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Lab Resource: Multiple Cell Lines

Generation of IBMS-iPSC-015, -016, -017 human induced pluripotent stem cells (IBMSi013-A, IBMSi014-A, and IBMSi015-A) derived from patients with atrial fibrillation

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A B S T R A C T

Atrial fibrillation is the most common heart disease in the world, with around 35 million patients in 2020. Here we reported the generation of IBMS-iPSC-015-06, IBMS-iPSC-016-06, and IBMS-iPSC-017-02 as human induced pluripotent stem cell (iPSC) lines from patients' peripheral blood mononuclear cells (PBMCs) with atrial fibrillation. The cell lines expressed properties of pluripotent stem cells, including pluripotent markers and the ability to differentiate into three germ layers. These cell lines served as suitable models for studying alternative therapies of atrial fibrillation.

Resource Table

Unique stem cell lines identifier	IBMSi013-A IBMSi014-A IBMSi015-A
Alternative names of stem cell lines	IBMS-iPSC-015-06 IBMS-iPSC-016-06 IBMS-iPSC-017-02
Institution	Institute of Biomedical Sciences, Academia Sinica, Taiwan
Contact information of distributor	Patrick Ching-Ho Hsieh, phsieh@ibms.sinica.edu.tw
Type of cell lines	Induced pluripotent stem cell lines (iPSC)
Origin	Human
Cell Source	Peripheral Blood Mononuclear Cells (PBMC)
Clonality	Clonal
Method of reprogramming	Transgene free (CytoTune™-iPS 2.0 Sendai Reprogramming Kit)
Multiline rationale	Derived from three patients with the same phenotype
Gene modification	No
Type of modification	N/A
Associated disease	Atrial fibrillation
Gene/locus	Unknown
Method of modification	N/A N/A
Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	June 2020
Cell line repository/bank	Bioresource Collection and Research Center, Taiwan, https://catalog.bcrc.firdi.org.tw/SubdomainSearch?id=&aType=4&bid=iPS%20cell
Ethical approval	The study was approved by the Institution Review Board on bio- medical science research at Academia Sinica Taiwan (AS-IRB-BM-15044) and National Taiwan University Hospital (201505121RIND).

1. Resource utility

Atrial fibrillation is a common cardiomyopathy worldwide (Anumonwo and Kalifa, 2016). The three patient-specific iPSC lines, IBMS-iPSC-015-06, IBMS-iPSC-016-06, and IBMS-iPSC-017-02, can be used for further mechanistic studies of alternative therapies.

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[1] Yueh-Ting Chiu and Ming-Heng Tsai shared dual-first authorship.

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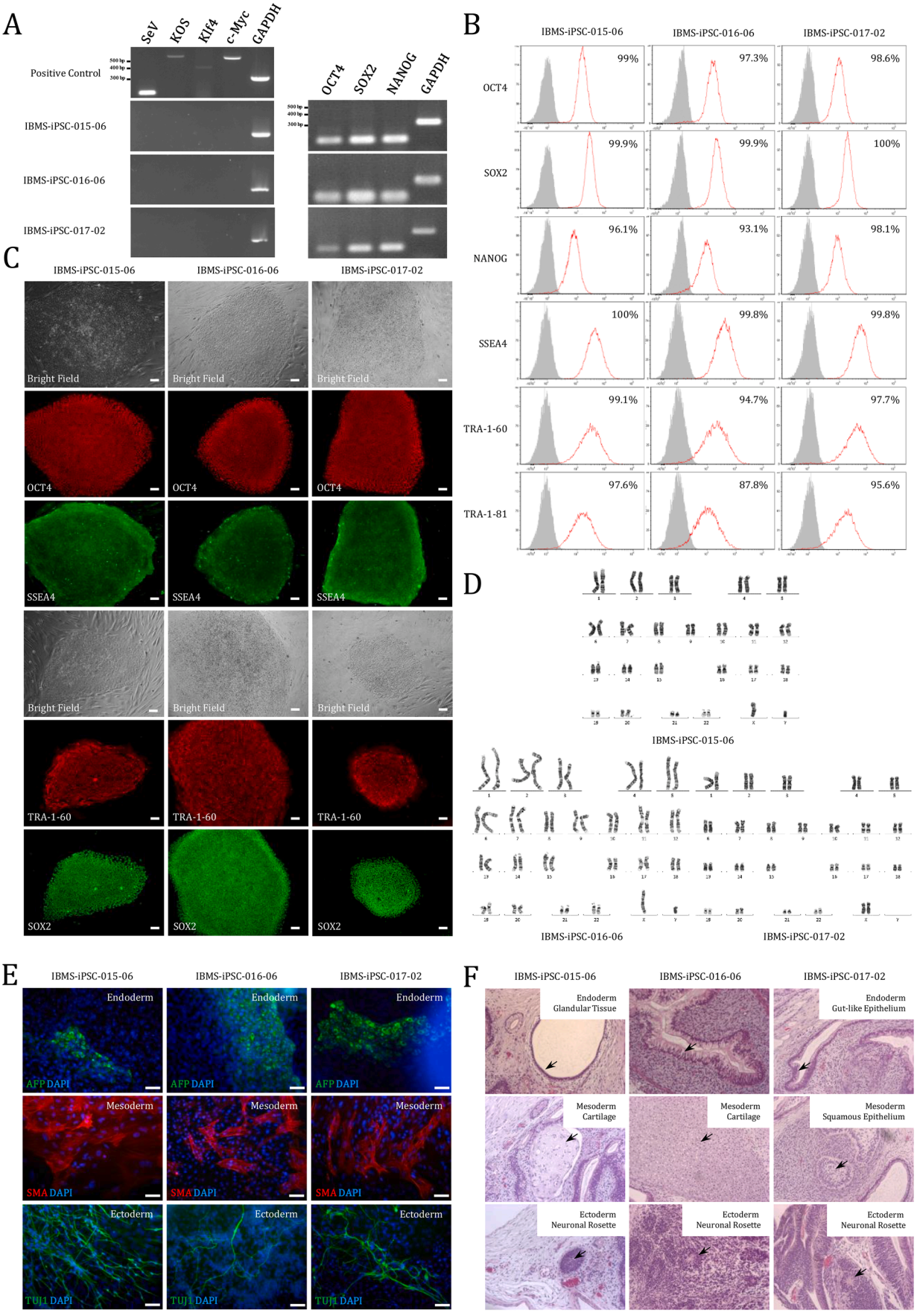

Fig. 1. Characterization of IBMS-iPSC-015-06, IBMS-iPSC-016-06, and IBMS-017-02.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
IBMS-iPSC-015-06		Male	53	Asian	Unknown	Atrial fibrillation
IBMS-iPSC-016-06		Male	34	Asian	Unknown	Atrial fibrillation
IBMS-iPSC-017-02		Female	32	Asian	Unknown	Atrial fibrillation

2. Resource details

Atrial fibrillation is one of the most prevalent cardiomyopathies in the world. It's characterized by irregular heart rates, leading to dizziness, chest pain, and heart failure. Although the pathological mechanism has been well-studied, novel and affordable treatments are essential to confronting the increasing occurrence rate worldwide (Munger et al., 2014). We identified two male and one female donors with atrial fibrillation and reprogrammed the donors' peripheral blood mononuclear cells (PBMCs) into induced pluripotent stem cells (iPSCs). Oct4, Sox2, Klf4, and c-Myc, the Yamanaka's factors, were delivered through the integration-free Sendai virus delivery method. All three cell lines, IBMS-iPSC-015-06, IBMS-iPSC-016-06, and IBMS-iPSC-017-02, were tested and presented to be exogenous transgene-free and expressed various endogenous pluripotent markers, such as OCT4, SOX2, and NANOG (Fig. 1A). Fig. 1B and C showed the flow cytometry staining and the immunofluorescence of all cell lines expressing pluripotent markers. Chromosomal integrity of the three cell lines (IBMS-iPSC-015-06 with 46XY, IBMS-iPSC-016-06 with 46XY, and IBMS-iPSC-017-02 with 46XX) by the end of the reprogramming process was demonstrated through G-band karyotyping (Fig. 1D). *In vitro* Embryoid bodies (EBs) formation and *in vivo* teratoma formation assays were carried out to determine the differentiation potential of three cell lines. All cell lines demonstrated capability in differentiating into three germ layers by expressing endodermal (alpha-fetoprotein, AFP), mesodermal (alpha-smooth muscle actin, α-SMA), and ectodermal (neuron-specific beta III Tubulin, TUJ1) markers (Fig. 1E). Besides, all cell lines exhibited pluripotency through teratoma formation after transplantation including ectodermal neural tissue, mesodermal cartilage, and endodermal glandular structure or gut-like epithelium (Fig. 1F). Also, the three engendered cell lines were mycoplasma-free (Fig. S1). To confirm cell lines identity, the STR-PCR assay was performed and concluded with a 100% match to the parental PBMCs, respectively. In summary, the three iPSC lines from different patients can be good models for further research on genetic causes of atrial fibrillation (Tables 1 and 2).

3. Materials and methods

3.1. Donor cells

The PBMCs were isolated from 10 mL of whole blood samples collected by Vacutainer® CPT™ (BD Biosciences) and maintained in Stempro-34 SFM completed medium (Thermo Fisher Scientific).

3.2. Reprogramming and hiPSC maintenance

CytoTune™-iPS 2.0 Sendai Reprogramming Kit was used to reprogram 5×10^5 PBMCs for individual donors (Thermo Fisher Scientific). Mitomycin-c treated mouse embryonic fibroblast cells served as feeder cells for selected colonies on Day 21–28. Cells were cultured with daily medium changes in DMEM/F12 medium supplemented with 20% KnockOut serum replacement (Thermo Fisher Scientific), 0.1 mM NEAA (Thermo Fisher Scientific), 1 mM Glutamine (Thermo Fisher Scientific), 0.1 mM 2-mercaptoethanol (Thermo Fisher Scientific), and 10 ng/mL bFGF (Thermo Fisher Scientific) in 5% CO_2 at 37 °C. hiPSC colonies were passaged mechanically every 5 days and plated onto MEF feeders. Cells were shifted to feeder-free conditions and cultured in StemFlex medium (Thermo Fisher Scientific). Cells were cultured in 5% CO_2 at 37 °C and were passaged every 3 days using Accutase (Stemcell Technologies) at a ratio of 1:16 and plated onto Matrigel-coated plates.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase Contrast Microscopy	ES-like morphology	Fig. 1 panel C
Pluripotency expression	Immunocytochemistry	Positive for pluripotency markers: OCT4, SSEA4, TRA-1-60, and SOX2.	Fig. 1 panel C
	Flow cytometry	IBMS-iPSC-015-06: OCT4 99%, SOX2 99.9%, NANOG 96.1%, SSEA4 100%, TRA-1-60 99.1%, and TRA-1-81 97.6% IBMS-iPSC-016-06: OCT4 97.3%, SOX2 99.9%, NANOG 93.1%, SSEA4 99.8%, TRA-1-60 94.7%, and TRA-1-81 87.8% IBMS-iPSC-017-02: OCT4 98.6%, SOX2 100%, NANOG 98.1%, SSEA4 99.8%, TRA-1-60 97.7%, and TRA-1-81 95.6%.	Fig. 1 panel B
Genome stability	Karyotype (G-banding) and resolution	IBMS-iPSC-015-06: 46XY, Resolution 450–500 IBMS-iPSC-016–06: 46XY, Resolution 450–500 IBMS-iPSC-017-02: 46XX, Resolution 450–500	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	Matched with PBMCs from the original donors. All 16 loci are tested and matched.	Available with authors
Mutation analysis	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Sterility	Mycoplasma	Mycoplasma tested by RT-PCR/Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation and Teratoma formation	Embryoid body formation: Expression of specific markers for endodermal (AFP), mesodermal (SMA), and ectodermal (TUJI) Teratoma formation: 3 germ layers, including neural tissue (ectoderm), cartilage (mesoderm), glandular structure (endoderm), and gut-like epithelium (endoderm)	Fig. 1 panel E and F
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C.	Negative	Available with authors
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Marker (Immunofluorescence staining)	Rabbit anti-OCT4	1:100	Thermo Fisher Scientific Cat# A24867, RRID: AB_2650999
Pluripotency Marker (Immunofluorescence staining)	Mouse anti-SSEA4 (IgG3)	1:100	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Pluripotency Marker (Immunofluorescence staining)	Mouse anti-TRA-1-60 (IgM)	1:100	Thermo Fisher Scientific Cat# A24868, RRID: AB_2651002
Pluripotency Marker (Immunofluorescence staining)	Rat anti-SOX2	1:100	Thermo Fisher Scientific Cat# A24759, RRID: AB_2651000
Pluripotency Marker (Flow cytometry)	PE mouse anti-OCT3/4 (IgG1)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560186, RRID: AB_1645331
Pluripotency Marker (Flow cytometry)	PE mouse anti-SOX2 (IgG2a)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560291, RRID: AB_1645334
Pluripotency Marker (Flow cytometry)	PE mouse anti-NANOG (IgG1)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560483, RRID: AB_1645522
Pluripotency Marker (Flow cytometry)	PE mouse anti-SSEA4 (IgG3)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560128, RRID:AB_1645533
Pluripotency Marker (Flow cytometry)	PE mouse anti-TRA1-60 (IgM)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560193, RRID: AB_1645539
Pluripotency Marker (Flow cytometry)	PE mouse anti-TRA1-81 (IgM)	20 μL/test (for $1x10^6$ cells)	BD Biosciences Cat# 560161, RRID: AB_1645540
Differentiation Marker	Mouse anti-AFP (IgG1)	1:500	Thermo Fisher Scientific Cat# A25530, RRID: AB_2651004
Differentiation Marker	Mouse anti-SMA (IgG2a)	1:100	Thermo Fisher Scientific Cat# A25531, RRID: AB_2651005
Differentiation Marker	Rabbit anti-TUJ1	1:500	Thermo Fisher Scientific Cat# A25532, RRID: AB_2651003
Secondary antibody	Alexa Fluor 555 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A24869, RRID: AB_2651006
Secondary antibody	Alexa Fluor 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877, RRID: AB_2651008
Secondary antibody	Alexa Fluor 555 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A24871, RRID: AB_2651009
Secondary antibody	Alexa Fluor 488 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A25535, RRID: AB_2651010
Secondary antibody	Alexa Fluor 488 goat anti-mouse IgG1	1:250	Thermo Fisher Scientific Cat# A25536, RRID: AB_2651011
Secondary antibody	Alexa Fluor 555 goat anti-mouse IgG2a	1:250	Thermo Fisher Scientific Cat# A25533, RRID: AB_2651012
Secondary antibody	Alexa Fluor 488 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A25535, RRID: AB_2651010

Primers		
	Target	Forward/Reverse primer (5′-3′)
Sendai virus detection	*SeV*/181 bp	F: GGATCACTAGGTGATATCGAGC/ R: ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai virus detection	*Klf4*/410 bp	F: TTCCTGCATGCCAGAGGAGCCC/ R: AATGTATCGAAGGTGCTCAA
Sendai virus detection	*KOS*/528 bp	F: ATGCACCGCTACGACGTGAGCGC/ R: ACCTTGACAATCCTGATGTGG
Sendai virus detection	c-Myc/532 bp	F: TAACTGACTAGCAGGCTTGTCG/ R: TCCACATACAGTCCTGGATGATGATG
Pluripotent stem cell marker	*OCT4*/149 bp	F: TGTACTCCTCGGTCCCTTTC/ R: TCCAGGTTTTCTTTCCTAGC
Pluripotent stem cell marker	*SOX2*/148 bp	F: GCTAGTCTCCAAGCGACGAA/ R: GCAAGAAGCCTCTCCTTGAA
Pluripotent stem cell marker	*NANOG*/143 bp	F: CAGTCTGGACACTGGCTGAA/ R: CTCGCTGATTAGGCTCCAAC
House-Keeping Gene	*GAPDH*/302 bp	F: AGCCACATCGCTCAGACACC/ R: GTACTCAGCGGCCAGCATCG

3.3. RT-PCr

Total RNA was collected by TRIzol reagent (Thermo Fisher Scientific) and reverse-transcribed by RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) for Sendai virus vector detection and endogenous pluripotent marker expression. The primers used are listed in Table 3. The PCR started with a denaturation step at 95 °C for 5 min, followed by a total of 35 cycles with 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s, and ended with a final elongation step of 10 min at 72 °C. Sterile dH_2O was used as a negative control.

3.4. Immunofluorescence staining

Cell samples were fixed by 4% formaldehyde (Sigma-Aldrich) for 15 min, 1% TritonX-100 (Sigma-Aldrich) in PBS for 10 min, 5% goat serum for 45 min, and stained by PSC 4-Marker Immunocytochemistry Kit (Thermo Fisher Scientific). The images were obtained under an inverted fluorescence microscope (Axio Observer).

3.5. Flow cytometry

Cells were fixed by the BD Cytofix/Cytoperm kit (BD Pharmingen™), and then stained with antibodies and isotype controls for 30 min (Table 3). The percentages were calculated on BD FACSDiva™ Software.

3.6. In vitro Embryoid body formation

Cells were seeded into ultralow attachment 6-well plates (Corning) and cultured in DMEM/F12 supplemented with 20% FBS for 7 days. EBs were then transferred onto 0.1% gelatin-coated plates. After additional 14 days, EBs were fixed by 4% formaldehyde. By the 3-Germ Layer Immunocytochemistry Kit (Thermo Fisher Scientific), each sample was stained with three germ layers markers respectively.

3.7. In vivo teratoma formation

1×10^6 cells were detached by Accutase, resuspended in 50% Matrigel, and then transplanted into the testis of the NOD/SCID mouse. The teratomas were fixed by 10% formaldehyde eight weeks after transplantation. The sections of tissues were embedded in paraffin and stained with hematoxylin and eosin for analysis.

3.8. Karyotyping

Cells were treated with 37 °C 10 μg/mL of Colcemid (Thermo Fisher Scientific) for 60 min, trypsinzed into single cells, then incubated with 0.075 M of hypotonic KCl solution and fixed with Carnoy's Fixative. The G-banding analysis was done in Centre for Medical Genetics of Changhua Christian Hospital in Taiwan.

3.9. Mycoplasma detection

iPSCs were cultured for two days without medium change. By Mycoplasma PCR Detection Kit (ABM Inc.), the medium was collected, purified, and tested for mycoplasma.

3.10. STR analysis

By PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific), DNA of iPSC lines and the corresponding parental PBMCs were collected and purified. Sixteen loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, FGA) were analyzed by AmpFlSTR™ Identifiler™ PCR Amplification Kit (Thermo Fisher Scientific).

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.2021.102419.

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