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Lab Resource: Stem Cell Line

Generation of refractory schizophrenia patient-derived induced pluripotent stem cell line UJSi001-A

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A B S T R A C T

Schizophrenia is considered one of the most serious mental disorders nowadays. Approximately 30–60% of people with schizophrenia do not present adequate response to drug treatment and persist with symptoms of the disease; they are known as refractory schizophrenic people. We generated induced pluripotent stem cells (iPSCs) from a refractory schizophrenia patient by electroporation of peripheral blood mononuclear cells (PBMC) with episomal plasmids encoding *OCT 4, SOX 2, NANOG, LIN 28, KLF 4* and *LMYC*. The resulting iPSCs had normal karyotype, were free of genomically integrated episomal plasmids, expressed pluripotency markers, and could differentiate into the three germ layers *in vivo*.

Resource table.

Unique stem cell line identifier	UJSi001-A
Alternative name(s) of stem cell line	WXZ-C3
Institution	Neurobiology & Mitochondrial Key Laboratory, School of Pharmacy, Jiangsu University, Zhenjiang, China
Contact information of distributor	Jing Sun, sjyancheng@aliyun.com
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 14 Sex: Female Ethnicity: Asian (China)
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free episomal plasmid vectors
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Refractory schizophrenia
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	May 2018
Cell line repository/bank	https://hpscreg.en/cell-line/UJSi001-A
Ethical approval	Patient informed consent was obtained. Ethics approval was received from the Ethics Committee of Bio-X Institutes in Shanghai Jiao Tong University (M16035)

Resource utility

This 14 years old patient-specific iPSC line is useful for modeling refractory schizophrenia *in vitro* and studying the molecular basis of the disease.

Resource details

Approximately 40% of people with schizophrenia do not present adequate response to drug treatment and persist with symptoms of the disease; they are known as refractory schizophrenic people (Okita et al., 2011). To uncover the refractory schizophrenia neuronal pathological processes patient-derived somatic cells can be reprogrammed to a pluripotent state and differentiated into relevant cell types for studying the disease *in vivo*. Peripheral blood mononuclear cells (PBMC) were derived from a 14-year-old refractory schizophrenia patient. The written informed consent was obtained from guardians. PBMC were reprogrammed to iPSC by transfecting 6 transcript factors (*OCT 4, SOX 2, NANOG, LIN 28, KLF 4* and *LMYC*) carried on an episomal plasmid (Pinto et al., 2018). After reprogramming, the UJSi001-A shows typical

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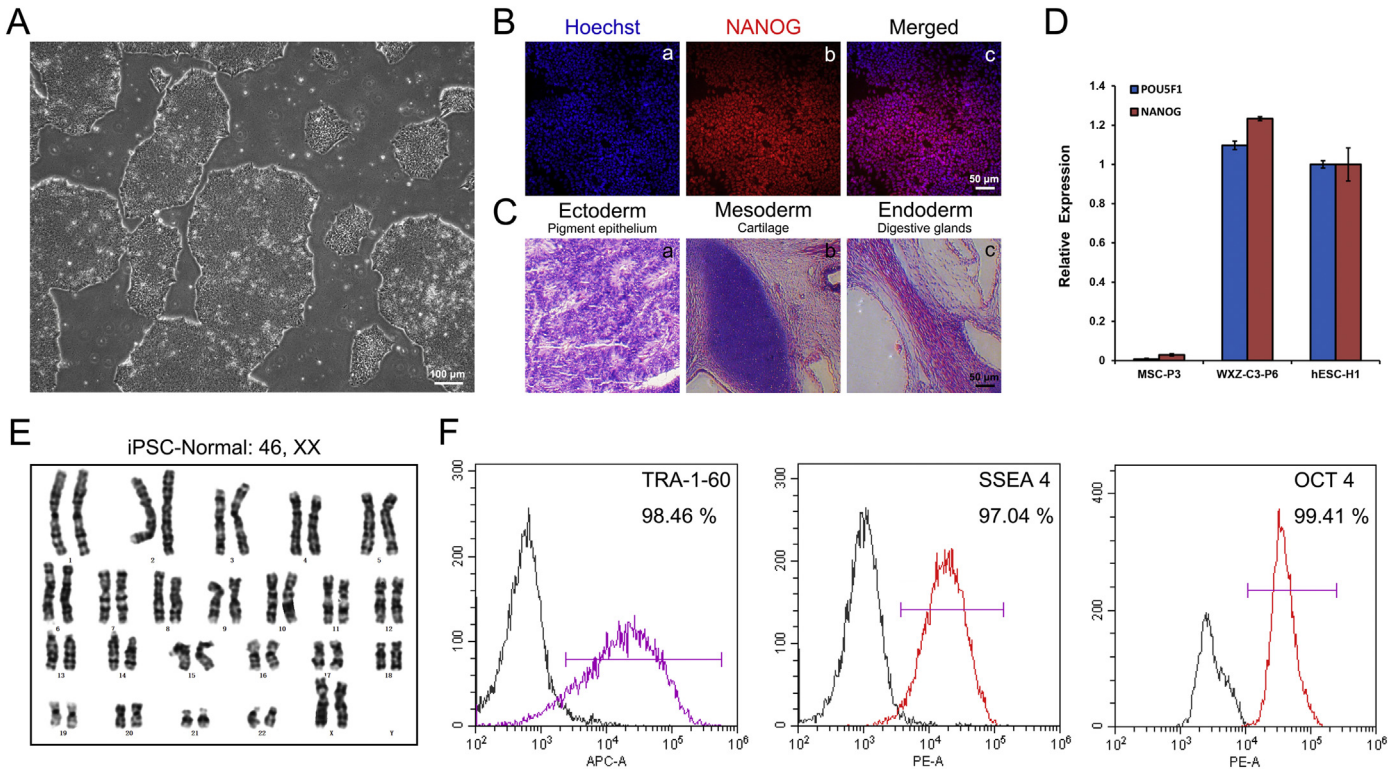

Fig. 1. Characterization of human UJSi001-A line generated from peripheral blood mononuclear cells (PBMCs). (A) Established UJSi001-A line shows typical round shape colony on the Matrigel. Scale bar: 100 μm (B) Immunofluorescence-based detection of human pluripotency-associated proteins NANOG. Hoechst was used for the nuclei staining. Scale bar: 50 μm. (C) H&E staining of teratoma sections identifying the three germ layers. Scale bar: 50 μm. (D)Quantitative PCR of relative gene expression of pluripotency genes compared to MSC and hESC. (E) UJSi001-A line shows normal karyotype after long term cultures. Presence of female karyogram 46, XX. (F) Flow cytometry profiles showing pluripotent surface markers of UJSi001-A cells.

embryonic stem cell-like morphology (Fig. 1A). To confirm the pluripotency of UJSi001-A, the expression of several pluripotent markers were evaluated by qRT-PCR, immunocytochemistry, and FACS analyses. Endogenous expression of *NANOG* was identified by qRT-PCR and immunocytochemistry, *TRA-1-60, SSEA 4, OCT 4* proteins were assessed by FACS analysis (Fig. 1B,D,F). Karyotype analysis showed no gain or loss of chromosomes, and no large chromosomal aberrations (Fig. 1E). Teratoma assay confirmed the pluripotency of the UJSi001-A *in vivo*, by detection of the presence of structures representing the three germ layers (Fig. 1C). The results of short tandem repeats (STR) analysis revealed that the UJSi001-A cell line matched to 17 loci tested of the reference STR profiles of PBMC. The Mycoplasma testing by RT-PCR was negative (Supplementary 2).

Materials and methods

PBMCs reprogramming (Meraviglia et al., 2015; Dowey et al., 2012)

Blood samples were collected from a patient into BD Vacutainer cell preparation tubes (CPT). The isolation of PBMCs from blood samples by using Ficoll-Paque Plus (GE, 17-1440-03, USA) for density-based centrifugal separation. PBMCs were grown in StemPro®-34 SFM (Gibco, 10639-011, USA) complete medium containing cytokines (SCF, IL-3, and GM-CSF) for 12 days, and 2×10^6 of PBMCs were electroporated with Episomal iPSC Reprogramming Vectors (Invitrogen, A14703, USA) by using the Neon Device (Thermo, MPK5000, USA) with the program setting: 1650 V, 10 msec, 3 pulses. After transfection, PBMCs were plated onto Matrigel®-coated 6 well culture-plates (Corning, 354,277, USA), and incubated overnight in complete StemPro™-34 medium containing cytokines. Next day the medium was changed into N2B27 medium supplemented with 100 ng/ml bFGF, and the medium was replaced every day. One week later, the medium was changed to Essential 8™ medium (Gibco, A15169–01, USA) and the medium was replaced every day. Colonies of iPSCs were manually picked 15–21 days after transfection, and transferred onto fresh Matrigel®-coated 6 well culture-plates for expansion. iPSCs were passaged by 0.5 mM Nuwacell™ EDTA (Nuwacell, RP01007, China) for 8 min at 37 °C. iPSCs were split at 1:10 twice a week, and the cells were cultured at 37 °C in 5% CO_2.

mRNA isolation and quantitative real-time PCR

Total mRNA was isolated using TRIzol reagent (Invitrogen, Cat# 15596026) according to the manufacturer's protocol. Then 1μg mRNA were subjected to cDNA synthesis using Reverse Transcription Kit (Takara, Cat# RR036A). In this study, amplification was done in a LightCycler 96 (Roche, Switzerland), and the results were analyzed using LightCycler software version 1.1.0.1320. The expression of pluripotent stem cell markers (*POUSF1, NANOG*) and endogenous control gene (*GAPDH*) were amplified using primers listed in Fig. 1D. hESC line H1 was used as positive control, WXZ-C3 line was alternative name of UJSi001-A, Mesenchymal stem cell (MSC) line P3 was used as negative control (Table 1).

Fluorescent immunocytochemistry

For immunofluorescence assay, the UJSi001-A clones were subseeded on Matrigel -coated coverslips. The iPSCs were permeabilized with 0.3% Triton X-100 for 5 min at room temperature (RT), and blocked with 2.5% albumin from bovine serum (BSA) for 1 h at RT. The antibodies were diluted in blocking solution. Then the coverslips were gently mounted on microscope slides using antifade reagent with DAPI (ThermoFisher, Cat# P36931). Images were captured under fluorescence microscope (Nikon, Ti-E Live Cell Imaging System, Japan).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Expression of pluripotency markers: Nanog	Fig. 1 panel B
	Flow cytometry	*OCT 4*: 99.41% *TRA 1-60*: 98.46% *SSEA-4*: 97.04%	Fig. 1 panel F G H
	qRT-PCR	Expression of pluripotency markers:*POUSF 1, NANOG*	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel E
Identity	STR analysis	17 loci tested, all matched	Available with the authors
Microbiology and virology	Mycoplasma	Mycoplasma testing by qRT-PCR or luminescence. Negative	Supplementary 2
Differentiation potential	Teratoma formation	Proof of three germlayers formation	Fig. 1 panel C
Donor screening (OPTIONAL)	HBsAg, HBsAb, HCV, TPPA, HIV	Negative	Supplementary 1
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	*NANOG* (D73G4) XP® Rabbit mAb	1:200	Cell Signaling Technology Cat# 4903, RRID:AB_956161
	TRA-1-60 Mouse mAb	1:400	Cell Signaling Technology Cat# 4746, RRID:AB_1186140
	OCT-4A (C30A3) Rabbit mAb	1:200	Cell Signaling Technology Cat# 2840, RRID: AB_1291399
Secondary antibodies	Goat anti Mouse IgG Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A-21124, RRID:AB_2535766
	Goat anti Rabbit IgG(H + L) Highly Cross Adsorbed Secondary Antibody, Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat # A11078, RRID:AB_2534122
	Goat anti Mouse IgM Heavy Chain Cross Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A21043, RRID:AB_2535712

Primers

	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers (qRT-PCR)	*NANOG*	CAGCCCCGATTCTTCCACCAGTCCC/CGGAAGATTCCCAGTCGGGTTCACC
House-Keeping Genes (qRT-PCR)	*GAPDH*	GGTTGTCTCCTGCGACTTCA
Pluripotency Markers (qRT-PCR)	*POU5F1*	CTTCTGCTTCAGGAGCTTGG/GAAGGAGAAGCTGGAGCAAA

Antibodies used are listed in Table 2.

Karyotype analysis

Cells were evaluated at passage 10. G-banding was used to karyotype our iPSC line. The iPSC at 60–80% confluency were treated with 50 ng/ml colchicine for 2 h at 37 °C, and detached by Accutase™ Cell Dissociation Reagent (Thermo, A1110501, USA) for 10 min at 37 °C, until obtaining single-cell dissociation. Cells were collected and centrifuged at 400*g* for 5 min at RT. Before fixation, iPSCs were incubated with 1 ml of a hypotonic solution (0.6% sodium citrate and 0.13% potassium chloride) for 20 min at RT, and then cells were fixed with Methanol: Acetic acid (3:1). The cell pellet was washed, resuspended, dropped on a slide and dried on a hotplate. Metaphase plates were analyzed using a Carl Zeiss Axioplan 2 imaging microscope and ISIS 3 (Meta Systems GmbH) software. There are about 500–800 metaphases on each plate, and 20–30 randomly metaphases were counted. Chromosome GTG band analysis was performed using an International System for Human Cytogenetic Nomenclature.

Teratoma assay

Teratoma assay was performed for confirmation of pluripotency *in vivo*. The iPSCs were cultured in feeder-free conditions, and 3×10^6 iPSCs were collected by Nuwacell™ EDTA (Nuwacell, RP01007, China) for 8 min at 37 °C. Before injection, cells were suspended in 50% Matrigel® (Corning, 354,277, USA) with PBS, and injected subcutaneously in the neck of NOD/SCID mice. After 1 month, teratomas were harvested and processed for haematoxylin and eosin staining.

Short tandem repeat (STR) analysis

DNA was extracted and purified from UJSi001-A cell line and the parental PBMCs by Chelex 100. AmpFLSTRTM Identifiler™ PCR Amplification Kit (Thermo Fisher Scientific) was used to determine genetic signature of 17 loci (D5S818, D21S11, D18S1364, D6S1043, D3S1358, D13S317, D7S820, D16S539, Penta D, D19S433, D22S1045, D11S2368, Penta E, D2S441, D12S391, D2S1338 and D13S325). PCR product was electrophoresed on an 3500xl Genetic Analyzer (Applied Biosystems, USA) and data were analyzed using GeneMapper v 4.0 software (Applied Biosystems).

Mycoplasma detection

Mycoplasma status was assessed using the MycoSEQ™ Mycoplasma Detection Kit (Thermo Fisher, 4460623, USA) following the manufacturer protocol. Data were analyzed by AccuSEQ™ v2.0 software.

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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.2019.101460.

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