Stem Cell Research 39 (2019) 101493

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Multiple cell lines

Generation of two human iPSC lines (MDCUi001-A and MDCUi001-B) from dermal fibroblasts of a Thai patient with X-linked osteogenesis imperfecta using integration-free Sendai virus

Siraprapa Tongkobpetch[a,b,c], Ruttachuk Rungsiwiwut[d,e], Kamthorn Pruksananonda[d], Kanya Suphapeetiporn[b,c,*], Vorasuk Shotelersuk[b,c]

[a] Interdisciplinary Program of Biomedical Sciences, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand
[b] Center of Excellence for Medical Genomics, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
[c] Excellence Center for Medical Genetics, King Chulalongkorn Memorial Hospital, The Thai Red Cross Society, Bangkok 10330, Thailand
[d] Human Embryonic Stem Cell Research Center, Reproductive Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand
[e] Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand

A B S T R A C T

Two clones of human induced pluripotent stem cells (iPSCs) were generated from dermal fibroblasts isolated from a one-year-old Thai patient with X-linked osteogenesis imperfecta. The patient harbored a mutation, p.N459S, in the *MBTPS2* gene. The cells were reprogrammed using an integration-free Sendai virus containing *KLF4*, *c-MYC*, *OCT4* and *SOX2*. Both of the established iPSC lines (MDCUi001-A and MDCUi001-B) maintained normal karyotype, expressed pluripotent markers and differentiated into all three germ layers.

Resource Table

Unique stem cell lines identifier	MDCUi001-A MDCUi001-B
Alternative names of stem cell lines	Th-XOI#1 Th-XOI#2
Institution	Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Contact information of distributor	Siraprapa Tongkobpetch, A_Siraprapa@yahoo.com Vorasuk Shotelersuk, vorasuk.s@chula.ac.th
Type of cell lines	iPSC
Origin	Human
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus
Multiline rationale	2 clones of a Thai patient with X-linked osteogenesis imperfecta
Gene modification	YES
Type of modification	Hereditary
Associated disease	X-linked osteogenesis imperfecta
Gene/locus	MBTPS2/p.N459S
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	December 2017
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/MDCUi001-A https://hpscreg.eu/user/cellline/edit/MDCUi001-B
Ethical approval	The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, IRB No. 193/56

Resource utility

We generated two human iPSC lines from dermal fibroblasts of a Thai patient with X-linked osteogenesis imperfecta containing p.N459S in *MBTPS2* using a non-integrating Sendai virus. These cell lines were used as a disease model to investigate the relevance of *MBTPS2* to bone morphogenesis.

Resource details

Osteogenesis imperfecta (OI) or brittle bone disease is a genetic disorder characterized by bone fragility and deformities associated with macrocephaly, blue sclerae, hearing loss, dentinogenesis imperfecta,

* Corresponding author at: Center of Excellence for Medical Genomics, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail address: kanya.su@chula.ac.th (K. Suphapeetiporn).

https://doi.org/10.1016/j.scr.2019.101493
Received 28 December 2018; Received in revised form 17 June 2019; Accepted 25 June 2019
Available online 29 June 2019

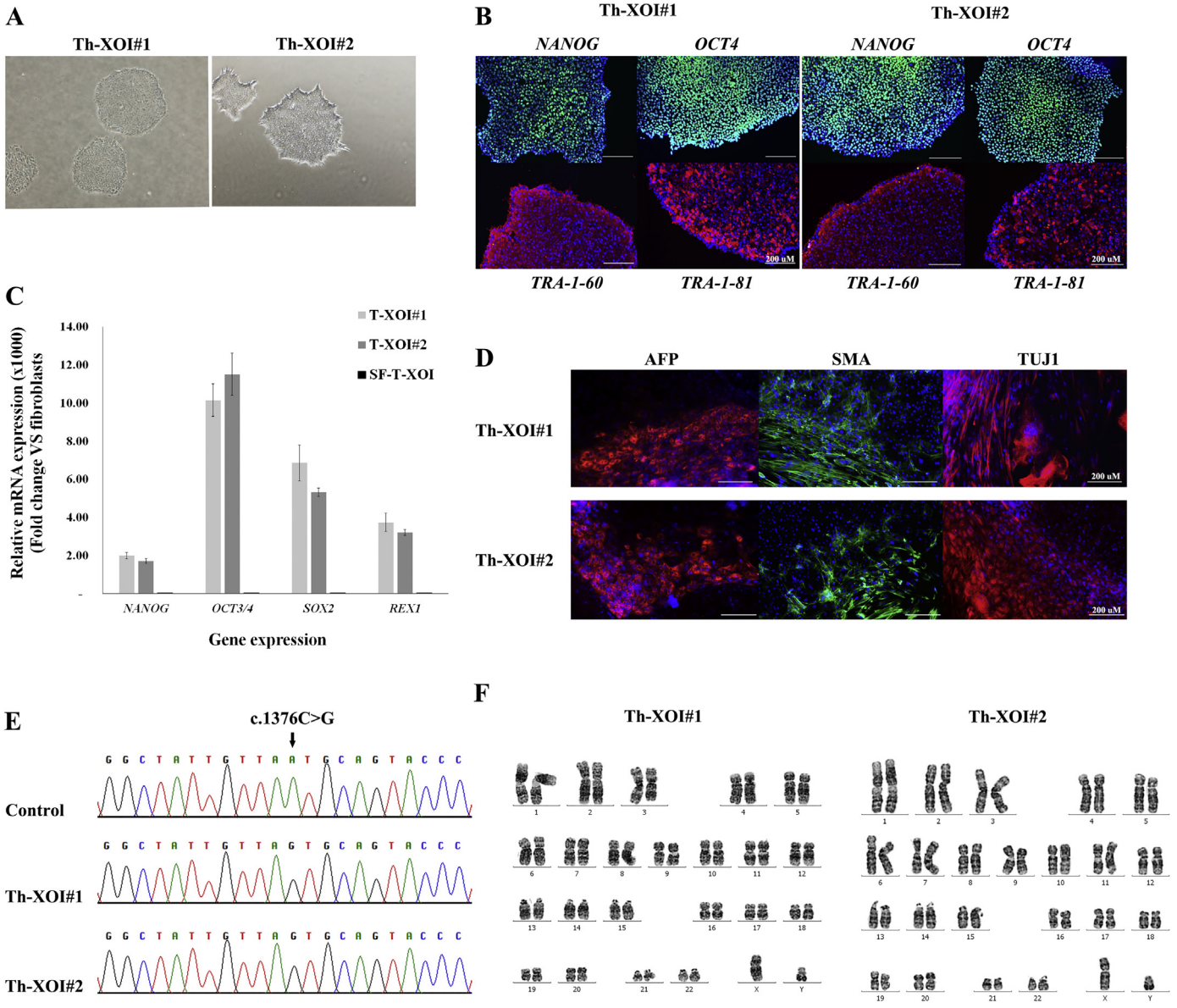

Fig. 1. Characterization of Th-XOI#1 and #2 cell lines.

and short stature. OI can be caused by mutations in at least 17 genes (Marini et al., 2017). Our patient harbored the c.1376A > G (p.N459S) mutation in the membrane-bound transcription factor peptidase, site 2 *(MBTPS2)* gene causing X-linked OI (Lindert et al., 2016). *MBTPS2* encodes site 2 protease (S2P) which is an enzyme playing a role in regulated intramembrane proteolysis (RIP) (Lal and Caplan, 2011). This is a new paradigm for bone morphogenesis. To better understand the MBTPS2 function relating to bone morphogenesis, we generated iPSCs from our patient and differentiated them into osteoblasts.

Dermal fibroblasts were collected from the one-year-old patient with X-OI. Sendai virus containing the Yamanaka reprogramming vectors (Takahashi and Yamanaka, 2006) was used to generate two iPSC lines, MDCUi001-A and MDCUi001-B (Fig. 1A). To verify their status, pluripotency and *in vitro* differentiation tests were performed. Expression of the four pluripotent genes, *OCT3/4, NANOG, TRA-1-60* and *TRA-1-81*, were evaluated in both lines using immunofluorescence staining (Fig. 1B). RNA expression of *REX1*, *SOX2*, *OCT3/4* and *NANOG* was identified by qRT-PCR (Fig. 1C). In addition, embryoid bodies were promoted to demonstrate the ability to generate all three germ layers. Alpha-fetoprotein (AFP), alpha-smooth muscle actin (SMA) and beta-tubulin III (TUJ1) represented endoderm, mesoderm and ectoderm, respectively (Fig. 1D). The Sendai virus-free status was investigated by RT-PCR. At the passage 15 and 17 of Th-XOI #1 and #2, respectively, showed the absence of virus vector (Fig. 1S). These cells were confirmed to harbor the point mutation, c.1376A > G, in *MBTPS2* using PCR-Sanger sequencing and the cell identity was analysed using STR (Fig. 1E and available with the authors). 16 loci were tested and 100% match to the parental fibroblasts was confirmed for both lines. Their normal karyotypes showed their genomic integrity (Fig. 1F). Mycoplasma contamination was not detected using MycoAlert (Table S1). The two iPSC clones, MDCUi001-A and MDCUi001-B, have been confirmed to be X-OI iPSCs and can be used for further investigations. (See Tables 1 and 2.)

Materials and methods

Cell culture and reprogramming

Dermal fibroblasts were isolated from a Thai X-OI patient and cultured in fibroblast culture medium (DMEM (GE Healthcare Life

Table 1
Summary of lines.

iPSC line name	Abbreviation in figure	Gender	Age	Ethnicity	Genotype of locus	Disease
MDCUi001-A	Th-XOI#1	Male	1	Thai	*MBTPS2:* c.1376A > G	X-linked osteogenesis imperfecta
MDCUi001-B	Th-XOI#1	Male	1	Thai	*MBTPS2* c.1376A > G	X-linked osteogenesis imperfecta

Sciences) supplemented with 10% fetal bovine serum (GE Healthcare Life Sciences) and 1 × penicillin/streptomycin). The CytoTune®-iPS 2.0 Sendai reprogramming vectors (Thermo Fisher Scientific) encoding *KLF4*, *c-MYC*, *OCT4* and *SOX2* were transduced into the dermal fibroblasts following the manufacturer's instruction. Individual iPSC colonies were manually picked and transferred onto mitomycin C treated human foreskin fibroblast (HFF) feeders that were cultured in fibroblast culture medium. After 24 h, the culture medium was changed to ESC medium (KO-DMEM (Gibco), 20% knockout serum replacement (KSR) (Gibco), 1 × GlutaMax® (Gibco), 1 × penicillin and streptomycin (P/S), 1 × non-essential amino acids (NEEA) (Gibco), 4 ng/ml basic-fibroblast growth factor (b-FGF) (R&D systems) and 55 μM beta-mercaptoethanol). About 7–10 days later, iPSC colonies were manually picked and transferred to the new feeder. At passage 5, they were incubated at 38 °C with 5% CO_2 for 7 days to eliminate Sendai virus. iPSC colonies were maintained on a Matrigel-coated plate in mTeSR-1 medium (Stem Cell Technologies). The medium was changed every day until the cells reached 80–90% confluency. Then, they were dissociated using CTK solution (0.2–5% trypsin, 10% collagenase type IV, 20% KSR and 1 mM calcium chloride) and passaged at 1:5 ratio.

Embryoid bodies (EBs) assay

Embryoid bodies (EBs) were formed from feeder-free iPSCs dissociated with CTK solution. Cell clumps were maintained on non-coated plates with ESC medium and EB medium (KO-DMEM, 10% FBS, 1 × GlutaMax®, 1 × P/S, 1 × NEAA, beta-mercaptoethanol) at 1:1 ratio. On day 7, EBs were transferred to Matrigel-coated plates with EB medium for 14 days. Throughout the differentiation, medium was changed every other day (Tables 1 and 2).

Immunofluorescence staining

The staining was used to investigate pluripotency and *in vitro* differentiation. Pluripotent markers are TRA-1-60, TRA-1-81, OCT3/4 and NANOG. The markers for *in vitro* differentiation are AFP, SMA and TUJ1 representing endoderm, mesoderm and ectoderm, respectively. Cells were fixed with 4% paraformaldehyde for 20 min. 0.1% Triton X-100 in PBS was used for 10 min at room temperature to increase membrane permeability for intracellular markers. Cells were blocked with 5% bovine serum albumin in PBS for 45 min at room temperature and stained with primary antibodies overnight at 4 °C. Subsequently, cells were washed with PBS for 5 min thrice. Staining with secondary antibodies was performed for 45 min at room temperature. The washing step was repeated and then nuclei were stained with Hoechst 33342 (Life Technologies). All antibodies are listed in Table 3. Cells were visualized under EVOS FLC Cell Imaging System (Thermo Fisher Scientific).

Gene expression analysis

RNA was isolated from iPSCs using QIAamp RNA Blood Mini Kit (Qiagen). 500 ng of RNA was reverse transcribed using ImProm-II™ Reverse Transcription System (Promega). The cDNA was amplified for *REX1*, *SOX2*, *OCT3/4*, *NANOG* and *GAPDH*, as a control using StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific) with SYBR™ Select Master Mix (Life Technologies). The PCR conditions were as follows: 50 °C for 2 min, 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s, 58 °C for 10 s and 72 °C for 30 s. To confirm the vector-free status of iPSC lines, the SeV genome was amplified using pro S Thermal Cyclers (Eppendorf) with Taq DNA Polymerase, recombinant (Thermo Fisher Scientific) and analysed by gel electrophoresis. The PCR condition was as follows: 94 °C for 5 min followed by 35 cycles of 94 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s. All primers are listed in Table 3.

Sequencing of the mutation site To confirm the presence of the c.1376A > G mutation in *MBTPS2* in MDCUi001-A and MDCUi001-B, DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen). The DNA was amplified using primers which were flanking the mutation (Table 3) using pro S Thermal Cyclers with Taq DNA Polymerase, recombinant. The PCR conditions were as follows: 94 °C for 5 min followed by 35 cycles of 94 °C for 15 s, 55 °C for 15 s and 72 °C for 20 s. The PCR products were analysed by gel electrophoresis. Then, the PCR products were sent to Macrogen (Seoul, Korea) for Sanger sequencing.

Mycoplasma contamination detection

MycoAlert™ PLUS Mycoplasma Detection kit (Lonza) was used to

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative Analysis immunocytochemistry	Expression of pluripotency markers: OCT4, NANOG, TRA-1-60 and TRA-1-81	Fig. 1 panel B
	Quantitative Analysis qRT-PCR	Cells express *REX1*, *SOX2*, *OCT3/4* and *NANOG*	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XY resolution: 400	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	16 sites were tested and 100% matched for parental fibroblasts and 2 iPSC clones	Available with author
Mutation analysis (IF APPLICABLE)	Sequencing	Hemizygous, point mutation, c.1376A > G	Fig. 1 panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	MycoAlert: Negative	Supplementary Table S1
Differentiation potential	Embryoid body formation	Expression of AFP, SMA, TUJ1	Fig. 1 panel D
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

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:200	Cell Signaling Technology Cat# 56159, RRID:AB_2799505 Cell Signaling Technology Cat# 4903, RRID:AB_10559205
	Rabbit anti-NANOG	1:200	Cell Signaling Technology Cat# 4746, RRID:AB_2119059 Cell Signaling Technology Cat# 4745, RRID:AB_2119060
	Mouse anti-TRA-1-60	1:200	
	Mouse anti-TRA-1-81	1:200	
Differentiation Markers	Mouse anti-AFP1	1:200	Abcam Cat# ab3980, RRID:AB_304203 Abcam Cat# ab32575, RRID:AB_722538
	Rabbit anti-SMA	1:150	Sigma-Aldrich Cat# T8660, RRID:AB_477590
	Mouse anti-TUJ1	1:150	
Secondary antibodies	Goat anti-Rabbit IgG, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11008, RRID:AB_143165
	Goat anti-mouse IgG, Cy3 conjugate	1:500	Millipore Cat# AP181C, RRID:AB_92578

Primers		
	Target	Forward/Reverse primer (5′-3′)
Episomal plasmid	*SeV* (181 bp)	F-GGATCACTAGGTGATATCGAGC R- ACCAGACAAGAGTTTAAGAGATATGTATC
Pluripotency Markers	*NANOG* (391 bp)	F-CAGCCCCGATTCTTCCACCAGTCCC
	OCT4 (142 bp)	R-CGGAAGATTCCCAGTCGGGTTCACC
	SOX2 (126 bp)	F-AGCGAACCAGTATCGAGAAC
	REX1 (306 bp)	R-TTACAGAACCACACTCGGAC F-AGCTACAGCATGATGCAGGA R-GGTCATGGAGTTGTACTGCA F-CAGATCCTAAACAGCTCGCAGAAT R-GCGTACGCAAATTAAAGTCCAGA
House-Keeping Genes	*GAPDH* (101 bp)	F-ATCACCATCTTCCAGGAGCGA R-TTCTCCATGGTGGTGAAGACG
e.g. Genotyping Targeted mutation sequencing	N/A *MBTPS2* c.1376A > G (620 bp)	N/A F- GCC CGG AAA GCA GTT GAA GC R- AGG AGC TCA GGG TTG TTG GC

detect mycoplasma. The principle of the test is to detect the mycoplasma enzymes. They react with substrates and change ADP to ATP. The ATP level was measured before and after addition of the substrate. The ratio of the ATP measurement indicates absence or presence of mycoplasma.

Karyotyping

The iPSCs at passage 10 were treated with 50 ng/ml colchicine for 3 h. Then cells were trypsinized, treated with 0.075 M KCl for 20 min and fixed with methanol and acetic acid at 3:1 ratio. The 20 metaphase spreads were examined by standard G-banding. The karyotype analysis was carried out at DNA Center, Bangkok, Thailand.

Short tandem repeat (STR) analysis

Cell identity was identified from genomic DNA by Investigator® IDplex GO! Kit (Qiagen), using 16 STR loci and performed at the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University.

Acknowledgements

This study was supported by the Thailand Research Fund (BRG5980001, DPG6180001) and the Royal Golden Jubilee Ph.D. Program (Grant No.PHD/0160/2553).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101493.

References

Lal, M., Caplan, M., 2011. Regulated intramembrane proteolysis: signaling pathways and biological functions. Physiology. 26 (1), 34–44.
Lindert, U., Cabral, W.A., Ausavarat, S., Tongkobpetch, S., Ludin, K., Barnes, A.M., et al., 2016. MBTPS2 mutations cause defective regulated intramembrane proteolysis in X-linked osteogenesis imperfecta. Nat. Commun. 7, 11920.
Marini, J.C., Forlino, A., Bachinger, H.P., Bishop, N.J., Byers, P.H., Paepe, A., et al., 2017. Osteogenesis imperfecta. Nat. Rev. Dis. Primers. 3, 17052.
Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 126 (4), 663–676.