Stem Cell Research 54 (2021) 102438

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of transgene-free iPSC lines from three patients with Friedreich's ataxia (FRDA) carrying GAA triplet expansions in the first intron of FXN gene

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A B S T R A C T

Friedreich's ataxia (FRDA) is a rare neurodegenerative disorder which is caused by triplet repeat expansion (GAA) in the first intron of *FXN* gene. In this present study, we generated induced pluripotent stem cells (iPSC) lines from fibroblasts of three unrelated FRDA patients using integration-free episomal vectors. All iPSC lines express the pluripotency markers such as OCT4 and SSEA4, display normal karyotypes and can differentiate into all three germ layers via *in vivo* teratoma formation assay.

Unique stem cell lines identifier	KUTTAMi001-A – https://hpscreg.eu/cell-line/KUTTAMi001-A KUTTAMi002-A – https://hpscreg.eu/cell-line/KUTTAMi002-A KUTTAMi003-A – https://hpscreg.eu/cell-line/KUTTAMi003-A
Alternative name(s) of stem cell lines	KU-FRDA3-1 KU-FRDA4-1 KU-FRDA5-2
Institution	Koç University/Research Center for Translational Medicine (KUTTAM)
Contact information of distributor	Tamer Onder/tonder@ku.edu.tr
Type of cell lines	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	KUTTAMi001-A Age: 38 Sex: Female Ethnicity if known: White KUTTAMi002-A Age: 38 Sex: Female Ethnicity if known: White KUTTAMi003-A Age: 32 Sex: Male Ethnicity if known: White
Cell Source	Human skin fibroblasts
Clonality	Clonal
Associated disease	Friedreich's Ataxia
Gene/locus	FXN 9q21.11 intron 1, triplet expansion
Date archived/stock date	September 2020
Cell line repository/bank	KUTTAMi001-A – https://hpscreg.eu/cell-line/KUTTAMi001-A KUTTAMi002-A – https://hpscreg.eu/cell-line/KUTTAMi002-A KUTTAMi003-A – https://hpscreg.eu/cell-line/KUTTAMi003-A
Ethical approval	This study was approved by the ethics committee of Koc University (2016.126.IRB2.080)

1. Resource utility

These iPSC lines generated from three different Friedreich's Ataxia (FRDA) patients with GAA triplet repeat expansion in the first intron of *FXN* gene will facilitate studies to understand molecular mechanisms related to FRDA pathology as well as therapeutic investigations such as drug screening or gene editing.

2. Resource details

Friedreich's ataxia (FRDA, OMIM#229300) is a hereditary neurodegenerative disorder and affects one in 50,000 people worldwide. It is

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https://doi.org/10.1016/j.scr.2021.102438
Received 12 April 2021; Received in revised form 15 June 2021; Accepted 17 June 2021
Available online 27 June 2021
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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal morphology	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive staining for OCT4, SSEA4 and Tra-1–60	Fig. 1A and D
	Quantitative analysis (RT-qPCR)	Positive expressions for pluripotency-related genes: OCT4, NANOG, SOX2, and LIN28A transcripts	Fig. 1B
Genotype	Karyotype (G-banding) and resolution	46XX, 46XY	Fig. 1C
Identity	STR analysis	STR Analysis matched with fibroblast samples	submitted in archive with journal
Mutation analysis	Sequencing	FXN PCR followed by fluorescence analysis of the size of the PCR products	Fig. 1G
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative.	Supplementary file
Differentiation potential	Teratoma formation	Histology for three germ layers in teratomas	Fig. 1E
List of recommended germ layer markers	NA	NA	NA
Donor screening	NA	NA	NA
Genotype additional info	NA	NA	NA
	NA	NA	NA

caused by a reduction in the *FXN* mRNA level and Frataxin protein level due to hyperexpanded GAA triplets in the first intron of *FXN* gene. Almost 96% of cases with FRDA are homozygous for GAA triplet expansion. Neurological symptoms include progressive gait and limb ataxia, spinocerebellar neurodegeneration, loss of position sense, dysarthria and scoliosis; whereas non-neurological symptoms include cardiomyopathy and diabetes (Campuzano et al., 1996). An unidentified silencing mechanism causes disease phenotype due to reduced frataxin protein level. Although a number of different approaches have been employed to study the mechanism of this disease, novel *in vivo* and *in vitro* models of FRDA are needed. There are a number of FRDA specific iPSCs that have been generated previously (Schreiber et al., 2019). In this study, we generated three additional iPSC lines from FRDA patients.

Three FRDA patients were diagnosed with GAA triplet expansion and they donated skin fibroblasts. We generated three iPSC lines by nucleofection of episomal vectors expressing Oct4, Sox2, L-Myc, Klf4, Lin28 and shp53 via Neon Transfection System (Invitrogen[1400 V, 20 ms, 2 pulses]) (Table 1). Three weeks after reprogramming, iPSC clones were picked and expanded on irradiated mouse embryonic fibroblasts (MEFs). The three expanded iPSC clones have embryonic stem cell-like morphology (Fig. 1A, top panel) and are positive for pluripotency surface marker protein TRA-1-60 (Fig. 1A, bottom panel). RT-PCR for pluripotency markers was performed and compared with one of the parental fibroblast cell lines. All iPSC lines have elevated expressions of pluripotency markers, *OCT4, NANOG, SOX2,* and *LIN28A* (Fig. 1B). Chromosomal analysis revealed the normal karyotypes for all iPSC lines (Fig. 1C). Immunofluorescence staining showed all iPSC lines have high protein levels of intracellular pluripotency markers (OCT4, NANOG) and surface pluripotency marker (SSEA4) (Fig. 1D). iPSCs were able to differentiate into three germ layers via *in vivo* teratoma formation assay (Fig. 1E). In addition, FRDA iPSC lines were integration-free as shown by the absence of EBNA gene in their genome (Fig. 1F). All FRDA lines exhibited repeat expansion at the FXN locus (Fig. 1G). This work was supported by TUBITAK Project 216S495.

3. Materials and methods

3.1. Generation of iPSCs

Skin fibroblasts derived via punch biopsy were maintained in DMEM high glucose with 10% FBS and 1% Penicillin/Streptomycin. Fibroblasts were nucleofected with plasmids containing reprogramming factors (pCXLE-hOct3/4-shp53, pCXLE-hSK, pCXLE-hUL, pCXWB-EBNA1) using Neon Transfection System (Okita et al., 2013). For the first 6 days, cells were treated 3 μM EPZ004777 (DOT1L inhibitor) to increase reprogramming efficiency (Ebrahimi et al., 2019). On the sixth day, transfected fibroblasts were transferred onto MEF-feeder cells and were maintained in human embryonic stem (hES) cell medium until visible iPSC colonies were obtained. Individual colonies were picked through manual passaging. hES medium composed of %20 KOSR (Thermofisher Scientific), 1% Penicillin/Streptomycin, 1% Non-essential amino acids (NEAA), 1% L-glutamine, 0.1 mM β-mercaptoethanol, 10 ng/ml FGF in DMEM/F-12. iPSCs were passaged with a 1:6 ratio with ReleSR (Stem Cell Technologies) every week and maintained on MEFs at 37 °C, 5% CO_2 without ROCK inhibitor.

3.2. Immunofluorescence staining

iPSCs were seeded on coverslips, fixed with 4% Paraformaldehyde (PFA) and permeabilized with 0.2% Triton-X solution, followed by blocking buffer composed of 3%BSA (Bovine serum albumin) and 5% donkey serum in DPBS. After blocking, cover slips were incubated primary antibody containing solution at 4 °C, overnight. For secondary antibody, the incubation was performed at 4 °C, 2 h. Nuclear staining was done by DAPI Vectashield (H-1200-10) (Table 2). The images were taken with LEICA DMI8-SP8. For Tra-1-60 staining HRP activity was visualized with addition of 1% DAB (3.3 diaminobenzidine), 1% Nickel ammonium sulfate, 0.3% H_2O_2.

3.3. Teratoma formation assay

All experiments were carried out under a protocol approved by Koç University Animal Experiments Ethics Committee. KU-FRDA3-1 (at passage 26), KU-FRDA4-1 (at passage 13) and KU-FRDA5-2 (at passage 17) iPSCs were injected intramuscularly in SCID mice as previously described (Fidan et al., 2015). Teratomas were collected 8–10 weeks after injection and analyzed histologically via hematoxylin and eosin staining (Fig. 1E).

3.4. Mycoplasma testing

Mycoplasma testing was performed using LT07-318 Myc Alert Detection Kit (100 T, Lonza) and LT07-518 Mycoalert Assay Control Set following the manufacturer's instructions (Supplementary File).

3.5. Testing episomal vector integration

Genomic DNAs were extracted by using MN Genomic DNA from tissue kit. PCR was performed using GAPDH and EBNA primers listed in Table 2.

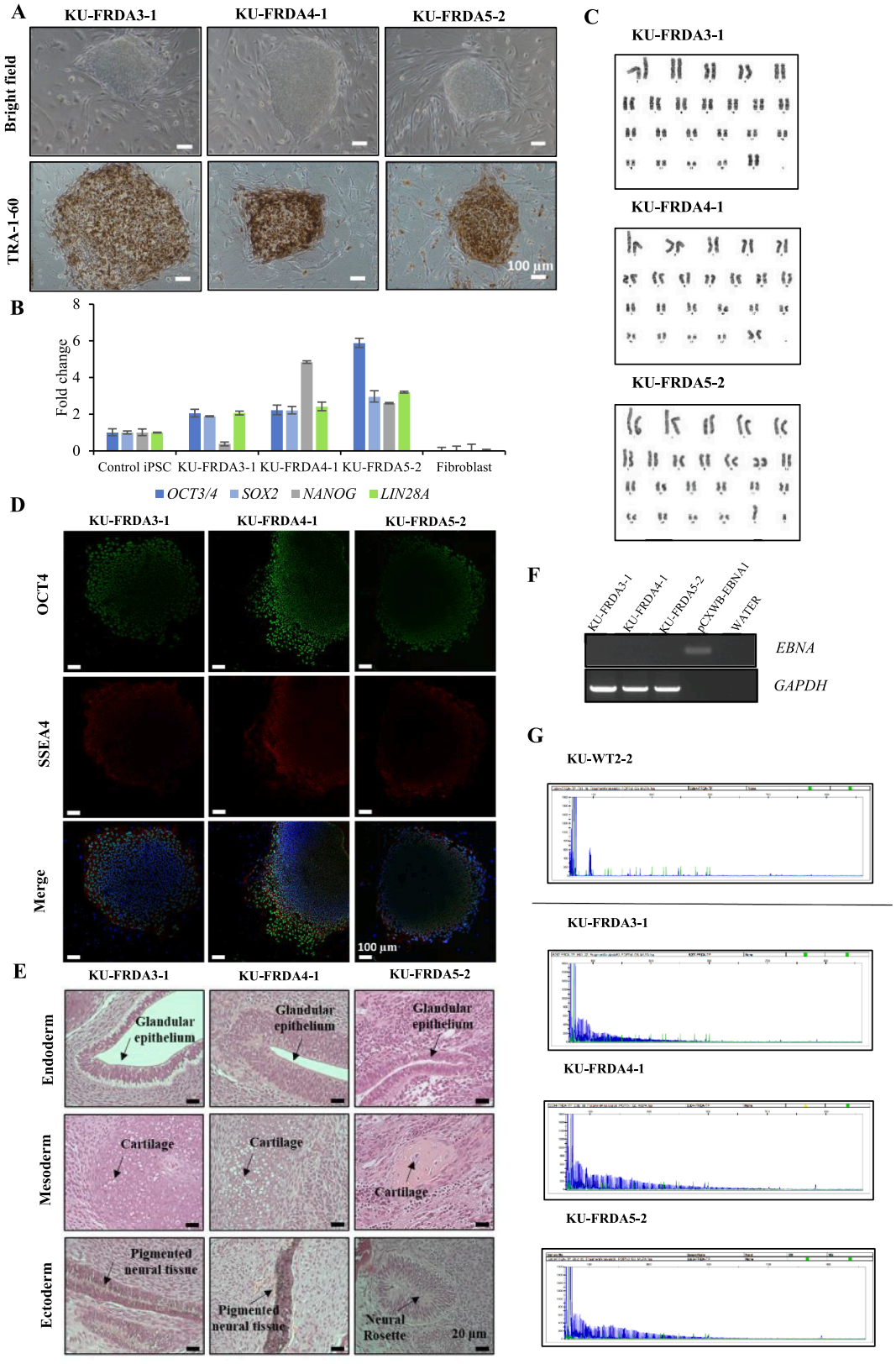


Fig. 1. Characterization of KU-FRDA3-1, KU-FRDA4-1 and KU-FRDA5-2 iPSC lines.

3.6. Genetic analyses

KU-FRDA3-1 and KU-FRDA4-1 iPSC (Passage 6) and KU-FRDA5-2 iPSC (Passage 8) were karyotyped at Koc University Diagnostic Centre for Genetic Diseases, Istanbul, Turkey. Promega PowerPlex® 16 HS system (Catalog number: DC2101) was used for STR analysis by following the manufacturer's instruction (Supplementary File). GAA repeat expansions were detected using Adellgene friedreich's ataxia kit

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse TRA-1-60 Ab.	1:200	BioLegend Cat.#330604	RRID: AB_1186140
Pluripotency Markers	Mouse SSEA Ab.	1:100	BD Biosciences Cat.#560218	RRID: AB_1645389
Pluripotency Markers	Rabbit OCT4 Ab.	1:100	Abcam Cat.#19857	RRID: AB_445175
Secondary antibodies	Alexa Fluor 488 Anti-Rabbit	1:500	Thermo Fischer Cat.#A27034	RRID: AB_2536097
	Primers			
	Target	Size of band	Forward/Reverse primer (5′–3′)	
Integration check-(PCR)	EBNA	426 bp	GTGGTTTGGAAAGCATCGTGGTCA/ATAGGTGGAAACCAGGGAGGCAAA	
Housekeeping gene-(PCR)	GAPDH	1596 bp	TCCCTGAGGGTTCTTTGTGC/GGGGCAGAGCATCTACACTC	
Housekeeping gene- (qPCR)	β-actin	151 bp	GGAGGAGCAATGATCTTGAT/TGAAGTGTGACGTGGACATC	
Reprogramming factor-(qPCR)	OCT4	142 bp	AGCGAACCAGTATCGAGAAC/TTACAGAACCACACTCGGAC	
Reprogramming factor-(qPCR)	NANOG	469 bp	TGATTTGTGGGCCTGAAGAAAA/TGGTGGTAGGAAGAGTAAAG	
Reprogramming factor-(qPCR)	SOX2	126 bp	AGCTACAGCATGATGCAGGA/GGTCATGGAGTTGTACTGCA	
Reprogramming factor-(qPCR)	LIN28A	134 bp	TGCGGGCATCTGTAAGTGG/GGAACCCTTCCATGTGCAG	

(Catalog no: AD-FA-16) following manufacturer's instructions. KU-WT2-2 is a control iPSC line generated from a healthy individual.

3.7. qPCR

Total RNAs were extracted by using MN Nucleospin RNA isolation kit. 1 μg of RNA was converted into cDNA using MMLV RT enzyme. For qPCR analysis β-actin was used as a housekeeping gene. Primers are listed in Table 2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102438.

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