Stem Cell Research 24 (2017) 25–28

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Derivation of a disease-specific human induced pluripotent stem cell line from a biliary atresia patient

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ARTICLE INFO

Article history:
Received 25 July 2017
Accepted 3 August 2017
Available online 8 August 2017

ABSTRACT

Biliary atresia (BA) is a common cause of pediatric end-stage liver disease. While its etiology is not yet clear, evidence has suggested that BA results from interactions between genetic susceptibility and environmental factors. Disease relevant human cellular models of BA will facilitate identification of both genetic and environmental factors that are important for disease prevention and treatment. Here we report the generation of a human induced pluripotent stem cell line from a BA patient using episomal vectors. Patient-specific BA iPSC lines provide valuable tools for disease mechanism study and drug development.

Resource table

Unique stem cell line identifier	JHUi001-A
Alternative name(s) of stem cell line	BA08.1
Institution	Johns Hopkins University, Baltimore, USA
Contact information of distributor	Yoon-Young Jang, yjang3@jhmi.edu
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 2 year Sex: M
Cell source	Peripheral blood
Method of reprogramming	Integration-free, episomal plasmid transfection
Genetic modification	NO
Type of modification	N/A
Associated disease	Biliary Atresia
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	The Johns Hopkins Medicine Institutional Review Boards (approval number: IRB00083753)

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http://dx.doi.org/10.1016/j.scr.2017.08.001

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal human pluripotent stem cell morphology	Not shown but available with author
Phenotype	Immunocytochemisty	Staining for expression of pluripotency markers: OCT4, NANOG and TRA-1-60	Fig. 1 panel A–C
	Flow cytometry	Assess cell surface marker expression: SSEA-3: 91%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46,XY Resolution 475–525	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR)	Not performed	–
	STR analysis	PowerPlex 16HS (Promega) 16 sites. Match with donor	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	–	–
	Southern Blot OR WGS	–	–
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative.	Fig. 1 panel H
Differentiation potential	Embryoid body formation	Expression of genes in embryoid bodies: smooth muscle actin, Tuj-1 β-tubulin and α-feto protein.	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 hepatitis B, Hepatitis C	–	–
Genotype additional info (OPTIONAL)	Blood group genotyping	–	–
	HLA tissue typing	–	–

Resource utility

We generated this patient-specific iPSC line to develop human cellular disease models of biliary atresia, a type of cholangiopathy possibly resulting from perturbed early development of the biliary system (Hartley et al., 2009). BA iPSC lines can be used to study molecular mechanisms underlying the disease and to discover contributing environmental factors (Tables 1 and 2).

Resource details

Peripheral blood cells isolated from a 2-year old biliary atresia patient were cultured under an erythroblast expansion condition before reprogramming. Non-integrating episomal plasmids expressing OCT4, SOX2, KLF4, c-MYC and BCL-XL were used to generate iPSCs. Five independent iPSC-like clones were picked and expanded. One iPSC line BA08.1 was expanded and characterized for its identity and stem cell property. When cultured in feeder-free conditions on plates coated with either matrigel or vitronectin, the iPSC line displays typical morphology of human pluripotent stem cells. The iPSC line expresses pluripotency-related transcription factors OCT4 (Fig. 1A) and NANOG (Fig. 1B) as well as cell surface markers TRA-1-60 (Fig. 1C) and SSEA3 (Fig. 1D). Pluripotency of BA08.1 was assessed by *in-vitro* embryoid body formation assay. Cells expressing markers for endoderm (α-fetoprotein), mesoderm (smooth muscle actin) and ectoderm (Tuj-1 β III-tubulin) were detected in day-10 embryoid bodies (Fig. 1E). Karyotyping analysis of the cell line demonstrates a normal male karyotype (46,XY) (Fig. 1F). We also examined the presence of episomal plasmid DNA in the established iPSC lines using a pair of PCR primers specific to the EBNA sequence that is common in all three reprogramming plasmids. PCR amplifications of plasmid DNA controls and iPSC genomic DNA show that the level of vector DNA in BA08.1 cell line is below detection limit by passage 11, even though EBNA DNA can be detected at passage 4 (Fig. 1G). To confirm the identity of this iPSC line, DNA profiling was conducted using a short tandem repeat (STR) typing assay that includes 15 STR loci and amelogenin. Data from STR analysis demonstrate a complete match between BA08 iPSC line and fibroblastic cells isolated from the patient (data archived but not shown). The iPSC culture is also shown to be free from mycoplasma contamination by MycoAlert™ mycoplasma detection kit (Fig. 1H).

Materials and methods

Peripheral blood mononuclear cell expansion and reprogramming

Mononuclear cells were isolated from patient blood by Ficoll gradient centrifugation and cultured for 10 days in serum-free medium containing SCF, IL-3, EPO and transferrin (Chou et al., 2015). At the end of expansion, the cells were transfected with plasmids MOS (expressing OCT4 and SOX2, addgene plasmid #64120), MMK (expressing c-MYC and KLF4, addgene plasmid #64121) and GBX (expressing BCL-XL, addgene plasmid #64123) using 4D Nucleofector (Lonza) (Chou et al.,

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-OCT4	1:200	Millipore Cat# MAB4401, RRID:AB_2167852
Pluripotency marker	Mouse anti-NANOG	1:100	BD Pharmingen Cat# 560109, RRID:AB_1645597
Pluripotency marker	Mouse anti-TRA-1-60	1:200	Millipore Cat# MAB4360, RRID:AB_11211864
Pluripotency marker	Alexa Fluor 488 anti-human/mouse SSEA-3	1:20	BioLegend Cat# 330306, RRID:AB_1279440
Differentiation marker	Mouse anti-SMA IgG2a	1:200	Sigma-Aldrich Cat# A5228, RRID:AB_262054
Differentiation marker	Mouse anti-Tuj-1 IgG, Alexa Fluor 488 labeled	1:800	Covance Research Products Inc. Cat# A488-435L, RRID:AB_10143904
Differentiation marker	Rabbit anti-AFP IgG	1:200	Dako Cat# A0008, RRID:AB_2650473
Secondary antibody	Alexa Fluor 488 goat anti-mouse IgG (H + L)	1:500	Invitrogen Cat #A11001, RRID:AB_2534069
Secondary antibody	Alexa Fluor 555 goat anti-mouse IgM	1:500	Invitrogen Cat# A21426, RRID:AB_2535847
Secondary antibody	AF555 Donkey anti-rabbit IgG	1:500	Thermo Fisher Scientific Cat# A-31572, RRID:AB_162543
Secondary antibody	AF555 Goat anti-mouse IgG2a	1:500	Thermo Fisher Scientific Cat# A-21137, RRID:AB_2535776

Primers		
	Target	Forward/reverse primer (5′–3′)
Episomal Plasmids (qPCR)	EBNA-1 sequence in episomal plasmids	TTTAATACGATTGAGGGCGTCT/GGTTTTGAAGGATGCGATTAAG