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Establishment of an induced pluripotent stem cell line (FDEENTi002-A) from a patient with Best's disease carrying c.888C > A mutation in *BEST1* gene

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Abstract

Best's disease (BD) is an inherited retinal degenerative disease caused by mutations in *BEST1* gene. A human induced pluripotent stem cell (iPSC) line has been generated with integration-free Sendai virus method from peripheral blood mononuclear cells (PBMCs) of a BD patient carrying c.888C > A mutation in *BEST1* gene. This cell line may serve as a model for the study of pathogenesis of BD.

Resource utility

Best's disease (BD) is an inherited degenerative maculopathy with no concrete therapies in human, which results in progressive loss of central vision. The FDEENTi002-A iPSC line may serve as a useful model for the pathogenesis study and drug screen of BD.

Resource details

Best's disease (BD) is an autosomal dominant form of macular degeneration characterized by abnormal accumulation of lipofuscin-like materials within and beneath retinal pigment epithelium (RPE), which leads to progressive loss of central vision. It has been proved to be caused by mutations in *BEST1* gene, which encodes BESTROPHIN1 (BEST1), a protein predominantly expressed in RPE (Petrukhin et al., 1998). Although a variety of mutations have been reported among BD patients, the mechanisms still remain unclear. In addition, different animal models reach a diversity of conclusions about the function of BEST1

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protein in RPE, all of which underlines the need for a more suitable model for BD (Johnson et al., 2017).

For this purpose, we established a BD-specific induced pluripotent stem cell (iPSC) line from a male patient carrying a heterozygous c.888C > A mutation in *BEST1* gene, which results in the substitution of lysine for asparagine at condon 296 (p.N296K) (Boon et al., 2007). Peripheral blood mononuclear cells (PBMCs) were collected and reprogrammed by Sendai viral vectors expressing OCT4, SOX2, KLF4 and C-MYC (Ban et al., 2011). The newly established iPSC line, which wastermed FDEENTi002-A, presented a typical human embryonic stem cell (hESC) morphology (Fig. 1A) and was positive for alkaline phosphatase staining (Fig. 1B). The mutation of *BEST1* gene in FDEENTi002-A was confirmed by Sanger sequencing of the PCR product (Fig. 1C). The iPSC line showed a normal diploid 46, XY karyotype (at passage 8) (Fig. 1D) and was genetically authenticated with the donor's PBMCs by short tandem repeat (STR) analysis. The expression of major pluripotent markers (OCT4, SOX2, NANOG) was examined by immunocytochemical staining (Fig. 1F). Quantitative polymerase chain reaction (qPCR) proved that OCT4, SOX2 and NANOG mRNA expression was at similar levels between FDEENTi002-A iPSC and hESC and was absent in the parental PBMCs (Fig. 1E). Trilineage differentiation potential was confirmed in vitro by the expression of ectoderm (PAX6), mesoderm (MESP1) and endoderm (FOXA2) markers (Fig. 1G). The absence of SeV genome and transgenes was confirmed at passage 11 by RT-PCR (Fig. 1H). Mycoplasma contamination was excluded by PCR testing (Fig. 1I, Table 1).

Materials and methods

Cell culture and reprogramming

PBMCs were isolated from the peripheral blood sample using Histopaque®−1077 (Sigma-Aldrich) and cultured in StemSpan SFEMIImedium (Stem Cell Technologies) supplemented with 100 ng/mL SCF, 100 ng/mL FLT-3 L, 20 ng/mL IL-3 and 20 ng/mL IL-6 cytokines (Peprotech). Five days later, the cells were counted and transduced using CytoTune®-iPS 2.0 Sendai reprogramming vectors (Thermo Fisher) following the manufacturer's instruction. The transduced cells were then plated onto irradiated mouse embryonic fibroblasts (MEFs) and cultured in mTeSR™1 medium (Stem Cell Technologies) which was changed every other day. Around day19 post-transduction, ESC-like colonies appeared and were manually picked on day25 post-transduction. The iPSCs were cultured on Matrigel (Corning)-coated plates in mTeSR™1 medium at 37 °C with 5% CO_2 and routinely passaged at 1:3 ratio using dispase (Stem Cell Technologies) every 4–6 days. The iPSCs were frozen in CryoStor® CS10 freezing medium and thawed with 10 μM Y-27632 (Stem Cell Technologies). hESC (H7 [Wi Cell Research Institute, Madison, WI, USA]) was cultured in parallel with FDEENTi002-A.

Alkaline phosphatase (AP) staining

Cells were fixed in 4% PFA for 1–2 min, and stained for alkaline phosphatase according to Alkaline Phosphatase Detection Kit protocol (Innovative Cellular Therapeutics, China).

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Immunocytochemistry

Cells were fixed in 4% PFA for 15 min, permeabilized with 0.3% Triton X-100 for 15 min, and blocked in 4% bovine serum albumin (BSA) for 30 min at room temperature. Then, they were treated with primary antibodies at 4 °C overnight and stained with secondary antibodies for 1 h at room temperature. Both primary and secondary antibodies were diluted in 1% BSA. Nuclei were stained with Hoechst (Thermo Fisher) for 5 min at room temperature. Images were taken by inverted fluorescence microscope (Leica Microsystems, Germany). Antibodies used are listed in Table 2.

In vitro spontaneous differentiation

The iPSCs ready for differentiation were cultured to around 70% confluency. Then, they were harvested using ACCUTASE™ (Stem Cell Technologies) and plated for trilineage differentiation according to the STEMdiff™ Trilineage Differentiation Kit protocol (Stem Cell Technologies). Five or seven days later, the cells were fixed for assessing lineage specific markers by immunocytochemistry.

RT-PCR and qPCR analysis

Total RNA was extracted using TRIzol® Reagent (Thermo Fisher). 500 ng RNA was reverse transcribed into cDNA using PrimeScript™ RT Master Mix (Takara). RT-PCR was performed on the S1000 Thermal Cycler (BIO-RAD) using EasyTaq® PCR SuperMix (Transgen, China) and analyzed by agarose gel electrophoresis. The cycle parameters were as follows: 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. qPCR was conducted on ViiA™ 7 Real-Time PCR System (Applied Biosystems) and analyzed using the ΔΔCT method. Primers used are listed in Table 2.

BEST1 mutation analysis

gDNA was extracted from FDEENTi002-A cells using a commercial kit from CORNING (AP-EMN-BL-GDNA-250G). The mutation region of BEST1 exon 8 was amplified by PCR on the S1000 Thermal Cycler (BIORAD) using EasyTaq® PCR SuperMix (Transgen, China). The primers used are listed in Table 2. Sequencing of the PCR products (531 bp) was performed by Sangon Biotech (Shanghai) Co., Ltd..

Karyotyping

FDEENTi002-A cells in the logarithmic phase at passage 8 were treated with colchicine for 4 h and then dissociated into single cells using Accutase (Stem Cell Technologies). The standard G-banding karyotyping was performed by KingMed Diagnostics, Shanghai and 20 metaphase spreads were counted.

Short tandem repeat (STR) analysis

gDNA was extracted using a commercial kit from CORNING (APEMN-BL-GDNA-250G). STR analysis was performed on the PBMCs and established iPSCs with detection of 20 loci (D3S1358, D8S1179, D19S433, AMEL, VWA, D21S11, TH01, D5S818, D7S820, D16S539, D13S317, D12S391, CSF1PO, D2S1338, TPOX, FGA, PENTAE, PENTAD, D18S51, D6S1043) by Shanghai Biowing Applied Biotechnology Co, LTD, China.

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Mycoplasma test

The absence of mycoplasma was regularly assessed by EZ-PCR Mycoplasma Test Kit (Biological Industries, BI) following the manufacturer's instruction.

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References

Ban H, Nishishita N, Fusaki N, Tabata T, Saeki K, Shikamura M, Takada N, Inoue M, Hasegawa M, Kawamata S, Nishikawa S, 2011. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc. Natl. Acad. Sci. U. S. A. 108, 14234–14239. [PubMed: 21821793]

Boon CJF, Klevering BJ, den Hollander AI, Zonneveld MN, Theelen T, Cremers FPM, Hoyng CB, 2007. Clinical and genetic heterogeneity in multifocal vitelliform dystrophy. Arch. Ophthalmol. 125, 1100–1106. [PubMed: 17698758]

Johnson AA, Guziewicz KE, Lee CJ, Kalathur RC, Pulido JS, Marmorstein LY, Marmorstein AD, 2017. Bestrophin 1 and retinal disease. Prog. Retin. Eye Res. 58, 45–69. [PubMed: 28153808]

Petrukhin K, Koisti MJ, Bakall B, Li W, Xie G, Marknell T, Sandgren O, Forsman K, Holmgren G, Aneasson S, Vujic M, Bergen AA, McGarty-Dugan V, Figueroa D, Austin CP, Metzker ML, Caskey CT, Wadelius C, 1998. Identification of the gene responsible for Best macular dystrophy. Nat. Genet. 19, 241–247. [PubMed: 9662395]

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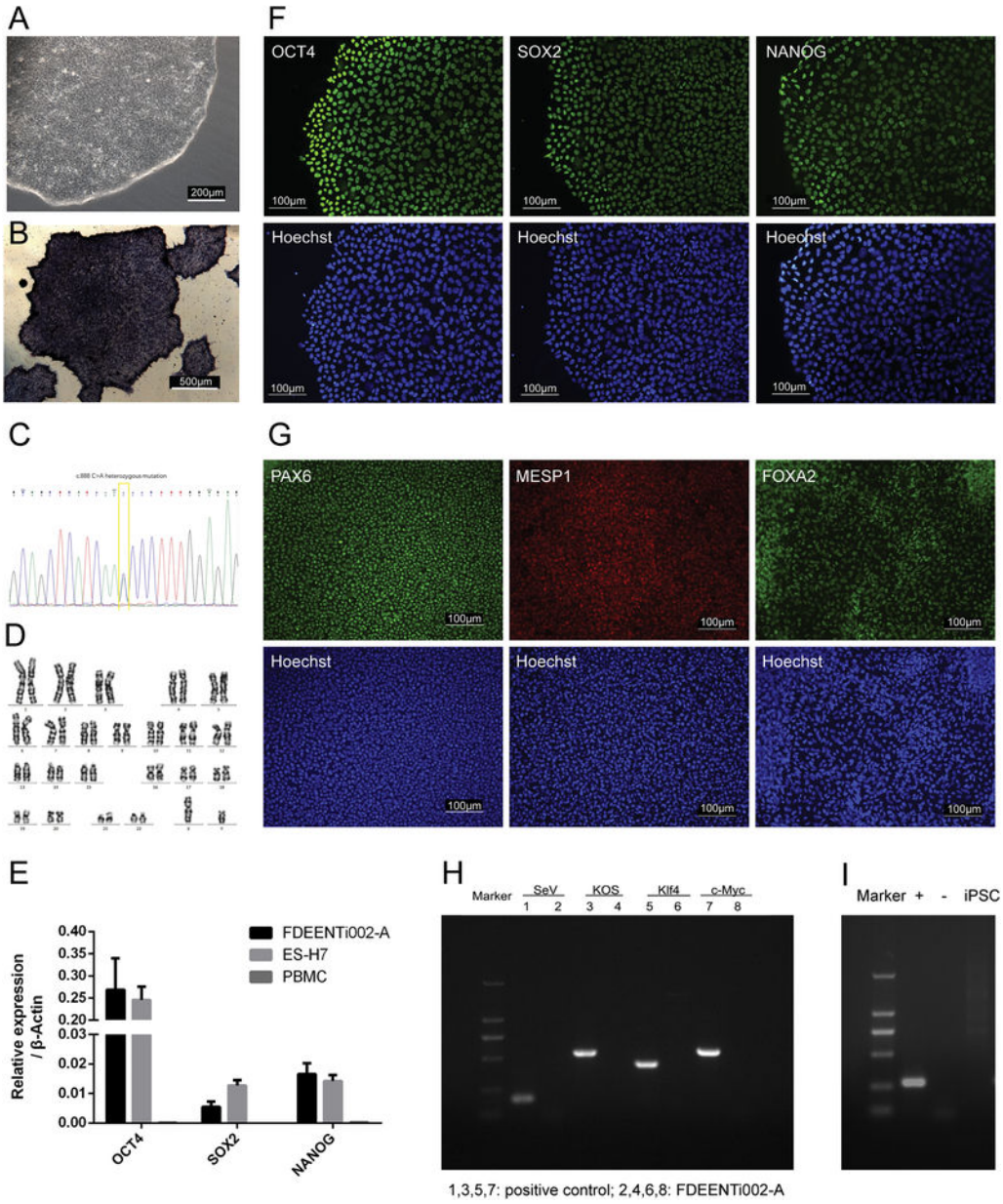

Fig. 1.
Characterization of iPSC line FDEENTi002-A.

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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 for pluripotency markers: OCT4, SOX2, NANOG	Fig. 1 panel F
	Quantitative analysis: RT-qPCR	Positive for pluripotency markers: OCT4, SOX2, NANOG	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 400	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	20 loci tested, 100% matched	Available with authors.
Mutation analysis (if applicable)	Sequencing	Heterozygous, c.888C > A mutation in *BEST1* gene	Fig. 1 panel C
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Fig. 1 panel I
Differentiation potential	Directed differentiation	Proof of three germ layers formation: ectoderm (PAX6), mesoderm (MESP1) and endoderm (FOXA2)	Fig. 1 panel G
Donor screening (optional)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (optional)	Blood group genotyping HLA tissue typing	Not performed	N/A
Genotype additional info (optional)	Blood group genotyping HLA tissue typing	Not performed	N/A

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Table 2

Reagents details.

	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency markers	Rabbit anti-OCT4	1:200	Abcam Cat# ab181557, RRID: AB_2687916
Pluripotency markers	Rabbit anti-SOX2	1:200	Abcam Cat# ab92494, RRID: AB_10585428
Pluripotency markers	Rabbit anti-NANOG	1:100	Abcam Cat# ab21624, RRID: AB_446437
Differentiation markers	Rabbit anti-PAX6	1:200	Abcam Cat# ab195045, RRID: AB_2750924
Differentiation markers	Mouse anti-MESP1	1:200	Abcam Cat# ab77013, RRID: AB_1566419
Differentiation markers	Rabbit anti-FOXA2	1:200	Abcam Cat# ab108422, RRID: AB_11157157
Secondary antibodies	AlexaFlour488 goat anti-mouse IgG	1:1000	Thermo Fisher Scientific Cat# A-11001, RRID: AB_2534069
Secondary antibodies	AlexaFlour488 goat anti-rabbit IgG	1:1000	Thermo Fisher Scientific Cat# A-11008, RRID: AB_143165
Secondary antibodies	AlexaFlour555 goat anti-mouse IgG	1:1000	Thermo Fisher Scientific Cat#A-21422, RRID: AB_2535844
Primers			
	Target	**Forward/Reverse primer (5'-3')**	
Sendai viral vector (RT-PCR)	SeV/181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS/528 bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG	
	KLF4/410 bp	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA	
	C-MYC/532 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
Pluripotency Markers (qPCR)	OCT4/169 bp	GCAAAGCAGAAACCCTCGTG/CACACTCGGACCACATCCTT	
	SOX2/171 bp	ATGGACAGTTACGCGCACAT/CGAGCTGGTCATGGAGTTGT	
	NANOG/179 bp	ACCCAGCTGTGTGTACTCAA/CCATTGCTATTCTTCGGCCA	
BEST1 mutation analysis (PCR)	BEST1/531 bp	TACACTCAGGGACAGCTGTG/TGCCTGTGAAATGGGGAGAT	
House-Keeping Genes (qPCR)	β-ACTIN/110 bp	ACTCTTCCAGCCTTCCTTCC/TGTTGGCGTACAGGTCTTTG	

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Resource table

Unique stem cell line identifier	FDEENTi002-A
Alternative name(s) of stem cell line	XTW-C5
Institution	Eye & ENT Hospital, Shanghai Medical School, Fudan University, Shanghai, China
Contact information of distributor	Xinyue Bai, xinyue_bai@126.com, Ling Chen, linglingchen98@hotmail.com
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 44
	Sex: male
	Ethnicity: Han Chinese
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free, Sendai viral vectors
Genetic modification	YES
Type of modification	Spontaneous mutation
Associated disease	Best's disease
Gene/locus	BEST1 gene/11q12.3, mutation: c.888C > A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	1st May 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/FDEENTi002-A
Ethical approval	This study was approved by the ethics committee of Fudan University affiliated Eye & ENT Hospital (KJ2011–04).