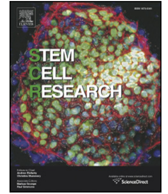
Stem Cell Research 16 (2016) 20–23

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



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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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 30 November 2015
Accepted 1 December 2015
Available online 02 December 2015

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.

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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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http://dx.doi.org/10.1016/j.scr.2015.12.002
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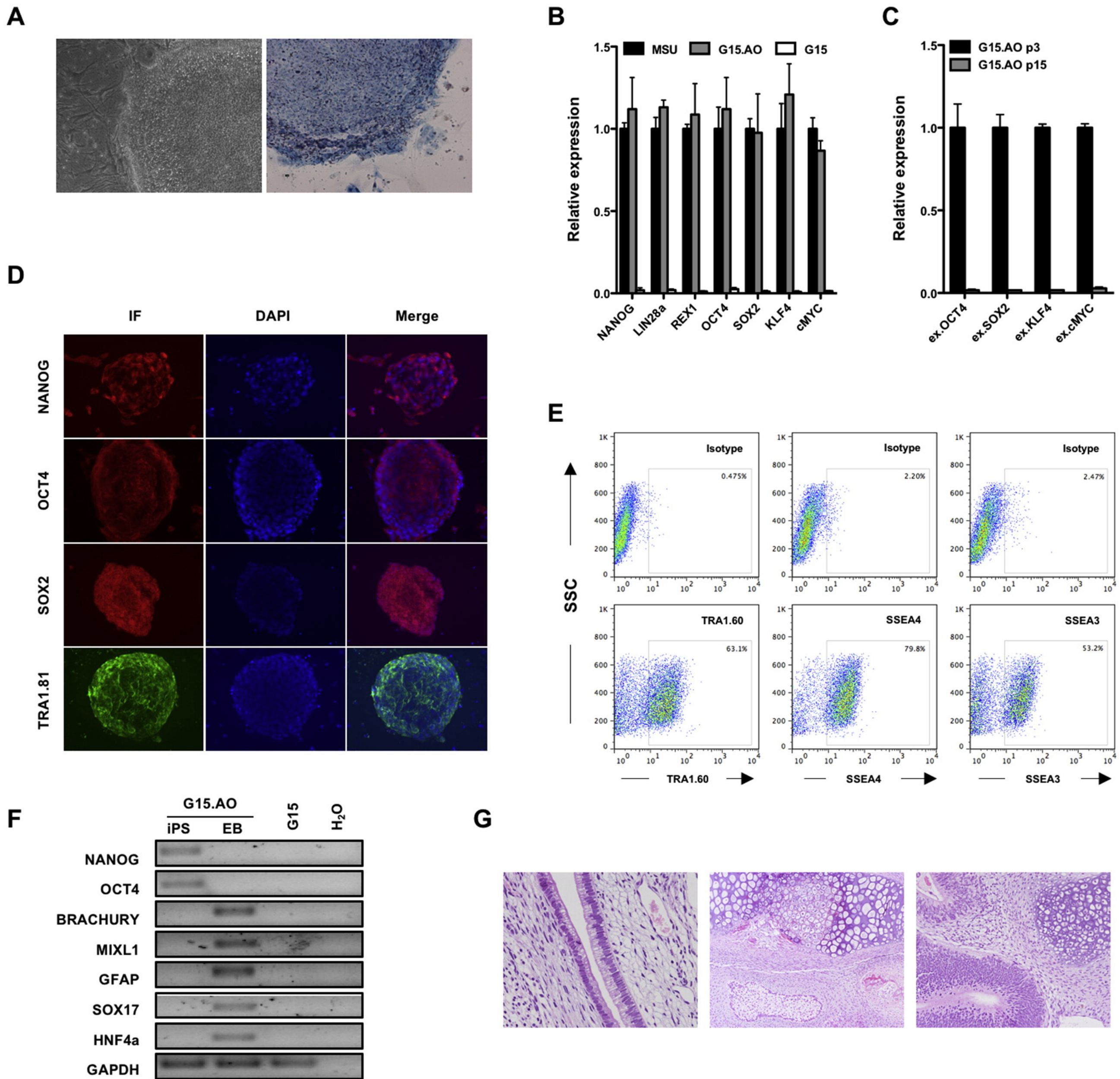

Fig. 1. Characterization of G15.AO hiPSC line. (A) G15.AO hiPSC line displays a typical round shape colony morphology with small, tightly packed cells (left panel). Alkaline phosphatase enzymatic activity (Right panel). (B) Endogenous pluripotency-associated markers NANOG, LIN28a, REX1, OCT4, SOX2, KLF4, 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

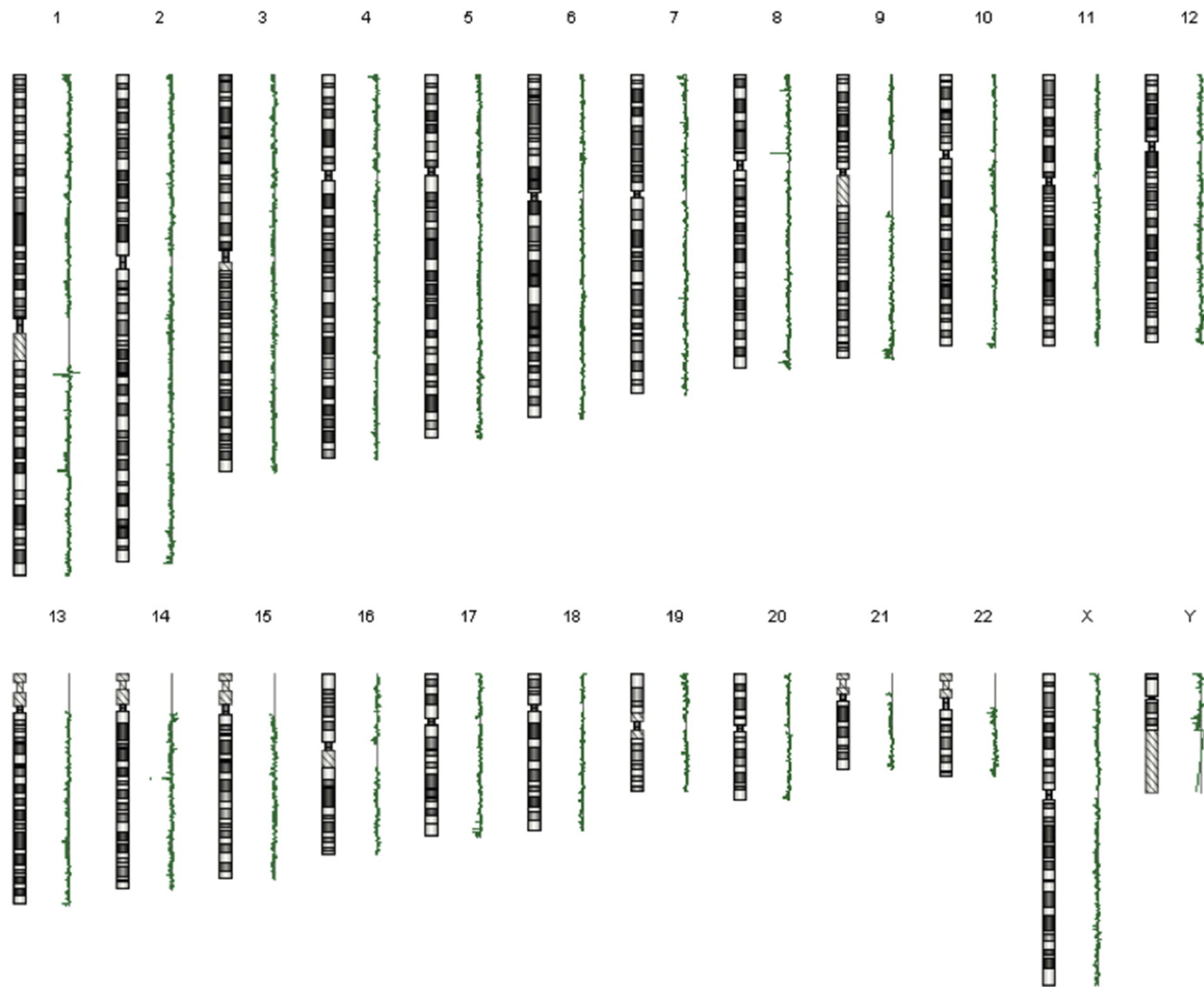

Fig. 2. CGH analysis of G15.AO hiPSC (passage >30) depicting a normal 46XY karyotype.

of 4 μg/ml polybrene (Sigma). Four days after the last infection, infected cells were detached using 0.05% trypsin solution (Lonza) and plated onto previously seeded MEF feeder layers. The following day, culture medium with Knockout DMEM (Gibco) supplemented with 20% Knockout serum replacement (KSR, Gibco), 0.1 mM NEAA (Lonza), 2 mM L-glutamine (Lonza), 100 UI/ml P/S (Lonza), 0.1 mM b-mercaptoethanol (Gibco), and 5 ng/mL bFGF (Peprotech) were added. In order to increase reprogramming efficiency, hiPSC media were supplemented with 1 mM of VPA (Sigma) during the first week of reprogramming. Three to four weeks after infection, emerging hiPSC colonies were picked individually and expanded on irradiated MEFs in the presence of 10 μM of ROCK inhibitor compound GSK269962A (AxonMedChem). Cells were routinely passaged at a splitting ratio of 1:3 or 1:6 every week when cells reached confluence (Fig. 1A).

RNA extraction and RT-qPCR

Total RNA was isolated using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific) and RNA quality was tested using Bioanalyzer (Agilent). Complementary DNA (cDNA) was synthesized using PrimeScript™ RT reagent Kit (Takara) according to the manufacturer's instructions after DNAse treatment (Fermentas). Quantitative PCR (qPCR) primers (Table 1) were designed using Primer3 input software and GAPDH was used as housekeeping gene. Expression of pluripotency-associated markers was evaluated by qPCR and expression levels were compared to previously established hiPSC line MSU (Ramos-Mejía et al. 2010) (Fig. 1B). Silencing of the exogenous reprogramming factors was analyzed by qPCR after 10–15 passages (Fig. 1C).

Table 1
Primer sequences used in this study.

Gene	Forward	Reverse
GAPDH	ctggtaaagtggatattgttgccat	tggaatcatattggaacatgtaaacc
LIN28A	ggaggccaagaaagggaatatga	aacaatcttgtggccactttgaca
NANOG	ccaacatcctgaacctcagc	tgcgtcacaccattgctatt
OCT4	ggaaggaattgggaacacaaagg	aacttcaccttccctccaacca
KLF4	acagtctgttatgcactgtggtttca	catttgttctgcttaaggcatacttgg
REX1	tggagcctgtgtgaacagaa	ccacctccaggcagtagtga
SOX2	tggcgaaccatctctgtggt	ccaacggtgtcaacctgcat
cMYC	acagaaatgtcctgagcaatcacct	gccaaggttgtgaggttgcat
SOX17	gaatccagacctgcacaacg	ctctgcctcctccacgaag
HNF4A	aacctgttgcaggagatgct	cgttggttcccatatgttcc
MIXL1	ggtaccccgacatccactt	gccaaaggttggaaggattt
BRACHURY	actcacctgcatgtttatcca	ccgttgctcacagaccacag
GFAP	tggaggttgagagggacaat	taggcagccaggttgttctc
ex.OCT4	ggctctcccatgcattcaaac	catggcctgcccggttatta
ex.SOX2	gcacactgcccctctcacac	caccagaccaactggtaatggtagc
ex.KLF4	cctcgccttacacatgaagagaca	caccagaccaactggtaatggtagc
ex.cMYC	gctacggaactcttgtgcgtga	caccagaccaactggtaatggtagc

Alkaline phosphatase (AP) staining and immunofluorescence (IF)

For AP staining, hiPSCs were fixed with Formaline (Merck) for 10 min, and Alkaline Phosphatase Blue Membrane Substrate Solution (Sigma) was used according to the manufacturer's instructions (Fig. 1A). For IF analysis, hiPSCs were fixed with 4% paraformaldehyde (PFA, Sigma), permeabilized for 10 min with 1% TritonX-100 (Sigma) in PBS and blocked with 5% bovine serum albumin (BSA) for 30 min at RT. SOX2 (R&D), OCT4 (Santa Cruz), NANOG (Abcam), and TRA1-81 (Chemicon) primary antibodies were diluted in PBS/TBS with 1% BSA and incubated for 1 h at RT. FITC-conjugated sheep anti-mouse and Cy3-conjugated sheep anti-rabbit secondary antibodies (Sigma) were incubated for 1–1.5 h at RT. Nuclei were counterstained with 1:4 dilution of DAPI mounting medium (Vector Labs). Samples were visualized under an inverted fluorescence microscope (Nikon Eclipse Ti-S) (Fig. 1D).

Flow cytometry analysis

Pluripotency-associated markers were analyzed by FACS as described (Montes et al. 2015). Briefly, hiPSCs were dissociated by incubation with TrypLE Express (Life Technologies) for 5 min. Then, hiPSCs were suspended in FACS buffer (5% FBS 2 mM EDTA in PBS) and incubated with PE-conjugated mouse anti-TRA1-60, PE-conjugated rat anti-SSEA-3, and FITC-conjugated mouse anti-SSEA-4 specific primary antibody (BD Biosciences) for 30 min at 4 °C. An irrelevant isotype-match antibody was used as a negative control. Then, the cells were washed with FACS buffer and stained with 7-aminoactinomycin D (7-AAD, BD Bioscience) for 5 min at RT. Stained cells were analyzed using a FACSCalibur (BD Bioscience) and FlowJo software (FlowJo Enterpirse) (Fig. 1E).

In vitro differentiation of G15.AO hiPSCs

G15.AO hiPSCs were grown on Matrigel until they reach confluence in E8 medium (Gibco). Embryoid bodies (EBs) were cultured in suspension in a 6-well ultralow-attachment plate (Falcon) in DMEM-KO supplemented with 20% FBS (Biochrom), 2 mM L-glutamine (Lonza), 100 UI/ml P/S (Lonza), 0.1 mM b-mercaptoethanol (Gibco). EBs samples were collected at day 21 of spontaneous differentiation to analyze induction of markers representative of the three germ layers by qPCR (Fig. 1F).

In vivo teratoma formation

Teratomas were generated by subcutaneous injection of $2–5 \times 10^6$ G15.AO hiPSCs cultured on Matrigel into the dorsal flanks of 4–6 week-old male immune-deficient $Rag2^{-/-}\gamma c^{-/-}$ mice according to the ethical guidelines observed by the University of Navarra. About 4–6 weeks after injection, tumors were dissected, fixed in 10% formalin (Sigma), and stored at 4 °C until histological analysis. Paraffin-embedded tumors were sectioned, stained with hematoxylin/eosin, and the presence of differentiated tissues representative of the three embryonic germ layers was analyzed (Fig. 1G).

Genomic stability by comparative genome hybridization (CGH)

Genomic DNA from G15.AO hiPSC line (passage number > 30) was hybridized into the human genome CGH microarray (Agilent Technologies). The resolution of the analysis was 200 kb for the majority of the regions and a minimum of five consecutive probes per DNA sample is considered as an alteration in the number of DNA copies. As shown in Fig. 2, G15.AO hiPSC line displays normal karyotype (46, XY).

DNA fingerprinting

DNA fingerprinting was performed at the Genomics Core Facility (CIMA, University of Navarra) in order to detect the pattern of short tandem repeats (STRs) of G15.AO hiPSC line and its parenteral ADSC using AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems). Multiplex PCR performed for the STRs Amelogenin, CSF1PO, D13S317, D16S539, D5S818, D7S820, THO1, TPOX, and vWA confirmed cell identity.

Acknowledgement

We thank the Genomics Core Facility of the CIMA, University of Navarra. This work was supported by the “Torres Quevedo” Subprogram, Ministry of Economy and Competitiveness (PTQ-11-04777) to JRRM; the Institute of Health Carlos III (PI13/00862) to JRRM, and RETIC (RD12/0019/0031) to FP; and the Fundación Bancaria Caja Navarra (70270) to JRRM.

Author disclosure statement

There are no competing financial interests in this study.

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