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Lab Resource: Stem Cell Line

Generation of an iPS cell line via a non-integrative method using urine-derived cells from a patient with USH2A-associated retinitis pigmentosa

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ABSTRACT

We have established an induced pluripotent stem (iPS) cell line using urine-derived cells from a 27-year-old male patient with retinitis pigmentosa associated with point mutations in the *USH2A* gene. Feeder-free culture conditions and the integration-free CytoTune™-iPS 2.0 Sendai Reprogramming Kit were used.

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Resource table

Unique stem cell line identifier	KLRMMEi001-A
Alternative name(s) of stem cell line	CJ127-iPS
Institution	Jinan University, China
Contact information of distributor	guoyonglong@163.com
Type of cell line	Induced pluripotent stem (iPS) cells
Origin	Human
Additional origin information	Age: 27 years Sex: male Ethnicity if known: Chinese
Cell source	Urine-derived cells
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic modification	None
Type of modification	N/A
Associated disease	Retinitis pigmentosa
Gene/locus	*USH2A*/1q41
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	8/20/17
Cell line repository/bank	Stem Cell Line Repository, Key Laboratory for Regenerative Medicine, Ministry of Education, Jinan University, China
Ethical approval	AIER2018IRB03

Resource utility

We have established an RP-UiPS cell line, derived from cells in the urine of a 27-year-old male patient with retinitis pigmentosa (RP). Patient-derived cells can be a good model for studying the pathogenesis of RP. They can also benefit the development of novel personalized drug, cell, and gene therapies.

Resource details

Recently, there have been various reports showing the efficient and feasible reprogramming of urine-derived cells (UCs) from healthy

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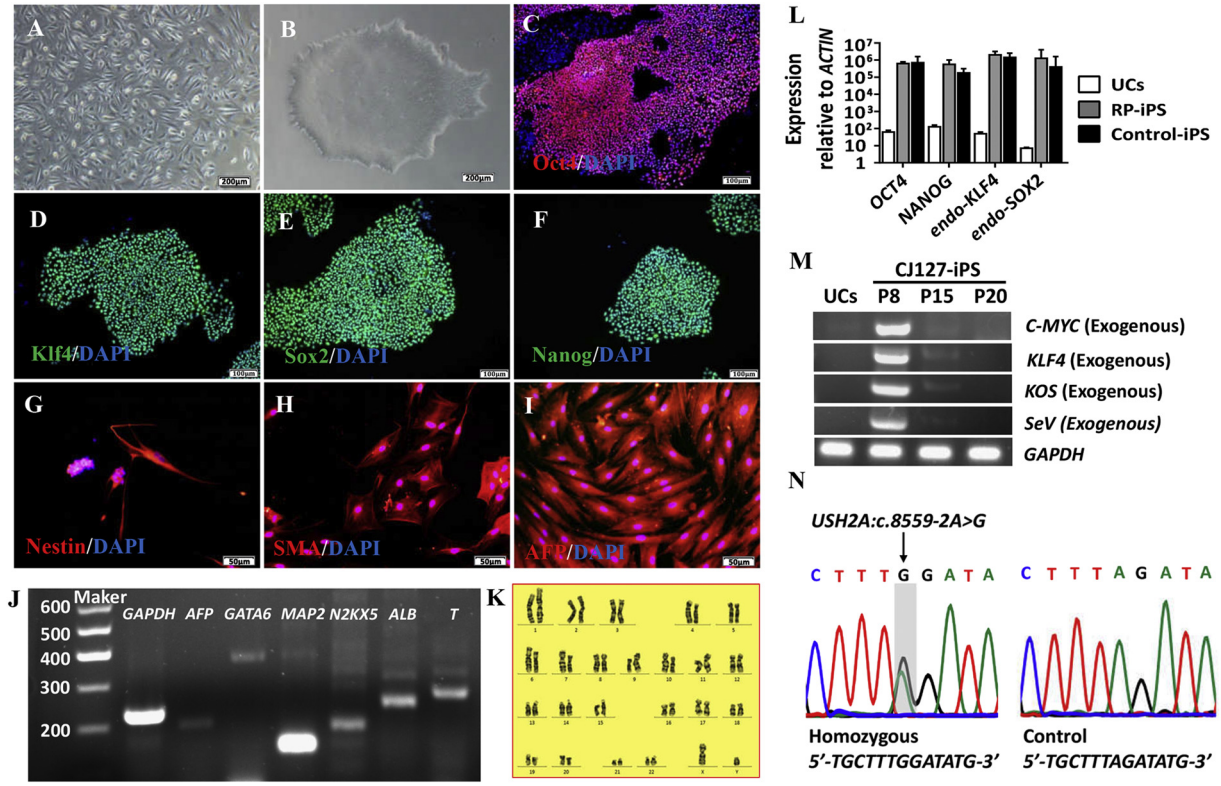

Fig. 1. Characterization of RP-UiPS cell line.

individuals or patients into induced pluripotent stem (iPS) cells, using the four Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc) (Szlachcic et al., 2017; Haase et al., 2017). In this present study, we generated the human iPS cell line CJ127-iPS (registered as KLRMMEi001-A at the Human Pluripotent Stem Cell Registry, http://hPSCreg.eu) from heterologous cells (Fig. 1A) that were isolated from the urine of a patient with retinal degeneration. The RP-UiPS cells were established with the integration-free CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, Waltham, MA, USA). The morphology of our RP-UiPS cell clones resembled that of typical iPS clones (Fig. 1B). The RP-UiPS cells were positive for the pluripotency markers Oct4, Klf4, Sox2, and Nanog, as verified by immunofluorescence staining (Fig. 1C–F). Real-time polymerase chain reaction (qPCR) results (Fig. 1L) indicated that the endogenous pluripotency genes *KLF4*, *SOX2*, *OCT4*, and *NANOG* were fully activated, with expression levels comparable to those of the commercial human control-iPS cell line (Nuwacell Biotechnology Co., Ltd., Anhui, China). Exogenous reprogramming of the transgenes *c-MYC*, *KLF4*, *KOS*, and *SeV* was determined to be gradually lost after multiple passages in our RP-UiPS cells, as verified by reverse-transcription PCR (RT-PCR) (Fig. 1M). In addition, embryoid body formation and spontaneous differentiation experiments showed that the RP-UiPS cells had the capacity to form three germ

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Microscopy	Normal morphology	Fig. 1 panel B
Phenotype	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency markers: Oct4, Nanog, Sox2, Klf4	Fig. 1 panels C–F
	Quantitative analysis (RT-qPCR, qPCR)	Expression of endogenous stemness markers: *OCT4*, *KLF4*, *SOX2*, *NANOG* Silenced transgene expression (from CytoTune Sendai virus): *c-MYC*, *KLF4*, *KOS*, *SeV*	Fig. 1 panels L and M
Genotype	Karyotype (G-banding) and resolution	46, XY Resolution 450–500	Fig. 1 panel K
Identity	Microsatellite PCR OR Short tandem repeat analysis	Not performed *Homo sapiens* (human), 10 sites tested, all matched	N/A Submitted in archive with journal
Mutation analysis	Sequencing Southern blot OR WGS	Heterozygous, USH2A:c.8559-2A>G Not performed	Fig. 1 panel N N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, negative	Fig. 1/supplementary
Differentiation potential	Embryoid body (EB) formation and directed differentiation	Expression of proteins (Nestin, SMA, AFP) and genes (*AFP, GATA6, MAP2, N2KX5, ALB* and *T*) in differentiated EB cells and proof of formation of three germ layers	Fig. 1 panels G–J
Donor screening (OPTIONAL)	HIV1 + 2, Hepatitis B, Hepatitis C	Negative	Not shown but available from author
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	N/A N/A

layers, which was verified by immunofluorescence staining with Nestin, SMA, and AFP (Fig. 1G–I), and by RT-PCR for determining *AFP*, *GATA6*, *MAP2*, *N2KX5*, *ALB*, and *T* expression (Fig. 1J). The RP-UiPS clones showed a normal karyotype (46, XY) (Fig. 1K) and the *USH2A* mutation was confirmed by genomic sequencing (Fig. 1N).

Materials and methods

Isolation of urine-derived cells

This study was approved by the Ethics Review Board of the Aier Eye Hospital of Changsha. Written informed consent was obtained from the patient. UCs were cultured as previously reported by Zhou et al. (Zhou et al., 2012). Briefly, 200 ml of fresh urine was centrifuged, and the pellet obtained was washed with PBS, resuspended in urine cell isolation (UCI; Cellapy Biotechnology, Beijing, China) medium, and plated onto a Vitronectin-coated plate. Six days post cell seeding, the medium was replaced with urine cell expansion (UCE; Cellapy Biotechnology) medium.

Non-integrative reprogramming of UCs into RP-UiPS cells

The UCs were reprogrammed using the integration-free CytoTune™-iPS 2.0 Sendai Reprogramming Kit. First, 2–3 × 10^5 UCs were plated onto one well of a Vitronectin-coated plate before viral transduction. At 24 h post transduction, the medium was replaced daily with fresh UCI medium containing 2.5 ng/ml basic fibroblast growth factor (FGF2). At 3 days post transduction, the medium was changed to a 1:1 (*v*/v) mix of UCE (no FGF2) and E6 (10 ng/ml FGF2) media. From Day 4 onward, the medium was replaced with E6 medium (10 ng/ml FGF2). Once colonies appeared, the medium was gradually

Table 2
Reagents used.

Antibodies used for immunocytochemistry/flow cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-OCT4	1:200	BioLegend Cat # 653701; RRID: AB_2561766
Pluripotency marker	Rabbit anti-KLF4	1:200	Proteintech Cat # 11880–1-AP; RRID: AB 2249452
Pluripotency marker	Rabbit anti-SOX2	1:200	Proteintech Cat # 11064–1-AP; RRID: AB 10782182
Pluripotency marker	Rabbit anti-NANOG	1:200	Thermo Fisher Scientific Cat # PA1–41577; RRID: AB_2150115
Differentiation marker	Mouse anti-Nestin	1:200	Novus Cat # NBP1-92717SS; RRID: AB_11013546
Differentiation marker	Mouse anti-SMA	1:200	Abbiotec Cat # 251813; RRID: AB_10637722
Differentiation marker	Mouse anti-AFP	1:200	Thermo Fisher Scientific Cat # MA5–12754; RRID: AB_10986624
Secondary antibodies	Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:1000	Life Sciences Cat # 11005; RRID: AB_11179634
Secondary antibodies	F(ab′)2-Goat Anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:1000	Life Sciences Cat # A11070; RRID: AB_11179983

Primers

	Target	Forward/Reverse primer (5′–3′)
Exogenous reprogramming of transgene (RT-PCR)	*SeV*	GGA TCA CTA GGT GAT ATC GAG C* ACC AGA CAA GAG TTT AAG AGA TAT GTA TC*
Exogenous reprogramming of transgene (RT-PCR)	*KOS*	ATG CAC CGC TAC GAC GTG AGC GC ACC TTG ACA ATC CTG ATG TGG
Exogenous reprogramming of transgene (RT-PCR)	*KLF4*	TTC CTG CAT GCC AGA GGA GCC C AAT GTA TCG AAG GTG CTC AA*
Exogenous reprogramming of transgene (RT-PCR)	*c-MYC*	TAA CTG ACT AGC AGG CTT GTC G* TCC ACA TAC AGT CCT GGA TGA TGA TG
Pluripotency marker (RT-qPCR)	*OCT4*	TCCGAGTCAAATCTTCCGCC TGTTGAGCGTCGAAGAACCA
Pluripotency marker (RT-qPCR)	*KLF4*	CACCTGCAGCTTCACCTATCCGA CCTTCAGCACGAACTTGCCCAT
Pluripotency marker (RT-qPCR)	*SOX2*	CGCATGGACAGTTACGCGCACA TCGGACTTGACCACCGAACCCA
Pluripotency marker (RT-qPCR)	*NANOG*	CCCCAGCCTCTACTCTTCCTAC CAAGTCACTGGCAGGAGAATTT
Differentiation potential marker (RT-PCR)	*AFP*	CCACTTGTTGCCAACTCAGTGA TGCAGGAGGGACATATGTTTCA
Differentiation potential marker (RT-PCR)	*GATA6*	CCTGCGGGCTCTACAGCAAGATGAAC CGCCCCTGAGGCTGTAGGTTGTGTT
Differentiation potential marker (RT-PCR)	*MAP2*	ACTACCAGTTTCACACCCCCTTT AAGGGTGCAGGAGACACAGATAC
Differentiation potential marker (RT-PCR)	*N2KX5*	GGGACTTGAATGCGGTTCAG CTCCACAGTTGGGTTCATCTGTAA
Differentiation potential marker (RT-PCR)	*ALB*	AAATGAAGATCAAAAGCTTAT TACCGAAGTGGAATAAGAGAGAA
Differentiation potential marker (RT-PCR)	Brachyury (*T*)	ACCTTCCATGTGAAGCAGCAA CTCCACAGTTGGGTTCATCTGTAA
GAPDH (RT-PCR)	*GAPDH*	GGTCGGAGTCAACGGATTTG TGGAAGATGGTGATGGGATT
Actin (RT-qPCR)	Actin	AGGCGGACTATGACTTAGTTGCGTTACACC AAGTCCTCGGCCACATTGTGAACTTTG

changed to E8 medium until colonies were ready to be picked (usually by Day 22) (Table 1).

Immunofluorescence staining

Pluripotency and differentiation were identified by immunofluorescence staining, following standard protocols (Dai et al., 2014). Briefly, the cells were fixed, permeabilized, incubated with primary and secondary antibodies (Table 2), and then stained with 4′,6-diamidino-2-phenylindole (Sigma-Aldrich, St. Louis, MO, USA). After washing, the cells were examined under a fluorescence microscope.

In vitro differentiation of RP-UiPS cells

The in vitro differentiation ability of the RP-UiPS cells was analyzed by spontaneous differentiation, according to our previous protocol (Zhao et al., 2012). First, dissociated RP-UiPS cells were cultured in ultralow adhesive plates with embryoid body induction medium (KO-DMEM, 20% KSR, penicillin/streptomycin, glutamax, β-mercaptoethanol, and non-essential amino acids) for 7 days. The embryoid bodies were then cultured in conventional culture plates with differentiation medium (DMEM/F12, 20% FBS, penicillin/streptomycin, glutamax, β-mercaptoethanol, and non-essential amino acids) for 6–7 days until confluent. An RT-PCR assay was performed on the differentiated cells.

Karyotype analysis

Chromosomes of at least 50 RP-UiPS cells in the proliferation phase were counted and analyzed by the First Affiliated Hospital of Jinan University (Guangzhou, China).

Assessment of USH2A mutation (targeted capture and next-generation sequencing, panel) and RT-PCR

Genomic DNA was extracted from RP-UiPS cells using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and DNA sequencing was performed by Clinbytes Inc. (Hangzhou, China). The mycoplasma test was performed by Cellapy Biotechnology. Short tandem repeat analysis of the RP-UiPS cells was performed by GENEWIZ, Inc. (Suzhou, China). Total RNA was isolated using TRIZOL, and cDNA was synthesized using a reverse-transcriptase kit (TOYOBO, Osaka, Japan) as per the manufacturer's instructions. RT-PCR was conducted in a thermal cycler (Eppendorf, Hamburg, Germany) as follows: denaturation (94 °C, 2 min), amplification (94 °C, 30 s, 30 cycles), annealing (60 °C, 30 s), and extension (72 °C, 1 min). The primer sequences used are shown in Table 2.

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Author disclosure statement

There are no competing financial interests with respect to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.03.022.

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