figures	Gender	Age when cells harvested (years)	Ethnicity	Genotype of locus	Disease
NIHTVBi024-A	182–5	Female	15	White	*STING1*_mutation c.461AAC > AGC (p.N154S)	SAVI
NIHTVBi025-A	198–2	Male	12	Hispanic	*STING1* mutation c.439AGT > ACT (p.V147L)	SAVI
NIHTVBi026-A	199–2	Male	26	White	*STING1*_mutation c.461AAC > AGC (p.N154S)	SAVI
NIHTVBi027-A	1131	Female	10	White	*STING1*_mutation c.461AAC > AGC (p.N154S)	SAVI

Table 2

Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase-contrast microscope	Normal	Fig. 1A
Phenotype	Qualitative analysis (immunofluorescence staining)	Expression of pluripotency markers: OCT4, NANOG, SSEA4 and TRA-1–60	Fig. 1A
	Quantitative analysis (RT-qPCR) Qualitative analysis (FACS)	Expression of pluripotency markers: *SOX2* and *NANOG* Expression of pluripotency markers: NANOG, SOX2, SSEA4, TRA-1–60, OCT4	Fig. 1C Fig. 1B
Genotype	Karyotype (G-banding) and resolution	46, XY or 46, XX; resolution 450–500 bands	Fig. 1E
Identity	Microsatellite PCR OR STR analysis	Not performed 15 sites tested, 100% match	N/A
Mutation analysis (IF APPLICABLE)	DNA sequencing	Heterozygous, *STING gene point mutation*	Fig. 1D
	Southern blot OR WGS	Not performed	N/A
Microbiology and virology	*Mycoplasma* testing by luminescence	Negative	Supplementary Table 2
Differentiation potential	Monolayer differentiation assay	Differentiating cells are expression of *HAND1*, *SOX17*, and *PAX6*; iPSC were able to differentiate into three germ layers	Fig. 1C
Donor screening (OPTIONAL)	HIV1 + HIV2, hepatitis B virus, hepatitis C virus	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	Not performed Not performed	N/A N/A

Table 3

Reagents Antibodies used for immunocytochemistry and FACS.

	Antibody	Dilution	Company	Cat#	RRID
Primary antibodies	Mouse anti-SSEA4	1:100	MilliporeSigma	MAB4304	AB_177629
	Mouse anti-TRA-1-60	1:150	MilliporeSigma	MAB4360	AB_2119183
	Alexa Fluor 488 anti-SSEA4 Antibody	1:10	BioLegend	330412	AB_1089198
	PE anti-TRA-1-60 Antibody	1:10	BioLegend	330610	AB_2119065
	Alexa Fluor 488 anti-SOX2 Antibody	1:10	BioLegend	656110	AB_2563957
	Alexa Fluor 488 anti-OCT4 Antibody	1:10	BioLegend	653,708	AB_2563184
	Alexa Fluor 647 anti-NANOG Antibody	1:10	BioLegend	674210	AB_2650619
Secondary antibodies	Alexa Fluor 594 Donkey anti-rabbit	1:300	Life Technologies	A21207	AB_141637
	Alexa Fluor 594 Donkey anti-mouse	1:300	Life Technologies	A21203	AB_141633
	Alexa Fluor 488 Donkey anti-mouse	1:300	Life Technologies	A21202	AB_141607
Primers used for RT-qPCR and PCR Target			Forward/reverse primer (5′–3′)		
NANOG			AGG GAA ACA ACC CAC TTC T/CCT TCT GCG TCA CAC CAT T		
OCT3/4			AGC GAA CCA GTA TCG AGA AC/TTA CAG AAC CAC ACT CGG AC		
SOX2			CCC AGC AGA CTT CAC ATG T/CCT CCC ATT TCC CTC GTT TT		
HAND1			CCA AGG ATG CAC AGT CTG G/AGG AGG AAA ACC TTC GTG CTG		
SOX17			GTG GAC CGC ACG GAA TTT G/GGA GAT TCA CAC CGG AGT CA		
PAX6			TGG GCA GGT ATT ACG AGA CTG/ACT CCC GCT TAT ACT GGG CTA		
STING			CAT GCC TTG GGA TTA AAG GA/CTG GGA GTG GGA CTG GTT TA		