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Lab Resource: Multiple Cell Lines

Generation of induced pluripotent stem cells (KSCBi009-A) from a patient with Prader–Willi syndrome (PWS) featuring deletion of the paternal chromosome region 15q11.2–q13

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A B S T R A C T

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder caused by the loss of paternally expressed genes in an imprinted region of chromosome 15q11.2–q13. We generated a human-induced pluripotent stem cell line, designated KSCBi009-A, from peripheral blood mononuclear cells of a 13-year-old male PWS patient exhibiting deletion of the paternal chromosome 15q11.2–q13 region. The deletion was confirmed via methylation-specific multiplex ligation probe amplification assay (MS-MLPA) of genomic DNA. The hiPSC line expressed pluripotency markers and differentiated into three germ layers. The cell line may serve as a valuable model of an imprinting PWS disorder useful in terms of drug discovery and development.

1. Resource table

Unique stem cell line identifier	KSCBi009-A
Alternative name(s) of stem cell line	KNIH-PWS003i-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: 13 years Sex: Male Ethnicity: Korean
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	Yes
Type of Modification	Congenital
Associated disease	Prader–Willi syndrome (PWS)
Gene/locus	*SNRPN*/15q11.2-q13, deletion
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	Deposited in the Korea Stem Cell Bank (KSCB) http://kscr.nih.go.kr
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).

2. Resource utility

Prader–Willi syndrome (PWS) is a multisystemic complex genetic disorder characterized by the gradual development of obesity, short stature /decreased growth velocity, and intellectual disabilities (Angulo et al., 2015). PWS-iPSCs are patient-specific neuronal cells allowing study of the molecular etiology of disease.

3. Resource details

PWS is caused by the absence of paternally expressed imprinted genes at 15q11.2–q13 and paternal deletion and maternal uniparental disomy of chromosome 15, or unbalanced paternal translocation. Most PWS patients (70%) exhibit large deletions of the paternal 15q11.2–q13 region (Ledbetter et al., 1981). We created PWS-iPSCs by reprogramming peripheral blood mononuclear cells (PBMCs) of a 13-year-old male PWS patient with a deletion of the paternal chromosome 15q11.2–q13 region (Fig. 1 and Table 1). The iPSC line, KSCBi009-A, exhibited normal human embryonic stem cell-like morphology and was positive for the pluripotency markers OCT4, SSEA-4, TRA-1-60, and

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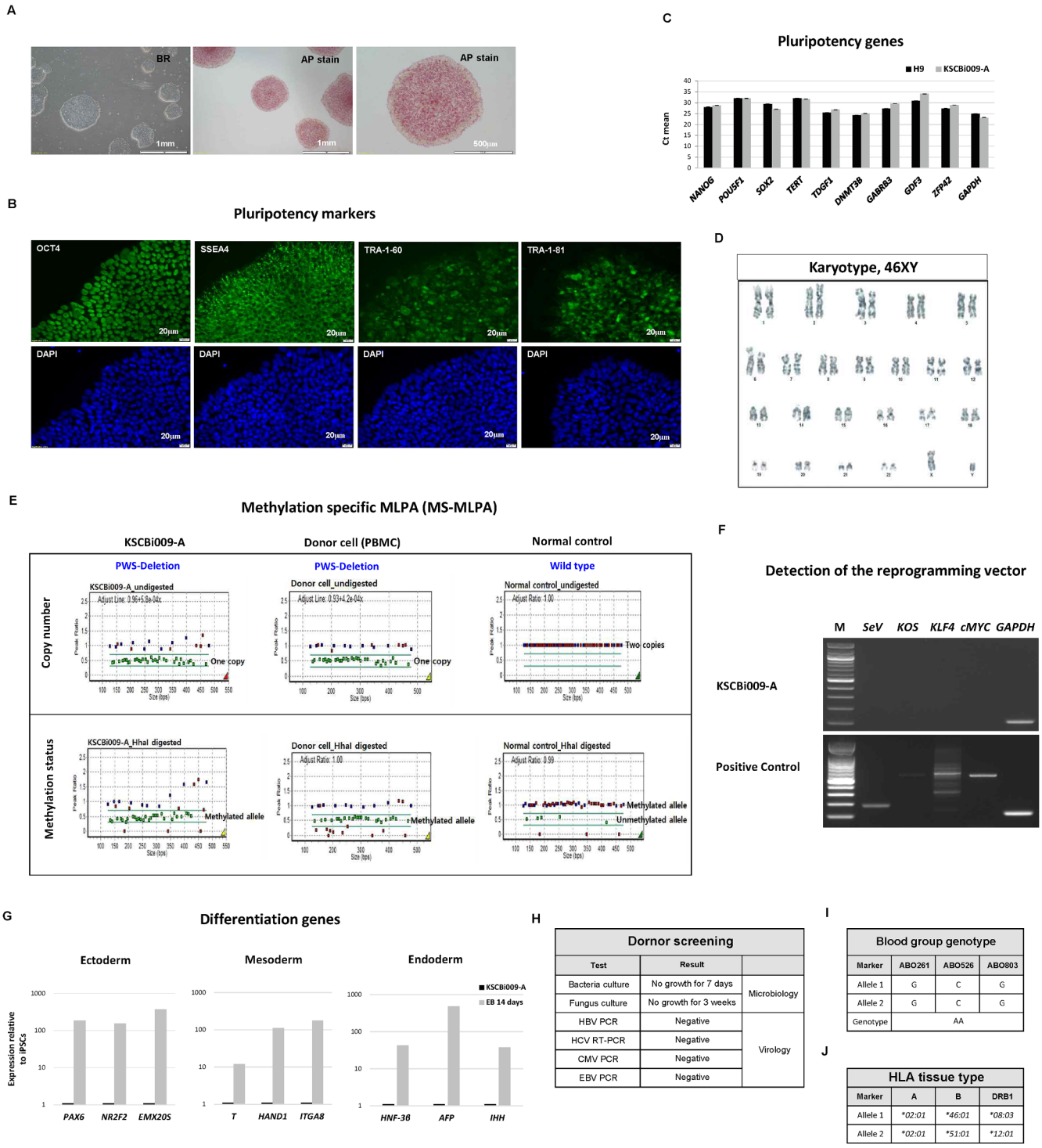

Fig. 1. Characterization of iPSC line KSCBi009-A. STR analysis.

TRA-1-81 on immunofluorescence staining (Fig. 1A and 1B). We used RT-qPCR employing TaqMan® probes to confirm endogenous expression of the pluripotent markers *NANOG, OCT4, SOX2, TERT, REX1, TDGF1, DNMT3B*, and *GDF3* (Fig. 1C). The hiPSC line exhibited a normal karyotype (46, XY) (Fig. 1D). Deletion of the unmethylated paternal allele was revealed using the methylation-specific multiplex ligation probe amplification assay (MS-MLPA), to evaluate genomic DNAs of both the donor cells and hiPSCs (Fig. 1E). At passage 10, the KSCBi009-A line evidenced a complete absence of all exogenous reprogramming factors (Fig. 1F). In vitro differentiation potential of the three germ layers were evaluated via embryoid body (EB) formation. Using TaqMan® expression probes, we confirmed that EBs expressed all three germ layer markers: ectodermal (*PAX6, NR2F2, EMX20S*), mesodermal (*T, HAND1, ITGA8*), and endodermal (*HNF-3β, AFP, IHH*) markers (Fig. 1G). In donor cells, the hepatitis B and C screening results were negative (Fig. 1H). The blood group genotype was AA (Fig. 1I). Human leukocyte antigen (HLA) class I and II typing data are shown in Fig. 1J. Short tandem repeat (STR) analysis revealed that the KSCBi009-

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Positive staining for OCT4, SSEA–4, TRA–1–60, TRA–1–81	Fig. 1 panel B
	RT-qPCR	Expression of the pluripotency markers: *NANOG, OCT4, SOX2, TERT, REX1, TDGF1, DNMT3B, and GDF3*	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY, 500 band resolution	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	N/A
	STR analysis	16 loci analyzed, all matched	STR analysis; the data are archived with the Journal
Mutation analysis	MS-MLPA	*SNRPN*, deletion	Fig. 1 panel E
	Southern blotting OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma-negative by RT-PCR	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Expression of ectodermal (*PAX6, NR2F2, EMX20S*), mesodermal (*T, HAND1, ITGA8*), and endodermal (*HNF-3β, AFP, IHH*) markers in embryoid bodies	Fig. 1 panel G
Donor screening	HIV 1 + 2, hepatitis B, hepatitis C	HIV 1 + 2: Not performed Hepatitis B, hepatitis C: Negative	Fig. 1 panel H
Genotype additional info	Blood group genotyping	DNA analysis AA	Fig. 1 panel I
	HLA tissue typing	A* 02:01/02:01 B* 46:01/51:01 DRB1* 08:03/12:01	Fig. 1 panel J

A genotype was identical to that of donor PBMCs. We used reverse transcription polymerase chain reaction (RT-PCR) to confirm that the hiPSC line was free of mycoplasma contamination (Supplementary Fig. 1).

4. Materials and methods

4.1. Ethics statements

The generation and use of human iPSCs were approved by the institutional review boards of the Severance Children's Hospital and the Korea National Institute of Health (KNIH). Formal informed consent was obtained from the patient's mother.

4.2. Reprogramming of PBMCs and hiPSC maintenance

Human peripheral blood mononuclear cells (PBMCs) from the PWS patient were reprogrammed to develop into iPSCs using the Cytotune™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies, A16518) according to the manufacturer's instructions. PBMCs (3×10^5 cells/mL placed in the middle section of a 24-well plate in complete PBMC medium) were transduced with each of four viruses (Oct3/4, KOS, KLF4, and cMyc). The generated hiPSCs were cultured under feeder-free conditions on Laminin-511 (Nippi, 892012)-coated plates with StemFit Basic02 medium (Ajinomoto, AJBASIC02) supplemented with 100 ng/ml bFGF (Sigma-Aldrich, F0291) at 37 °C in a 5% CO_2 atmosphere. The cells were fed every other day for a week. Cells were passaged at a 1:4 ratio using 0.5 mM EDTA (Invitrogen, 15575–038) with 10 μM Y-27632 (Sigma-Aldrich, Y-0503).

4.3. Immunofluorescence staining

The generated hiPSCs were fixed in 4% paraformaldehyde (Wako, 163-20145) for 20 min, blocked with 5% bovine serum albumin (Sigma-Aldrich, A9647) with 0.25% Triton X (Sigma-Aldrich, A8787), and incubated with primary antibodies targeting OCT4, SSEA4, TRA-1-60, and TRA-1-81 (Table 2). Images were acquired using a fluorescence microscope (Olympus, JP/IX83) (scale bar: 20 μm).

4.4. 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 purified using a Maxwell RSC simplyRNA Cells kit (Promega, AS1390), and converted to cDNA using cDNA EcoDry Premix (Clontech, 639543). Real-time PCR was performed using TaqMan® Gene Expression Master Mix (Applied Biosystems, 4369510). All gene expression data were normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The TaqMan® probes used are listed in Table 2.

4.5. Karyotyping

The karyotype was analyzed by standard cytogenetic procedures using 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). After Giemsa-trypsin banding, the karyotype was analyzed according to the International System for Human Cytogenetic Nomenclature using the standard G-banding method by the Molecular Diagnostics Laboratory Service (SMLab Inc., Seoul).

4.6. MS-MLPA assay of copy number and DNA methylation status of SNRPN

We used MS-MLPA to quantify DNA levels and determine the methylation status of the PWS/AS region. Genomic DNA (gDNA) was isolated from donor cells and iPSCs using a Maxwell RSC Blood DNA kit (Promega, AS1400) and analyzed using the MS-MLPA ME028 Prader–Willi/Angelman probe set (MRC-Holland). The probes contain recognition sites for HhaI, a methylation-sensitive restriction enzyme that digests only unmethylated DNA. We evaluated copy number changes using undigested samples and digested other samples with HhaI to evaluate methylation status. A peak ratio of 1.0 (two copies) associated with a methylation ratio of 0.5 (both the maternal and paternal alleles) is the wild-type scenario; a peak ratio of 0.5 (one copy) with a methylation ratio of 0.5 (maternal allele only) indicates PWS deletion. The x-axes show fragment sizes (bp), and the y-axes the probe

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_2167703
	Mouse anti-SSEA4	1:200	Millipore Cat# MAB4304, RRID:AB_177629
	Mouse anti-TRA–1–60	1:200	Millipore Cat# MAB4360, RRID:AB_2119183
	Mouse anti-TRA–1–81	1:200	Millipore Cat# MAB4381, RRID:AB_177638
Secondary antibodies	Alexa Fluor 488 donkey anti-rabbit IgG (H + L)	1:500	Thermo Fisher Scientific, Cat# A21206, RRID:AB_2535792
	Alexa Fluor 488 donkey anti-mouse IgG (H + L)	1:500	Thermo Fisher Scientific, Cat# A21203, RRID:AB_2535789

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
	NR2F2	TaqMan Probe ID Hs01047078-m1
	EMX2OS	TaqMan Probe ID Hs01393139-m1
	T	TaqMan Probe ID Hs00610080-m1
	HAND1	TaqMan Probe ID Hs02330376-s1
	ITGA8	TaqMan Probe ID Hs00233321-m1
	HNF-3β	TaqMan Probe ID Hs00232764-m1
	AFP	TaqMan Probe ID Hs00173490-m1
	IHH	TaqMan Probe ID Hs00745531-s1
Housekeeping gene (qPCR)	*GAPDH*	TaqMan Probe ID Hs99999905-m1

peak ratios. Blue: internal control probes; red: PWS/AS probes; green: the five HhaI methylation-sensitive probes (Procter et al. (2006)).

4.7. Detection of the reprogramming vector

To test whether transgenes were silenced, hiPSCs at passage 10 were confirmed by RT-PCR. Total RNA was isolated using a Maxwell RSC simplyRNA Cells Kit (Promega, AS1390), and cDNA was synthesized by reverse transcription of RNA employing cDNA EcoDry Premix (Clontech, 639543). Primers specific to the SeV genome are listed in Table 2. *GAPDH* was amplified concurrently and used as an internal control.

4.8. In vitro differentiation assay

The *in vitro* differentiation potential of KSCBi009-A cells was evaluated using an EB formation assay. EBs were cultivated in EB medium comprised of Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Gibco, 11320-082) with 20% knockout serum replacement (Gibco, 10828-028), 1% nonessential amino acids (Gibco, 11140-050), and 0.1 mM β-mercaptoethanol (Gibco, 21985-023) for 14 days. Differentiated cells were analyzed by RT-qPCR using TaqMan® Gene Expression Master Mix (Applied Biosystems, 4369510). The TaqMan® probes used are listed in Table 2.

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

Hepatitis B and C screening of donor cells, blood group genotyping, and human leukocyte antigen (HLA) tissue typing were performed by the Molecular Diagnostics Laboratory Service (SMLab Inc., Seoul).

4.10. STR analysis

Short tandem repeat (STR) analysis was performed on generated hiPSCs and donor cells using the PowerPlex®16 System (Promega) to detect 16 loci: D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA, and Amelogenin by the Molecular Diagnostics Laboratory Service (SMLab Inc., Seoul).

4.11. Mycoplasma test

Mycoplasma contamination status in culture medium was evaluated using a PCR Mycoplasma Detection Set (Takara, 6601) according to the manufacturer's instructions. Amplified DNA was subjected to 1.5% agarose gel electrophoresis.

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.2020.101847.

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