Stem Cell Research 16 (2016) 105–109

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem cell line

Induced pluripotent stem cells (iPSCs) derived from a pre-symptomatic carrier of a R406W mutation in microtubule-associated protein tau (MAPT) causing frontotemporal dementia

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ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form 9 December 2015
Accepted 18 December 2015
Available online 23 December 2015

ABSTRACT

Skin fibroblasts were obtained from a 28-year-old pre-symptomatic woman carrying a R406W mutation in microtubule-associated protein tau (MAPT), known to cause frontotemporal dementia. Induced pluripotent stem cell (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.1216C>T substitution in exon 13 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.

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Resource table:

Name of stem cell construct	H236 C6
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 R406W 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 (Fig. 1).
Link to related literature (direct URL links and full references)	http://onlinelibrary.wiley.com/doi/10.1111/j.1468-1331.2008.02069.x/abstract;jsessionid=175DB65708CDD60137A0E1D39E93D3D6.f04t01 The MAPT R406W carrier is a pre-symptomatic daughter of a patient diagnosed with frontotemporal dementia which is also heterozygous for the MAPT R406W mutation http://www.sciencedirect.com/science/article/pii/S1873506115001865
Information in public databases	Link to any data or information about this resource in a database if applicable

1. Resource details

Fibroblasts were obtained from a 28-year old woman heterozygous for a R406W mutation in microtubule-associated protein tau (MAPT), which can cause frontotemporal dementia. However, the woman was pre-symptomatic at the time of biopsy (Lindquist et al., 2008). 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

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http://dx.doi.org/10.1016/j.scr.2015.12.012
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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 32 colonies per 1×10^5 fibroblasts (0.03%) 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.1216C>T substitution in one of the alleles of exon 13 in the *MAPT* gene corresponding to a R406W 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 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).

2. Materials and methods

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

2.1. Establishment of iPSC

A skin biopsy was obtained from a 28-year old woman carrying a R406W 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

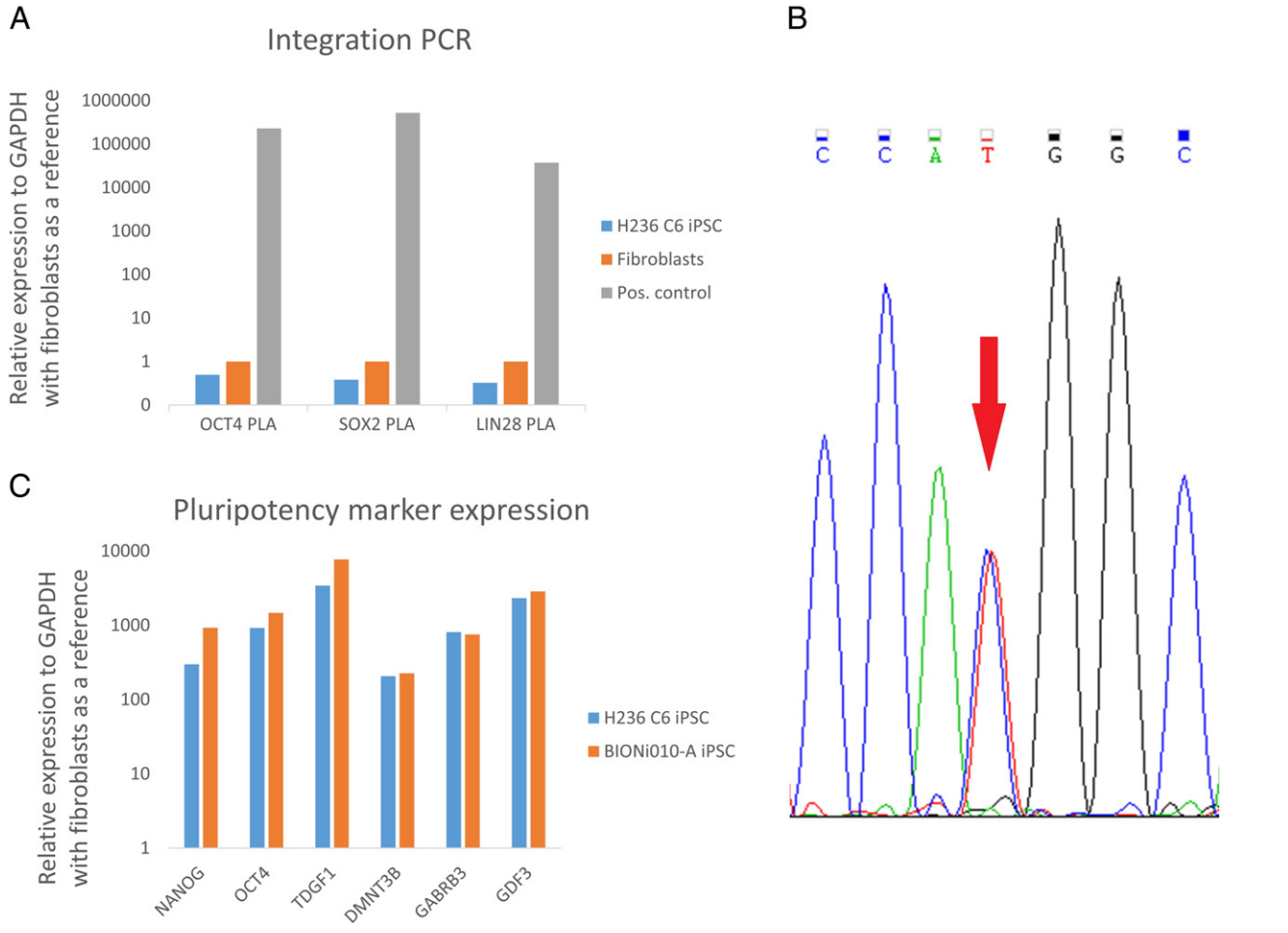

Fig. 1. A. Integration analysis. Quantitative PCR (qPCR) on genomic DNA from H236 C6 induced pluripotent stem cells (iPSC), fibroblasts and a pool of fibroblasts electroporated with episomal plasmids (positive control) with plasmid-specific primers of *hOCT4*, *hSOX2*, and *hLIN28*. Data is shown as 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 H236 C6 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 H236 C6 induced pluripotent stem cells (iPSC), 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-ΔΔCt) with *GAPDH* and fibroblasts as references. D. Immunofluorescence staining. Immunocytochemical detection of H236 C6 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 H236 C6 induced pluripotent stem cells on day 28 with smooth muscle actin (SMA), alpha-fetoprotein (AFP) and betaIII-tubulin (TUJI). Scale bars correspond to 100 μm. F. Karyotyping. Representative karyotype of H236 C6 induced pluripotent stem cells.

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