Stem Cell Research 44 (2020) 101748

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line (UQACi001-A) from a severe epidermolysis bullosa simplex patient with the heterozygous mutation p.R125S in the *KRT*14 gene

Mbarka Bchetnia[a], Laurie Martineau[b], Véronique Racine[b], Julie Powell[c], Catherine McCuaig[c], Jack Puymirat[b], Catherine Laprise[a,*]

[a] Département des Sciences Fondamentales, Université du Québec à Chicoutimi, 555 Boulevard de l'Université, Chicoutimi, QC G7H 2B1, Canada
[b] CHU de Québec-Université Laval Research Center, Quebec City, QC G1V 4G2, Canada
[c] Hôpital Ste-Justine, Montréal, QC H3T 1C5, Canada

A B S T R A C T

We have generated UQACi001-A, a new induced pluripotent stem cell (iPSC) line derived from skin fibroblasts of a male patient with the generalized severe epidermolysis bullosa simplex phenotype (EBS-gen sev) and carrying the keratin 14 (K14) R125S mutation. Fibroblasts were reprogrammed using non-integrating Sendai virus vectors. The iPSC line displayed normal molecular karyotype, expressed pluripotency markers, is capable of differentiating into three embryonic germ layers and is genetically identical to the originating parental fibroblasts. The established iPSC model provides a valuable resource for studying the rare disease of epidermolysis bullosa simplex and developing new therapies as DNA editing by CRISPR/Cas9 technology.

Resource table

Unique stem cell line identifier	UQACi001-A
Alternative name(s) of stem cell line	iPSC-EBS21
Institution	Université du Québec à Chicoutimi (Québec), Canada
Contact information of distributor	Catherine Laprise, Catherine.Laprise@uqac.ca
Type of cell line	iPSC
Origin	Human skin cells
Additional origin info	Induced pluripotent stem cell line (iPSC) Age: 33 years old Sex: male Ethnicity: Canadian
Cell Source	Human fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrating SeV-mediated delivery of OCT4, SOX2, c-MYC, and KLF4
Genetic Modification	YES
Type of Modification	Hereditary
Associated disease	Epidermolysis bullosa simplex
Gene/locus	Keratin14 gene (*KRT14*), locus 17q21.2, Autosomal dominant mutation NM_000526.5 (*KRT14*):c.373C>A (K14 p.R125S)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	November 2019
Cell line repository/bank	N/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)

1. Resource utility

The iPSCs line was established from an EBS patient with the severe missense mutation p.R125S, not reported elsewhere (Bchetnia et al., 2012). This line might provide a cellular model to investigate the biological pathways altered by this mutation as well as to construct *in vitro* 3D skin models useful for novel personalized therapies.

2. Resource details

Epidermolysis bullosa simplex (EBS) is a rare skin disease characterized by skin fragility and blistering upon minor mechanical trauma. This disease is primarily caused by dominantly autosomal mutations in the keratin 5 (*KRT5*) or 14 genes (*KRT14*). These mutations lead to a collapse of the keratin cytoskeleton into cytoplasmic protein aggregates and the appearance of the EBS phenotype. To date,

* Corresponding author.
E-mail address: catherine_laprise@uqac.ca (C. Laprise).

https://doi.org/10.1016/j.scr.2020.101748
Received 2 December 2019; Received in revised form 15 February 2020; Accepted 18 February 2020
Available online 06 March 2020
1873-5061/ © 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

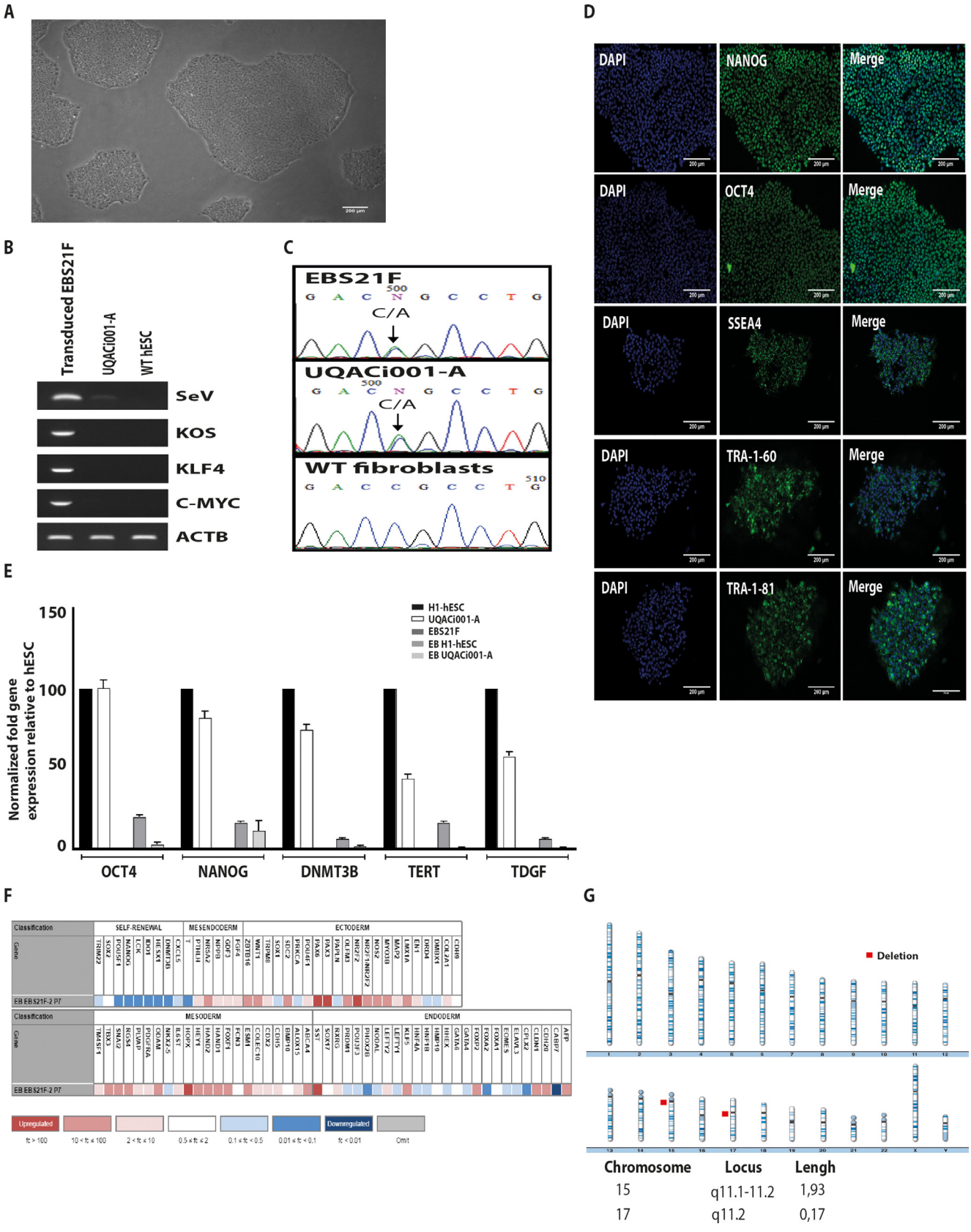

Fig. 1. Characterization of the UQACi001-A iPSC line.

close to 200 distinct pathogenic mutations have been identified (http://www.interfil.org) and variants in the *KRT14* gene that encodes keratin 14, are responsible for approximately 37% of all cases (Fine et al., 2014). A particular arginine codon within the helix initiation peptide in K14 (R125) is the most commonly mutated residue occurring in >30% of EBS-gen-sev cases, probably because it contains a hypermutable CpG dinucleotide. As a result, many studies in the literature reported severe affected patients with the cysteine (TGC) or histidine (CAC) in the place of the arginine codon (CGC) (Uitto et al., 2007). Our patient with the EBS-gen-sev phenotype is carrying a serine (AGC) at the 125 position (p.R125S) which is until now not reported elsewhere (Bchetnia et al., 2012).

Here we reprogrammed primary fibroblasts obtained from a skin biopsy of this patient (EBS21F) on iPSCs using non-integrative Sendai virus containing the human reprogramming factors, OCT4, SOX2, C-MYC and KLF4 (Takahashi et al., 2007) following instructions by manufacturer. Four weeks post transduction, colonies with a typical morphology of pluripotent stem cells appeared. These clones were subsequently manually picked and expanded to establish feeder-free iPSC cells (Fig. 1A). After two months' culture, the clearance of the virus and the exogenous reprogramming factor genes was confirmed in the resulting UQACi001-A cell line by PCR using specific primers (Fig. 1B). The presence of the heterozygous (K14 p.R125S) mutation was confirmed in the iPSC line by Sanger sequencing (Fig. 1C). Pluripotency was assessed by specific immunofluorescence staining for OCT4, NANOG, SSEA4, TRA-1-60 and TRA-1-81 (Fig. 1D) as well as by qRT-PCR for OCT4, NANOG, DNM3TB, hTERT, and TDGF (Fig. 1E). The iPSC line showed robust expression of all tested pluripotency markers. UQACi001-A cell line formed embryoid bodies that spontaneously differentiated into three germ layers. Using scorecard analysis, we observed expression of specific markers for ectoderm, mesoderm and endoderm (Fig. 1F). Examination of the genomic integrity of our iPSC line using array CGH, after six passages, showed a normal karyotype with no gain or loss that would be detected in a traditional karyotype (>5 MB) (Fig. 1G). STR analysis for 16 short tandem repeat markers showed identical profiles for iPSC line with the parental fibroblasts (available with the authors). Mycoplasma testing was negative proving that our iPSC line is free from mycoplasma contamination (Fig. S1). The current data proves that stable EBS-gen-sev patient specific iPSC line have been successfully generated. UQACi001-A cell line can provide a powerful tool for: 1) establishing an iPSC-derived skin equivalent; 2) identifying the biological pathways altered by the R125S mutation; 3) innovative drug screening and genome editing for EBS.

3. Materials and methods

3.1. Reprogramming patient's fibroblasts

Skin fibroblasts were cultured at 37 °C, 5% CO2, in the fibroblast media consisted of DMEM with 10% FBS and 1% Penicillin/Streptomycin (Thermo Fisher Scientific). 3×10^5 cells, at passage 2, were transduced with the genome integration-free SeV virus kit (CytoTune™ − 2.0, ThermoFisher Scientific) following manufacturer's guidelines. When clones with hESC like appearance reached a sufficient size, they were manually picked and passaged for expansion (Table 1). The iPSCs were maintained on Matrigel (Corning) with mTeSR1 medium (StemCell Technologies™) at 37 °C and 5% CO2. Cells were mechanically passaged every 6–8 days.

3.2. Mutation verification

Genomic DNA was extracted from primary fibroblasts and iPSCs cells using QuickExtract™ DNA Extraction Solution (Epicentre). Primers used for amplification and Sanger sequencing of K14p.R125S flanking region are described in Table 2.

3.3. Immunofluorescence analysis

The pluripotency status of UQACi001-A cell line was evaluated by immunostaining for NANOG, OCT4, SSEA4, TRA-1-60, and TRA-1-81. Briefly, the iPSCs were fixed with 4% para formaldehyde for 15 min at room temperature and washed with DPBS. They were permeabilized with 0.1% Triton™X-100, and blocked with 1%BSA, 0.3% Triton™X-100 in DPBS at room temperature. Cells were then stained with specific antibodies (Table 1). Images were captured under the fluorescent microscope (Zeiss Axio Observer Microscope).

3.4. PCR and qRT-PCR analysis

PCR was carried out on genomic DNA using HotStarTaq DNA polymerase kit (Qiagen) using specific primers to assess the presence of remaining Sendai virus vectors (Table 2). Total RNA was isolated from iPSC cells using Direct-zol™ RNA miniprep and reverse transcribed into cDNA using the Quantitect Reverse transcription kit. Pluripotency markers expression was performed by qRT-PCR using SYBR Green I Master hot start reaction mix. RPL13A and ACTIN were used as normalization controls. Markers characterizing the three germ layers were assessed by scorecard assay using the scorecard™ Kit 384w (Applied Biosystems) following manufacturer's protocol (Fergus et al., 2016).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for TRA-1-81, TRA-1-60, SSEA4, OCT4 and NANOG	Fig. 1 panel D
	Quantitative analysis qRT-PCR	Expression of pluripotent markers OCT4, NANOG, DNM3TB, hTERT and TDGF	Fig. 1 panel E
Genotype	Karyotype (ArrayCGH)	46XY Resolution: 41 kb overall and 33 kb in RefSeq genes	Fig. 1 panel G
Identity	STR analysis 16 sites tested, all matched	submitted in archive with journal	
Mutation analysis	Sequencing	Heterozygous for p.R125S mutation in *KRT14*	Fig. 1 panel C
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Supplementary Fig. S1
Differentiation potential	Embryoid body formation	Expression of genes of all three germ layers	Fig. 1 panel F
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

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	Forward/Reverse primer (5′ − 3′)
Sendai virus detection (PCR)	SeV	GGATCACTAGGTGATATCGAGC
		GGATCACTAGGTGATATCGAGC
	KOS	ATGCACCGCTACGACGTGAGCGC
		ACCTTGACAATCCTGATGTGG
	KLF4	TTCCTGCATGCCAGAGGAGCCC
		AATGTATCGAAGGTGCTCAA
	C-MYC	TAACTGACTAGCAGGCTTGTCG
		TCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (qRT-PCR)	NANOG	CCTGGAACAGTCCCTTCTATAAC
		TCACTCATCTTCACACGTCTTC
	OCT4	GTGGAGGAAGCTGACAACAA
		CAGGTTTTCTTTCCCTAGCT
	DNM3TB	TGCTGCTCACAGGGCCCGATACTTC
		TCCTTTCGAGCTCAGTGCACCACAAAAC
	hTERT	TGTGCACCAACATCTACAAG
		GCGTTCTTGGCTTTCAGGAT
	TDGF	TCCTTCTACGGACGGAACTG
		AGAAATGCCTGAGGAAAGCA
Internal control gene (qRT-PCR)	ACTB	GGACTTCGAGCAAGAGATGG
		AGCACTGTGTTGGCGTACAG
Internal control gene (qRT-PCR)	RPL13A	ACCGCCCTACGACAAGAAAA
		TGCACAATTCTCCGAGTGCT
Targeted mutation analysis (Sanger sequencing)	*KRT14* (exon 1)	AAAGTGCCAGACCCGCCC
		CTGGGGAAGGGAAAGCATCT

3.5. In vitro differentiation

To evaluate the ability of UQACi001-A line to form three germ layers, spontaneous formation of embryoid body (EB) *in vitro* was assessed. iPSCs were harvested with accutase and plated in non-adherent dishes in EB medium consisting of DMEM/F12, 20% KnockOut™ Serum Replacement 1% non-essential amino acids and 1% GlutaMAX™ (ThermoFisher Scientific), 0.1 mM 2-mercaptomethanol and 50 μM rock inhibitor Y-27632. Forming EBs were transferred, after 8 days in suspension, onto gelatin coated plate and cultured for another 8 days.

3.6. Molecular karyotyping

Array comparative genomic hybridization (aCGH) at passage 6 was performed for UQACi001-A cell line at the Cell Line Genetics Inc laboratories (Madison, WI, USA). aCGH does not detect translocations or inversions, alterations in chromosome structure, mosaicism or polyploidy.

3.7. STR analysis

This also was performed at the Cell Line Genetics Inc laboratories (Madison, WI, USA). Briefly, fibroblasts and generated iPSCs DNA was extracted and PCR amplification of 16 distinct STRs (vWA, D8S1179, TPOX, FGA, D3S1358, THO1, D21S11, D8S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D) was carried out. PCR products were separated and analyzed.

3.8. Mycoplasma contamination detection

The absence of mycoplasma contamination was detected using Venor®GeM Mycoplasma PCR Detection Kit (Cederlane).

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.

Acknowledgments

We wish to thank the participant recruited in the epidermolysis bullosea UQAC biobank for his valuable participation in this study. This work was supported by la fondation du grand défi Pierre Lavoie, 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/).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101748.

References

Bchetnia, M., Tremblay, M.L., Leclerc, G., Duperee, A., Powell, J., McCuaig, C., Morin, C., Legendre-Guillemin, V., Laprise, C., 2012. Expression signature of epidermolysis bullosa simplex. Hum. Genet. 131 (3), 393–406.

Fergus, J., Quintanilla, R., Lakshmipathy, U., 2016. Characterizing pluripotent stem cells using the TaqMan(R) hPSC Scorecard(TM) panel. Methods Mol. Biol. 1307, 25–37.

Fine, J.D., Bruckner-Tuderman, L., Eady, R.A., Bauer, E.A., Bauer, J.W., Has, C., Heagerty, A., Hintner, H., Hovnanian, A., Jonkman, M.F., Leigh, I., Marinkovich, M.P., Martinez, A.E., McGrath, J.A., Mellerio, J.E., Moss, C., Murrell, D.F., Shimizu, H., Uitto, J., Woodley, D., Zambruno, G., 2014. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J. Am. Acad. Dermatol. 70 (6), 1103–1126.

Takahashi, K., Okita, K., Nakagawa, M., Yamanaka, S., 2007. Induction of pluripotent stem cells from fibroblast cultures. Nat. Protoc. 2 (12), 3081–3089.

Uitto, J., Richard, G., McGrath, J.A., 2007. Diseases of epidermal keratins and their linker proteins. Exp. Cell Res. 313 (10), 1995–2009.