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Lab Resource: Multiple Cell Lines

Generation of two induced pluripotent stem cell lines (RCMGi005-A/B) from human skin fibroblasts of a cystic fibrosis patient with homozygous F508del mutation in *CFTR* gene

Irina Panchuk [a,*], Ekaterina Kondrateva [a], Anna Demchenko [a], Olga Grigorieva [a], Anastasija Erofeeva [a], Elena Amelina [b], Vyacheslav Tabakov [a], Maria Orlova [a], Ekaterina Voronina [a], Victoria Pozhitnova [a], Alexander Lavrov [a], Svetlana Smirnikhina [a], Sergey Kutsev [a]

[a] *Research Centre for Medical Genetics, Moscow, Russian Federation*
[b] *The Research Institute of Pulmonology, Moscow 115682, Russian Federation*

A R T I C L E I N F O

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Induced pluripotent stem cells (iPSC)
Cell reprograming
cystic fibrosis (CF)
Pluripotency

A B S T R A C T

Induced pluripotent stem cells (iPSCs) was successfully generated from skin fibroblast obtained from patient with cystic fibrosis by using non-integrating, viral CytoTune™-iPS 2.0 Sendai Reprogramming Kit, which contain three vectors preparation: polycistronic Klf4–Oct3/4–Sox2, cMyc, and Klf4. Created iPSC lines showed a normal karyotype, expressed pluripotency markers and demonstrated the potential to differentiate into three germ layers in spontaneous differentiation assay.

1. Resource table

Unique stem cell lines identifier	RCMGi005-A RCMGi005-B
Alternative name(s) of stem cell lines	P5L1, P5L5
Institution	Research Centre for Medical Genetics, Moscow, Russia
Contact information of distributor	MD Ekaterina Kondrateva, ekaterina.kondratyeva@gmail.com
Type of cell lines	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age: 29 Sex: male Ethnicity: Caucasian
Cell Source	skin fibroblast
Clonality	clonal
Method of reprogramming	Sendai virus (4 transgenes: OCT3/4, SOX2, KLF4, and c-MYC)
Genetic Modification	YES
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Cystic Fibrosis
Gene/locus	Gene: CFTR Locus: 7q31.2 Mutation:NM_000492.3:1521_1523delCTT (Phe508del)
Date archived/stock date	September 2018
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/RCMGi005-A https://hpscreg.eu/user/cellline/edit/RCMGi005-B
Ethical approval	This study was approved by FSBSI RCMG Ethics Committee (Protocol # 1 from 28.01.2016)

2. Resource utility

Pluripotent stem cell lines were derived from a cystic fibrosis donor who had homozygous F508del mutations in *CFTR* gene (Table 1). iPSC may be used for personalized therapy (genome editing), potential use in regenerative medicine, as modeling studies: researching intercellular interactions for further differentiation, 3D cell organization, drug screening and in toxicological studies.

* Corresponding author at: Research Centre for Medical Genetics, Moskvorechie str.,1, Moscow 115478, Russian Federation.
E-mail address: irina.panchuk42@gmail.com (I. Panchuk).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemisty)	Positive for pluripotency markers: SSEA-4, NANOG, SOX2, OCT-4	Fig. 1 panel B
	Quantitative analysis (Immunocytochemisty counting)	RCMGi005-A: SSEA-4: 98.8 %, NANOG: 96.2 %, SOX2: 96.5 %, OCT-4: 97.1 % RCMGi005-B: SSEA-4: 99,8%, NANOG: 95,9%, SOX2: 96,9%, OCT-4: 99,5%	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46XY Resolution; 400	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 STR sites analyzed, 15 matching	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sanger sequencing	Homozygous type of mutation	Fig. 1 panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by end point PCR Negative	Supplementary Fig. 1
Differentiation potential	In vitro directed differentiation	Positive expression of differentiation markers: Endoderm: FOXA2 Ectoderm: beta III tubulin Mesoderm: brachyury	Fig. 1 panel C
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

3. Resource details

Cystic fibrosis (CF) is inherited monogenic autosomal recessive multisystem disease that clinical affected airways, pancreas, biliary tract, gastrointestinal system, internal genitalia, heart (Cystic Fibrosis, n.d.). Mutations in *CFTR* gene drive disorder, the most frequent mutation is F508del (NM_000492.3:1521_1523delCTT(Phe508del)) which amount 85.8 % (Homozygous 44,5% and Heterozygous 41,2% variant) of disease alleles (Annual Data Report. Cystic Fibrosis Foundation Patient Registry, 2020). Disorder affecting 1 of 2000–3000 newborns is associated with Caucasian populations with Northern European ancestors (Chen Qionghua Shen Yuelin, and Zheng Jingyang 2021)). Clinical features start in early childhood, progress rapidly, and rather difference person to person. Average age of diagnosis of disease is about 3 years old (Chen Qionghua et al., 2021). Newborn screening improves quality of medical care, as in 2020, 88.5 % new cases were detected among people younger than 6 months (Annual Data Report. Cystic Fibrosis Foundation Patient Registry, 2020). Despite all approaches to therapy such as pathogenesis-based and palliative therapy, the development of new treatment considerations like regenerative medicine with combination of genome editing technology looks also promising. In this paper we present obtained reprogrammed iPSC from fibroblasts for continued use by developing gene therapy. Here, we established two iPSCs lines derived from a 28-year-old man with CF, clinically manifested and genetically proven (mutation F508del). Somatic cells, fibroblasts, were transformed into iPSCs by using non-integrating viral CytoTune™-iPS 2.0 Sendai Reprogramming Kit expressing the Yamanaka factors hc-Myc, hKLF4, hSox2 and hOct3/4. iPSC clones named RCMGi005-A and RCMGi005-B were purified (Fig. 1 A, scale bar is 200 μm). Positive expression of pluripotency markers SSEA4, OCT4, SOX2, and NANOG by provided immunocytochemistry confirmed pluripotency of the generated iPSCs lines (Fig. 1 B, scale bar is 200 μm) at passage 6–16 for RCMGi005-A and RCMGi005-B. At passage 17 RCMGi005-A and RCMGi005-B showed a normal 46,XY karyotype (Fig. 1 D). *In vivo* differentiation into the three germ layers at passage 16 and 18 was showed by directed differentiation in a monolayer from iPSC and were analyzed by immunocytochemistry assay on ectodermal marker (beta III tubulin), mesodermal markers (brachyury) and endodermal marker (FOXA2) (Fig. 1C; scale bar is 200 μm). Fibroblasts and the iPSC lines carry F508del mutation in homozygous state (Fig. 1E). In addition, all iPSC at 16 and 18 passage cell lines were mycoplasma-free was confirmed by end-point PCR based on amplification of species-specific regions of 16S rRNA genes with presence of UDG and Sendai virus was eliminated (according manufacturer's protocol) (Supplementary Fig. 1 and Fig. 2, respectively). STR analysis was performed using AmpFLSTR Identifiler Direct PCR Amplification Kit (Applied Biosystems) which evaluates the genetic profile of 16 microsatellite markers to detect and compare the genetic marks of cells, results shown coincided with the origin of fibroblasts and obtained iPSC line from same patient (Listed in STR report). Cell lines were obtained and collected in Moscow Branch of the Biobank "All-Russian Collection of Biological Samples of Hereditary Diseases".

4. Materials and methods

4.1. iPSCs derivation and cultivation

Fibroblast were obtained from patient skin cultivated in Amniocar medium (PanEco). On 2nd-3rd passage cells were transferred to fibroblast medium (User Guide: CytoTune-iPS 2.0 Sendai Reprogramming Kit). Method of cell cultivation was exactly the same as described in CytoTune-iPS 2.0 Sendai user guide. In brief, on day 1 100.000 cells/well were reprograming using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen™) based on the manufacturer's recommendation. On day 1–6 cells were cultured in fibroblast media, which were changed every day. On day 7, transduced cells were reseed on Vitronectin (Gibco) pre-coated culture plates. Then cells, on day 8 to 28, were switched to complete Essential 8™ (Gibco) medium, replace the spent medium every day thereafter. Colonies appeared by day 18–28, were passaged every 4–10 days with Cell Dissociation Reagent, Versen (PanEco), in ratio split of 1:6 to 1:10 in Essential 8™ Medium with 5mkM Y27632 (Stemcell Technologies). Cells were cultivated at 37 °C, 5 % CO_2.

4.2. Immunocytochemistry counting

IPSCs were fixed with 4 % paraformaldehyde solution and blocking with 0.25 % Triton X-100 in PBS and 1 % bovine serum albumin (BSA) (Gibco), respectively. Fixed cells were incubated with primary antibodies at room temperature (RT) one hour and then with secondary antibodies at RT for 1 h in the dark, diluted in 1 % BSA, respectively. Nuclei was stained with DAPI (Abcam) at RT for 10 min. Fluorescent images were captures with Lionheart FX Automated Microscope (Bio-Tek). Images were processed and analyzed using open-source software CellProfiler 3.0.0. This program can possible count percentage of positive cell of stained cell cytoplasm and nuclei by antibody and evaluate fluorescence intensity of it in green, red, blue channel.

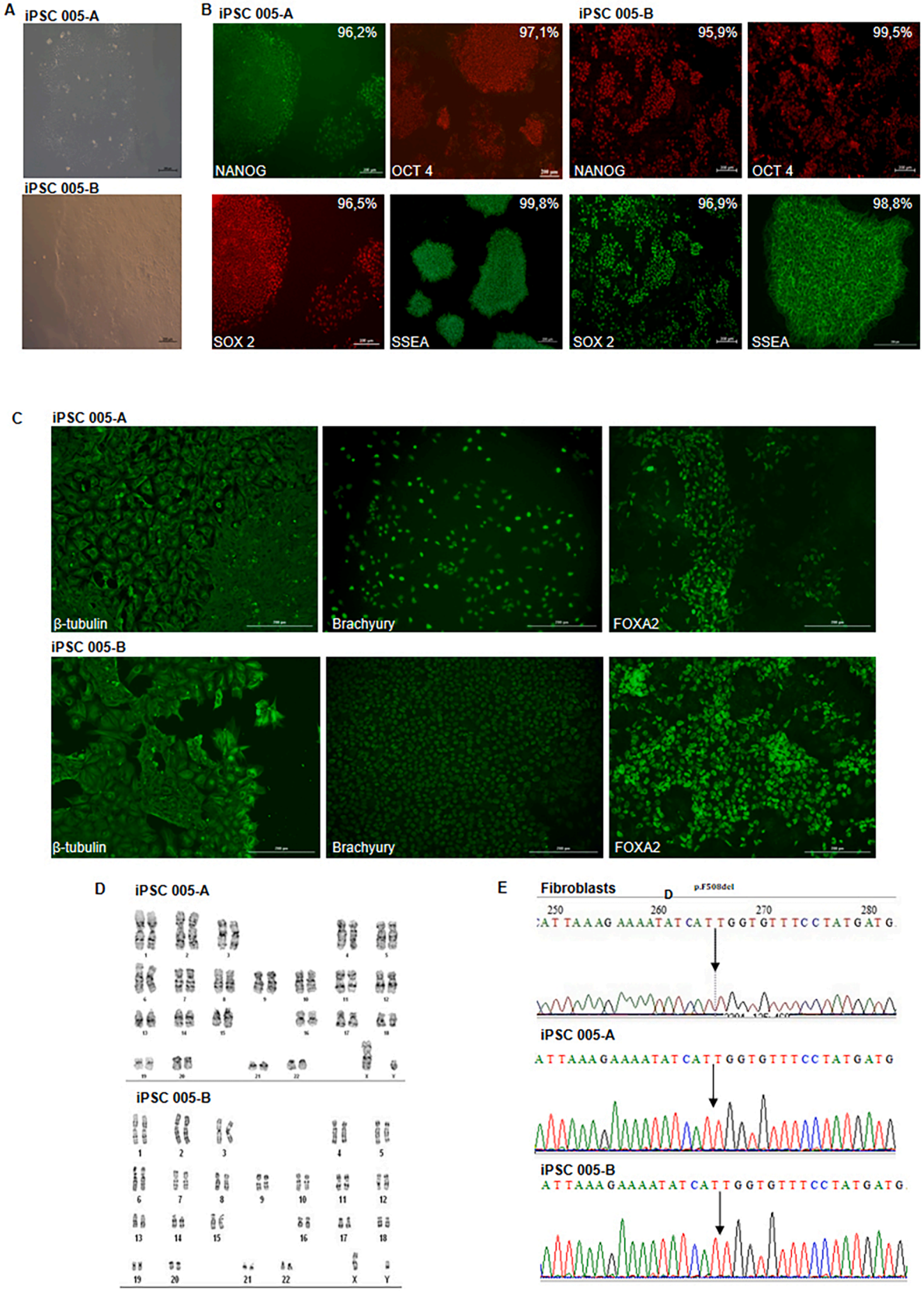

Fig. 1. Characterization of iPSC lines RCMGi005-A and -B from patient with cystic fibrosis.

Table 2
Reagents details.

	Antibody name	Dilution	Company Cat # and 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	1:100	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	Brachyury Monoclonal Antibody (X1AO2), Mouse	1:100	eBioscience™, Cat#:2028029 RRID: AB_2573016
	Anti-beta III Tubulin antibody [2G10], Mouse	1:100	Abcam, Cat #: 78078, RRID:AB_2256751
	Anti-FOXA2 antibody [EPR4465], Rabbit	1:400	Abcam, Cat #: ab108396, RRID:AB_10863255
Secondary antibodies	Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:300	Abcam, Cat #: A11037, RRID:AB_2534095
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:100	Abcam, Cat #: A11029, RRID:AB_297683
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:150	Cat# A-11032, RRID:AB_2534091
	Goat anti-Rabbit IgG (H&L) Secondary Antibody, Alexa Fluor® 488	1:500	Abcam, Cat#:150077, RRID:AB_2630356
Primers	Target		Forward/Reverse primer sequence (5′-3′)
Sanger sequencing	CFTR F508del locus (Supplementary Fig. 1 panel B)	503 bp	TGCATAGCAGAGTACCTGAAACAGGA TTGATCCATTCACAGTAGCTTACCCA
Primers for Sendai virus elimination			
	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 TGA TG

4.3. Karyotyping

Cells at 17th passage and approximately 70 % confluency were arrested by 0,1 μg/mL demecolcine (Sigma), harvested by trypsinization, hypotonized 13 min in 0.075 M KCl at 37 °C and fixed using standard cytogenetic procedures. Slides were stained by Vectashield mounting medium with DAPI (Vector Laboratories) contrasted with 0,3 mg/mL Actinomycin D (Serva). At least 15–20 metaphase images were analyzed according to ISCN 2020 nomenclature.

4.4. Sanger sequencing

The original fibroblasts and the resulting iPSCs were used to isolate genomic DNA using Quick-DNA Miniprep Kit (Zymo Research) according to manufacturer's protocol. The mutation locus in *CFTR* gene was amplified using original primers for PCR (Table 2). Amplifications were performed using Taq DNA polymerase (Evrogen) and Eppendorf Mastercycler Personal thermal cycler (Eppendorf). PCR conditions were as follows: 95 °C for 3 min; 35 cycles of 95 °C for 30 *sec*, 60 °C for 30 *sec*, and 72 °C for 60 *sec*; 72 °C for 5 min. ABI Prism 3130XL Genetic Analyzer (Applied Biosystems) was used for Sanger sequencing. Cycle parameters were as follows: 95 °C for 3 min; 25 cycles of 95 C for 15 *sec*, 50 C for 15 *sec*, and 60 C for 2 min.

4.5. Directed differentiation

For directed differentiation iPSC at passage 16 and 18 were detached using Versen, then dissociated into single cell suspension with TESR-E8 medium with 5 mkM Y27632 (ROCK inhibitor, Stemcell) and seed in 500.000 cell on pre-coated vitronectin 6 plate well. After reaching 60 % confluence, the following induce Endoderm: RPMI 1640, 1 % B27 (Gibco), 2 mM Glutamax, 100 ng/ml Activin A (Stemcell) and 3 μM CHIR99021(Stemcell) for 1st day and for 2–3 days without CHIR99021. Ectoderm: DMEM/F12, 2 %KOSR, 0,1mM NEAA, 2 mM Glutamax, 1 % N2 supplement (PanEco), 50 μM DAPT, 35 ng/ml BMP4 and culturing 3 days after all. Mesoderm: RPMI 1640, 1 % B27 without vitamin A and 3 μM CHIR 99021 for 24 h and RPMI 1640, 1 % B27 only for another 2 days. The expression of markers was verified by immunocytochemistry method (Table 2).

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.2022.102896.

References

Annual Data Report. Cystic Fibrosis Foundation Patient Registry. (2020).
Cystic Fibrosis. (n.d.). Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) 04/01/2020, World Wide Web URL: Https://Www.Omim.Org/Entry/219700.
Qionghua, C., Yuelin, S., Jingyang, Z., 2021. A review of cystic fibrosis: Basic and clinical aspects. Animal Models and Experimental Medicine 4 (13).