

Thymic derived iPS cells can be differentiated into cardiomyocytes

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1. ABSTRACT

Ventricular septal defect (VSD) is a common congenital heart malformation. Several factors lead to the development of VSD, including familial causes, exposure to certain drugs, infectious agents, and maternal metabolic disturbances. We hypothesized that induced pluripotent stem (iPS) cells can be obtained from VSD patients to generate cardiomyocytes. Here, we show the generation and cardiomyocyte differentiation of iPS cells from the thymic epithelial cells of a patient with VSD (TECs-VSD) by overexpressing four transcription factors: *OCT4*, *SOX2*, *NANOG*, and *LIN28* using lentiviral vectors. The self-renewal capacity and pluripotency of iPS cells was verified *in vitro* by expression of pluripotency markers and formation of teratoma *in vivo*. The results show that iPS cells can be derived from patients with VSD and they can be differentiated into cardiomyocytes. These cells can be used for understanding the pathogenesis of defect that causes VSD.

2. INTRODUCTION

Ventricular septal defects (VSDs) constitute the most frequent types of congenital heart disease

(CHD), occurring in 50% of all children with CHD and as an isolated lesion in 20% cases (1). VSD, a type of CHD present at birth, occurs due to problems early in the development of the heart, but the underlying molecular mechanisms in humans remain to be elucidated. Advances in imaging technologies have led to considerable increases in the incidence of VSD. An extensive literature review has estimated a median incidence of VSD of up to 5% in newborn babies (2,3). To our knowledge, no genes in mice or humans have been linked primarily to isolated VSD without other associated congenital cardiac malformations. Genetics and environmental factors probably both play a role. Several known risk factors, including a family history of CHD, exposure to teratogens, and untreated maternal metabolic illnesses (e.g., phenylketonuria and pregestational diabetes) are associated with VSD (3). It sometimes occurs with other genetic problems, such as Down syndrome (4). Although monogenic defects that initiate the development of VSDs are less well defined at present, studies have implicated the roles of *TBX5*, *GATA4*, and *NKX2.5* in septal defects (5,6).

To investigate the events driving heart development and to determine the molecular mechanisms that cause myocardial diseases in humans, it is essential to generate functional human cardiomyocytes (7). Induced pluripotent stem (iPS) cells are one of the most promising sources of cardiomyocytes (8). iPS cells possess the developmental potential of human embryonic stem (hES) cells. They can be derived directly from readily accessible patient tissue; therefore, these cells have been heralded as a powerful tool to study development (9, 10), and particularly human disease; moreover, they can be differentiated into all cell types (11,12). As a tool to develop new cardioactive compounds or to screen compounds for potential cardiotoxicity, access to abundant populations of human cardiomyocytes is of particular interest to scientists. The generation of pluripotent stem cells from an individual patient would enable the large-scale production of the cell types affected by the specific disease (13). More importantly, human iPS cells could be utilized to generate patient-specific lineages for studying early developmental biology, disease modeling, drug discovery, and translational research by overcoming the ethical issues and immunological problems associated with hES cells. Cardiac therapy requires the development of patient-specific and disease-specific drugs, new cell resources for replacement therapy, and ultimately, engineering of whole human heart organs for transplantation (14). The limited availability of human cardiomyocytes is, however, a major obstacle for such progress.

Here, we generated iPS cells from a 24-month-old boy diagnosed with VSD. These patient-specific iPS cells possess properties of hES cells and were successfully directed to differentiate to cardiomyocytes, which serve as a model system for studying the pathophysiology of VSD, toxicity testing, and determining underlying genetic factors.

3. METHODS

3.1. Animals and ethical approval

Male CF1 mice were obtained from SiDanSai Biotechnology Co., Ltd., Shanghai. Nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai. All animals were housed and maintained in pathogen-free conditions. All animal experimental procedures and protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Biomedical Research Ethics Committee of Fudan University. Written approval for human thymus tissue collection, subsequent iPS cell generation, and genome/gene analyses performed in the current study was obtained from the Ethics Committee for Human Research at Fudan University (approval number: 048). The patient involved in the study was presented

with VSD at age 24 months. Written informed consent was obtained from the patient's guardian.

3.2. Cell dissociation

Human thymic tissues were obtained via operative incision, when the patient underwent cardiac surgery. Dissected thymic tissues were harvested and stored in cold Dulbecco's modified Eagle medium (DMEM, Invitrogen) complemented with 10% fetal bovine serum (FBS, Gibco/Invitrogen) during dissection. Thymic tissues were minced with scalpels into smaller pieces, and tissue fragments were placed into a 100-mm tissue culture dish and washed in PBS without Ca^{2+} or Mg^{2+} twice before being dissociated with 0.25% trypsin solution containing 0.1% EDTA, at 37°C in a humidified incubator with 5% CO_2 . Thymic epithelial cells (TECs) were maintained in complete DMEM containing 10% FBS and 1% penicillin/streptomycin (Invitrogen). A dense outgrowth of cells appeared after 7–14 d, and the cells were passaged and expanded.

3.3. Lentivirus production

The 293T cells were seeded overnight at 2×10^6 cells per 100-mm dish with DMEM supplemented with 10% FBS. The lentiviral vectors pCDH-OCT4, pCDH-SOX2, pCDH-NANOG, and pCDH-LIN28 were packaged in 293T cells with packaging mix TAT, GAG, REC, and VSVG, using Lipo2000 reagent (Invitrogen) according to the manufacturer's instructions. At approximately 48 hours after transfection, the medium containing the lentivirus was collected, and the cellular debris was removed by centrifugation. The supernatant was filtered through a 0.45- μm filter (Millipore), and the lentivirus was pelleted by ultracentrifugation at 4°C. Thereafter, the lentivirus particles were resuspended in DMEM and stored at -80°C.

3.4. VSD-specific iPS cell culture

Human iPS cells were reprogrammed by lentiviral-mediated transduction of 4 transcription factors (OCT4, SOX2, NANOG, and LIN28) were previously described (10). Briefly, the TECs were infected with pCDH vectors encoding exogenous OCT4, SOX2, NANOG, and LIN28 in the presence of 10 $\mu\text{g/mL}$ of polybrene. The iPS cells derived from TECs were maintained on a mitotically inactivated mouse embryonic fibroblast (MEF) feeder layer as previously described. The media was changed on day 3 to DMEM/F12 (Invitrogen) supplemented with 20% KnockOut Serum Replacement (Invitrogen), 1% nonessential amino acids (NEAA, Invitrogen), 1 mM l-glutamine (Invitrogen), 0.1% β -mercaptoethanol (β -ME, Sigma), 4 ng/mL basic fibroblast growth factor (bFGF), and 1% penicillin/streptomycin. On approximately day 20, hES cell-like colonies were selected based on morphology.

3.5. Teratoma formation from iPS cells

For the teratoma formation, 5×10^6 iPS-VSD cells of each iPS cell line were harvested and subcutaneously injected into NOD-SCID mice with

Matrigel (BD Biosciences). Nine weeks after injection, teratomas were dissected, rinsed with PBS, and fixed in 4% paraformaldehyde. The paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin (HE staining) (15,16).

3.6. Short tandem repeat analysis

Genomic DNA from parental peripheral blood, parental TECs, iPS01, and iPS02 was purified according to the manufacturer's recommendations (Qiagen). The commercial identifier kit from Applied Biosystems enables co-amplification of 19 STR loci and the sex-typing marker amelogenin with four different dye labels (Applied Biosystems). Once data was collected in the ABI 3100 multi-capillary Genetic Analyzer, the raw data was converted into the appropriately colored peaks and the STR genotype information was determined using the GeneScan and Genotyper programs of Applied Biosystems.

3.7. Human stem cell culture

hES cells were maintained in DMEM/F12 supplemented with 20% KnockOut Serum Replacement (Invitrogen), 4 ng/mL bFGF, 2 mM L-glutamine, 1% NEAA, 0.1 mM β -ME, 50 U/mL penicillin, and 50 g/mL streptomycin. The cells were passaged approximately once a week.

3.8. Cardiac differentiation

To induce differentiation, human iPS cells were dispersed into small clumps with 1 mg/mL collagenase IV (Life Technologies) for 30 min and then seeded onto a Matrigel-coated cell-culture dish at 100,000–200,000 cell/cm². To form embryoid bodies (EBs), human iPS cell aggregates generated by dispase treatment were cultured in low-attachment plates in DMEM plus 10% FBS, where they aggregated to form EBs and were cultured in suspension. After 4 d of cultivation, the iPS cells formed ball-shaped structures. In the absence of serum to induce cardiogenesis, the chemically defined medium with a range of growth factors involved in normal cardiac development (BMP4, activin A, bFGF, and Wnt agonists and antagonist) was used (17, 18). EBs were then plated onto 0.1% (wt/vol) gelatin-coated six-well culture plates at 50–100 EBs per well and were cultured in RPMI 1640/B27 medium. Cells then were treated with 25 ng/mL BMP4 (R&D) and 6 ng/mL bFGF, in RPMI/B27-insulin. After 24 h, the medium was changed to RPMI/B27-insulin supplemented with 100 ng/mL activin A (R&D) for another day followed by 250 ng/mL Noggin. At day 5, the medium was changed to RPMI/B27-insulin supplemented with 200 ng/mL DKK1 for another 8 d, and the medium was changed every 3 d. The cells were transferred to RPMI/B27 at the appearance of spontaneous contractions.

3.9. Alkaline phosphatase staining and immunocytochemistry

Alkaline phosphatase (AP) staining was performed using the Alkaline Phosphatase Detection Kit (Sidansai, China) according to the manufacturer's

instructions. For immunocytochemistry, cells were washed with PBS and fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 30 min, and blocked in 3% BSA in PBS for 2 hr. The primary antibodies used in this study included OCT4 (1:200, Santa Cruz), NANOG (1:200, Santa Cruz), tumor-related antigen (TRA)-1-60 (1:150, Santa Cruz), SSEA4 (1:150, Santa Cruz), sarcomeric α -actinin (1:100, Bioworld), troponin T (1:800, Sigma), GATA4 (1:150, Bioworld), myosin light chain (MLC)-2a (1:200, Synaptic Systems), and MLC-2v (1:200, Synaptic Systems). The secondary antibodies used were rhodamine-conjugated AffiniPure goat anti-mouse IgM (1:200, Bioworld), rhodamine-conjugated AffiniPure goat anti-mouse IgG (1:200, Bioworld) and rhodamine-conjugated AffiniPure goat anti-rabbit IgG (1:200, Bioworld). Nucleuses were stained with DAPI (1:1000, Sigma). An epifluorescence microscope (DM IRB; Leica) with a QImaging Retiga 4000R camera was used for the imaging analysis.

3.10. Analysis of gene expression

Total RNA was extracted using an RNeasy Kit (QIAGEN) according to the manufacturer's instructions. RNA (1 mg) was reverse transcribed into cDNA via Oligo (dT) with SuperScript III Reverse Transcriptase (Invitrogen). Quantitative RT-PCR in triplicate for each sample and each gene was performed with SYBR Green PCR Master Mix (Qiagen) to determine the expression of endogenous pluripotency genes and cardiac-specific genes. The primer sequences are listed in Table 1. The transcript levels were determined using the 7500 Real-Time PCR System (Applied Biosystems). The target gene expression was normalized using GAPDH expression as an endogenous control.

4. RESULTS

4.1. Reprogramming of TECs from patients with VSD

To generate VSD-iPS, TECs-VSD were obtained from thymic tissues samples of the patient with VSD when he underwent cardiac surgery (Figure 1A). The optimized lentiviruses containing *OCT4*, *SOX2*, *NANOG*, and *LIN28* were introduced into TECs, and the cells were plated onto Matrigel-coated plates and maintained in high glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. The day after transduction, cells were plated on 0.1% Matrigel-coated dishes containing irradiated CF1 MEF. Approximately 10 d later, some atypical granulated colonies were visible, which were unlike hES cells in morphology (Figure 1B). VSD-iPS cell colonies with morphologies similar to those of hES cells appeared around 20 d after the first infection (Figure 1C). The hES cell-like colonies were selected and amplified by passage culture. Established VSD-iPS cell lines were maintained on MEF in conventional iPS medium (Figure 1D). After several generations, iPS cell clones (iPS01 and iPS02) at

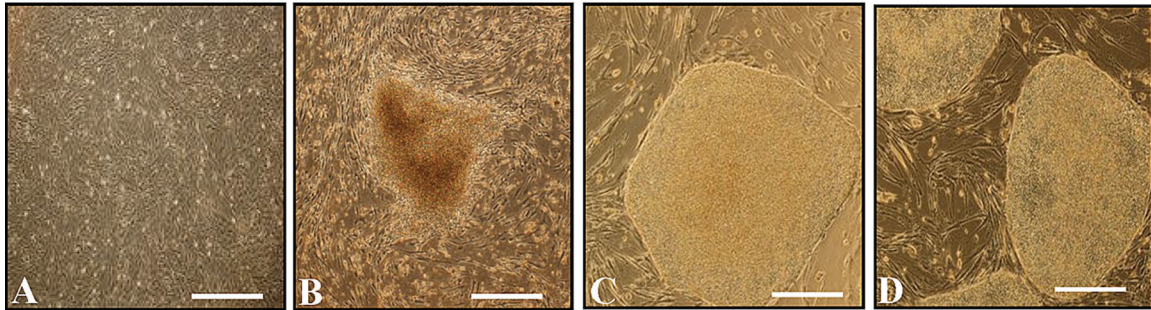


Figure 1. Induction of human iPS cells from TECs-VSD. (A) Primary cultured TECs-VSD. (B) Image of atypical granulated colony. (C) Image of VSD-iPS cell colony with morphology similar to those of hES cells. (D) Established VSD-iPS cell lines. Scale bars: 200µm.

Table 1. Primers for semi-quantitative PCR analysis

Gene	Primer	
	Forward (5'- 3')	Reverse (5'- 3')
OCT4 (total)	AGCTCTGCAGAAAGAACTCG	GCTTCCTCCACCCACTTCT
OCT4 (Endo)	GGGAGGAGCTAGGGAAAGAAAACCT	GAACTTCACCTTCCCTCCAACCAGT
SOX2 (total)	CCAAGATGCACAACCTCGGAG	GGGCAGCGTGTACTTATCCT
SOX2 (Endo)	TACGGTAGGAGCTTTCAGG	ACTGTCCTAAATTTTCAGCTGCA
NANOG (total)	ATAGCAATGGTGTGACGCAG	CTGTTCCAGGCCTGATTGT
NANOG (Endo)	ATGGAGGGTGGAGTATGGTTGG	AGGCTGAGGCAGGAGAATGG
CRIPTO (total)	AATTTGCTCGTCCATCTCGG	GACCCACAGTTCTCTTTGCG
CRIPTO (Endo)	CTTCTTTGCCCTGCCCTCT	GCAGGAGAATGGCTTGAACC
MLC-2a	AAGCCCCTCCTCAATGACTC	TGTCACCAAGGTCACAAGGA
MLC-2v	CCATGTTTGAGCAGACCCAG	TGGTCTCTTCAGGATCAGCC
ACTN2	GTCATCTCAGGGGAAAGGCT	CAGCAGACCTTCTTTGGCAG
GATA4	TCCTACTCCAGCCCCTACC	CAGATAGTGACCCGTCCTCAT
IRX4	TTCCGTTCTGAAGCGTGGTC	TGAAGCAGGCAATTATTGGTGT
cTnT	TTCACCAAAGATCTGCTCCTCGCT	TTATTACTGGTGTGGAGTGGGTGTGG
cTnI	CTCCAACCTACCGCGCTTATG	ACAAGTCCTGCAGCTCCG

passage 8 (P:8) were selected for further characterization and differentiation experiments. Human ES cell line X-01 cells (19) were used as the control.

4.2. Human VSD-iPS cells expressed pluripotent-specific markers

The expression of the pluripotent markers in the two iPS cell clones, iPS01 and iPS02, was examined using AP staining and immunostaining. The reprogrammed cells stained positive for AP (Figure 2A, image of iPS01) and expressed endogenous pluripotency markers (SSEA3, OCT4, TRA-1-60, and SSEA4) at the protein level, as demonstrated by immunocytochemistry (Figure 2B, images of iPS01). We also analyzed the total expression of four pluripotency genes (SOX2, OCT4, NANOG, and CRIPTO/TDGF1), as well as expression of

endogenous transcripts. The difference between total and endogenous gene expression is attributable to expression of the transgene. Quantitative RT-PCR showed that the pluripotency-associated genes expressed in VSD-iPS cells were consistently upregulated relative to the parental TECs (Figure 3). In hES cells, the total and endogenous transcripts of the four pluripotency-associated genes did not differ, indicating the lack of transgenes. The VSD-iPS cells exhibited significantly greater expression of total than of endogenous pluripotency genes, indicating residual transgene expression. The endogenous loci in the iPS cells were reactivated to levels comparable to hES cells line X-01 in VSD-specific iPS cell lines.

DNA from parental peripheral blood, parental TECs, iPS01, and iPS02 was analyzed by STR profiling

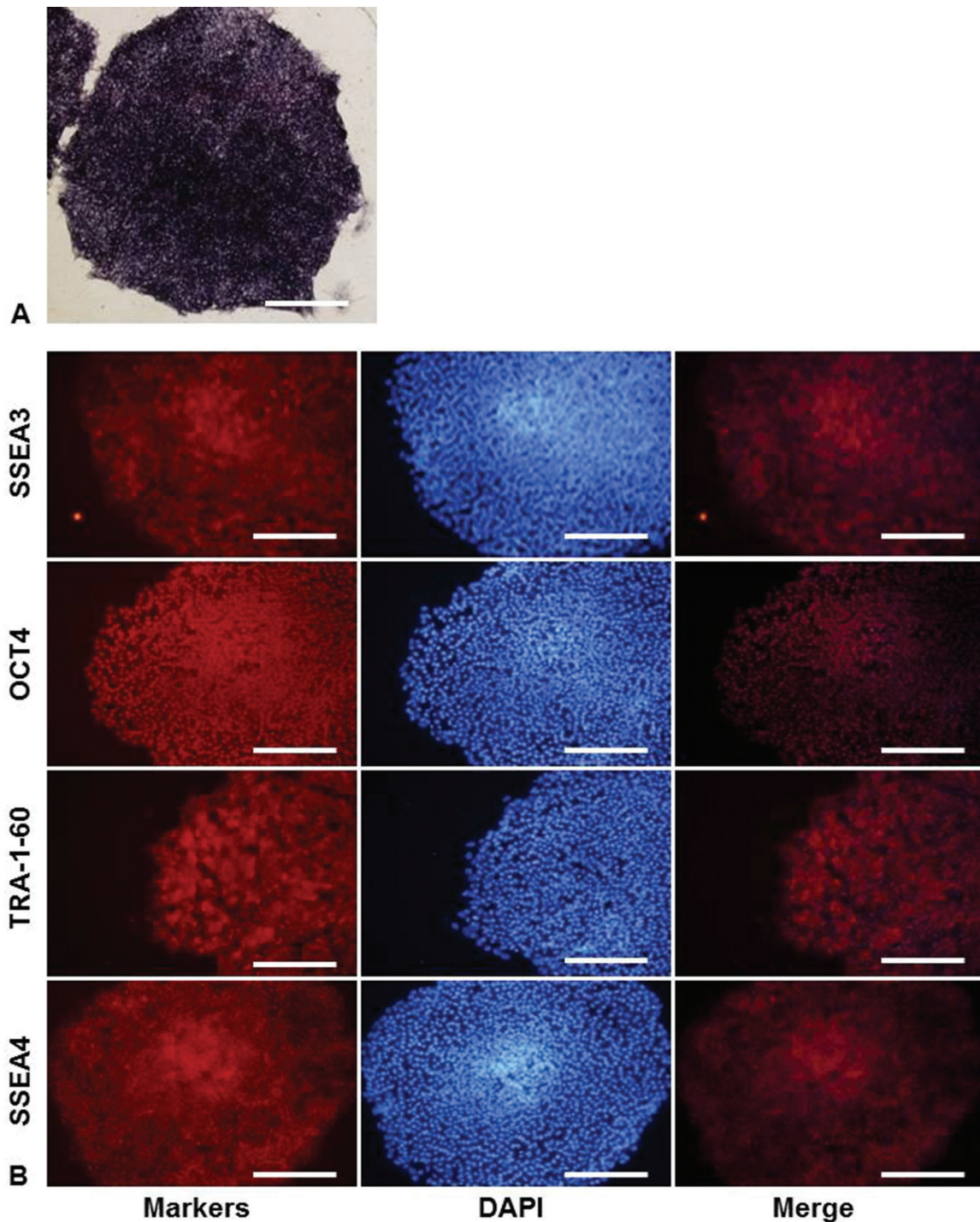


Figure 2. Human iPS-VSD cell line, iPS01, expressed pluripotency markers. (A) The iPS cells expressed alkaline phosphatase. (B) As shown by the immunostaining results, the cells expressed pluripotency markers, including SSEA3, OCT4, TRA-1-60 and SSEA4. Nuclei were stained with DAPI (blue). Scale bars: 200µm.

to confirm the origin of the human VSD-specific iPS cells from TECs-VSD, free of contamination by laboratory hES cells (20). The patterns of 19 STRs between iPS cells and parental TECs completely matched. The two

selected iPS cell clones were genetically matched to their parental TECs, ruling out the possibility of cross-contamination from existing cultured human pluripotent cells (Figure 4).

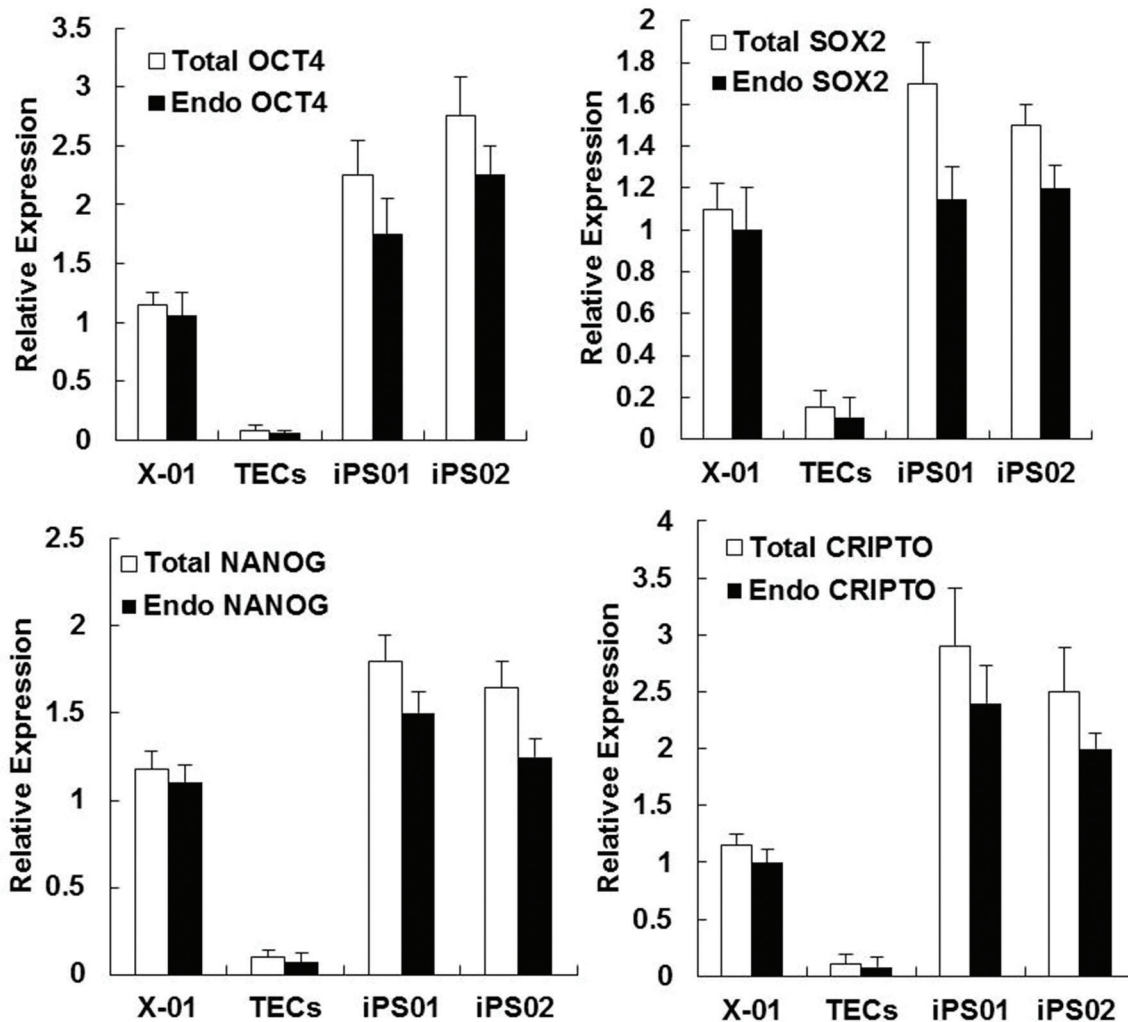


Figure 3. The VSD-iPS cells expressed pluripotency-associated genes. The relative expression levels between total and endogenous (Endo) gene-expression of *OCT4*, *SOX2*, *NANOG* and *CRIPTO* were determined with semi-quantitative PCR in iPS01 and iPS02; parental TECs and human ES cells, X-01 cell line, were used as control.

4.3. Teratoma formation with human iPS cells

The ultimate standard of pluripotency for the fully reprogrammed cells is teratoma formation in immunodeficient murine hosts. The VSD-iPS cells were injected into NOD/SCID mice. Eight weeks after injection, the teratomas were harvested for HE staining. The teratomas contained derivatives of the endoderm (gut-like structures), mesoderm (cartilage), and ectoderm (neuroepithelium) (Figure 5). These results suggest that VSD-iPS can spontaneously differentiate into derivatives of all three germ layers in vivo.

4.4. Cardiomyocyte differentiation from induced pluripotent stem cells

Our studies showed that the iPS cell clones (iPS01 and iPS02) characterized in this study could form EBs with spontaneous contractions. At approximately day 17 after

EBs adherented, beating EBs were observed in VSD-iPS cell clones. The VSD-specific iPS cell clones showed spontaneously contracting areas in the following days. The experiments then focused on EBs (DF01 and DF02) derived from the two iPS cell clones (iPS01 and iPS02, respectively). To evaluate the expression of myofilament and cardiac-specific proteins in DF01 and DF02, we performed immunolabeling with antibodies against GATA4, sarcomeric α -actinin, troponin T, MLC-2a, and MLC-2v using enzymatically dissociated and replated monolayer cells from the beating areas. An immunofluorescence analysis clearly revealed the distribution and localization of these cardiac markers in the dissociated cells from beating foci. GATA-4, a transcription factor involved in myocardial differentiation and function, showed strong nuclear localization, while α -actinin, cTnT, MLC-2a, and MLC-2v, which are myofilament proteins, exhibited cytoplasmic

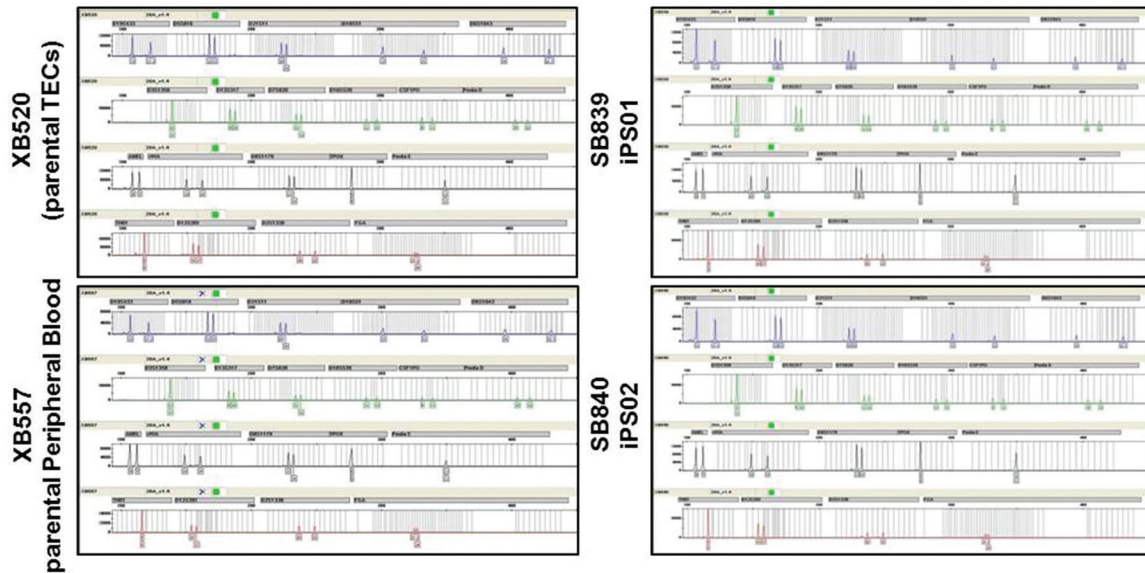


Figure 4. Human VSD-iPS cells were derived from parental TECs, not from a contamination of laboratory human ES cells. Fully analyzed data derived from the DNA of parental TECs, parental peripheral blood and VSD-iPS cells, showing the blue, green, brown and red amplified STR peaks and allele classifications.

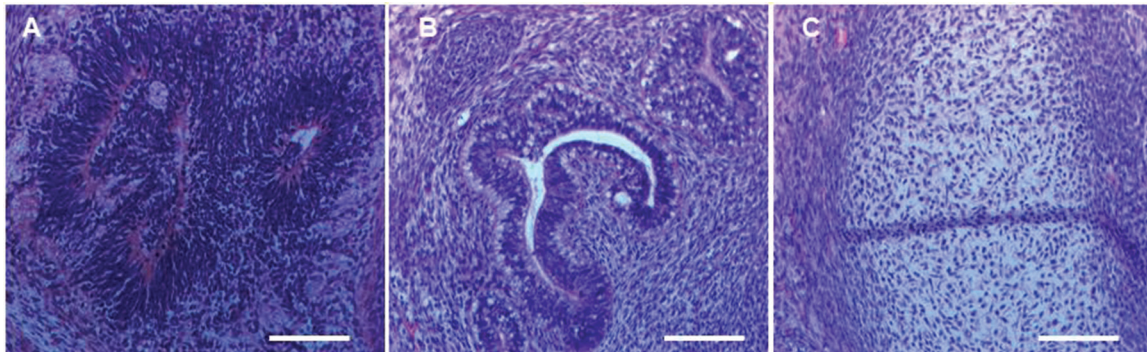


Figure 5. The induced pluripotent stem cells (iPS-VSD) maintain pluripotency in vivo. Teratomas were collected and stained with hematoxylin and eosin. The tissues originating from the three embryonic germ layers were present in the teratomas (A. ectoderm (neuroepithelium), B. endoderm (gut-like structures), C. mesoderm (cartilage)). Scale bars: 200µm.

localization. These data suggest that beating areas isolated from differentiating iPS cell lines showed features similar to cardiac myocytes (Figure 6A).

We examined the gene expression patterns present in contracting regions from DF01 and DF02 using RT-PCR. Overall, there was a strong increase in cardiac gene expression following cardiac differentiation. Expression of *SOX2*, *OCT4*, and *NANOG* was high in the two undifferentiated iPS cells (iPS01 and iPS02), and the expression greatly decreased following differentiation (Figure 6B). Meanwhile, we examined a variety of cardiac genes: the zinc-finger transcription factor *GATA4*; a marker of ventricular myocardium, *IRX4*; myofilament protein genes including cardiac troponin T (*cTNT*), cardiac troponin I (*cTNI*), α -actinin (*ACTN2*), *MLC-2a*, and *MLC-2v*. The

cardiac genes showed no detectable expression in the two undifferentiated iPS-VSD cell lines. In contrast, by day 25, there was robust expression of the full range of cardiac genes in cardiomyocytes derived from EBs (DF01 and DF02), comparable to the level of expression observed in undifferentiated iPS cells, iPS01 and iPS02 (Figure 6C).

5. DISCUSSION

Factor-based reprogramming of somatic cells generates pluripotent stem cell lines that are effectively immortal in culture and can be differentiated into almost all cell types. They are therefore valuable for studying early developmental biology, cell-based therapy, and modeling of human diseases (9,10,21). Specifically, human iPS cells could be utilized to generate patient-specific lineages

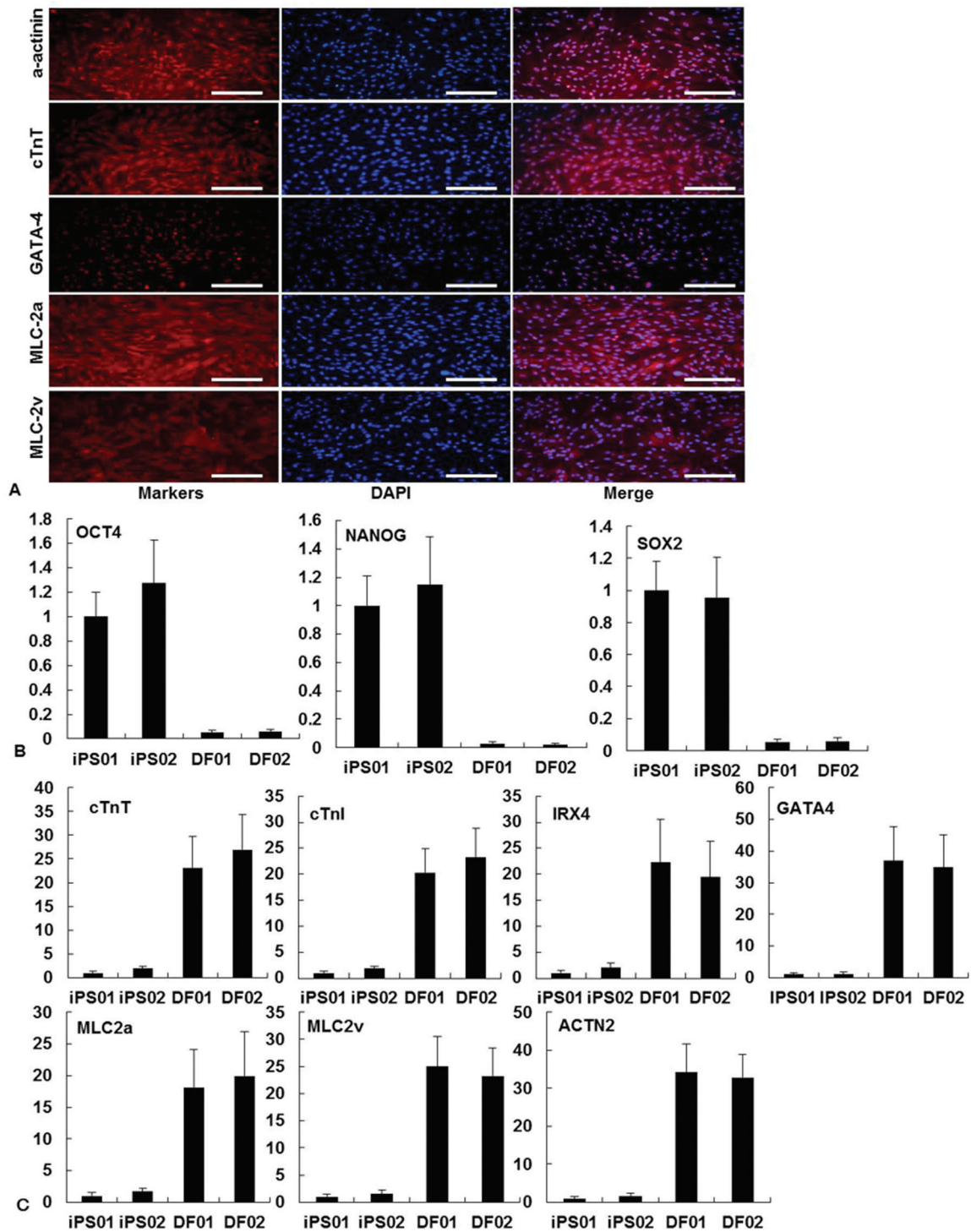


Figure 6. Expression of cardiac markers in iPS-VSD cell-derived cardiomyocytes. (A) Immunocytochemical analysis of myofilament and cardiac-specific proteins (α -actinin, cTnT, MLC-2a, MLC-2v and GATA4). Nuclei were counterstained with DAPI (blue). Scale bars: 200 μ m. (B and C) Quantitative RT-PCR analyses of the pluripotency (B) and cardiac-specific (C) gene expression in two of iPS-VSD cells and two of cardiomyocytes derived from them. The iPS-VSD cell-derived cardiomyocytes expressed cardiac transcripts, which were not detected in undifferentiated iPS cells. *GAPDH* was shown as a positive amplification and loading control.

for a variety of translational research programs (12). Such disease-specific stem cells offer an unprecedented

opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, and a new technology

platform for drug screening, thereby enabling disease investigation and drug development (13). Consistent with previously described iPS cell lines, the exogenous expression of only four factors, *OCT4*, *SOX2*, *NANOG*, and *LIN28*, was sufficient to reprogram human TECs to a pluripotent state in our study. Our results confirmed a previous report that TECs may have an improved ability to become multipotent keratinocyte stem cells following reprogramming (22). We also demonstrated that it is possible to produce patient-specific pluripotent stem cells. The use of defined reprogramming factors for the generation of patient-specific iPS cells has allowed us to study disease pathophysiology under controlled conditions *in vitro* in a manner specific to the genetic lesions, whether known or unknown.

Formation of the heart tube, looping, septation, and resultant systemic and pulmonary circulation is a complex process, and involves a multitude of genes, most of which are not cardiac specific. The course of fetal heart formation has been known for decades; however, until recently, the genes regulating its development were not known (23). Furthermore, it is known that disruption at any point during primary morphogenesis leads to a large spectrum of CHD being treated today (1, 24). VSD is the most common CHD in human infants. Understanding and investigating the mechanisms underlying VSD is challenging because of the genetic complexity and individual variability of VSD phenotypes. Many recent insights into the pathophysiology of VSD come from the study of familial forms of this disease. Studies of families with Holt-Oram syndrome indicate that a *TBX5* mutation causes atrial septal defects and VSD (1). Trisomy 21 (Down syndrome) in humans is commonly associated with incomplete septation of the atrioventricular valves, but the gene(s) on chromosome 21 responsible for valve development remains unknown (25,26). The genetics of ventricular septation are poorly understood, and in human populations, no single gene disorder primarily associated with isolated VSD exists. In the mouse model, a number of gene disruptions, such as *NFATc*, *FOG-2*, *endothelin-1*, *Tbx5*, *GATA4*, *BMP6*, and *BMP7*, have been shown to affect ventricular septation as part of a larger constellation of congenital cardiac abnormalities (27). Although murine models of human congenital and acquired diseases are invaluable, considerable differences exist between the human and mouse genomes, and there are many human-specific genetic modulators. Therefore, murine models are a limited representation of human pathophysiology (13,28).

Essential for modeling cardiac disease are reproducible differentiation protocols to obtain functional cardiomyocytes *in vitro*. Recent advances in pluripotent stem cell biology now make it possible to generate human cardiomyocytes *in vitro* from both healthy individuals and patients with cardiac abnormalities, which may provide new avenues for understanding cardiac

disease development and establish novel platforms for drug discovery to prevent disease progression or to reverse it (11,17,29). Here we describe the generation of iPS cells from a patient with VSD. The VSD-specific iPS cells produced here will be important tools for further studies of VSD pathogenesis and for elucidating the molecular mechanisms involved herein. Cardiac development is a very elaborate process, the successful completion of which requires precise operations. Purely stochastic events could also have an important role, which are likely to have deleterious consequences occasionally. Environmental factors such as teratogens, maternal infections, and untreated maternal metabolic illnesses have been associated with VSD (2, 3). iPS cell-derived cardiomyocytes generated from individuals with VSD would carry the precise constellation of genetic information associated with the pathology of that person. This approach would allow studies of cardiomyocytes generated from VSD in cases with unknown genetic lesions, providing insight into their interactions with other cell types, and their susceptibility to the environmental conditions that are considered to play an important role in VSD pathogenesis.

Human TECs from patients with VSD were successfully reprogrammed into iPS cells. The VSD-iPS retained the ability of self-renewal and pluripotent potential *in vitro* and *in vivo*. With the combination of human genetics and biochemical analyses, VSD-derived iPS cells may facilitate the identification of unrecognized genetic etiology for VSD and potential mechanisms through which certain cardiac defects may occur.

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Abbreviations: VSD: Ventricular septal defect; TECs-VSD: thymic epithelial cells of VSD patient; CHD: congenital heart disease; TECs: Thymic epithelial cells; DMEM: Dulbecco's modified Eagle medium; bFGF: basic fibroblast growth factor; HE staining: hematoxylin and eosin staining

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