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Lab Resource: Stem Cell Line

Induced pluripotent stem cells (iPSCs) derived from a patient with frontotemporal dementia caused by a P301L mutation in microtubule-associated protein tau (MAPT)

Mikkel A. Rasmussen [a,*], Lena E. Hjermind [b], Lis F. Hasholt [c], Gunhild Waldemar [b], Jørgen E. Nielsen [b], Christian Clausen [a], Poul Hyttel [d], Bjørn Holst [a]

[a] Bioneer A/S, Kogle Alle 2, 2970 Hoersholm, Denmark
[b] Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark
[c] Institute of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3B, 2200 N, Denmark
[d] Department of Veterinary Clinical and Animal Sciences, Section for Anatomy & Biochemistry, University of Copenhagen, Groennegårdsvej 7, 1870 Frb C, Denmark

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ABSTRACT

Skin fibroblasts were obtained from a 57-year-old woman diagnosed with frontotemporal dementia. The disease is caused by a P301L mutation in microtubule-associated protein tau (MAPT). Induced pluripotent stem cells (iPSCs) were established by electroporation with episomal plasmids containing *hOCT4*, *hSOX2*, *hKLF2*, *hL-MYC*, *hLIN-28* and *shP53*. iPSCs were free of genomically integrated reprogramming genes, contained the expected c.902C>T substitution in exon 10 of the *MAPT* gene, expressed the expected pluripotency markers, displayed *in vitro* differentiation potential to the three germ layers and had normal karyotype. The iPSC line may be useful for studying hereditary frontotemporal dementia and TAU pathology *in vitro*.

Resource table

Name of stem cell construct	H251 C3
Institution	Bioneer A/S
Person who created resource	Mikkel Aabech Rasmussen, Bjørn Holst
Contact person and email	Bjørn Holst, bho@bioneer.dk
Date archived/stock date	July 1, 2012
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from a MAPT P301L mutation carrier
Sub-type	Induced pluripotent stem cell
Key transcription factors	Episomal plasmids containing *hOCT4*, *hSOX2*, *hL-MYC*, *hKLF4*, *hLIN28* and *shP53* (Addgene plasmids 27077, 27078 and 27080; Okita et al. 2011)
Authentication	Identity and purity of cell line confirmed by integration analysis, sequencing of mutation, pluripotency analysis, karyotyping and in vitro differentiation (Figure 1).
Link to related literature (direct URL links and full references)	http://brain.oxfordjournals.org/content/138/11/3345.long
Information in public databases	Link to any data or information about this resource in a database if applicable

Resource details

Fibroblasts were obtained from a 57-year old woman diagnosed with frontotemporal dementia and heterozygous for a P301L mutation in microtubule-associated protein tau (MAPT). iPSC-derived neurons from a patient with a P301L mutation were recently shown to undergo faster maturation and display altered mitochondrial transport and contorted processes compared with controls (Iovino et al. 2015). Reprogramming was performed by electroporation with three episomal plasmids containing *hOCT4* with or without a short hairpin to TP53 (*shp53*), *hSOX2* and *hKLF4*, and *hL-MYC* and *hLIN28* (Okita et al. 2011). This method had previously been used to establish integration-free iPSC from an 18-year old healthy male (Rasmussen et al. 2014). Four weeks after reprogramming, an average of 20 colonies per 1×10^5 fibroblasts (0.02%) emerged with the inclusion of *shp53*, whereas, no colonies were observed without *shp53*. Integration analysis with plasmid-specific primers showed that *hOCT4*, *hSOX2* and *hLIN28*, present on each of the three plasmids, had not integrated into the genome (Fig. 1A) and sequencing confirmed the presence of a c.902C>T substitution in one of the alleles of exon 10 in the *MAPT* gene corresponding to a P301L mutation (Fig. 1B). Pluripotency analysis showed that transcription from the endogenous pluripotency genes *NANOG*, *POU5F1* (*OCT4*), *TDGF1*, *DNMT3B*, *GABRB3* and *GDF3* were between 100 and 10,000 times upregulated compared with fibroblasts (Fig. 1C) and

* Corresponding author. Tel.: +45 45160463.
E-mail address: mar@bioneer.dk (M.A. Rasmussen).

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immunocytochemical (ICC) analysis demonstrated the presence of the pluripotency markers OCT4, NANOG, TRA1-60, TRA1-81, SSEA3 and SSEA4 at the protein level (Fig. 1D). Finally, *in vitro* differentiation followed by ICC analysis with the mesodermal marker smooth muscle actin (SMA), the endodermal marker alpha-feto protein (AFP) and the ectodermal marker beta-III-Tubulin (TUJI) demonstrated the differentiation potential into all three germ layers (Fig. 1E).

Materials and methods

Unless otherwise stated, consumables and reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Establishment of iPSC

A skin biopsy was obtained from a 57-year old woman carrying a P301L mutation in MAPT. The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (RH), and written informed consent was obtained from the donor. Fibroblasts were cultured in fibroblast medium, consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Pen/Strep). 1×10^5 fibroblasts were electroporated with a total of 1 μg of episomal plasmids containing *hOCT4* with or without a short hairpin to TP53 (*shp53*; Addgene plasmids 27077 and 27076, respectively), *hSOX2* and *hKLF4* (Addgene plasmid 27078), and *hL-MYC* and *hLIN28* (Addgene plasmid 27,080; Okita et al. 2011) and cultured in fibroblast medium.

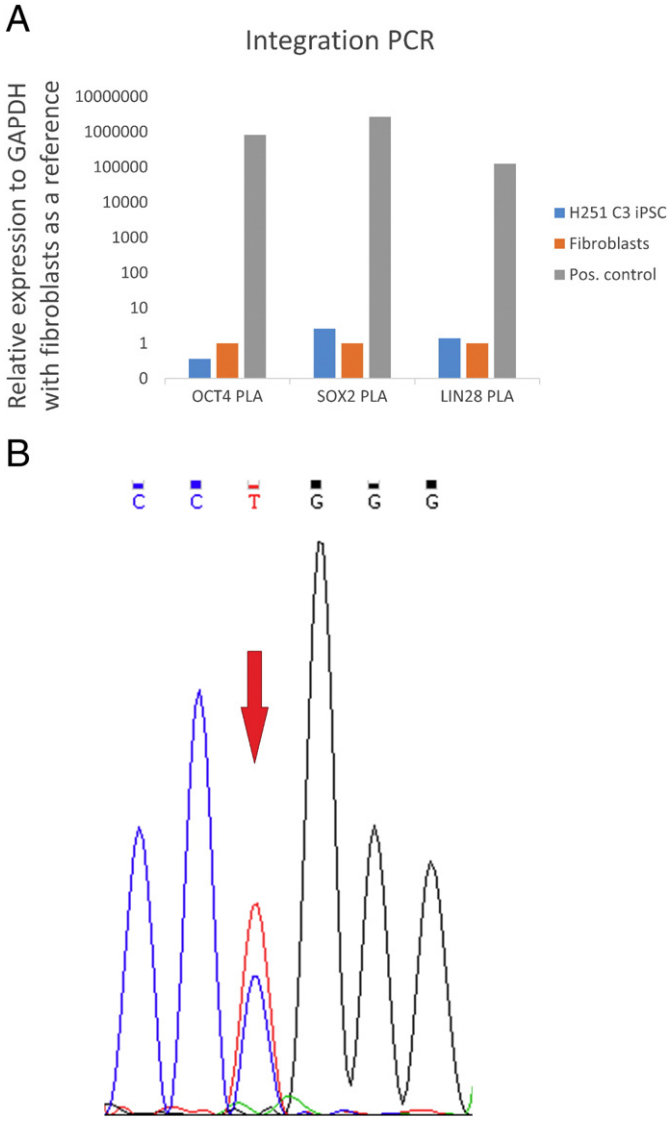

Fig. 1. A. Integration analysis. Quantitative PCR (qPCR) on genomic DNA from H251 C3 induced pluripotent stem cells (iPSCs), fibroblasts and a pool of fibroblasts electroporated with episomal plasmids (positive control) with plasmid-specific primers of *hOCT4*, *hSOX2*, and *hLIN28*. Data is shown as the fold change ($2^{-\Delta\Delta Ct}$) with *GAPDH* and fibroblasts as references. B. Sequencing of mutation. Sequencing of exon 13 of the *MAPT* gene in H251 C3 induced pluripotent stem cells showing a c.1216C>T substitution in one of the alleles marked with a red arrow. C. Pluripotency expression analysis. Quantitative reverse-transcriptase PCR (qRT-PCR) expression analyses on cDNA from H251 C3 induced pluripotent stem cells (iPSCs), fibroblasts and the iPSC line BIONi010-A (Rasmussen et al. 2014) as a positive control with the endogenous pluripotency genes *NANOG*, *POU5F1* (*OCT4*), *TDGF1*, *DNMT3B*, *GABRB3* and *GDF3*. Relative expression is shown as the fold change ($2^{-\Delta\Delta Ct}$) with *GAPDH* and fibroblasts as references. D. Immunofluorescence staining. Immunocytochemical detection of H251 C3 induced pluripotent stem cells with the pluripotency markers OCT3/4, TRA1-81, NANOG, TRA1-60, SSEA3, and SSEA4. Scale bars correspond to 400 μm. E. *In vitro* differentiation. Immunocytochemical staining of plated embryoid bodies (EBs) from H251 C3 induced pluripotent stem cells on day 28 with smooth muscle actin (SMA), alpha-fetoprotein (AFP) and betaIIItubulin (TUJI). Scale bars correspond to 100 μm. F. Karyotyping. Representative karyotype of H251 C3 induced pluripotent stem cells.