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Lab resource

Generation of iPS cell lines from schizophrenia patients using a non-integrative method

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ABSTRACT

Skin biopsies were collected from three male patients (age 45, 44 and 44) with clinically diagnosed schizophrenia. The patients were diagnosed according to DSM-5 criteria by a trained psychiatrist. Dermal fibroblast cell lines were established and expanded for subsequent reprogramming procedures. Induced pluripotent stem (iPS) cells were derived using the integration-free CytoTune®-iPS 2.0 Sendai Reprogramming Kit, containing Sendai virus particles of the four Yamanaka factors Oct3/4, Sox2, Klf4 and c-Myc.

Resource table

Name of stem cell construct	EZQ-3 iPSC, EZQ-4 iPSC, EZQ-9 iPSC
Institution	D'Or Institute for Research and Education
Person who created resource	Jaroslaw Sochacki, PhD
Contact person and email	Stevens Rehen, PhD srehen@lance-ufrj.org
Date archived/stock date	November 7th, 2015
Origin	Human skin fibroblasts
Type of resource	Induced pluripotent stem (iPS) cell; derived from human skin fibroblasts
Sub-type	Induced pluripotent stem cell (iPS)
Key transcription factors	Oct3/4, Sox2, Klf4, *c*-Myc
Authentication	Identity and purity of cell line confirmed (Figs. 1, 2 and 3)
Link to related literature	Not available
Information in public databases	Not available
Ethics	Patient informed consent obtained/Ethics Review Board-competent authority approval obtained

Resource details

Skin fibroblasts were reprogrammed using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, USA), which contains reprogramming vectors with the four Yamanaka factors, Oct3/4, Sox2, Klf4 and *c*-Myc. These factors have been repeatedly shown to be sufficient for efficient reprogramming (Lieu et al., 2013; Takahashi et al., 2007). The identity of derived human iPS cell lines was confirmed by immunohistochemistry, using the following antibodies for pluripotency markers, TRA-1-60, Sox2, Oct3/4 and SSEA4 (Fig. 1A). To confirm trilineage differentiation potential, *in vitro* embryonic body (EB) formation assay was performed (Fig. 3A). Spontaneous differentiation induced the transcription of the following genes: AFP (endoderm), MSX1 (mesoderm) and Pax6 (ectoderm) (Fig. 3B). Additionally, the formation of the three germ layers was confirmed at the protein level by immunocytochemistry, which showed the expression of Nestin, TUJ1, SMA and AFP (Fig. 3C). Ploidy of the derived iPS cell lines was analyzed by low-pass whole-genome sequencing (Fig. 2) (Wells et al., 2014).

Materials and methods

Derivation of human fibroblast cell lines EZQ-3, EZQ-4 and EZQ-9

Procedures for sample collection and iPS cell lines generation were approved by the Institutional Ethics Committee and informed consent was obtained from the patients and/or their legal tutors. Tissue fragments were collected and cultured as described previously (Chen et al., 2011) with minor modifications. Briefly, fresh small skin biopsies

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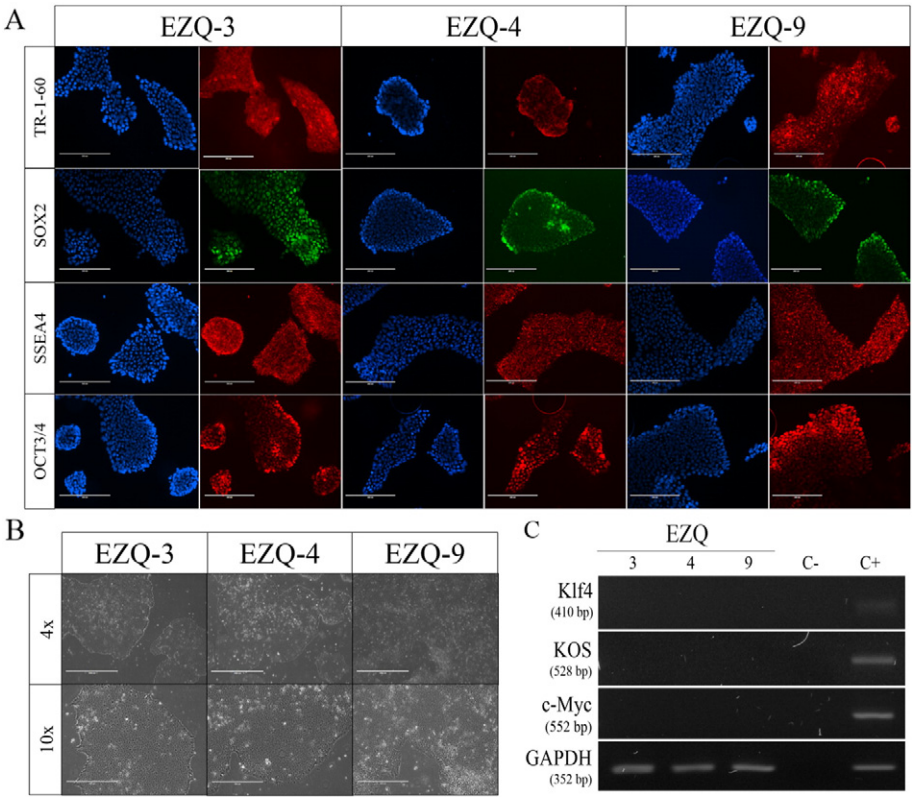

Fig. 1. Characterization of three EZQ iPS cell lines. A) Expression of pluripotency markers: TRA-1-60, SOX2, SSEA4 and OCT3/4. Immunofluorescence staining, red/green: pluripotency markers, Blue: DAPI, nuclei staining. Scale bars, 200 μm. B) Feeder-free EZQ iPS cell lines cultures. C) Reverse transcription PCR for the detection of Sendai transgenes after passage 7. Transduced cell pool at passage 0 was used as positive control. C–, non-template control.

were collected and directly plated in DMEM high glucose medium with 1% penicillin-streptomycin (Thermo Fisher Scientific, USA). Human fibroblasts spontaneously migrated after two weeks and were expanded in standard culture conditions (37 °C in 5% CO_2) for 3 passages. The cells were tested for Mycoplasma (MycoAlert™ PLUS, Lonza, USA) before any further manipulation. Cells at passage 3 were used for iPS cell generation.

iPS cell lines generation and expansion

Cells were reprogrammed using the integration-free CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, USA), which contains Sendai virus particles of the four Yamanaka factors (Takahashi et al., 2007). Briefly, 3.0–3.5 × 10^5 human dermal fibroblasts were plated onto one well of a gelatin-coated 6-well plate 24 h before virus transduction. The cells were transduced according to the manufacturer's protocol. Four days after transduction, cells were plated onto MEF feeder cells, and fed with iPS cells medium supplemented with 30 ng/mL freshly added bFGF (Thermo Fisher Scientific, USA). About 7 days after plating, the first appearing colonies were picked for expansion into individual iPS cell lines and transferred onto Matrigel (BD Biosciences, USA)-coated plates and cultured with E8 medium (Thermo Fisher Scientific, USA). After successful adaptation to feeder-free conditions of at least 3 iPS colonies from each patient, the cells were expanded in E8 medium using 0.5 mM EDTA passaging solution (both from Thermo Fisher Scientific, USA) (Fig. 1B).

Immunofluorescence staining of iPS cell lines

Oct3/4, Sox2, SSEA4 and Tra-1-60 immunofluorescence staining confirmed pluripotency genes expression in all EZQ iPS cell lines (Fig. 1A). iPS colonies were plated onto 96-well plates (CellCarrier, Perkin-Elmer, USA) and fixed with 4% paraformaldehyde for 20 min at room temperature. Fixed cells were permeabilized with 0.3% Triton-X and blocked with 2% BSA. Cells were then washed with PBS, incubated with primary antibodies at 1:100 dilution, at 4 °C, overnight. Primary antibodies were then washed with PBS and cells were incubated with secondary antibodies at 1:400 dilutions at room temperature for 1 h. After washing and incubation with DAPI for 5 min, cells were covered with glycerol, the plates were sealed with AlumaSeal CS film (Excel Scientific, USA) and stored at 4 °C until visualization. Images were acquired using the EVOS XL Cell Imaging System (Thermo Fisher Scientific, USA) (Fig. 1A). The antibodies used in the experiments are listed in Table 2.

In vitro differentiation of iPS cell lines

The differentiation ability of three EZQ iPS cell lines was analyzed by *in vitro* embryonic body (EB) formation, as published previously (Itskovitz-Eldor et al., 2000). Briefly, 100 mm cell culture dishes with EZQ iPS cell lines were treated with Collagenase IV (Thermo Fisher Scientific, USA) for 30–40 min at 37 °C, washed with PBS and seeded onto 60 mm culture dishes (Corning, USA). The cultures were maintained on horizontal shaker in DMEM supplemented with 20% KSR, non-essential amino acids, L-glutamine, penicillin/streptomycin and sodium pyruvate (all from Thermo Fisher Scientific, USA) and without bFGF to allow for formation of embryonic bodies (EBs) (Fig. 3A). After 7 days, EBs were seeded onto gelatin-coated 100 mm culture dishes and grown for another 21 days with medium change every other day. Subsequently, the cultures were harvested for total RNA isolation using the GeneJET RNA Purification kit (Thermo Fisher Scientific, USA), followed by