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Lab Resource: Stem Cell Line

Generation of induced pluripotent stem cells (iPSCs) from an Alzheimer's disease patient carrying a M146I mutation in *PSEN1*

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ABSTRACT

Skin fibroblasts were obtained from a 46-year-old symptomatic man carrying a M146I mutation in the presenilin 1 gene (*PSEN1*), responsible for causing Alzheimer's disease (AD). Induced pluripotent stem cells (iPSCs) were derived *via* transfection with episomal vectors carrying *hOCT4*, *hSOX2*, *hKLF2*, *hL-MYC*, *hLIN28* and *shTP53* genes. M146I-iPSCs were free of genomically integrated reprogramming genes, had the specific mutation but no additional genomic aberrancies, expressed the expected pluripotency markers and displayed *in vitro* differentiation potential to the three germ layers. The reported M146I-iPSCs line may be a useful resource for *in vitro* modeling of familial AD.

Resource Table

Name of stem cell construct	M146I-hiPSC
Institution	University of Copenhagen
Person who created resource	Tong Li, Carlota Pires, Kristine K Freude
Contact person and email	Kristine K Freude: kkf@sund.ku.dk
Date archived/stock date	September 2015
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cells (iPSCs); derived from *PSEN1* M146I heterozygous mutation patient
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	*hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN28*, and shRNA against TP53 (Addgene plasmids 27,077, 27,078 and 27,080; Okita et al., 2011, Okita et al., 2011)
Authentication	Identity and purity of cell line confirmed by analysis of plasmid integration, mutation sequencing, karyotyping, pluripotency markers and *in vitro* differentiation potential (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

Resource Details

The study was approved by the "De Videnskabsetiske Komiteer for Region Hovedstaden" (protocol number H-4-2011-157), Copenhagen, Denmark and written informed consent was obtained in all cases. To protect patient family privacy, no personal patient information is presented here. A skin biopsy was obtained from a 46-year-old man carrying a heterozygous mutation in exon 5 of presenilin 1 (*PSEN1*) gene causing a change in amino acid M146I. Mutations in *PSEN1* are the most common cause of inherited Alzheimer's disease (AD). The mother of the patient died of AD at the age of 42, and DNA for molecular genetic testing from her was not available. He had had cognitive symptoms since the age of 41, blurred by affective symptoms. He was diagnosed with AD at the age of 43. Episomal plasmids carrying gene sequences for *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN28* and a short hairpin against TP53 (Okita et al., 2011) were used to reprogram the fibroblasts into iPSCs. This technique was previously successfully established for generation of integration and feeder-free iPSCs (Rasmussen et al., 2014). 24 days after reprogramming, several iPSC colonies were picked for further selection and expansion as single cells. Episomal plasmid integration was analyzed by qPCR with DNA from iPSCs at passage 10 with plasmid-specific primers, using DNA from human fibroblasts as control. The analysis confirmed that the reprogramming genes *hOCT4*, *hSOX2* and *hLIN28* were absent and had, consequently, not integrated into the genome (Fig. 1A). The clones were karyotypically normal (Fig. 1E) and the mutation was sequenced and confirmed by the presence of a c.438 G > C change in exon 5 of the *PSEN1* gene corresponding to a

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heterozygous M146I mutation (Fig. 1B). qPCR was also performed to analyze the pluripotency marker expression at the mRNA level, which showed that the endogenous pluripotency genes *OCT4*, *DMNT3B*, *GABRG3*, *NANOG*, *TDGF1*, *GDF3*, *SOX2* and *ZFP42* were slightly upregulated compared to human embryonic stem cells (hESCs) (Fig. 1C). At the protein level, immunocytochemical (ICC) analysis confirmed the expression of the pluripotency markers OCT4, NANOG, SSEA3, SSEA4, TRA-1-60 and TRA-1-81 (Fig. 1D). Furthermore, *in vitro* differentiation followed by ICC analysis of the endodermal marker α-feto protein (AFP), the mesodermal marker smooth muscle actin (SMA) and the ectodermal marker β-III tubulin (TUJI) confirmed the ability of the iPSCs to differentiate into all three germ layers (Fig. 1F).

Materials and methods

Reprogramming of fibroblasts and establishment of iPSC lines

A skin biopsy was obtained from a 46-year-old man carrying a M146I mutation in exon 5 of presenilin 1 (*PSEN1*) gene. The biopsies were cut into small pieces and left undisturbed in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% of penicillin and streptomycin (Pen/Strep) for 10 days to allow fibroblasts to grow out from the biopsy. After 10 days, media was changed every 2–3 days to expand culture before fibroblasts were frozen for storage. 1×10^5 fibroblasts were electroporated with a total of 1 μg carrying the sequences for *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC* and *hLIN28* with or without a short hairpin against TP53 (*shp53*) (Addgene, Ca, USA plasmids 27,076, 27,077, 27,078 and 27,080; Okita et al., 2011) and cultured in fibroblast medium. Electroporation was performed with Neon™ electroporation device with two pulses at 1200 V for 20 ms (Life Technologies, Carlsbad, CA, USA). 7 days after electroporation, the fibroblasts were trypsinized and split 1:2 onto hESC-qualified Matrigel-coated dishes (BD Biosciences, NJ, USA) and cultured in E8 medium (Life Technologies) in 5% O_2, 5% CO_2 in N_2 with the medium replenished every other day. After 24 days, primary iPSC colonies were dissected out manually and transferred to new Matrigel-coated 6-well dishes and cultured in E8. The iPSC lines were split 1:6 every 5–6 days with dispase (Stem Cell Technologies). At passage 10, the iPSC lines were harvested for subsequent analyses or frozen in E8 medium with 10% DMSO (Sigma-Aldrich, MO, USA) in liquid nitrogen.

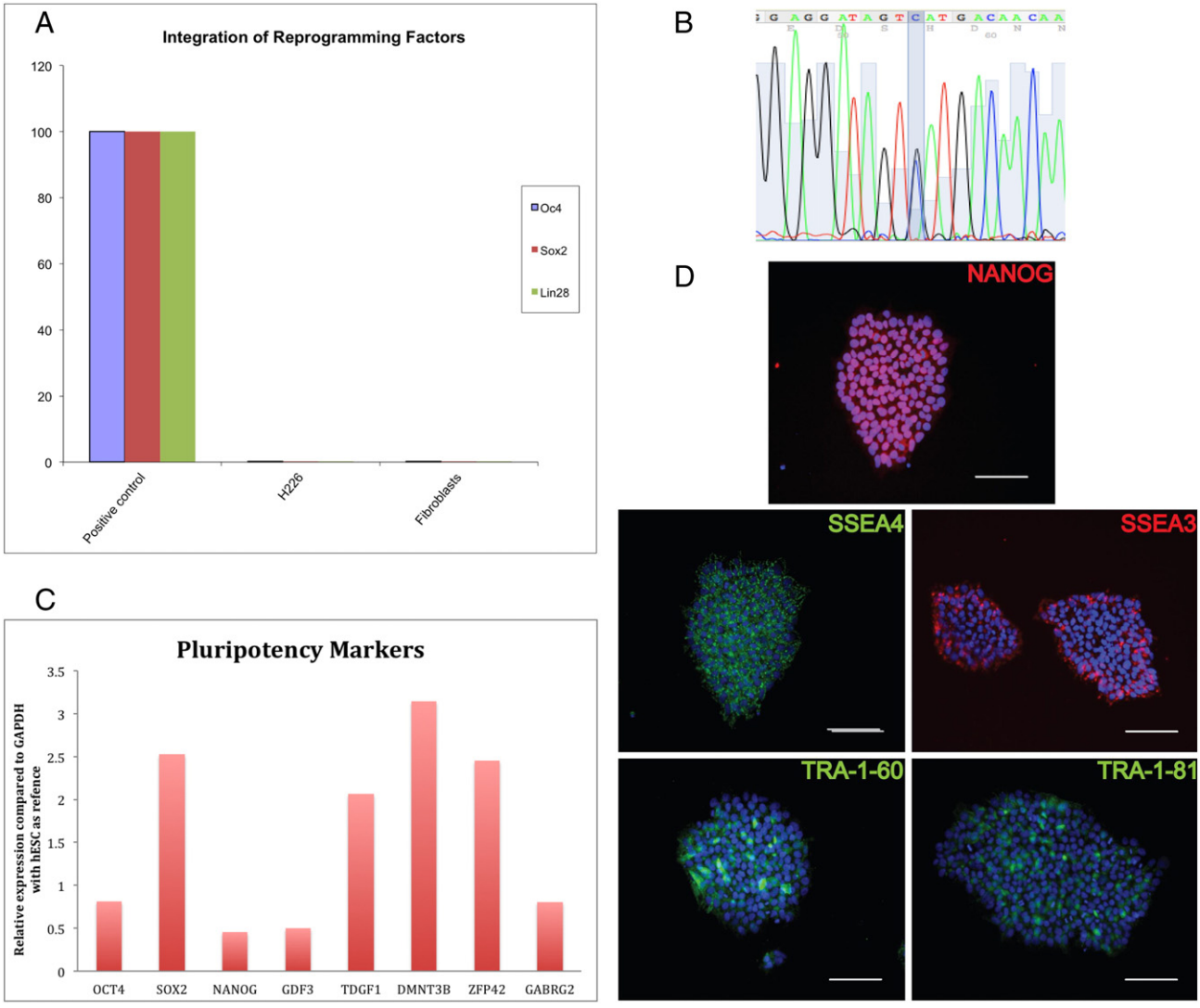

Fig. 1. A. Quantitative PCR (qPCR) with DNA from fibroblasts and M146I-iPSCs with plasmid-specific primers for *hOCT4*, *hSOX2* and *hLIN28*. Relative expression is shown as the fold change with *GAPDH* as reference, fibroblasts as negative control and a fibroblast line with all factors genomically integrated as positive control. B. Sequencing of exon 5 of the *PSEN1* gene in iPSCs showing a heterozygous c.438 G > C substitution. C. Quantitative reverse-transcriptase PCR (qRT-PCR) with cDNA from M146I-iPSCs *vs.* hESCs with the endogenous pluripotency genes *OCT4*, *DMNT3B*, *GABRG3*, *NANOG*, *TDGF1*, *GDF3*, *SOX2 and ZFP42* normalized to *GAPDH* and hESCs. Expression levels of pluripotency markers are comparable to the gene expression in hESCs (hESCs = 1). D. Immunocytochemical detection in iPSCS of the pluripotency markers, NANOG, SSEA3, SSEA4, TRA1-60 and TRA1-81. Scale bars correspond to 100 μm.

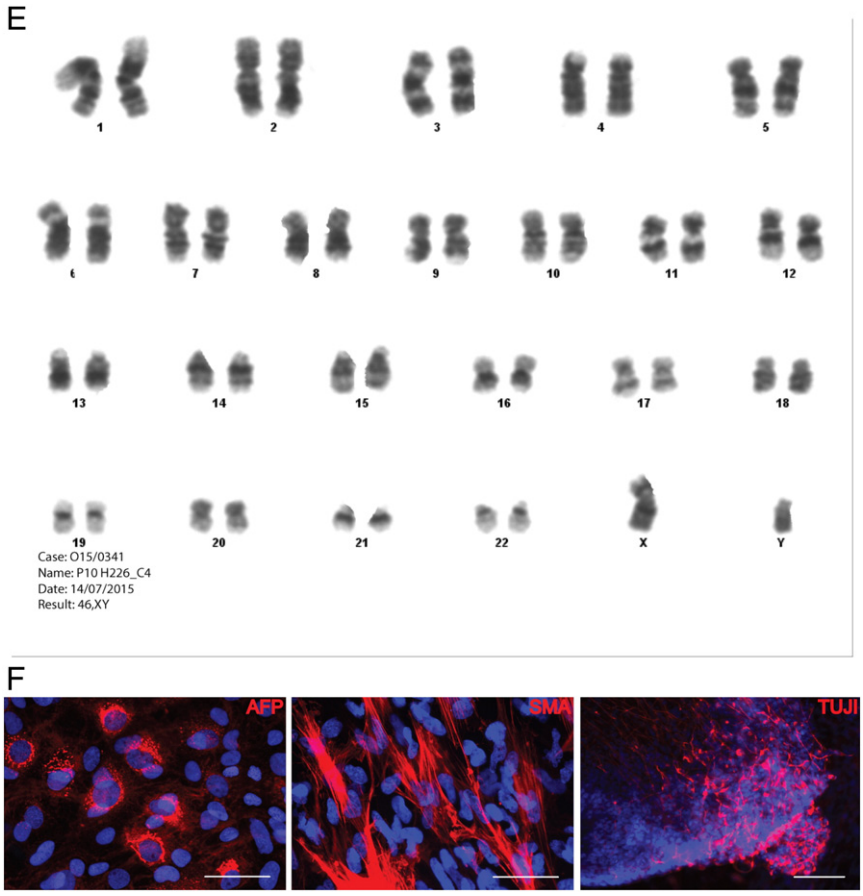

Fig. 1 (*continued*).

Quantitative polymerase chain reaction (qPCR).

DNA and total RNA was purified from fibroblasts, hESCs and M146I-iPSCs using DNeasy Blood and Tissue kit and RNeasy mini kit, respectively (Qiagen, Hilden, Germany). Conversion to cDNA was performed with RevertAid First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA). Primers for integration qPCR were all from Okita et al., 2011 (Table 2). For plasmid integration analysis, relative quantification was calculated using $2^{-\Delta\Delta Ct}$ normalized to *GAPDH* as reference gene. As controls, fibroblasts were used with and without integration. Several primers were designed for pluripotency markers expression (Table 3) and relative quantification was calculated using $2^{-\Delta\Delta Ct}$ with hESCs as reference and *GAPDH* as reference gene.

Table 1
Primary antibodies used for immunocytochemistry.

	Antibody and host species	Dilution	Company and catalog number
Pluripotency	rabbit anti-NANOG	1:500	Peprotech, 500-P236
	goat anti-OCT4	1:500	Santa Cruz, sc-8628
	rat anti-SSEA3	1:100	Biolegend, 330,302
	mouse anti-SSEA4	1:100	Biolegend, 330,402
	mouse anti-Tra-1-60	1:200	Biolegend, 330,602
	mouse anti-Tra-1-81	1:200	Biolegend, 330,702
In vitro differentiation	rabbit anti-α-1-fetoprotein (AFP)	1:500	DAKO, A0008
	mouse anti-smooth muscle actin (SMA)	1:500	DAKO, M0851
	mouse anti-β-III tubulin (TUJI)	1:4000	Sigma-Aldrich, T8660

Sequencing

Sanger sequencing of exon 5 of *PSEN1* gene was carried out on an ABI PRISM 310 Genetic Analyzer using a designed set of primers that flank the mutation site according to standard procedures.

Immunofluorescence staining

iPSCs were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min and stained by standard immunofluorescence staining procedures. The primary antibodies (Table 1) were visualized with the secondary antibodies Alexa488 or Alexa594 diluted 1:400 (Life technologies) and counterstained with Hoechst bisbenzimide 33,258. Images were acquired with a Leica DMRB-fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

In vitro differentiation

Embryoid body (EB) formation was performed by transferring EDTA-treated iPSCs to low attachment plates (Corning, NY, USA) in E8.

Table 2
Primers (Okita et al., 2011) used for integration analysis by qPCR.

	Forward sequence	Reverse sequence
OCT4	5′-CATTCAAACTGAGGTAAGGG-3′	5′-TAGCGTAAAAGGAGCAACATAG-3′
SOX2	5′-TTCACATGTCCCAGCACTACCA GA-3′	5′-TTTGTTTGACAGGAGCGACAAT-3′
L-MYC	5′-GGCTGAGAAGAGGATGGC TAC-3′	5′-CAGGGGGTCTGCTCGCACCGTG ATG-3′

Table 3
Primers used for pluripotency analysis by qPCR.

	Forward sequence	Reverse sequence
OCT4	5′-CCCCAGGGCCCCATTTTGGTACC-3′	5′-ACCTCAGTTTGAATGCATGGGAGAGC-3′
NANOG	5′-AAAGAATCTTCACCTATGCC-3′	5′-GAAGGAAGAGGAGAGACAGT-3′
SOX2	5′-TTCACATGTCCAGCACTACCAGA-3′	5′-TCACATGTGTGAGAGGGGCAGTGTGC-3′
DMNT3B	5′-GTGAAGGGGAGGATGGAGAT-3′	5′-TCTGTGAGGTCGATGGTGAG-3′
GABRG3	5′-ACAGCATTGGTCCTGTGTCA-3′	5′-GTGAGCCTCTGCGGTTTTAG-3′
TDGF1	5′-ACAGAACCTGCTGCCTGAAT-3′	5′-ATCACAGCCGGGTAGAAATG-3′
GDF3	5′-TGACCATCTCCCTCAACAGC-3′	5′-TACCCACACCCACACTCATC-3′
ZFP42	5′-CACTGTGCTGCCTCCAAG-3′	5′-GCCTATGACTCACTTCCA-3′

5′-ACAGCATTGGTCCTGTGTCA-3′.

After 2 days of culture, the medium was changed to DMEM/F12 containing 20% knockout serum replacement (Life Technologies), 1× non-essential amino acids, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol and 1% PenStrep. After 7 days, the EBs were plated on 0.5 μg/cm^2 vitronectin (Life Technologies) coated dishes and cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1% Pen/Strep for up to three weeks. EBs were fixed for 15 min in 4% PFA for ICC analyses with the antibodies AFP, SMA and TUJI (Table 1).

Karyotyping

Fibroblasts and M146I-hiPSCs were treated for 45 min with KaryoMAX colcemid (Life Technologies), harvested in fresh fixative consisting of 25% acetic acid and 75% methanol and sent for G-band karyotyping (Cell Guidance Systems, UK).

Verification and Authentication

Karyotyping was performed by Cell Guidance Systems (UK) and a minimum of 20 metaphases were analyzed. The results showed a normal 46, XY karyotype, without any detectable abnormalities (Fig. 1E). M146I-iPSC line identity and purity was confirmed by sequencing of *PSEN1* (Fig. 1B) and ICC for pluripotency genes expression (Fig. 1D).

E. Representative karyotype of M146I-iPSCs.

F. Immunocytochemical staining of plated embryoid bodies (EBs) on day 28 with α-fetoprotein (AFP), smooth muscle actin (SMA) and β-III tubulin (TUJI). Scale bars correspond to 50 μm in AFP and SMA and to 100 μm in TUJI.

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