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Lab Resource: Stem Cell Line

Integration-free erythroblast-derived human induced pluripotent stem cells (iPSCs) from an individual with Ataxia-Telangiectasia (A-T)

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ABSTRACT

Peripheral blood was obtained from a 12-year old male carrying bialleleic inactivating mutations at the ATM locus, causing Ataxia-Telangiectasia (A-T). Blood erythroid cells were briefly expanded *in vitro* and induced pluripotent stem cells (iPSCs) were generated *via* transfection with episomal vectors carrying *hOCT4*, *hSOX2*, *hKLF4*, *hMYC* and *hBCL2L1*. SF-003 iPSCs were free of genomically integrated reprogramming genes, had the specific compound heterozygous mutations, stable karyotype, expressed pluripotency markers and formed teratomas in immunodeficient (NOD scid gamma; NGS) mice. The SF-003 iPSC line may be a useful resource for *in vitro* modeling of A-T.

Resource table

Name of Stem Cell construct	SF-003
Institution	Johns Hopkins University School of Medicine
Person who created resource	Niraj Bhatt, Yongxing Gao, Linzhao Cheng, Sonia Franco
Contact person and email	Sonia Franco; sfranco2@jhmi.edu
Date archived/stock date	May 2015
Origin	Human peripheral blood cells
Type of resource	Biological reagent: induced pluripotent stem cell (iPSC); derived from an Ataxia-Telangiectasia (A-T) patient
Sub-type	Induced pluripotent stem cells (iPSC)
Key transcription factors	*hOCT4*, *hSOX2*, *hC-MYC*, *hKLF4*, *hBCL2L1* Addgene plasmids 64120 (MOS; *hOCT4*-2A-*hSOX2*), 64121 (MMK; *hMYC*-2A-*hKLF4*) and 64123 (GBX; eGFP-2A-*hBCL2L1*)
Authentication	Identity and purity of cell line confirmed by analysis of plasmid integration, mutation sequencing, karyotyping, indirect immunofluorescence for pluripotency markers and teratomas formation (Fig. 1)
Link to related literature (direct URL links and full references)	http://www.ncbi.nlm.nih.gov/pubmed/25742692
Information in public databases	N/A

Resource details

This study was approved by the Johns Hopkins University Institutional Review Board (IRB; protocol number, IRB#CR00007000), Baltimore MD. To protect patient privacy, no personal patient information is presented here. Peripheral blood (PB) was obtained from a 12-year-old boy compound heterozygous for null frameshift mutations in *ATM* exon 23 (3369delA in codon 1123) and exon 26 (3754delTATinsCA in codon 1252). Homozygous mutations in *ATM* cause the autosomal recessive syndrome Ataxia-Telangiectasia (A-T) and, accordingly, the patient clinical presentation was consistent with the "classical" form of A-T. Peripheral blood mononuclear cells (PB MNC) were briefly expanded *in vitro* with a cytokine cocktail that stimulates erythroblast proliferation and nucleofected with three plasmid vectors encoding *hOCT4*, *hSOX2*, *hC-MYC*, *hKLF4* and *hBCL2L1*, as we had described (Chou et al., 2015). Emerging TRA-1-60^{+} colonies were pooled and expanded. This technique successfully established feeder-free, xeno-free iPSC. Episomal plasmid integration was analyzed by qPCR on iPSC DNA at passage 22 with plasmid-specific primers, using S18 primers as loading control (Fig. 1A). These analyses confirmed lack of integration of the reprogramming plasmids. The cells were karyotypically normal (Fig. 1B) and Sanger sequencing confirmed the presence of the 3369delA in exon 23 and 3754delTATinsCA in exon 26 (Fig. 1C). Indirect immunofluorescence confirmed the expression of stem cells markers TRA-1-60, OCT4, SOX2 and SEEA4 in single cells (Fig. 1D). Finally, the SF-003 iPSC line formed teratomas *in vitro* with high efficiency (Fig. 1E), further confirming pluripotency.

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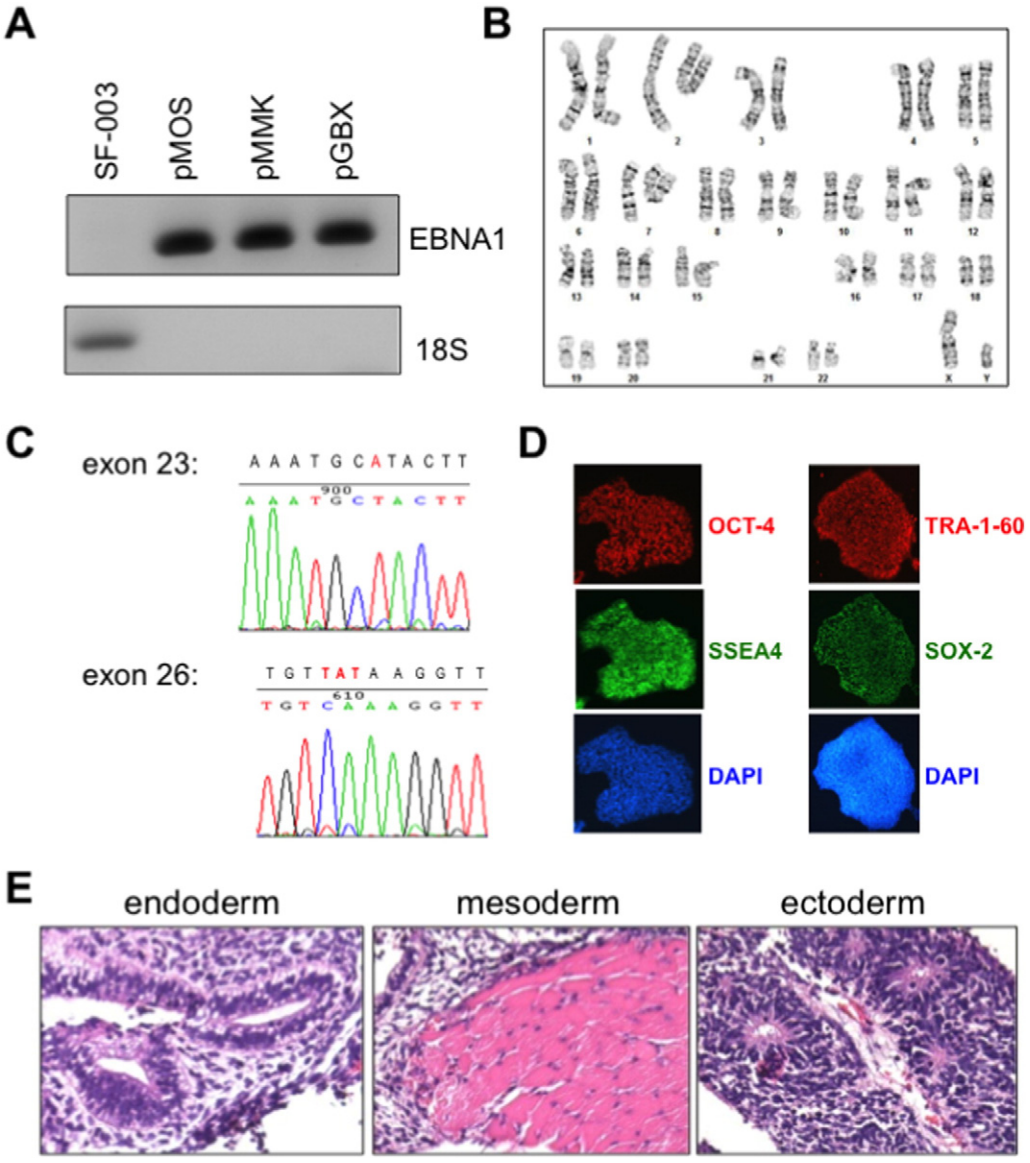

Fig. 1. Characterization of the SF-003 iPSC line. A) PCR for vector integration. EBNA1 primers amplified a fragment common to all three plasmids used for expression of reprogramming factors (pMOS, pMMK and pGBX). As a loading control, we amplified 18S. B) Karyotypic analyses by G-banding. A representative metaphase out of 20 is shown. C) Chromatogram of SF-003 gDNA after amplification of exons 23 and 26 containing mutations. The consensus sequence is shown on top with mutated nucleotides highlighted in red. D) Indirect immunofluorescence for pluripotency markers OCT-4, SSEA4, TRA-1-60 and SOX-2. Nuclei were counterstained with DAPI. E) Sections of SF-003-derived teratomas after H/E staining. Representative examples of differentiation to endoderm, mesoderm and ectoderm are shown.

Materials and methods

Reprogramming of peripheral blood mononuclear cells and establishment of iPSC lines

Peripheral blood (PB) was obtained by venipuncture from a 12-year old male known to carry frameshift mutations in exon 23 (3369delA, codon 1123) and exon 26 (3754delTATinsCA in codon 1252) of *ATM*. Mononulcear cells were purified in a Ficoll gradient (Ficoll-Paque Premium, GE Healthcare) and cultured in serum-free medium (SFM) supplemented with Stem Cell Factor (SCF), interleukin (IL)-3, human holotransferrin, erythropoietin, insulin-like growth factor 1 (IGF-1) and dexamethasone to expand erythroid progenitors. Cells were nucleofected with three plasmids encoding reprogramming factors *hOCT4*, *hKLF4*, *hCMYC*, *hSOX2* and *hBCL2L1* and stained for TRA-1-60 after 7 days. TRA-1-60 + colonies (over 95% of total colonies) were pooled and expanded in E8 media (Life Technologies) on vitronectin plates.

PCR for integration analyses

PCR was done on genomic DNA using primers that detect the common backbone for all three plasmids: EBNA-Fw1: 5′-

Table 1
Primers used for *ATM* mutational analyses.

ATM exon	Forward sequence	Reverse sequence
Exon 23	5′- TTTGTTCTGGAATATGCTTTGG-3′	5′- TGGTGAAGTAATTTATGGGATATTCA-3′
Exon 26	5′- CTTTAATGCTGATGGTATTAAAACAG-3′	5′- GCCATACCTGTTTTCCCAAT-3′

ACGATGCTTTCCAAACCACC-3′ and EBNA-Rev1: 5′-CATCATCATCCGGG TCTCCA-3′. As a control, we amplified the same DNA with primers to 18S: 18S-F: 5′-GCGAGTACTCAACACCAACATCG-3′ and 18S-R: 5′-TCAAGTCTCCCCAGCCTTGC-3′.

Karyotyping

SF-003 iPSCs at passage 8 were sent to the WiCell Genetic Laboratory for metaphase preparation and G banding. For each line, 20 metaphases were analyzed.

Mutation analysis

Genomic DNA from early passage SF-003 cells was sequenced using primers specific for exons 23 and 26 containing the mutations (see Table 1 for primer sequences). PCR products were cloned into TOPO-TA and sequenced at the Johns Hopkins University Genetic Resources Core Facility using Sanger sequencing.

Indirect immunofluorescence for pluripotency markers

iPSCs were incubated with antibodies to pluripotency markers OCT4, SOX2, SSEA4 and TRA-1-60 using the Pluripotent Stem Cells 4-marker Immunocytochemistry Kit (Life Technologies, A24881), following manufacturer's instructions. Images were taken using an EVOS FL Auto Cell Imaging System.

Teratoma formation

iPSCs (pool of 6 wells of a 6-well plate, or approximately 10 million cells) were mixed 1:1 with Matrigel (Cornig Matrigel hESC-qualified Matrix, #354277) to a total volume of 200 μl and injected into the subcultaneous space in the flanks of NOD scid gamma (NGS) mice. Formalin-fixed teratomas were paraffin-embedded at the Johns Hopkins Histopathology Core Facility and five noncontiguous (H/E)-stained sections were analyzed.

Verification and authentication

For karyotyping, SF-003 cells at passage 10 were sent to WiCell Genetic Laboratory for metaphse preparation and G banding. A total of 20 metaphases were analyzed. No karyotypic abnormalities were detected. To confirm line identity, iPSC genomic DNA was sequenced using primers specific for exons 23 and 26 containing the mutations (see Table 1 for primer sequences). To assess purity, we confirmed expression of pluripotency markers TRA-1-60, OCT4, SOX4 and SSE4 in single cells by indirect immunofluorescence.

Acknowledgements

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Reference

Chou, B.K., Gu, H., Gao, Y., Dowey, S.N., Wang, Y., Shi, J., Li, Y., Ye, Z., Cheng, T., Cheng, L., 2015. A facile method to establish human induced pluripotent stem cells from adult blood cells under feeder-free and xeno-free culture conditions: a clinically compliant approach. Stem Cells Transl. Med. 4, 320–332.