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Lab Resource: Multiple Cell Lines

Establishment of induced pluripotent stem cells from schizophrenia discordant fraternal twins

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A B S T R A C T

Schizophrenia (SCZ) is one of the major psychiatric disorders. The genetic factor is certainly influential in the onset of the disease but is not decisive. There is no identified molecular/cellular marker of the disease, and the pathomechanism is still unknown. In this study, we generated human induced pluripotent stem cells (iPSCs) derived from SCZ-discordant fraternal twins, and they could contribute to elucidation of the pathomechanism of SCZ.

1. Resource table

Unique stem cell line identifiers	BRCi015-A BRCi003-A
Alternative names of stem cell lines	HPS3918 HPS3920
Institution	RIKEN BioResource Research Center (BRC), Kyoto, Japan
Contact information of distributor	Haruhisa Inoue h
Type of cell lines	iPSC
Origin	Human
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal vectors (Oct3/4, mp53DD, Sox2, KLF4, L-MYC, LIN28, EBNA1)
Multiline rationale	Fraternal twins
Gene modification	None
Type of modification	Not available
Associated disease	Schizophrenia
Gene/locus	Not identified
Method of modification	Not available
Name of transgene or resistance	Not available
Inducible/constitutive system	Not available
Date archived/stock date	June 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/BRCi015-A https://hpscreg.eu/cell-line/BRCi003-A
Ethical approval	Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University (approved No. R00 91, G259) Ethics Committee of the RIKEN BioResource Research Center (approved No. Tsukuba 29–1)

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2. Resource utility

Schizophrenia (SCZ) is a major inheritable psychiatric disorder. However, the genetic cause remains unknown and the molecular marker has not been identified. Human induced pluripotent stem cells (hiPSCs) from a SCZ patient and from his fraternal twin brother may offer an opportunity for advancing research to reveal the pathomechanism.

3. Resource details

Schizophrenia (SCZ) is one of the major psychiatric disorders, and it has been known since the beginning of recorded human history, although just being recognized as a disorder in the recent era. An individual with SCZ lacks interpretation of reality and shows abnormal behavior. Symptoms of SCZ are categorized into 3 types: positive symptoms, which are typically represented as hallucination, delusion

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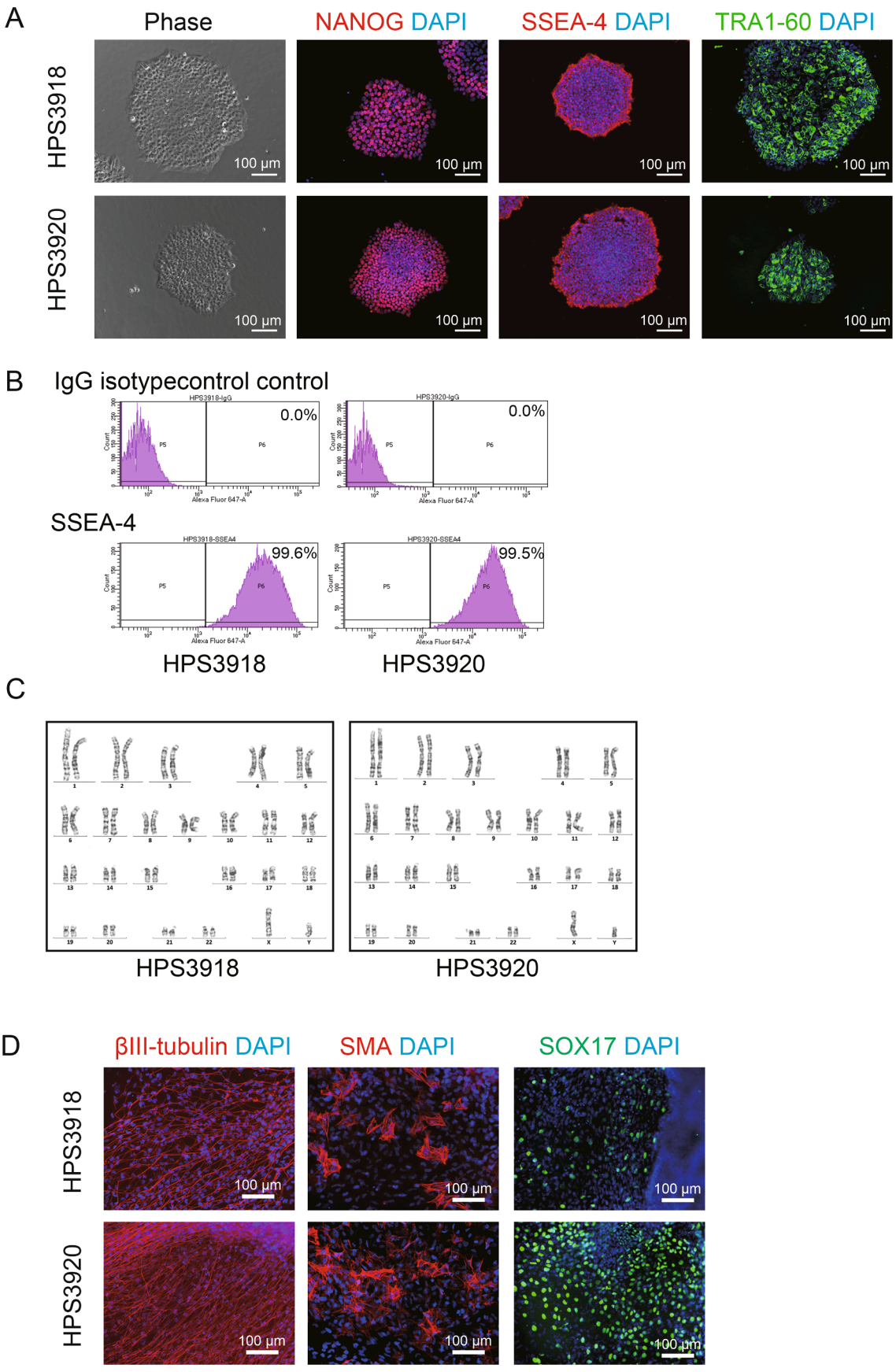

Fig. 1. Characterization of the iPSC lines.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal, human ESC-like morphology	Fig. 1 panel A
Phenotype	Immunocytochemistry	Positive staining of pluripotency markers: NANOG, SSEA-4, TRA1-60	Fig. 1 panel A
	Flow cytometry	SSEA-4 HPS3918: 99.6% positive HPS3920: 99.5% positive	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 400	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Not shown but available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	not performed	Not performed
	Southern Blot OR WGS	not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by Vero-Hoechst method and PCR. Negative	Not shown but available with the authors
Differentiation potential	Embryoid body formation OR Teratoma formation OR Scorecard	Describe expression of genes in embryonic bodies: βIII-Tubulin, SMA, and SOX17	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV, HTLV-1, Hepatitis B, Hepatitis C	Negative	Not shown but available with the authors
Genotype additional info (OPTIONAL)	Blood group genotyping	Not available	Not available
	HLA tissue typing	Not available	Not available

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:500	Cosmo Bio Co Cat# RCAB0003P, RRID: AB_1962353
Pluripotency Markers	Mouse anti-TRA1-60	1:200	Cell Signaling Technology Cat# 4746S, RRID: AB_2119059
Pluripotency Markers	Mouse anti-SSEA-4	1:1,000	Millipore Cat# MAB4304, RRID: AB_177629
Ectoderm differentiation Markers	Mouse anti-βIII-tubulin	1:1,000	Millipore Cat# CBL412X, RRID: AB_1977541
Mesoderm differentiation Markers	Mouse anti-SMA	1:500	DAKO Cat# M0851, RRID: AB_2223500
Endoderm differentiation Markers	Goat anti-SOX17	1:1,000	R and D Systems Cat# AF1924, RRID: AB_355060
Secondary antibodies	Donkey anti-Goat IgG Alexa Fluor 488	1:1,000	Molecular Probes Cat# A-11055, RRID: AB_142672
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 546	1:1,000	Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 488	1:1,000	Thermo Fisher Scientific Cat# A-32723 RRID: AB_2633275
Secondary antibodies	Goat anti-Mouse IgM Alexa Fluor 488	1:1,000	Thermo Fisher Scientific Cat# A-21042 RRID: AB_141357
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 546	1:1,000	Thermo Fisher Scientific Cat# A11010 RRID: AB_2534077
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Nested-PCR, 1st step	mycoplasma detection	*ACACCATGGGAG(C/T) TGGTAAT/CTTC(A/T)TCGACTT*	
Nested-PCR, 2nd step	mycoplasma detection	*(C/T)CAGACCCAAGGCAT GTG(C/G)GG(A/C) TGGATCACCTCCT/ GCATCCACCA(A/T)A(A/T)AC (C/T)CTT*	

and paranoia; cognitive dysfunctions, which are deterioration of essential cognitive skills like attention and working memory; negative symptoms, which include anhedonia and social withdrawal. SCZ is inheritable; 8–10% of an individual with a SCZ sibling, and 40–50% of those with a SCZ identical twin show the symptoms (Tamminga and Holcomb, 2005). Thus, the genetic factor obviously contributes to the risk of the disorder. GWAS-studies identified risk common variants with low effect and risk copy number variants. However, molecular/cellular markers of the disease have not been identified, and the pathomechanism is also still unknown. Abnormality during brain development could be the potential mechanism of the disease (Birnbaum and Weinberger, 2017). Human induced pluripotent stem cells (iPSCs) from a SCZ patient and a healthy fraternal twin of the patient, who may have relatively similar genetic and environmental backgrounds, are likely to offer a substrate to elucidate the mechanism of the onset of the disease. In this report, we established hiPSC lines, HPS3918 from a SCZ patient and HPS3920 from his healthy fraternal twin brother (Table 3). First, reprogramming factors were transduced to peripheral blood mononuclear cells (PBMCs) of donors by using episomal vectors. The transduced cells were reprogrammed and presented pluripotency markers: NANOG, SSEA-4 and TRA1-60 (Fig. 1A). Flow cytometry analysis demonstrated that 99.6% of the patient iPSCs and 99.5% of the healthy fraternal twin iPSCs were positive for SSEA-4, respectively (Fig. 1B). The results of karyotyping showed that a normal karyotype was maintained after the reprogramming in both clones (Fig. 1C). Finally, embryonic bodies (EBs) were formed from both iPSC clones, and they spontaneously differentiated into three germ layers showing ectodermal marker βIII-Tubulin, mesodermal marker smooth muscle actin (SMA), and endodermal marker (SOX17), respectively (Fig. 1D, Table 1).

4. Materials and methods

4.1. Ethics statements

The generation and use of human iPSCs were approved by the Ethics Committees of RIKEN BioResource Research Center and the Department of Medicine and Graduate School of Medicine, Kyoto University. Formal informed consent was obtained from the patient. All experiments in this study were performed in accordance with the approved guidelines.

Table 3
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
BRCi015-A (HPS3918)	HPS3918	Male	49	Japanese	–	Schizophrenia
BRCi003-A (HPS3920)	HPS3920	Male	49	Japanese	–	Healthy

4.2. Generation of iPSCs

The reprogramming factors (Oct3/4, mp53DD, Sox2, KLF4, L-MYC, LIN28, EBNA1) on episomal vectors (Okita et al., 2013) were transduced into PBMCs from SCZ-discordant fraternal twins by in-house constructed episomal vectors with electroporation (4D-Nucleofector X-unit; Lonza, Basel, Switzerland). The generated iPSCs were seeded on laminin 511 E8-coated plates and cultivated with StemFit AK02N medium (Ajinomoto, Tokyo, Japan) in an incubator conditioned with 37 °C / 5% CO_2 (Nakagawa et al., 2014). The cells were isolated by TrypLE Select diluted 1:1 with 0.5 mM EDTA solution (Thermo Fisher Scientific, Waltham, MA, US) and passaged every 6–8 days to maintain pluripotency. 10 μM Y-27632 (Nacalai Tesque, Kyoto, Japan) was added to the culture medium after passaging and replaced with fresh medium on the following day.

4.3. In vitro differentiation into three-germ layer cells

For differentiation into three-germ layers, embryonic bodies (EBs) were formed from approximately 9,000 iPSCs in U-bottom 96-well plates (Thermo Fisher) under KSR medium [Knockout DMEM with 20% Knockout Serum Replacement (KSR; Thermo Fisher), 10 mM Non-Essential Amid Acid (NEAA; Thermo Fisher), 2-mercaptoethanol (2-ME; Thermo Fisher), 200 mM L-glutamate] for two weeks. Then EBs were transferred onto Matrigel-coated 24-well plates and cultivated in DMEM/F12 with Glutamax and 10% fetal bovine serum (FBS; Thermo Fisher) for 7 additional days.

4.4. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde, then treated with 0.2% Triton X-100 (Nacalai Tesque) for permeabilization. After blocking with 5% Blocking One Histo (Nacalai Tesque), the cells were stained with the primary and secondary antibodies listed in Table 2. 4′,6-diamidino-2-phenylindole (DAPI; Thermo Fisher) was used for nuclei staining. Fluorescence images were obtained by fluorescence microscope BZ-X710 (Keyence, Osaka, Japan).

4.5. Karyotype analysis

Karyotype of the iPSC lines at passage number 5 was analyzed. 80–90% confluent cells were treated with colcemid solution for more than 2 h at 37 °C and harvested. The cells were exposed to hypotonic treatment and fixed. A Giemsa banding karyotype analysis of a total of 20 metaphases was conducted by LSI Medience (Tokyo, Japan).

4.6. Flow cytometry analysis

iPSCs were treated with Accumax (Innovative Cell Technologies, San Diego, CA, US) and dissociated into single cells. Then, the cells were incubated at 1.0×10^6 cells/ml concentration in PBS containing 2% FBS and 20 μl SSEA-4 APC conjugated monoclonal antibody (BD Biosciences, Franklin Lakes, NJ, US) for 30 min at 4 °C. The cells were washed twice with PBS containing 2% FBS and analyzed by FACSAria (BD Biosciences). Unstained negative controls were also analyzed to exclude signals from non-specific fluorescence.

4.7. DNA fingerprinting

The cell identity was verified by short tandem repeat (STR) analysis with PowerPlex 16 HS system (Promega, Madison, WI, US) implemented on 3500 × L Genetic Analyzer (Themo Fisher), which was calibrated by PowerPlex 4C Matrix Standard (Promega).

4.8. Mycoplasma test

The generated iPSCs were tested by Vero-Hoechst method and nested-PCR method, confirming no contamination by mycoplasma. For the Vero-Hoechst method, the iPSC culture medium was co-cultured with VERO cells (RCB0001, RIKEN BRC Cell Bank) and then stained using Hoechst33258 (Thermo Fisher). Primers listed in Table 2 were used in nested-PCR.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Haruhisa Inoue reports financial support was provided by Japan Agency for Medical Research and Development.

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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.2021.102504.

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