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Lab Resource: Stem Cell Line

Generation of urine iPS cell line from a patient with obsessive-compulsive disorder using a non-integrative method

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ABSTRACT

Urine sample was collected from a 29-year-old male patient with an early onset form of DSM-5 obsessive-compulsive disorder (OCD), comorbid body dysmorphic and excoriation (skin-picking) disorders, and a positive family history for OCD. Urine cell line was established and expanded for the reprogramming procedure. Induced pluripotent stem (iPS) cells were derived using the integration-free CytoTune®-iPS 2.0 Sendai Reprogramming Kit, which includes Sendai virus particles of the four Yamanaka factors Oct3/4, Sox2, Klf4 and c-Myc.

Resource table

Name of stem cell construct	TOC-4 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 urine epithelial cells
Type of resource	Induced pluripotent stem cell (iPS); derived from human urine epithelial cells
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

Urine epithelial cells underwent the reprogramming procedure using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, USA) containing 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 line was confirmed by immunocytochemistry, using the following antibodies for pluripotency markers, TRA-1-60, Sox2, Oct3/4 and SSEA4 (Fig. 1a). Sendai virus transgenes were undetectable from passage 7 (Fig. 1c). 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 line was analyzed by low-pass whole-genome sequencing (Fig. 2) (Wells et al., 2014).

Materials and methods

Derivation of human urine epithelial cell line TOC-4

Procedures for sample collection and iPS cell line generation were approved by the Institutional Ethics Committee and informed consent was obtained from the patients and/or their legal tutors. Urine cells (UCs) were collected and cultured as described previously (Zhou et al., 2011) with small modifications. Briefly, fresh urine samples (250–

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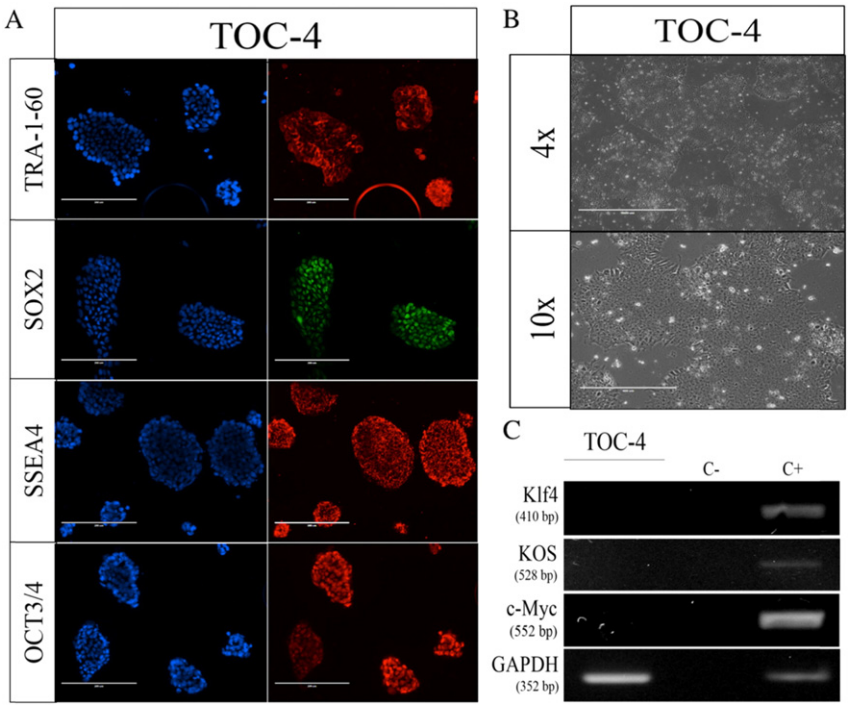

Fig. 1. Characterization of the derived TOC-4 iPS cell line. A) Expression of pluripotency markers: TRA-1-60, SOX2, SSEA4 and OCT3/4. Immunofluorescence staining, red/green: pluripotency markers, Blue: DAPI, nuclei staining. Scale bars, 200 μm. B) Feeder-free TOC-4 iPS cell line cultures. C) Reverse 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.

300 mL) were collected from the patients, centrifuged for 10 min at 1700 rpm and washed once with 1xPBS with penicillin/streptomycin and then plated in 1 mL urine renal epithelial growth medium (REGM, Lonza, USA) on a gelatin-coated 12-well plate. The cells were expanded in standard culture conditions (37 °C, 5% CO_2) and tested for Mycoplasma (MycoAlert™ PLUS, Lonza, USA) before any further manipulation. Cells at passage 3 were used for reprogramming.

iPS cell line 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, 3.5–4 × 10^5 urine epithelial cells were plated onto one well of a gelatin-coated 6-well plate 24 h before viral 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 line, 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 cells

Oct3/4, Sox2, SSEA4 and Tra-1-60 immunofluorescence staining confirmed pluripotency gene expression in TOC-4 cell line. iPS colonies were plated onto 96-well plates (CellCarrier, Perkin-Elmer, USA) and fixed with 4% paraformaldehyde for 15 min at room temperature. Fixed cells were permeabilized with 0.3% Triton-X and blocked with

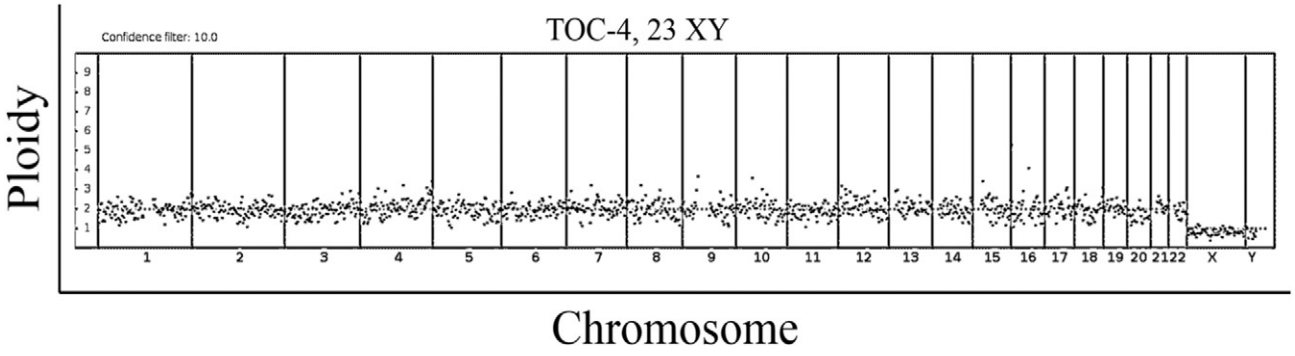

Fig. 2. Aneuploidy analysis of TOC-4 iPS cell line. Analysis of chromosome copy number was carried out using low-pass whole genome sequencing. Snapshot of IGV Light Whole Genome View screen depicts overviews of the derived iPS cell line chromosome sets. Dots correspond to sequencing tiles approximately 2 Mb long each. MAPD: 0.222; read count: 514,581; total number of bases: 94.8 Mb; total number of bases(AQ20): 83.8 Mb; % bases(AQ20): 88.4; mean coverage depth(fold): 0.035. MAPD, = median absolute pairwise difference.