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Lab resource: Single Cell Line

Generation of patient-specific induced pluripotent stem cells (KSCBi007-A) derived from a patient with Prader–Willi syndrome retain maternal uniparental disomy (UPD)

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A B S T R A C T

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder caused by loss of paternally expressed genes in an imprinted region of 15q11.2–q13. We established a human-induced pluripotent stem cell (hiPSC) line, KSCBi007-A, from the peripheral blood mononuclear cells of a 5-month-old girl with PWS that retained maternal uniparental disomy (UPD). Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) of genomic DNA revealed the maternal UPD in the hiPSCs. The generated hiPSC line expressed pluripotency markers and showed the ability to differentiate into three germ layers in vitro. This hiPSC line could be used as a cellular model of an imprinting disorder in humans.

Resource table

Unique stem cell line identifier	KSCBi007-A
Alternative name(s) of stem cell line	KNIH-PWS001i-A
Institution	Korea National Institute of Health (KNIH)
Contact information of distributor	Soo Kyung Koo, skkoo@korea.kr
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 5 month-olds Sex: Female Ethnicity: Asian
Cell Source	Human peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus (CytoTune™-iPS 2.0 Sendai Reprogramming Kit, Life Technologies)
Genetic Modification	Congenital
Type of Modification	N/A
Associated disease	Prader-Willi syndrome (PWS)
Gene/locus	*SNRPN*/15q11.2-q13, maternal uniparental disomy (UPD)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	October 2017
Cell line repository/bank http://kscr.nih.go.kr	Deposited in the Korea Stem Cell Bank (KSCB)
Ethical approval	KNIH Institutional Review Board (IRB) approval obtained (2017-03-05-P-A). Severance Children's Hospital IRB approval obtained (IRB No. 4-2015-0404).

1. Resource utility

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder that causes physical, mental, and behavioral problems, and a constant urge to eat. The PWS patient-specific iPSC line with maternal uniparental disomy (UPD) described herein could be used as cellular model of PWS and should serve as a valuable tool to assess the efficacy of new treatments.

2. Resource details

Prader–Willi syndrome is caused by an epigenetic phenomenon known as imprinting, in turn caused by loss of expression of genes in the paternally inherited chromosome 15q11.2–q13 (Angulo et al., 2015). The syndrome affects 1 in 25,000 live births and about 25% of PWS cases are due to maternal UPD (Cassidy and Driscoll, 2009). We generated a human-induced pluripotent stem cell (hiPSC) line,

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KSCBi007-A, from a 5-month-old girl with PWS and maternal UPD. This iPSC line showed typical colony formation, similar to human embryonic stem cells (hESCs), and was positive for the pluripotency markers OCT4, SSEA-4, TRA-1-60, and TRA-1-81 on immunofluorescence staining (Fig. 1A and B). The hiPSCs expressed pluripotency genes according to TaqMan reverse transcription polymerase chain reaction

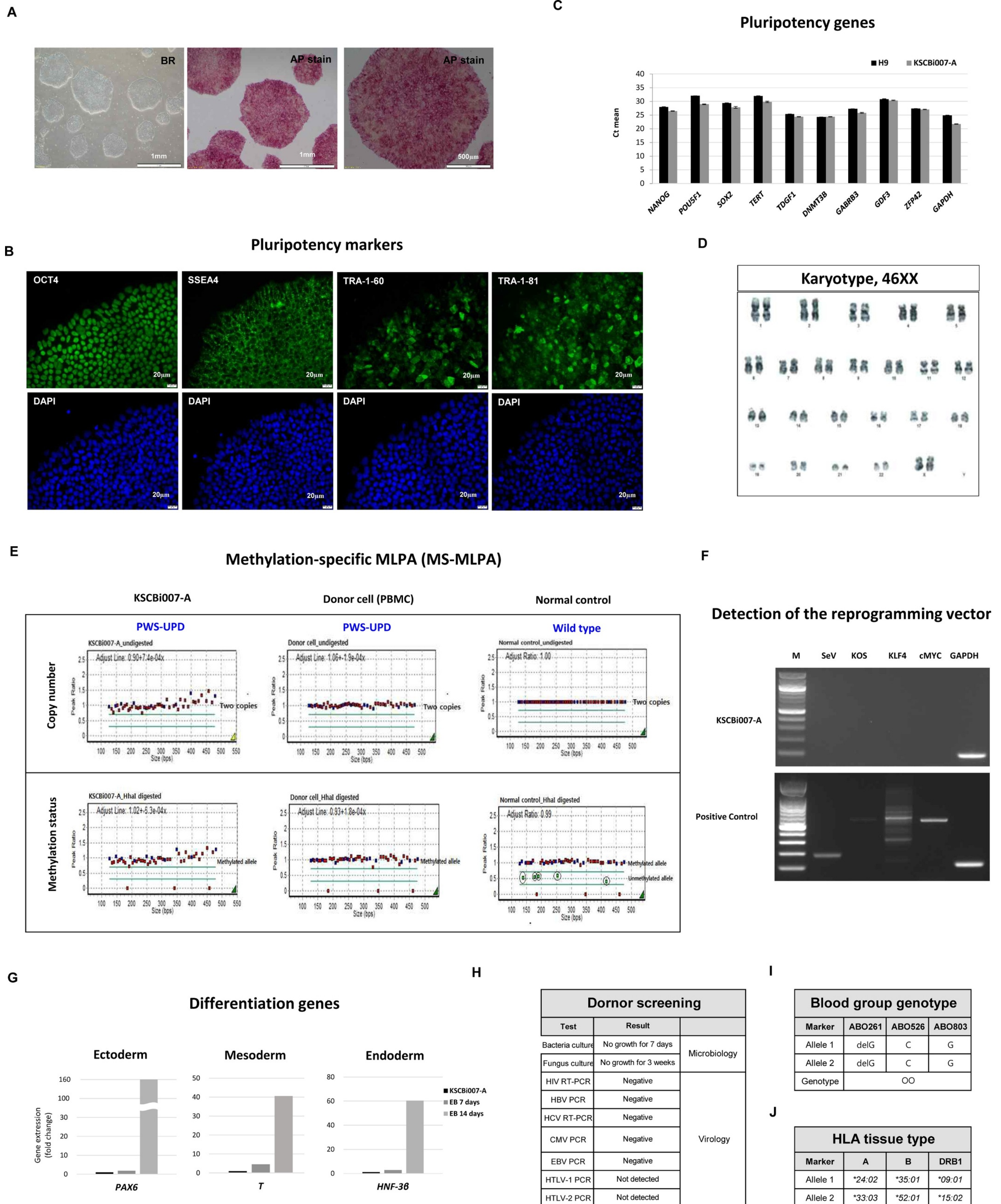

Fig. 1.

(RT-PCR) based on the hESC line H9 (Fig. 1C). G-banding analysis showed a normal karyotype (46, XX) in the hiPSC line (Fig. 1D). Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), using genomic DNA from the donor cell and hiPSCs, confirmed the methylated maternal allele of 15q11.2-q13 (Fig. 1E). Using TaqMan® probes, embryoid body (EB) formation demonstrated that the hiPSC line expressed all three germ layer markers: paired box 6 (*PAX6*) for ectoderm, brachyury (*T*) for mesoderm, and hepatocyte nuclear factor-3 beta (*HNF-3β*) for endoderm (Fig. 1G). At passage 10, the KSCBi007-A line showed complete removal of all exogenous reprogramming factors (Fig. 1F). In donor cells, HIV-1/2 and hepatitis B and C screening results were negative (Fig. 1H). Blood group genotyping revealed blood group O (Fig. 1I). Human leukocyte antigen (HLA) class I and II typing is available (Fig. 1J). Short tandem repeat (STR) analysis of 16 allele loci of KSCBi007-A revealed results identical to those of the donor cells (STR analysis). The hiPSC line was free from mycoplasma contamination, as determined by RT-PCR (Supplementary Fig. 1).

3. Materials and methods

3.1. Ethics statements

The generation and use of iPSCs, were approved by the Institutional Review Boards of Severance Children's Hospital and the Korea National Institute of Health (KNIH). Formal informed consent was obtained from the patient's mother.

3.2. Reprogramming of PBMCs and hiPSC maintenance

Human peripheral blood mononuclear cells (PBMCs) from the PWS patient were reprogrammed using Sendai virus vectors (SeV), which were obtained using the CytoTune®-iPS Sendai Reprogramming Kit (Life Technologies), following the manufacturer's protocol. The generated hiPSCs were cultured under feeder-free conditions on iMatrix-511 (Nippi)-coated plates with StemFit Basic02 medium (Ajinomoto) (Table 1).

3.3. Immunofluorescence staining

The cells were fixed with 4% paraformaldehyde (Wako) for 20 min, blocked with 5% (v/v) bovine serum albumin (Sigma–Aldrich) with 0.25% (v/v) Triton X (Sigma–Aldrich), and incubated with primary antibodies for OCT3/4, SSEA-4, TRA-1-60, and TRA-1-81. The primary antibodies are listed in Table 2. Images were acquired with fluorescence microscope (scale bar: 20 μm).

3.4. Real-time RT- PCR analysis

The hiPSCs were screened for endogenous expression of the pluripotency markers *NANOG, OCT4, SOX2, ZFP42, DMMT3B, TERT, and TDGF1* using real-time RT-PCR. Total RNA was isolated from hiPSCs using the Maxwell RSC simplyRNA Cells kit (Promega), and converted to cDNA using cDNA EcoDry Premix (Clontech). Real-time PCR was performed using TaqMan® Gene Expression Master Mix (Applied Biosystems). The TaqMan® probe IDs are listed in Table 2.

3.5. Karyotyping

Generated hiPSCs were karyotyped using standard cytogenetic procedures by the GTG-band method. The cells were treated with colcemid for 45 min, incubated in hypotonic solution and fixed with methanol-acetic acid solution (3:1; Merck). After Giemsa-trypsin banding, the karyotype was analyzed according to the International System for Human Cytogenetic Nomenclature by G-banding.

3.6. MS-MLPA assay

MS-MLPA can detect copy number changes as well as determining methylation status of the PWS/AS region. Genomic DNA was isolated from donor cells and iPSCs using the Maxwell RSC Blood DNA kit (Promega). The genomic DNA was then analyzed using the MS- MLPA ME028 Prader–Willi/Angelman probe (MRC-Holland). These probes contain a recognition site for HhaI, a methylation sensitive restriction enzyme used to digest only unmethylated genomic DNA. One is an undigested sample to detect copy number changes and the other is a digested sample (with HhaI endonuclease enzyme) for methylation status detection. A peak ratio of 1.0 (two copies) with a methylation ratio of 0.5 (both maternal and paternal alleles) represents a wild type; a peak ration of 1.0 (two copies) with a methylation ratio of 1 (maternal alleles only) represents PWS-UPD. x -axes represent fragment size in bps and y -axes represent probe peak ratios. Blue, internal control probes; red, PWS/AS region probes; green, five HhaI methylation sensitive probes (Procter et al., 2006).

3.7. Detection of the reprogramming vector

To test whether transgenes were silenced, hiPSCs from passage 10 were confirmed by RT-PCR. Total RNA was isolated using the Maxwell RSC simplyRNA Cells kit (Promega), and cDNA was synthesized by reverse transcription using RNA to cDNA EcoDry Premix (Clontech). Primers specific to the SeV genome are listed in Table 2. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was amplified concurrently and used as an internal control.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Figure 1 panel A
Phenotype	Immunocytochemistry	Positive staining for OCT4, SSEA-4, TRA-1-60, and TRA-1-81	Figure 1 panel B
	RT-qPCR	Expression of the pluripotency markers *NANOG, OCT4, SOX2, TERT, REX1, TDGF1, DNMT3B,* and *GDF3*	Figure 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XX, 500 band resolution	Figure 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	N/A
	STR analysis	16 loci analyzed, all matched	STR analysis is archived with the journal
Mutation analysis	MS-MLPA	*SNRPN*, uniparental disomy (UPD)	Figure 1 panel E
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma-negative by RT-PCR	Supplementary Figure 1
Differentiation potential	Embryoid body formation	Expression of ectodermal (*PAX6*), mesodermal (*T*), and endodermal (*HNF-3β*) markers in embryoid bodies	Figure 1 panel G
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C	Negative	Figure 1 panel H
Genotype additional info	Blood group genotyping	DNA analysis OO	Figure 1 panel I
	HLA tissue typing	A* 24:02/33:03 B* 35:01/52:01 DRB1* 09:01/15:02	Figure 1 panel J

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT3/4	1:200	Santa Cruz Cat# sc-9081, RRID:AB_2,167,703
	Mouse anti-SSEA-4	1:200	Millipore Cat# MAB4304, RRID:AB_177,629
	Mouse anti-TRA-1-60	1:200	Millipore Cat# MAB4360, RRID:AB_2,119,183
	Mouse anti-TRA-1-81	1:200	Millipore Cat# MAB4381, RRID:AB_177,638
Secondary antibodies	Anti-rabbit IgG	1:500	Vector Lab Cat# FI-1000, RRID:AB_2,336,197
	Anti-mouse IgG	1:500	Vector Lab Cat# FI-2000, RRID:AB_2,336,176

Primers		
	Target	Forward/Reverse primer (5′−3′)
SeV transgene detection (RT-PCR)	*SeV*/181 bp	GGA TCA CTA GGT GAT ATC GAG C*/ACC AGA CAA GAG TTT AAG AGA TAT GTA TC*
	KOS/528 bp	ATG CAC CGC TAC GAC GTG AGC GC/ACC TTG ACA ATC CTG ATG TGG
	KLF4/410 bp	TTC CTG CAT GCC AGA GGA GCC C/AAT GTA TCG AAG GTG CTC AA*
	c-MYC/532 bp	TAA CTG ACT AGC AGG CTT GTC G*/TCC ACA TAC AGT CCT GGA TGA TG
Housekeeping gene (RT-PCR)	*GAPDH*	CAT GTT CGT CAT GGG TGT GAA/GGA CTG TGG TCA TGA GTC CTT

Pluripotency markers (qPCR)		
	NANOG	TaqMan Probe ID Hs02387400-g1
	OCT4	TaqMan Probe ID Hs00742896-s1
	SOX2	TaqMan Probe ID Hs00602736-s1
	TERT	TaqMan Probe ID Hs00162669-m1
	TDGF1	TaqMan Probe ID Hs02339499-g1
	DNMT3B	TaqMan Probe ID Hs00171876-m1
	REX1	TaqMan Probe ID Hs00399279-m1
Differentiation markers (qPCR)	*PAX6*	TaqMan Probe ID Hs00240871-m1
	T	TaqMan Probe ID Hs00610080-m1
	HNF-3β	TaqMan Probe ID Hs00232764-m1
Housekeeping gene (qPCR)	*GAPDH*	TaqMan Probe ID Hs99999905-m1

3.8. In vitro differentiation assay

The in vitro differentiation potential of KSCBi007-A cells was evaluated using the EB formation assay. hiPSCs on iMatrix-511 (Nippi)-coated plates were detached using 0.5 mM EDTA. Cells were gently spun down and re-suspended in EB medium comprised of Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Gibco), 20% knockout serum replacement (Gibco), 1% nonessential amino acids (Gibco), and 0.1 mM β-mercaptoethanol (Invitrogen) for 14 days. Differentiated cells were analyzed by RT-qPCR using TaqMan® Gene Expression Master Mix. Table 2 lists the TaqMan® probe IDs.

3.9. Donor screening, blood group genotyping and HLA tissue typing

HIV-1/2, hepatitis B, and C screening of donor cells, blood group genotyping, and HLA tissue typing were carried out by Molecular Diagnostics Co.

3.10. STR analysis

The STR analysis was performed on the generated hiPSCs and donor cells using the PowerPlex®16 System (Promega) and 16 loci were detected.

3.11. Mycoplasma test

Cell culture medium was examined for mycoplasma using the PCR Mycoplasma Detection Set (Takara) following the manufacturer's instruction.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.scr.2019.101647

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