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Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cells derived from a 77-year-old healthy woman as control for age related diseases

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ABSTRACT

Induced pluripotent stem cells (iPSCs) hold great promise to model diseases, where the disease affected cell type is difficult to access. A major obstacle for the development of disease models is the lack of well characterized control iPSCs from old people not affected by such a disease. Furthermore, gene-editing approaches often require iPSCs from healthy donors, where pathogenic mutations can be inserted if patient material is not available. Here, we report the generation of an iPSC line (16423 #6) from a 77-year-old woman, who did not display any disease symptoms at the time, when the skin biopsy was taken.

Resource table.

Name of stem cell line	hiPSC-16423 #6
Institution	Bioneer A/S
Person who created resource	Natakarn Nimsanor, Ida Jørring, Bjørn Holst, Benjamin Schmid
Contact person and email	Benjamin Schmid, bsc@bioneer.dk
Date archived/stock date	July 2016
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cells (iPSCs) derived from a 77-year-old healthy woman
Sub-type	Induced pluripotent stem cell
Key transcription factors	Episomal plasmids containing hOCT4, hSOX2, hL-MYC, hKLF4 and hLIN28 (Addgene plasmids 27,077, 27,078 and 27,080; Okita et al., 2011)
Authentication	Identity and purity of cell line confirmed by integration analysis, pluripotency analysis, karyotyping and *in vitro* differentiation (Fig. 1).

Resource details

Fibroblasts were obtained from a 77-year-old healthy woman, who did not display any disease related symptoms. Reprogramming was performed by electroporation with 3 episomal plasmids containing hOCT4, hSOX2 and hKLF4, and hL-MYC and hLIN28 (Okita et al., 2011). The line was termed hiPSC-16423 #6. qPCR analysis with plasmid-specific primers showed that none of the plasmids were present anymore after reprogramming (Fig. 1A). qRT-PCR analysis showed that the endogenous pluripotency genes OCT4, NANOG, GABRB3, TDGF1 and DNMT38 were expressed in the same range as in the control iPSC line BIONi010-C iPSCs (Rasmussen et al., 2014) (Fig. 1B). Immunocytochemistry (ICC) analysis demonstrated the presence of the pluripotency markers OCT4, NANOG, TRA1-60, TRA1-81 and SSEA4 on protein level (Fig. 1C). *In vitro* differentiation followed by ICC analysis with the mesodermal marker smooth muscle actin (SMA), the endodermal marker alpha-1-fetoprotein (AFP) and the ectodermal marker beta-III-Tubulin (TUJI) demonstrated the differentiation potential into all three germ layers (Fig. 1D). In addition, the iPSC-16423 #6 line maintained a normal, female karyotype after the reprogramming process (Fig. 1E).

Materials and methods

Generation of iPSCs

A skin biopsy was obtained from a 77-year-old healthy woman. Fibroblasts were cultured in fibroblast medium, consisting of Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 2 mM L-glutamine, and 1% penicillin and streptomycin. 1×10^5 fibroblasts were

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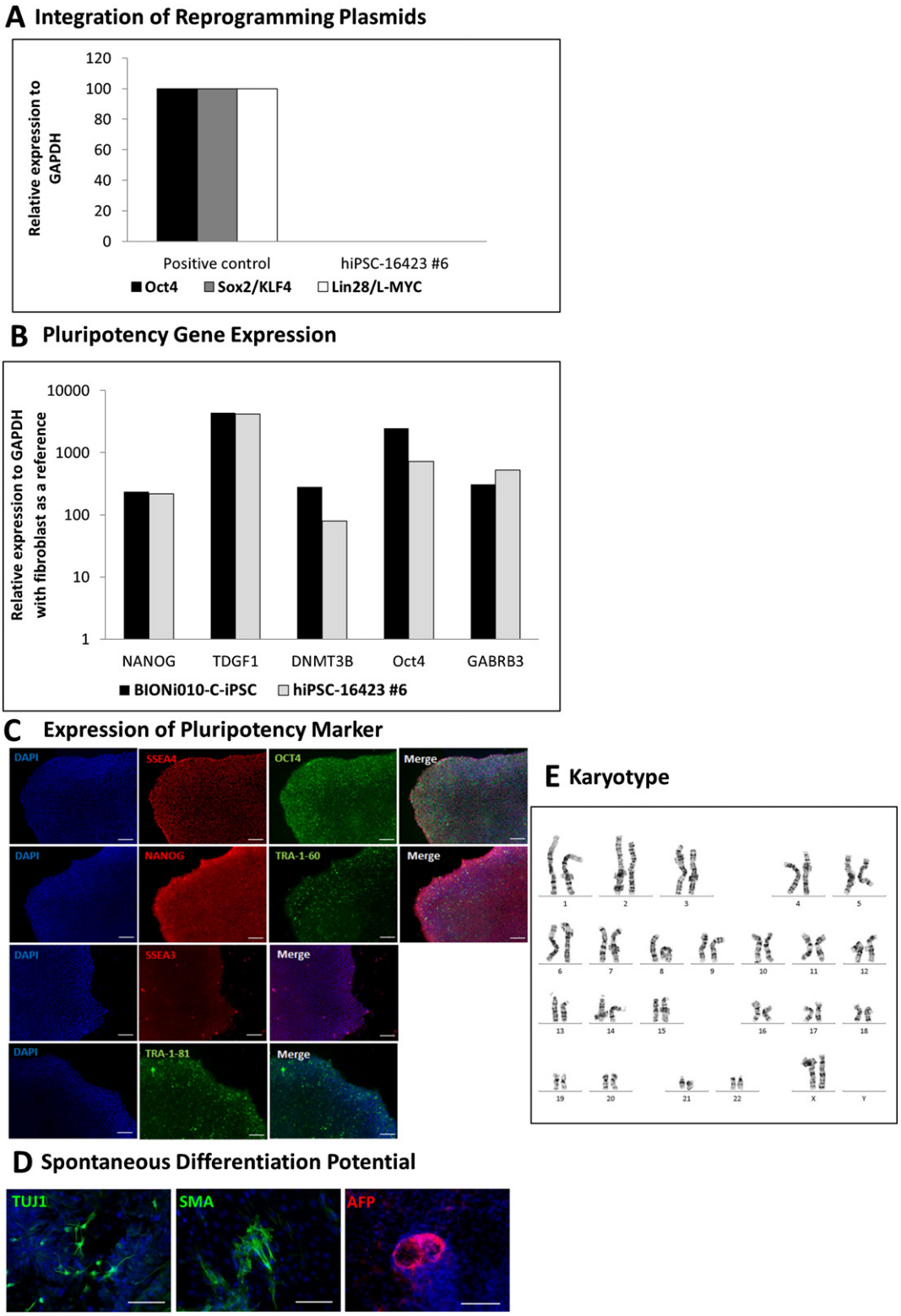

Fig. 1. (A) qPCR on genomic DNA from the 16423 #6 iPSC line and a pool of fibroblasts 20 days after electroporation (positive control) with primers specific for each of the 3 plasmids. Values were standardized relative to GAPDH. (C) qRT-PCR expression analyses of the endogenous pluripotency genes NANOG, OCT4, TDGF1, DNMT3B and GABRB3 on RNA from the hiPSC-16423 #6 line and the iPSC line BIONi010-C as positive control. Data is shown as the fold expression change ($2^{-\Delta\Delta Ct}$) to fibroblasts. Values were standardized relative to GAPDH. (D) Immunocytochemical staining of the stem cell markers OCT4, TRA1-81, NANOG, TRA1-60, SSEA3 and SSEA4 for the hiPSC-16423 #6 line. Scale bars correspond to 100 μm. (E) Immunocytochemistry analysis for proteins representative of the three germ layers, Tuj1 (ectoderm), SMA (mesoderm) and AFP (endoderm), after *in vitro* differentiation by embryoid body formation, scale bars = 200 μM. (F) Representative karyotype for the hiPSC-16423 #6 line (46, XX).

electroporated with a total of 1 μg of episomal plasmids containing hOCT4 (Addgene plasmids 27076), hSOX2 and hKLF4 (Addgene plasmid 27078), and hL-MYC and hLIN28 (Addgene plasmid 27080; Okita et al., 2011). Electroporation was carried out using a Neon™ electroporation device with two pulses at 1200 V for 20 ms (Life Technologies). After electroporation, the cells were cultured on ESC grade matrigel-

Table 1
Antibodies used for immunochemistry.

	Antibodies and host species	Dilution	Company and catalog number
Pluripotency	Goat *anti*-OCT4	1:500	Santa Cruz, sc-8628
	Rabbit anti-NANOG	1:100	Millipore, AB5731
	Mouse anti-SSEA4	1:500	BioLegend, 330,402
	Mouse anti TRA-1-60	1:500	BioLegend, 330,602
	Mouse anti TRA-1-81	1:500	BioLegend, 330,702
	Rat anti SSEA3	1:500	BioLegend, 330,302
In vitro differentiation	Mouse anti-Smooth muscle actin (SMA)	1:500	Dako, M0851
	Rabbit anti-Alpha-1-fetoprotein (AFP)	1:500	Dako, A0008
	Mouse anti-Beta-III-tubulin (TUJ1)	1:500	Sigma-Aldrich, T8660

coated dishes (BD Biosciences) in fibroblast medium. After 3–4 days, depending on confluency, the medium was changed to E8 medium containing 100 μM NaB. Individual iPSC colonies were manually picked and transferred to a new matrigel-coated dish. The iPSCs were split once a week with 0.5 mM EDTA and frozen in liquid nitrogen in E8 medium containing 10% DMSO and thawed in the presence of E8 medium supplemented with 1:200 diluted Revita cell supplement (Gibco).

Integration analysis

DNA was purified from hiPSC-16423 #6 and a pool of fibroblasts electroporated with episomal plasmids and grown for 20 days as a positive control using the DNeasy Blood and Tissue kit (Qiagen). qPCR analyses were carried out with the primers OCT4, SOX2, Lin28 and KLF4, which are specific to the three plasmids (Okita et al., 2011), and data was analyzed using the $2^{-\Delta\Delta Ct}$ method standardized on GAPDH with fibroblasts as a reference.

qRT-PCR analysis of stem cell markers

Total RNA was purified from hiPSC-16423 #6, fibroblasts and the control iPSC line BIONi010-C using the RNeasy mini kit from Qiagen. Conversion to cDNA was performed with RevertAid First Strand cDNA synthesis kit (Thermo Scientific). qPCR analysis was carried out using the TaqMan primers GAPDH Hs03929097_g1, NANOG Hs02387400_g1, OCT4 Hs00999632_g1, TDGF1 Hs02339497_g1, DMNT3B Hs00171876_m1 and GABRB3 Hs00241459_m1 (Thermo Scientific, International Stem Cell Banking Initiative). Data was analyzed using the $2^{-\Delta\Delta Ct}$ method standardized on GAPDH with fibroblasts as a reference.

Immunostaining of pluripotency markers

hiPSC-16423 #6 cells were seeded on matrigel coated coverslips. After 2 days, the cells were fixed at room temperature with 4% paraformaldehyde in PBS for 10 min and permeabilized with 0.1% Triton X-100 and blocked with 2% BSA in PBS for 1 h. Immunostaining was performed with primary antibodies against Oct4, Nanog, Tra-1-60, Tra-1-81, SSEA3 and SSEA4 (Table 1). After incubation with the primary antibody, the cells were washed 3 times with PBS and then incubated with fluorescence-conjugated secondary antibodies Alexa fluor 488 (goat *anti*-mouse, 1:1000) or Cyanine 3 (goat anti-rabbit, 1:1000) for 1 h (both Life technologies). The cells were covered with mounting solution containing DAPI (Life Technologies) and transferred to an object plate.

In vitro differentiation

hiPSC-16423 #6 were dissociated with EDTA (Gibco) and allowed to form aggregates in none-coated cell culture dishes with E8 medium. On day 2, the E8 medium was changed to E6 medium (E6 medium = E8 medium without FGF2 and TGF-β1). On day 7, the aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium. For mesodermal differentiation: DMEM/F12 containing 10% FBS, 1% L-glutamine, and 1% non-essential amino acids (all Gibco). For endodermal differentiation: MCDB131–1 containing 0.5% BSA, 0.1% pen/strep, 3 μM CHIR99021 (Selleckchem), 100 ng/ml Activin A (Cell Guidance). For ectodermal differentiation: DMEM/F12 mixed with neurobasal medium in a ratio of 1:1, 1× B27, 1× N2, and 1% L-glutamine (all Gibco), 10 μM SB 431542 and 0.1 μM LDN 193189 (both Selleckchem). Cells were fixed for immunocytochemistry on day 21. ICC analysis was performed with the antibodies TUJI, SMA and AFP (Table 1).

Karyotyping

hiPSC-16423 #6 were treated for 1 h with KaryoMAX colcemid (Life Technologies) and harvested in fresh fixative containing 25% acetic acid and 75% methanol. Karyotyping was performed on G-banded metaphase chromosomes using standard cytogenetic procedures. An intact genome was demonstrated by karyotyping using G-banding of 15 mitoses. The analysis was performed at the Institute of Medical Genetics and Applied Genomics, University of Tübingen, Germany. The results showed a normal 46, XX karyotype, free of any discernible abnormalities (Fig. 1F).

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