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Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line, BRCi009-A, derived from a patient with glycogen storage disease type 1a

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A B S T R A C T

Glycogen storage disease type 1a (GSD1a) is an autosomal recessive disorder caused by mutations of the glucose-6-phosphatase (*G6PC*) gene. Mutations of the *G6PC* gene lead to excessive accumulation of glycogen in the liver, kidney, and intestinal mucosa due to the deficiency of microsomal glucose-6-phosphatase. Human induced pluripotent stem cells (iPSCs) enable the production of patient-derived hepatocytes in culture and are therefore a promising tool for modeling GSD1a. Here, we report the establishment of human iPSCs from a GSD1a patient carrying a *G6PC* mutation (c.648G > T; p.Leu216 =).

1. Resource Table:

Unique stem cell line identifier	BRCi009-A
Alternative name(s) of stem cell line	HPS3926
Institution	RIKEN BioResource Research Center (BRC), Kyoto, Japan
Contact information of distributor	Haruhisa Inoue haruhisa.inoue@riken.jp
Type of cell line	iPSC
Origin	Human
Additional origin info	Applicable for human iPSC Age: 31 Sex: female Ethnicity if known: Japanese
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus vectors (KLF4, OCT3/4, SOX2, c-Myc)
Genetic Modification	None
Type of Modification	None
Associated disease	Glycogen storage disease type 1a (GSD1a)
Gene/locus	*G6PC* gene/Chromosome 17
Method of modification	Not available
Name of transgene or resistance	Not available
Inducible/constitutive system	Not available
Date archived/stock date	January 2020
Cell line repository/bank	RIKEN BioResource Research Center (BRC), Japan http://en.brc.riken.jp/index.htmlcellbank. brc@riken.jp
Ethical approval	Ethics Committee of the RIKEN BioResource Research Center (Approval No. Tsukuba 29–1)

1.1. Resource utility

GSD1a is an autosomal recessive disorder caused by mutations of the *G6PC* gene. The disease pathophysiology is not well understood. iPSCs were generated from a GSD1a patient with a *G6PC* gene mutation (c.648G > T; p.Leu216 =). This disease-specific iPSC line will be used to study the pathological mechanisms of GSD1a (Table 1).

1.2. Resource details

Glycogen storage disease type 1a (GSD1a) is an autosomal recessive

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry)	Positive staining of the pluripotency markers NANOG and SSEA-4	Fig. 1 panel A
	Quantitative analysis (Flow cytometry)	99.8% of cells positive for pluripotency marker, SSEA-4	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 500	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Available from the authors
Mutation analysis	Sequencing	Homozygous *G6PC* mutation (c.648 G > T, p. Leu216 =)	Fig. 1 panel E
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by indirect staining and RT-PCR. Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Proof of formation of the three germ layer type cells: expression of βIII-Tubulin (ectoderm), alpha smooth muscle actin (mesoderm) and SOX17 (endoderm)	Fig. 1 panel C
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

disorder caused by mutations of the glucose-6-phosphatase (*G6PC*) gene. Mutations of the *G6PC* gene lead to excessive accumulation of glycogen in the liver, kidney, and intestinal mucosa due to the deficiency of microsomal glucose-6-phosphatase (Akanuma et al., 2000; Kanungo et al., 2018). The clinical manifestations of GSD1a include short stature, doll-like face, hepatomegaly, hypoglycemia, hyperuricemia, and lactic acidemia. However, the disease pathophysiology is largely unknown. GSD 1a-specific iPSCs have the potential for investigation of the pathophysiological mechanisms of GSD 1a. We established and characterized an iPSC line from a patient with attenuated GSD 1a who carried a *G6PC* gene mutation (c.648G > T; p.Leu216 =). GSD1a-specific iPSCs were generated from fibroblasts of the GSD1a patient using Sendai virus vectors carrying reprogramming factors (Fujie et al., 2014). The GSD1a-specific iPSCs (HPS3926) had a human ES cell-like morphology (Fig. 1A). The iPSCs were immunocytologically positive for the pluripotency markers NANOG and SSEA4 (Fig. 1A); 99.8% of the iPSCs were positive for the pluripotency marker SSEA4 by flow cytometry (Fig. 1B). The capacity of the iPSCs to differentiate into the three germ layers was confirmed using an *in vitro* embryoid body (EB) formation assay (Fig. 1C; βIII-tubulin, ectoderm; smooth muscle actin (SMA), mesoderm; SOX17, endoderm). Furthermore, the iPSCs retained a normal karyotype (Fig. 1D), and carried the *G6PC* gene mutation (Fig. 1E). The identity of the cell line was verified with an STR analysis (information available from the authors). Mycoplasma contamination was not detected in the cell culture (Supplementary Fig. 1). Established iPSCs were able to differentiate into hepatocytes that showed positive staining for alpha-fetoprotein (AFP) (Fig. 1F)

2. Materials and methods

2.1. Ethics statement

The generation and use of human iPSCs was approved by the Ethics Committees of RIKEN BioResource Research Center (BRC). Formal informed consent was obtained from the patient.

2.2. Generation of iPSCs

Human cDNAs for reprogramming factors were transduced into the fibroblasts using Sendai virus vectors (KLF4, OCT3/4, SOX2, c-Myc). The generated iPSCs were cultured under feeder-free conditions on iMatrix-511 (Nippi, Tokyo, Japan)-coated plates with StemFit (AK02N, Ajinomoto, Tokyo, Japan) (Nakagawa et al., 2015).

2.3. Karyotyping

A G-band analysis was performed by LSI Medience (Tokyo, Japan) to determine the karyotype of the iPSC line at passage number 6. Twenty metaphase plates were analyzed.

2.4. Genotyping

Genomic DNA from undifferentiated iPSCs at passage number 27 was extracted using a PureLink Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific) and amplified by PCR using the enzyme KOD Plus Neo (TOYOBO, Japan). The targeted PCR product was directly sequenced.

2.5. In vitro three germ layer differentiation assay

Embryoid bodies (EBs) were produced by culturing 9,000 dissociated iPSCs in DMEM/F12 medium supplemented with 20% knockout serum replacement (KSR), 2 mM L-glutamine, 0.1 mM non-essential amino acid (NEAA) supplement, 0.1 mM 2-mercaptoethanol (Thermo Fisher Scientific), 10 μM Y-27632 (Nacalai Tesque, Kyoto, Japan) for 11 days, followed by culture in DMEM containing 10% FBS (Thermo Fisher Scientific) on a Matrigel (BD Bioscience)-coated tissue culture plate for 7 days.

2.6. Hepatocyte differentiation

Dissociated iPSCs were plated onto a Matrigel coated plate, and cultured in RPMI 1640 Medium, GlutaMAX™ supplement, with B27 supplement (Minus Vitamin A: $B27^{VitA-}$) (Thermo Fisher Scientific), 5% KSR, 1% DMSO, 100 ng/ml of human recombinant Activin A (Wako Pure Chemical Industries Ltd.), 3 μM CHIR99021 (STEMCELL Technologies) and 10 μM Y-27632 for 3 days. The cells were then cultured in RPMI 1640 medium, GlutaMAX™ supplement with $B27^{VitA-}$, 5% KSR, 1% DMSO, 2 mM valproic acid, 20 ng/ml of BMP4 and 20 ng/ml of FGF4 (Wako Pure Chemical Industries Ltd.) for 3 days. Next, the cells were cultured in RPMI1640 medium, GlutaMAX™ supplement with $B27^{VitA-}$, 5% KSR, 20 ng/ml hepatocyte growth factor (HGF; R&D Systems) and 2 mM valproic acid for 4 days. Finally, the cells were cultured in hepatocyte culture medium (Lonza) with 20 ng/ml of oncostatin M (Wako Pure Chemical Industries Ltd.) for 5 days.

2.7. Immunocytochemistry

Cells were fixed in 4% paraformaldehyde (Nacalai Tesque). The fixed cells were incubated with blocking buffer composed of PBS containing 5% Blocking-One-histo (Nacalai Tesque) and 0.1% Triton-X 100 (Nacalai Tesque). Nuclei were stained with DAPI (Invitrogen, Thermo

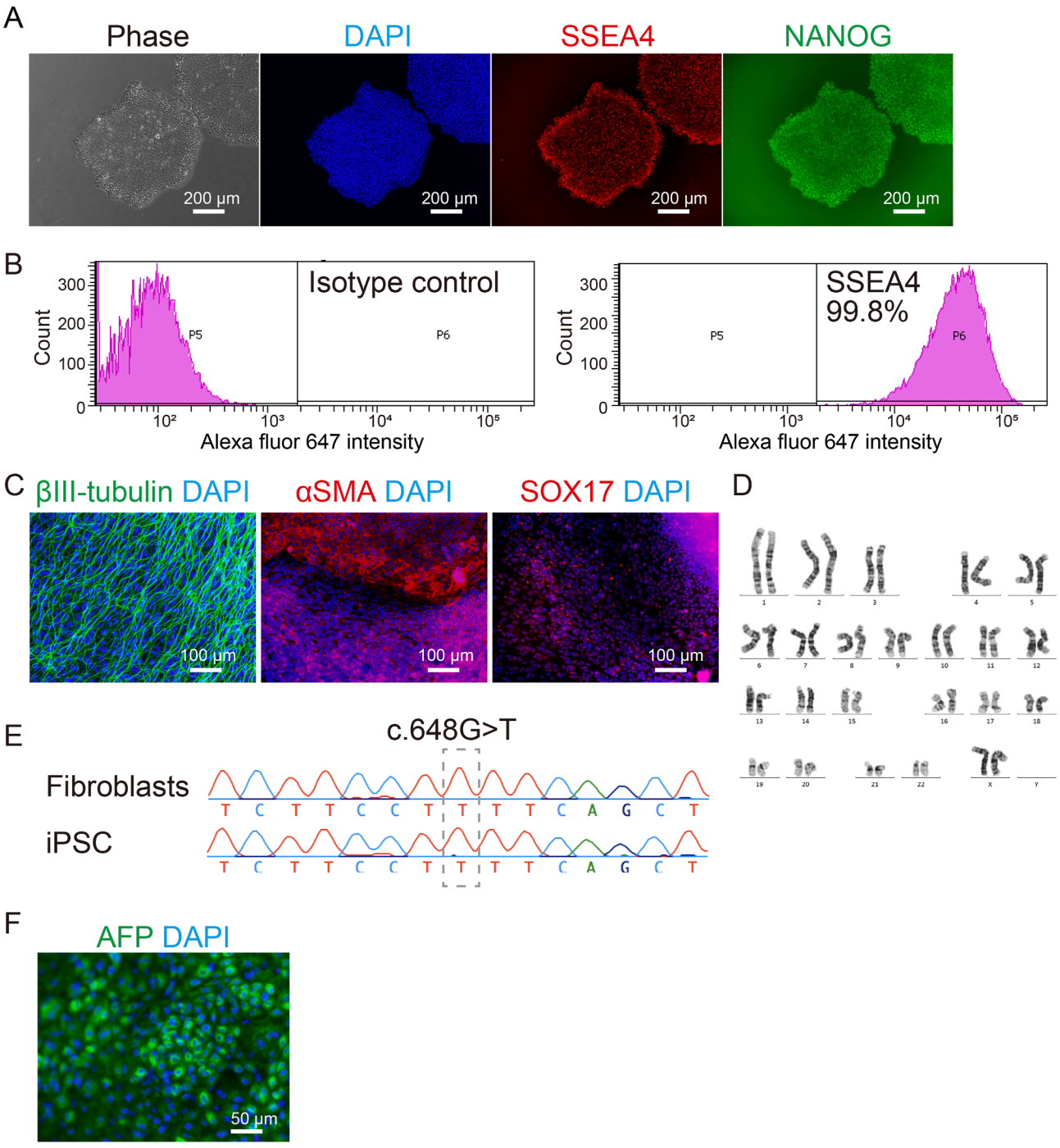

Fig. 1.

Fisher Scientific). Images were obtained with a BZ-X710 microscope (Keyence, Osaka, Japan).

2.8. Flow cytometry

The iPSCs were dissociated with Accumax solution (Sigma Aldrich, St. Louis, MO). The dissociated iPSCs were suspended in PBS containing 2% FBS (Stain buffer, BD Bioscience, San Jose, CA) at a density of 1.0×10^6 cells/ml. The cells were stained for 30 min on ice and analyzed using a FACS Aria (BD Bioscience). The Alexa Fluor 647-labeled antibodies used for flow cytometry are listed in Table 2. The FlowJo software program (ver.10, FLOWJO, BD Bioscience) was used for data analysis.

2.9. STR analysis

STR analysis was performed using a PowerPlex® 16 System (Promega, Madison, WI).

2.10. Mycoplasma test

iPSC culture medium was tested by staining with Hoechst33258 (Thermo Fisher Scientific) after 6 days of co-culture with VERO cells (RCB0001, RIKEN BRC Cell Bank) as mycoplasma negative indicator cells. Nested-PCR was performed using AmpliTaq Gold 360 DNA Polymerase (Thermo Fisher Scientific) to detect mycoplasma contamination.

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-Stage-Specific Embryonic Antigen-4 (SSEA-4)	1:1,000	Millipore Cat# MAB4304 RRID: AB_177629
Pluripotency marker	Rabbit anti-NANOG	1:500	Cell Signaling Technology Cat# 3580 RRID:AB_2150399
Differentiation marker (Ectoderm)	Mouse anti-Tubulin βIII	1:1,000	Millipore Cat# MAB1637 RRID: AB_2210524
Differentiation marker (Mesoderm)	Mouse anti-alpha-Smooth Muscle Actin (αSMA)	1:500	DAKO Cat# MAB1637 RRID: AB_2210524
Differentiation marker (Endoderm)	Goat anti-SOX17	1:1,000	R&D systems Cat# AF1924 RRID: AB_355060
Differentiation marker (Hepatocyte)	Mouse anti-α-Fetoprotein (AFP) Clone C3	1:1,000	Sigma-Aldrich Cat# A8452 RRID: AB_258392
Secondary antibody	Goat Anti-Mouse IgG Alexa Fluor Plus 488	1:1,000	Thermo Fisher Scientific Cat# A32723 RRID: AB_2633275
Secondary antibody	Goat Anti-Rabbit IgG Alexa Fluor 488	1:1,000	Thermo Fisher Scientific Cat# A11034 RRID: AB_2576217
Secondary antibody	Chicken Anti-Mouse IgG Alexa Fluor 647	1:1,000	Thermo Fisher Scientific Cat# A21463 RRID: AB_2535869
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Genotyping	*G6PC* Exon 5 (839 bp)	CACATGGGAATAAGCCAGGC/ ATGGGAATAAGCCAGGCGAC	
Nested-PCR, 1st step PCR (MCGpF11/ MCGpR1)	Mycoplasma detection (200–400 bp)	ACACCATGGGAG(C/T)TGGTAAT/ CTTC(A/T)TCGACTT(C/T) CAGACCCAAGGCAT	
Nested-PCR, 2nd step PCR (R16–2/ MCGpR21)	Mycoplasma detection (200–400 bp)	GTG(C/G)GG(A/C) TGGATCACCTCCT/ GCATCCACCA (A/T)A(A/T)AC(C/T)CTT	

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102095.

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