Generation of two heterozygous *MYBPC3* mutation-carrying human iPSC lines, SCVIIi001-A and SCVIIi002-A, for modeling hypertrophic cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is an inherited heart disease that can cause sudden cardiac death and heart failure. HCM often arises from mutations in sarcomeric genes, among which the *MYBPC3* is the most frequently mutated. Here we generated two human induced pluripotent stem cell (iPSC) lines from a HCM patient who has a familial history of HCM and his daughter who carries the pathogenic non-coding mutation. All lines show the typical morphology of pluripotent cells, a high expression of pluripotency markers, normal karyotype, and in vitro capacity to differentiate into all three germ layers. These lines provide a valuable resource for studying the molecular basis of HCM and drug screening for HCM.

1. Resource utility

The two iPSC lines generated from individuals carrying a pathogenic mutation in *MYBPC3* provide an unlimited source for differentiating iPSC-derived cardiomyocytes (iPSC-CMs) in vitro. Thus, they are an excellent tool for modeling HCM to elucidate the underlying molecular mechanisms of the disease and for drug screenings that may provide treatment.

2. Resource details

Hypertrophic cardiomyopathy (HCM) is a heritable disorder of cardiomyocytes that can cause sudden cardiac death (SCD) and heart failure (Marian and Braunwald, 2017). Unfortunately, the efficacy of conventional HCM treatments is variable and unpredictable....
due to vast differences in variant-specific HCM mechanisms (Mosqueira et al., 2019; Wu et al., 2019). MYBPC3 is one of the most common mutated genes found in HCM patients (Seeger et al., 2019). MYBPC3 encodes the myosin binding protein C (MyBP-C), a sarcomeric protein that regulates cardiac contractility by modulating myofilament sliding velocity (Seeger et al., 2019). The non-coding variant (c.3330 + 2 T > G) in MYBPC3 is known to be both pathogenic and prevalent (Morita et al., 2008). Peripheral blood mononuclear cells (PBMCs) were isolated from a 60-year-old male HCM patient with this pathogenic variant in one allele and his 24-year-old daughter who was genotype-positive but phenotype-negative at that time (Table 1). The PBMCs were reprogrammed using the Sendai virus. The iPSC clones displayed typical morphology and normal karyotype (passages 12 and 13, respectively) as assessed by the KaryoStat assay. Immunostaining showed a high expression of pluripotency markers OCT3/4, NANOG, and SOX2. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) confirmed the comparable expression of OCT3/4 (also named as POU5F1) in these two iPSC lines with that of the widely used iPSC line SCVI273, which is 100-fold higher than that in differentiated cardiomyocytes. Additionally, the iPSCs were able to differentiate into derivatives of all three germ layers. The heterozygous c.3330 + 2 T > G mutation was confirmed by Sanger sequencing (Fig. 1 and Table 2). All lines were mycoplasma-negative. The origin of these lines was confirmed by short tandem repeat (STR) analysis showing that the profile of the lines fully matched that of the donors’ somatic cells.

3. Materials and methods

3.1. Reprogramming

Peripheral blood mononuclear cells (PBMCs) were isolated from blood by Percoll separation (GE Healthcare), purified by multiple rounds of washing using DPBS buffer (Thermo Fisher), and plated in a 24-well plate. Then, 1–2 × 10^6 PBMCs were cultured in 1 mL of complete PBMC medium made up of complete StemPro-34 medium (Thermo Fisher) combined with supplements including 100 ng/mL SCF (Peprotech), 100 ng/mL FLT3 (Thermo Fisher), 20 ng/mL IL-3 (Peprotech), 20 ng/mL IL-6 (Thermo Fisher), and 20 ng/mL EPO (Thermo Fisher). The medium was replaced every other day until the cell number could remain stable for few days (PBMCs include a heterogeneous population of cells, the medium is targeting a small population). About 2 × 10^5 PBMCs resuspended in 300 μL of complete PBMC medium were infected with a Sendai virus reprogramming cocktail based on the instructions of the CytoTune®-iPSC Sendai Reprogramming Kit (Thermo Fisher). The following day, the cells were washed, resuspended in 1 mL of complete PBMC medium, and plated in one well of a Matrigel-coated 12-well plate. On day 3, the cells were resuspended in 1 mL of StemPro-34 medium (Thermo Fisher) consisting of the StemPro™-34 SFM basal medium supplied with the StemPro™-34 SFM supplement and plated in one well of a Matrigel-coated 12-well plate. The StemPro™-34 medium was replaced every two days. On Day 7, 1 mL of StemMACS medium (Miltenyi Biotec) was added on top of the StemPro™-34 medium and the medium was replaced with fresh StemMACS on Day 8. The medium was replaced every other day with 1 mL of fresh StemMACS until Day 10–15 after Sendai virus infection when colonies appeared and were
ready to be picked up. The picked colonies were expanded when they grew out and were frozen.

3.2. Cell culture

The iPSCs were cultured in StemMACS medium (Miltenyi Biotec), and they were passaged in StemMACS medium combined with 10 μM Y27632 (Selleck Chemicals). Subsequently, the medium was changed every other day with StemMACS medium. All cells were cultured at 37 °C, 5% CO₂, and 85% relative humidity in an incubator.

3.3. Trilineage differentiation

The ability of iPSCs to differentiate into the three germ layers (ectoderm, mesoderm, and endoderm) was assessed using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies), following the manufacturer’s instructions.

3.4. Immunofluorescent staining

Cells were fixed in 4% PFA (EMD Millipore) for 15 min at room temperature, permeabilized with 50 μg/mL digitonin for 10 min, and blocked for 30 min at room temperature with 1% of BSA (Sigma-Aldrich) plus 5% of serum (Thermo Fisher) from host species raised for secondary antibodies. Primary antibodies were incubated with 1% of BSA overnight at 4 °C followed by an incubation with secondary antibodies in 1% of BSA solution for 30 min at room temperature. Nuclei were counterstained with DAPI (Vector Laboratories).

3.5. RT-qPCR

Total RNA from iPSCs and differentiated iPSC-CMs was extracted using the Direct-zol RNA Miniprep Kit (ZYMO RESEARCH). Reverse transcription of RNA was performed using iScript™ cDNA Synthesis Kit (Bio-Rad). cDNA production was amplified on CFX96 Real-Time PCR Detection System using Applied Biosystems TaqMan Fast Advanced Master Mix (Thermo Fisher). Pou5F1 was amplified using POU5F1 (OCT3/4) TaqMan™ Gene Expression Assay (Thermo Fisher, Hs00999634). Other primers are shown in Table 3.

3.6. Mycoplasma detection

Mycoplasma was assessed using the MycoAlert Detection Kit (Lonza) following the manufacturer’s instructions.

3.7. Short tandem repeat analysis

Genomic DNA was isolated from iPSCs and PBMCs using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer’s instructions. STR-analyses were performed by Stanford PAN Facility, using CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits (Thermo Fisher).

3.8. Karyotyping

iPSCs at passages 12 and 13 were analysed using KaryoStat™ assay (Thermo Fisher).
3.9. Sanger sequencing

Genomic DNA was isolated from iPSCs using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer’s instructions. MYBPC3 mutation analysis was performed on a purified PCR product obtained from genomic DNA amplification using Phusion High-Fidelity PCR Kit (Thermo Fisher). The PCR product was purified using QIAquick PCR Purification Kit (Qiagen) and sent for Sanger sequencing. Primers are shown in Table 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Characterization of iPSC lines SCVIi001-A and SCVIi002-A. A. Morphology of the iPSC colonies. B. Immunofluorescent staining for pluripotency markers OCT3/4, SOX2, and NANOG. C. Immunofluorescent staining for ectoderm, mesoderm, and endoderm markers after differentiation. D. Sanger sequencing results. E. Relative expression of pluripotency marker POU5F1 (OCT3/4) in iPSCs and cardiomyocytes. F. Results of KaryoStat assay.
## Table 1

Summary of lines.

| iPSC Line names | Abbreviation in figures | Gender | Age | Ethnicity               | Genotype of locus          | Clinical disease                 |
|-----------------|-------------------------|--------|-----|-------------------------|---------------------------|----------------------------------|
| SCVI001-A       | SCVI550                 | Male   | 60  | Not Hispanic or Latino  | c.3330 + 2 T > G heterozygous | Hypertrophic cardiomyopathy    |
| SCVI002-B       | SCVI591                 | Female | 24  | Not Hispanic or Latino  | c.3330 + 2 T > G heterozygous | –                               |
## Table 2

**Characterization and validation.**

| Classification       | Test                                      | Result                                                                 | Data       |
|----------------------|-------------------------------------------|------------------------------------------------------------------------|------------|
| Morphology           | Photography                               | Visual record of the line: normal                                       | Fig. 1 panel A |
| Phenotype            | Qualitative analysis: immunofluorescence staining | Positive expression of pluripotency markers: Oct4, Nanog, Sox2          | Fig. 1 panel B |
|                      | Quantitative analysis: RT-qPCR             | Fold-change for OCT4: SCVIi001-A: 130 ± 6                              | Fig. 1 panel E |
|                      |                                           | SCVIi002-A: 146 ± 9                                                   |            |
| Genotype             | Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb | Normal karyotype: 46, XY for SCVIi001-A; 46, XX for SCVIi002-A         | Fig. 1 panel F |
| Identity             | Microsatellite PCR                         | N/A                                                                   | N/A        |
|                      | (mPCR) or STR analysis                     | 16 loci tested match well                                              | Available with the authors |
| Mutation analysis     | Sequencing                                | Heterozygous MYBPC3 c.3330 + 2 T > G                                  | Fig. 1 panel D |
| (IF APPLICABLE)      |                                           | for the two iPSC lines                                                |            |
|                      | Southern blot or WGS                       | N/A                                                                   | N/A        |
| Microbiology and      | Mycoplasma                                 | Luminescence: Negative                                                | Supplementary Table 1 |
| virology             |                                           |                                                                        |            |
| Differentiation       | Trilineage in vitro differentiation by     | Positive staining of three germ layer markers: ectodermal (OTX2), mesoderm (BRACHYURY), endoderm (SOX17) | Fig. 1 panel C |
| potential            | immunofluorescence analysis                |                                                                        |            |
| Donor screening       | HIV 1 + 2 Hepatitis B, Hepatitis C        | Not performed                                                         | Not performed |
| (OPTIONAL)            |                                           |                                                                        |            |
| Genotype additional   | Blood group genotyping                     | Not performed                                                         | Not performed |
| info (OPTIONAL)       | HLA tissue typing                          | Not performed                                                         | Not performed |
## Table 3

### Antibodies used for immunocytochemistry/flow-cytometry

| Antibody               | Dilution | Company Cat # and RRID                                |
|------------------------|----------|-------------------------------------------------------|
| Pluripotency Marker    | Mouse IgGκ Anti-OCT-3/4 1:200 | Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051 |
| Pluripotency Marker    | Rabbit Anti-NANOG 1:200       | Proteintech Cat# 142951-1-AP, RRID: AB_1607719          |
| Pluripotency Marker    | Mouse IgGκ Anti-SOX 2 1:200   | Santa Cruz Biotechnology Cat# sc-365823, RRID: AB_10842165 |
| Ectoderm Marker        | Goat Anti-OTX2 1:200          | R&D Systems Cat# 963273, RRID: AB_2157172               |
| Endoderm Marker        | Goat Anti-SOX17 1:200         | R&D Systems Cat# 963121, RRID: AB_355060                 |
| Mesoderm Marker        | Goat Anti-BRACHYURY 1:200     | R&D Systems Cat# 963427, RRID: AB_2200235               |
| Secondary Antibody     | Alexa Fluor 488 Goat Anti-Mouse IgGκ 1:1000 | Thermo Fisher Scientific #A-21121 RRID: AB_2535764 |
| Secondary Antibody     | Alexa Fluor 647 Goat Anti-Mouse IgGκ 1:250 | Thermo Fisher Scientific #A-21242 RRID: AB_2535811 |
| Secondary Antibody     | Alexa Fluor 555 Goat Anti-Rabbit IgG (H + L) 1:500 | Thermo Fisher Scientific #A-21428 RRID: AB_141784 |
| Secondary Antibody     | Alexa Fluor 488 Donkey Anti-GOat IgG (H + L) 1:1000 | Thermo Fisher Scientific #A-11055 RRID: AB_2534102 |

### Primers

| Target                      | Forward/Reverse primer (5′-3′) |
|-----------------------------|--------------------------------|
| Pluripotency Markers (qPCR) | OCT4                          |
|                             | Hs00999634_gH                  |
| House-keeping genes         | 18S                            |
|                             | F: AGAAACGGCTACCACATCCA        |
|                             | R: CCCTCAATGGATCTCGTT          |
| Genotyping                  | MYBPC3                         |
|                             | F: CCACAGCTCACATTTCCAGTCCAC    |
|                             | R: TAATGCTCCAAGCGTGAAACCAC    |
# Resource Table

| **Unique stem cell lines identifier** | SCVIi001-A  
SCVIi002-A |
|-------------------------------------|------------------|
| **Alternative names of stem cell lines** | SCVI550  
SCVI591 |
| **Institution** | Stanford Cardiovascular Institute |
| **Contact information of distributor** | Dr. Joseph C. Wu; joewu@stanford.edu |
| **Type of cell lines** | iPSC |
| **Origin** | Human |
| **Cell Source** | Blood |
| **Clonality** | Clonal |
| **Method of reprogramming** | Integration-free Sendai virus expressing human OCT4, SOX2, KLF4, and c-MYC |
| **Multiline rationale** | Non-isogenic cell lines obtained from one patient and one healthy relative with the same mutation in MYBPC3 |
| **Gene modification** | Yes |
| **Type of modification** | Spontaneous mutation |
| **Associated disease** | Hypertrophic cardiomyopathy |
| **Gene/locus** | MYBPC3/chr11 (p11.2)  
Heterozygous MYBPC3 c.3330 + 2 T > G |
| **Method of modification** | N/A |
| **Name of transgene or resistance** | N/A |
| **Inducible/constitutive system** | N/A |
| **Date archived/stock date** | SCVIi001-A: 4/26/2016  
SCVIi002-A: 8/16/2016 |
| **Cell line repository/bank** | https://hpscreg.eu/cell-line/SCVIi001-A  
https://hpscreg.eu/cell-line/SCVIi002-A |
| **Ethical approval** | The generation of the lines was approved by the Administrative Panel on Human Subjects in Medical Research, Stanford University under IRB #29904 for working with human subjects |