Stem Cell Research 16 (2016) 304–307

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of human induced pluripotent stem cell line from a patient with a long QT syndrome type 2

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ARTICLE INFO

Article history:
Received 31 December 2015
Accepted 31 December 2015
Available online 6 January 2016

ABSTRACT

We report here the generation of human iPS cell line UKKi009-A from dermal fibroblasts of a patient carrying heterozygous mutation c.3035-3045delTCCCTCGATGC, p.Leu1012Pro (fs*55) in KCNH2 gene leading to long QT syndrome type 2 (LQT2). We used the Sleeping Beauty transposon-based plasmids expressing OSKM along with microRNAs 307/367 to reprogram the fibroblasts. The iPS cells possess pluripotent stem cell characteristics and differentiate to cell lineages of all three germ layers. This cell line can serve as a source for in vitro modeling of LQT2. This cell line is distributed by the European Collection of Authenticated Cell Cultures (ECACC).

Resource Table

Name of stem cell line	UKKi009-A
Alternative name	NP0011-8
Institution	Institute for Neurophysiology, Medical Faculty, University of Cologne, Germany
Person who created resource	Dina Ivanyuk
Contact person and email	Tomo Saric, tomo.saric@uni-koeln.de
Date archived/stock date	29th August 2014
Origin	Dermal fibroblasts
Sex	Female
Disease status	Long QT syndrome type 2 (LQT2; OMIM entry # 613688)
Mutation	Heterozygous mutation c.3035-3045delTCCCTCGATGC, p.Leu1012Pro (fs*55) in KCNH2 gene
Type of resource	iPS cell line for disease modeling, drug and toxicity testing
Sub-type	hiPSC, derived from dermal fibroblast, no genetic modification
Key transcription factors	POU5F1, SOX2, KLF4, MYC
Authentication	Identity was confirmed by DNA sequencing (Fig. 1C) SNP genotyping (Table 1) and STR analysis of six markers (Fig. 2)
Other clonal lines available	UKKi009-B (NP0011-19) iPS cell line; http://hpscreg.eu/cell-line/UKKi009-B
Link to related literature	None
Information in public databases	http://hpscreg.eu/cell-line/UKKi009-A
Ethics	Consent from the donor of the tissue has been obtained, Ethics Review Board/competent authority approval has been obtained

1. Resource details

The human iPSC line was generated by reprogramming of fibroblasts cultured from skin biopsy of a 32 year old patient suffering from LQT syndrome Type 2 (LQT2). LQT syndrome is a medical condition, either genetic or acquired, described after its characteristic electrocardiogram showing a prolonged cardiac repolarization phase resulting in a long QT interval. The clinical symptoms of LQTS include palpitations, syncope, seizures leading to sudden cardiac death. The patient carried a heterozygous mutation in KCNH2 gene c.3035_3045delTCCCTCGATGC leading to amino acid frame shift beginning at Leucine 1012 (p.Leu1012Pro, fs*55). Fibroblasts were reprogrammed by transposition of OCT4, SOX2, KLF4 and c-MYC (OSKM) and miRNA302/367 expression cassettes mobilized by the SB100X hyperactive transposase (Grabundzija et al., 2013). The reprogramming was performed on murine embryonic fibroblast (MEFs) in the presence of valproic acid and vitamin C for 7 days after transfection. After isolation and initial expansion, the iPS cells were maintained in a feeder-free system on vitronectin-coated plates in

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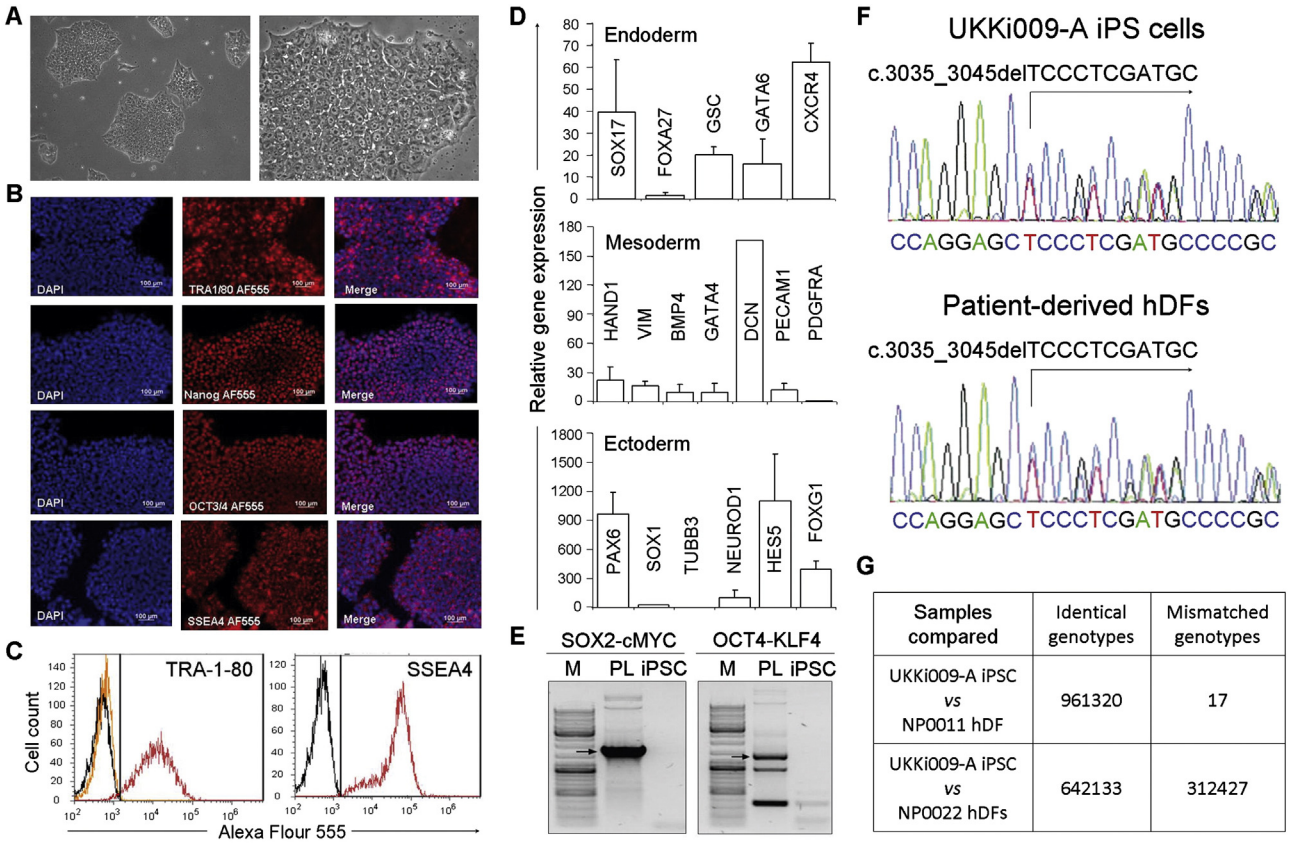
http://dx.doi.org/10.1016/j.scr.2015.12.039



Samples compared	Identical genotypes	Mismatched genotypes
UKKi009-A iPSC *vs* NP0011 hDF	961320	17
UKKi009-A iPSC *vs* NP0022 hDFs	642133	312427

Fig. 1. Characterization of human iPS cell line UKKi009-A. A. Morphology of iPS cell colonies as observed by bright field microscopy (left image: 10×, right image: 32×). B. Immunostaining for OCT4, NANOG, TRA-1-80 and SSEA4 showing the iPS colonies uniformly expressing the markers. C. Expression of pluripotent stem cell markers TRA-1-80 and SSEA4 on the surface of UKKi009-A iPS cells as measured by flow cytometry. D. Assessment of pluripotency of iPS cells by RT-qPCR analysis of expression of indicated germ layer-specific transcripts at day 14 of differentiation compared against undifferentiated cells as a control using the ΔΔCt method. E. RT-PCR showing absence of transgenes SOX2 and c-MYC (left panel) and OCT4 and KLF4 (right panel) expression in iPS cells. Plasmid encoding OSKM cassette was used as a positive control. Arrows indicate the expected band size (1848 bp and 1364 bp for left and right panel, respectively). M = DNA-size marker. F. DNA sequence showing the presence of mutation c.3035-3045delTCCCTCGATGC in the KCNH2 gene of patient-derived dermal fibroblasts (hDFs) and UKKi009-A iPS cells derived from them. The nucleotide sequence indicated beneath the sequencing chromatogram is the NCBI Reference Sequence: XM_011516185.1. G. Single nucleotide polymorphism (SNP) genotyping reveals identity of UKKi009-A iPS cells with NP0011 patient-derived hDFs abut not with hDFs derived from an unrelated donor NP0022.

Essential 8 (E8) medium. They exhibited morphology similar to those of human embryonic stem (ES) cells (Fig. 1A) and expressed endogenous self-renewal genes OCT4, NANOG and the pluripotent stem cell markers TRA-1-80 and SSEA4 at the protein level as demonstrated by immunocytochemistry (Fig. 1B) and flow cytometry (Fig. 1C). The pluripotency was further demonstrated in vitro by embryoid body (EB)-based differentiation to endodermal, mesodermal and ectodermal cell lineages (Fig. 1D). Cardiac differentiation was confirmed by identification of spontaneously beating cells (Supplemental video 1). This iPS cell line did not express transcripts of exogenous reprogramming factors as shown by semi-quantitative RT-PCR (Fig. 1E). The identity of the UKKi009-A iPS cell line with parental fibroblasts was confirmed by identification of the disease-associated mutation in the KCNH2 gene by DNA sequencing (Fig. 1F), SNP genotyping (Fig. 1G) and short tandem repeat (STR) analysis (Fig. 2). Molecular karyotyping revealed a normal female karyotype (Fig. 3). The cell line was also confirmed to be free of mycoplasma and viral (HIV-1, HCV and HBV) sequences.

2. Materials and methods

Human dermal fibroblasts (hDFs) were isolated from the skin biopsy of the patient and cultured in human fibroblast culture medium [Dulbecco's modified Eagle medium (DMEM)-GlutaMAX supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 100 U/mL penicillin, 100 μg/mL streptomycin and 100 μM β-mercaptoethanol (β-ME)]. Reprogramming was achieved by transfecting hDFs with plasmids expressing SB100× transposase, OKSM-mCherry, and microRNA 307/367 as described in (Grabundzija et al., 2013). Briefly, 3×10^6 hDFs were transfected with 3 μg of SB-OKSM, 1 μg SB100× and 1 μg of microRNA plasmid using Neon Transfection device and a 100 μL Neon Tip according to the manufacturer's instructions (ThermoFischer Scientific). Cells were then cultured in human fibroblast culture medium for the first 7 days. Later, the cells were re-plated on irradiated MEFs and cultured in reprogramming medium (DMEM/F-12 + GlutaMAX supplemented with 20% KnockOut Serum Replacement (KOSR), 8 ng/mL basic fibroblast growth factor (bFGF), 1% NEAA, and 100 μM β-ME) containing vitamin C (50 μg/ml) and valproic acid (1 mM) at 37 °C in normoxic conditions and 5% CO_2. The cells were treated with small molecules for 7 days. Later, the cells were cultured on MEFs in reprogramming media without small molecules until iPS cell colonies appeared which were then picked and cultured. Each picked clone was expanded on MEFs in human iPS cell medium (DMEM/F-12 + GlutaMAX supplemented with 20% KOSR, 1% NEAA, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM β-ME and 10 ng/ml bFGF) and later adapted to culture on vitronectin in E8 medium. iPS cell culture was repeatedly checked for mycoplasma contamination using MycoAlert before making frozen stocks.

3. In vitro differentiation

Media was removed and the cells were washed with PBS. TrypLE was added for 3 min. DMEM containing 20% FCS was added to quench the TrypLE. Cells were centrifuged at 300 ×g for 3 min and

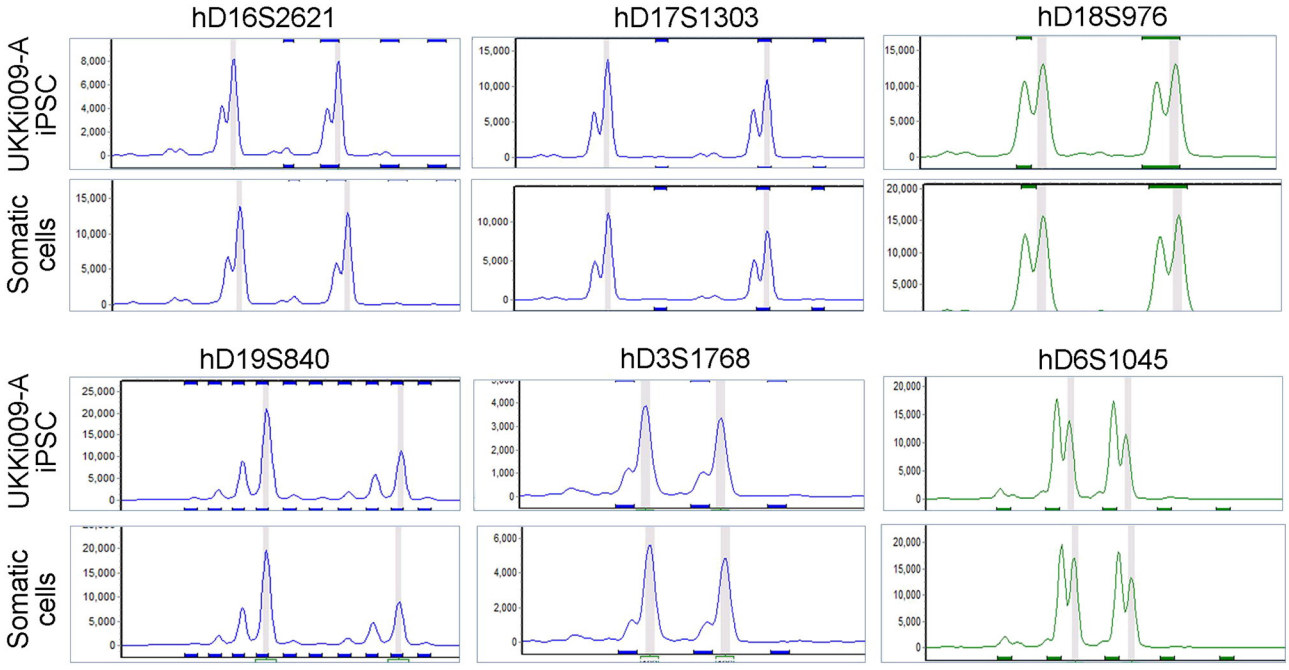


Fig. 2. Confirmation of genetic identity of UKKi009-A iPSC line with the corresponding patient-derived somatic cells. Microsatellite analysis shows matching profiles of short tandem repeats (STR) amplified from a genomic DNA of UKKi009-A iPS cells and NP0011 patient-derived keratinocytes.

the cell pellet was resuspended in APEL medium (Stemcell Technologies) containing 10 μM Rock inhibitor at 30,000 cells per ml. 100 μl was then added to each well of a U-bottom 96 well plate and centrifuged at 300 ×g for 3 min. The plates were placed in an incubator at 37 °C, 5% CO_2. EB's formed overnight. Samples were collected at day 14. RNA was extracted using Maxwell RSC machine and kit, according to manufactures instructions (Promega). Three biological replicates were carried out with one passage between each experiment. Directed cardiac differentiation was performed according to (Lian et al., 2013).

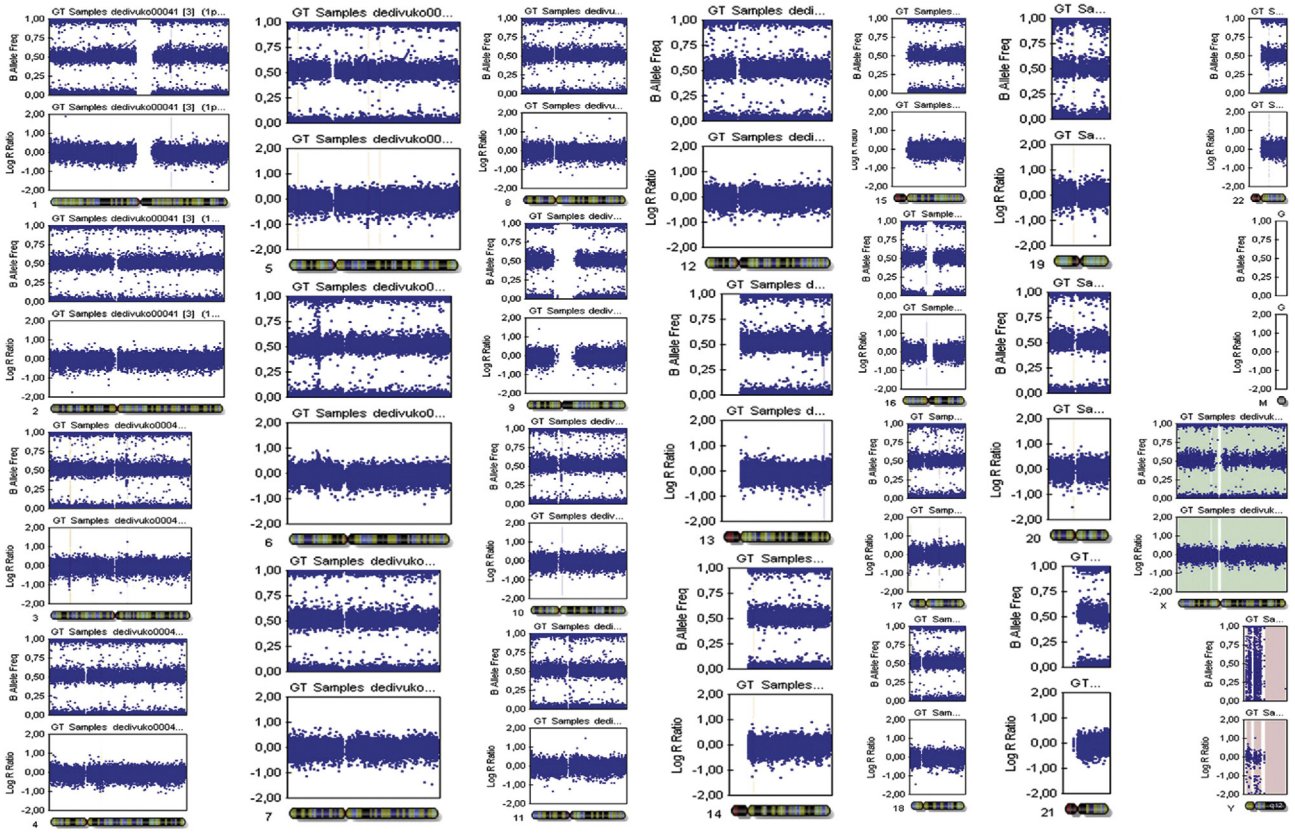


Fig. 3. Molecular karyotype of UKKi009-A iPS cells. SNP genotyping shows that iPS cells have a normal female karyotype.

4. Comparative Real-Time PCR

cDNA was produced using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions and analyzed with gene-specific probes (Applied Biosystems) by standard methods and run on Quantstudio thermocycler. For assessment of cell line pluripotency each differentiated sample was compared against its undifferentiated counterpart as a normalized control using the ΔΔCt method, to give relative quantitation (RQ) values using GAPDH and ACTB as reference genes.

5. Semi-quantitative RT-PCR

Total RNA was isolated using TRIzol Reagent from confluent growing cells. DNase I-treated total RNA (500 ng) was reverse-transcribed using Superscript II RTase and random hexamers (Life Technologies). cDNA was diluted 1:4 with sterile tri-destilled water and 5 μl were amplified using JumpStart™ RedTaq ReadyMix™ PCR Reaction Mix (Sigma). Negative controls were generated in RT reactions in which all reaction components were included except RTase. Reactions were terminated at the exponential phase of amplification and products were analyzed by agarose gel electrophoresis.

6. Immunocytochemistry

Undifferentiated iPS cells were fixed at 80% confluency with 4% paraformaldehyde (PFA), permeabilized with 0.1% Triton X-100 and blocked with 5% FBS. The cells were then stained overnight at 4 °C with primary antibodies specific for OCT4 (Santa Cruz Biotechnology, Cat. No. Sc-5279, 1:100), NANOG (R&D Systems, Cat. No. AF1997, 1:100) TRA-1-80 (Santa Cruz, Cat. No. Sc-21706, 1:200) and SSEA4 (Santa Cruz, Cat. No. Sc-21704, 1:800). Samples were visualized after staining with Alexa Fluor 488- or Alexa Fluor 555-conjugated secondary antibodies (Life Technologies) for 1 h at room temperature. Nuclei were counterstained with Hoechst 33342. Samples were embedded in ProLong Gold Antifade Reagent (Life Technologies) and observed on Axiovert Microscope (Carl-Zeiss Microimaging, Oberkochen, Germany) equipped with the image processing software Axiovision 4.5.

7. Flow cytometry

Cells were dissociated with Trypsin/EDTA and incubated for 30 min at 4 °C with Alexa Flor 555-conjugated antibodies against cell surface markers TRA-1-80 and SSEA4. Alexa Flor 555-conjugated mouse IgM and IgG antibodies served as isotype controls for TRA-1-80 and SSEA4, respectively. Detection of labeled cells was accomplished on Attune® Acoustic Focusing Cytometer (Applied Biosystems) using Attune® Cytometric Software.

8. Microsatellite analysis

Genotype analysis of cell lines was performed using 6 highly informative microsatellite markers. Fluorescently labeled PCR products were electrophoresed and detected on an automated 3730 DNA Analyzer and data were analyzed using Genemapper software version 3.0 (Applied Biosystems).

9. Molecular karyotyping

The molecular karyotype was analyzed by SNP genotyping using Illumina's HumanOmniExpressExome-8-v1.2 BeadArray (Illumina, Inc., San Diego, CA, USA) at the Institute for Human Genetics (Department of Genomics, Life & Brain Center, University of Bonn, Germany). Processing was performed on genomic DNA following the manufacturer's procedures. LogR ratio and B allele plots were generated in GenomeStudio V2011.1 (Illumina, Inc., San Diego, CA, USA) using the provided manifest and cluster files version 1.2-B. Copy number regions were detected using the cnvPartition version 3.1.6. A visual inspection had been performed for mosaicism states.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.12.039.

Acknowledgments

This work was supported by the Innovative Medicines Initiative (IMI) project “European Bank for induced Pluripotent Stem Cells” (EBiSC) and the grants from the Bundesministerium für Bildung und Forschung (Federal Ministry of Education and Research, grant No. 01 GN 0824) and Köln Fortune Programm.

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