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Generation of a human Juvenile myelomonocytic leukemia iPSC line, CHOPi001-A, with a mutation in *CBL*

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Abstract

Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative disorder of early childhood characterized by expansion of clonal myelomonocytic cells and hyperactive Ras/MAPK signaling. The disorder is caused by somatic and/or germline mutations in genes involved in the Ras/MAPK and JAK/STAT signaling pathways, including *CBL*. Here we describe the generation of an iPSC line with a homozygous *CBL* c.1111T- > C (Y371H) mutation, designated CHOPJMML1854.

Resource table.

Unique stem cell line identifier	CHOPi001-A
Alternative name(s) of stem cell line	CHOPJMML1854
Institution	The Children's Hospital of Philadelphia (CHOP), Philadelphia, Pennsylvania, USA
Contact information of distributor	Deborah L. French, frenchd@email.chop.edu
Type of cell line	iPSC
Origin	Human

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Additional origin info	Age: 7 months Sex: female
Cell Source	Bone marrow
Clonality	Clonal
Method of reprogramming	Lentivirus
Genetic Modification	No
Type of Modification	N/A
Associated disease	Juvenile myelomonocytic leukemia (JMML)
Gene/locus	CBL/c.1111T- >C
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	May 2017
Cell line repository/bank	N/A
Ethical approval	UCSF Benioff Children's Hospital IRB approval 10–04212 with written informed consent

Resource utility

Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disorder that mainly affects young children, making primary pathological specimens rare and difficult to obtain. The generation of an iPSC line harboring a JMML-associated *CBL* mutation provides a new resource for mechanistic studies and drug testing.

Resource details

Approximately 85% of JMML patients harbor somatic and/or germline mutations in either *CBL, NRAS, KRAS, PTPN11 or NF1* (Stieglitz et al., 2015). These mutations confer myeloid cell hypersensitivity to granulocyte macrophage colony-stimulating factor (GM-CSF). Previously, we showed that this hallmark diagnostic feature is recapitulated in myeloid cells derived from *PTPN11* mutant iPSCs generated from a JMML patient (Gandre-Babbe et al., 2013). In this report, bone marrow mononuclear cells were collected from a JMML patient who was diagnosed with a homozygous *CBL* c.1111T- > C p.Y371H mutation due to loss of heterozygosity (Loh et al., 2009). These cells were transduced with the STEMCCA polycistronic lentivirus expressing OCT4, KLF4, SOX2, cMYC (Somers et al., 2010) and iPSC colonies were generated by standard methods. Data shown represent one of three independent clones that were expanded and characterized. Typical cellular morphology was observed and expression of NANOG, OCT4, and SOX2 was shown by immunocytochemistry (ICC) (Fig. 1A and B). Stem cell surface marker expression, performed at single cell resolution by flow cytometry, included SSEA3, SSEA4, Tra-1–60, and Tra-1–81 (Fig. 1C) and gene expression, performed using QRT-PCR, included *OCT4, NANOG, SOX2,* and *DNMT3B* (Fig. 1D). A normal karyotype (46,XX) was demonstrated by G-band analysis (Fig. 1E) and transgene silencing of OCT4 and KLF4 was demonstrated by QRT-PCR (Fig. 1F). The *CBL* Y371H mutation in the iPSC line was confirmed by DNA sequencing (Fig. 1G), and pluripotency was verified *in vitro* using the Human Pluripotent Stem Cell Functional Identification kit (Fig. 1H). DNA fingerprinting by STR analysis was

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used to authenticate the identity of the iPSC line to the primary patient cells. The cells tested negative for *Mycoplasma* (Supplementary Fig. 1). Myeloid colonies derived from this line showed hypersensitivity to GM-CSF (Tasian et al. in press).

Materials and methods

Cell culture

The iPSCs were cultured at 37 °C in an environment of 5% CO_2, 5% O_2, and 90% N_2. Cells were maintained on irradiated mouse embryonic fibroblasts (MEFs) in human embryonic stem cell (HES) medium consisting of DMEM/F12 (50:50; Mediatech) supplemented with 20% knock-out serum replacement (ThermoFisher Scientific), 10^{-4} M mercaptoethanol (Sigma), and 10 ng/mL bFGF (R&D Systems) in 6-well tissue culture plates. Cells were split every 5–7 days at an approximate ratio of 1:15 in HES media with 10 μM Y-27632, using TrypLE cell dissociation reagent.

Primary cell source and iPSC reprogramming

Ficoll-purified mononuclear cells from bone marrow were transduced using a DOX-inducible version of the human STEMCCA lentivirus expressing OCT4, KLF4, SOX2, and cMYC (Somers et al., 2010) in DMEM containing 10% FBS. After 24 h, 2 μg/mL of DOX was added and cells were plated on MEFs. Four days after transduction, cells were cultured in HES medium containing 5 ng/mL of bFGF and 2 μg/mL of DOX. The medium was replenished every 2–3 days for 3 weeks. Colonies were then mechanically isolated and expanded on MEFs in HES medium without DOX. Analysis for silencing of lentiviral genes was performed on clones between passage 5–7.

Mutation verification

PCR amplification was performed on genomic DNA extracted using the Purelink genomic DNA extraction kit (Invitrogen). Cycling parameters were as follows: 95°C for 10 min, 30 cycles of 95°C 30 s, 55°C 30 s, 72°C 2 min, and a 4 °C hold in a Biometra T3000 Thermocycler (#050–723). Products were sequenced by GENEWIZ, Inc. (South Plainfield, NJ).

Karyotype analysis

Chromosomal G-band analyses were performed at passage 28 by Cell Line Genetics, Inc. (Madison, WI). Twenty metaphase spreads were counted, and nine were analyzed with a 400–450 band resolution reported as fair.

Flow cytometry

Trypsin-dissociated single cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter) and the FlowJo software program (Tree Star, CA). Expression of pluripotency markers were evaluated using the following antibodies: Alexa-Fluor®-647 α-human SSEA4 and Tra-1–81; Alexa-Fluor®-488 SSEA3 and Tra-1–60 (BioLegend). Cells were incubated for 15 min at room temperature, washed, and analyzed.

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Immunocytochemistry

Cells were plated on 1:3 matrigel-coated coverslips at 90% confluency and fixed in 4% paraformaldehyde for 15 min at room temperature. Permeabilization was performed using ice cold methanol at −20°C for 10 min. Blocking and antibody buffers were prepared in 0.3% TritonX-100/PBS with 5% goat serum and 1% BSA, respectively. Cells were blocked at room temperature for 1 h. Primary antibody incubation was performed at 4°C overnight, and secondary antibody incubation at room temperature for 2 h. Coverslips were mounted in Vectashield mounting media with DAPI (Vector Laboratories, Burlingame, CA). Staining was visualized on a Leica DMI4000 B inverted fluorescent microscope.

QRT-PCR

RNA was isolated using the PureLink RNA Mini Kit (Invitrogen), according to the manufacturer's instructions. Reverse transcription was performed on total RNA (500 ng) using random hexamers and Superscript III Reverse Transcriptase (Invitrogen). QRT-PCR was performed on a LightCycler-480II (Roche, IN). All experiments were performed in triplicate using SYBR Select Master Mix (Applied Biosystems), according to the manufacturer's instructions. Human genomic DNA, diluted in 10-fold increments from 1 to 100 ng/μl, was used to generate a standard curve to determine PCR efficiency and relative gene expression compared to the housekeeping gene TBP (TATA Binding Box Protein) or Cyclophilin. Relative expression of pluripotency genes in the patient iPSC line was compared to embryonic stem cells (H9 and CHB8) and a control iPSC line (CHOPWT6). All cells were maintained in HES media as described in the cell culture section.

In vitro germ layer differentiation

The Human Pluripotent Stem Cell Functional Identification Kit (R& D Systems, #SC027) was used to differentiate the CHOPJMML1854 iPSCs *in vitro* into the three germ layers. The germ layers were identified by immunocytochemistry using SOX17 for endoderm, Otx2 for ectoderm, and Brachyury for mesoderm, per manufacturer's instructions (Tables 1 and 2).

STR analysis

The genetic integrity of the iPSC line was confirmed by DNA fingerprinting. PCR amplification was performed on genomic DNA extracted from patient cells and the iPSC line. Products were separated on a 3.0% agarose gel and visualized with ethidium bromide.

Mycoplasma testing

Testing for mycoplasma contamination was performed using the Sigma LookOut Mycoplasma PCR Detection Kit according to manufacturer's instructions.

Supplementary Material

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References

Gandre-Babbe S, Paluru P, Aribeana C, et al. , 2013. Patient-derived induced pluripotent stem cells recapitulate hematopoietic abnormalities of juvenile myelomonocytic leukemia. Blood 121, 4925–4929. [PubMed: 23620576]

Loh ML, Sakai DS, Flotho C, et al. , 2009. Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood 114, 1859–1863. [PubMed: 19571318]

Somers A, Jean JC, Sommer CA, et al. , 2010. Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. Stem Cells 28, 1728–1740. [PubMed: 20715179]

Stieglitz E, Taylor-Weiner AN, Chang TY, et al. , 2015. The genomic landscape of juvenile myelomonocytic leukemia. Nat. Genet. 47, 1326–1333. [PubMed: 26457647]

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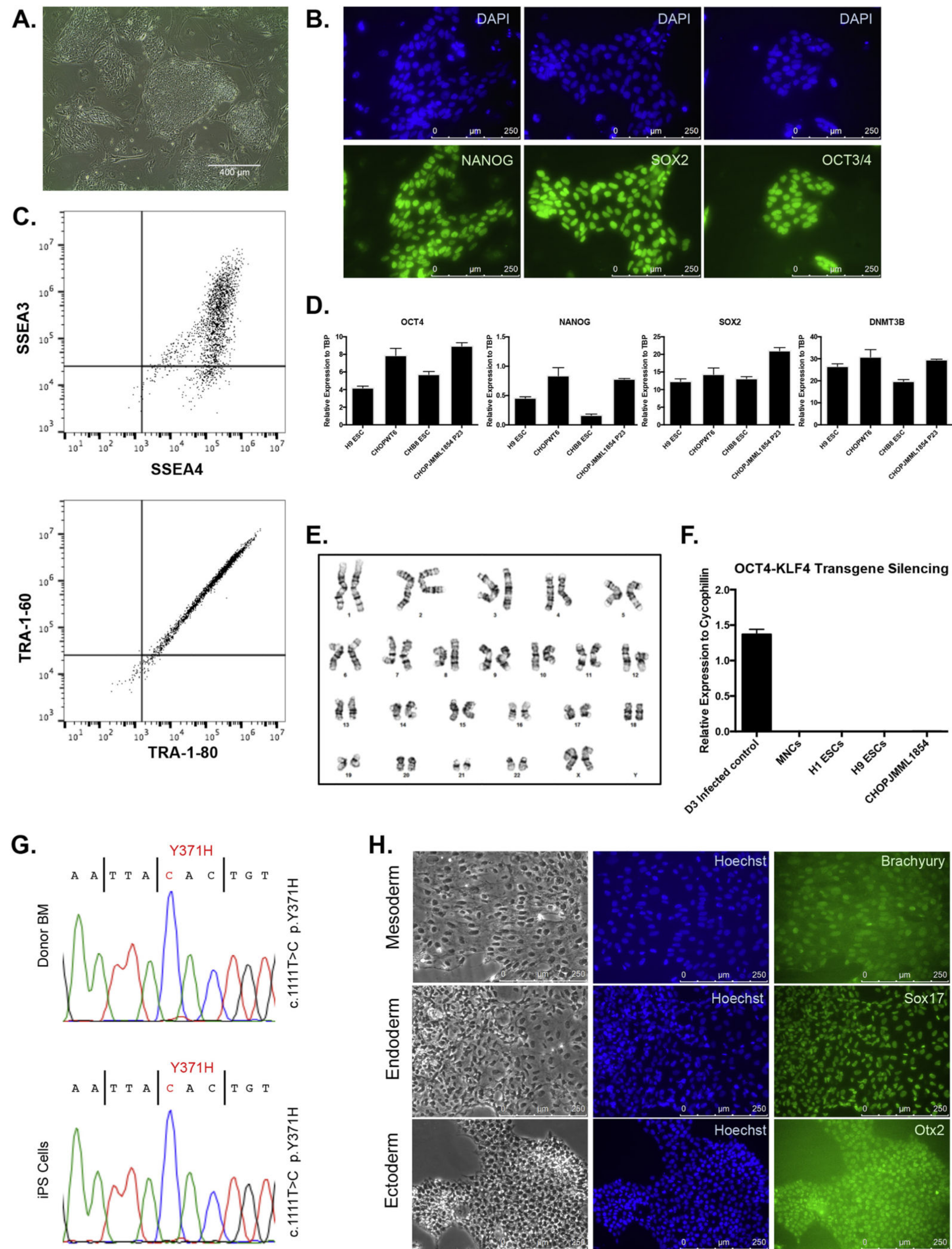

Fig. 1.

Characterization of human iPSC line CHOPJMML1854

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Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Positive for OCT4, NANOG, and SOX2	Fig. 1B
	Flow cytometry	SSEA3/4: 87%, Tra-1–60/81: 95%	Fig. 1C
	QRT-PCR	*OCT4, NANOG, SOX2, DNMT3B*	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	46,XX	Fig. 1E
		Resolution: 400–450	
Identity	STR analysis	4 sites tested and matched for iPSCs and primary bone marrow cells	Submitted in archive with journal
Mutation analysis	Sequencing	Homozygous c.1111T > C p.Y371H in donor bone marrow cells and iPSCs	Fig. 1G
Microbiology and virology	Mycoplasma	Negative by LookOut Mycoplasma PCR Detection Kit (Sigma, #MP0035)	Supp Fig. 1
Differentiation potential	*In vitro* differentiation	AF488-positive staining of markers Brachyury, Sox17, and Otx2 for mesoderm, endoderm, and ectoderm, respectively	Fig. 1H
Donor screening	HIV 1+2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

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Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers (Flow cytometry)	SSEA-3	1:50	Biolegend, Cat# 330306, RRID: AB_1279440
	SSEA-4	1:400	Biolegend, Cat# 330408, RRID: AB_1089200
	Tra-1-60	1:50	Biolegend, Cat# 330614, RRID: AB_2119064
	Tra-1-81	1:50	Biolegend, Cat# 330706, RRID: AB_1089242
Stemness Markers (ICC)	OCT3/4	1:200	Santa Cruz, Cat# sc-5279, RRID: AB_628051
	Nanog	1:400	Cell Signaling, Cat# 4903S, RRID: AB_10559205
	SOX2	10μg/mL	R&D, Cat# AF2018, RRID: AB_355110
Differentiation Markers (ICC)	Brachyury for mesoderm	1:40	R&D, Cat# AF2085, RRID: AB_2200235
	Sox17 for endoderm	1:40	R&D, Cat# AF1924, RRID: AB_355060
	Otx2 for ectoderm	1:50	R&D, Cat# AF1979, RRID: AB_2157172
	Hoechst 33342	1 μg/μl	Thermo Fisher Scientific, Cat# 62249
Secondary Antibodies (ICC)	Goat anti-Rabbit IgG (H+L) AF647	1:400	Thermo Fisher Scientific, Cat# A-21245, RRID: AB_2535813
	Goat anti-mouse IgG (H+L) AF488	1:400	Thermo Fisher Scientific, Cat# A-11029, RRID: AB_2534088
	Donkey anti-goat IgG FITC	1:800	Santa Cruz, Cat# sc-2024, RRID: AB_631727 (discontinued)
	Donkey anti-goat IgG (H+L) AF488	1:800	Thermo Fisher Scientific, Cat# A-11055, RRID: AB_2534102

Primers		
	Target	Forward/reverse primer (5′–3′)
Pluripotency Markers (qRT-PCR)	*OCT4*	AACCTGGAGTTTGTGCCAGGGTTT/TGAACTTCACCTTCCCTCCAACCA
	NANOG	CCTGAAGACGTGTGAAGATGAG/GCTGATTAGGCTCCAACCATAC
	SOX2	ATGACCAGCTCGCAGACCTACA/GGACTTGACCACCGAACCCA
	DNMT3B	TACAGACGTGTGCAGTTGTAGGCA/GTGCAGACTCCAGCCCTTGTATTT
House-Keeping Gene (qRT-PCR)	*TBP*	TTGCTGAGAAGAGTGTGCTGGAGATG/CGTAAGGTGGCAGGCTGTTGTT
Targeted mutation analysis/sequencing	*CBL* (PCR, 315 bp)	GGACCCAGACTAGATGCTTT/CGCTGTTTAGATCCGTAC
	CBL (sequencing)	TGTCCACAGGGCTCAATCTT
STR analysis (PCR)	MCT118 (DS180)	GAAACTGGCCTCCAAACACTGCCCGCCG/GTCTTGTTGGAGATGCACGTGCCCCTTGC

	D17S5	AAACTGCAGAGAGAAAGGTCGAAGAGTGAAGTG/AAAGGATCCCCCACATCCGCTCCCCAAGTT
	D10S1214	ATTGCCCCAAAACTTTTTTG/TTGAAGACCAGTGTCGGAAG
	D17S1290	GCCAACAGAGCAAGACTGTC/GGAAACAGTTAAATGGCCAA

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