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Lab resource: Stem Cell Line

Generation of human induced pluripotent stem cells from peripheral blood mononuclear cells of a Senior-Loken syndrome patient

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A B S T R A C T

Senior-Loken syndrome (SLS) is a rare disorder primarily associated with kidney and retinal dysfunction. We generated a human induced pluripotency stem cell (hiPSC) line, designated DKHi005-A, from peripheral blood mononuclear cells of a patient with SLS using a Sendai virus reprogramming method. We confirmed that DKHi005-A cells harbor the same mutation as the patient and show a normal karyotype. DKHi005-A also has pluripotency and the capacity for differentiation into the three germ layers. This cell line is registered and available at the National Stem Cell Bank, Korea National Institute of Health.

Resource Table

Unique stem cell line identifier	DKHi005-A
Alternative name(s) of stem cell line	DKH005i-A
Institution	Korea National Institute of Health
Contact information of distributor	Soo Kyung Koo, skkoo@nih.go.kr
Type of cell line	iPSC
Origin	Human
Additional origin info	Applicable for human Ipsc Age: 10 Sex: female Ethnicity if known: Korean
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus (CytoTune™-iPS 2.0 Sendai Reprogramming Kit, Thermo Fisher Scientific)
Genetic Modification	Congenital
Type of Modification	N/A
Associated disease	Senior-Loken syndrome
Gene/locus	NM 025132.3(WDR19): c.3533G > A/Chr4: 39274649(GRCh37)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	June 2018
Cell line repository/bank	Korea National Stem Cell Bank(KSCB)
Ethical approval	Yonsei University Health System, Severance Hospital, Institutional Review Board(IRB) approval obtained (IRB No. 4-2016-1158) IRB approval obtained (IRB No. 2017-03-05-C-A)

1. Resource utility

Senior-Loken syndrome(SLS) is rare and early-onset retinal dystrophy (Haghighi et al., 2011). This generated hiPSC line with a WDR19 R1178E mutation could facilitate investigations of the pathogenic mechanism underlying SLS and should serve as a valuable tool to screen new drugs for improving the symptoms of SLS patients'.

2. Resource details

SLS accounts for less than 1% of all infantile-onset retinal dystrophies. However, patients presenting with early-onset retinal dystrophy are not routinely screened for renal dysfunction. To date, six causative genes of SLS have been reported, including WDR19, the least frequent overall (Coussa et al., 2013). A recent study showed that eupatilin rescued the ciliary transition zone defects from ameliorating ciliopathy-related phenotypes in a mouse model (Kim et al., 2018); however, the efficacy of eupatilin for SLS treatment needs to be confirmed in a humanized model. Thus, we established an iPSC line from a patient with SLS to serve as a retinal model. The patient, first presented at the clinic at the age of 1 year with symmetric pendular nystagmus. She could fix and follow objects, and cycloplegic refraction was +8.00 in both eyes. Dilated fundus examination was normal at the posterior pole. At 2 years, she was admitted to hospital due to dyspnea and fever. Blood

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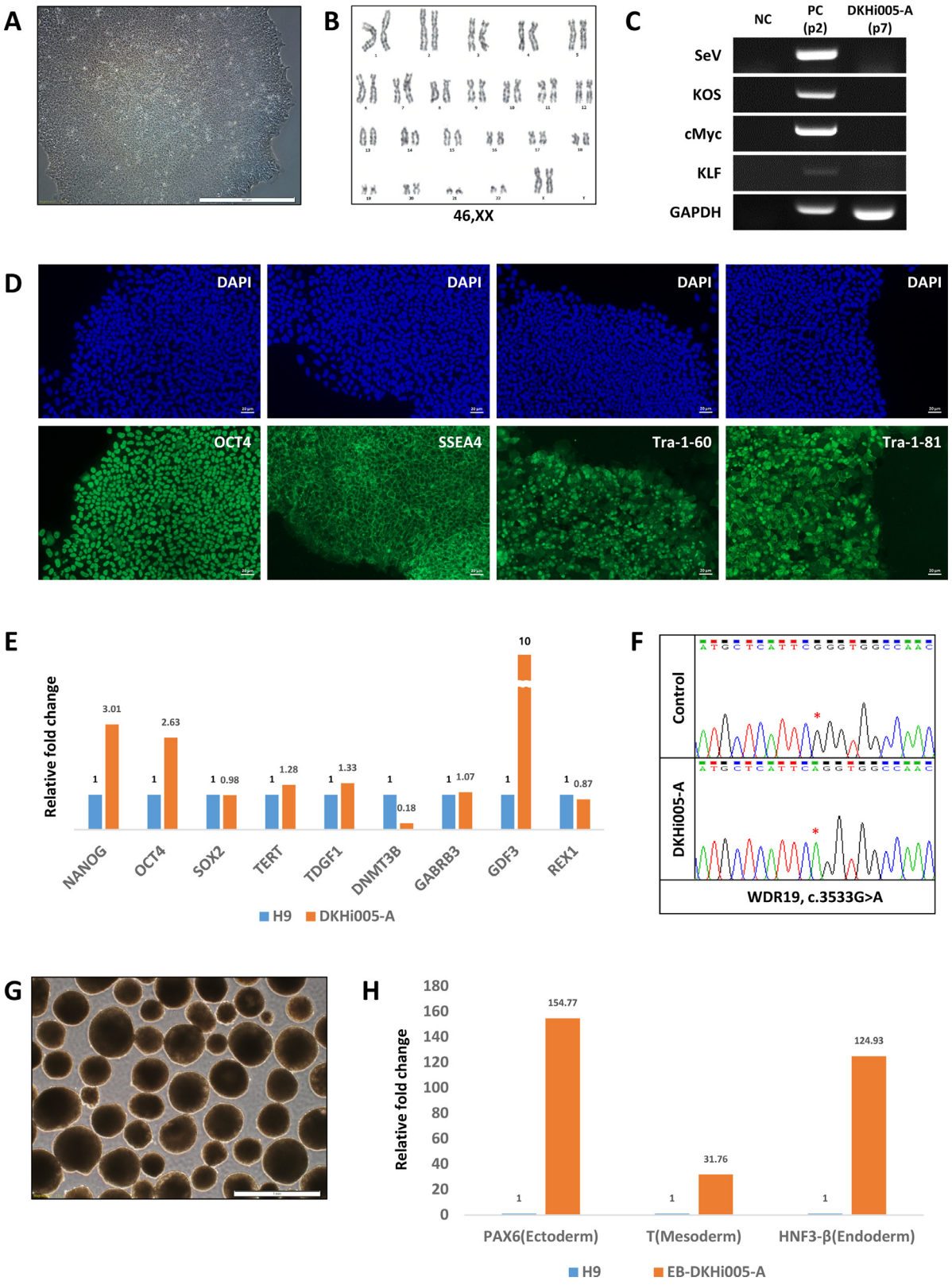

Fig. 1. Characterization of DKHi005-A cell line.

urea nitrogen was 95 mg/dL (normal: 7–17 mg/dL) and the serum creatinine level was 4.3 mg/dL (normal: 0.68–1.19 mg/dL). Hemodialysis was performed, and the ultrasonogram showed poor corticomedullary differentiation of both kidneys with high echogenicity of the parenchyma. Intrahepatic duct dilation and splenomegaly were also noted. A diagnosis of nephronophthisis was made, and she was re-evaluated for possible retinal involvement. Dilated fundus examination showed a depigmented patch-like lesion at the temporal to the macula. At the age of 2 years and 6 months, living-donor kidney transplantation from her mother and liver transplantation from a deceased donor were

performed. At the age of 7 years, dilated fundus examination showed diffuse pigmentary retinopathy and optic atrophy. Her best visual acuity was 20/400 in both eyes. Full-field electroretinogram showed extinguished responses. Targeted panel next-generation sequencing revealed a homozygous c.3533G > A (p.Arg1178Glu) WDR19 mutation. The patient's peripheral blood mononuclear cells (PBMCs) were reprogrammed into DKHi005-A using a Sendai virus-based gene delivery system. The morphology of DKHi005-A cells was similar to that of typical human embryonic stem cells (Fig. 1A). G-banding analysis exhibited a normal karyotype (46, XX) in the DKHi005-A hiPSC line (Fig. 1B). The DKHi005-A hiPSC line was reverse-transcribed using reprogramming factor-targeted primers, demonstrating that all factors to induce reprogramming were silenced (Fig. 1C). The pluripotency of the generated hiPSC line was identified by immunocytochemistry staining with pluripotency markers OCT4, SSEA4, Tra-1-60, and Tra-1-81 (Fig. 1D). We also identified pluripotency at the mRNA level with Taqman® Expression Probes based on the embryonic stem cell line H9 (Fig. 1E). The DKHi005-A hiPSC line harbors the same G3533A homozygous mutation confirmed in the patient (Fig. 1F). Embryonic body formation assessment (Fig. 1G) demonstrated that DKHi005-A hiPSC line expressed all three germ layer markers, PAX6 (ectoderm), T (medoderm) and HNF3-β (endoderm), using Taqman® Expression Probes (Fig. 1H). Short tandem repeat (STR) analysis showed that 16 allele loci of DKHi005-A were consistent with those of donor cells. Detailed information on STR analysis is presented in the supplementary data. DKHi005-A was confirmed to be free from Mycoplasma infection (Supplementary Fig. S1)(Table 1).

3. Materials and methods

3.1. Reprogramming and hiPSC maintenance

Human iPSCs were generated by reprogramming PBMCs using a CytotuneiPS™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies) following the manufacture's protocol (Fusaki et al., 2009). Obtained clones were cultured onto vitronectin (Gibco) coated plates with TeSR™-E8™ medium (Stemcell Technologies). The culture medium was changed every day.

3.2. Karyotyping

The cultured cells were treated with colcemid for 45 min and then harvested in hypotonic solution with fixative, followed by the preparation of metaphase slides. After Giemsa-trypsin banding, the karyotype was analyzed according to the International System for Human Cytogenetic Nomenclature using the standard GTG-banding method.

3.3. Detection of the reprogramming vector

Total RNA was isolated using a Maxwell® RSC simplyRNA Cells Kit (Promega), and cDNA was synthesized by reverse transcription using RNA to cDNA EcoDry Premix (Clontech). hiPSCs at passage 2 were used as a positive control. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified concurrently and used as an internal control. A negative control was included in all runs with all of the same components except the template to detect contamination with nucleic acids.

3.4. Mutation sequencing

Genomic DNA was isolated using a Maxwell® RSC Blood DNA Kit (Promega) and sent for Cosmogenetech (Seoul, Korea) with designed primers. The control cell line is the KSCBi002-B which is already registered in the hPSCreg.

3.5. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde (Wako) for 20 min, blocked with 5% bovine serum albumin (Sigma-Aldrich) with 0.25% Triton X (Sigma-Aldrich), and incubated with primary antibodies for Oct3/4, SSEA4, Tra-1-60, and Tra-1-81 (Table 2). Images were acquired using a fluorescence microscope (scale bar: 20 μm).

3.6. Real-time PCR analysis

Real-time PCR was performed using Taqman® Gene Expression Master Mix (Applied Biosystems). All quantitative gene expressions were normalized to the expression level of GAPDH. The probes used are listed in Table 2.

3.7. In vitro differentiation into the three germ layers

To determine the capacity of the hiPSCs to differentiate into the three germ layers, we induced embryoid bodies using hiPSCs harvested by dispase (Gibco). The culture medium for embryoid bodies was DMEM/F12 (Gibco) supplemented with 20% Knockout Serum Replacement (Gibco), 0.1 mol/L Minimal Essential Media non-essential amino acids solution (Gibco), 0.1 mmol/L 2-mercaptoethanol (Gibco), 1% (v/v) penicillin-streptomycin (Gibco) and MycoZap plus-PR (Lonza). The culture medium was changed every other day. The embryoid bodies were harvested at 14 days and real-time PCR analysis for the three germ layers was performed. The probes used are listed in Table 2.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	OCT4, SSEA4, Tra-1-60 and Tra-1-81	Fig. 1 panel D
	Real-time PCR	NANOG, OCT4, SOX2, TERT, TDGF1, DNMT3B, GABRB3, GDF3 and REX1	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed Tested 16 loci, all matched	Not performed STR analysis
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous	Fig. 1 panel F
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR negative	Supplementary figure 1
Differentiation potential	Embryoid body formation	Paired box 6 (PAX6) for ectoderm, Brachyury (T) for mesoderm and Hepatocyte nuclear factor-3 beta (HNF-3β) for endoderm	Fig. 1 panel G and H
Donor screening (OPTIONAL)	HIV 1 + +2 Hepatitis B, Hepatitis C	Not performed	Not performed
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

Table 2
Reagents details.
RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Marker	Rabbit anti-OCT3/4	1:200	Santa Cruz Biotechnology Cat# sc-9081, RRID:AB_2167703
Pluripotency Marker	Mouse anti-SSEA4	1:200	Millipore Cat# MAB4304, RRID:AB_177629
Pluripotency Marker	Mouse anti-TRA-1-60	1:200	Millipore Cat# MAB4360, RRID:AB_2119183
Pluripotency Marker	Mouse anti-TRA-1-81	1:200	Millipore Cat# MAB4381, RRID:AB_177638
Secondary antibody	Anti-rabbit IgG(H + L) Fluorescein conjugated	1:500	Vector Laboratories Cat# FI-1000, RRID:AB_2336197
Secondary antibody	Anti-mouse IgG(H + L) Fluorescein conjugated	1:500	Vector Laboratories Cat# FI-2000, RRID:AB_2336176

Primers		
	Target	Forward/Reverse primer (5′-3′)
Sendai virus test(RT-PCR)	SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai virus test(RT-PCR)	KOS	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
Sendai virus test(RT-PCR)	Klf4	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
Sendai virus test(RT-PCR)	c-Myc	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
Targeted mutation analysis/sequencing	3533G > A	GGCCATCATCAAGGAGTTGT/GGGTGAGAGCTCTGGTCAGT
House-Keeping Gene(RT-PCR)	GAPDH	CATGTTCGTCATGGGTGTGAA/GGACTGTGGTCATGAGTCCTT
Pluripotency Marker (qPCR)	NANOG	Hs02387400-g1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	OCT4	Hs00742896-s1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	SOX2	Hs00602736-s1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	TERT	Hs00162669-m1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	TDGF1	Hs02339499-g1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	DNMT3B	Hs00171876-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	GABRB3	Hs00241459-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	GDF3	Hs00220998-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	REX1	Hs00399279-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	PAX6	Hs00240871-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	T (Brachyury)	Hs00610080-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	HNF-3β	Hs00232764-m1 (Taqman® Probe ID)
House-Keeping Gene (qPCR)	GAPDH	Hs999999905-m1 (Taqman® Probe ID)

3.8. STR analysis

STR analysis of the generated hiPSCs and parental cells was performed using a PowerPlex 16 System (Promega) to detect 16 loci.

3.9. Mycoplasma test

The Mycoplasma test of the cell culture medium was performed using a PCR Mycoplasma Detection Set (TAKARA).

Declaration of Competing Interest

There are no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101648.

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