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Published in:
Stem Cell Research

DOI:
10.1016/j.scr.2015.12.031

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Stem Cell Research 16 (2016) 162–165

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem cell line

Generation of an isogenic, gene-corrected control cell line of the spinocerebellar ataxia type 2 patient-derived iPSC line H196

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ARTICLE INFO

Article history:
Received 24 December 2015
Accepted 29 December 2015
Available online 3 January 2016

ABSTRACT

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease primarily affecting the cerebellum. Very little is known about the molecular mechanisms underlying the disease and, to date, no cure or treatment is available. We have successfully generated *bona fide* induced pluripotent stem cell (iPSC) lines of SCA2 patients in order to study a disease-specific phenotype. Here, we demonstrate the gene correction of the iPSC line H196 clone 7 where we have exchanged the expanded CAG repeat of the *ATXN2* gene with the normal length found in healthy alleles. This gene corrected cell line will provide the ideal control to model SCA2 by iPSC technology.

1. Resource table

Name of stem cell construct	H196 clone7 GC
Institution	University of Copenhagen and Bioneer A/S
Person who created resource	Adele G. Marthaler, Benjamin Schmid
Contact person and email	Adele G. Marthaler, adele.marthaler@sund.ku.dk
Date archived/stock date	July 2015
Origin	Human induced pluripotent stem cell line H196 clone 7
Type of resource	Gene-corrected induced pluripotent stem cells; originally derived from skin fibroblasts of patient with spinocerebellar ataxia type 2
Sub-type	Cell line
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 stem cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	
Information in public databases	

2. Resource details

An induced pluripoten stem cell (iPSC) line had been generated from human skin fibroblasts of a male, symptomatic 52-year-old spinocerebellar type 2 (SCA2) patient (anonymized as H196) using episomal vectors carrying transcripts for human *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, and small hairpin RNA for *TP53* (Okita et al. 2011). This cell line, H196 clone (c) 7, has been described as a *bona fide* iPSC line with a normal karyotype (Marthaler et al., submitted to Stem Cell Research).

We have generated a gene-corrected clone of H196 c7 using the CRISPRs/Cas9 system (Ran et al. 2013), where the expanded 36 CAG region in the *ATXN2* gene has been replaced with a wildtype 22 CAG repeat (Fig. 1A). Successful exchange was validated by sequencing (Fig. 1B). We have furthermore confirmed that the DNA sequence stayed intact and no frameshift or other mutation had been introduced into the gene edited site, by analyzing the region around the CRISPR cutting site (nucleotide 119–141 in Fig. 1A).

Subsequently, we confirmed that the gene corrected clone of H196 c7, termed H196 c7 GC, remained truly pluripotent. This was demonstrated by expression of key pluripotency markers on RNA, as well as protein level (Fig. 1C and D). Additionally, H196 c7 GC retained the potential to differentiate into cell types of the three germ layers upon embryoid body formation (Fig. 1E). More importantly, no genetic chromosomal aberrations were introduced by the gene editing process and the cells still exhibit a normal karyotype (Fig. 1F).

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http://dx.doi.org/10.1016/j.scr.2015.12.031

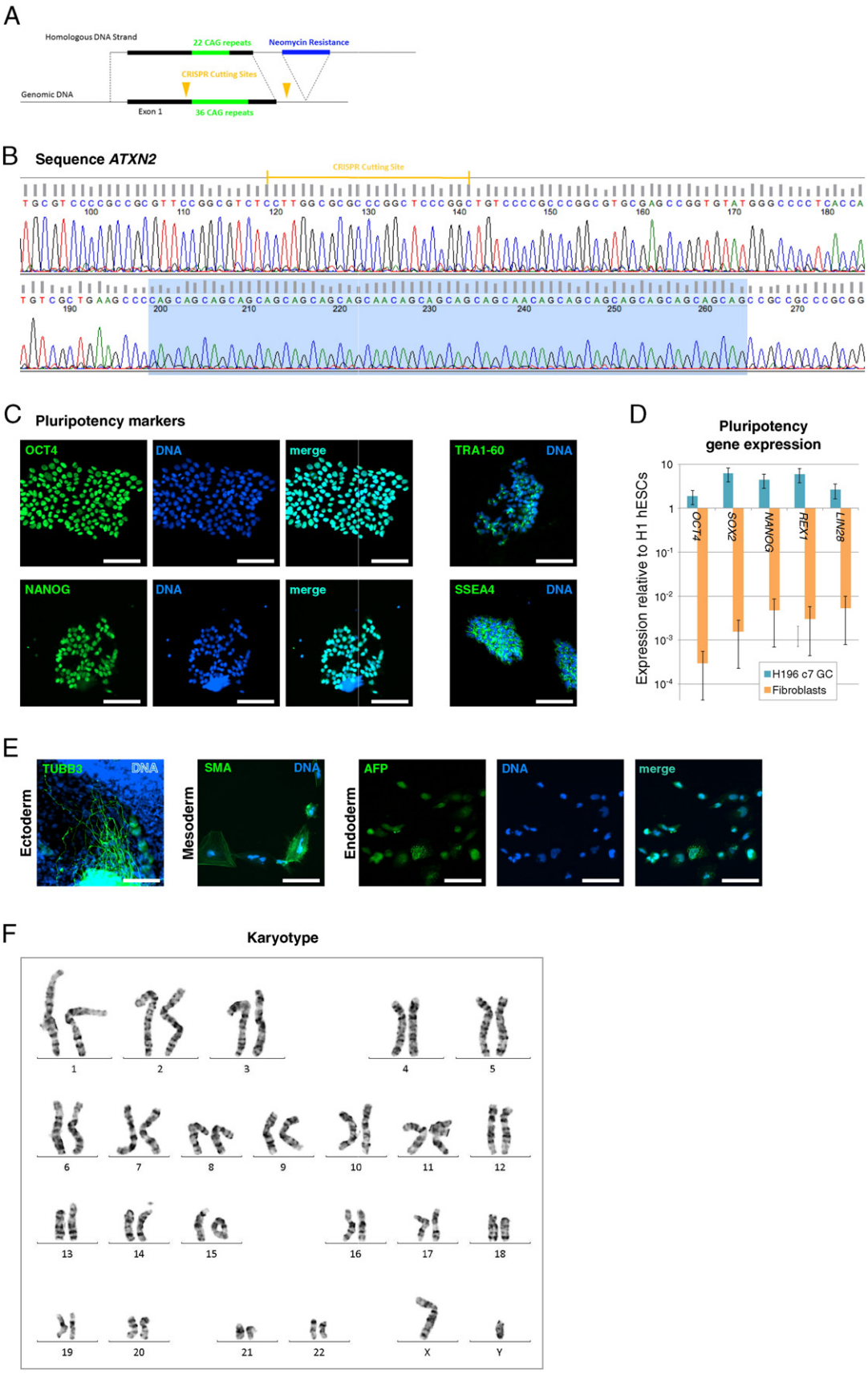

Fig. 1. (*caption on page 164*).

In summary, we have generated an isogenic, gene-corrected iPSC line of an existing SCA2 iPSC line. Together with two more SCA2 patient-derived iPSC lines and their corresponding isogenic, gene-corrected controls (Marthaler et al., submitted to Stem Cell Research), they will serve as an ideal study tool for *in vitro* disease modeling of SCA2.

3. Materials and methods

3.1. CRISPR design

Isogenic gene-corrected controls were obtained using the CRISPRs/Cas9 system in combination with a homologous construct in the pEasy Flox II vector. CRISPRs targeting each site of the CAG repeat of the *ATXN2* locus were designed at http://crispr.mit.edu/. The CRISPRs were generated following the protocol from Ran et al. (Ran et al. 2013) in a single plasmid containing both sgRNA and the Cas9 (Addgene plasmid ID 48139).

3.2. Nucleofection

iPSCs growing on dishes coated with matrigel (Corning Bioscience) in E8 medium (Gibco) were detached using Accutase (Gibco). 10^6 cells were co-nucleofected with 2 μg of each CRISPR/Cas9 plasmid and 1 μg of the resistance marker using the Amaxa 4D Nucleofector (program CA167) and P3 Primary Cell Kit according to the manufacturer's instructions (Lonza). iPSCs were subsequently transferred back to a matrigel-coated dish in E8 medium supplemented with 1 mM ROCK inhibitor (Sigma). 24 h post nucleofection, cells were subjected to neomycin selection and allowed to recover for a week. Resistant colonies were then picked and expanded for genotyping.

3.3. Genotyping

DNA for genotyping was extracted using the FlexiGene Kit (Qiagen). PCR genotyping was performed using TEMPase Hot Start DNA Polymerase (Ampliqon) according to the manufacturer's instructions at an annealing temperature of 58 °C. The following primers were designed 300 base pairs up- and downstream of the CRISPR cutting site to ensure detection of insertion at homologous site: SCA2 long1 forward 5′-CAGA CCCGCCTTGAGGAAG-3′ and SCA2 long1 reverse 5′-GAGGAGACCGAG GACGAGG-3′. Clones where the expanded SCA2 allele was successfully replaced with the *ATXN2* wildtype construct were subjected to sequencing to exclude introduction of frameshifts or other mutations.

3.4. Sequencing

Sanger sequencing of a 300 base pair region around the CAG repeat region of the *ATXN2* gene was carried out in an ABI PRISM 310 Genetic Analyzer using the primers SCA2 seq2 forward 5′-CTTGGTCTCGGCGG GC-3′ and SCA2 seq2 reverse 5′-GAGGAGACCGAGGACGAGG-3′.

3.5. RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

RNA was extracted using RNeasy Mini (QIAGEN) and cDNA synthesis was performed using Revert Aid First Strand cDNA synthesis kit (Thermo Scientific) according to the manufacturers' protocols. qRT-PCR was carried out using LightCycler 480 SYBR Green I Master (Roche). Data was plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$. The following primers were used:

OCT4 for	5′-CCCCAGGGCCCCATTTTGGTACC-3′
OCT4 rev	5′-ACCTCAGTTTGAATGCATGGGAGAGC-3′
SOX2 for	5′-TTCACATGTCCAGCACTACCAGA-3′
SOX2 rev	5′-TCACATGTGTGAGAGGGGCAGTGTGC-3′
NANOG for	5′-AAAGAATCTTCACCTATGCC-3′
NANOG rev	5′-GAAGGAAGAGGAGAGACAGT-3′
REX1 for	5′-TTTCTGAGTACGTGCCCAGGCAA-3′
REX1 rev	5′-CTCTGAGAAAGCATCTCTCCTTC-3′
LIN28 for	5′-AGCCATATGGTAGCCTCATGTCCGC-3′
LIN28 rev	5′-TCAATTCTGTGCCTCCGGGAGCAGGGTAGG-3′
ACTB for	5′-TCAAGATCATTGCTCCTCCTGAG-3′
ACTB rev	5′-ACATCTGCTGGAAGGTGGACA-3′
RPL13A for	5′-TTCCAAGCGGCTGCCGAAGA-3′
RPL13A rev	5′-TTCCGGCCCAGCAGTACCTGT-3′
HSP90AB1 for	5′-TCCGGCGCAGTGTTGGGAC-3′
HSP90AB1 rev	5′-TCCATGGTGCACTTCCTCAGGC-3′
GAPDH for	5′-CTGGTAAAGTGGATATTGTTGCCAT-3′
GAPDH rev	5′-TGGAATCATATTGGAACATGTAAACC-3′

3.6. Immunocytochemistry

Immunocytochemistry was performed as previously described (Marthaler et al. 2013). The following primary antibodies were used: Anti-OCT4 (Santa Cruz, sc 8628); anti-NANOG (Peprotech, 500P236); anti-TRA1–60 (BioLegend, 330,602); anti-SSEA4 (BioLegend, 330,402); anti-TUBB3 (Millipore, MAB1637); anti-SMA (Dako, M0851), anti-AFP (Dako, A0008); all 1:500. Secondary antibodies used were: Alexa Fluor 488 donkey anti-rabbit (A21206), donkey anti-goat (A11055), and goat anti-mouse (A11017), all 1:2000 (Invitrogen).

3.7. Embryoid body differentiation

iPSCs growing in E8 medium (Gibco) on matrigel (Corning Bioscience) were dissociated with EDTA (Gibco) and allowed to form aggregates in none-coated cell culture dishes. On day 3, aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium: DMEM/F12 containing 20% FBS, L-glutamine, and non-essential amino acids (all Gibco) for meso- and endoderm induction, or DMEM/F12 containing 50% neurobasal medium, B27, N2, and L-glutamine (all Gibco) for ectoderm induction. Cells were fixed for immunocytochemistry on day 14.

3.8. Verification and authentication

An intact genome was demonstrated by karyotyping using G-banding (Fig. 1F). Analysis was performed at the Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany.

Fig. 1. The gene-corrected H196 c7 GC is a *bona fide* iPSC line with a normal karyotype. (A) Schematic representation of the gene editing strategy using the CRISPR/Cas9 system. (B) Simultaneous sequencing of both *ATXN2* alleles showing 22 CAGs, highlighted in blue. The CRISPR cutting site upstream of the CAG repeat is indicated in yellow. (C) OCT4, NANOG, TRA1–60, and SSEA4 immunofluorescence images of H196 c7 GC, counterstained with Hoechst. Scale bars, 100 μm. (D) Expression levels of pluripotency genes measured by qRT-PCR. Data were plotted relative to H1 human ESCs. Fibroblasts served as a negative control. Error bars indicate standard error of calculations based on ΔΔCt-values obtained from four housekeeping genes, *ACTB*, *RPL37a*, *HSP90AB1*, *and GAPDH*. (E) Immunocytochemistry for marker proteins representative of the three germ layers, TUBB3 (ectoderm), SMA (mesoderm), and AFP (endoderm), after *in vitro* differentiation of H196 c7 GC by embryoid body formation. Scale bars, 100 μm. (F) Chromosome analysis of H196 c7 GC showing a normal 46, XY karyotype.

Acknowledgments

We would like to thank Ida Jørring and Anita Pacht for their excellent technical assistance. This project was funded by the European Union 7th Framework Program (PIAPP-GA-2012-324451-STEMMAD) and Innovation Fund Denmark through BrainStem.

References

Marthaler, A.G., Tiemann, U., Arauzo-Bravo, M.J., Wu, G., Zaehres, H., Hyun, J.K., Han, D.W., Scholer, H.R., Tapia, N., 2013. Reprogramming to pluripotency through a somatic stem cell intermediate. PLoS One 8, e85138.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., et al., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8, 409–412.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8, 2281–2308.