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Generation of an integration-free induced pluripotent stem cell line (FDEENTi003-A) from a patient with pathological myopia

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Abstract

Pathological myopia (PM) is a major cause of irreversible vision impairment worldwide. We have successfully reprogrammed the peripheral blood mononuclear cells (PBMCs) from a PM patient to induced pluripotent stem cells and characterized their pluripotency and genetic stability, as well as the potential to differentiate to retinal pigment epithelium (RPE). This line may serve as a useful tool to explore the pathogenesis of PM.

Resource Utility

Pathological myopia (PM) is a major cause of irreversible vision impairment worldwide. A lack of elaborated etiology and suitable animal models makes it an incurable disease. The FDEENTi003-A iPSC line may serve as a useful tool to explore the pathogenesis of PM.

Resource Details

Pathological myopia (PM) is a major cause of irreversible vision impairment and blindness worldwide, especially in Asia. It refers to highly myopic eyes with characteristic chorioretinal atrophy, which often leads to progressive and irreversible vision loss (Ohno-Matsui et al., 2018). Pathogenesis of PM is complex and still unclear, partly due to the limited access to patients' atrophic retina tissues and a lack of animal models to recapitulate the disease phenotypes.

To provide a source of patient retinal tissue of PM for study, we generated a patient-derived PM-specific induced pluripotent stem cell (iPSC) line and differentiated it into

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retinal pigment epithelium (RPE), one of the ten layers of retina. A 55-year-old female patient presented with high myopia accompanied by characteristic chorioretinal atrophy in both eyes. Peripheral blood was collected and freshly isolated for mononuclear cells. The cells were reprogrammed by Sendai viral vectors expressing OCT4, SOX2, KLF4 and C-MYC(Ban et al., 2011). The established FDEENTi003-A line exhibited a typical morphology of human embryonic stem cell (hESC) colonies (Fig. 1A, Table 1) and was positive for alkaline phosphatase staining (Fig. 1B). The expression of major pluripotent markers was examined by immunocytochemical staining using antibodies against human OCT4, SOX2, NANOG (Fig. 1G). Quantitative polymerase chain reaction (qPCR) showed that OCT4, SOX2 and NANOG mRNA was expressed at similar levels between FDEENTi003-A iPSC and hESC and was hardly expressed /absent in the parental PBMCs (Fig. 1C). The FDEENTi003-A line showed a normal diploid 46, XX karyotype (at passage 10) (Fig. 1D) and was genetically authenticated with the patient's peripheral blood cells by short tandem repeat (STR) analysis. SeV genome and transgenes were absent at passage 11 as confirmed by RT-PCR (Fig. 1E). PCR testing demonstrated the absence of mycoplasma contamination (Fig. 1F). Trilineage differentiation potential was detected in vitro and confirmed by the expression of ectoderm (PAX6), mesoderm (MESP1), and endoderm (FOXA2) markers (Fig. 1H). Furthermore, the potential for differentiation into retina was confirmed by directed differentiation into RPE, one of the ten layers of human retina. Patient-derived iPSC-RPE exhibited a typical polygonal morphology similar to that of human RPE cells, pigmented, and expressed major RPE markers (ZO-1, BEST1, MITF). (Fig. 1).

Materials and methods

Cell culture and reprogramming

PBMCs were isolated from the whole 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 collected and transduced with CytoTune®-iPS 2.0 Sendai reprogramming vectors (Thermo Fisher) following the manufacturer's instruction. The transduced cells were plated onto irradiated mouse embryonic fibroblasts (MEFs) and maintained in mTeSR™1 medium (Stem Cell Technologies) which was changed every other day. Around day16 post-transduction, ESC-like colonies appeared and were manually picked on day20 post-transduction. The established 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 FDEENTi003-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).

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.2% Triton X-100 for 15 min, and blocked in 4% bovine serum albumin for 30 min at room temperature. Afterwards, they were incubated with primary antibodies at 4 °C overnight and visualized with secondary antibodies for 1 h at room temperature. Both primary and secondary antibodies were diluted in PBS with 1% bovine serum albumin. 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.

IPSC differentiation

For trilineage differentiation, iPSCs were cultured to approximately 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 in 4% paraformaldehyde for assessing lineage specific markers by immunocytochemistry.

For directed differentiation into RPE cells, we followed a previously published protocol (Foltz and Clegg, 2017). After differentiation and maturation for 3 months, iPSC-RPE were tested for morphology and pigmentation and immunostained for RPE markers.

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, 55 °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.

Karyotyping

FDEENTi003-A cells in the logarithmic phase at passage 10 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 isolated using a commercial kit from CORNING (AP-EMN-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, D1S1656) by Shanghai Biowing Applied Biotechnology Co, LTD, China.

Mycoplasma test

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

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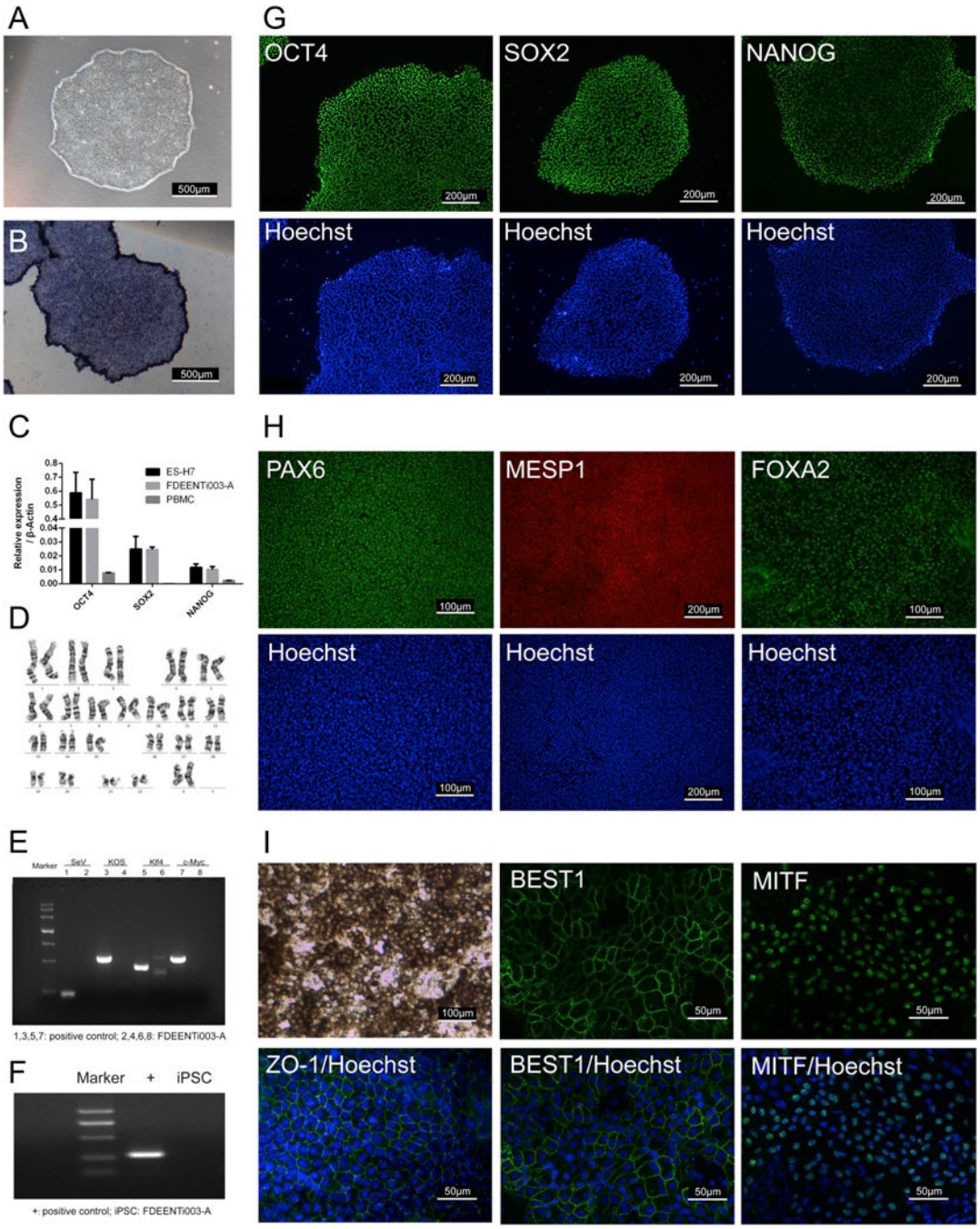

Fig. 1.
Characterization of iPSC line FDEENTi003-A.

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 G
	Quantitative analysis: RT-qPCR	Positive for pluripotency markers: OCT4, SOX2, NANOG	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 400	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	21 loci tested, 100% matched	Available with authors.
Mutation analysis (IF APPLICABLE)	Sequencing	Not performed	N/A
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Fig. 1 panel F
Differentiation potential	Directed differentiation	Proof of three germ layers formation: ectoderm (PAX6), mesoderm (MESP1) and endoderm (FOXA2)	Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	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
RPE Markers	Rabbit anti-ZO-1	1:100	Thermo Fisher Scientific Cat# 402200, RRID: AB_2533456
RPE Markers	Mouse anti-BEST1	1:100	Abcam Cat# ab2182, RRID: AB_302880
RPE Markers	Mouse anti-MITF	1:100	Abcam Cat# ab3201, RRID: AB_303601
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
House-Keeping Genes (qPCR)	β-ACTIN/110 bp	ACTCTTCCAGCCTTCCTTCC/TGTTGGCGTACAGGTCTTTG

Resource Table

Unique stem cell line identifier	FDEENTi003-A
Alternative name(s) of stem cell line	ZSY-IPS
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: 55
	Sex: female
	Ethnicity: Han Chinese
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free, Sendai viral vectors
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Pathological myopia
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	1st October 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/FDEENTi003-A
Ethical approval	This study was approved by the ethics committee of Fudan University affiliated Eye & ENT Hospital (KJ2011-04).