Stem Cell Research 17 (2016) 285–288

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of a gene-corrected isogenic control cell line from an Alzheimer's disease patient iPSC line carrying a A79V mutation in *PSEN1*

Carlota Pires [a], Benjamin Schmid [b], Carina Petræus [a], Anna Poon [a], Natakarn Nimsanor [c], Troels T. Nielsen [d], Gunhild Waldemar [d], Lena E. Hjermind [d], Jørgen E. Nielsen [d], Poul Hyttel [a], Kristine K. Freude [a,*]

[a] Faculty of Health and Medical Sciences, University of Copenhagen, Gronnegaardsvej 7, 1870 Frederiksberg C, Denmark
[b] Bioneer A/S, Hoersholm, Denmark
[c] Faculty of Medical Technology, Mahidol University, Bangkok, Thailand
[d] Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark

ARTICLE INFO

Article history:
Received 15 July 2016
Accepted 3 August 2016
Available online 07 August 2016

ABSTRACT

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease causing neural cell degeneration and brain atrophy and is considered to be the most common form of dementia. We previously generated an induced pluripotent stem cell (iPSC) line from an AD patient carrying an A79V mutation in *PSEN1* as an *in vitro* disease model. Here we generated a gene-corrected version from this hiPSC line by substituting the point mutation with the wild-type sequence. The reported A79V-GC-iPSCs line is a very useful resource in combination with the A79V-iPSC line in order to study pathological cellular phenotypes related to this particular mutation.

Resource table

Name of stem cell construct	A79V-GC-hiPSC
Institution	University of Copenhagen
Person who created resource	Carlota Pires, Kristine K Freude
Contact person and email	Kristine K Freude: kkf@sund.ku.dk
Date archived/stock date	February 2016
Origin	Human induced pluripotent cell line (hiPSCs)
Type of resource	Biological reagent: gene-corrected induced pluripotent stem cells, originally derived from skin fibroblasts of a *PSEN1* A79V 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 27077, 27078 and 27080; Okita et al., 2011)
Authentication	Identity and purity of cell line confirmed by mutation sequencing, karyotyping and pluripotency markers (Fig. 1)
Link to related literature (direct URL links and full references)	http://dx.doi.org/10.1016/j.scr.2016.01.002 Li T, Pires C, Nielsen TT, Waldemar G, Hjermind LE, Nielsen JE, Dinnyes A, Hyttel P, Freude KK. Generation of induced pluripotent stem cells (iPSCs) from an Alzheimer's disease patient carrying an A79V mutation in PSEN1, Stem Cell Research. 2016; 16: 229–232
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 the patient family privacy, no personal patient information is presented here. Mutations in presenilin 1 (*PSEN1*) gene are the most common known causes of inherited Alzheimer's disease (AD).

A human induced pluripotent stem cell (hiPSC) line was previously generated from a skin biopsy obtained from a 48-year-old woman carrying a heterozygous mutation in exon 4 of the *PSEN1* gene, which causes a change in amino acid A79V. 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, successfully establishing integration and feeder-free iPSCs. This cell line has been described previously as a *bona fide* iPSC line with normal karyotype (Li et al., 2016).

The A79V-hiPSC (c.236 C > T) was gene-corrected with the CRISPR/Cas9 system (Ran et al., 2013) by replacing the point mutation "T" with the wild-type nucleotide "C" (Fig. 1A). The nucleotide substitution/correction was validated by sequencing (Fig. 1B). Gene-integrity was also checked by confirming that the DNA sequence surrounding the mutation and the CRISPR cutting site were not altered, with no frameshifts or other mutation(s) being generated upon gene editing, confirming successful gene correction of A79V-hiPSC, named A79V-GC-hiPSC ("GC" = gene corrected). The sequencing of the isogenic line confirmed the c.236 T > C replacement in exon 4 of the *PSEN1*

DOI of original article: http://dx.doi.org/10.1016/j.scr.2016.01.002.
* Corresponding author.
E-mail address: kkf@sund.ku.dk (K.K. Freude).

http://dx.doi.org/10.1016/j.scr.2016.08.002

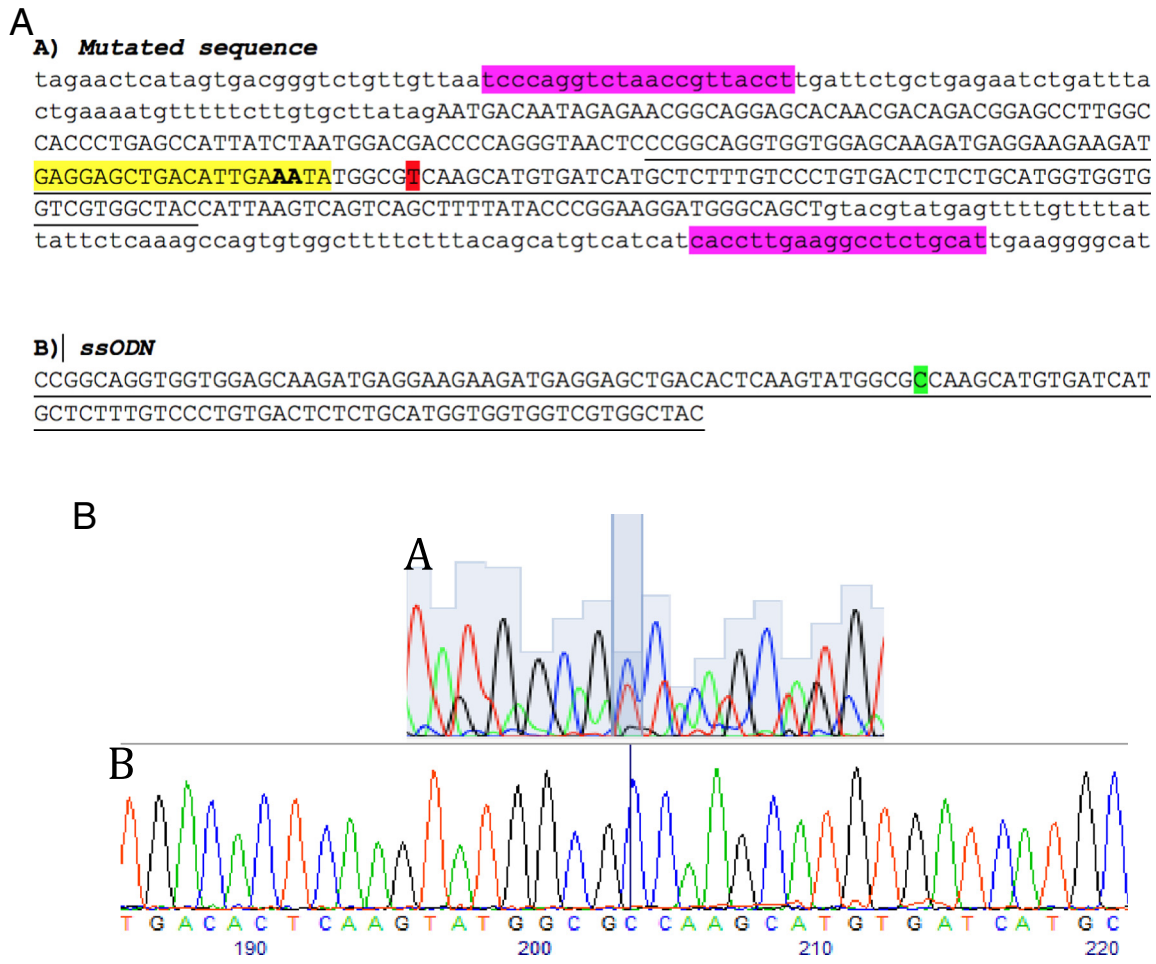

Fig. 1. A. CRISPR/Cas9 and ssODN used to repair the point mutation in A79V-hiPSC. A) Genomic sequence surrounding the mutation site: mutated nucleotide (T, red); sgRNA recognition site containing 20 bp (yellow); CRISPR cutting site between the 17th and 18th bp (bold); forward and reverse primers (pink). B) ssODN with 120 bp, 60 bp upstream and 60 bp downstream the mutation site containing the WT nucleotide (C, green). B. Sequencing of exon 4 of the *PSEN1* gene in hiPSCs. A) Heterozygous c.236C > T substitution in the mother line previously published. B) Successful correction of the point mutation (WT nucleotide – C; Mutated nucleotide – T). C. Immunocytochemical detection in hiPSCs of the pluripotency markers OCT4, NANOG, SSEA3, SSEA4, TRA1-60 and TRA1-81. Scale bars correspond to 200 μm. D. Representative karyotype of A79V-GC-iPSCs.

gene corresponding to the wild-type (healthy) sequence (Fig. 1B). Immunocytochemical (ICC) analysis of the pluripotency markers OCT4, NANOG, SSEA3, SSEA4, TRA-1-60 and TRA-1-81 (Fig. 1C) confirmed the gene-edited line maintained its pluripotency like the mother line previously published. Finally, karyotyping was performed to confirm that no chromosomal aberrations were introduced in the isogenic line upon gene correction (Fig. 1D).

Materials and methods

CRISPR design

Isogenic gene-corrected controls were obtained using the CRISPR/Cas9 technology in combination with single-stranded oligonucleotides (ssODNs), which provided the wild-type template for gene-correction.

CRISPRs were designed with the help of the software available at http://crispr.mit.edu/. The single-guide RNAs (sgRNA) were ordered from Sigma and the cloning of CRISPRs followed the protocol from Ran et al. (2013), with a single plasmid containing both the sgRNA and the Cas9 (pSpCas9(BB)-2A-Puro (PX459) V2.0; addgene ID 48139).

ssODNs were designed to contain 120 bp in total, with 60 bp upstream and 60 bp downstream of the cutting site. Screening of clones was performed with restriction digestions where the wild-type sequence was recognized (and cut) by specific restriction enzymes (EheI, 5′-GGC↓GCC-3′, ThermoScientific), while the mutated sequence was not, which means EheI only digested clones where the ssODN was successfully integrated.

Nucleofection

A79V-hiPSCs growing on matrigel-coated (Corning Bioscience) dishes with E8 medium (Gibco) were detached with Accutase (Gibco). 2×10^6 cells were nucleofected with 10 μg of CRISPR/Cas9 plasmid and 100 pmol of ssODN using the Amaxa 4D Nucleofector (program CA137, Lonza) and P3 Primary Cell Kit (Lonza) according to the manufacturer's instructions. Nucleofected hiPSCs were plated back in a matrigel-coated dish with E8 medium supplemented with 1 mM ROCK inhibitor (Sigma). hiPSCs were subjected to puromycin selection 4 h after nucleofection and allowed to recover for 4–5 days. Surviving (resistant) colonies were manually picked into 96 well plates coated with matrigel with E8 and then expanded for genotyping and sequencing.

Genotyping and restriction assays

DNA for genotyping was extracted using the prepGem™ Kit (ZyGEM, PTI0500) (Qiagen). PCR genotyping was performed using TEMPase Hot Start DNA Polymerase (Ampliqon) according to the manufacturer's instructions, using an annealing temperature of 60 °C. The following primers were designed to cover the mutation and