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Lab resource: Stem Cell Line

Generation of CSi001-A, a transgene-free, induced pluripotent stem cell line derived from a Parkinson Disease (PD) patient

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A B S T R A C T

Peripheral blood mononuclear cells (PBMC) were collected from a 70-year old Parkinson Disease patient. The PBMCs were reprogrammed with the human OKSM transcription factors using the non-integrating episomal vector system. The transgene-free iPSC showed pluripotency verified by immunocytochemistry for pluripotency markers and differentiated directly toward the 3 germ layers in vitro. Furthermore, the iPSC line showed normal karyotype.

Resource table

Unique stem cell line identifier	CSi001-A
Alternative name(s) of stem cell line	N/A
Institution	Changshu No.2 Hospital, Yangzhou University School of Medicine, Suchow, PR China
Contact information of distributor	Hui Liu, liusci7912@126.com
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 70 Sex: male Ethnicity: Chinese Han
Cell source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free, Episomal plasmids
Genetic modification	Yes
Type of modification	Spontaneous mutation
Associated disease	Parkinson Disease
Gene/locus	MAPT, Chr 17, rs242562 A > G
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	16th April,2018
Cell line repository/bank	hPSCreg, https://hpscreg.eu/user/cellline/edit/CSi001-A
Ethical approval	Participants informed consent obtained/Ethics Review Board-competent authority approval obtained (EC.2016-LW-023).

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Resource utility

The iPSC line can be used as a platform to further study disease mechanisms of Parkinson Disease (PD).

Resource details

Peripheral blood mononuclear cells (PBMCs) were donated by a 70-year old male volunteer. Volunteers were recruited by the Department of Neurology and Institute of Neurology, Changshu No.2 Hospital, Yangzhou University School of Medicine, China. Genetic screening of the PBMCs (Fig. 1G) revealed MAPT gene SNP (Table 1) which might be associated with the Parkinson Disease in China (Yu et al., 2014). The SNP still existed in iPSCs (Fig. 1H) we made.

To generate the CSi001-A iPSC line, the four "Yamanaka reprogramming factors" OCT3/4, SOX2, KLF4 and C-MYC (Ban et al., 2011) were delivered into PBMCs using the non-integrating episomal vectors previously described (Chou et al., 2011) (Fig. 1A). The iPSC-like colonies were selected 27–35 days after transduction. From the 5th passage of iPSCs, the absence or presence of the vector was analyzed by RT-PCR using KOS, C-MYC and KLF4 primers (Table 2). After the 7th passage, the elimination of reprogramming factors was confirmed (Fig. 1C) in the CSi001-A iPSC line, which was selected for further analysis. The karyotype of the CSi001-A iPSC line was inspected by Giemsa-banding, and the results showed normal diploid 46, XY karyotype, without any detectable abnormalities (Fig. 1B). Flow cytometry and immunofluorescent staining were performed to examine the expression of pluripotent markers (Fig. 1D and E) using the antibodies against human NANOG, SOX2, SSEA4 and OCT3/4. The expression of ectoderm (βIII-TUBULIN), mesoderm (SMA) and endoderm (AFP) markers (Carpenter et al., 2003) was detected and demonstrated the directed differentiation potential toward the three germ-layers of the CSi001-A iPSC line in vitro (Fig. 1F).

Materials and methods

Reprogramming of peripheral blood mononuclear cells (PBMC)

Human peripheral blood was added to the superstratum of Histopaque®-1077 (Sigma-Aldrich) and separated by centrifugation (400 *g*, 20 min, room temperature) to obtain the peripheral blood mononuclear cells (PBMCs). The freshly isolated PBMCs were seeded in StemPro®-34 SFM Complete Medium (Gibco™) with Human PBMC Culture Supplement (1000×) (IxCell, Shanghai). Four days later (Day 0) the cells were collected by centrifugation (100 *g*, 5 min) and transduced by non-integrating oriP/EBNA-1 based episomal plasmids (OCT3/4, SOX2, C-MYC, KLF4 transcription factors, A14703, Invitrogen). 1×10^6 PBMCs were electroporated with 1 μg of each episomal vectorwith the following program: 1650 V, 10 ms, 3 pulses. (100 μL DNA and cells mixture per electroporation with 100 μL electroporation tip from Neon Transfection System). From the following day to the 7th day, StemPro®-34 SFM Complete Medium was changed every 48 h. On the 7th day, the cells were collected by centrifugation (100 *g*, 5 min) and seeded (3×10^4 cells/cm^2) on Matrigel (BD Biosciences). On the 8th day, the medium was changed to Essential 8™ Medium (Gibco™), and the cells were observed closely for the formation of colonies with an ES-like appearance. The colonies were manually picked based on the morphology between Day 27 to Day 35 post-transduction, and cultured and passaged as iPSCs thereafter. Human iPSC cultures were maintained on plates coated with Matrigel (BD Biosciences) in mTESR-1 medium (Stem Cell Technologies). All cells were cultured at 37 °C in humidified atmosphere containing 5% CO2, feeding daily with mTESR-1 medium until the cells reached 80–90% confluency. Normally in 3–7 days the cells were dissociated using Gentle Cell Dissociation Reagent (Stem Cell Technologies) and passaged at 1:8 ratio.

Reverse transcription polymerase chain reaction (RT-PCR) and Quantitative Real-Time Polymerase Chain Reaction (QPCR)

The cell samples were lysed and total RNA was extracted using TRIzol® Reagent (Life, 15596018). 1 μg of RNA was reverse transcribed into first-strand cDNA using Fast Quant RT kit (TIANGEN, Beijing, China) in a 20 μl reaction mixture containing 5 μl 4 × FQ-RT Super Mix. The qPCR was conducted on Applied Biosystem 7500 Fast Real-Time PCR System with 2 × Hot Start SYBR Green qPCR Master Mix (ExCell Bio,Jiangsu,China) according to the manufacturer's instructions. The reaction conditions were as follows: 95 °C for 5 min followed by 50 cycles of 95 °C for 10 s, 56 °C for 10 s, and 72 °C for 10 s. To confirm the transgene-free status of the iPSC lines, the KOS, C-MYC and KLF4 primers were used and the PCR products were confirmed by the electrophoresis on agarose gel.

In vitro directed differentiation

The iPSCs were cultured to logarithmic phase and harvested and plated for differentiation according to the STEMdiff™ Trilineage Differentiation Kit protocol (Stem Cell Technologies). Five to seven days later, the cells were fixed with 4% PFA for the IF staining of the lineage specific markers.

Immunofluorescence (IF) staining

The expression of pluripotency markers was studied using IF staining. For all immunostainings, the cells were fixed in 4% PFA for 15 min and incubated in blocking buffer (5% bovine serum albumin with 0.1% Triton X-100 in PBS) for 30mins at room temperature. Afterwards, cells were incubated with primary antibodies diluted in PBS with 1% bovine serum albumin at 4 °C overnight and then incubated with secondary antibodies for 1 h at room temperature. Finally, nuclei were stained with DAPI for 15 min at room temperature. Then cells were visualized under inverted fluorescence microscope (OLYMPUS1 ×71) (Fig. 1A). OCT-3/4 (Santa Cruz), NANOG (Cell Signaling Technology), SOX2 (Cell Signaling Technology) and SSEA-4 (Abcam) antibodies were used to identify the characteristics of the hiPSC line. AFP (Sigma), SMA (Sigma) and βIII-TUBULIN (Abcam) antibodies were used to assess directed differentiation.

Flow cytometry

Human iPSCs were dissociated using Gentle cell Dissociation Reagent (Stem Cell Technologies) and permeabilized with Fixation/Permeabilization reagents (catalog number: 5123 and 5223-56, eBioscience). The cells were then incubated 30 min at room temperature with the antibodies against OCT-3/4 (Santa Cruz), NANOG (Cell Signaling Technology) and SSEA4 (Abcam), following with a 30-min incubation of Alexa fluor Donkey-anti-Mouse or Rabbit 647 nm (Thermo Fisher Scientific) at room temperature. Finally, the cells were analyzed using a FACS Aria flow cytometer (BD). Flow cytometry data analysis was performed using Flowing Software 2.5.1.

Karyotyping

CSi001-A cells at passage 15 were cultured in a feeder-free culture system with mTeSR™1 medium and treated with 0.1 μg/ml KaryoMAX® Colcemid™ solution (Life Technologies) for 3 h. They were then

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