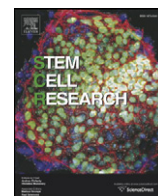


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Lab resource: stem cell line

Peripheral blood mononuclear cell-converted induced pluripotent stem cells (iPSCs) from an early onset Alzheimer's patient

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ABSTRACT

Improvement in transduction efficiency makes it possible to convert blood cells into induced pluripotent stem cells (iPSC). In this study, we generated an iPSC line from peripheral blood mononuclear cells (PBMC) donated by a patient who exhibited memory deficit at age 59; outcome of positron emission tomography scan is consistent with a diagnosis of Alzheimer's disease. Integration-free CytoTune-iPS Sendai Reprogramming factors which include Sendai virus particles of the four Yamanaka factors Oct4, Sox2, Klf4, and c-Myc were introduced to PBMC to convert them to iPSCs without retention of virus. Three germ layer differentiation was induced to demonstrate the pluripotency of these iPSCs.

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1. Resource table

Name of stem cell construct	I2-12F
Institution	Edith Nourse Rogers Memorial Veterans Hospital
Person who created resource	Han-Kyu Lee, Weiming Xia
Contact person and email	Weiming Xia, Weiming.Xia@va.gov
Date archived/stock date	May 14, 2015
Origin	Human peripheral blood mononuclear cells (PBMCs)
Type of resource	Induced pluripotent stem cell (iPS); derived from human peripheral blood mononuclear cells (PBMCs)
Sub-type	Induced pluripotent stem cell (iPS)
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 2)
Link to related literature (direct URL links and full references)	Not available
Information in public databases	Not available

2. Resource details

The blood sample was donated by the patient who was hospitalized at the Bedford VA Hospital Special Dementia Care Unit, which is a

hospice unit staffed and managed by the Bedford Geriatric Research, Education and Clinical Center (GRECC).

The patient exhibited memory deficit at age 59 that prevented him from performing job-related function. Subsequent neurological tests and positron emission tomography (PET) scan presented clinical diagnosis consistent with Alzheimer's disease. The patient also suffered two witnessed seizures which were controlled by anti-epileptic drug levetiracetam. The study was approved by the Bedford Veterans Affairs Hospital Institutional Review Board under the project title, "iPSC for Neurodegenerative diseases".

Peripheral blood mononuclear cells (PBMC) were separated from whole blood and transduced by reprogramming vectors carrying four Yamanaka factors, Oct4, Sox2, Klf4, and c-Myc included in the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Invitrogen). The four Yamanaka factors have been well documented for efficient reprogramming of skin fibroblasts and blood cells (Trokovic et al., 2014; Lieu et al., 2013; Takahashi et al., 2007).

We confirmed development of human iPSC by karyotyping (Fig. 1), which is a standard procedure to authenticate normal karyotypes of cells (Li et al., 2011). To validate bona fide iPSCs in our culture that is distinguishable from only partially transduced cells, we examined the expression of cell surface proteins, such as sialylated keratan sulfate antigens Tra-1-81 and Tra-1-60, which defines a human embryonic stem cell (Badcock et al., 1999; Nethercott et al., 2011; Sommer et al., 2012). Immunocytochemical staining using antibody against stem cell marker TRA-1-81 (Fig. 2A) and Tra-1-60 (not shown) was performed to confirm the purity and pluripotency of our iPSC.

Many approaches have been developed to produce footprint-free iPSC (Ovchinnikov et al., 2015), and we applied the CytoTune-iPS 2.0 Reprogramming System for our studies (Seki et al., 2010). This system

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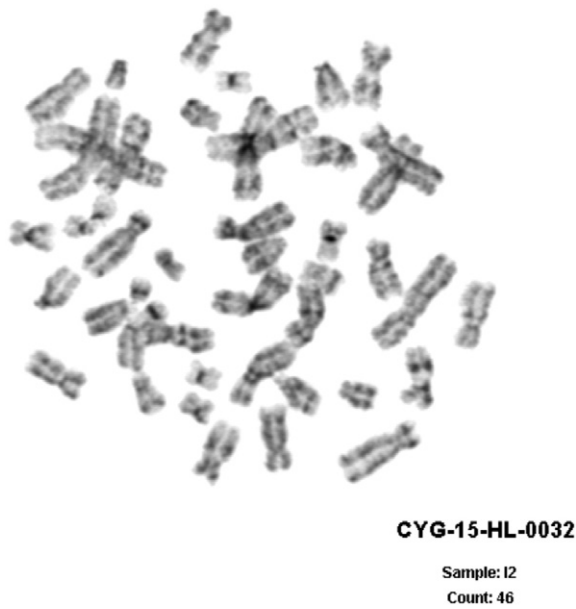


Fig. 1. Karyotyping of Induced pluripotent stem cell I2-12F. Cells were stained with Giemsa and the metaphase chromosome number from individual nuclei were imaged and counted. All ten metaphase cells had 46 chromosomes and XY sex chromosomes characteristic of a male patient.

uses vectors that remain in the cytoplasm (Li et al., 2000) and do not integrate into host genome (Fusaki et al., 2009). Therefore, the host cell can be cleared of the vectors and reprogramming factor genes (Li et al., 2000). We searched for any remaining SeV protein by immunocytochemical staining using Anti-SeV antibody (MBL international) and confirmed that there is no trace of viral protein (Fig. 2B).

To demonstrate capacity of iPSC for three germ layer differentiation, we tested formation of embryoid body (EB) (Hribar et al., 2015). We performed immunostaining using three different antibodies corresponding to three germ layers, i.e., ectoderm: β III tubulin antibody, mesoderm: smooth muscle actin (SMA) antibody, endoderm: α -fetoprotein (AFP) antibody. Our results demonstrated differentiation capacity of newly converted iPSC (Fig. 3).

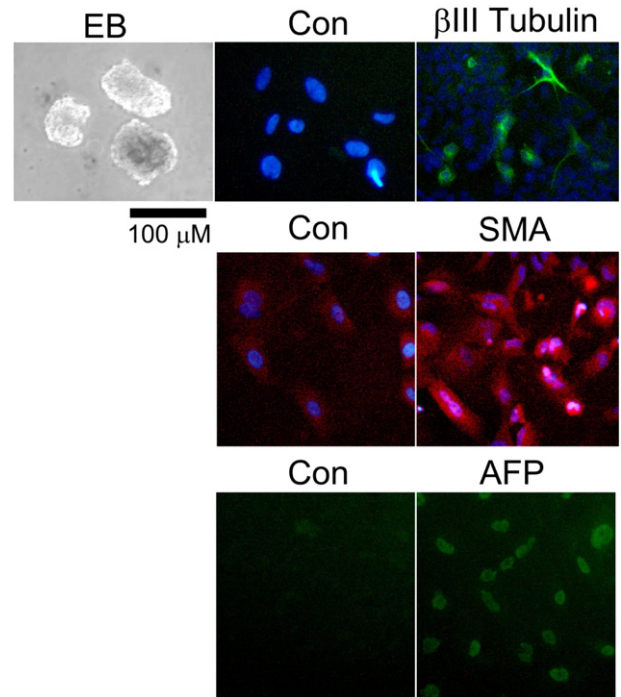


Fig. 3. Characterization of embryoid body (EB) formation. EBs were fixed with 4% PFA and immunostained with three different antibodies (ectoderm: β III tubulin, green; mesoderm: smooth muscle actin (SMA), red; endoderm: α -fetoprotein (AFP) green). Con: cells were stained in the absence of primary antibodies.

3. Materials and methods

3.1. Human blood sample

Blood was collected in Vacutainer cell preparation tube (CPT) (Becton, Dickinson and Company, Franklin Lakes, NJ). PBMCs were prepared within 1 h of blood collection. Blood samples were centrifuged at 1500 rcf for 20 min at room temperature, and the PBMC were collected

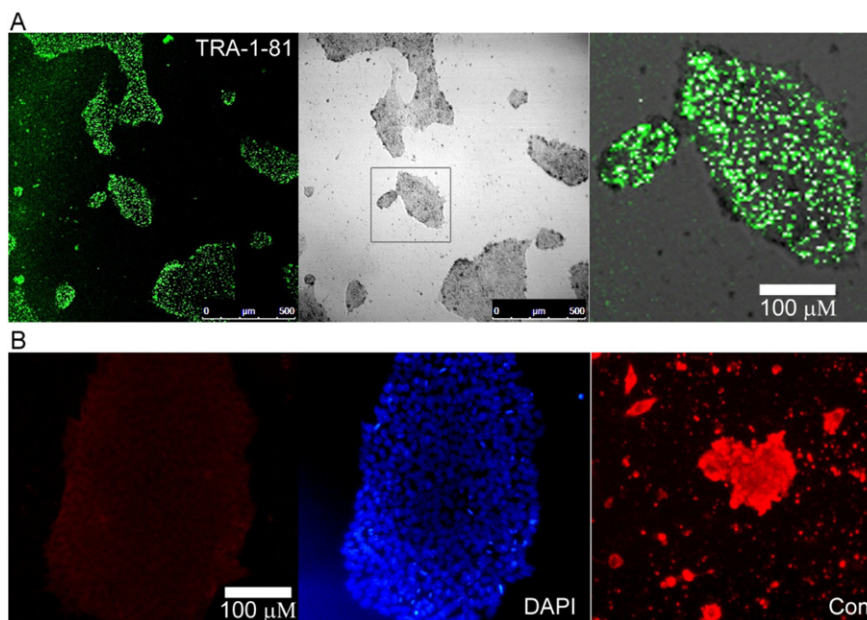


Fig. 2. Characterization of iPSC I2-12F. Stem cell surface protein Sialylated Keratan Sulfate Antigen Tra-1-81 was present in all iPSC I2-12F (A, left; bright field: right). There is no trace of Sendai virus protein, detected by antibody against SeV protein (B, left), compared to positive cells of early stage (at passage five) when SeV protein was present.

and transferred to a fresh tube, washed with PBS, and centrifuged at 300 rcf for 10 min at room temperature. Cell pellets were re-suspended in PBMC medium (Invitrogen) containing growth factors for transduction.

3.2. iPSC generation and expansion

Induced PSCs were generated from freshly collected, peripheral blood-derived PBMCs (Chou et al., 2011). Cells were transduced with Sendai virus particles containing four Yamanaka factors using the integration-free CytoTune-iPS Sendai Reprogramming Kit (Lieu et al., 2013; Takahashi et al., 2007). Transduced cells were plated with mouse embryonic fibroblast (MEF) feeder cells. Cells were fed with iPSC medium containing bFGF (Invitrogen) until small colonies were formed in 2 weeks. Growth of small colonies was maintained for another two weeks before each colony was picked for expansion into individual iPSC lines. After successful formation of iPSC colonies, iPSCs were transferred to plates coated with Geltrex (Invitrogen) and expanded in E8 medium (Invitrogen).

3.3. Live staining of iPSC

Purity and pluripotency of iPSCs grown on both MEF feeder cells and Geltrex coated plates were confirmed by live staining with Tra-1-81 and Tra-1-60 antibodies (Invitrogen). Cells were washed with Knockout DMEM and stained with Tra-1-81 or Tra-1-60 antibodies at 1:200 dilutions for 1 h in the CO₂ incubator. Cells were subsequently washed twice with Knockout DMEM and incubated with secondary anti-mouse antibody conjugated with Alexa Fluor 488 for one hour. Finally, cells were washed with Knockout DMEM and replenished with iPSC growth medium for microscopic imaging. Images were acquired using a Leica TCS SP5 Confocal Laser Scanning Microscope.

3.4. iPSC karyotyping

Karyotyping of the iPSC was carried out at the CytoGenomics Core Laboratory (Brigham and Women's Hospital, Harvard Medical School, Boston, MA). G-banding was used to karyotype our iPSC line. Cells in a stage of active division in metaphase were arrested, harvested and fixed with methanol:acetic acid (3:1). The cell pellet was washed, re-suspended, dropped on a slide and dried on a hotplate. Cells were stained with Giemsa and the metaphase chromosome number from individual nuclei were imaged (Olympus BX51, 100×) and counted using the Cyto Vision Software.

3.5. Three germ layer *in vitro* differentiation and characterization

The differentiation capability of iPSC lines was analyzed by the formation of EB, based on the previously published protocol (Carpenter et al., 2003; Itskovitz-Eldor et al., 2000). Briefly, undifferentiated iPSC colonies were transferred into the non-adherent poly-HEMA (Sigma) coated petri dish and were cultured in E6 medium without bFGF for 7 days. The resulting EB, which theoretically comprise three embryonic germ layers, were plated onto Geltrex coated tissue culture dishes and cultured for spontaneous differentiation. After cells were differentiated in EB culture for a total of 19 days, cells were fixed with 4% PFA and immunostained with three different antibodies (Ectoderm: β III tubulin, Sigma; Mesoderm: Smooth muscle actin, Invitrogen; Endoderm: Alpha fetoprotein, Invitrogen). Images were acquired using the Nikon TMS,

10×; the Leica TCS SP5 Confocal Microscope, 10×; and the AmScope microscope digital camera.

4. Verification and authentication

Karyotyping of our iPSC was performed and revealed by the microscope scan. All ten metaphase cells had 46 chromosomes and had XY sex chromosomes characteristic of a male patient (Fig. 1).

We examined embryonic stem cell makers, Tra-1-81 and Tra-1-60 by live staining of cells and found all cells are Tra-1-81 (Fig. 2A) and Tra-1-60 (not shown) positive, thus confirming iPSC purity and pluripotency.

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