Stem Cell Research 42 (2020) 101701

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem Cell Line

The mRNA-based reprogramming of fibroblasts from a *SOD1*^{E101G} familial amyotrophic lateral sclerosis patient to induced pluripotent stem cell line UOWi007

Rachelle Balez[a,b], Tracey Berg[a,b], Monique Bax[a,b], Sonia Sanz Muñoz[a,b], Mauricio C. Cabral-da-Silva[a,b], Martin Engel[a,b], Dzung Do-Ha[a,b], Claire H. Stevens[a,b], Dominic Rowe[c], Shu Yang[c], Ian P. Blair[c], Lezanne Ooi[a,b,*]

[a] Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia
[b] School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW 2522, Australia
[c] Centre for Motor Neuron Disease Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

A B S T R A C T

Dermal fibroblasts were donated by a 43 year old male patient with clinically diagnosed familial amyotrophic lateral sclerosis (ALS), carrying the *SOD1*^{E101G} mutation. The induced pluripotent stem cell (iPSC) line UOWi007-A was generated using repeated mRNA transfections for pluripotency transcription factors Oct4, Klf4, Sox2, c-Myc, Lin28 and Nanog. The iPSCs carried the *SOD1*^{E101G} genotype and had a normal karyotype, expressed expected pluripotency markers and were capable of *in vitro* differentiation into endodermal, mesodermal and ectodermal lineages. This iPSC line may be useful for investigating familial ALS resulting from a *SOD1* ^{E101G} mutation.

Resource table

Unique stem cell line identifier	UOWi007-A
Alternative name(s) of stem cell line	MQ54-2
Institution	Illawarra Health and Medical Research Institute, University of Wollongong
Contact information of distributor	Lezanne Ooi, lezanne@uow.edu.au
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 43 Sex: Male
Cell Source	Dermal fibroblast
Clonality	Clonal
Method of reprogramming	Transgene free, mRNA based reprogramming with Oct4, Klf4, Sox2, c-Myc, Lin28 and Nanog.
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Amyotrophic lateral sclerosis
Gene/locus	*SOD1*^{E101G}
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	12.04.2018
Cell line repository/bank	N/A
Ethical approval	HE13/272 University of Wollongong Human Research Ethics Committee

1. Resource utility

This iPSC line can be used to investigate familial ALS resulting from a *SOD1*^{E101G} mutation. This resource is available under request owing to the Materials Transfer Agreement in place.

2. Resource details

Dermal fibroblasts were obtained from a 43 year old male patient by Macquarie University, Australia. The patient was clinically diagnosed with familial amyotrophic lateral sclerosis (ALS), heterozygous for the *SOD1*^{E101G} mutation (also termed *SOD1*^{E100G} due to N-terminal methionine cleavage). The *SOD1* gene encodes the cytosolic antioxidant enzyme, copper-zinc superoxide dismutase (SOD1). Mutant SOD1^{E101G} retains the wild-type-like dismutase activity of the enzyme with pathology hypothesized to be due to misfolding and subsequent aggregation of mutant SOD1 (Valentine et al., 2005; Farrawell et al.,

* Corresponding author: Dr Lezanne Ooi, Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia.
E-mail address: lezanne@uow.edu.au (L. Ooi).

https://doi.org/10.1016/j.scr.2020.101701
Received 30 August 2019; Received in revised form 20 December 2019; Accepted 5 January 2020
Available online 16 January 2020

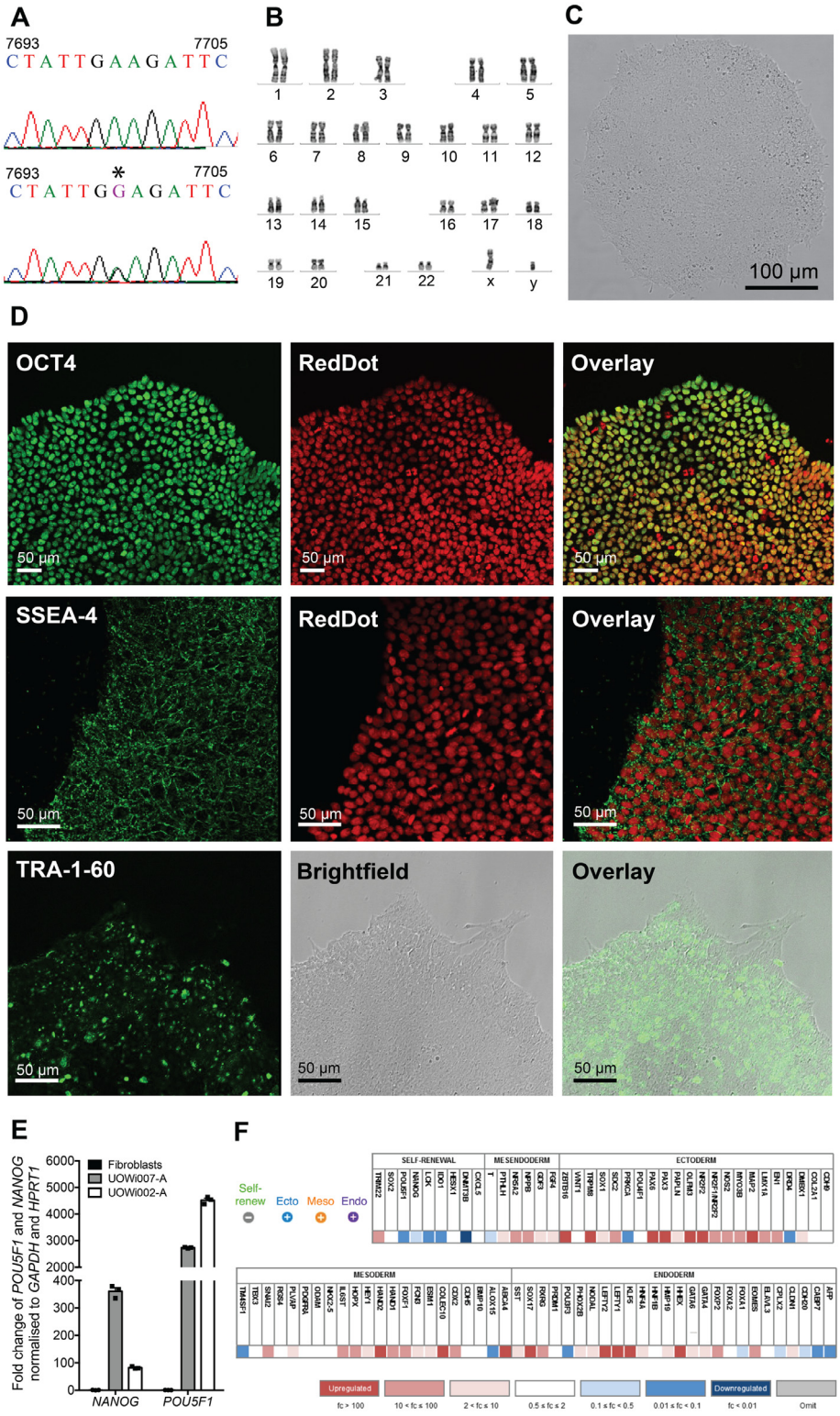

Fig. 1. Characterization of iPSC line UOWi007-A.

2019).

Reprogramming was performed using repeated mRNA transfections for pluripotency transcription factors Oct4, Klf4, Sox2, c-Myc, Lin28 and Nanog. The first iPSC colonies appeared on day 14 and were isolated on day 17 for expansion and characterisation, with clone 2 (UOWi007-A) selected following confirmation of pluripotency. The UOWi007-A line was authenticated against its parental fibroblast line via short tandem repeat (STR) profiling, with sequencing confirming the presence of the *SOD1*^{E101G} mutation (Fig. 1A). The UOWi007-A colonies had a normal karyotype (Fig. 1B), with no abnormalities detected in 15 cells at 400 bands per haploid set and had characteristic stem cell morphology (Fig. 1C). Immunocytochemical analysis confirmed expression of pluripotency markers Oct4, SSEA-4 and TRA-1-60 (Fig. 1D). Transcription of endogenous pluripotency genes *NANOG* and *POU5F1* (Oct4) by quantitative RT-PCR (RT-qPCR) showed a 360 and 2700 fold increase, respectively, in UOWi007-A in comparison to

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1C
Phenotype	Immunocytochemistry	Expression of pluripotency markers Oct4, SSEA-4, TRA-1-60	Fig. 1D
	RT-qPCR	Cells express *POU5F1, NANOG*	Fig. 1E
Genotype	Karyotype (G-banding) and resolution	46XY, resolution: 400 bphs	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	18 sites tested, matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Yes - $SOD1^{E101G}$ mutation	Fig. 1A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence, negative	Supplementary file 1
Differentiation potential	TaqMan hPSC scorecard	Passed	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

parental fibroblasts (Fig. 1E) and similar expression levels compared to another iPSC line UOWi002-A (Muñoz et al., 2018). In addition, pluripotency was assessed using the hPSC Scorecard assay, with an upregulation of mesodermal, endodermal and ectodermal specific genes and a downregulation of genes associated with pluripotency (Fig. 1F).

3. Materials and methods

3.1. Reprogramming of human dermal fibroblasts

Dermal fibroblasts were cultured in Dulbecco's Modified Eagle Medium F12 (DMEM/F12, Thermo Fisher Scientific) with 1x Non-Essential Amino Acids (Thermo Fisher Scientific) and 10% foetal bovine serum (Interpath) at 37 °C and 5% CO_2. Fibroblasts were reprogrammed using StemMACS mRNA Reprogramming Kit (Myltenyi Biotec #130-104-460) following the manufacture's protocol. Spontaneous iPSC colonies were isolated and expanded into individual iPSC lines (clones). Established iPSC clones were maintained in TeSR-E8™ (Stemcell Technologies) on Matrigel-coated plates (Corning) at 37 °C and 5% CO_2 and passaged (1:5) using dispase (Stemcell Technologies) (See Table 1). The study was approved by the University of Wollongong Human Ethics Committee (HE 13/272).

3.2. Sequencing

Genomic DNA was extracted using the ISOLATE II Genomic DNA Kit (Bioline), with DNA amplified by PCR using MyTaq HS DNA Polymerase (Bioline) with the *SOD1* primers (Table 2). Sequencing reactions were performed using the ABI BigDye Terminator v3.1 Ready Reaction Mix and separated using the 3500xL Genetic Analyzer (Applied Biosystems). BioEdit (Ibis Therapeutics) was used to analyse the sequences.

3.3. Karyotyping

Karyotyping was performed at Sullivan Nicolaides Pathology Pty Ltd (Brisbane, Australia), on iPSCs at passage 24, with 15 metaphase spreads counted at 400 bphs.

3.4. Immunofluorescence staining

The iPSCs were fixed in 4% paraformaldehyde for 10 min, permeabilised with 0.05% Triton-X for 7 min (SSEA-4) or 15 min (Oct4) and blocked in 10% goat serum for 1 h at 22 °C. Primary antibodies Oct4 and SSEA-4 (Table 2) were incubated overnight at 4 °C, followed by secondary antibody (Table 2) for 1 h at 22 °C. Cultures were counter stained with Reddot2 (1:200, Biotium) for 10 min at 22 °C. For TRA-1-60 staining, cultures were incubated with TRA-1-60 live stain following manufacture's protocol. Immunocytochemical preparations were imaged with a Leica DMI6000B confocal microscope and acquired using LAS AF 2.6 software (Leica Microsystems).

3.5. Quantitative polymerase chain reaction (qPCR)

Fibroblast and iPSC RNA was extracted using Tri-Reagent (MRC Gene) as per manufacture's protocol. Genomic DNA was removed using the Turbo DNAse kit (Thermo Fisher Scientific) and RNA purity determined using a Nanodrop 2000C. Tetro Reverse Transcriptase kit was used for cDNA synthesis (Bioline). The expression of *POU5F1* and *NANOG* was assessed via qPCR using SensiFast SYBR (Bioline) on the

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Oct4	1:000	Stemcell Technologies Cat# 01550, RRID: AB_1118539
	SSEA-4	1:200	Abcam Cat# 16286, RRID: AB_778073
	TRA-1-60	1:200	Stemcell Technologies Cat# 60064A, RRID: AB_2686905
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG (H + L)	1:1000	Thermo Fisher Scientific Cat# A11001, RRID: AB_2534069

Primers		
	Target	Forward/reverse primer (5′ − 3′)
Sequencing	*SOD1*	CATCAGCCCTAATCCATCTGA/ CGCGACTAACAATCAAAGTGA
Pluripotency Genes (qPCR)	*POU5F1*	GATCACCCTGGGATATACAC/ GCTTTGCATATCTCCTGAAG
	NANOG	CCAGAACCAGAGAATGAAATC/ TGGTGGTAGGAAGAGTAAAG
House-Keeping Genes (qPCR)	*GAPDH*	GAGCACAAGAGGAAGAGAGAGACCC/ GTTGAGCACAGGGTACTTTATTGATGGTACATG
	HPRT1	TGACACTGGCAAAACAATGCA/ GGTCCTTTTCACCAGCAAGCT

RRID requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

Corbett RotorGene 3000 (Thermo Fisher Scientific) and normalised to the expression of housekeeper genes *HPRT1* and *GAPDH*. Difference in expression was calculated via the ΔΔct method.

3.6. Scorecard

Mesodermal and endodermal differentiation was induced using the STEMdiff Mesoderm Induction Medium (Stemcell Technologies) and STEMdiff Definitive Endoderm Kit (Stemcell Technologies), respectively, following the manufacture's protocols. Induction of ectodermal differentiation was achieved using Neural Induction medium (DMEM/F12, 1% N2, 0.4% B27, 1% MEM Non-essential amino acids, 1% GlutaMAX) supplemented with 1 μM LDN193189 (Focus Bioscience) for 2 days. The iPSC colonies were detached with dispase (Thermo Fisher Scientific) to form embryoid bodies and cultured in Neural Induction medium supplemented with 3 μM CHIR99021 (Focus Bioscience) and 2 μM SB431542 (Focus Bioscience) for 7 days. From each culture, cDNA was mixed at a ratio of 1:1:1, with 1 μg analysed with the TaqMan hPSC Scorecard (Thermo Fisher Scientific) following the manufacturer's protocol.

3.7. STR analysis

STR analysis of 18 locations was performed at Garvan Molecular Genetics Institute (Darlinghurst, Australia).

Declaration of Competing Interest

None.

Acknowledgements

This work was supported by research grants from the Motor Neurone Disease Research Institute of Australia (Grant-in-Aid awarded to LO), and the National Health and Medical Research Council of Australia (NHMRC, APP1095215). LO is supported by an NHMRC Boosting Dementia Research Leadership Fellowship (APP1135720). RB is supported by the Australian Government Research Training Program Award (AGRTP). The authors wish to acknowledge the fibroblast donor that made this research possible.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101701.

References

Farrawell, N.E., Yerbury, M.R., Plotkin, S.S., McAlary, L., Yerbury, J.J., 2019. CuATSM protects against the *in vitro* cytotoxicity of wild-type-like copper–zinc ouperoxide dismutase mutants but not mutants that disrupt metal binding. ACS Chem. Neurosci. 10 (3), 1555–1564.

Muñoz, S.S., Balez, R., Cabral-da-Silva, M.e.C., Berg, T., Engel, M., Bax, M., Do-Ha, D., Stevens, C.H., Greenough, M., Bush, A., Ooi, L., 2018. Generation and characterization of human induced pluripotent stem cell lines from a familial Alzheimer's disease PSEN1 A246E patient and a nondemented family member bearing wild-type PSEN1. Stem Cell Res 31, 227–230.

Valentine, J.S., Doucette, P.A., Potter, S.Z., 2005. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. Annu. Rev. Biochem. 74, 563–593.