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Lab Resource: Single Cell Line

Generation of induced pluripotent stem cell line (RCMGi009-A) from urine cells of patient with fibrodysplasia ossificans progressiva

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A B S T R A C T

Urine cells obtained from a 14-year-old man with genetically proven (*ACVR1*: c.6176G > A) and clinically manifested fibrodysplasia ossificans progressiva were successfully transformed into induced pluripotent stem cells by using Sendai virus-based reprogramming vectors including the four Yamanaka factors such as OCT3/4, SOX2, KLF4, and c-MYC. These iPSCs expressed pluripotency markers, exhibited the potential to differentiate into three germ layers in spontaneous differentiation assay and had a normal karyotype. The iPSC line may provide a model for development of a personalized treatment including genome editing and drug screening, may be used for disease modelling, cell differentiation and pharmacological investigations.

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Resource Table:

Unique stem cell line identifier	RCMGi009-A https://hpscreg.eu/cell-line/RCMGi009-A
Alternative name(s) of stem cell line	P14L1
Institution	Research Centre for Medical Genetics, Moscow, Russia
Contact information of distributor	MD Ekaterina Kondrateva, ekaterina.kondratyeva@gmail.com
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 14 Sex: male Ethnicity: Caucasian
Cell Source	Urine cells
Clonality	Clonal
Method of reprogramming	Sendai virus (OCT3/4, SOX2, KLF4, and c-MYC)
Genetic Modification	Yes
Type of Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Fibrodysplasia ossificans progressiva
Gene/locus	Gene: ACVR1 Locus: 2q24.1 Mutation: NM_001111067.4(ACVR1):c.617G > A (p.Arg206His)
Date archived/stock date	August 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/RCMGi009-A
Ethical approval	The study was approved by the local ethics committee of the Federal State Budgetary Institution "Research Centre for Medical Genetics" (the approval number 4/2 from 29.06.2020)

1. Resource utility

Induced pluripotent stem cells (iPSCs) line was derived from donor with fibrodysplasia ossificans progressiva who is heterozygous for c.6176G > A (p.Ary206His) mutation in the *ACVR1* gene. These iPSCs may be subsequently used for disease modelling, cell differentiation and organoid formation, development of a treatment including genome editing and pharmacological investigations (Table 1).

2. Resource details

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic

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autosomal dominant disorder caused by mutations in the *ACVR1* gene. In most cases it's a single nucleotide transition c.617G > A resulted in substitution R206H in the intracellular GS domain of Activin A receptor, type I (Kaliya-Perumal et al., 2020). FOP is characterized by congenital malformations of the big toes and progressive heterotopic endochondrial ossification, most often in response to trauma (Pignolo et al., 2013). Most people with FOP become wheelchair-bound by the third decade of life and die around age 40 due to complications of thoracic insufficiency syndrome. There are currently no treatments for FOP other than symptomatic therapy, and it's not highly effective and do not alleviate the progressive nature of this disease (Wentworth et al., 2019). That is why obtaining iPSCs for further modeling and development of treatment methods can be very promising. The use of urine cells (UCs) to obtain iPSCs is advantageous due to their low cost and non-invasiveness (important for dangerous skin diseases and some genetic diseases such as FOP), less exposure to UV radiation, and high reprogramming efficiency compared to fibroblasts (Shi et al., 2016). Here, we established an iPSC line derived from a 14-year-old man with FOP, clinically manifested and genetically proven (c.6176G > A mutation leading to p. Ary206His). UCs were transformed into iPSCs by using Sendai virus-based reprogramming vectors expressing the four Yamanaka factors, OCT3/4, SOX2, KLF4, and c-MYC, with 5:5:4 MOI ratio for KOS, c-Myc and Klf4 viruses, respectively. iPSC clone named RCMGi009-A was purified (Fig. 1A). At passage 15 the expression of pluripotency markers SSEA4, OCT4, SOX2, and NANOG was detected and counted by immunocytochemistry assay (Fig. 1B, C). Sanger sequencing confirmed the presence of the c.6176G > A mutation in heterozygous state in UCs and iPSC line (Fig. 1D). iPSCs karyotype was inspected by in-house Giemsa-banding of at least 15 metaphase spreads based on ISCN 2016. At passage 17 RCMGi008-A showed a normal diploid 46,XY karyotype, without any detectable abnormalities (Fig. 1E). Potential of iPSCs to differentiate into three germ layers was proven in spontaneous differentiation assay by formation of embryoid bodies (EB) and analyzing them by immunocytochemistry staining which was positive for alpha fetoprotein (endodermal marker), tubulin beta (ectodermal marker), and vimentin (mesodermal marker) (Fig. 1F). Identical DNA profiles of the patient's UCs and the generated line RCMGi009-A were verified by short tandem repeat (STR) analysis of 18 microsatellite markers (listed in the STR report). Sendai virus genome and transgenes elimination was confirmed on passage 11 according to the manufacturer' protocol for CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) using the provided primers (listed in Table 2). Moreover, mycoplasma testing was carried out using MycoReport kit (Evrogen) which is based on end-point PCR with amplification of species-specific regions of 16S rRNA genes. It revealed that these iPSCs at passage 13 were not infected by mycoplasma. Stable iPSCs line was collected in Moscow Branch of the Biobank "All-Russian Collection of Biological Samples of Hereditary Diseases".

3. Materials and methods

3.1. iPSCs derivation and cultivation

UCs were obtained from urine and cultivated as described previously (Zhou et al., 2011). On day 1 reprogramming of 100,000 cells/well was performed using mentioned reprogramming kit. On days 5–6 we observed the appearance of colonies. On the 8th day the cells were reseeded into culture plates pre-coated with Matrigel (Corning). A day later, cells were transferred to TeSR™-E8™ medium (E8) (Stemcell Technologies) with daily replacement thereafter. On day 14, some colonies were picked manually and replated. One iPSC clone was then cultured in E8 at 37 °C, 5% CO_2 with passaging every 4–7 days (split ratio 1:6) using Gentle Cell Dissociation Reagent (GCDR) and with addition of Y27632 ROCK inhibitor (Stemcell Technologies) at 5 μM for 24 h.

3.2. Immunocytochemistry counting

Cells were fixed with 4% paraformaldehyde at 4 °C for 20 min, permeabilized with 0,25% Triton X-100 in PBS at RT for 10 min and blocked with 1% BSA in PBS at RT for 30 min. Antibodies were diluted in 1% BSA. Samples were incubated with primary antibodies at 4 °C overnight and with secondary antibodies at RT for 1 h in the dark. Nuclei were counterstained using DAPI (Abcam) at RT for 10 min. Fluorescent images were captures using Lionheart FX Automated Microscope (BioTek). Images were processed and analyzed using CellProfiler 3.0.0. Cell cytoplasm and nuclei were determined and then the average fluorescence intensity of staining per cell was measured and the percentage of positives cells in the green and red channels was counted.

3.3. Karyotyping

iPSCc were cultured as described until reaching 70–80% confluency. 10 μg/ml colchicine solution (PanEco) was added to cells for 40 min to

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for pluripotency markers: SSEA-4, NANOG, SOX2, OCT-4	Fig. 1 panel B
	Quantitative analysis (Immunocytochemistry counting)	100% SSEA-4, 90,2% NANOG, 100% SOX2, 100% OCT-4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XY Resolution: 1000	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	18 STR sites analyzed, 18 matching	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sanger sequencing	Heterozygous type of mutation *ACVR1*: c.6176G > A	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Testing by MycoReport (Evrogen) Negative	Supplementary files, Fig. 1
Differentiation potential	In vitro spontaneous differentiation: embryoid bodies formation and immunocytochemisty	Positive expression of differentiation markers: Endoderm: alpha fetoprotein Ectoderm: Tubulin beta Mesoderm: Vimentin	Fig. 1 panel F
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

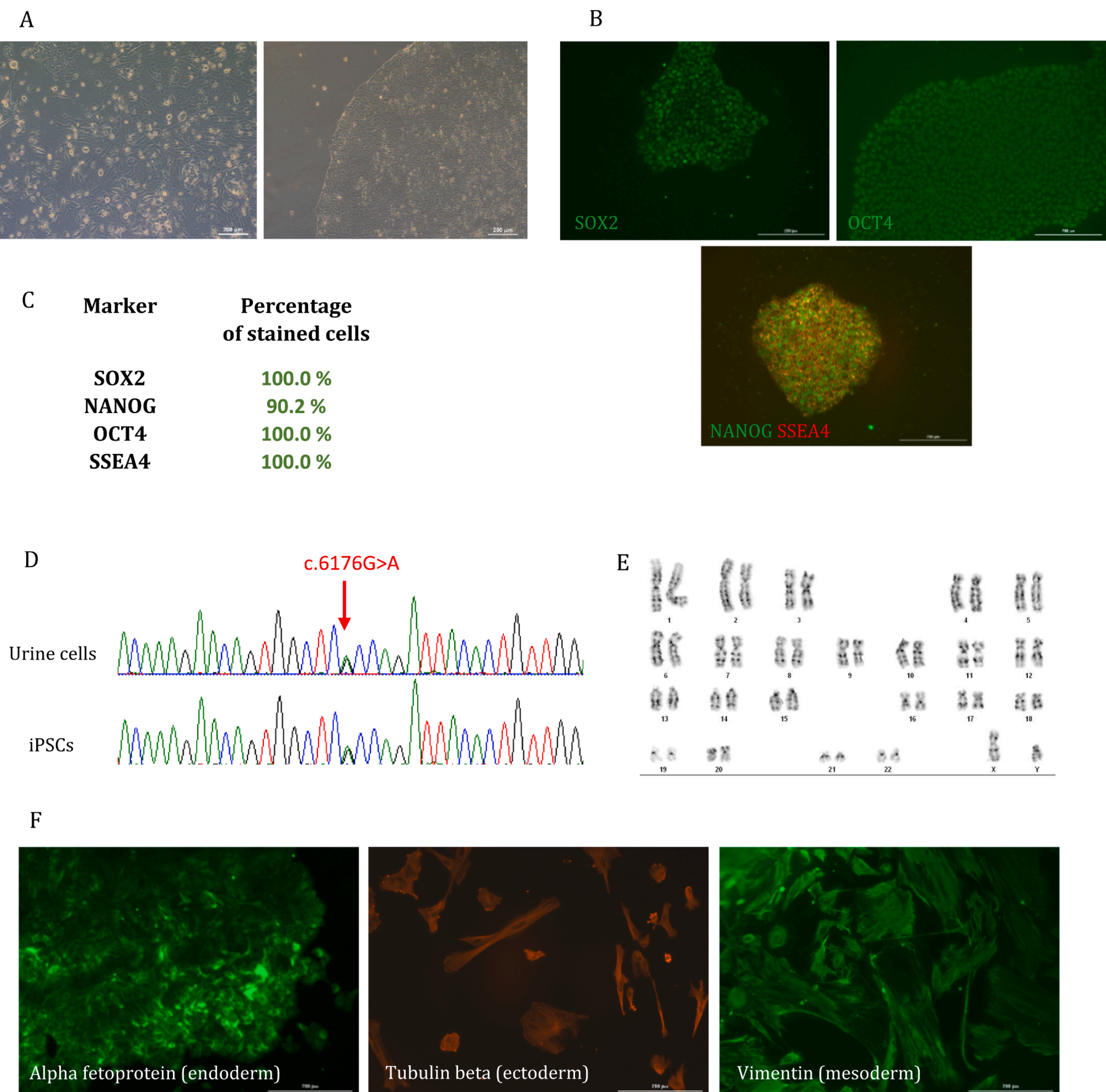

Fig. 1. Characterization of RCMGi009-A cell line.

arrest mitosis. Then cells were treated by hypotonic solution (0.075 M KCl) at 37C for 13 min and fixed by twice incubation in 3:1 cooled solution made of 3 parts methanol and 1 part glacial acetic acid for 30 and 20 min. The chromosome spread was analyzed under the Axio Imager 2 microscope (ZEISS).

3.4. Sanger sequencing

Genomic DNA was isolated from UCs and iPSCs using Quick-DNA Miniprep Kit (Zymo Research). *ACVR1* locus was amplified by PCR (Protocol: 95 °C 3′; 35x (95 °C 20″, 58 °C 20″, 72 °C for 25″); 72 °C 3′) using Taq DNA polymerase (Evrogen) in Eppendorf Mastercycler (Eppendorf). PCR products were analyzed by Sanger sequencing (Protocol: 95 °C 3′; 25x (95 °C 15″, 50 °C 15″, 60 °C for 2′) using ABI Prism 3130XL Genetic Analyzer (Applied Biosystems).

3.5. EB formation

On the 12th passage, the iPSCs were detached using GCDR and transferred in E8 with Y27632 to ultra-low attachment plate (Corning). On day 3 the half of medium was replaced and on the day 6 all the medium was replaced with EB medium consisting of Advanced DMEM/F12 (Gibco), 20% KO Serum replacement (Gibco), 2 mM L-glutamine (Gibco), 1% MEM NEAA, 50 U/ml penicillin, 50 ug/ml streptomycin. Thereafter, the medium was replaced every 2–3 days with an addition of 1% FBS and then stepwise increase it from 1 to 10%. In about 3 weeks EB were transferred onto gelatin-coated plates in EB medium with 10% FBS. After 7–14 days the expression of markers was analyzed using immunocytochemistry.

Declaration of Competing Interest

The authors declare that they have no known competing financial

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	SSEA4 Monoclonal Antibody (MC813-70), Mouse	1:100	Thermo Fisher Scientific, Cat # 41–4000	RRID: AB_2533506
	Anti-Oct4 Antibody, Rabbit	1:100	Abcam, Cat # ab18976	RRID:AB_444714
	Nanog Polyclonal Antibody, Rabbit	1:200	Thermo Fisher Scientific, Cat # PA1-097X	RRID: AB_2539868
	Anti-SOX2 Antibody [9–9–3], Mouse	1:200	Abcam, Cat # ab79351	RRID: AB_10710406
Differentiation Markers	Recombinant Anti-Vimentin Antibody [EPR3776], Rabbit	1:250	Abcam, Cat # Ab92547	RRID: AB_10562134
	anti-AFP antibody, Rabbit	1:50	FineTest, Cat # FNab00203	RRID: AB_2938663
	Anti-beta Tubulin antibody [1E1-E8-H4], Mouse	1:300	Abcam, Cat # ab131205	RRID: AB_11156121
Secondary antibodies	Goat Anti-Mouse IgG H&L (Alexa Fluor® 488)	1:200	Abcam, Cat # ab150113	RRID: AB_2576208
	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647)	1:200	Abcam, Cat # 150,079	RRID: AB_2722623
	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:150	Abcam, Cat #: A21206	RRID: AB_2535792
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:150	Cat# A-11032,	RRID: AB_2534091
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sanger sequencing	*ACVR1* c.6176G > A locus	394 bp	ACATGTACACTAACAGGCCA CAGGTGCTCCAACATTAGTCAT	
Sendai virus elimination	SeV	181 bp	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG	

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.2023.103133.

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