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Lab Resource: Stem Cell Line

Generation of human iPSC line *GRX-MCiPS4F-A2* from adult peripheral blood mononuclear cells (PBMCs) with Spanish genetic background

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ABSTRACT

We have generated iPSCs from peripheral blood mononuclear cells (PBMCs) of a healthy man using heat sensitive and non-integrative Sendai virus containing Sox2, Oct3/4, c-Myc and Klf4. Human *GRX-MCiPS4F-A2* cell line was established and characterized through this study.

Resource Table: *GRX-MCiPS4F-A2*

Name of Stem Cell construct	CytoTune® 2.0 Sendai Reprogramming Kit (Life Technologies)
Institution	Otology & Neurotology Group CTS495, Department of Genomic Medicine, GENYO, -Centre for Genomics and Oncological Research– Pfizer/Universidad de Granada/ Junta de Andalucía, PTS
Person who created resource	Sonia Cabrera, Ae-Ri Ji
Contact person and email	Sonia Cabrera, sonia.cabrera@genyo.es; Ae-Ri Ji, aeri.ji@genyo.es
Date archived/stock date	20, June, 2015
Origin	Healthy human male peripheral blood mononuclear cells
Type of resource	Biological reagent: induced pluripotent stem cell (iPSC)
Sub-type	Cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	
Information in public databases	No

1. Resource details

Heat sensitive Sendai virus containing Sox2, Oct3/4, c-Myc and Klf4 were used to generate human iPSC line *GRX-MCiPS4F-A2*. The exogenous reprogramming factors were eliminated from the established line at passage 7 (p7) in normal growth cultures.

To confirm the pluripotency of *GRX-MCiPS4F-A2*, the expression of several pluripotent markers were evaluated by RT-PCR, immunocytochemistry, and FACS analyses. Endogenous expression of OCT4, SOX2, KLF4, and NANOG were identified at mRNA level by RT-PCR. OCT4, SSEA4, TRA-1-60, and Tra-1-81 proteins were assessed by immunocytochemistry and FACS analysis.

Moreover, *GRX-MCiPS4F-A2* has shown normal karyotype (46, XY) after 23 passages by mechanical transfers (Fig. 1). Differentiation capacity into three germ layers was demonstrated by *in vitro* differentiation of embryoid bodies and teratoma formation *in vivo* (Fig. 2). *GRX-MCiPS4F-A2* cell line has been adapted to be cultured in feeder-free culture (on Matrigel) and it has been maintained over 40 passages without morphological alterations.

2. Materials and methods

2.1. Reprogramming lymphocytes to iPSCs

Blood samples were obtained from a healthy man with Spanish genetic background, who gave written informed consent for the generation of iPSCs. Mononuclear cells from peripheral blood were

[1] These authors contributed equally to this work.

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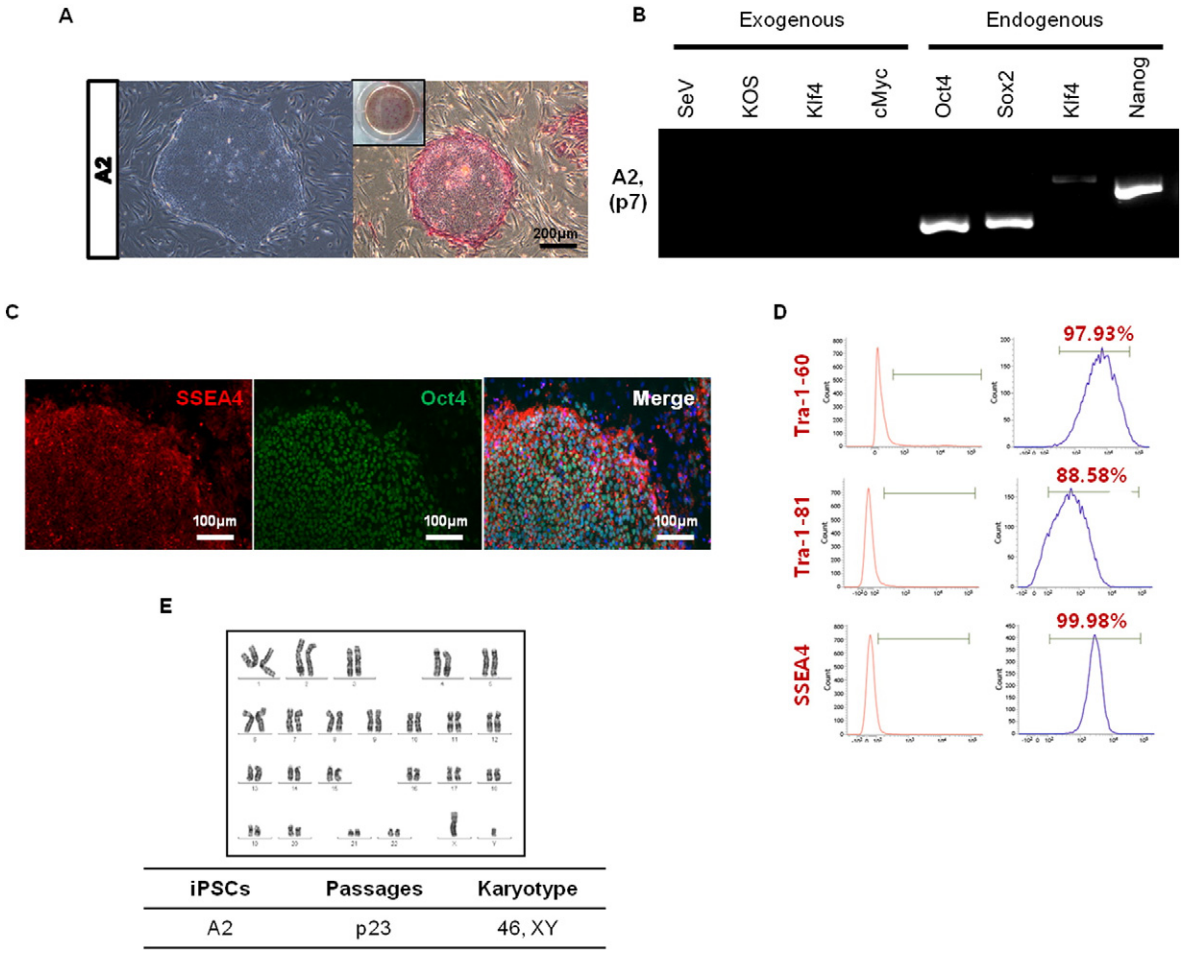

iPSCs	Passages	Karyotype
A2	p23	46, XY

Fig. 1. Characterization of human *GRX-MCiPS4F-A2* line generated from peripheral blood mononuclear cells (PBMCs). (A) Established *GRX-MCiPS4F-A2* line shows typical round shape colony on the human foreskin fibroblast feeder cells and strong alkaline phosphatase (AP) positive cells. (B) Exogenous reprogramming factors and Sendai virus vector (SeV) were disappeared after several passages in *GRX-MCiPS4F-A2*, and the expression of endogenous pluripotent markers, OCT4, SOX2, KLF4, and Nanog, were confirmed by RT-PCR. (C, D) Representative data show the expression of pluripotent markers in protein levels by immounocytochemistry and FACS analysis. (E) *GRX-MCiPS4F-A2* line shows normal karyotype after long term cultures.

separated by Ficoll gradients (Lymphosep, Lymphocyte Separation Media, Biowest, France). Isolated mononuclear cells were cultured for 4 days in PMBC media: StemSpan™ SFEM (StemCell Technologies Inc, Bâtiment Sirocco, France) supplemented with 100 ng/ml hSCF, 100 ng/ml hFLT3L, 20 ng/ml hTPO, 10 ng/ml G-CSF and 2 ng/ml hIL3 (GIBCO). Then, mononuclear cells were transferred to new culture plates and exposed to Sendai viral particles included in the CytoTune cellular reprogramming kit (Invitrogen, San Diego, CA). The next day, the CytoTune® 2.0 Sendai viral particles were removed by centrifuging, and transduced cells were grown for 4 days in PMBC media. Then, cells were transferred on irradiated human foreskin fibroblast (iHFF, ATCC®CRL-2429) feeder cells in human iPSC medium: KO-DMEM (Life Technology, CA, USA) supplemented with 20% knockout serum replacement (Fisher Scientific, MA, USA), 8 ng/ml FGF2 (Miltenyl Biotec, Cologne, Germany), 10 μM Y-27632 (Deltaclon, Madrid, Spain), 1 mM glutamine (Fisher Scientific), 1% MEM non-essential amino acids (Fisher Scientific) and 0.1 mM 2-mercaptoethanol (Sigma Aldrich, MO, USA). The emerging iPSC colonies were selected and transfered to new culture plates covered by fresh iHFF feeder cells.

2.2. Ethical approval

This study was approved by the Andalusian Ethics Review Board for cellular reprogramming, according to Spanish and EU legislation.

2.3. RT-PCR

Total RNA was isolated using High Pure RNA Isolation kit (Roche Applied Science, Germany) according to the manufacturer's instructions. The concentration and quality of isolated RNA was determined using a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. Aliquots of cDNA were used as a template for polymerase chain reaction (PCR) amplification with specific primer sets. The primers used for exogenous reprogramming factors (SeV, KOS, Klf4, and cMyc) provided by the company, and endogenous pluripotency markers (Oct4, Sox2, Nanog, Klf4, and cMyc) are listed in Table 1. 5 μl of PCR products were separated on a 1.5% agarose gel and visualized by GelRed™ Gel Stain (Biotium, CA, USA).

2.4. Immunocytochemistry

Cultured *GRX-MCiPS4F-A2* cells were fixed with 4% paraformaldehyde (PFA) in PBS buffer and then permeabilized with 0.03% Triton-X 100 (Sigma-Aldrich) for 20 min. Next, the cells were blocked with 3% BSA (Sigma-Aldrich) in PBS for 30 min at room temperature (RT). After that, cells were incubated with rabbit anti-human Oct 4 antibody (1:1000, Abcam, Cambridge, UK), mouse anti-human Tra-1-60 (1:200, Merck Millipore), or SSEA4 (1:200,

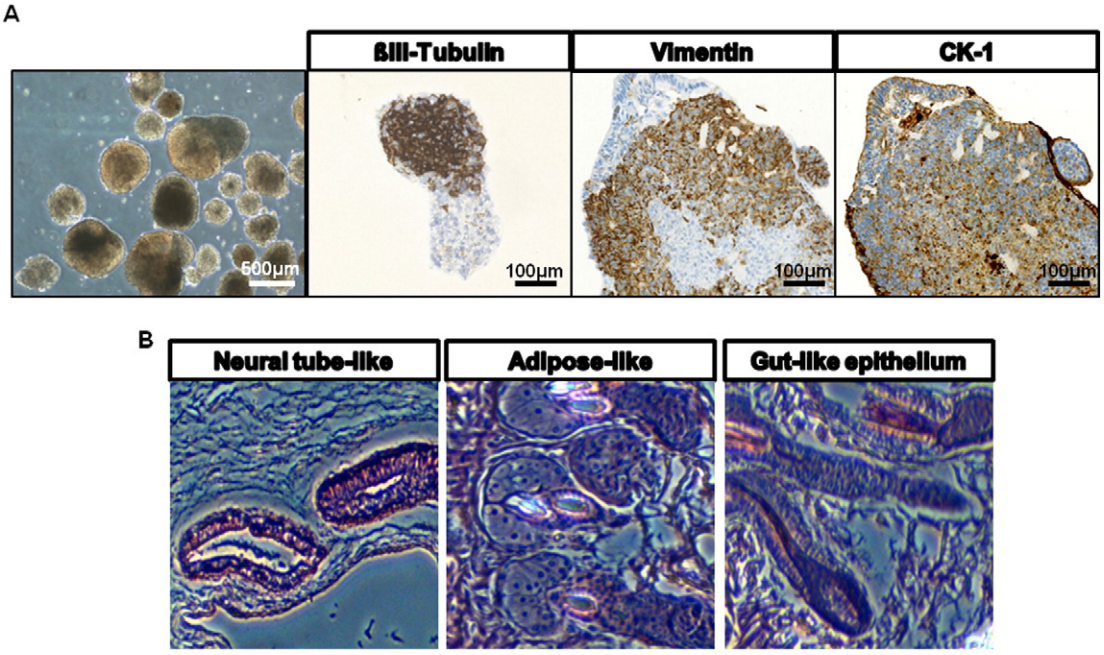

Fig. 2. *In vitro* and *in vivo* potential differentiation of *GRX-MCiPS4F-A2* line. (A) Embryonic bodies (EBs) of *GRX-MCiPS4F-A2* were grown for 24 days and demonstrated the differentiation into ectoderm (ßIII-tubulin), mesoderm (Vimentin), and endoderm (Ctokeratin AE1/AE3) by immunohistochemistry *in vitro*. (B) Hematoxylin and eosin (HE) stains show neural tube-like, adipose-like, and gut-like epithelium structures by teratoma formation *in vivo*.

Abcam) for 2 h at RT and then Alexa Fluor® 488 donkey anti-rabbit IgG (1:500, Life Technology, CA, USA) or Alexa Fluor® 555 goat anti-mouse IgG (1:500, Biomol S.L, Madrid, Spain) as a secondary antibody for 1 h at RT. Cells were examined using a confocal microscope (Nikon, Yokohama, Japan) equipped with Zeiss LSM 710 (Zeiss, Jena, Germany) using Axio Imager (Zeiss).

2.5. Flow cytometry analysis

Expression of pluripotency-associated markers was determined by flow cytometry using Alexa Fluor® 647 Mouse anti-SSEA-4 (1:100), Anti-Human TRA-1-60 Podocalyxin (1:100) and Alexa Fluor® 647 Mouse anti-Human TRA-1-81 (1:100). An irrelevant isotype-match antibody was always used. *GRX-MCiPS4F-A2* colonies were washed with PBS and were incubated for 5 min with TrypLE Express (Life Technologies, CA, USA). Tripsin-dissociated cells were suspended in 3%FBS in PBS at a concentration of $1–2 \times 10^5$ cells per 100 µL and incubated with the specific primary antibody for 1 h at 4 °C. After washing with 3%FBS in PBS, cells were stained with 7-aminoactinomycin D (7-AAD) (BD Bioscience) for 5 min at RT. Stained cells were analyzed using a FACSCanto (BD Bioscience) and FACSDiva™ software program (BD Bioscience).

2.6. Karyotype analysis

Chromosomal G-band analyses were performed at the Andalusian Public Health System Biobank, Spain. The study was conducted according to the recommendations published by the International System Cytogenetics Nomenclature.

2.7. In vitro differentiation of human iPSCs

Embryoid bodies (EBs) were generated from colonies of *GRX-MCiPS4F-A2* grown in feeder-free cultures. To make EBs, 1 ml of 1:6 diluted Matrigel was added into iPSC cultures one day before. The iPSCs colonies were harvested using 2 mg/ml Collagenase IV and moved to Ultra-low attachment multiwell plate (Sigma-aldrich). Embryoid bodies were grown in human iPSC medium without FGF2 to induce spontaneous differentiation for 24 days. Potential differentiation of EBs was examined into three germ-layers.

2.8. In vivo teratoma formation assay by human iPSCs

GRX-MCiPS4F-A2 cells were grown in feeder-free culture and dissociated by collagenase IV treatment into single cell suspensions. Dissociated single cell pellets were re-suspended in 20 µl PBS containing 2×10^5 cells and injected subcutaneously into dorsal flanks of SCID mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ). Teratomas were formed around 1 month after transplantations; teratomas were collected after 2 months and fixed in 10% formalin. Then fixed samples were processed for paraffin embedding and H&E staining using standard procedures (Gropp et al., 2012). Animal care and experimental procedures for generation of teratomas were approved by the Animal Experimentation Ethics Committee of the University of Granada, Spain.

Table 1
Primer sets used to amplify endogenous pluripotent genes by reverse transcription-polymerase chain reaction (RT-PCR).

Gene	5′ primer sequence	3′ primer sequence	Annealing temp. (°C)	Size (bp)
Endogenous pluripotent markers				
hOct4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC	56	144
hSox2	GGGAAATGGGAGGGGTGCAAAAGAGG	TTGCGTGAGTGTGGATGGGATTGGTG	56	151
hKlf4	ACGATCGTGGCCCCGGAAAAGGACC	TGATTGTAGTGCTTTCTGGCTGGGCTCC	56	397
hNanog	CAGCCCCGATTCTTCCACCAGTCCC	CGGAAGATTCCCAGTCGGGTTCACC	56	343

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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Reference

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