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Lab resource: Stem Cell Line

Generation of sibling-matched induced pluripotent stem cell lines from spinal and bulbar muscular atrophy patients

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ABSTRACT

Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disease caused by the expansion of CAG repeats in the *Androgen Receptor* gene (*AR*). We report the generation of induced pluripotent stem cell (iPSC) lines from two SBMA patients and their healthy siblings. The SBMA and healthy iPSC lines retain the number of *AR* CAG repeats, express pluripotency markers and are able to differentiate into the three germ layers. The iPSC lines are also free of Sendai virus transgenes and have normal karyotypes. The SBMA iPSC lines with their sibling-matched controls would serve as useful tools to study SBMA disease mechanism.

Resource table

Names of stem cell lines	GIS2014-WT2C15 (healthy sibling line of GIS2014-SB2C20) GIS2014-WT3C16 (healthy sibling line of GIS2014-SB3C16) GIS2014-WT4C10 (non-related healthy control line) GIS2014-SB2C20 (Diseased sibling line of GIS2014-WT2C15) GIS2014-SB3C16 (Diseased sibling line of GIS2014-WT3C16)
Institution	Genome Institute of Singapore
Person who created resource	Gunaseelan Narayanan, Marianne Sheila
Contact person and email	Lawrence W. Stanton, stantonl@gis.a-star.edu.sg
Date archived/stock date	December 2014 for all five lines
Origin	human peripheral blood mononuclear cells (PBMCs) for all five lines
Type of resource	Induced pluripotent stem cells derived by reprogramming PBMCs
Sub-type	Pluripotent stem cell lines
Key transcription factors	OCT4, SOX2, CMYC, KLF4
Authentication	Identity and purity of iPSC lines confirmed by Sanger sequencing of *AR* CAG repeat region, analysis of pluripotency markers, differentiation into the three germ layers, karyotyping (Fig. 1), RT-PCR of Sendai virus transgenes (Fig. S1A) and DNA fingerprinting (Table S1)
Link to related literature	NA
Information in public databases	NA
Ethics	Work is approved by SingHealth Centralised Institutional Review Board (CIRB) with informed consent of all PBMC donors

Resource details

Spinal and bulbar muscular atrophy (SBMA) is a neuromuscular disorder caused by the expansion of the CAG repeat region in the first exon of the *Androgen Receptor* gene (*AR*) (Spada et al., 1991). Healthy individuals have about 9–36 CAG repeats while SBMA patients have greater than 38 CAG repeats (Andrew et al., 1997). SBMA is characterized by the selective degeneration of lower motor neurons within the spinal cord and brainstem which results in gradual weakness in the bulbar and limb muscles. The disease mechanisms underlying SBMA are not fully understood. iPSC lines from SBMA patients would serve as excellent tools to model and understand SBMA disease mechanism. In addition, iPSC lines from healthy siblings of the SBMA patients could be used as appropriate wild-type controls with similar genetic background.

We reprogrammed peripheral blood mononuclear cells (PBMCs) from two SBMA patients and their respective healthy sibling. We also generated iPSC line from a healthy individual who is not related to the SBMA patients and could serve as a non-related healthy control. Clinical information of the PBMC donors is summarized in Table 1. The PBMCs were reprogrammed to iPSCs using a protocol described previously (Seki et al., 2012) with modifications. Firstly, PBMCs were cultured over a period of five days in the presence of IL-2 to activate the T-cells

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Table 1
Summary of iPSC lines generated.

iPSC Line	Gender	Age	Ethnicity	Number of AR CAG repeats	PBMC donor symptoms	Relationship
GIS2014-WT2C15	Male	50	Asian	26	Apparently healthy	Sibling of GIS2014-SB2C20
GIS2014-WT3C16	Male	61	Asian	22	Apparently healthy	Sibling of GIS2014-SB3C16
GIS2014-WT4C10	Male	29	Asian	24	Apparently healthy	
GIS2014-SB2C20	Male	55	Asian	47	Limb weakness, Dysarthria, Dysphagia, Tongue and chin fasciculations, Gynaecomastia	Sibling of GIS2014-WT2C15
GIS2014-SB3C16	Male	58	Asian	43	Limb weakness, Dysphagia, Dysarthria, Tongue and chin fasciculations, No gynaecomastia	Sibling of GIS2014-WT3C16

Abbreviations: iPSC, induced pluripotent stem cell; AR, androgen receptor; PBMC, peripheral blood mononuclear cell.

to form cell clusters (Fig. 1A, Day 0–Day 5). At Day 5, the T-cells were infected with Sendai virus carrying transgenes for the four Yamanaka factors. 48 h post-infection, the T-cells were replated onto MEF feeder layer. As early as seven days post-infection, small colonies were observed (Fig. 1A, Day 12). By 25 days post-viral infection, tightly packed pluripotent colonies were formed (Fig. 1A, Day 25). Selected colonies were transferred onto MEF feeder layer (Fig. 1A, Day 26) and expanded further (Fig. 1A, Day 47). iPSC colonies were subsequently adapted to Matrigel (Fig. 1A, Day 54) and maintained in feeder-free conditions (Fig. 1A, Day 68). All lines were named according to the guidelines proposed in (Luong et al., 2011). The wild-type line GIS2014-WT2C15 and SBMA line GIS2014-SB2C20 were generated from siblings. The wild-type line GIS2014-WT3C16 and SBMA line GIS2014-SB3C16 were generated from siblings. The wild-type line GIS2014-WT4C10 was generated from a healthy individual who is not related to the SBMA patients.

All iPSC lines express pluripotency markers including SOX2, OCT3/4 and NANOG at the protein (Fig. 1B) and mRNA level (Fig. 1C). Embryoid bodies (EBs) generated from all the iPSC lines differentiated into the three germ layers, mesoderm, endoderm and ectoderm (Fig. 1D). Sequencing of the *AR* CAG repeat region ensured that the number of CAG repeats were unaltered during reprogramming (Fig. 1E). Furthermore, all iPSC lines have normal male karyotypes (Fig. 1F), did not retain the Sendai virus transgenes (Fig. S1A), and are free of mycoplasma (Fig. S1B). DNA fingerprinting of 24 genomic loci including 22 short tandem repeat (STR) loci confirmed that each iPSC line was derived from its respective patient PBMC sample (Table S1).

Materials and methods

Isolation of PBMCs

All work with PBMCs are approved by SingHealth Centralised Institutional Review Board (CIRB) with informed consent of the donors. For PBMC donors, three healthy individuals and two SBMA patients were recruited at the National Neuroscience Institute. Each of the SBMA patients has a sibling who is among the three healthy PBMC donors. 10 ml of venous blood was drawn from each individual and collected in K2EDTA tubes (BD Biosciences). Equal volume of PBS was added to the blood sample. For every 6 ml of blood, 4 ml of Ficoll-Paque Plus (GE Healthcare) was added dropwise in a 15 ml conical centrifuge tube. The mixture was then centrifuged at 400 × *g* for 35 min. The buffy coat layer containing PBMCs was carefully transferred into a 15 ml conical centrifuge tube. The PBMCs were washed twice with PBS and subsequently resuspended in freezing medium consisting of 90% heat inactivated fetal bovine serum (FBS) and 10% DMSO. PBMCs were kept in liquid nitrogen for long term storage.

Reprogramming PBMCs to iPSCs

PBMCs from SBMA patients and healthy individuals were reprogrammed to iPSCs using a protocol described previously (Seki et al., 2012) with modifications. Each of the SBMA patient has a healthy sibling. PBMCs were seeded at 500,000 cells per well in a 24-well culture dish coated with 10 μg/ml CD3 antibody (BD Biosciences). The T cells were activated by incubation at 37 °C for five days in T-cell medium containing 10 ng/ml IL-2 (Peprotech). The T cell clumps were then mechanically dissociated and plated at 50,000 cells per well in a 24-well culture dish. Immediately after plating, the cells were infected with Sendai virus harbouring SOX2, OCT4, C-MYC and KLF4 transgenes (CytoTune™ 2.0 kit, Invitrogen) for 48 h at 37 °C. The infected cells were then transferred onto mitomycin-inactivated mouse embryonic fibroblasts (MEF) feeder layer and cultured in hiPSC medium for 18 days for iPSC colonies to emerge. Individual iPSC colonies were picked and transferred to MEF feeder layer for expansion. Each iPSC clone was passaged up to five times in MEF feeder layer before adapting to feeder-free Matrigel.

Feeder-free maintenance of iPSC lines

All iPSC lines were maintained in feeder-free conditions in mTeSR1 medium (STEMCELL Technologies) on Matrigel-coated tissue culture dishes. Medium was replenished daily and the iPSCs were passaged every five to seven days.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 25 min at room temperature and washed thrice with PBS. The cells were then simultaneously blocked and permeabilized with 10% FBS and 0.01% Triton-X in PBS for one hour at room temperature. Subsequently, the cells were incubated at 4 °C overnight with primary antibodies. The primary antibodies and their dilutions were anti-SOX2 (Santa Cruz, Cat# SC17320, 1:250), anti-OCT3/4 (Santa Cruz, Cat# SC8628, 1:200), anti-SSEA4 (Millipore, Cat# MAB4304, 1:500), anti-TRA1-60 (Invitrogen, Cat# 41-1000, 1:100), anti-TRA1-81 (Invitrogen, Cat# 41-1100, 1:100), anti-NANOG (Santa Cruz, Cat# SC33759, 1:100), anti-SMA (Sigma, Cat# A5228, 1:500), anti-vimentin (Sigma, Cat# V2258, 1:500), anti-AFP (DAKO, Cat# A0008, 1:100), anti-GFAP (Sigma, Cat# G9269, 1:1000), anti-Tuj1 (Covance, Cat# MMS435P, 1:5000). The following day, the cells were washed thrice with PBS and incubated with Alexa Fluor-conjugated secondary antibodies (Invitrogen) (1:1000 dilution) for one hour at room temperature in the dark. The cells were then washed thrice with PBS and nuclei stained with Hoechst 33342 (Invitrogen). Images were acquired using Axio Observer. D1 inverted microscope (Zeiss) and analysed using AxioVision software (Zeiss).

EB assay

To initiate EB formation, iPSCs were seeded in low-attachment 6-well culture dishes (Corning) and cultured for three days in hiPSC medium containing 10 ng/ml bFGF (Peprotech). EBs were further maintained for one week in MEF medium and then transferred to gelatin-coated 6-well culture dishes. The EBs were spontaneously differentiated for one week in MEF medium and then immunostained using antibodies specific for TUJ1 (ectoderm lineage marker), smooth muscle actin (mesoderm