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Lab Resource: Multiple Cell Lines

Generation and characterization of a control and patient-derived human iPSC line containing the Hermansky Pudlak type 2 (HPS2) associated heterozygous compound mutation in *AP3B1*

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A B S T R A C T

Induced pluripotent stem cells (iPSCs) were generated from blood outgrowth endothelial cells (BOECs) obtained from a healthy donor and from a patient diagnosed with Hermansky Pudlak Syndrome type 2 (HPS2), caused by compound heterozygous *AP3B1* mutations (c.177delA and c.1839-1842delTAGA). BOECs were reprogrammed with a hOKSM self-silencing polycistronic lentiviral vector, where the generated iPSCs showed normal karyotype, expression of pluripotency associated markers and *in vitro* spontaneous differentiation towards the three germ layers. The generated iPSCs can be used to study HPS2 pathophysiology and the basic functions of AP3B1 protein in different cell types.

1. Resource table

Unique stem cell lines identifier	1) SANi009-A 2) SANi010-A
Alternative names of stem cell lines	1) PBL.HPS241.cl2 HPS2-90 2) PBL.5486.cl3 WT-30
Institution	Sanquin, Amsterdam, The Netherlands
Contact information of distributor	Emile van den Akker e.vandenakker@sanquin.nl
Type of cell lines	iPSC
Origin	Human
Cell Source	Blood outgrowth endothelial cells (BOECs)
Clonality	Clonal
Method of reprogramming	Lentivirus, integrative viral vector
Multiline rationale	Control and disease pair
Gene modification	Yes (only SANi009-A)
Type of modification	Hereditary (only SANi009-A)
Associated disease	Hermansky Pudlak Syndrome type 2
Gene/locus	Gene *AP3B1*, Locus 5q14, Mutation c.177delA and c.1839-1842delTAGA
Method of modification	–
Name of transgene or resistance	–
Inducible/constitutive system	–
Date archived/stock date	13-06-2017
Cell line repository/bank	https://hpscreg.eu/cell-line/SANi009-A https://hpscreg.eu/cell-line/SANi010-A
Ethical approval	Informed consent was given in accordance with the Declaration of Helsinki and Dutch national and Sanquin internal ethic boards. February 2015

2. Resource utility

HPS2 is a rare autosomal recessive disorder and is caused by mutations in the *AP3B1* gene. Since patient material can be scarce, the established cell line is a good alternative to study the disease mechanism in different cell types. In addition, iPSC line SANi010-A can function as control cell line.

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Table 1
Characterization of iPSC lines derived from a HPS2 patient and a (unrelated) healthy donor.

iPSC line names	Abbreviation in figures	Gender	Genotype of locus	Disease
SANi009-A	HPS2	Female	c.177delA, c.1839-1842delTAGA	Hermansky-Pudlak syndrome type 2
SANi010-A	WT	Female	–	Healthy

3. Resource details

Peripheral blood mononuclear cells (PBMC) were collected from a patient with mutations in the *AP3B1* gene that encodes for the β1 subunit of the heterotetrameric AP-3 complex. The patient is a compound heterozygote where mutations (exon 2: c.177delA; exon 17: c.1839-1842delTAGA) lead to a frame shift and a premature stop codon (de Boer et al., 2017). These mutations have been described to cause Hermansky Pudlak syndrome type 2, which is a rare autosomal recessive disorder, affecting 1 in 500,000 people worldwide. HPS2 patients suffer from severe neutropenia and other immunodeficiencies (de Boer et al., 2017).

Blood outgrowth endothelial cells (BOECs) were derived from

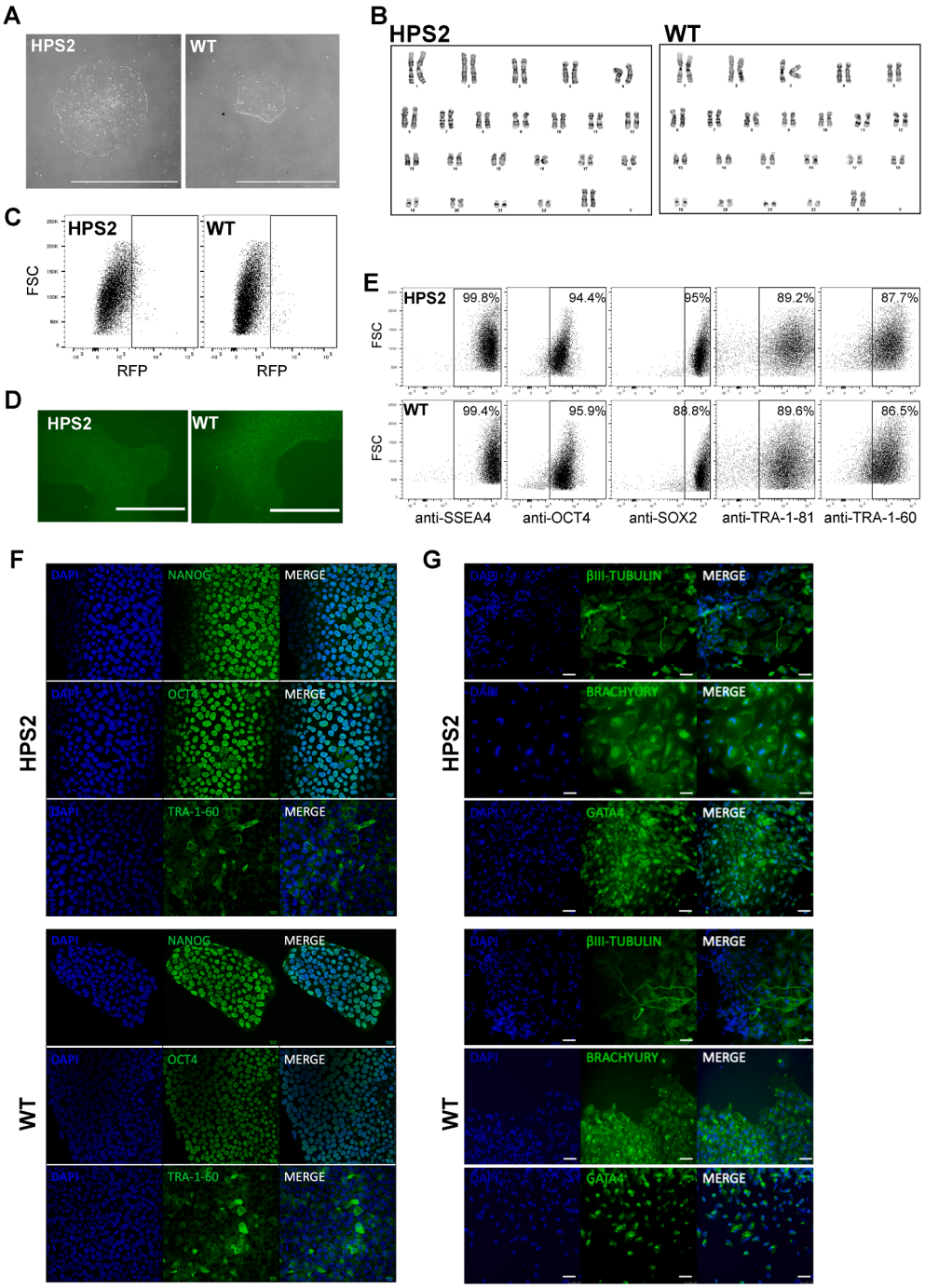

Fig. 1.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Alkaline phosphatase positive	Fig. 1D
		NANOG, OCT4, TRA-1–60 positive	Fig. 1F
	Flow Cytometry	SANi009-A SSEA4: 99.8% OCT4: 94.4% SOX2: 95% TRA-1–81: 89.2% TRA-1–60: 87.7%	Fig. 1E
		SANi010-A SSEA4: 99.4% OCT4: 95.9% SOX2: 88.8% TRA-1–81: 89.6% TRA-1–60: 86.5%	
Genotype	Karyotype	SANi009-A: 46XX, resolution 500–550 SANi010-A: 46XX, resolution 500–550	Fig. 1B
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	16 loci tested, all matched	Submitted in archive with journal
Mutation analysis	Sequencing	Compound heterozygous mutation: c.177delA c.1839-1842delTAGA	Suppl. Fig. 1
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Suppl. Fig. 2
Differentiation potential	Embryoid body formation and spontaneous differentiation	Embryoid body formation to all three germ layers	Fig. 1G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency FACS Markers	TRA-1–81-APC	1:100	Stem Cell Technologies, # 60065AZ.1, AB_1118559
	TRA-1–60-FITC	1:100	Millipore, FCMAB115F
	OCT4-APC	1:10	R&D Systems, FMC001
	SOX2-PE	1:10	R&D Systems, FMC001
	SSEA4-CFS	1:10	R&D Systems, FMC001
Pluripotency ICC Markers	Goat anti-NANOG	1:50	R&D System, AF1997
	Mouse anti-OCT4	1:50	Santa Cruz, sc-5279
	TRA-1–60-FITC	1:25	Millipore, FCMAB115F
Differentiation Markers	Mouse Neuronal Class III B-TUBULIN	1:2000	Covance, # MMS-435P, AB_2313773
	Rabbit anti-Brachyury	1:50	Santa Cruz, #sc-20109, AB_2255702
	Mouse anti-GATA4	1:50	Santa Cruz, #sc-25310, AB_627667
Secondary antibodies	Donkey anti-mouse IgG (H + L) Secondary Antibody, AF488	1:2000	Thermo Fisher Scientific, A-21202, AB_141607
	Goat anti-rabbit IgG (H + L) Secondary antibody, AF488	1:2000	Thermo Fisher Scientific, A-11008, AB_143165
	Rabbit anti-goat IgG (H + L) Secondary antibody, AF488	1:2000	Thermo Fisher Scientific, A11078, AB_2534122

Primers		
	Target	Forward/Reverse primer (5′–3′)
Targeted mutation analysis/ sequencing	AP3B1, exon 2	Forward: 5′AAT GCC TGG GAG TAC AAG ATC AAG 3′ Reverse: 5′ CCT CAT TCT CAT AGT AGA AAT ACC CTG 3′
	AP3B1, exon 17	Forward: 5′ TGA GGT AAT ATG CCT ATT ACC TTG AGC 3′ Reverse: 5′ CTA TCC TAG AGT CTT CCA CTG CAT TG 3′
Mycoplasma test	Mycoplasma (270 bp)	Forward: 5′ GGGAGCAAACCAGATTAGATACCC 3′ Reverse: 5′ TGCACCATCTGTCACTCTGTTAACCTC 3′

PBMCs of a HPS2 patient and a (unrelated) healthy donor (Table 1). Both BOECs were transduced with the self-inactivating pRRL.PPT.SF. hOct34co.hKlf4co. hSox2co.hmyc.idTomato.preFRT lentiviral vector (Voelkel et al., 2010; Warlich et al., 2011). Reprogramming was performed on an irradiated mouse embryonic fibroblast (iMEF) feeder layer, where the iPSC-like colonies were individually picked 17–21 days post-transduction. The iPSC lines SANi009-A (HPS2) and SANi010-A (WT) iPSC showed embryonic stem cell-like morphology (Fig. 1A, scale bar 1000 μm (Table 2)) and showed normal karyotype after reprogramming with no chromosomal abnormalities (Fig. 1B). Both lenti-virally reprogrammed iPSC lines were negative for dTomato, indicating the silencing of the reprogramming cassette (Fig. 1C). The presence of the disease causing compound heterozygous mutation (exon 2: c.177delA; exon 17: c.1839-1842delTAGA) was confirmed by Sanger DNA sequencing, while the wild type iPSC line SANi010-A did not show these mutations (Supplementary Fig. 1). The SANi009-A and SANi010-A cells also showed a 100% match by Short Tandem Repeat (STR) analysis between iPSCs and parental cells (Supplementary STR file). Alkaline phosphatase staining (Fig. 1D, scale bar 400 μm) and positive expression of SSEA4, OCT4, SOX2, TRA-1–60, TRA-1–81 and NANOG markers (Fig. 1E, F) by flow cytometry and immunostaining, confirmed pluripotentcy of the generated iPSC lines. The *in vitro* spontaneous differentiation potential of the iPSC lines towards the three germ layers was demonstrated by the expression of ectodermal (βIII-TUBULIN), mesodermal (BRACHYURY) and endodermal (GATA4) markers (Fig. 1G) using Immunocytochemistry staining. Both iPSC lines were negative for mycoplasma (Supplementary Fig. 2).

In conclusion, we have generated a human iPSC line SANi009-A from BOECs of a HPS2 patient, carrying compound heterozygous *AP3B1* mutations, and an unrelated wild type human iPSC line SANi010-A. The iPSC lines can be used in pairs for investigation of disease-related pathophysiology as well as disease modeling studies.

4. Materials and methods

4.1. Experimental procedures

All used chemicals were purchased from Sigma-Aldrich (Munich,

Germany) and all culture reagents from Thermo Fisher Scientific (Waltham, MA, USA), unless otherwise specified.

4.2. Isolation of primary cell source and reprogramming of BOECs

Blood outgrowth endothelial cells were isolated as previously described and cultured in EGM-2 (Lonza, Basel, Switzerland, CC-3162) supplemented with 18% fetal calf serum (FCS, Bodinco, Alkmaar, The Netherlands) (EGM-18) (Karampini et al., 2019). 2.5×10^6 BOECs were transduced with lentivirus derived from pRRL.PPT.SF.hOct34co.hKlf4co.hSox2co.hmyc.idTomato.preFRT lentivirus vector (Voelkel et al., 2010; Warlich et al., 2011). Three days post-transduction the cells were seeded onto irradiated-MEF (GlobalStem, Gaitherburg, USA) in EGM-8 medium. From day 6 post-transduction, media was changed to essential-8 medium (E8) supplemented with 2 mM valproic acid. From day 10 post-transduction the medium was changed to E8 and refreshed every other day hereafter. On day 17–21 post-transduction, observed iPSC colonies were individually picked and cultured on Matrigel-coated (Corning, NY, USA) plates in E8 media at 37 °C in humidified atmosphere containing 5% CO_2. Passaging was performed weekly using ReLeSR (Stem Cell Technologies, Köln, Germany), where the culture media was supplemented with Revitacell.

4.3. Genomic mutation

Sanger DNA sequencing was applied to validate the compound heterozygous mutations (exon 2: c.177delA; exon 17: c.1839-1842delTAGA) in the *AP3B1* gene. Genomic DNA was extracted from the generated iPSCs and the patient-derived EBLs using QIAamp DNA mini kit (Qiagen). Amplification was performed by using primers (Table 3) flanking the *AP3B1* gene. After the purification, the PCR products were labelled with BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) for sequencing on Abiprism 3700 genetic analyzer (Applied Biosystems).

4.4. Karyotype analysis

Routine G-banding method was applied for the karyotyping analysis, with a minimum of 20 metaphase spreads. HPS2-derived and WT-derived iPSCs at passage 32 were treated with Demecolcine solution (10 μg/mL in HBSS) and processed according to standard cytogenic protocols. The cells were defined according to the International System for Human Cytogenetic Nomenclature (ISCN 2016).

4.5. Pluripotency analysis

Pluripotency of the iPSCs was confirmed by alkaline phosphatase (AP)- and ICC staining using methods described in Varga et al. (2017). In addition, pluripotency marker expression was quantified by flow cytometry for SSEA4, OCT4, SOX2, TRA-1–81 and TRA-1–60. Single cell suspension was obtained by TrypLE at cell passage 28. For intracellular staining, cells were permeabilized with fixation/permebilization buffer (R&D systems) for 30 min according to manufacturer's instructions. Subsequently, cells were incubated with the specific conjugated antibodies against pluripotency markers and isotype controls. Analysis of expression level was performed using LSR-II (BD Bioscience) and FlowJo software (FlowJo, Ashland, USA). All applied antibodies are listed in Table 3.

4.6. In vitro spontaneous differentiation

The *in vitro* spontaneous differentiation was carried out according to previously described method (Varga et al., 2017) using cells at passage 29. After 14 days of differentiation, the cells were fixed with 4% PFA and stained for the three germ layer markers as described previously (Varga et al., 2017). The antibodies specific for the three germ layers that were used are listed in Table 3.

4.7. STR analysis

STR analysis was performed by extracting genomic DNA from the HPS2 patient generated iPSC line (SANi009-A), the HPS2 patient-derived BOECs, healthy donor generated iPSC line (SANi010-A) and the healthy donor-derived BOECs using the PowerPlex® 16 System (Promega).

4.8. Mycoplasma

Via PCR amplification the mycoplasma contamination was evaluated of culture media samples at passage 24. The PCR reaction was performed on T3 Thermal Cycler (Biometra) using using Salsa polymerase (MRC-Holland), Taqstart Antibody (Clontech, Saint-Germain-en-laye, France) and specific mycoplasma primers listed in Table 3.

CRediT authorship contribution statement

Cathelijn E.M. Aarts: Data curation, Formal analysis, Methodology, Writing - original draft. **Ellie Karampini:** Data curation, Methodology. **Tatjana Wust:** Data curation, Methodology. **Steven Webbers:** Data curation, Methodology. **Eszter Varga:** Methodology, Writing - review & editing. **Judy Geissler:** Data curation, Methodology. **Jan Voorberg:** Supervision. **Marieke von Lindern:** Supervision Ruben Bierings: Conceptualization, Supervision **Emile van den Akker:** Conceptualization, Supervision, Writing - review & editing. **Taco Kuijpers:** Conceptualization, Supervision, Writing - review & editing,

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.2021.102444.

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