Characterization of an iPSC line NCHi006-A from a patient with hypoplastic left heart syndrome (HLHS)

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Abstract

Hypoplastic left heart syndrome (HLHS) is a severe congenital heart defect characterized by underdeveloped structures on the left side of the heart, including hypoplasia of the left ventricle and stenosis or atresia of the aortic and mitral valves. Here, we generated an iPSC line from the peripheral blood mononuclear cells of a male patient with HLHS through Sendai virus-mediated transfection of 4 Yamanaka factors. This iPSC line exhibited normal morphology, expressed pluripotency markers, had a normal karyotype, and could differentiate into cells of three germ layers. This iPSC line can be used for studying cellular and developmental etiologies of HLHS.

1. Resource Table

| Unique stem cell line identifier | NCHi006-A |
|----------------------------------|-----------|
| Alternative name(s) of stem cell line | NCH023 (NCHi006-A) |
| Institution | Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children’s Hospital, Columbus, OH, USA Mingtao Zhao, PhD |
| Contact information of distributor | Mingtao.Zhao@nationwidechildrens.org |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info required for human ESC or iPSC | Age: 5 months Ethnicity: Caucasian Sex: male |

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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.2022.102892.
2. **Resource utility**

Derived from a patient with hypoplastic left heart syndrome (HLHS), this iPSC line can be differentiated into cardiac cell lineages for modeling congenital heart defects. It may serve as a useful in vitro biological system to study underlying mechanisms of HLHS, screen for candidate therapeutics, and increase our understanding of human cardiac development.

3. **Resource details**

Hypoplastic left heart syndrome is a severe congenital heart defect where the left ventricle of the heart is underdeveloped, causing aberrant hemodynamics (Grossfeld et al., 2019). The cause of HLHS is still poorly understood due to the lack of experimental models. Here, we established and characterized an iPSC line derived from a male infant with HLHS to provide a biological system that retains the genetic information of the proband and can be utilized to model stages of human development through differentiation into the three germ layers. We envision this iPSC line to be used as a patient-specific biological model to study human cardiac development, especially to interrogate the mechanisms that govern the pathogenesis of HLHS (Hall et al., 2022; Lin et al., 2021).

To generate iPSC line NCHi006-A, blood was drawn from a patient clinically diagnosed with HLHS and mitral/aortic stenosis, with no other observed heart defects (see Table 1). The peripheral blood mononuclear cells (PBMCs) were isolated and transfected with 4 Yamanaka factors to produce an iPSC line that exhibited normal morphology and colony formation (Fig. 1A). The majority of cells displayed pluripotency markers TRA-1–60, SOX2, NANOG, and OCT3/4, as detected by immunofluorescence staining (Fig. 1B & D). Genetically, the iPSCs displayed a normal male karyotype (46, XY) as confirmed by a whole-genome array (Fig. 1C), and their identity was confirmed using STR analysis to prove their origin from the patient’s PBMCs (in archive with the journal). This iPSC line
had the ability to differentiate into cells of all three germ layers, as established by positive immunofluorescence staining of germ layer-specific markers. Ectodermal-like cells showed expression of PAX6 and OTX2, mesodermal-like cells displayed TBX6 and Brachyury, while endodermal-like cells expressed FOXA2 and SOX17 (Fig. 1E). The iPSCs were also tested negative for mycoplasma contamination (Supplementary Fig. 1A).

4. Materials and methods

4.1. Reprogramming

Patient PBMCs were isolated and incubated for one week in StemPro-34 SFM medium (Thermo Fisher Scientific) supplemented with 100 ng/mL SCF (PeproTech), 100 ng/mL FLT3 (Thermo Fisher Scientific), 20 ng/mL IL3 (PeproTech), 20 ng/mL IL6 (Gibco), 20 ng/mL EPO (Thermo Fisher Scientific), and 1× GlutaMAX (Thermo Fisher Scientific). PBMCs were then transfected using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. Transfected cells were resuspended in supplemented StemPro-34 SFM medium and transferred into a Matrigel-coated plate for one week. Cells were then switched to complete E8 medium (Thermo Fisher Scientific). After two weeks, emerging iPSC clones were picked, expanded over multiple passages, and stored in liquid N\textsubscript{2}.

4.2. iPSC maintenance and passaging

Cells were maintained in complete E8 media at 37 °C with 5% CO\textsubscript{2}. Upon reaching 90% confluency, cells were washed with DPBS then dissociated with 0.5 mM EDTA for 5–8 min. EDTA was then removed, and iPSCs were manually dislodged with complete E8 media plus ROCK inhibitor (Y-27632, Selleck Chemicals). To split, the cell suspension was replated at a 1:6–1:10 ratio.

4.3. Immunofluorescent staining

The pluripotency of iPSCs (passages 12–13) was assessed by immunofluorescence staining and manual counting. Cells were fixed with 4% paraformaldehyde solution (Electron Microscopy Sciences) for 15 min, then permeabilized with 0.1% Triton X-100 solution (Sigma) for 20 min at room temperature. Cells were then blocked with 0.2% BSA (Sigma) in DPBS, and incubated with primary antibodies (dilution: 1:200) overnight at 4 °C. The next day, secondary antibodies (dilution: 1:2000) in 0.2% BSA were added at room temperature for 1 h, then counterstained with DAPI (dilution: 1:2000) in DPBS at room temperature for 10 min (see Table 2). Stained coverslips were mounted onto glass slides using SlowFade Gold Antifade (Thermo Fisher Scientific) and imaged with a fluorescence microscope (Keyence).

4.4. Karyotyping

To detect chromosomal abnormalities using whole genome array, 2 × 10\textsuperscript{6} iPSCs (passages 12–13) from were harvested and analyzed using the KaryoStat Assay (Thermo Fisher Scientific).
4.5. **Short tandem repeat (STR) analysis**

Genomic DNA was extracted from iPSCs (passages 14–15) and PBMCs using the Quick-DNA Miniprep Plus Kit (Zymo Research). The PowerPlex 16 System (Promega) was then utilized to amplify genomic materials according to the manufacturer’s instructions. Samples were sent for capillary sequencing using an ABI 3730xl Genetic Analyzer (Thermo Fisher Scientific). GeneMapper 5.0 (Thermo Fisher Scientific) software was used to analyze the sequencing data for allele callings for 16 loci per sample. Only strong allele calling signals were considered for analysis.

4.6. **Germ layer differentiation**

Pluripotency was confirmed by differentiating iPSCs (passages 11–12) into endoderm and ectoderm cells using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) according to the manufacturer’s instructions. Mesoderm differentiation was induced through two-day application of 6 μM CHIR99021 (Selleck Chemicals) in RPMI 1640 media (Thermo Fisher Scientific) with B27 minus insulin supplement (Thermo Fisher Scientific). Samples were fixed and stained with respective germ layer-specific markers.

4.7. **Mycoplasma detection**

Mycoplasma contamination was checked using the MycoAlert™ Detection Kit (Lonza) on iPSC passage 12 supernatant following the manufacturer’s protocol.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Characterization of an iPSC line derived from an HLHS patient.
Table 1

Characterization and validation.

| Classification         | Test                                | Result                                           | Data                                      |
|------------------------|-------------------------------------|-------------------------------------------------|-------------------------------------------|
| Morphology             | Photography Bright field            | Normal                                          | Fig. 1A                                   |
| Phenotype              | Qualitative analysis: Immunocytochemistry | Expression of TRA-1–60, NANOG, SOX2, OCT3/4 | Fig. 1D                                   |
|                        | Quantitative analysis: Immunocytochemistry counting | NANOG: 91 ± 2%, OCT3/4: 97 ± 2%, SOX2: 95 ± 2% | Fig. 1B                                   |
| Genotype               | Whole genome array (KaryoStat+ Assay) | Normal karyotype: 46, XY; Resolution 1–2 Mb    | Fig. 1C                                   |
| Identity               | Microsatellite PCR (mPCR)           | Not performed                                   | N/A                                       |
|                        | OR                                  | 16 loci tested with matching identity           | Submitted in archive with journal         |
| Mutation analysis      | Sequencing                          | N/A                                             | N/A                                       |
|                        | Southern Blot OR WGS                | N/A                                             | N/A                                       |
| Microbiology and virology | Mycoplasma                           | Negative                                        | Supplementary Figure 1A                   |
| Differentiation potential | Trilineage in-vitro differentiation | Positive immunofluorescence staining of three germ layers | Fig. 1E                                   |
|                        | 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                                       |
### Table 2

Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | Antibody | Dilution | Company Cat # | RRID          |
|--------------------------------------------------------|----------|----------|---------------|---------------|
| Pluripotency Marker                                    | Rabbit anti-NANOG | 1:200 | Cell Signaling Technology, Cat# 4903P | AB_10559205   |
| Pluripotency Marker                                    | Alexa Fluor 488 Mouse anti-OCT3/4 | 1:2000 | BD Biosciences, Cat# 561628 | AB_10895977   |
| Pluripotency Marker                                    | Mouse anti-TRA-1–60 | 1:200 | Thermo Fisher Scientific, Cat# MA1-023X | AB_2536705   |
| Pluripotency Marker                                    | Rabbit anti-SOX2 | 1:200 | Thermo Fisher Scientific, Cat# PA1-094X | AB_2539862   |
| Ectoderm Marker                                        | Goat anti-OTX2 | 1:200 | R&D Systems, Cat# AF1979 | AB_2157172   |
| Ectoderm Marker                                        | Rabbit anti-PAX6 | 1:200 | Thermo Fisher Scientific, Cat# 42-6600 | AB_2533534   |
| Mesoderm Marker                                        | Goat anti-Brachyury | 1:200 | R&D Systems, Cat# AF2085 | AB_2200235   |
| Mesoderm Marker                                        | Rabbit anti-TBX6 | 1:200 | Thermo Fisher Scientific, Cat# PA5-35102 | AB_2552412   |
| Endoderm Marker                                        | Goat anti-SOX17 | 1:200 | R&D Systems, Cat# AF1924 | AB_355060    |
| Endoderm Marker                                        | Mouse anti-FOXA2 | 1:200 | Abnova, Cat# H00003170-M10 | AB_534871    |
| Secondary Antibody                                     | Goat anti-Mouse IgG (H + L), Alexa Fluor 594 | 1:2000 | Thermo Fisher Scientific, Cat# A-11032 | AB_2534091   |
| Secondary Antibody                                     | Goat anti-Mouse IgG (H + L), Alexa Fluor 488 | 1:2000 | Thermo Fisher Scientific, Cat# A-11001 | AB_2534069   |
| Secondary Antibody                                     | Goat anti-Rabbit IgG (H + L), Alexa Fluor 594 | 1:2000 | Thermo Fisher Scientific, Cat# A-11012 | AB_2534079   |
| Secondary Antibody                                     | Donkey anti-Mouse IgG (H + L), Alexa Fluor 594 | 1:2000 | Thermo Fisher Scientific, Cat# R37115 | AB_2556543   |
| Secondary Antibody                                     | Donkey anti-Rabbit IgG (H + L), Alexa Fluor 594 | 1:2000 | Thermo Fisher Scientific, Cat# R37119 | AB_2556547   |
| Secondary Antibody                                     | Donkey anti-Goat IgG (H + L), Alexa Fluor Plus 488 | 1:2000 | Thermo Fisher Scientific, Cat# A32814 | AB_2762838   |

### Primers

| Target (RT-PCR)       | Band Size | Primer Sequence (5’-3’):  |
|-----------------------|-----------|---------------------------|
| Housekeeping Gene     | GAPDH     | 452 bp | F: ACCACAGTTCATGCAATCAC  |
|                       |           |     | R: TCCACACCCTGTTGCTGTA   |
| Transgene (RT-PCR)    | KOS       | 528 bp | F: ATGCACCGCTACGACGTGAGC  |
|                       |           |     | R: ACCTTGACAATCTGAGTGGG   |

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