Stem Cell Research 41 (2019) 101584

Contents lists available at ScienceDirect

Stem Cell Research

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

Generation of VCCRIi001-A, a human induced pluripotent stem cell line, from a patient with spontaneous coronary artery dissection

Ketan Mishra[a], Keerat Junday[a], Claire M.Y. Wong[a,1], Andrea Y. Chan[a], Stephanie Hesselson[a], David W. Muller[a,b], Siiri E. Iismaa[a,b], Ashish Mehta[a,b], Robert M. Graham[a,b,*]

[a] Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales 2010, Australia
[b] St. Vincent's Clinical School, University of New South Wales, Kensington, New South Wales 2052, Australia

ARTICLE INFO

Keywords:
SCAD
Induced pluripotent stem cells
Disease modeling
Cardiovascular biology
Reprogramming

ABSTRACT

Spontaneous coronary artery dissection (SCAD) is a non-atherosclerotic form of coronary artery disease of unknown cause that predominantly affects women (>90%; mean age 44–55 years) and can be fatal. The finding of familial clustering, including the concordant involvement of monozygotic twins, and its association with the *PHACTR1/EDN1* genetic locus, indicate a genetic predisposition to its pathophysiology. A human induced pluripotent stem cell line (hiPSC) was generated from a patient who had survived an episode of SCAD. This disease-specific hiPSC line will be useful for the study of SCAD after differentiation into blood vessel-forming cells.

1. Resource table

Unique stem cell line identifier	VCCRIi001-A
Alternative name(s) of stem cell line	N/A
Institution	Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia.
Contact information of distributor	Robert M. Graham; b.graham@victorchang.edu.au
Type of cell line	hiPSC
Origin	Human
Additional origin info	Age: 46 Sex: female Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai Virus
Genetic Modification	No
Type of Modification	N/A
Associated disease	Spontaneous coronary artery dissection
Gene/locus	rs9349379 variant in *PHACTR1*/Chromosome 6q24
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	28 June 2017
Cell line repository/bank	N/A
Ethical approval	St Vincent's Hospital Human Research Ethics Committee (HREC/16/SVH/338); SVH File number: 16/245

2. Resource utility

SCAD is a vasculopathy of unknown cause. However, consistent with a genetic predisposition, we recently showed highly significant association between SCAD and the rs9349379 A-allele of the *PHACTR1/EDN1* genetic locus (odds ratio: 1.67, $p < 1.67 \times 10^{-21}$) (Adlam et al., 2019). To aid in understanding the pathophysiological mechanisms of SCAD, hiPSCs were generated from a SCAD survivor.

3. Resource details

SCAD is a non-atherosclerotic form of CAD that predominantly affects young to middle-aged women, accounting for 23–36% of acute myocardial infarcts, who have few traditional cardiovascular risk factors, such as obesity, diabetes, dyslipidemia or high blood pressure, and can recur in up to 30% of cases (Graham et al., 2018; Saw et al., 2017).

* Corresponding author.
E-mail addresses: k.junday@victorchang.edu.au (K. Junday), claire.wong@health.nsw.gov.au (C.M.Y. Wong), a.chan@victorchang.edu.au (A.Y. Chan), s.hesselson@victorchang.edu.au (S. Hesselson), dmuller19@me.com (D.W. Muller), s.iismaa@victorchang.edu.au (S.E. Iismaa), b.graham@victorchang.edu.au (R.M. Graham).
[1] Current address: Department of Clinical Genetics, The Children's Hospital at Westmead, Westmead, New South Wales 2145, Australia.

https://doi.org/10.1016/j.scr.2019.101584
Received 5 September 2019; Accepted 14 September 2019
Available online 08 October 2019

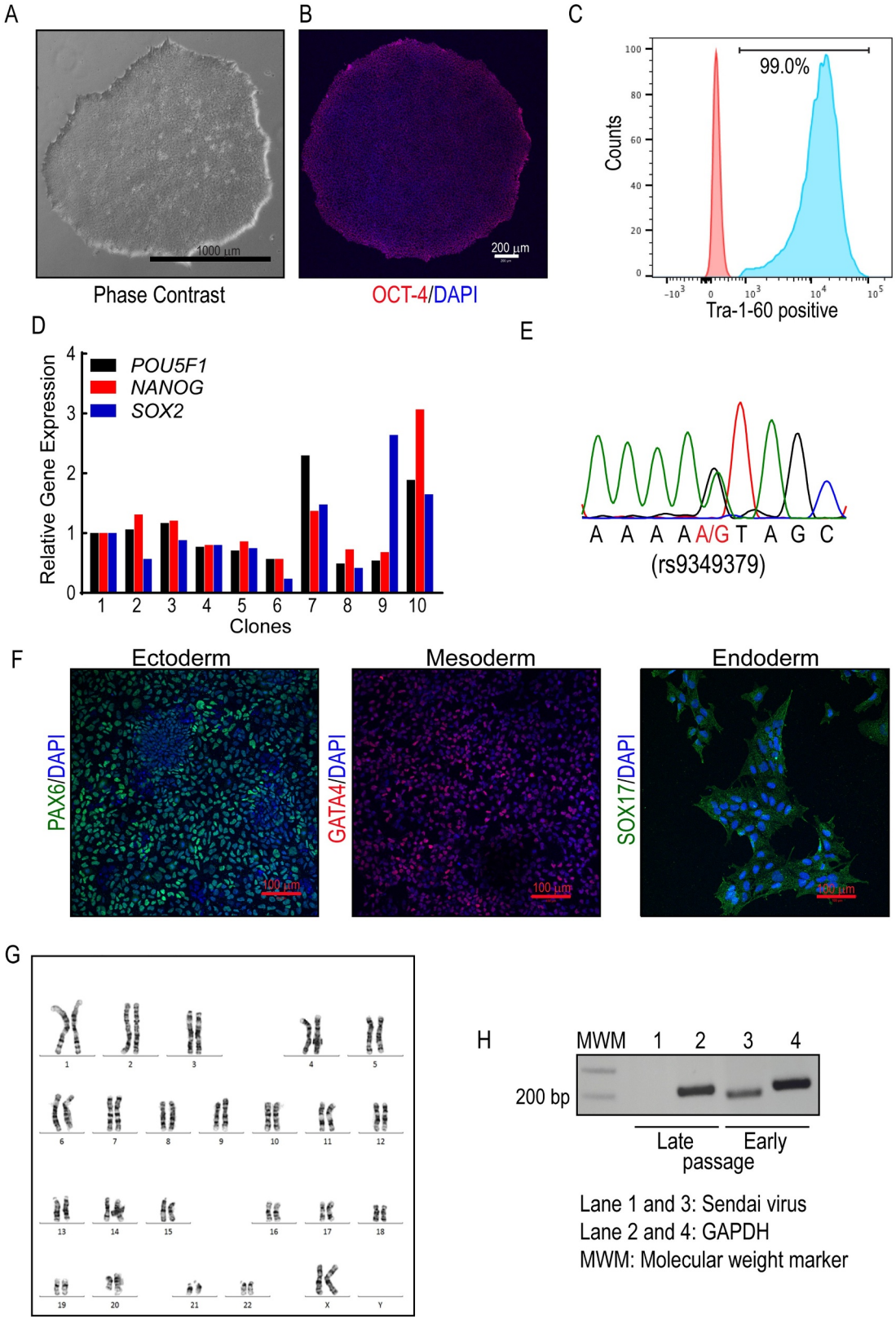

Fig. 1. Characterization of the VCCRIi001-A hiPSC line.

It is likely a systemic vasculopathy, associated not infrequently with other vascular disorders (fibromuscular dysplasia, cervical artery dissection, migraine), but evidence of inflammatory processes is lacking and associated genetic causes of vasculopathy, such as Ehlers Danlos and Loeys-Dietz, are rarely found. The rs9349379 A-allele of the *PHACTR1/EDN1* genetic locus is strongly associated with SCAD, and a lower incidence of atherosclerotic coronary artery disease (AsCAD) (Adlam et al., 2019), whereas the rs9349379 G-allele is associated with AsCAD and hypertension (Gupta et al., 2017). SCAD causes myocardial ischemia or infarction due to the development of an intramural

Table 1
Characterization and validation of the VCCRIi001-A hiPSC line.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig 1, panel A
Phenotype	Immunocytochemistry	Positive staining of pluripotency marker, OCT4; counterstained with DAPI	Fig. 1, panel B
	Flow cytometry	Positive for pluripotency marker, Tra-1-60	Fig. 1, panel C
	Quantitative analysis qRT-PCR	qRT-PCR shows expression of endogenous *POU5F1, NANOG, SOX-2*	Fig. 1, panel D
Differentiation potential	Directed differentiation	Positive staining for PAX6 (ectoderm), GATA4 (mesoderm), SOX17 (endoderm), counterstained with DAPI	Fig. 1, panel F
Genotype	Karyotype (G-banding)	46XX (15)	Fig. 1, panel G
Identity	Microsatellite PCR	Not performed	Not available
	STR analysis	18 loci analyzed, all matching	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous at PHACTR1 locus for the rs9349379-AG variant	Fig. 1, panel E
	Southern Blot or whole genome sequencing	Not available	Not available
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Data not shown but available from authors
	Sendai Virus	Elimination of Sendai Virus with repeated passaging	Fig. 1, panel H
Donor screening	HIV 1 + 2; Hepatitis B; Hepatitis C	All negative	Not shown but available from authors
Genotype additional info	Blood group genotyping	Not available	Not available
	HLA tissue typing	Not available	Not available

hematoma (IMH) of an epicardial coronary artery that compresses the true lumen, with or without a luminal tear. However, the pathophysiology of IMH-development and the vascular cell type(s) involved, have not been elucidated. Here, we generated and characterized a SCAD-specific hiPSC from a 46-year-old female patient heterozygous (AG) for the rs9349379 variant. hiPSCs were generated from the patient's peripheral blood mononuclear cells (PBMCs) using Sendai virus encoding the reprogramming factors, OCT3/4, SOX2, KLF4 and c-MYC, under feeder-free conditions(Viswanathan et al., 2018). Ten clones were generated. VCCRIi001-A showed typical human embryonic stem cell-like morphology (Fig. 1A), expressed the pluripotent marker, OCT-4, by immunofluorescence (Fig. 1B) and maintained 99.0% Tra-1-60 positivity by flow cytometry (Fig. 1C). VCCRIi001-A also expressed high *POU5F1, NANOG* and *SOX2* levels by qRT-PCR (Fig. 1D, clone 7), retained the rs9349379-AG variant (Fig. 1E), differentiated into all three germ layers (ectoderm: PAX6, mesoderm: GATA4, endoderm: SOX17, Fig. 1F), and was karyotypically normal (Fig. 1G). Identity was verified by short tandem repeat (STR) analysis and the cells were mycoplasma negative. After repeated passaging, Sendai virus was eliminated from the hiPSCs (Fig. 1H), making them foot-print free.

4. Materials and methods

4.1. Ethics statement

The generation and use of hiPSC were approved by the St Vincent's Hospital Human Research Ethics Committee (HREC/16/SVH/338); SVH File number: 16/245. All studies were performed according to approved guidelines after obtaining informed consent.

4.2. PBMCs collection and reprogramming

Blood (5 ml) was collected into an EDTA Vacutainer blood collection tube. PBMCs were isolated using a Ficoll (GE Healthcare) density gradient and cultured in StemSpan II medium with Erythroid expansion supplement (Stemcell Technologies). Reprogramming was performed using the Cytotunes 2.0 Kit (Thermo Fisher Scientific), as per manufacturer's instructions. Colonies exhibiting hiPSC-like morphology were manually selected 3–4 weeks post-transduction and maintained on Matrigel and mTeSR1™ plus (StemCell Technologies) with a 7-day passage cycle as described previously (Viswanathan et al., 2018).

4.3. In vitro differentiation assay

Cells were differentiated in vitro into the three germ layers using STEMdiff Trilineage Differentiation kit (StemCell Technologies) as per manufacturer's instructions, fixed and stained for germ layer markers.

4.4. Immunofluorescence assay and flow cytometry

Cells were fixed with 4% paraformaldehyde (15 min, room temperature). For permeabilization and blocking, fixed cells were incubated for 30 min with PBS containing 10% donkey serum (Sigma) and 0.2% Triton X-100 (Sigma). Primary (overnight incubation, 4 °C) and secondary (1 h, room temperature) antibodies were used as detailed in Table 1. Samples were mounted with Prolong Gold antifade containing DAPI (Thermo Fisher Scientific) and images were captured by Zeiss LSM 700 confocal microscopy. For flow cytometry, cells were fixed in 4% paraformaldehyde (15 min, room temperature), blocked with PBS with 2% FBS for 30 min, incubated with primary (overnight, 4 °C) and secondary (1 h, room temperature) antibodies and run on Canto II (BD). A total of 10,000 events were acquired.

4.5. qRT-PCR

RNA was isolated using RNeasy Plus Mini kit (Qiagen). cDNA was generated using Superscript IV Vilo (Invitrogen) and amplified with SYBR Green Master mix on a LightCycler 480 II thermal cycler (Roche) under the following conditions: denaturation (95 °C, 5 min), amplification (95 °C, 15 s; 60 °C, 30 s; 72 °C, 30 s for 35 cycles) and final extension (72 °C, 5 min). Ct values were calculated, and values normalized to GAPDH as the reference gene.

4.6. Elimination of Sendai virus

Sendai virus (SeV) was amplified by PCR using cDNA (10uL, generated from 1 μg of hiPSC RNA) and AccuPrime SuperMix 1 (Thermo Fisher Scientific) under the following conditions: denaturation (95 °C, 30 s), amplification (95 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s for 30 cycles). Primers used are listed in Table 2. PCR products were analyzed using 2% agarose gel electrophoresis.

4.7. Karyotyping and mycoplasma detection

hiPSCs were karyotyped by G-banding metaphase analysis

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse IgM anti-TRA-1-60	1:1000	Cell Signaling Technology Cat. No. 4746, RRID: AB_211905
	Rabbit IgG anti-OCT4	1:400	Cell Signaling Technology Cat. No. 2840, RRID: AB_2167691
Differentiation markers	Rabbit IgG anti-PAX6	1:100	Thermo Fisher Scientific Cat. No. 42–6600, RRID: AB_2533534
	Rabbit IgG anti-GATA-4	1:400	Cell Signaling Technology Cat. No. 36966, RRID: AB_2799108
	Rabbit IgG anti-SOX17	1:100	Thermo Fisher Scientific Cat. No. 703063, RRID: AB_2784556
Secondary antibodies	Alexa 594 Goat Anti-rabbit IgG	1:000	Invitrogen Cat. No. A_11012, RRID: AB_2534079
	FITC Conjugate goat anti-mouse IgM	1:2000	Sigma Cat. No. F9259, RRID: AB_259799

Primers		
	Target	Forward/reverse primer (5′ – 3′)
Sendai virus (SeV)-specific primers	SeV, 181bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
Reference gene for PCR	*GAPDH,* 203bp	TGAAGGTCGGAGTCAACGGA/CCAATTGATGACAAGCTTCCCG
Endogenous Pluripotency Markers (qRT-PCR)	*POU5F1*	AGTTTGTGCCAGGGTTTTTG/ACTTCACCTTCCCTCCAACC
	NANOG	CTCCATGAACATGCAACCTG/GAGGAAGGATTCAGCCAGTG
	SOX2	AAAAATCCCATCACCCACAG/GCGGTTTTTGCGTGAGTGT
Reference gene (qRT-PCR)	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT
Genotyping	*PHACTR1*, rs9349379	CCTGGCCTTTGCCCTTAGAA/CCCAGTTGAATTCCTGACCCAT

(Queensland Diagnostics, Brisbane, Australia), and confirmed to be mycoplasma-negative using the MycoAlert Mycoplasma detection kit (Lonza Biosciences), as per manufacturer's instructions.

4.8. Sanger sequencing and STR analysis

Genomic DNA was isolated from the patient's buccal cells using Purelink Genomic DNA kit (Invitrogen), and subjected to sequence analysis (Garvan Molecular Genetics Facility, NSW, Australia) using DNASTAR software. For STR analysis, genomic DNA was extracted from hiPSCs using DNAEasy Kit (Qiagen) and evaluated by the Garvan Molecular Genetics Facility.

Declaration of Competing Interest

None.

Acknowledgments

Supported in part by grants from the Cardiac Society of Australia and New Zealand, National Health and Medical Research Council, Australia (APP1161200), St Vincent's Clinic Foundation, Catholic Archdiocese of Sydney, Perpetual Philanthropy and SCAD Research Inc.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101584.

References

Adlam, D., Olson, T.M., Combaret, N., Kovacic, J.C., Iismaa, S.E., Al-Hussaini, A., O'Byrne, M.M., Bouajila, S., Georges, A., Mishra, K., Braund, P.S., d'Escamard, V., Huang, S., Margaritis, M., Nelson, C.P., de Andrade, M., Kadian-Dodov, D., Welch, C.A., Mazurkiewicz, S., Jeunemaitre, X., Consortium, D., Wong, C.M.Y., Giannoulatou, E., Sweeting, M., Muller, D., Wood, A., McGrath-Cadell, L., Fatkin, D., Dunwoodie, S.L., Harvey, R., Holloway, C., Empana, J.P., Jouven, X., Group, C.A.D.S., Olin, J.W., Gulati, R., Tweet, M.S., Hayes, S.N., Samani, N.J., Graham, R.M., Motreff, P., Bouatia-Naji, N., 2019. Association of the PHACTR1/EDN1 genetic locus with spontaneous coronary artery dissection. J. Am. Coll. Cardiol. 73 (1), 58–66. https://doi.org/10.1016/j.jacc.2018.09.085.

Graham, R.M., McGrath-Cadell, L., Muller, D.W.M., Holloway, C.J., 2018. The mystery and enigma of spontaneous coronary artery dissection. Heart Lung Circ. 27 (4), 401–405. https://doi.org/10.1016/S1443-9506(18)30060-X.

Gupta, R.M., Hadaya, J., Trehan, A., Zekavat, S.M., Roselli, C., Klarin, D., Emdin, C.A., Hilvering, C.R.E., Bianchi, V., Mueller, C., Khera, A.V., Ryan, R.J.H., Engreitz, J.M., Issner, R., Shoresh, N., Epstein, C.B., de Laat, W., Brown, J.D., Schnabel, R.B., Bernstein, B.E., Kathiresan, S., 2017. A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. Cell 170 (3), 522–533.e515. https://doi.org/10.1016/j.cell.2017.06.049.

Saw, J., Humphries, K., Aymong, E., Sedlak, T., Prakash, R., Starovoytov, A., Mancini, G.B.J., 2017. Spontaneous coronary artery dissection: clinical outcomes and risk of recurrence. J. Am. Coll. Cardiol. 70 (9), 1148–1158. https://doi.org/10.1016/j.jacc.2017.06.053.

Viswanathan, S.K., Puckelwartz, M.J., Mehta, A., Ramachandra, C.J.A., Jagadeesan, A., Fritsche-Danielson, R., Bhat, R.V., Wong, P., Kandoi, S., Schwanekamp, J.A., Kuffel, G., Pesce, L.L., Zilliox, M.J., Durai, U.N.B., Verma, R.S., Molokie, R.E., Suresh, D.P., Khoury, P.R., Thomas, A., Sanagala, T., Tang, H.C., Becker, R.C., Knoll, R., Shim, W., McNally, E.M., Sadayappan, S., 2018. Association of cardiomyopathy with MYBPC3 D389V and MYBPC3Delta25bpIntronic deletion in South Asian descendants. JAMA Cardiol. 3 (6), 481–488. https://doi.org/10.1001/jamacardio.2018.0618.