An induced pluripotent stem cell line (TRNDi006-A) from a MPS IIIB patient carrying homozygous mutation of p.Glu153Lys in the NAGLU gene

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Abstract

Mucopolysaccharidosis type III B (MPS IIIB) is a lysosomal storage disorder caused by mutations in the \textit{NAGLU} gene encoding N-acetylglucosaminidase. Here, we report the generation of a human induced pluripotent stem cell (iPSC) line from dermal fibroblasts of a MPS IIIB patient. The iPSC line has homozygous mutations of G > A transversion at nucleotide 457 of the \textit{NAGLU} gene (457G > A), resulting in the substitution of lysine for glutamic acid at codon 153 (Glu153Lys). This iPSC line allows for the study of disease phenotypes and pathophysiology as well as disease modeling in human cells.

Resource table

| Unique stem cell line identifier | TRNDi006-A |
|---------------------------------|------------|
| Alternative name(s) of stem cell line | HT527A |
| Institution | National Institutes of Health  
National Center for Advancing Translational Sciences  
Bethesda, Maryland, USA |
| Contact information of distributor | Dr. Wei Zheng  
Wei.Zheng@nih.gov |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age: 1-year-old  
Sex: Female  
Ethnicity: Caucasian |

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Resource utility

This TRNDi006-A iPSC line is a valuable resource for elucidating the disease phenotype and pathophysiology of MPS IIIB. It can be differentiated into various mature cell types for use as cell-based disease models of MPS IIIB for compound screening and drug development.

Resource details

MPS IIIB, also known as Sanfilippo syndrome type B, is an inherited lysosomal storage disease caused by mutations in the NAGLU gene, which encodes N-acetylgluosaminidase, which normally degrades heparin sulfate (Genger et al., 2018). Heparin sulfate proteoglycans can bind to many ligands to modulate various cellular activities and maintain tissue architecture and physiology. Deficiency of N-acetylgluosaminidase’s function causes lysosomal accumulation of heparin sulfate resulting in neurological dysfunction in MPS IIIB patients (Andrade et al., 2015), though the exact mechanism of MPS IIIB disease is unclear. Children with MPS IIIB have severe neurological and behavioral defects, leading to death in the second or third decade of life. Currently, there are no effective treatments for MPS IIIB. The clinical treatments for this disease are symptomatic and palliative that do not improve patient prognosis.

In this study, an iPSC line was established from skin fibroblasts of a 1-year-old female patient carrying a homozygous gene mutation of p.Glu153Lys (c.457 G > A) in the NAGLU gene by using a non-integrating Sendai viral vector kit (A16517, ThermoFisher) containing OCT3/4, KLF4, SOX2 and C-MYC pluripotency transcription factors (Beers et al., 2012; Beers et al., 2015). Mutations of the NAGLU gene in the newly generated iPSC line, designated as TRNDi006-A, were confirmed by Sanger sequencing of the PCR product harboring the single nucleotide variation (SNV) (Fig. 1D). The patient-derived iPS cells exhibited classical embryonic stem cell morphology (Fig. 1A) and a normal karyotype (46, XX) that was confirmed by G-banded karyotyping at passage 15 (Fig 1C). The cells

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expressed the major pluripotent protein markers of NANOG, SOX2, OCT4, SSEA4 and TRA-1–60 (Fig. 1 A, B) as evidenced by both immunofluorescence staining and flow cytometry analysis. Sendai virus vector (SeV) clearance was detected with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers with no virus present by passage 15 (Fig. 1E). Mycoplasma testing was performed for the TRNDi006-A cell line and a negative result was obtained (Supplementary Fig. S1). The iPSC line was authenticated using STR DNA profiling analysis which demonstrated matching genotypes at all 18 loci examined (information available from the authors). Furthermore, a teratoma formation experiment demonstrated pluripotency of this iPSC cell line, as it exhibited its ability to differentiate into tissues of all three germ layers (ectoderm, neural tube; mesoderm, cartilage; endoderm, gut) in vivo (Fig. 1F). (See Table 1.)

Materials and methods

Cell culture

Patient skin fibroblasts, obtained from Coriell Institute (GM01426), were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100μg/ml streptomycin in a humidified incubator with 5% CO₂ at 37 °C. The iPSCs were cultured in StemFlex medium (ThermoFisher) on matrigel (Corning, 354277)-coated plates at 37 °C in humidified incubator with 5% CO₂ and 5% O₂. Cells were passaged with the dissociation agent of 0.5 mM ethylenediaminetetraacetic acid (EDTA) at 80% confluency.

Reprogramming of human skin fibroblasts

Non-integrating Sendai virus was used to reprogram patient-derived fibroblasts into iPSC cells. Methods were described previously (Beers et al., 2012; Beers et al., 2015).

Genome analysis

Genomic analysis of NAGLU variants was performed by Applied StemCell (Milpitas, California). Genomic DNA was extracted from TRNDi006-A using QuickExtract™ DNA Extraction Solution (Lucigen) and PCR amplifications using MyTaq™ Red Mix (Bioline) were carried out using a previously defined protocol (Li et al., 2018). Sanger sequencing analysis was used for genotyping of the homozygous mutation for a p. Glu153Lys variant (c.457 G > A) of the NAGLU gene. The specific primers used in these protocols are listed in Table 2.

Immunocytochemistry

The iPSCs were fixed with 4% paraformaldehyde, and permeabilized with 0.3% Triton X-100. Cells were treated overnight at 4 °C with SOX2, OCT4, NANOG, SSEA4 and TRA-1–60 primary antibodies (Table 2). After washing, cells were incubated with secondary antibodies conjugated with Alexa Fluor 488 or Alex Fluor 594. Cells were stained with Hoechst 33342 and imaged with an INCell Analyzer 2200 imaging system (GE Healthcare) using 20× objectives and Texas Red, FITC, and DAPI filter sets.
Flow cytometry analysis

Cells were dissociated using TrypLE (ThermoFisher), fixed with 4% paraformaldehyde. Prior to fluorescence-activated cell sorting, cells were permeabilized with 0.2% Tween-20 in Dulbecco’s Phosphate Buffered Saline (DPBS) and stained with fluorophore-conjugated antibodies for 1 h at 4°C on a shaker. Relative fluorophore-conjugated animal nonimmune immunoglobulin were used as the negative control (Antibodies and nonimmune immunoglobulin used are listed in Table 2). Cells were then analyzed on Accuri™ C6 Flow Cytometry system (BD Biosciences).

G-banded karyotyping

The G-banding karyotype analysis was conducted at WiCell Research Institute (Madison, WI, USA). A total of 20 randomly selected metaphases were analyzed by G-banding for each cell line.

Short tandem repeat (STR) analysis

Samples for cell line authentication were analyzed by the Johns Hopkins University Genetic Resources core facility using a PowerPlex 18D Kit (Promega) and the PCR product was electrophoresed on an ABI Prism® 3730xl Genetic Analyzer. Data were analyzed using GeneMapper® v 4.0 software (Applied Biosystems).

Mycoplasma detection

Mycoplasma status was assessed using the Lonza MycoAlert kit following the manufacturer protocol. A ratio of B/A < 0.9 indicates a mycoplasma negative sample.

Sendai virus detection

For positive controls, human fibroblasts (Coriell, GM05659) were transfected with Sendai virus for 4 days, and total RNAs were extracted using RNeasy Plus Mini Kit (Qiagen). The cDNA was reverse transcribed using 1 μg of RNA with the Superscript™ III First-Strand Synthesis SuperMix kit and amplification was performed using Platinum II Hot-Start PCR Master Mix (ThermoFisher) with the following program: 94 °C, 2 mins; 30 cycles of [94 °C, 15 s, 60 °C, 15 s and 68 °C, 15 s] on the Mastercycler pro S (Eppendorf) and primers are listed in Table 2. Products were loaded to the E-Gel® 1.2% with SYBR Safe™ gel, and imaged using the G: Box Chemi-XX6 gel doc system (Syngene).

Teratoma formation assay

The iPSCs were cultured in 6 well plates prior to dissociation with 0.5 mM EDTA in DPBS. Dissociated cells (1 × 10^7) were resuspended in 400 μl culture medium supplemented with 25 mM HEPES (pH 7.4) and cooled on ice. Cold matrigel (Corning, 354277) was added and mixed with the cells at 50% volume (200 μl), then injected subcutaneously into NSG mice (JAX No. 005557) at 150 pi per injection site. Visible tumors were removed 6–18 weeks post injection, fixed in 10% Neutral Buffered Formalin, and then embedded in paraffin for staining with hematoxylin and eosin.
**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgement**

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Fig. 1.
Characterization of TRNDi006-A iPSC line A) Left panel shows phase contrast imaging of TRNDi006-A colonies grown on Matrigel at passage 10; Right panels show immunofluorescent staining of TRNDi006-A iPSCs, demonstrating expression of SOX2, OCT4, TRA-1-60, NANOG and SSEA4. Hoechst (blue) was used to label the nucleus. B) Pluripotency protein markers TRA-1-60, NANOG and SSEA4 were assessed by flow cytometry analysis. C) Normal karyotype was confirmed through G-banding karyotype analysis (46, XX). D) A homozygous gene mutation of p.Glu153Lys (c.457 G > A) in
the NAGLU gene was confirmed by Sanger sequencing. E) RT-PCR verification of the clearance of Sendai virus from the reprogrammed cells. Fibroblasts transfected with Sendai virus were used as positive control. F) Histological characterization of teratoma formation from TRNDi006-A, showing formation of three germ layers (Ectoderm, Mesoderm, and Endoderm).
Characterization and validation.

| Classification       | Test                                      | Result                                      | Data                        |
|----------------------|-------------------------------------------|---------------------------------------------|-----------------------------|
| Morphology           | Photography                               | Normal                                      | Fig. 1 Panel A              |
|                      | Immunocytochemistry                        | SOX2, OCT4, NANOG, SSEA-4, TRA-1-60          | Fig. 1 Panel A              |
|                      | Flow cytometry                            | TRA-1-60 (99.98%); NANOG (96.08%); SSEA-4 (99.98%) | Fig. 1 Panel B              |
|                      | Karyotype (G-banding) and resolution       | 46XX Resolution: 350–400                   | Fig. 1 Panel C              |
|                      | Microsatellite PCR (mPCR) OR               | Not performed                               | N/A                         |
|                      | STR analysis                              | 18 sites tested, all sites matched          | Available with the authors  |
|                      | Sequencing                                | Homozygous mutation of NAGLU                | Fig. 1 Panel D              |
|                      | Southern Blot OR WGS                      | N/A                                         | N/A                         |
|                      | Mycoplasma                                | Mycoplasma testing by luminescence. Negative Teratoma with three germ layers formation. Ectoderm (neural tube); Mesoderm (cartilage); Endoderm (gut) | Supplementary Fig. SI       |
|                      | Teratoma formation                        |                                             |                             |
|                      | 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                         |
Table 2

## Table 2

### Reagents details

#### Antibodies used for immunocytochemistry/flow-cytometry

| Antibody | Dilution | Company Cat # and RRID |
|----------|----------|------------------------|
| Pluripotency Markers Mouse anti-SOX2 | 1:50 | R & D systems, Cat# MAB2018, RRID: AB_358009 |
| Pluripotency Markers Rabbit anti-NANOG | 1:400 | Cell Signaling, Cat# #903, RRID: AB_10559205 |
| Pluripotency Markers Rabbit anti-OCT4 | 1:400 | Thermo Fisher, Cat# A13998, RRID: AB_2534182 |
| Pluripotency Markers Mouse anti-SSEA4 | 1:1000 | Cell Signaling, Cat# 4755, RRID: AB_1264259 |
| Pluripotency Markers Mouse anti-TRA-1-60- Alexa Fluor 488 | 1:10 | BD Biosciences, Cat# 560173, RRID: AB_1645379 |
| Secondary Antibodies Donkey anti-Mouse IgG (Alexa Fluor 488) | 1:400 | Thermo Fischer, Cat# A21202, RRID: AB_141607 |
| Secondary Antibodies Donkey anti-Rabbit IgG (Alexa Fluor 594) | 1:400 | Thermo Fischer, Cat# A21207, RRID: AB_141637 |
| Flow Cytometry Antibodies Anti-Tra-1-60-DyLight 488 | 1:50 | Thermo Fischer, Cat# MA1-023-D488X, RRID: AB_2536700 |
| Flow Cytometry Antibodies Anti-Nanog-Alexa Fluor 488 | 1:50 | Millipore, Cat# FCABS352A4, RRID: AB_10807973 |
| Flow Cytometry Antibodies anti-SSEA-4-Alexa Fluor 488 | 1:50 | Thermo Fischer, Cat# 53-8843-41, RRID: AB_10597752 |
| Flow Cytometry Antibodies Mouse-IgM-DyLight 488 | 1:50 | Thermo Fischer, Cat# MA1-194-D488, RRID: AB_2536969 |
| Flow Cytometry Antibodies Rabbit IgG-Alexa Fluor 488 | 1:50 | Cell Signaling Technology, Cat# 4340S, RRID: AB_10694568 |
| Flow Cytometry Antibodies Mouse IgG3-FITC | 1:50 | Thermo Fischer, Cat# 11-4742-42, RRID: AB_2043894 |

#### Primers

| Target | Forward/Reverse primer (5′-3′) |
|--------|--------------------------------|
| Sev specific primers (RT-PCR) | Sev/181 bp |
| Sev specific primers (RT-PCR) | KOS/528 bp |
| Sev specific primers (RT-PCR) | Klf/4410 bp |
| Sev specific primers (RT-PCR) | C-Myc/525 bp |
| House-Keeping gene (RT-PCR) | GAPDH/197 bp |
| Targeted mutation analysis (PCR) | NAGLU (c.457 G > A)/290 bp |