Generation of two induced pluripotent stem cell lines carrying the phospholamban R14del mutation for modeling ARVD/C

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Abstract

The phospholamban (PLN) R14del mutation is associated with arrhythmogenic right ventricular dysplasia (ARVD/C). ARVD/C is a cardiac disease characterized by arrhythmias and structural abnormalities in the right ventricle. Because PLN is a regulator of calcium release, this mutation can have deleterious effects on tissue integrity and contraction. This mutation is a trinucleotide (AGA) deletion that leads to an arginine deletion at position 14 of the PLN structure. Here we show two lines carrying this mutation with typical iPSC morphology, pluripotency, karyotype, ability to differentiate into the three germ layers \textit{in vitro}, and readily availability for studying pathological mechanisms or ARVD/C.

Keywords

Arrhythmogenic right ventricular dysplasia; PLN; Induced pluripotent stem cells

1. Resource utility

Two iPSC lines (Resource Table) have been generated to study the underlying mechanism of arrhythmogenic right ventricular dysplasia cardiomyopathy (ARVD/C). These lines provide an unlimited and valuable resource to derive cardiomyocytes and other cell types \textit{for in vitro} disease modeling and therapeutics screening.

2. Resource Table:

| Unique stem cell lines identifier | SCVIi030-A |
|----------------------------------|------------|
| SCVIi031-A                      |            |

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102834.
2.1. Resource details

ARVD/C is an inheritable disease with a pathological hallmark of a defective right ventricle and a prevalence ranging from 1:2,000 to 1:5,000, depending on the patient cohort (Corrado et al., 2017). Typically, patients have mutations in desmosomal proteins, which are conventionally present at intercellular junctions and are essential for the tissue integrity (Groeneweg et al., 2014). PLN is a regulator of the sarcoplasmic reticulum Ca\(^{2+}\) (SERCA2) pump in cardiac muscle and thus crucial for maintaining Ca\(^{2+}\) homeostasis. PLN mutations are associated with dilated cardiomyopathy (DCM) and ARVD/C as several histopathological features overlap in the diagnosis (van der Zwaag et al., 2012). The R14del mutation in PLN has been studied extensively for cardiomyopathy and is present in 13–43% of ARVD/C patients (Groeneweg et al., 2014; Karakikes et al., 2015; van der Zwaag et al., 2012). The consensus mechanism of PLN mutations is irregular Ca\(^{2+}\) homeostasis due to disrupted regulation of SERCA2, which leads to disassembly of the desmosomal proteins and loss of myocardial tissue integrity (Groeneweg et al., 2014; van der Zwaag et al., 2012). It is essential to properly understand the disease progression to manage DCM and ARVD/C patients with this mutation. Here we present two resource lines that can enable researchers to study those intricacies and identify potential interventions Table 1.

Two human iPSC lines (SCVIi030-A & SCVIi031-A) were derived from peripheral blood mononuclear cells (PBMCs) of two patients diagnosed with ARVC/D. The lines were derived from a 54-year old female and a 27-year old male (c.40_42 AGAdel), both with family histories of cardiac disease (Resource Table). To reprogram the PBMCs into iPSCs,
we utilized the Sendai virus to deliver the Yamanaka factors. The two iPSC lines had normal morphology. The scale bar = 930 μm (Fig. 1A). The expression of pluripotent markers NANOG, OCT3/4, and SOX2 was verified by immunostaining. The scale bar = 70 μm (Fig. 1B). Both iPSC lines could differentiate into all three germ layer lines. The scale bar = 70 μm (Fig. 1C). Expression levels of NANOG and SOX2 were measured through mRNA and detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (Fig. 1D). For comparison, a healthy control SCVI15 was also measured at both iPSC and cardiomyocyte states to show comparable levels of NANOG and SOX2 for iPSCs, but exhibited low levels for cardiomyocytes (Fig. 1D) (Manhas et al., 2022). Sendai virus was absent in SCVIi030-A and SCVIi031-A at passage ~20 but still present at a low passage (P4) in healthy control iPSC culture (Fig. 1E). The PLN R14del mutation was confirmed by Sanger sequencing and was absent in the control SCVI15 (Fig. 1F). Moreover, karyotyping confirmed the biological sex and showed no chromosomal aberrations (Fig. 1G). Mycoplasma testing showed proper culturing of these lines (Supp. Fig. 1A). Finally, a short tandem repeat (STR) analysis confirmed that the iPSCs were derived from their respective PBMC origins.

3. Materials and methods

3.1. Reprogramming

PBMCs were isolated from blood by Percoll density gradient medium (GE Healthcare #17089109), washed with DPBS, and plated in a 24-well plate. The culture medium for the PBMCs consisted of Stem-Pro™−34 medium (Thermo Fisher #14190144) supplemented with 100 ng/mL FLT3 (Thermo Fisher #PHC9414), 20 ng/mL IL-6 (Thermo Fisher #PHC0063), 20 ng/mL EPO (Thermo Fisher #PHC9631), 20 ng/mL IL-3 (Peprotech #200–3), and 100 ng/mL SCF (Peprotech #300–07). To reprogram PBMCs into iPSCs, we used the Sendai virus reprogramming cocktail according to the CytoTune™-iPSC 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific #A16517). The transduced cells were resuspended and plated in a Matrigel-coated plate using the PBMC culture media. On day-7 after transduction, the medium was switched to StemMACS™ iPS-Brew XF medium (Miltenyi Biotec #130–104–368) until day 10–15 post-transduction, when colonies appeared. Colonies were picked and expanded (Manhas et al., 2022).

3.2. Cell culture

iPSCs were cultured in StemMACS™ iPS-Brew XF medium with supplement at 37 °C and 5% CO₂ until 90% confluency. Cells were further passaged using 10 μM Y-27632, a potent inhibitor of ROCK1 (Selleck Chemicals #S1049). The inhibitor was withdrawn after 24 hr.

3.3. Trilineage differentiation

The ability of iPSCs to differentiate into the three germ layers (ectoderm, endoderm, and mesoderm) was assessed using the STEM-diff™ Trilineage Differentiation Kit (STEMCELL Technologies, #05230) following the manufacturer’s instructions.
3.4. Immunofluorescent staining

We performed a qualitative analysis of pluripotency and trilineage differentiation. At room temperature, cells were fixed in 4% paraformaldehyde for 15 min, permeabilized with 50 μg/mL digitonin for 10 min, and blocked for 30 min with 1% BSA plus 5% FBS in PBS. The cells were incubated overnight at 4 °C with primary antibodies diluted in 1% BSA-PBS for staining. The following day, the cells were incubated with secondary antibodies in 1% BSA-PBS for 30 min at room temperature. Nuclei were counter-stained using NucBlue™ from Invitrogen™.

3.5. RT-qPCR

According to the manufacturer’s protocol, total RNA was extracted and isolated using the Direct-zol™ RNA Miniprep Kit (ZYMO RESEARCH #3R2061). RT-PCR was performed using the iScript™ cDNA Synthesis Kit (BioRad # 1708891) following the manufacturer’s protocol of 5 min at 25 °C, 20 min at 46 °C, and 1 min at 95 °C. Target molecules were amplified using commercial primers (Table 2) and TaqMan™ Gene Expression Assay from Applied Biosystems™.

3.6. Mycoplasma detection

Mycoplasma detection was analyzed using MycoAlert Detection Kit (Lonza #LT07–318) following the manufacturer’s protocol.

3.7. Short tandem repeat analysis

Genomic DNA was isolated from iPSCs and PBMCs using DNeasy Blood & Tissue Kit (Qiagen #69504). STR analyses were performed by the Stanford PAN facility using CLA Identifier™ Plus and Identifier™ Direct PCR Amplification Kits (Thermo Fisher #A44661).

3.8. Karyotyping

The whole-genome array to detect chromosomal abnormalities was performed at passage 12 with KaryoStat™ (Thermo Fisher Scientific) on 2 × 10^6 cells.

3.9. Sequencing

PCR primers were designed to amplify the region of interest in the PLN sequence (Table 2). iPSC genomic DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen #69504) and served as the PCR template, with NEB Phusion High Fidelity PCR Kit (Thermo Fisher) being used to amplify the template. The PCR reaction was performed under the following conditions: 98 °C for 30 min, 98 °C for 10 s, 68 °C for 15 s, and 72 °C for 1 min for 35 cycles. Sanger sequencing was performed on ABI3130xl by the Stanford PAN Facility. The WT and mutant alleles were parsed out using the web-based Poly Peak Parser tool from http://yosttools.genetics.utah.edu/PolyPeakParser/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
Acknowledgements

This work was supported by National Institutes of Health 75N92020D00019, R01 HL126527, R01 HL130020, and P01 HL141084 (JCW).

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Fig. 1.
Characterization of two iPSC lines from ARVC patients carrying PLN R14del mutation.
Table 1

Characterization and validation.

| Classification | Test | Result | Data |
|----------------|------|--------|------|
| Morphology     | Bright field photography | Visual record of the line: normal | Fig. 1 panel A |
| Phenotype      | Qualitative analysis Immunofluorescence staining | Positive expression of pluripotency markers: Oct3/4, Nanog, Sox2 | Fig. 1 panel B |
|                | Quantitative analysis RT-qPCR | mRNA expression of NANOG and SOX2 that is absent in differentiated cardiomyocytes | Fig. 1 panel D |
| Genotype       | Whole genome array (KaryoStat™ Assay) Resolution 1-2 Mb | Normal karyotype, SCVi090-A: 46XX, SCVi091-A: 46XY | Fig. 1 panel G |
| Identity       | STR analysis | 16 loci tested, 100% matching identity | Submitted in archive with journal |
| Mutation analysis | Sequencing | Heterozygous (c.40_42 AGAdel) for both iPSC lines | Fig. 1 panel F |
| Microbiology and virology | Mycoplasma | Luminescence: negative | Supplementary Fig. 1A |
| Differentiation potential | Directed differentiation | Positive IF staining of three germ layer markers | Fig. 1 panel C |
| List of recommended germ layer markers | Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer | Ectoderm: PAX6, OTX1/2; Endoderm: SOX17, FOXA2; Mesoderm: BRACHYURY, TBX6 | Fig. 1 Panel C |
| Donor screening | HIV 1 + 2, Hepatitis B, Hepatitis C | N/A | N/A |
| Genotype additional info | Blood group genotyping | N/A | N/A |
|                  | HLA tissue typing | N/A | N/A |
### Reagents Details

| Antibodies used for immunocytochemistry | Dilution | Company Cat # | RRID   |
|----------------------------------------|----------|---------------|--------|
| Pluripotency marker                     | 1:200    |               |        |
| Mouse IgGκ x Anti OCT-3/4              | 1:200    | Santa Cruz Biotechnology Cat# sc-5279 | AB_628051 |
| Rabbit Anti-NANOG                      | 1:200    | Proteintech Cat# 142951-1-AP            | AB_1607719 |
| Mouse IgGκ x Anti-SOX2                 | 1:200    | Santa Cruz Biotechnology Cat# sc-365823 | AB_10842165 |
| Ectoderm marker                        | 1:200    |               |        |
| Goat Anti-OTX2                         | 1:200    | R&D Systems Cat# 963,273                | AB_2157172 |
| Rabbit Anti-PAX6                       | 1:200    | Thermo Fisher Cat# 42,6600              | AB_2533534 |
| Endoderm marker                        | 1:200    |               |        |
| Goat Anti-SOX17                        | 1:200    | R&D Systems Cat# 963,121                | AB_355060  |
| Rabbit Anti-FOXA2                      | 1:200    | Thermo Fisher Cat# 701,698              | AB_2576439 |
| Mesoderm marker                        | 1:200    |               |        |
| Goat Anti-BRACHYURY                    | 1:200    | R&D Systems Cat# 963427                | AB_2200235 |
| Rabbit Anti-TBX6                       | 1:200    | Thermo Fisher Cat# PA5-35102            | AB_2552412 |
| Secondary Antibody                     | 1:200    |               |        |
| Alexa Fluor 488 Goat, Anti-mouse IgG1  | 1:200    | Thermo Fisher Cat# A-21121             | AB_2535764 |
| Alexa Fluor 488 Donkey, Anti-goat IgG (H + L) | 1:200    | Thermo Fisher Cat# A-11055             | AB_2534102 |
| Alexa Fluor 647 Goat, Anti-mouse IgG2b | 1:200    | Thermo Fisher Cat# A-21242             | AB_2535811 |
| Alexa Fluor 555 Goat, Anti-rabbit IgG (H + L) | 1:200    | Thermo Fisher Cat# A21428             | AB_141784  |
| Alexa Fluor 555 Donkey, Anti-rabbit IgG (H + L) | 1:200    | Thermo Fisher Cat# A-31572             | AB_162543  |

| Primers Target                        | Size of band | Forward/Reverse primer (5'-3') |
|---------------------------------------|--------------|--------------------------------|
| Sendai virus plasmids (qPCR)          | 181 bp       | Mr04269880_mr                   |
| Pluripotency marker (qPCR)            | 327 bp       | Hs02367400_g1                   |
| SOX2                                  | 256 bp       | Hs04234836_g1                   |
| House-keeping gene (qPCR)             | 471 bp       | Hs02786624_g1                   |
| Antibody                      | Dilution | Company Cat #     | RRID          |
|------------------------------|----------|-------------------|---------------|
| 18S                          | 207 bp   | Hs03003631_g1     |               |
| Genotyping                   |          |                   |               |
| For PLN mutation             |          |                   |               |
| PLN (c.40_42 AGAdel)         | 350 bp   |                   |               |
| FWD: TACATTCCAGGCTACCTAAAAGAAG |          |                   |               |
| REV: TTTCCTGTCTGCAAGGGATGAC  |          |                   |               |