Stem Cell Research 50 (2021) 102137

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem Cell Line

Establishment human induced pluripotent stem cell line from idiopathic non-familial Parkinson's disease patient using self-replicating RNA vector

Harry Murti [a,e,1], Kelvin Pieknell [b,c,d,1], Indra Bachtiar [a,e], Siska Yuliana Sari [a,e], Yanuar Alan Sulistio [c,*], Sang-Hun Lee [b,c,d]

[a] Department of Translational Research Stem Cell, Stem Cell and Cancer Institute, PT. Kalbe Farma Tbk., Jakarta, Indonesia
[b] Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul, Republic of Korea
[c] Hanyang Biomedical Research Institute, Hanyang University, Seoul, Republic of Korea
[d] Graduate School of Biomedical Science and Engineering, Hanyang University, Seoul, Republic of Korea
[e] ReGeniC Laboratory, PT. Bifarma Adiluhung, Jakarta, Indonesia

A B S T R A C T

Induced pluripotent stem cell (iPSC) line HUi002-A was reprogrammed from skin fibroblasts via non-integrating, virus free self-replicating RNA. Skin fibroblasts from a 53-year-old male Caucasian, non-familial Parkinson's disease patient, idiopathic (clinical summary confirmed Parkinson's disease) was obtained from the Coriell Institute (AG20442). Generated iPSCs were characterized and pluripotency was confirmed.

Resource Table

Unique stem cell line identifier	HUi002-A
Alternative name(s) of stem cell line	iPSC AG20442R3
Institution	Hanyang Biomedical Research Institute, Hanyang University, Seoul, Korea
Contact information of distributor	Yanuar Alan Sulistio, Ph.D
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 53 Sex: Male Ethnicity: Caucasian
Cell Source	Skin Fibroblast
Clonality	Single Clone
Method of reprogramming	Transgene free, non-integrating & self-replicating RNA
Genetic Modification	NO
Type of Modification	NO
Associated disease	Parkinson disease, non-familial, idiopathic
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	09/07/2020
Cell line repository/bank	N/A
Ethical approval	The Coriell Institute for Medical Research (Camden, NJ), provider of the fibroblast line (AG20442), guarantees that the skin sample was collected under IRB approval and patient informed consent.

1. Resource utility

The iPSC line generated in this study can be utilized for in vitro modelling to study cellular and molecular mechanisms of Parkinson's Disease.

2. Resource details

Parkinson's Disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD), affecting 2% of the population over the age of 60 (Dauer and Przedborski, 2003). The clinical features of the disease include cardinal motor dysfunctions: postural instability, resting tremor, bradykinesia, and rigidity. Non-monogenic form of PD covers over 85% of PD cases, suggesting that sporadic form of the late-onset disease results from a complex interaction between genetic and environmental risk factors (Wu and Farrer,

* Corresponding author at: Hanyang Biomedical Research Institute, Hanyang University, Seoul, Republic of Korea.
E-mail address: sulistio@hanyang.ac.kr (Y.A. Sulistio).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scr.2020.102137
Received 28 October 2020; Received in revised form 1 December 2020; Accepted 17 December 2020
Available online 24 December 2020

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal (human ESC-like morphology). Scale bar, 100 μm	Fig. 1B
Phenotype	Immunocytochemistry	Expression of pluripotency markers OCT4, NANOG, SOX2. Scale Bar, 100 μm	Fig. 1B
	Alkaline Phosphatase (AP) activity	AP-positive colonies	Fig. 1B
	RT-qPCR	Cells express POU5F1 (OCT4), NANOG, LIN28A, CDH1 (E-CAD)	Fig. 1C
Genotype	Karyotype (G-banding) and resolution	46,XY,del(6),der(8)t(6;8),t(13;15),der(16)t(8;16),inv(17), resolution: 550 bands	Fig. 1A
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	100% match in 16 tested sites	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR was Negative	Supplementary Fig. 1
Differentiation potential	Directed differentiation (Immunocytochemistry)	FOXA2 (endoderm), BRACHYURY/T (mesoderm), and TUJ-1 (ectoderm) positive cell. Scale Bar, 100 μm.	Fig. 1F
	Directed differentiation (RT-qPCR)	GATA4 (endoderm), FOXC2 (mesoderm) & PAX6 (ectoderm)	Fig. 1G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

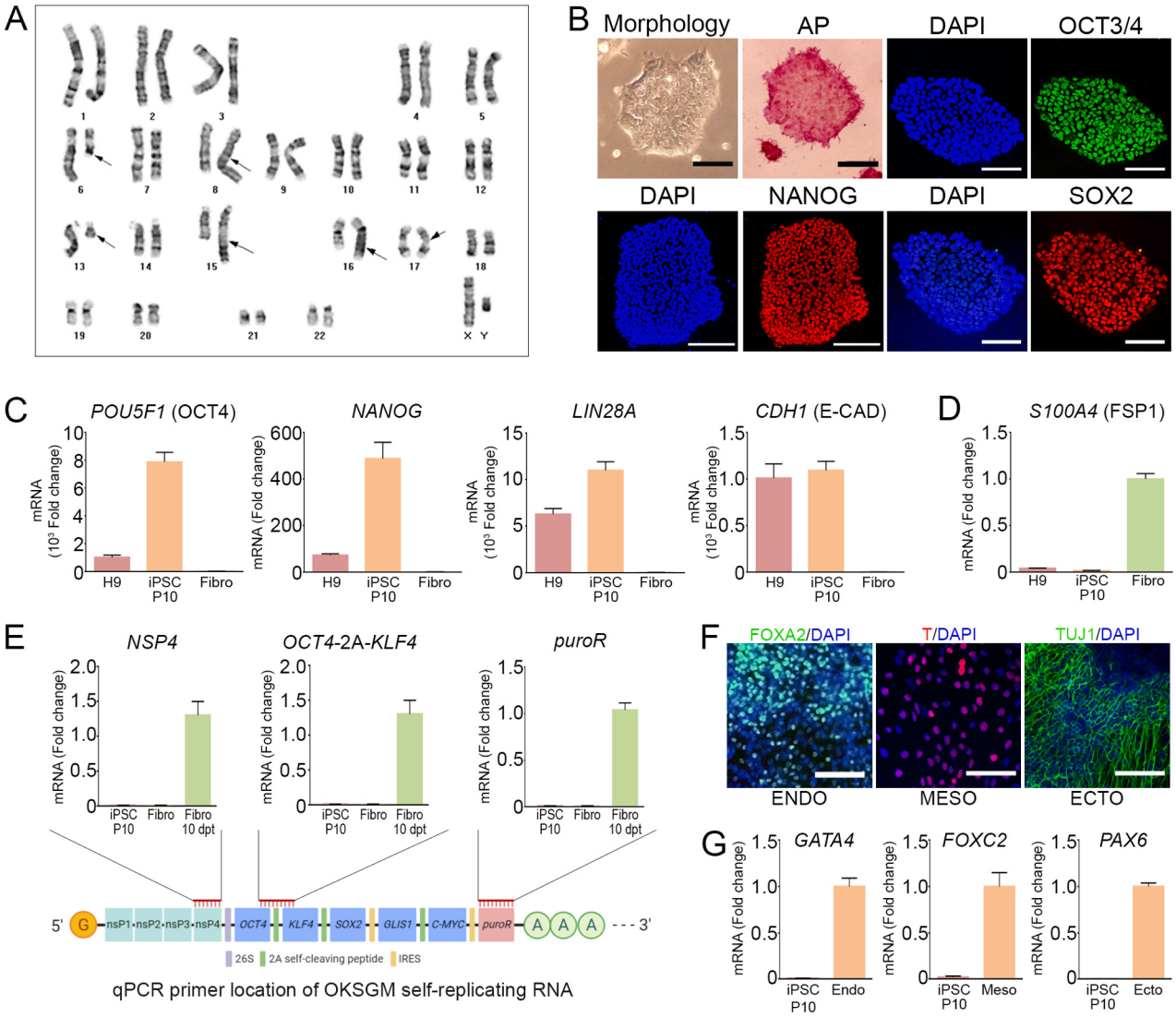

Fig. 1.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:200	BD Biosciences Cat# 561555, RRID: AB_10715577
Pluripotency Markers	Rabbit anti-SOX2	1:200	Millipore Cat# AB5603, RRID:AB_2286686
Pluripotency Markers	Rabbit anti-NANOG	1:200	Cell Signaling Technology Cat# 4903, RRID: AB_10559205
Differentiation Markers (Endoderm)	Mouse anti-FOXA2	1:200	Santa Cruz Biotechnology Cat# sc-374376, RRID: AB_10989742
Differentiation Markers (Mesoderm)	Rabbit anti-BRACHYURY	1:200	Abcam Cat# ab20680, RRID:AB_727024
Differentiation Markers (Ectoderm)	Rabbit anti-TUBULIN-β-3	1:200	BioLegend Cat# 802001, RRID:AB_2564645
Secondary antibodies	Goat anti-mouse Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A28175, RRID: AB_2536161
Secondary antibodies	Goat anti-rabbit Cy™3	1:500	Jackson ImmunoResearch Labs Cat# 111–165-144, RRID:AB_2338006
Secondary antibodies	Donkey anti-rat Cy™3	1:500	Jackson ImmunoResearch Labs Cat# 712–165-153, RRID:AB_2340667

qRT-PCR Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers	POUF51 (OCT4)	Forward: GGG AGA TTG ATA ACT GGT GTG TT Reverse: GTG TAT ATC CCA GGG TGA TCC TC
Pluripotency Markers	NANOG	Forward: TGC CTC ACA CGG AGA CTG Reverse: GCT ATT CTT CGG CCA GTT
Pluripotency Markers	LIN28A	Forward: CGG GCA TCT GTA AGT GGT TC Reverse: CAG ACC CTT GGC TGA CTT CT
Pluripotency Markers	CDH1 (E-CAD)	Forward: TGG TTC AAG CTG CTG ACC TTC Reverse: ACG ACG TTA GCC TCG TTC T
Residual self-replicating RNA reprogramming vector	Puromycin Resistance Gene (puroR)	Forward: GCA ACC TCC CCT TCT ACG AGC Reverse: TCA GGC ACC GGG CTT
Residual self-replicating RNA reprogramming vector	NSP4	Forward: CCA CAA TAC GAT CGG CAG TG Reverse: ATG TCC TGC AAC ATA TTC AAA
Residual self-replicating RNA reprogramming vector	OCT4-2a-KLF4	Forward: CGG CGC CAG AAG GGC AAG CG Reverse: CAC CTG CTT GAC GCA GTG TC
Fibroblast specific marker gene	S100A4 (FSP1)	Forward: TCT TGG TTT GAT CCT GAC TGC T Reverse: GCC CGA GTA CTT GTG GAA GG
Differentiation Markers (Endoderm)	GATA4	Forward: CTG GCC TGT CAT CTC ACT ACG Reverse: GGT CCG TGC AGG AAT TTG AGG
Differentiation Markers (Mesoderm)	FOXC2	Forward: CGG CGG CGC TTC AAA AA Reverse: TCT TGA TCA CCA CCT TCT TCT CG
Differentiation Markers (Ectoderm)	PAX6	Forward: AAC AGA CAC AGC CCT CAC AAA CA Reverse: CGG GAA CTT GAA CTG GAA CTG AC
House-Keeping Genes	GADPH	Forward: TTG AGG TCA ATG AAG GGG TC Reverse: GAG GTG AAG GTC GGA GTC A

2020). GWAS analysis from several population groups have discovered several dozens of genetic loci associated with PD, signifying that genetics play a major but not decisive role (Foo et al., 2020). The ability to generate neuron from iPSC of PD patients provide access for mechanistic studies and modelling real cases of human genetic diseases in vitro. A study by Tran et al. estimated that only 20% of PD-iPSC lines were derived from sporadic PD patients and 80% of PD-iPSC lines were derived from PD patients with monogenic mutations, despite in reality over 85% of PD cases are sporadic (Tran et al., 2020). Accordingly, the authors estimated that 10–30 PD-iPSC lines are needed in each study to achieve desirable statistical power to reliably model PD using iPSC. To this end, generation of sporadic PD patient-derived iPSC is important to capture the diverse heterogeneity of PD genetics. The iPSC line established in this study can be used for in vitro PD model to uncover underappreciated mechanism in PD pathogenesis. This approach, for example, has been used to shed light on LIN28A mutation in PD (Chang et al., 2019).

Skin fibroblasts from 53-year-old male late-onset sporadic Parkinson's disease patient were obtained from the 0442) and were reprogrammed to PD-specific iPSC. We generated patient-derived iPSC line (HUi002-A) using self-replicating RNA reprogramming vector, ReproRNA-OKSGM Kit (STEMCELL Technologies) which was followed by isolation and expansion as individual clone (Table 1). Donor fibroblast for this iPSC line has abnormal karyotype and iPSC reprogramming did not change the karyotype (Fig. 1A). HUi002-A showed typical PSC morphology and positive staining for pluripotent markers OCT4, NANOG, SOX2 and alkaline phosphatase (Fig. 1B). Pluripotent gene expressions were further confirmed in passage 10 of iPSC using POU5F1 (OCT4), NANOG, LIN28A, CDH1 (E-CAD) mRNA expression which are comparable or higher than H9 hESC (Fig. 1C), while fibroblast marker gene S100A4 (FSP1) expression disappeared after reprogramming (Fig. 1D). Traces of reprogramming vector was not detected in the passage 10 of iPSC as indicated by NSP4, OCT-2A-KLF4 junction region, and puromycin resistance (puroR) mRNA which expression was absent when compared to positive control (reprogramming vector-transfected fibroblast, 10-day post transfection) to the level comparable to untransfected donor fibroblast (Fig. 1E). In vitro trilineage differentiation potential of HUi001-A was confirmed by directed differentiation into endoderm, mesoderm, and ectoderm, as indicated by FOXA2, BRACHYURY (T), and TUJ1 immunofluorescence staining (Fig. 1F) and GATA4, FOXC2, PAX6 qPCR (Fig. 1G), respectively. The generated HUi002-A iPSC did not contain mycoplasma contamination (Fig. S1). The generated iPSC line was authenticated against its donor fibroblast line using short tandem repeat (STR) profiling.

3. Materials and methods

3.1. Reprogramming of skin fibroblasts into iPSCs

Late-onset PD patient-derived fibroblast were obtained from Coriell (AG20442-A). Donor fibroblast was plated on Matrigel-coated dish and cultured in DMEM/F12 (Gibco, #11330-032) with 10% FBS (Gibco, #26140-079), 1X Glutamax (Gibco, #35050-061). Reprogramming were done using intregration-free ReproRNA-OKSGM Kit (STEMCELL Technologies, #05930) according to the manufacturer's protocol. iPSC colonies were manually transferred to another Matrigel-coated dish for isolation and expansion and maintained in mTeSRTM until stable

Table 2 (*continued*)

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID

(STEMCELL Technologies, #85850) at 37 °C and 5% CO_2.

3.2. Karyotyping

Karyotyping of generated iPSC was performed by GenDix (Seoul, Korea) at 550 band resolution.

3.3. Immunofluorescence staining

iPSC cultured cells were cultured on matrigel-coated coverslips and fixed with 4% paraformaldehyde (PFA) in PBS and blocked in 0.3% Triton X-100 with 1% BSA for 1 h, then incubated with primary antibodies (Table 2) overnight at 4 °C following by secondary antibody for 1 h at RT. The stained cells were mounted with VECTASHIELD antifade mounting medium with DAPI (Vector Laboratories). Images were taken by epifluorescence microscope (Leica) or confocal microscope (Leica).

3.4. Alkaline phosphatase staining

iPSC cultured cells were fixed with 4% paraformaldehyde (PFA) in PBS, then stained with alkaline phosphatase staining solution (Sigma, #10713023001). Phase contrast images were taken using inverted microscope (Olympus).

3.5. Quantitative reverse transcription polymerase chain reaction

Total RNA was prepared using the Trizol Reagent (Invitrogen) according to manufacturer's instruction. cDNA synthesis was carried out using a Superscript kit (Invitrogen). Quantitative RT-PCR was performed on a CFX96TM Real-Time System using iQ™ SYBR green supermix (Bio-Rad) normalized to GAPDH expression. Primers used in this study are shown in Table 2.

3.6. In vitro trilineage differentiation

Pluripotency of iPSC was confirmed using directed differentiation protocol into each of three germ layer which then assayed using immunostaining and qPCR analysis. Endoderm differentiation was induced by basal differentiation media containing 20 ng/mL Activin A (Peprotech), and 3 μM CHIR (Stemgent) for 7 days. Mesoderm was induced using basal differentiation media containing 2 ng/mL Activin A (Peprotech) and 40 ng/mL BMP4 (Peprotech) for 7 days. Ectoderm differentiation was induced by basal differentiation media containing 10 μM SB-431542 (TOCRIS) and 100 ng/mL Noggin (Peprotech) for 12 days. For basal differentiation media, 1X N2 supplement (Gibco), 1X B27 without retinoic acid (Gibco), 1X Glutamax (Gibco, #35050-01), and 200 μM Ascorbic acid were supplemented into DMEM/F12 (Gibco).

3.7. STR analysis

STR analysis of 16 sites was performed by Macrogen (Seoul, Korea) using AmpFISTR®Identifiler® PCR Amplification KIT.

3.8. Mycoplasma test

Mycoplasma contamination was conducted using e-MycoTM Mycoplasma PCR detection kit (iNtRON Biotechnology) according to the manufacturer's protocol.

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.

Acknowledgements

This work was supported by 2017R1A5A2015395, 2020M3A9D8039925, 2017M3A9B4062415, funded by the National Research Foundation of Korea (NRF) of the Ministry of Science and ICT, Republic of Korea and PT Kalbe Farma, Tbk, Indonesia.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102137.

References

Chang, Mi-Yoon, et al., 2019. LIN28A loss of function is associated with Parkinson's disease pathogenesis. The EMBO J 38 (24), e101196.
Dauer, William, Przedborski, Serge, 2003. Parkinson's disease. Neuron 39 (6), 889–909.
Foo, Jia Nee, et al., 2020. Identification of risk loci for parkinson disease in asians and comparison of risk between asians and europeans: a genome-wide association study. JAMA Neurol.
Tran, Jenne, Anastacio, Helena, Bardy, Cedric, 2020. Genetic predispositions of Parkinson's disease revealed in patient-derived brain cells. NPJ Parkinson's Dis. 6 (1), 1–18.
Wu, Ruey-Meei, Farrer, Matthew J., 2020. Parkinson disease risk variants in East Asian populations. Nat. Rev. Neurol. 16 (9), 461–462.