Establishment of a human iPSC line (IISHDOi001-A) from a patient with McArdle disease

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ABSTRACT

Human iPSC line IISHDOi001-A was generated from fibroblasts of a patient with McArdle disease harbouring the mutation, c.148C→T; p.Arg50Ter, in the PYGM gene. Reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc were delivered using Sendai virus.

Resource utility

McArdle disease is a disorder of carbohydrate metabolism inherited in an autosomal recessive way, associated with mutations in the PYGM gene. Patients with this disease experience exercise intolerance including, sometimes, rhabdomyolysis and myoglobinuria. The line IISHDOi001-A will be very useful for modelling this disease and searching for therapeutic approaches.

Resource details

The generation of the human iPSC line, IISHDOi001-A, was performed using non-integrative Sendai viruses containing the reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc (Takahashi et al., 2007). For this purpose, fibroblasts from a patient with McArdle disease were employed. These fibroblasts harbour the most prevalent mutation among the Caucasian population, located in the PYGM gene (c.148C→T; p.Arg50Ter) (Nogales-Gadea et al., 2016). The presence of this mutation in the iPSCs was confirmed (Fig. 1A). IISHDOi001-A iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B). We confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after twelve culture passages (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors OCT4, SOX2, KLF4, NANOG, CRIPTO and REX1 was evaluated by quantitative real time polymerase chain reaction (qPCR) (Fig. 1E). Immunofluorescence analysis revealed expression of transcription factors OCT4, NANOG, SOX2 and surface markers SSEA3.
SSEA4, TRA-1-60 and TRA-1-81 characteristics of pluripotent ES cells (Fig. 1F). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1G). We also confirmed by DNA fingerprinting analysis that the line IISHDO001-A was derived from the patient's fibroblasts. In addition, the line was confirmed by PCR.
analysis to be mycoplasma-negative (Fig. 1H). Finally, the capacity of the generated iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) was tested in vitro using an embryoid body based assay (Fig. 1I).

Materials and methods

Reprogramming of McArdle fibroblasts into iPSCs

Human McArdle fibroblasts harbouring the mutation p.Arg50Ter in the PYGM gene were reprogrammed using the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer. IISHDOi001-A was maintained and expanded both on feeder and feeder-free layers as described in Galera et al., 2016.

Phosphatase alkaline analysis

The iPSC line IISHDOi001-A was seeded on a feeder layer plate. After one week, direct phosphatase alkaline activity was determined using the phosphatase alkaline blue membrane substrate solution kit (Sigma, AB0300) (Table 1).

Mutation analysis

Total DNA from patient’s fibroblasts and iPSCs was extracted using a standard phenol-chloroform protocol. Subsequently, a PCR was carried out with the primers listed in Table 2. Following PCR amplification, direct sequencing of amplicons was performed in an ABI 3730 sequencer (Applied Biosystems).

qPCR analysis

Total mRNA was isolated using TRIZOL and 1 μg was used to synthesize cDNA using the QuantiTect RT cDNA synthesis kit. One microliter of the reaction was used to quantify by qPCR the expression of the endogenous pluripotency associated genes (OCT4, SOX2, KLF4, NANOG, CRIPTO and REX1). Primers are listed in Table 2 (Aasen et al., 2008). All the expression values were normalized to the GAPDH gene. Plots are representative of at least three independent experiments.

Karyotype analysis

Karyotype analyses were carried out using cells with more than twenty culture passages. Briefly, cells were treated with 10 μg/mL of Colcemid (Gibco) for 90 min at 37 °C, trypsinized, treated with hypotonic solution KCl 0.075 M, and fixed with Carnoy’s fixative. Cells were then dropped on a microscope glass slide and dried. Metaphase cells were G banded using Wright staining. At least 20 metaphases were karyotyped.

Immunofluorescence analysis

Cells were grown on 0.1% gelatin-coated 35 mm culture plates (81,156, Ibidi), fixed with 4% paraformaldehyde for 30 min at RT and permeabilized using TBS + (0.1% Triton X-100 in Tris-buffered saline, TBS) for 45 min. Then the cells were incubated in TBS + (3% donkey serum, 0.3% Triton X-100 in TBS) for 2 h at RT. Primary antibodies were applied overnight at 4 °C. Secondary antibodies for 2 h at RT. Nuclei were stained with DAPI (Sigma, 28718-90-3). All the antibodies are listed in Table 2.

In vitro differentiation assay

The in vitro pluripotency capacity of the line IISHDOi001-A was tested by spontaneous embryoid body differentiation. The protocol we have used has been described in detail by Galera et al., 2016.
Author disclosure statement

There are no competing financial interests in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.020.
References


