Stem Cell Research 61 (2022) 102750

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of two induced pluripotent stem cell lines (UQACi002-A and UQACi005-A) from two patients with *KRT14* epidermolysis bullosa simplex mutations

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A B S T R A C T

More than 107 pathogenic variations were identified in Keratin 14 gene (*KRT14*) in patients affected by epidermolysis bullosa simplex (EBS), a rare skin disease with still no curative treatment. Disease models as human induced pluripotent stem cells (hiPSCs) are promising tool for further advance the knowledge about this disorder and accelerate therapies development. Here, two hiPSC lines were reprogrammed from skin fibroblasts of two EBS patients carrying mutations within *KRT14* by using CytoTune®Sendai virus. These iPSCs display pluripotent cell morphology, pluripotent markers expression, and the capability to differentiate into the three germ layers.

1. Resource table

Unique stem cell lines identifier	1- UQACi002-A 2- UQACi005-A
Alternative name(s) of stem cell lines	iPSC-EBS1 iPSC-EBS10
Institution	Université du Québec à Chicoutimi (Québec), Canada
Contact information of distributor	Catherine Laprise, Catherine.Laprise@uqac.ca
Type of cell lines	iPSC
Origin	Human skin cells
Additional origin info required *for human ESC or iPSC*	UQACi002-A, Age: 30, Sex: Male, Ethnicity: Canadian UQACi005-A, Age: 5, Sex: Female, Ethnicity: Canadian
Cell Source	Human fibroblasts
Clonality	Clonal
Associated disease	Epidermolysis bullosa simplex
Gene/locus	Keratin14 gene (*KRT14*), locus 17q21.2Autosomal dominant mutations NM_000526.5 (*KRT14*): UQACi002-A: Heterozygous (c.1130 T > C), UQACi005-

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	1- UQACi002-A 2- UQACi005-A
	A: Heterozygous (c.1234 A > T)
Date archived/stock date	October 2021
Cell line repository/bank	https://hpscreg.eu/user/cellline/UQACi002-A https://hpscreg.eu/user/cellline/UQACi005-A
Ethical approval	Le comité d'éthique de la recherche avec des êtres humains de l'Université du Québec à Chicoutimi, Canada (Approval number 602.162.05)

2. Resource utility

The two iPSC lines (UQACi002-A and UQACi005-A) generated from two EBS patients carrying pathogenic variants within exon 6 of *KRT14* can be differentiated into keratinocytes, the affected cells in EBS, and hence serving as precious cellular model to further understand the disease pathogenesis and develop new therapies.

Abbreviations: *KRT14*, Keratin 14; EBS, epidermolysis bullosa simplex; hiPSCs, human induced pluripotent stem cells; *KRT5*, keratin 5; FACS, fluorescence-activated cell sorting; RT-PCR, reverse transcription polymerase chain reaction; STR, short tandem repeat; hESC, human embryonic stem cell; bFGF, fibroblast growth factor; chr, chromosome.

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https://doi.org/10.1016/j.scr.2022.102750
Received 6 December 2021; Received in revised form 27 February 2022; Accepted 11 March 2022
Available online 14 March 2022
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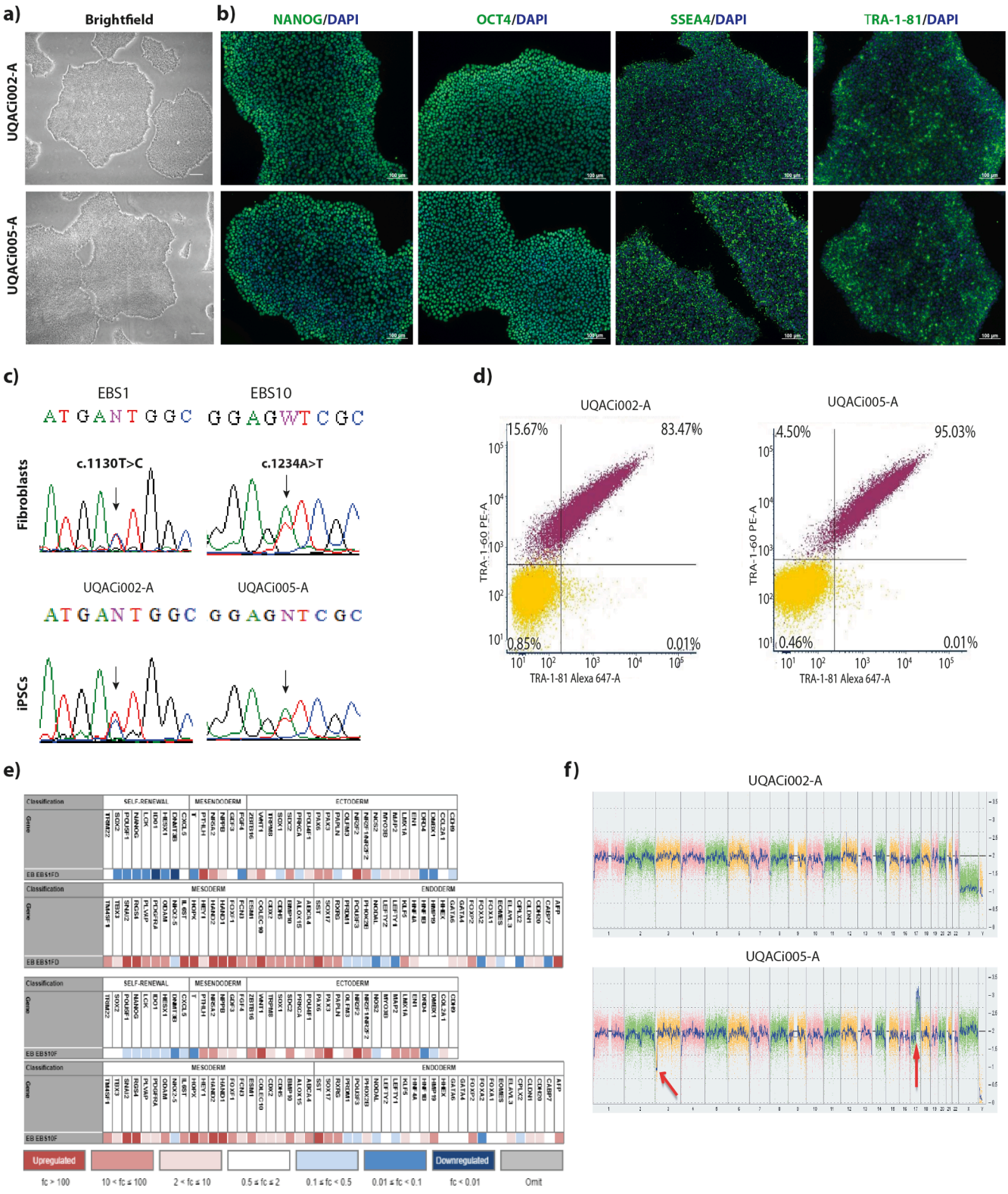

Fig. 1. Generation and characterization of human induced pluripotent stem cells (iPSCs) from two patients with epidermolysis bullosa simplex. a) Typical embryonic stem cell-like colony morphology was observed after fibroblasts reprogramming. b) Immunofluorescence analysis showed expression of pluripotent markers OCT4, NANOG, TRA1-81 and the surface marker SSEA4 (scale bars: 100 μm). c) Electropherograms showed the presence of the mutations in the patient's fibroblasts and in the iPSCs lines. d) Expression analysis of pluripotent markers TRA-1-81 and TRA-1-60 by flow cytometry analysis showed expression in 83–95% of cells. e) Scorecard analysis showed the expression of many genes of the three germ layers (endoderm, mesoderm and ectoderm). f) KaryoStat assay analysis showed normal karyotype for UQACi002-A and abnormalities in ch17 and chr3 for two clones of UQACi005-A among five characterized ones.

3. Resource details

Epidermolysis bullosa simplex (EBS) is an autosomal dominant inherited dermatological disease, characterized by recurrent bullous lesions and intraepidermal blisters after exposure to mechanical trauma. EBS first occurs in childhood and can be subdivised on three major clinical forms that may be generalized or localized (Bolling et al., 2011). The causative genes, keratin 5 (*KRT5*) and keratin 14 (*KRT14*) are encoding the main structural proteins of keratin intermediate filaments in the epidermal keratinocytes (Tan et al., 2021). The pathogenesis mechanism of EBS is not fully elucidated, and up to now, there are only limited palliative support for patients affected by this cutaneous disease. Therefore, it is necessary to uncover the disease process and explore new ideas for therapy and potential approaches for treatment as reprogramming patient's cells into iPSCs and correcting the pathogenic variations by the new developing genome edition tools.

In this study, we successfully generated two iPSC lines from skin fibroblasts of two mildly affected EBS patients heterozygous for pathogenic variations within exon 6 of *KRT14*. UQACi002-A is from a patient (EBS1) aged of thirty years and holding the mutation c.1130 T > C (p.Ileu377Thr). UQACi005-A derived from a patient (EBS10) aged of five years and carrying the variation c.1234 A > T (p.Ile412Phe). We previously published the identification of these two pathogenic variations (Bchetnia et al., 2012; Bchetnia et al., 2020). We used the Cytotune Sendai Virus System to deliver the reprogramming factors KOS, KLF4, and C-MYC into EBS patient skin fibroblasts. Single iPSC colonies were isolated and observed under phase contrast microscopy. They displayed a standard pluripotent stem cell morphology characterized by a large nucleus and densely packed cells that grow in colonies (Fig. 1a). Immunofluorescence showed the expression of pluripotent marker genes including NANOG, OCT3/4, SSEA-4, and TRA-1-81 (Fig. 1b). Reverse transcription polymerase chain reaction (RT-PCR) further confirmed the expression of OCT3/4, NANOG, REX1, DNMT3b and hTERT (Supplementary Fig. S1a). Fluorescence-activated cell sorting (FACS) for the pluripotency makers TRA-1-60 and TRA-1-81 showed expression in 83–95% of cells (Fig. 1d), RT-PCR showed the absence of Sendai virus four weeks after reprogramming, indicating the successful activation of endogenous pluripotent genes (Supplementary Fig. S1b). Scorecard analysis showed the expression of several genes of the three germ layers (endoderm, mesoderm and ectoderm) (Fig. 1e). Sanger sequencing validated the presence of the two *KRT14* mutations c.1130 T > C and c.1234 A > T carried by the studied patients in the parental fibroblasts and in the generated iPSCs (Fig. 1c). Karyotype was assessed by KaryoStat assay and was normal with no clonal abnormalities for UQACi002-A. However, among five characterized UQACi005-A clones, two are showing abnormalities in chromosome 17 (chr17) and chr3 and the other are normal (Fig. 1f, Supplementary Fig. S1d). The chromosomal gain on ch17 is frequently observed in iPSCs (Kyriakides et al., 2018) however, the chromosomal loss on chr3 seems to be a rare aberration. Cell identities were verified by comparing short tandem repeat (STR) analyses of fibroblasts and their derived iPSCs for nine STR markers with amelogenin for sex determination. The iPSCs were not contaminated with mycoplasma (Supplementary Fig. S1c).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel a
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for NANOG, OCT4, SSEA4, and TRA-1–81,	Fig. 1 panel b
	Quantitative analysis RT-PCR and Flow cytometry	Expression of pluripotent markers OCT4, NANOG, REX1, DNM3TB, HTERT, TRA-1-81 and TRA-1-60	Fig. 1 panel d and Supplementary Fig. S1a
Genotype	Molecular karyotyping using Karyostat™	46XY for UQACi002-A Aberration on chr3 and chr17 for UQACi005-A	Fig. 1 panel f
Identity	STR analysis	10 sites tested, all matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	UQACi002-A is heterozygous for the mutation c.1130 T > C in *KRT14*. UQACi005-A is heterozygous for the mutation c.1234 A > T in *KRT14*	Fig. 1 panel c
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Supplementary Fig. S1c
Differentiation potential	Scorecard	Expression of genes of all three germ layers	Fig. 1 panel e
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

4. Materials and methods

4.1. Skin fibroblasts reprogramming

Skin fibroblasts were cultured in standard DMEM contgaining 10% FBS and 1% Penicillin/ Streptomycin (Thermo Fisher Scientific) at 37 °C, 5% CO2. The genome integration-free SeV virus kit (CytoTune™-2.0, ThermoFisher Scientific), containing c-MYC, KLF4, OCT3/4, and SOX2 pluripotency transcription markers, was used to reprogram these fibroblasts to iPSCs in mTeSR™ Plus (StemCell Technologies; 05826). Fibroblasts, at passage 2, were reprogrammed into iPSCs on irradiated mouse embryonic fibroblasts (Amsbio) and feed daily with KnockDMEM/F-12, 20% KnockOut Serum Replacement, Glutamax, 1% MEM Non-Essential Amino Acids, 55 μM 2-Mercaptoethanol and basic fibroblast growth factor (bFGF) at 10 μg/ml). The generated cell lines UQACi002-A and UQACi005-A were cultured and maintained on Matrigel-coated plates (Corning) in mTeSR1 medium (StemCell Technologies™), at 37°C in humidified air with 5% CO2. Medium changes were performed every other day until iPSC colonies reached appearance like embryonic stem cell (hESC, line H1) and were ready to pick and expansion. The iPSCs were enzymatically passaged using 0,5 mM EDTA. Medium was changed daily, and cells were subcultured once every 6 to 8 days.

4.2. Mutation analysis

Genomic DNA was extracted from primary fibroblasts, at passage 2, and iPSCs using QuickExtract™ DNA Extraction Solution (Epicentre). Exon 6 of *KRT14* was amplified by PCR and analyzed by Sanger sequencing with primers listed in Table 2.

Table 2
Reagents details.

	Antibodies		
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Mouse anti-OCT3/4	1:500	Santa Cruz Cat# sc-5279, RRID: AB_628051
	Mouse anti-NANOG	1:2000	Millipore Cat# MABD24, RRID: AB_11203829
	Mouse anti-SSEA4	1:100	ThermoFisher Scientific Cat# 41-4000, RRID: AB_2533506
	Mouse anti-TRA1-60	1:100	StemCell Technologies Cat# 60064, RRID: AB_2686905
	Mouse anti-TRA1-81	1:500	ThermoFisher Scientific Cat# MA1-24, RRID: AB_2356706
Secondary antibodies	Rabbit anti-mouse IgG (H + L), Alexa-Fluor 488	1:500	ThermoFisher Scientific Cat# A-11001, RRID: AB_2534069
	Primers		
	Target	**Size of band (pb)**	**Forward/Reverse primer (5′-3′)**
Sendai virus detection (PCR)	SeV	181	GGATCACTAGGTGATATCGAGCACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	528	ATGCACCGCTACGACGTGAGCGCACCTTGACAATCCTGATGTGG
	KLF4	410	TTCCTGCATGCCAGAGGAGCCCAATGTATCGAAGGTGCTCAA
	C-MYC	532	TAACTGACTAGCAGGCTTGTCGTCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (RT-PCR).	NANOG	225	CCTGGAACAGTCCCTTCTATAAC TCACTCATCTTCACACGTCTTC
	OCT4	1070	GTGGAGGAAGCTGACAACAA CAGGTTTTCTTTCCCTAGCT
	DNM3TB	230	TGCTGCTCACAGGGCCCGATACTTC TCCTTTCGAGCTCAGTGCACCACAAAAC
	hTERT	200	TGTGCACCAACATCTACAAG GCGTTCTTGGCTTTCAGGAT
	REX1	220	CGCAATCGCTTGTCCTCAGAG GCTCTCAACGAACGCTTTCCCA
Reference gene	ACTB	329	GGACTTCGAGCAAGAGATGG AGCACTGTGTTGGCGTACAG
Targeted mutations analysis (Sanger sequencing)	*KRT14* (exon 6)	1016	GGCCTAAGGAACACCAATCC CTGTCACCCAGTTTCCCT

4.3. Immunofluorescence staining

Immunostaining of markers NANOG, OCT4, SSEA4, and TRA-1–81 was performed in order to verify the pluripotency status of the two generated cell lines. Briefly, Cells were fixed with 4 % paraformaldehyde for 15 min at room temperature and permeabilized with 0.2% Triton X-100 for 10 min. Samples were blocked with 1%BSA, 0.3% TritonTMX-100 in DPBS for 30 min at room temperature. Then, they were incubated overnight at 4 ∘C with the primary antibodies in 1% BSA. Afterwards, they were incubated with the secondary antibodies for 1 h at room temperature. The used antibodies and their dilution rate were listed in Table 1. Nuclei were counterstained with NucBlue Reagent DAPI and images visualization was taken using the fluorescent microscope Zeiss Axio Observer Microscope.

4.4. Flow cytometric analysis

After dissociation into single cells with accutase, iPSCs were count and incubated with a fixable viability stain (BD Horizon, Cat# 565388; dilution 1:1000) for 30 min on ice. Then, cells were washed two times with stain buffer (BD Phamingen Cat#554656) and incubated with TRA-1-81 and TRA-1-60 antibodies and their isotype controls, 30 min at room temperature, in obscurity. Analysis was performed using the flow cytometer BD FACSMelody.

4.5. RT-PCR analysis

RNA was harvested from iPSCs as well H1-hESC using a Direct-zol™ RNA miniprep and RNA integrity and yield were assessed using Agilent BioAnalyser RNA 2100 and on 2% agarose gel. The Quantitect Reverse transcription kit was used to synthesize cDNA. PCR was performed using with the primers listed in Table 2 to confirm expression of the pluripotency markers OCT3/4, NANOG, REX1, DNMT3b and hTERT (at passage 15) as well as the absence of Sendai virus vectors SEV, KOS, KLF4, and C-MYC (at passage 6). Expression of pluripotency genes was also further compared to it levels in H1-hESC.

4.6. Trilineage differentiation

To evaluate the differentiation potential of the two iPSC lines into the three germ layers, we used the scorecard™ Kit 384w (Applied Biosystems) following manufacturer's instructions.

4.7. Karyotype analysis

The KaryoStat™ assay (Thermo Fisher Scientific) was used to analyze the karyotype of iPSC lines at passage 5. The hPSC Genetic Analysis Kit was used for detecting the majority of karyotypic abnormalities reported in human pluripotent stem cells (hPSC) (StemCell Technologies).

4.8. The short tandem repeat (STR) analysis

STR analysis of original patient fibroblasts and derived iPSCs was carried out by Genome Quebec laboratories.

4.9. Detection of mycoplasma contamination

The Venor®GeM Mycoplasma PCR Detection Kit (Cederlane) was used to test for mycoplasma contamination following the manufacturer's protocol.

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.

Acknowledgements

We wish to thank the participants recruited in the epidermolysis bullosa UQAC biobank for their valuable participation in this study. This work was supported by *la Fondation du grand défi Pierre Lavoie, le Réseau de thérapie cellulaire, tissulaire et génique du Québec – ThéCell (*www.reseauthecell.qc.ca*)*, the rare disease foundation, and Debra Canada. Catherine Laprise (C.L) is the director of the *Centre intersectoriel en santé durable de l'UQAC* and the chairholder of the Canada Research Chair in the Environment and Genetics of Respiratory Disorders and Allergies (www.chairs.gc.ca). C.L. and Mbarka Bchetnia are scientific members of the CERMO-FC (www.cermofc.uqam.ca/) and members of ThéCell

Network of the Fonds de recherche en santé du Québec. François Gros-Louis holds a tier 2 Canada research Chair in brain diseases modelling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102750.

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