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Lab Resource: Stem Cell Line

Generation of urine iPS cell lines from patients with Attention Deficit Hyperactivity Disorder (ADHD) using a non-integrative method

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ABSTRACT

Urine samples were collected from three patients (2 males, 1 female) with clinically diagnosed Attention Deficit Hyperactivity Disorder (ADHD) according to DSM-5 criteria using semi-structured interviews (K-SADS adapted for adults) by a trained psychiatrist. Urine epithelial cell lines were established and expanded for subsequent reprogramming procedure. Induced pluripotent stem cells (iPSCs) were derived using 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	ADHD-4 iPSC, ADHD-5 iPSC, ADHD-10 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, Oct4, Sox2, Klf4 and c-Myc. These factors have been repeatedly shown to be sufficient for very efficient reprogramming (Lieu et al. 2013; Takahashi et al. 2007). The identity of derived human iPS cell lines 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 3 derived iPS cell lines was analyzed by low-pass whole-genome sequencing (Fig. 2) (Wells et al. 2014).

Materials and methods

Derivation of human urine epithelial cell lines ADHD-4, ADHD-5 and ADHD-10

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. 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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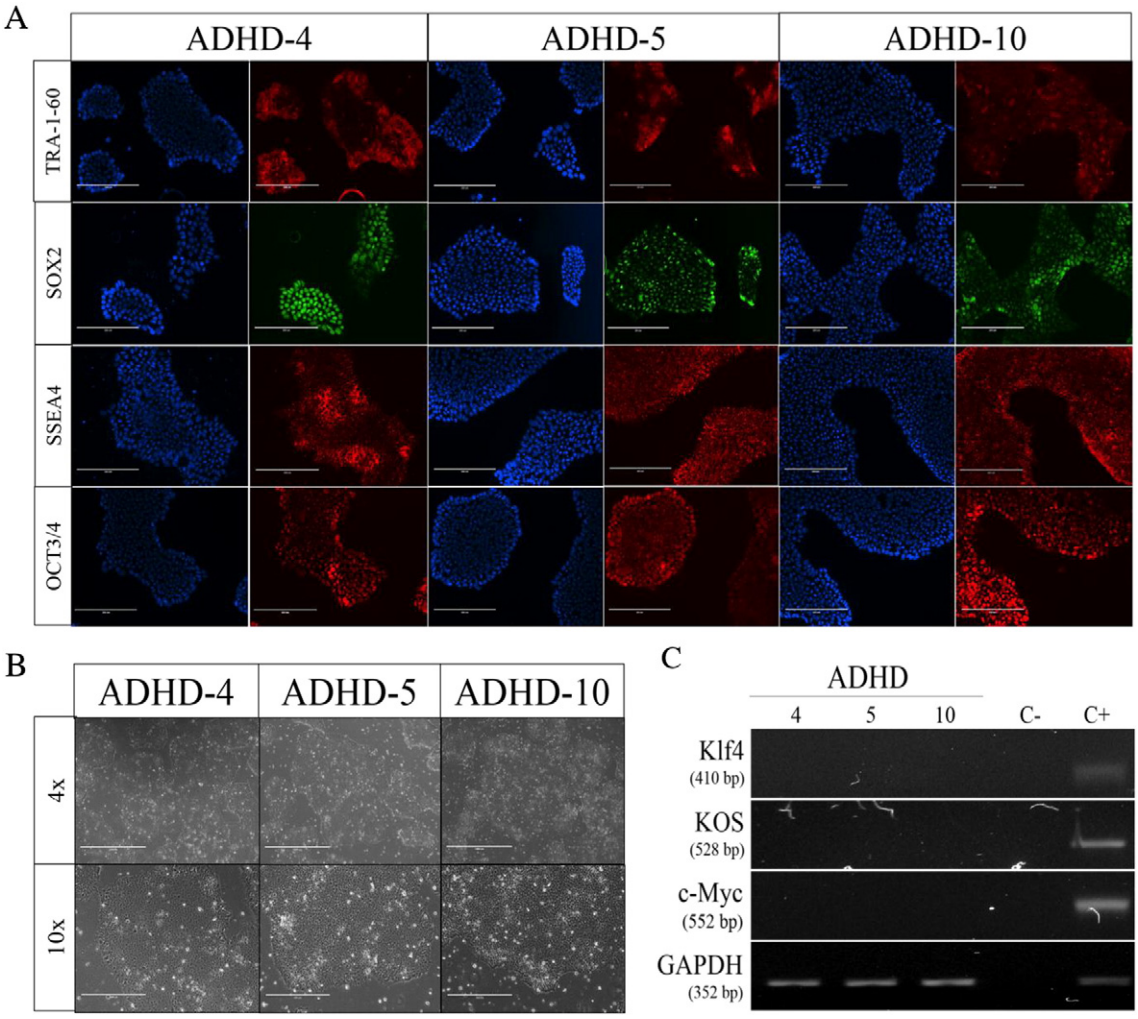

Fig. 1. Characterization of the three ADHD iPS cell lines. A) Expression of pluripotency markers: TRA-1-60, SOX2, SSEA4 and OCT3/4. Immunofluorescence staining, red/green: pluripotency markers, Blue: DAPI, nuclei staining. Scale bars, 200 μm. B) Feeder-free ADHD iPS cell lines cultures. C) Reverse transcription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

300 mL) were collected from the patients, centrifuged for 10 min at 1700 rpm and washed once with 1× PBS 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 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 protocol. Four days after transduction, cells were plated onto MEF feeder cells, and fed with iPS cell's 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, transferred to 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 ADHD iPS cell lines (Fig. 1a). The cells 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 2% BSA. Cells were then washed with PBS, incubated with primary antibodies at 1:100 dilution, at 4 °C, overnight, in a humid chamber. 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 plate was sealed with AlumSeal 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.