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Lab Resource: Stem Cell Line

# Generation of Mucopolysaccharidosis type II (MPS II) human induced pluripotent stem cell (iPSC) line from a 3-year-old male with pathogenic IDS mutation

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ABSTRACT

Peripheral blood was collected from a 3-year-old male patient with an X-linked recessive mutation of Iduronate 2-sulfatase (IDS) gene (NM_000202.7(IDS):c.85C>T) causing MPS II (OMIM 309900). Peripheral blood mononuclear cells (PBMCs) were reprogrammed by lentiviral delivery of a self-silencing hOKSM polycistronic vector. The pluripotency of the iPSC line was confirmed by the expression of pluripotency-associated markers and *in vitro* spontaneous differentiation towards the 3 germ layers. The iPSC line showed normal karyotype. The cell line offers a good platform to study MPS II pathophysiology, for drug testing, early biomarker discovery and gene therapy studies.



Resource table.

| | |
|---|---|
| Name of stem cell line | MPSII-2.5 |
| Institution | BioTalentum Ltd. |
| Person who created resource | Prof. András Dinnyés, Eszter Varga |
| Contact person and email | andras.dinnyes@biotalentum.hu |
| Date archived/stock date | May, 2013 |
| Origin | Peripheral blood mononuclear cells |
| Type of resource | Induced pluripotent stem cell; derived from a patient with hemizygous NM_000202.7(IDS):c.85C>T mutation |
| Sub-type | Induced pluripotent stem cell |
| Key transcription factors | OCT3/4, KLF4, SOX2, C-MYC |
| Authentication | Identity and purity of the cell line was confirmed by: Sanger sequencing of the pathogenic mutation, expression analysis of dTomato and pluripotency markers, *in vitro* differentiation potential, karyotyping |
| Link to related literature | http://www.ncbi.nlm.nih.gov/pubmed/20385817 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3070104/ http://www.ncbi.nlm.nih.gov/pubmed/18038146 |
| Information in public databases | N/A |
| Ethics | Patient informed consent obtained/Ethics Review Board-competent authority approval obtained |

## Resource details

Peripheral blood was collected from a 3-year-old male patient with genetically characterized MPS II disorder diagnosed by Department of Pediatrics, University of Pécs (Hungary). Based on the clinical symptoms of the patient, the disorder was determined to be severe MPS II. The patient carries a pathogenic, X-linked, hemizygous mutation of the IDS gene (NM_000202.7(IDS):c.85C>T, p.Gln29Ter). The single nucleotide variation (SNV) results a premature termination codon, leading to Iduronate 2-sulfatase enzyme deficiency and to the severe accumulation of glycosaminoglycans. Mutations of IDS gene have been shown to cause MPS II disorder (Wraith et al. 2008). In the patient-derived iPSCs the presence of the pathogenic mutation was confirmed by Sanger sequencing of the PCR product harboring the SNV (Table 1, Fig. 1A).

To generate the MPSII-2.5 iPSC line the pRRL.PPT.SF.hOKSMco.idTomato.preFRT lentiviral vector was used, which was shown that under certain condition is self-silencing shortly after transduction (Voelkel et al. 2010; Warlich et al. 2011). TRA-1-60 expressing iPSC-like colonies were picked 18–21 days post-transduction, based on *in vivo* immunocytochemistry staining (ICC) (Fig. 1B). Five stable lines were maintained and based on morphological criteria the MPSII-2.5 was chosen for further examination.

The pluripotency of MPSII-2.5 line was confirmed by alkaline phosphatase staining (ALP) and by ICC for endogenous NANOG and E-CADHERIN (Fig. 1B) after silencing of the exogenous hOKSM construct.

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**Table 1**
Primers used in the study.

| Gene symbol | Nucleotide change | Protein change | Fwd and Rvs primers (5′–3′) | PCR product (bp) |
|---|---|---|---|---|
| IDS | c.85C>T (NM_000202.7) | p.Gln29Ter | TTCCCGACGAGGAGGTCTCT ATGCAGGAAAGGACAGATGG | 653 |

The *in vitro* spontaneous differentiation potential of the iPSC line towards the three germ layers was demonstrated by the expression of ectodermal (βIII-TUBULIN), mesodermal (BRACHYURY) and endodermal (GATA4) markers (Fig. 1B) using ICC.

The karyotype of the MPSII-2.5 iPSC line was determined by Giemsa-banding, proving normal diploid 46, XY karyotype, without any detectable abnormalities (Fig. 1B).

## Materials and methods

### *Experimental procedures*

The chemicals were purchased from Sigma-Aldrich and all the culture reagents from Thermo Fisher Scientific, unless otherwise specified.

### *Cell culture*

All cells were cultured at 37 °C in humidified atmosphere containing 5% $CO_2$. The iPSCs were cultured on Matrigel (BD Biosciences) in mTeSR-1 medium (Stem Cell Technologies) following the manufacturer's instructions.

### *Reprogramming of PBMCs*

PBMCs were isolated using the Vacutainer® CPT™ Cell Preparation Tube with Sodium Heparin (BD Biosciences). PBMCs were seeded ($1 \times 10^6$ cells) in Expansion Medium (EM): QBSF-60 medium (Quality Biological) supplemented with 50 μg/ml Ascorbic acid, 1% Pen/Strep, 50 ng/ml SCF (R&D systems), 10 ng/ml IL-3 (R&D systems), 2 U/ml EPO (R&D systems), 40 ng/ml IGF-1 (R&D systems) and 1 μM Dexamethasone. Three days later the cells were transduced with pRRL.PPT.SF.hOKSMco.idTomato.preFRT (Voelkel et al. 2010; Warlich et al. 2011) vector using conventional lentivirus transduction procedure at 0,2–0,4 MOI. Three days post-transduction the cells were seeded onto Mitomycin C treated-MEF in IMDM MEF medium (IMDM, 10% FBS, 1% 100× MEM Non-Essential Amino Acid Solution, 0.1 mM β-mercaptoethanol, 1% Pen/Strep) supplemented with 50 μg/ml Ascorbic acid and growth factors applied in EM medium. From day seven post-transduction cells were cultured in HESC medium: DMEM/F12, 20% Knockout Serum Replacement, 1% 100× MEM Non-Essential Amino Acid Solution, 0.1 mM β-mercaptoethanol, 0.5% Pen/Strep, 10 ng/ml bFGF, supplemented with 50 μg/ml Ascorbic acid. The appeared ES-like colonies were manually picked (Days 18–21) and cultured as iPSCs thereafter.

### *Alkaline phosphatase staining*

Following 4% PFA fixation the cells were incubated with ALP solution for 30 min at RT. The stained cells were observed under inverted

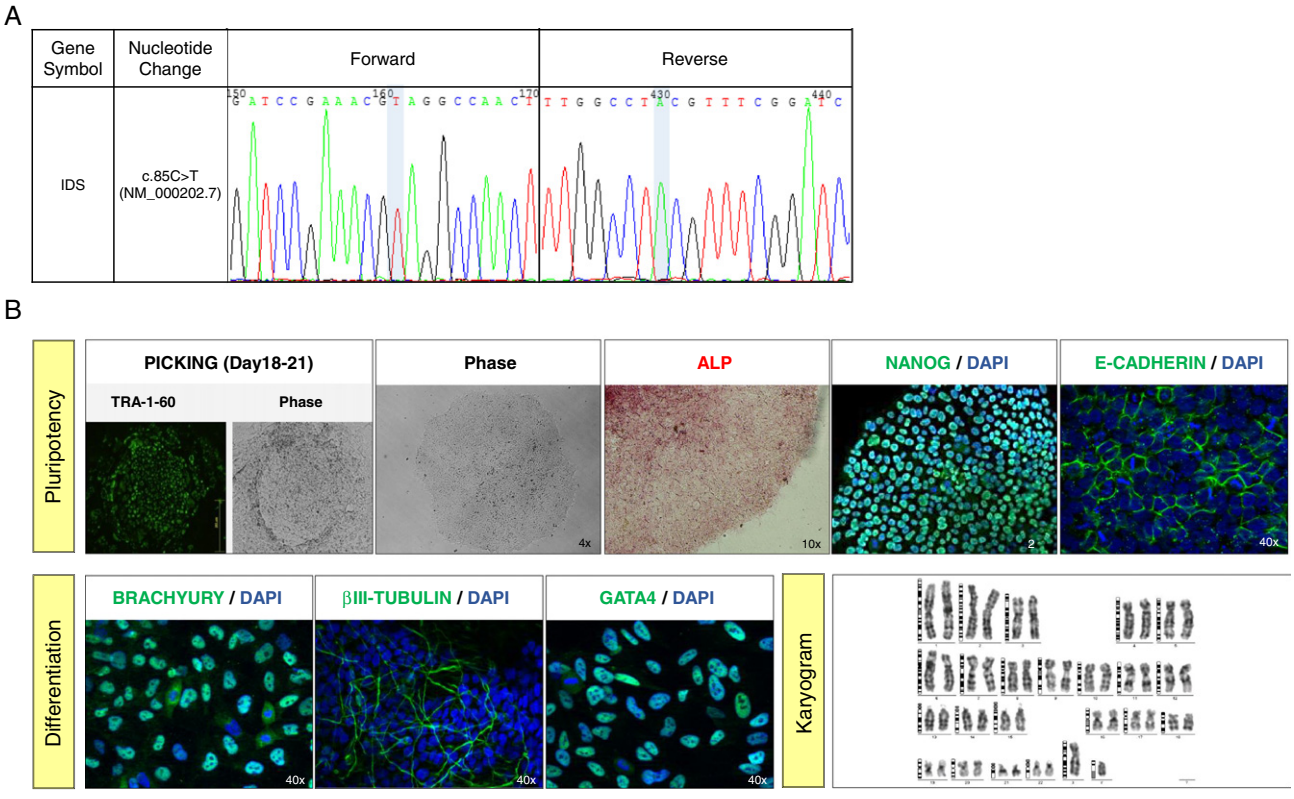


**Fig. 1.** Characterization of MPSII-2.5 iPSC line. A) Sanger sequencing of the region containing the NM_000202.7(IDS):c.85C>T SNV in the newly established iPSC line. B) Top: Picking of iPSC colonies based on *in vivo* TRA-1-60 ICC (4×), morphology of the generated iPSC (4×), the iPSC line showed Alkaline Phosphatase activity (ALP) (10×) and was positive for pluripotency-associated markers: NANOG (20×), E-CADHERIN (40×) (in green), nucleus was labelled with DAPI (in blue). Bottom: Spontaneous differentiation potential (Day 14) was confirmed by ICC for mesodermal (BRACHYURY), ectodermal (βIII-TUBULIN) and endodermal (GATA4) germ layers (40×) (in green), the nucleus was labelled with DAPI (in blue), karyogram showing normal 46 chromosomes (XY).

**Table 2**
Antibodies used in this study.

| | Manufacturer | Dilution |
|---|---|---|
| Pluripotency markers | | |
| Mouse anti-Tra-1-60, IgM | Santa Cruz | 1/50 |
| Mouse anti-E-Cadherin (human) | Thermo Fischer Sci. | 1/1000 |
| Goat anti-hNanog | R&D Systems | 1/50 |
| Three germ layer markers | | |
| Rabbit anti-βIII-tubulin | Covance | 1/2000 |
| Rabbit anti-BrachyuryT | Santa Cruz | 1/50 |
| Mouse anti-GATA4 | Santa Cruz | 1/50 |
| Secondary antibodies | | |
| Alexa Fluor 488 donkey anti-mouse IgM | Jackson ImmunoResearch | 1/100 |
| Alexa Fluor 488 donkey anti-goat IgG | Thermo Fischer Sci. | 1/2000 |
| Alexa Fluor 488 donkey anti-mouse IgG | Thermo Fischer Sci. | 1/2000 |
| Alexa Fluor 488 donkey anti-rabbit IgG | Thermo Fischer Sci. | 1/2000 |

microscope. The ALP solution consisted of 29.7 mM TRIS base and 6 mM maleic acid (Fluka) pH 8.5–9.0; supplemented with 0.08% MgCl2, 10.8 mM Naphtol-As MX Phosphate and 23 μM Fast Red TR Salt.

*Immunocytochemistry staining*

The expression of specific pluripotency and germ layer markers were analyzed using conventional ICC staining. The cells were stained *in vivo* or fixed in 4% PFA. The antibodies and applied dilutions are listed in Table 2. The cells were observed under fluorescent microscope equipped with 3D imaging module (AxioImager system with ApoTome, Carl Zeiss) controlled by AxioVision 4.8.1 Microscope software (Carl Zeiss).

*Karyotyping*

Cells were treated with Demecolcine solution (10 μg/ml in HBSS), and processed with standard methods. Giemsa-banded karyotype was performed and a minimum of 20 metaphases were analyzed. The chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN).

*Sanger sequencing*

To confirm the presence of the pathogenic IDS mutation in the newly established iPSC line PCR primers were designed to the region of interest (Table 1). PCR reactions were performed using Phusion Hot Start II High-Fidelity DNA Polymerase and MPSII-2.5 gDNA as the template. The PCR product was purified with GenElute PCR cleanup kit and sequenced directly using an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems) and BigDye Terminator Cycle Sequencing v3.1 Kit (Applied Biosystems).

*In vitro spontaneous differentiation*

Cell clumps were cultured in suspension for five days in mTeSR-1. The formed EBs were plated on 0.1% gelatin covered cover slips in differentiation medium: DMEM, 20% FBS, 1% 100× MEM Non-Essential Amino Acid Solution, 0.1 mM β-mercaptoethanol, 1% Pen/Strep. The cells were fixed on Day 14 of differentiation with 4% PFA and stained for the 3 germ layer markers (Table 2).

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