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Lab Resource: Single Cell Line:

Generation of human induced pluripotent stem cell line (NIDCRi001-A) from a Muenke syndrome patient with an FGFR3 p.Pro250Arg mutation

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Abstract

Muenke syndrome is the leading genetic cause of craniosynostosis and results in a variety of disabling clinical phenotypes. To model the disease and study the pathogenic mechanisms, a human induced pluripotent stem cell (hiPSC) line was generated from a patient diagnosed with Muenke syndrome. Successful reprogramming was validated by morphological features, karyotyping, loss of reprogramming factors, expression of pluripotency markers, mutation analysis and teratoma formation.

1. Resource utility

Muenke syndrome (MS), caused by a p.Pro250Arg (c.749C>G) gain-of-function mutation in the *FGFR3* gene (Muenke et al., 1996; Wilkie et al., 2013), is a common form of craniosynostosis. Human induced pluripotent stem cells (hiPSCs) from an MS patient may serve as a disease model to elucidate underlying pathogenetic mechanism(s).

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Declaration of competing interest
The authors have no conflicts of interest.

Appendix: Supplementary Materials
1. Mycoplasma test analysis certificate
2. Karyotyping analysis certificate

2. Resource details

The clinical manifestations of MS patients are highly variable with incomplete penetration, ranging from developmental delay and hearing impairment to increased intracranial pressure often requiring surgery for decompression (Kruszka et al., 2016; Twigg et al., 2009). Through surface plasmon resonance and X-ray crystallography, one study demonstrated that fibroblast growth factor receptor 3 (FGFR3) with the MS mutation has enhanced affinity for atypical ligands, such as FGF2 and FGF9 (Ibrahimi et al., 2004). Loss of ligand specificity may potentially be a unique mechanism that separates MS from other FGFR3-related diseases that tend to severely affect long bone growth. However, a detailed description of the aberrant molecular signalling pathways involved in this disease has not yet been reported. The iPSC line, NIDCRi001-A, was generated from a 7-year-old male patient previously diagnosed with MS. Peripheral blood mononuclear cells (PBMCs) were expanded in StemSpan $CD34^+$ Expansion Supplement (Cat# 02691, STEMCELL Technologies Inc) for 9 days to expand $CD34^+$ cells. Reprogramming into iPSCs was accomplished with Sendai virus containing the factors OCT4, SOX2, KLF4 and c-MYC. The resulting line showed appropriate morphology (Fig. 1A); a normal diploid male karyotype (46, XY) (Fig. 1B); and clearance of reprogramming factors by passage 10, confirmed by RT-PCR compared with passage 1 (Fig 1C). NIDCRi001-A expressed pluripotency markers, evaluated by nuclear staining for OCT3/4 and NANOG and by FACS analysis for SSEA4, Tra-1-60, and Tra-1-81 (Fig. 1D, E). Short Tandem Repeat (STR) analysis confirmed that the iPSCs had an identical genetic background as the donor PBMCs (data available from the authors), and Sanger sequencing showed that the *FGFR3* heterozygous missense mutation was present in both cell types (Fig. 1F). Pluripotency was further confirmed by differentiation of hiPSCs into derivatives of all three germ layers through a teratoma assay in immunocompromised mice (Fig. 1G). Mycoplasma testing was negative (Supplementary Data).

3. Materials and Methods

3.1 Reprogramming

PBMCs isolated using lymphocyte separation medium (Lonza) were expanded (9d at 37°C in 5% CO_2) in StemSpan SFEM II/$CD34^+$ Expansion Supplement (STEMCELL Technologies Inc) and reprogrammed with CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific). After 3d, cells were plated on 12-well plates coated with Matrigel® Matrix (Corning) or Vitronectin XF™ (Nucleus Biologics) in complete E8™ medium (ThermoFisher Scientific). Clones were picked (~21d) and expanded in 6-well plates for 10 passages. Subculture (1:6) used 50mM EDTA in calcium-magnesium-free phosphate buffered saline (PBS) for 4-5min every 4-5 days.

3.2 Karyotype analysis

Karyotyping (passage 10) was performed on twenty G-banded metaphase cells at 475 band resolution (Cell Line Genetics).

3.3 Mutation analysis

DNA (100ng/35μL) was PCR-amplified with primers flanking exon 7 of *FGFR3* using high fidelity AccuPrime™ *Taq* DNA Polymerase (ThermoFisher Scientific). Reactions were run in a 96-well thermal cycler: 95°C for 5min, 40 cycles at 95°C for 30sec, 66°C for 30sec, 72°C for 30sec, and a final step at 72°C for 7min. Products were separated on a 1% agarose gel, purified using ExoSAP-IT™ *Express* (ThermoFisher Scientific) and sequenced (Eurofins Clinical Molecular Testing Services).

3.4 STR analysis

Identity analysis was performed by Cell Line Genetics on PBMCs and the iPSC line using the PowerPlex 16 System (Promega).

3.5 RNA isolation and RT-PCR analysis

Clearance of Sendai virus and reprogramming factors was confirmed by RT-PCR after 10 passages. Total RNA (1×10^6 cells) was obtained using the RNeasy Mini Kit (Qiagen). cDNA was generated through iScript™ cDNA Synthesis Kit (Bio-Rad) with *GAPDH* as the control. PCR was performed using GoTaq® Green Master Mix (25μl, Promega) and Proflex PCR System (ThermoFisher Scientific). A three-step PCR for *GAPDH* was performed: denaturation for 2min at 95°C; 35 cycles of 45sec at 95°C, 45sec at 58°C, and 1min at 72°C; 30sec at 72°C). For Sendai virus genome and reprogramming factors: denaturation for 2min at 95°C; 40 cycles of 30sec at 95°C, 30sec at 55°C, and 30sec at 72°C. cDNA from passage 1 was used as a positive control.

3.6 Immunocytochemistry

Cells fixed with 4% paraformaldehyde for 20min at RT and washed 3x with PBS were blocked with 10% normal goat serum (NGS), permeabilized with 0.3% TX-100 for 40min, and washed. Cells were incubated with primary antibodies in 5% NGS/PBS for 1hr, washed, and incubated with secondary antibodies (1hr). After a final wash, nuclei were stained with 0.2μg/ml bisbenzimide in PBS for 10min. An Axiovert 200 (Zeiss) was used for imaging.

3.7 Flow cytometry

Cells released using Accutase™ (STEMCELL Technologies) were passed through a 30μm cell strainer and incubated in 96-well plates with primary antibodies and isotype controls for 30min, followed by incubation with secondary antibodies for another 30min. Analysis was performed using a FACSCalibur™ analyzer, and CellQuest™ Pro software (BD Bioscience).

3.8 Teratoma Assay

iPSCs (1×10^6), released using 50mM EDTA and suspended in 50% Matrigel, were injected subcutaneously into NOD.Cg-*Prkdcscid Il2rgtm1Wjl*/SzJ mice (Jackson Laboratory). After 6wk, teratomas were histologically analyzed to identify derivatives of all three germ layers.

3.9 Mycoplasma detection

Mycoplasma (19 species) and *Acholeplasma* (1 species) were tested for by Cell Line Genetics.

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References

1. Muenke M, Gripp KW, McDonald-McGinn Met al.A unique point mutation in the fibroblast growth factor receptor 3 gene (FGFR3) defines a new craniosynostosis syndrome. Am. J. Hum. Genet, 60 (3) 1997, pp. 555–564 [PubMed: 9042914]
2. Wilkie AO, Byren JC, Hurst JAet al.Prevalence and complications of single-gene and chromosomal disorders in craniosynostosis. Pediatrics, 126 (2) 2010, pp. e391–400 [PubMed: 20643727]
3. Kruszka P, Addissie YA, Yarnell CMet al.Muenke syndrome: An international multicenter natural history study. Am. J. Med. Genet. A170A (4) 2016, pp. 918–929 [PubMed: 26740388]
4. Twigg SR, Healy C, Babbs Cet al.Skeletal analysis of the Fgfr3(P244R) mouse, a genetic model for the Muenke craniosynostosis syndrome. Dev Dyn. 238 (2) 2009, pp. 331–342 [PubMed: 19086028]
5. Ibrahimi OA, Zhang F, Eliseenkova AVet al.Proline to arginine mutations in FGF receptors 1 and 3 result in Pfeiffer and Muenke craniosynostosis syndromes through enhancement of FGF binding affinity. Hum Mol Genet. 13 (1) 2003, pp. 69–78 [PubMed: 14613973]

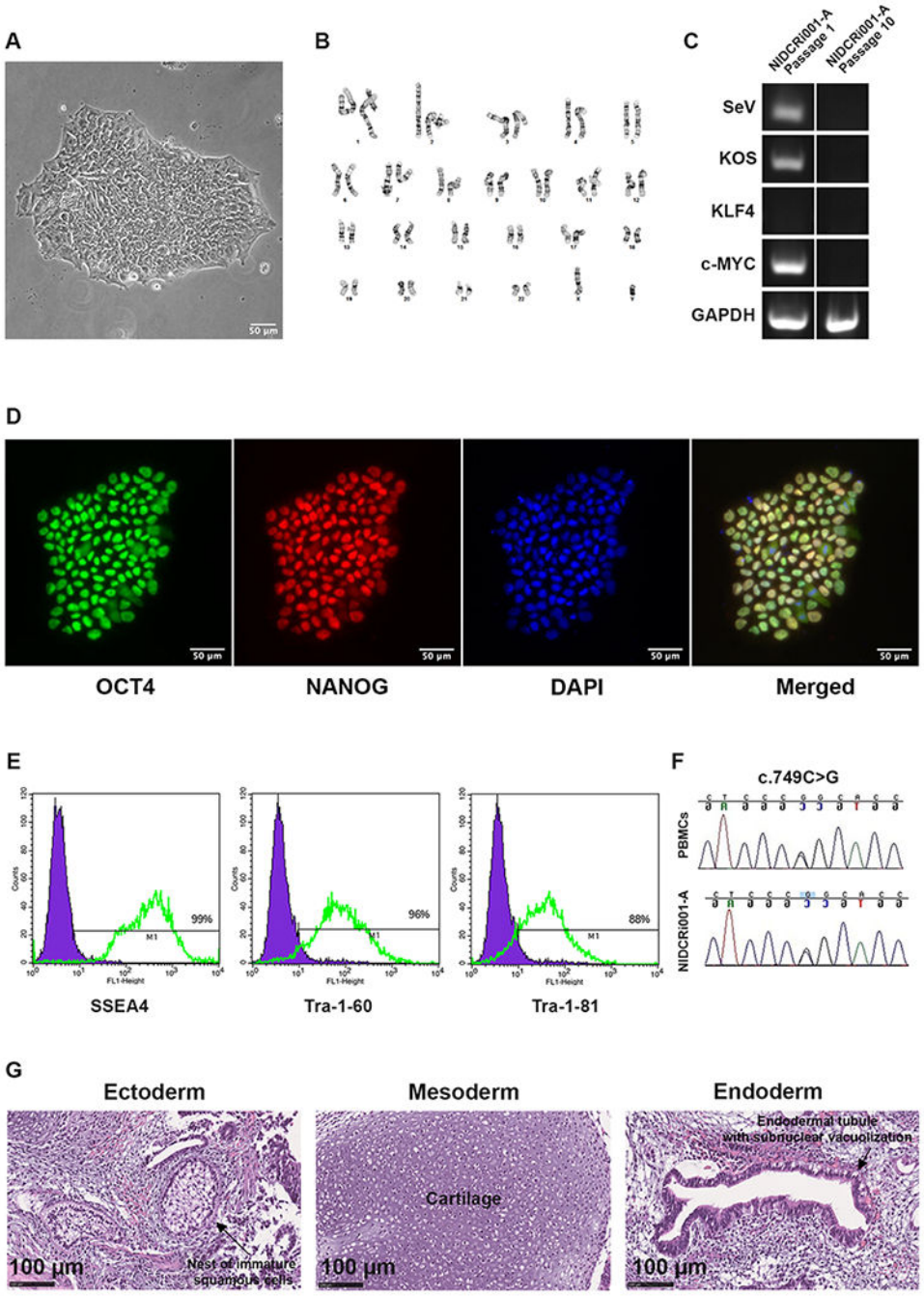

Figure 1:

Table 1:

Characterization and validation

Classification	Test	Result	Data
Morphology	Photography	normal	Figure 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	Positive for expression of pluripotency markers: OCT4, NANOG	Figure 1 panel D
	Quantitative analysis Flow cytometry	SSEA-4 99% Tra-1-60 96% Tra-1-81 88%	Figure 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 475	Figure 1 panel B
Identity	STR analysis	16 *loci* analyzed, all matching	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous missense mutation	Figure 1 panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing negative	Supplementary
Differentiation potential	Teratoma formation	Presence of three embryo germ layers by histology	Figure 1 panel G
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 2:

Reagents details

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Mouse anti-TRA-1-60 (IgM)	1:100	Santa Cruz Biotechnology cat# sc-21705, RRID: AB_628385
	Mouse anti-TRA-1-81 (IgM)	1:100	Santa Cruz Biotechnology cat# sc-21706, RRID: AB_628386
	Mouse anti-SSEA4 (IgG_3)	1:100	Santa Cruz Biotechnology cat# sc-21704, RRID: AB_628289
	Mouse anti-Oct3/4 (IgG_{2b})	1:400	Santa Cruz Biotechnology cat# sc-5279, RRID: RRID: AB_628051
	Rabbit anti-Nanog	1:200	Reprocell cat# RCAB004P-F; RRID: RRID: AB_1560380
Secondary Antibodies	Alexa Fluor 488 goat anti-mouse (IgG)	1:100	ThermoFisher Scientific cat# A-11001, RRID: AB_2534069
	Alexa Fluor 555 rabbit anti-mouse (IgG)	1:500	ThermoFisher Scientific cat# A-21428, RRID: AB_2535849
	Alexa Fluor 488 goat anti-mouse (IgM)	1:1000	ThermoFisher Scientific cat# A-21042, RRID: AB_141357
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Sendai virus detection	*SeV*, 181bp	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC GenBank Accession #NC_001552.1	
Transgene detection	*KOS*, 528bp	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG GenBank Accession #NM_002701.4, NM_003106.2, BC029923.1	
Transgene detection	*KLF4*, 410bp	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA GenBank Accession #BC029923.1	
Transgene detection	*c-MYC*, 532bp	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG GenBank Accession #K02276.1	
House-keeping gene	*GAPDH*, 816bp	AGCCGCATCTTCTTTTGCGTC TCATATTTGGCAGGTTTTTCT GenBank Accession #NG_007073.2	
Mutation sequencing	*FGFR3* (c.749C>G), 191bp	CGGCAGTGGCGGTGGTGGTGA GACCCAAATCCTCACGCAACC GenBank Accession #NG_012632.1	

Resource Table:

Unique stem cell line identifier	NIDCRi001-A
Alternative name(s) of stem cell line	MS-iPSC-1a
Institution	National Institutes of Health, Bethesda, USA
Contact information of distributor	Pamela G. Robey; probey@dir.nidcr.nih.gov Fahad Kidwai; fahad.kidwai@nih.gov
Type of cell line	hiPSC
Origin	human
Additional origin info	Age: 7 years Sex: Male Ethnicity: unknown
Cell Source	PBMCs
Clonality	Clonal
Method of reprogramming	Sendai Virus
Genetic Modification	Yes
Type of Modification	Sporadic mutation
Associated disease	Muenke syndrome
Gene/locus	Gene: *FGFR3* Locus: 4p16.3 Mutation: heterozygous p.Pro250Arg (c.749C>G)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	November 25th, 2019
Cell line repository/bank	https://hpscreg.eu/browse/provider/954
Ethical approval	NIH Combined Neuroscience Institutional Review Board (IRB) (Protocol 16-D-0040)