Stem Cell Research 15 (2015) 694–696

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem cell line

Generation of integration-free induced pluripotent stem cells from a patient with Familial Mediterranean Fever (FMF)

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ARTICLE INFO

Article history:
Received 28 October 2015
Accepted 30 October 2015
Available online 2 November 2015

ABSTRACT

Fibroblasts from a Familial Mediterranean Fever (FMF) patient were reprogrammed with episomal vectors by using the Neon Transfection System for the generation of integration-free induced pluripotent stem cells (iPSCs). The resulting iPSC line was characterized to determine the expression of pluripotency markers, proper differentiation into three germ layers, the presence of normal chromosomal structures as well as the lack of genomic integration. A homozygous missense mutation in the *MEFV* gene (p.Met694Val), which lead to typical FMF phenotype, was shown to be present in the generated iPSC line.

Resource table

Name of the stem cell Construct	KU_FMF_iPSC_1
Institution	Koc University, School of Medicine
Person who created the resource	Kerem Fidan, Tamer T Onder
Contact person and email	Tamer Onder, tonder@ku.edu.tr
Date archived/stock date	April 26, 2014
Origin	Human Skin Fibroblasts
Type of resource	Induced pluripotent stem cell (iPSC); derived from fibroblasts of a FMF patient carrying homozygous p.Met694Val mutation in the *MEFV* gene
Sub-type	Induced pluripotent stem cell (iPSC)
Key transcription factors	Oct4, Sox2, L-Myc, Klf4, Lin28, shp53
Authentication	Identity and purity of cell line confirmed
Link to related literature (direct URL links and full references)	Not available
Information in public databases	Not available

1. Resource details

We reprogrammed fibroblasts obtained from a patient with Familial Mediterranean Fever (FMF), an autosomal recessively inherited autoinflammatory disease caused by mutations in the *MEFV* gene (The International FMF Consortium, 1997; French FMF Consortium, 1997). Patient-specific fibroblasts were transfected with episomal factors expressing, Oct4, Sox2, L-Myc, Klf4, Lin28 and shp53 by using the Neon Transfection System (Invitrogen) based on a previously published protocol (Fidan et al., 2015; Okita et al., 2011). The expression of pluripotency genes in the resulting iPSC line was tested by immunofluorescense staining, using antibodies against Oct4 and Nanog (Fig. 1).

To demonstrate the capacity of differentiation into three germ layers, KU_FMF_iPSC_1 cells were injected intramuscularly into SCID mice (Lensch et al., 2007). The resulting teratomas displayed differentiated cells from three germ layers (Fig. 2).

The three non-integrating episomal vectors were introduced by using the Neon Transfection System. The presence of non-integrating episomal vectors was tested by a PCR-based detection method from genomic DNA of KU_FMF_iPSC_1 (Fig. 3).

The presence of homozygous p.Met694Val mutations in exon 10 of *MEFV* gene in the starting fibroblasts and iPSCs was analyzed by genomic DNA sequencing. Results confirmed the presence of p.Met694Val mutation in the KU_FMF_iPSC_1 line (Fig. 4).

2. Materials and methods

2.1. Patient fibroblast culture

A skin punch biopsy from arm was obtained from an FMF patient by a physician (AG) after the patient have given informed consent under a protocol approved by the relevant Institutional Review Board. The biopsy sample of 3 mm skin was washed with DPBS and cut into small pieces using sterile razor blades. Pieces were cultured in complete DMEM medium (90% DMEM, 10% FBS). Cell line was established in 2 weeks by changing the medium every 3 days. Confluent cells were passaged

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http://dx.doi.org/10.1016/j.scr.2015.10.018

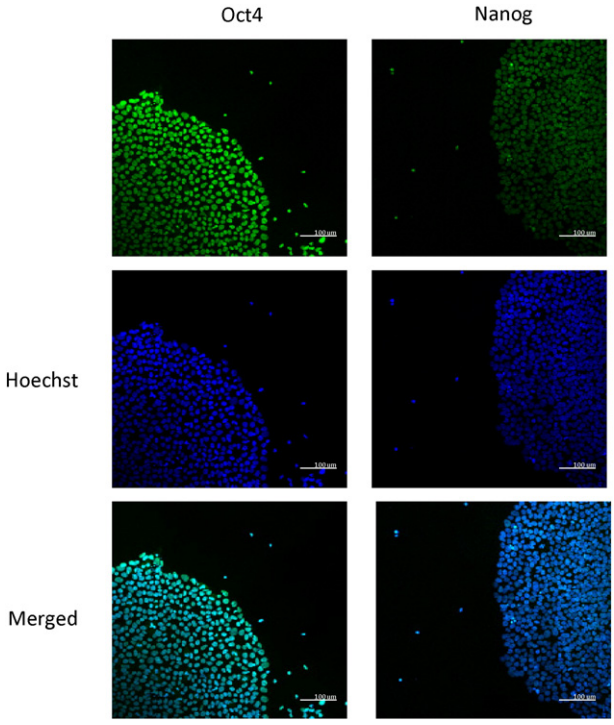

Fig. 1. Characterization of an FMF patient induced pluripotent stem cells (iPSC) KU_FMF_iPSC_1. Immunofluorescence stainings of FMF patient iPSCs with Oct4 and Nanog. Hoechst was used for nucleus staining.

with 0.05% trypsin/EDTA solution. Established cells were frozen in 10% DMSO, 50% complete DMEM and 40% FBS.

2.2. iPSC generation and expansion

Established FMF patient fibroblasts were transfected with 1 μg of the three plasmids encoding the reprogramming factors (pCXLE-hOCT3/4-shp53-F, Addgene plasmid 27077; pCXLE-hUL, Addgene plasmid 27080; pCXLE-hSK, Addgene plasmid 27078) using the Neon Transfection System (Fidan et al., 2015; Okita et al., 2011). Transfected fibroblasts were cultured in DMEM medium until day 7. On day 7, patient fibroblasts were passaged onto mitotically arrested MEFs (Mouse Embryonic Fibroblasts) in hES medium (20% KOSR, 1% L-glutamine, 1% non-essential aminoacids, 0.1 mM β-mercaptoethanol, 10 ng/ml bFGF in DMEM/F12). In order to increase the reprogramming efficiency, 3 μM Dot1L inhibitor (EPZ004777, iDot1L) was added during the first 2 weeks of reprogramming (Onder et al., 2012). After the successful formation of iPSC colonies, iPSCs were expanded both on MEFs and on Matrigel in mTeSR1 medium.

2.3. Immunofluorescence staining of iPSC

Pluripotent gene expression in KU_FMF_iPSC_1 was confirmed by Oct4 and Nanog immunofluorescence staining. iPSC colonies were fixed in 4% paraformaldehyde for 30 min at room temperature. Fixed cells were blocked with 5% donkey serum and permeabilized with 0.2% Triton-X. Cells were then washed with PBS and stained with Oct4 (Abcam) and Nanog (Abcam) primary antibodies at a 1:200 dilution and left at +4 °C overnight. Primary antibodies were then washed with PBS and cells were treated with 488 Alexafluor secondary antibody (Invitrogen) at a 1:500 dilution and incubated at room temperature for 1 h. Cells were mounted with Hoechst 33342 prepared in 50% glycerol/PBS. Images were acquired using a confocal microscope (Nikon Eclipse 90i) (Fig. 1).

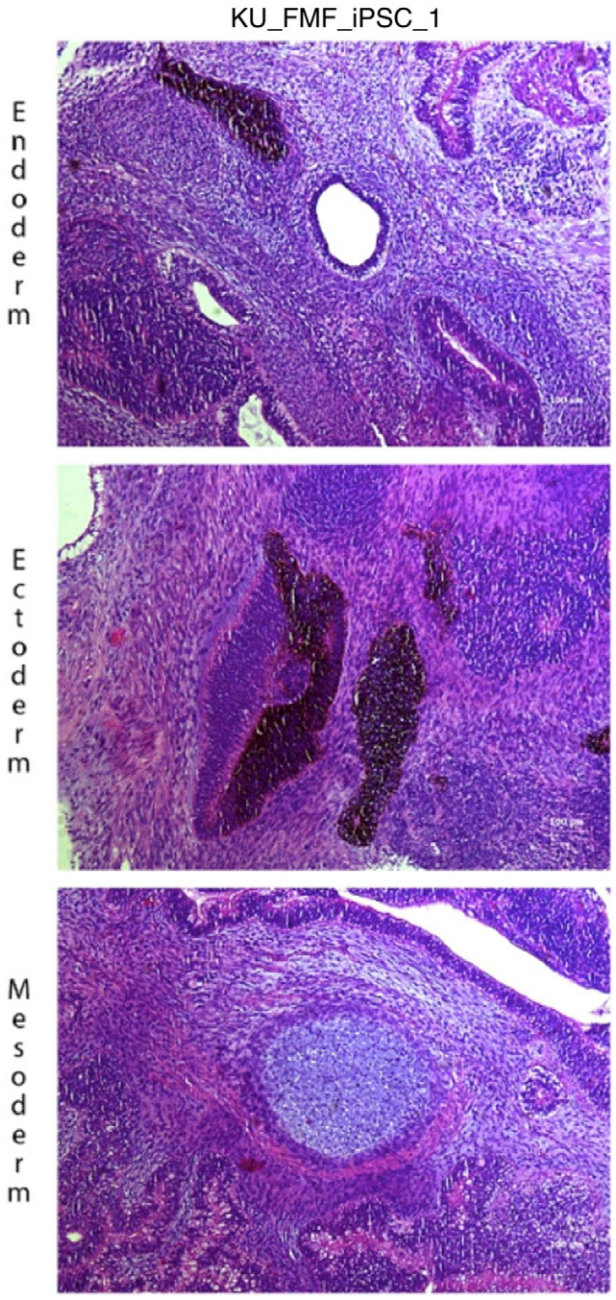

Fig. 2. Teratoma formation of KU_FMF_iPSCs. Injection of iPSC into SCID mice resulted in teratoma formation. Isolated teratomas were stained with hematoxylin and eosin to visualize three germ layer derivatives. All three germ layer structures were observed.

2.4. Teratoma formation and characterization

We tested the differentiation ability of iPSCs by teratoma formation. Undifferentiated iPSCs were expanded on Matrigel (Corning) in mTesR1 medium (Stem Cell Technologies) followed by collection and subsequent intramuscular injection into SCID mice. Teratoma formation was observed 10 weeks after the injection. Resulting teratomas were isolated, fixed with 10% formalin and stained with hematoxylin and eosin. Derivatives of three germ layers were observed including cartilage, glandular structures, melanin pigmented cells and neural rosettes, all of which demonstrate the pluripotency of the injected iPSCs (Fig. 2).

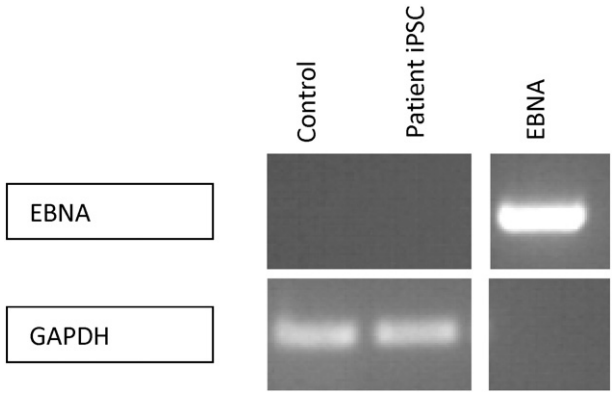

Fig. 3. Integration assay. PCR-based method demonstrated that episomal vectors were not integrated into the genomic DNA of KU_FMF_iPSCs. pCXLE-SK plasmid containing EBNA sequence was used as positive control.

2.5. Testing episomal vector integration

Episomal vectors are considered non-integrating, however, a low probability of integration still exists. To test the integration of episomal vectors, genomic DNA was isolated from KU_FMF_iPSCs using a commercial genomic DNA isolation kit (Qiagen). An EBNA sequence, encoded by all three vectors, was used to analyze the genomic integration of episomal vectors by PCR. Results demonstrate that iPSCs were established as integration-free cell line (Fig. 3). The following primers were used for PCR: EBNA-Fwd: AGGGCCAAGACATAGAGATG, EBNA-Rev: GCCAATGCAACTTGGACGTT, GAPDH-Fwd: ATCACCATCTTCCAGG AGCGA, GAPDH-Rev: TTCTCCATGGTGGTGAAGACG.

2.6. Mutation analysis of iPSCs

The FMF patient whose fibroblasts were reprogrammed carried a homozygous p.Met694Val mutation in the *MEFV* gene. Genomic DNA from KU_FMF_iPSCs were isolated, and exon 10 of *MEFV* gene was amplified by PCR using the following primers: Exon10-Fwd: TTCCAGAAGA ACTACCCTGT, Exon10-Rev: TGCATTTCCCATAGCAGCTA. Sanger sequencing was performed which indicated the presence of homozygous single nucleotide mutation that leads to p.Met694Val in patient's iPSCs (Fig. 4).

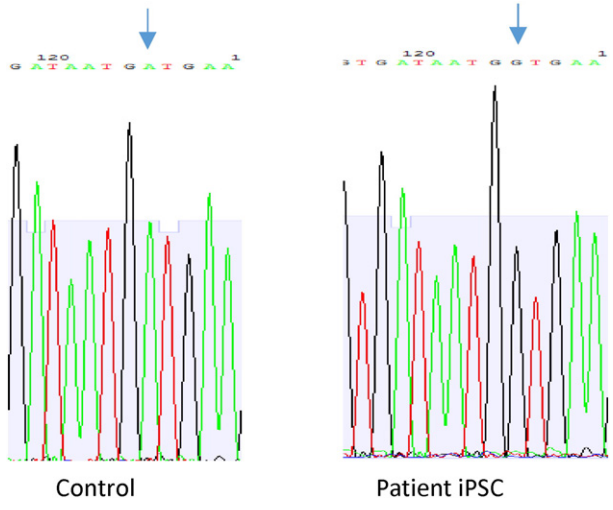

Fig. 4. Mutation detection in patient iPSCs. Mutation in the *MEFV* gene leading to M694V is indicated in iPSCs (blue arrows point the mutated nucleotide in patient iPSCs).

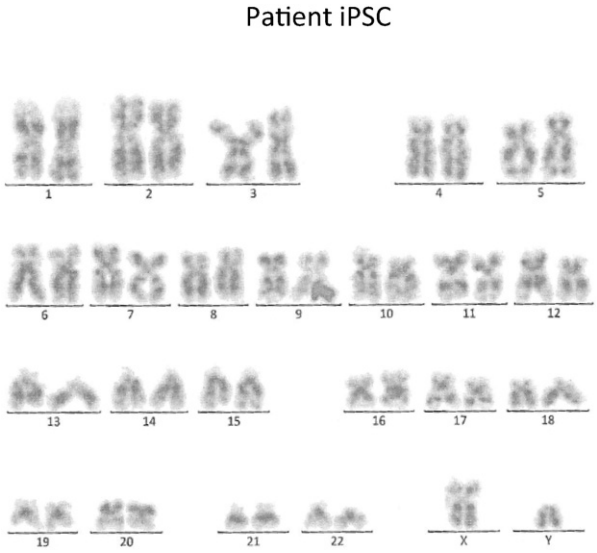

Fig. 5. Karyotype analysis. Patient iPSC has normal karyotype, 44 + XY.

2.7. Karyotyping

Karyotyping of KU_FMF_iPSCs was carried out at a clinical diagnostic center (Nesiller Genetics, Turkey). G-banding was utilized to karyotype the FMF patient's iPSC line. Cells were arrested in metaphase and analyzed. All ten metaphase cells counted had 44 + XY chromosomes as shown in Fig. 5.

3. Verification and authentication

Identity and purity of KU_FMF_iPSC_1 was confirmed by immunofluorescence for the expression of stem cell markers Oct4 and Nanog, karyotyping, and in vivo teratoma formation. In addition, genomic DNA from KU_FMF_iPSC_1 carried no detectable integration of the reprogramming plasmids. The presence of the disease-relevant mutation in the *MEFV* gene was confirmed by DNA sequencing.

Acknowledgments

Work in our lab is supported by an EMBO installation grant (2543), an FP7 Marie Curie Career Integration Grant and by TUBITAK (The Scientific and Technological Research Council of Turkey).

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