Lab resource: Stem Cell Line

Generation and characterization of a human induced pluripotent stem cell line UOWi005-A from dermal fibroblasts derived from a CCNF$^{621G}$ familial amyotrophic lateral sclerosis patient using mRNA reprogramming

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ABSTRACT

Dermal fibroblasts from a 59 year old male patient with amyotrophic lateral sclerosis (symptomatic at the time of collection), attributed to a mutation in the cyclin F gene ($CCNF^{621G}$), were reprogrammed using mRNA and microRNA-delivered OSKM factors to induced pluripotent stem cells (iPSCs). The generated iPSCs were confirmed pluripotent, expressing typical pluripotency markers and were capable of three germ layer differentiation. This is the first reported reprogramming of cells with a mutation in the cyclin F gene, and represents a novel resource for the study of amyotrophic lateral sclerosis.

Resource utility

This iPSC line serves as a novel cell line to study the effects of the $CCNF^{621G}$ mutation. This resource is available under request owing to the Materials Transfer Agreement in place.

Resource details

Dermal fibroblast samples were collected from a 59 year old male patient with amyotrophic lateral sclerosis (symptomatic at the time of collection) attributed to a mutation in the cyclin F gene ($CCNF^{621G}$). The fibroblasts were provided by the Macquarie University Centre for Motor Neuron Disease Research under a Materials Transfer Agreement with the University of Wollongong. The iPSC line UOWi005-A (Fig. 1) was reprogrammed using mRNA transfections of OSKM factors, LIN28, and nGFP using an microRNA-enhanced mRNA reprogramming kit donated by Stemgent (00-0071 and 00-0073), according to the manufacturer’s instructions. Stem cell colonies arising within the reprogrammed culture were transferred and cultured as separate clones. Successful passaging and morphology assessments were used to shortlist clones to clone 1 (Fig. 1A). Karyotyping revealed no chromosomal abnormalities present (Fig. 1B), and sequencing indicated retention of the $CCNF^{621G}$ mutation (Fig. 1C). Expression of pluripotency marker mRNA transcripts $POUSF1$ and $NANOG$ were significantly elevated compared to fibroblast levels as assessed via qRT-PCR (Fig. 1D). Protein expression of pluripotency markers Oct4, SSEA4 and TRA-1-60 were confirmed via immunocytochemistry (Fig. 1E, F). To confirm the capacity of the reprogrammed iPSCs to generate all three germ layers, cells were differentiated into cells of each lineage. Mesodermal differentiation generated cells with myoblast morphology and which were positive for sarcomeric α-actinin (Fig. 1G). Endodermal directed differentiation generated cells with endodermal progenitor morphology which also positively expressed FOXA2 (Fig. 1H). The UOWi005-A were also differentiated to an ectodermal lineage, generating cells with neuronal morphology and which were positive for neurofilament heavy (SMI32) (Fig. 1I). Differentiation potential of the iPSCs into the three germ layers was assessed by TaqMan hPSC Scorecard (Fig. 1 J) STR profiling confirmed the authentication of the iPSCs with the fibroblasts. Mycoplasma testing was negative for infection (Supplementary Fig. 1).

Materials and methods

Reprogramming of dermal fibroblasts and maintenance of induced pluripotent stem cells

Dermal fibroblasts from a 59 year old male symptomatic for...
Fig. 1. Characterization of UOWi005-A iPSC line.
amyotrophic lateral sclerosis at the time of collection, with mutation in the cyclin F gene (CCNF<sup>S621G</sup>) were cultured in fibroblast medium, consisting of Dulbecco’s Modified Eagle Medium F12 (Thermo Fisher Scientific, 12,500-096) supplemented with 10% foetal bovine serum (Interpath SFBS-F), L-Glutamine (Life Technologies, 25,030) and PenStrep (Sigma Aldrich, P4333) at 37 °C, 5% CO₂. Fibroblasts were reprogrammed with the Stemgent microRNA-enhanced mRNA reprogramming kit (Stemgent, 00-0073, 00-0071), following the manufacturer’s protocol. Manual clonal selection was performed as per Muñoz et al., 2018. The iPSCs were cultured in TesR-E8 (Stem Cell Technologies), passaged 1:8 using 1× dispase (Stem Cell Technologies) and maintained at 37 °C, 5% CO₂. (See Table 1.)

Karyotyping

Karyotyping of the cells was performed by StemCore (University of Queensland, Australia) at passage 24, 15 metaphase spreads were counted and cells were assessed at a resolution of 400 bands per haploid set.

Sequencing

Sequencing was as described in Muñoz et al., 2018 with CCNF primers (Table 2).

Quantitative real time polymerase chain reaction (qRT-PCR)

Cultures were sampled with Tri-Reagent (MRC Gene, TR118) and RNA extracted as per manufacturer’s instructions. Genomic DNA was removed (Ambion Turbo DNase, Thermo Fisher Scientific AM1907) and cDNA was generated (Tetro Reverse Transcriptase, Bioline BIO-65050). The expression of POU5F1 and NANOG was assessed via qRT-PCR (SYBR Green, Bioline, BIO-98020) using LightCycler 480 (Roche) and normalised to housekeeper genes U6 and HPRT1. Expression calculated via the ΔΔCt method, averaging across the two housekeeper genes.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 10 min, permeabilised with 0.05% v/v Triton-X (Sigma-Aldrich, T9284) for 15 min and blocked with 10% v/v goat serum for 1 h (Thermo Fisher Scientific, 16,210-064). Cultures were incubated with primary antibodies (Table 2) or IgG control (Thermo Fisher Scientific, 10400C) at 4 °C for 16 h, followed by secondary antibody incubation (Table 2), and nuclear staining with Reddot2 (1:200, Biotium, 40,061-1), or Hoechst 33342 (1 μg/mL, Life Technologies) for 10 min. Images were captured on an epifluorescence microscope (Leica DMI8 or DMI6000B) acquired using LAS AF (Leica Microsystems).

Differentiation to mesodermal germ layer

Myoblasts were differentiated as per Caron et al., 2016.

Differentiation to endodermal germ layer

Endodermal progenitors were differentiated using STEMdif Definitive Endoderm Kit (Stemcell Technologies, 05115) following the manufacturer’s protocol.

Differentiation to ectodermal germ layer

Motor neurons were generated as described in Zeineddine et al. (2015). These neural precursors were caudalized via the addition of retinoic acid (0.3 μM) and bFGF (2.5 ng/mL) to the neural precursor media for three consecutive days. Cells were transitioned to motor neuron precursors via the addition of purmorphamine (Stem Cell Technologies, 72,204) and a lower concentration of retinoic acid (0.1 μM) to the precursor media and matured for 6 weeks.

TaqMan hPSC scorecard

iPSCs were differentiated into the three germ layer. Endoderm and mesoderm were generated with STEMdiff Definitive Endoderm Kit and STEMdiff Mesoderm Induction Medium (Stem Cell Technologies, 05115 and 05220) following the manufacturer's instructions. Ectoderm was generated as in Muñoz et al., 2018. cDNA of the three germ layers was mixed in a 1:1:1 ratio to analyse 1 μg by TaqMan hPSC Scorecard (Müller et al., 2011).

STR analysis

STR analysis of 18 locations was performed at Garvan Molecular Genetics Institute (Darlinghurst, Australia).
Table 2
Reagent details

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<th>Antibodies used for immunocytochemistry/flow-cytometry</th>
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<tr>
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<td>SSEA4</td>
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<th>Primers</th>
<th>Target</th>
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<td>CCNF</td>
<td>Forward: CTGACACAGCTCCTACCTC&lt;br&gt;Reverse: GGAAGCTGTGGAGGCATC</td>
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<td>POU5F1</td>
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<td>HPRT1</td>
<td>Forward: TGACACTGGAAAGAAATGCA&lt;br&gt;Reverse: GGTCTCTTTTACCAGCAAGC</td>
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Acknowledgements

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References


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