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Lab resource: Stem cell Line

Generation and characterization of human iPSC line generated from mesenchymal stem cells derived from adipose tissue

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ABSTRACT

In this work, mesenchymal stem cells derived from adipose tissue (ADSCs) were used for the generation of the human-induced pluripotent stem cell line G15.AO. Cell reprogramming was performed using retroviral vectors containing the Yamanaka factors, and the generated G15.AO hiPSC line showed normal karyotype, silencing of the exogenous reprogramming factors, induction of the typical pluripotency-associated markers, alkaline phosphatase enzymatic activity, and *in vivo* and *in vitro* differentiation ability to the three germ layers.

Resource Table: G15.AO

Name of stem cell construct:	G15.AO
Institution:	Cell Therapy Program. Center for Applied Medical Research (CIMA). University of Navarra.
Person who created resource:	Juan R. Rodriguez-Madoz, Felipe Prosper
Contact person and email:	jrrodriguez@unav.es; fprosper@unav.es
Date archived/stock date:	November 6, 2013.
Origin:	Adipose derived mesenchymal stem cells (ADSCs).
Type of resource:	Biological reagent: human induced pluripotent stem cell (hiPSC) line.
Sub-type:	Cell line
Key transcription factors:	SOX2, OCT4, cMYC, KLF4.
Authentication:	Identity and purity of cell line confirmed
Link to related literature (direct URL links and full references)	Not available
Information in public databases:	http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml

Resource Details

G15.AO human-induced pluripotent stem cell (hiPSC) line has been generated from mesenchymal stem cells derived from adipose tissue (ADSCs) using retroviral vectors coding for the reprogramming factors SOX2, OCT4, cMYC, and KLF4. G15.AO hiPSC line displayed a typical small, round shape, and tightly packed ESC-like morphology with a high nucleus/cytoplasm ratio with prominent nucleoli and was positive for alkaline phosphatase activity (Fig. 1A). The expression of several pluripotent markers was confirmed by qPCR (Fig. 1B), immunofluorescence (Fig. 1D), and FACS analyses (Fig. 1E). Moreover, the silencing of exogenous reprogramming transgenes was observed by RT-PCR after 10–15 passages (Fig. 1C). Differentiation capacity into three germ layers was demonstrated by *in vitro* embryoid bodies formation (Fig. 1F) and *in vivo* teratoma formation (Fig. 1G). Finally, G15.AO hiPSC line showed normal karyotype (46, XY) after more than 30 passages (Fig. 2).

Materials and methods

Ethical approval

All procedures described in this work were approved by the University of Navarra Ethical Committee as well as by the Advisory Committee for Human Tissue and Cell Donation and Use, according to Spanish and EU legislation. ADSCs used for generation of the induced pluripotent stem cell line were isolated from a healthy donor after written informed consent.

Cell culture

ADSCs were obtained from the stromal vascular phases of adipose tissue from an adult donor. Adipose tissue was carefully separated

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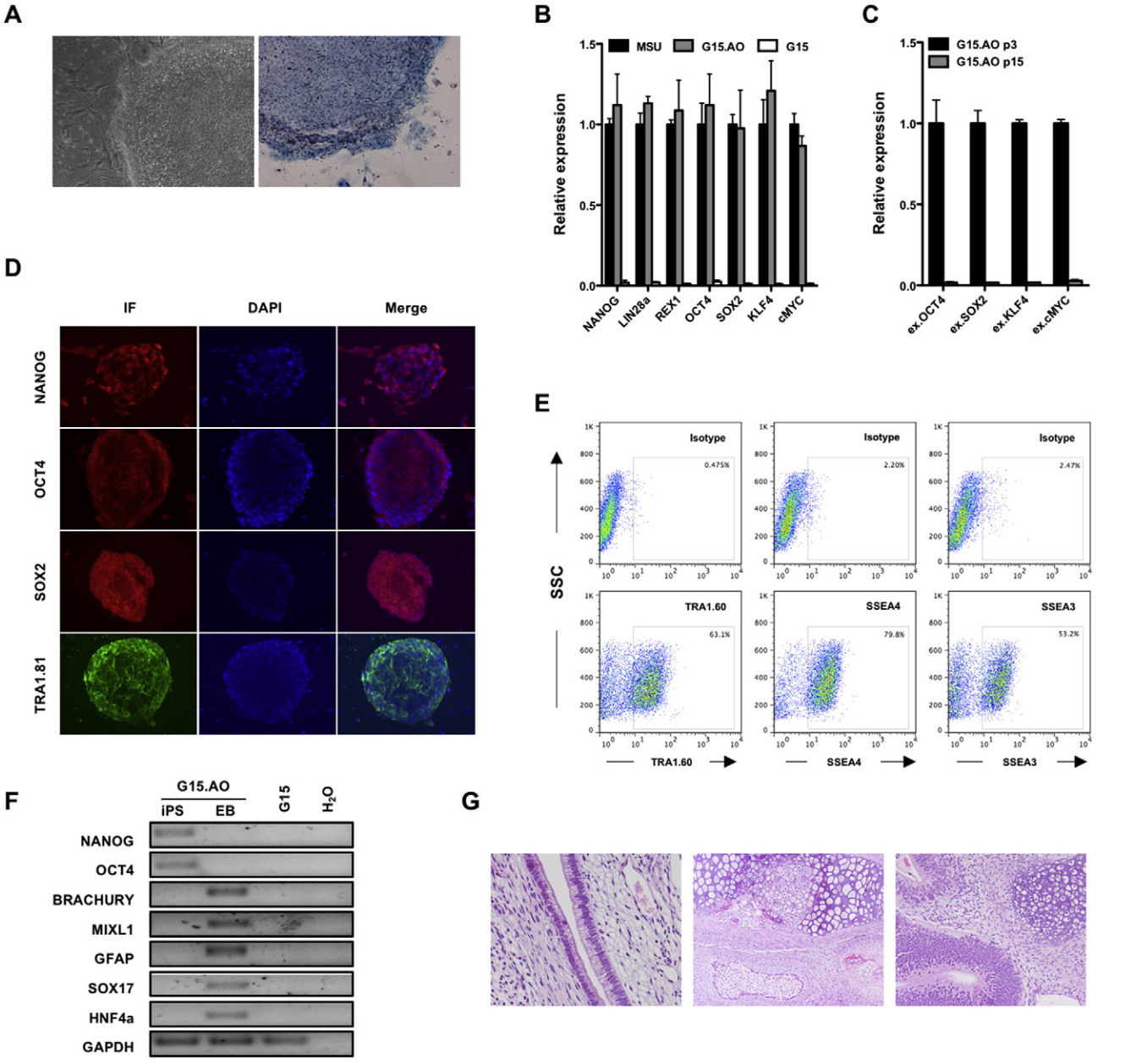

Fig. 1. Characterization of G15.AO hiPSC line. (A) G15.AO hiPSC line displays a typical round shape colony morphology with small, tightly packed cells (left panel). Alkaline phosphatase enzymatic activity (Right panel). (B) Endogenous pluripotency-associated markers NANOG, LIN28a, REX1, OCT4, SOX2, KLF4, and cMYC were confirmed by qPCR. MSU hiPSC line and parental ADSCs (G15) were used as positive and negative controls, respectively. (C) Silencing of exogenous reprogramming factors was confirmed by qPCR after passage 15. (D) Expression of pluripotency-associated markers NANOG, OCT4, SOX2, and TRA1-81 at protein level by immunofluorescence. (E) Expression of pluripotency-associated markers TRA1-60, SSEA4, and SSEA3 by FACS analysis. The upper panel shows the staining using the corresponding irrelevant isotype-matched antibody. (F) *In vitro* differentiation study by EB formation. EBs were grown for 21 days and induction of genes representative of the three germ layers were analyzed by RT-PCR. Parental ADSCs (G15) were used as negative control. (G) *In vivo* differentiation test by teratoma formation assay. The pictures show hematoxylin/eosin staining (H&E) with representative tissues from the three germ layers. Pseudostratified epithelium similar to trachea (left), cartilage tissue (middle), and glandular tissues (right).

from skin and vessels, minced until getting a semi-solid paste, and digested with 2 mg/mL of collagenase type I (Gibco) until getting two phases. The lower phase containing the mesenchymal stem cells was filtered through a 100 μm and a 40 μm mesh (Falcon) and seeded in gelatin-coated culture plates in Alpha Minimum Essential Medium (αMEM) supplemented with 10% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine (Lonza), 100 UI/ml penicillin/streptomycin (P/S, Lonza), and 1 ng/mL of bFGF (Peprotech). ADSCs were expanded for a maximum of five passages before use.

G15.AO hiPSC generation

To induce cell reprogramming, isolated ADSCs were infected with VSVG-coated MMLV retroviral vectors coding for the human reprogramming factors OCT4, SOX2, c-MYC, and KLF4 (Addgene plasmids 17217, 17218, 17219, and 17220) as described (Takahashi et al. 2007). Briefly, ADSCs were seeded at 5×10^4 cells/well of a 6-well plate (Falcon) and infected for three consecutive days with freshly produced retroviral vectors using a ratio 2:1:1:1 (O:S:K:M) in the presence