Stem Cell Research 53 (2021) 102345

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

Establishment of a Vietnamese ethnicity induced pluripotent stem cell line (VRISGi001-A) from umbilical cord blood hematopoietic stem cells under a feeder-free system

Thi-Tuyet-Trinh Tran [a], Thi-Hong-Nhung Nguyen [a], Thi-Thao Nguyen [a], Xuan-Hung Nguyen [a,b,c,*]

[a] *Vinmec Institute of Applied Sciences and Regenerative Medicine (VIASRM), Vinmec Healthcare System, Hanoi, Viet Nam*
[b] *Vinmec Research Institute of Stem Cell and Gene Technology (VRISG), Vinmec Healthcare System, Hanoi, Viet Nam*
[c] *College of Health Sciences, VinUniversity, Hanoi, Viet Nam*

ARTICLE INFO

Keywords:
Vietnamese human induced pluripotent stem cell
Yamanaka factor
Sendai virus

ABSTRACT

We have established the original footprint-free Vietnamese human induced pluripotent stem cell line, VRISGi001-A, from cord-blood derived CD34 + hematopoietic stem cells (HSCs) of a 35 year old healthy woman under cGMP-compliant process. For the hiPSC induction, three Sendai virus vectors carrying four reprogramming factors including c-MYC, SOX2, KLF4, and OCT3/4 were delivered into $CD34^+$ HSCs. The VRISGi001-A cell line expresses the majority of the pluripotent markers and differentiate *in vitro* into derivatives of three germ layers. The availability of Vietnamese hiPSC line could contribute to the improvement of inadequate genetic diversity in the currently available hiPSC lines.

1. Resource utility

The original established Vietnamese human-specific iPSC line can be used in studying the genetic contribution to diseases, in drug screening, and in therapeutic personalized medicine.

2. Resource table

Unique stem cell line identifier	VRISGi001-A
Alternative name of stem cell line	UCB01-hiPS-CL2
Institution	Vinmec HiTech Center
Contact information of distributor	Dr. Xuan-Hung, Nguyen; *v.hungnx@gmail.com*
Type of cell line	iPSC
Origin	Human
Additional origin information	Age: 35, Sex: female, Ethnicity: Vietnamese
Cell source	Umbilical cord blood (UCB)
Clonality	Clonal cell line
Method of reprogramming	Integration-free Sendai virus vectors
Genetic modification	No
Type of Modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archive/ stock date	Passage 6 stock vials (July 15, 2020)
Cell line repository	Vinmec HiTech Center Cell Storage System
Ethical approval	The study was approved by the Vinmec International hospital. Informed consent was obtained from the blood donor.

3. Resource details

Generation of hiPSC using footprint-free Sendai virus vectors carrying four Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc) have been reported in a myriad of studies with high efficiency (Haase et al., 2017; Soares et al., 2015). In this report, we used CytoTune™-iPS 2.0 Sendai Reprogramming vector system (Thermo Fisher Scientific, Waltham, MA,

* Corresponding author at: Vinmec Institute of Applied Sciences and Regenerative Medicine (VIASRM), Vinmec Healthcare System, 10000 Hanoi, Viet Nam.
E-mail address: v.hungnx@gmail.com (X.-H. Nguyen).

https://doi.org/10.1016/j.scr.2021.102345
Received 6 March 2021; Received in revised form 1 April 2021; Accepted 5 April 2021
Available online 17 April 2021
1873-5061/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

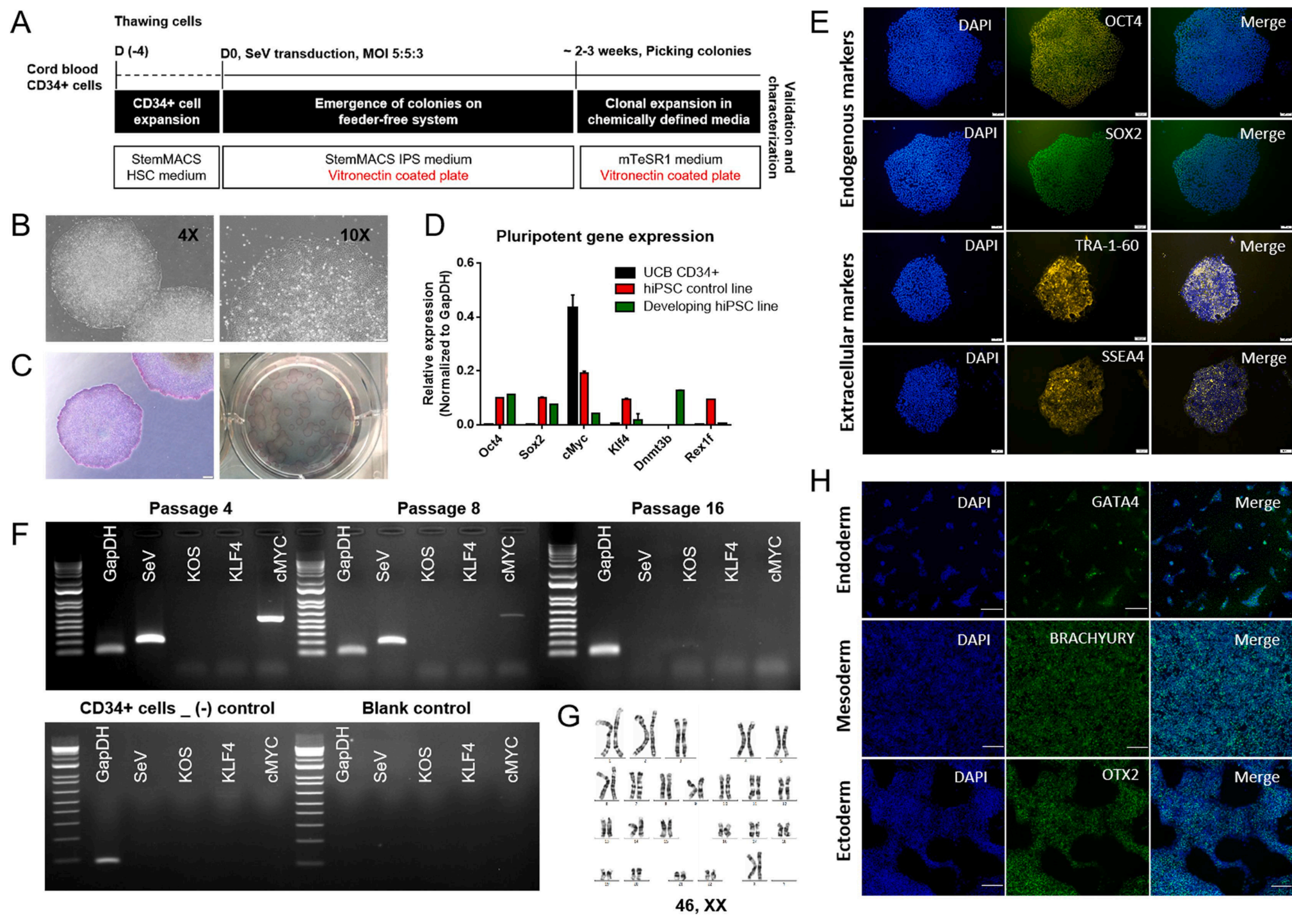

Fig. 1. xv

USA) to generate the first Vietnamese human iPSC line. The VRISGi001-A hiPSC line, specialized by UCB01-hiPS-CL2, was derived from healthy donor cord blood CD34 + HSCs and further characterized and validated. Detailed procedure and time schedule for hiPSC generation is presented in the Fig. 1A. The hiPS cells grew in Table 1.defined border colonies with typical round and compact morphology, composed a monolayer of homogeneous cells with high nucleus-cytoplasm ratio and prominent nucleoli. Fig. 1B showed the colony and cell morphorlogy after 6 passages at 4X (top left) and 10X magnification (Phase contrast). Positive alkaline phosphatase staining of colonies at passage 6 indicated the pluripotent nature of the colonies (Fig. 1C). In comparison to non-transduced CD34 + cells and hiPSC control line, quantitative real-time PCR proved the activation of pluripotency gene markers in the developed iPS cell line (Fig. 1D). In addition, immunocytochemistry analysis of pluripotency protein markers (OCT4, SOX2, TRA-1-60, and SSEA4) confirmed again the pluripotency of this clone (Fig. 1E, scale bar: 100 μm). Subsequently lost of Sendai virus vectors expression after continuous passaging was confirmed by RT-PCR at passage 16 (Fig. 1F), using GAPDH as an internal control. G-banded karyotyping showed a normal chromosome pattern after a long culture period of 20 passages (Fig. 1G). The iPS cells also possessed the ability to differentiate into cells of all three germ layers (Fig. 1H, scale bar: 200 μm) as indicated by positive staining for OTX2 (ectoderm), BRACHYURY (mesoderm) and GATA-4 (endoderm).

4. Materials and methods

4.1. Reprogramming of umbilical cord blood CD34$^+$ HSCs using Sendai virus system

The study was approved by the Hospital Board of Vinmec International Hospital in Hanoi, Vietnam. Mononuclear cells (CB-MNCs) were isolated from a unit of umbilical cord blood of 35 year-old Vietnamese healthy female donor who gave written informed consent before withdrawing the cord blood. Umbilical cord blood CD34 + cells were isolated using CD34 immunomagnetic microbeads (Miltenyi 130–097-047) and expanded in StemMACS HSC expansion medium (Miltenyi, 130-100-473) in the humidified 37 °C CO_2 incubator. 2×10^5 cord blood CD34$^+$ cells were transduced using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer's instructions, with a multiplicity of infection (MOI) of 5:5:3 (KOS MOI = 5, c-Myc MOI = 5, Klf4 MOI = 3). Three days after transduction, the cells were transferred onto vitronectin-coated wells of a 6-well plate in Stem MACS iPS medium (Miltenyi). Three weeks after transduction, primary colonies were picked and expanded in the defined medium mTeSR1 (StemCELL Tech) into different clones. The cell clones were daily fed, and weekly passaged when reaching the confluence of 70–80 percent in the culture vessels. The clone VRISGi001-A which displaying a typical ESC-like morphology was selected for further characterization.

4.2. Alkaline phosphatase (ALP) staining

Early determination of hiPS colonies was done with Alkaline Phosphatase staining kit (Abcam, ab242286) according to the

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Alkaline phosphatase staining	Positive	Fig. 1 panel C
	Quantitative analysis RT-qPCR	Positive for Oct4, Sox2, c-Myc, Klf4, Dnmt3b, and Rex1f transcription factors	Fig. 1 panel D
	Qualitative analysis Immunocytochemistry	Positive for OCT4, SOX2, TRA-1–60, and SSEA4 markers	Fig. 1 panel E
Genotype	G-banded karyotype	46, XX	Fig. 1 panel G
Identity	STR analysis	24 loci tested, completely matched	Submitted in archive with journal
Mutation analysis	Sequencing	N/A	N/A
Microbiology and virology	Colorimetric test	Mycoplasma testing by luminescence, negative	Supplementary Fig. S1
Differentiation potential	In vitro trilineage differentiation	Proof of three germ-layers formation by positive immunostaining for OTX2 (Endoderm), Brachyury (Mesoderm), and GATA4 (Ectoderm)	Fig. 1 panel H
Donor screening	HIV, Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional information	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

manufacturer's instruction.

4.3. Evaluation of pluripotency markers by RT-PCR

RNA was extracted from iPSCs at different passages using RNeasy kit from QIAGEN. 1ug of extracted total RNA were converted into cDNA using qScript cDNA synthesis kit (Quantabio, 95047). Real-time PCR was performed to evaluate the expression of pluripotent genes using PerfeCTa SYBR Green SuperMix (Quantabio, 95055). Table 2 list the target genes and primers used in our study.

4.4. Sendai virus genome and transgene detection

To detect the SeV genome and transgenes expression in cultured iPSCs, 500 ng of total RNA were isolated and converted into cDNA as described above. The PCR reactions were then performed using GoTaq Green Master Mix (Promega, M7123) following the manufacturer's protocol on a thermocycler (Proflex PCR system, Thermo Fisher). Thermocycling amplification programs with different annealing temperatures were applied for different primer pairs, specifically 5 m at 95 °C, 30 s at 95 °C, 30 s at 55–60 °C, 42 s at 72 °C (30 cycles), 5 min at 72 °C and hold at 4 °C. The presence of interest genes in PCR products was then analyzed by running these products on 1% agarose gel at the voltage of 100 in 30 min.

Table 2
Reagent details.

Primers			
	Target	Forward/Reverse primer (5′–3′)	
	Oct4	Forward: CCTCACTTCACTGCACTGTA Reverse: CAGGTTTTCTTTCCCTAGCT	
	Sox2	Forward: ATGTCCCAGCACTACCAGAG Reverse: GCACCCCTCCCATTTCCC	
	Myc	Forward: CTGAAGAGGACTTGTTGCGGAAAC Reverse: TCTCAAGACTCAGCCAAGGTTGTG	
	Klf4	Forward: GGTCGGACCACCTCGCCTTACAC Reverse: CTCAGTTGGGAACTTGACCA	
	DNMT3B	Forward: ATAAGTCGAAGGTGCGTCGT Reverse: GGCAACATCTGAAGCCATTT	
	Rex1F/ZFP42	Forward: CCGAGACCACGTCTGTGCGG Reverse: AGCGCTTTCCGCACCCTTCA	
	PBDG	Forward: GGAGCCATGTCTGGTAACGG Reverse: CCACGCGAATCACTCTCATCT	
	SeV	Forward: GGATCACTAGGTGATATCGAG C Reverse: ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	Forward: ATG CAC CGC TAC GAC GTG AGC GC Reverse: ACC TTG ACA ATC CTG ATG TGG	
	Klf4	Forward: TTC CTG CAT GCC AGA GGA GCC C Reverse: AAT GTA TCG AAG GTG CTC AA	
	c-Myc	Forward: TAA CTG ACT AGC AGG CTT GTC G Reverse: TCC ACA TAC AGT CCT GGA TGA TGA TG	
Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody name	Dilution	Company Cat # and RRID
Cell viability and purity	Anti-human CD34 PE	1:100	130–081-002 (Myltenyi), RRID: AB_244351
	Anti-human CD45 FITC	1:100	130–080-202 (Myltenyi) RRID: AB_244234
	7AAD staining solution	1:100	130–111-568 (Myltenyi) RRID:
Pluripotency markers	Anti-human OCT3/4 PE	1:200	130–105-606 (Myltenyi) RRID: AB_2653084
	Anti-human SOX2 FITC	1:200	130–104-993 (Myltenyi) RRID: AB_2653499
	Anti-human TRA-1–60 PE	1:200	130–100-350 (Myltenyi) RRID: AB_2654226
	Anti-human SSEA4 PE	1:200	130–100-635 (Myltenyi) RRID: AB_2653518
Differentiation marker	Anti-human GATA4 NL493	1:10	Human Three Germ Layer 3-Color Immunocytochemistry Kit (R&D, sc022)
	Anti-human BRACHYURY NL557	1:10	
	Anti-human OTX2 NL557	1:10	

4.5. Immunoflourescence

The cells were fixed, permeabilized, and stained with primary and secondary antibodies (Table 2) as previously described (Khan et al., 2020). The cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) before visualization under the ImageXpress® Micro Confocal Imaging System (Molecular Devices LLC).

4.6. Karyotyping

G- banded karyotype analysis of the VRISGi001-A iPSCs at passage 20 was carried out by Medical Genetic Department at Vinmec hospital (Hanoi, Vietnam). In short, cells were collected, arrested at metaphase by colcemide, inflated by hypotonic 0.56% KCl solution to separate the chromosomes. Chromosomes were then spread on the slide for Giemsa stainning and analyzed with the

Carl Zeiss auto-imaging microscope, Metafer slide scanning and Ikaros karyotyping platforms. At least 20 metaphase spreads were counted.

4.7. Short tandem repeat (STR) analysis

STR analysis of VRISGi001-A clone was carried out at the Institute of DNA Technology and Genetics Analysis (GENLAB, Hanoi, Vietnam) using the PowerPlex® Fusion System (Promega – USA).

4.8. Differentiation into 3 germ layers

After 6 passages in culture, the clone VRISGi001-A was induced to differentiate separately into cells derived from three germ layers (ectoderm, mesoderm or endoderm) using the commercial STEMdiff Trilineage Differentiation Kit (STEMCELL Technology, #05230), following manufacturer's instructions.

4.9. Mycoplasma screening

The absence of mycoplasma in the culture medium was analyzed at passage 32 using MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza), according to the manufacturer's instructions.

5. Financial support

This study was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) No.108.06-2018.309 and the International Centre for Genetic Engineering and Biotechnology (ICGEB) No. CRP/VNM18-05_EC.

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 thank Professor Ludovic Vallier and Ms. Ying Shao for providing the technical training regarding IPSC generation at the Core Facility of Cambridge Biomedical Research Center and Professor Thanh-Liem Nguyen for his insightful comments on the manuscript. We thank Ms. Thi-Thao Le for providing the umbilical cord blood unit with donor consent attached and Dr. Quynh-Mai Trinh for sharing the experience in handling human iPSCs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102345.

References

Haase, A., G. Göhring, and U.J.S.c.r. Martin, Generation of non-transgenic iPS cells from human cord blood CD34+ cells under animal component-free conditions. 2017. 21: p. 71-73.

Soares, F.A., Pedersen, R.A., Vallier, L., 2015. Generation of human induced pluripotent stem cells from peripheral blood mononuclear cells using Sendai virus. In: Induced Pluripotent Stem (iPS) Cells. Springer, pp. 23–31.

Khan, M., et al., Generation and characterization of three clones (NCCSi007A, NCCSi007B and NCCSi007C) of an integration free induced Pluripotent Stem Cell line from a patient with alcoholic liver cirrhosis of Indian ethnicity. 2020. 42: p. 101678.