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Modeling of Catecholaminergic Polymorphic Ventricular Tachycardia With Patient-Specific Human-Induced Pluripotent Stem Cells

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Objectives The goal of this study was to establish a patient-specific human-induced pluripotent stem cells (hiPSCs) model of catecholaminergic polymorphic ventricular tachycardia (CPVT).

Background CPVT is a familial arrhythmogenic syndrome characterized by abnormal calcium (Ca^{2+}) handling, ventricular arrhythmias, and sudden cardiac death.

Methods Dermal fibroblasts were obtained from a CPVT patient due to the M4109R heterozygous point *RYR2* mutation and reprogrammed to generate the CPVT-hiPSCs. The patient-specific hiPSCs were coaxed to differentiate into the cardiac lineage and compared with healthy control hiPSCs-derived cardiomyocytes (hiPSCs-CMs).

Results Intracellular electrophysiological recordings demonstrated the development of delayed afterdepolarizations in 69% of the CPVT-hiPSCs-CMs compared with 11% in healthy control cardiomyocytes. Adrenergic stimulation by isoproterenol (1 μM) or forskolin (5 μM) increased the frequency and magnitude of afterdepolarizations and also led to development of triggered activity in the CPVT-hiPSCs-CMs. In contrast, flecainide (10 μM) and thapsigargin (10 μM) eliminated all afterdepolarizations in these cells. The latter finding suggests an important role for internal Ca^{2+} stores in the pathogenesis of delayed afterdepolarizations. Laser-confocal Ca^{2+} imaging revealed significant whole-cell [Ca^{2+}] transient irregularities (frequent local and large-storage Ca^{2+}-release events, broad and double-humped transients, and triggered activity) in the CPVT cardiomyocytes that worsened with adrenergic stimulation and Ca^{2+} overload and improved with beta-blockers. Store-overload–induced Ca^{2+} release was also identified in the hiPSCs-CMs and the threshold for such events was significantly reduced in the CPVT cells.

Conclusions This study highlights the potential of hiPSCs for studying inherited arrhythmogenic syndromes, in general, and CPVT specifically. As such, it represents a promising paradigm to study disease mechanisms, optimize patient care, and aid in the development of new therapies. (J Am Coll Cardiol 2012;60:990–1000) © 2012 by the American College of Cardiology Foundation

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a familial arrhythmogenic disorder caused by unstable sarcoplasmic reticulum (SR) calcium (Ca^{2+}) storage leading to exercise- or emotion-induced ventricular tachyarrhythmias and sudden cardiac death (1,2). The majority of CPVT cases are associated with dominant mutations in the cardiac ryanodine receptor gene (*RyR2*) with variable penetrance (CPVT1) (3,4), whereas the minority of cases result from recessive mutations in the cardiac calsequestrin isoform 2 (*CASQ2*) gene (5). Investigation of the mutations giving rise to CPVT provided important insights into the mechanisms underlying this disease and to the processes governing cardiomyocyte Ca^{2+} handling in general (6). Nevertheless, among the hurdles in studying this disease process has been the lack of appropriate human cardiac tissue models and the inability to study patient-specific disease variations.

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The development of the groundbreaking human- induced pluripotent stem cells (hiPSCs) technology (7,8) may provide a possible solution to the aforementioned challenges. The iPSCs approach, pioneered by Takahashi and Yamanaka, allows the reprogramming of adult somatic cells into pluripotent stem cells by ectopic expression of a set of transcription factors (9). Subsequent studies showed the robust ability to generate hiPSCs and to coax their differentiation into cardiomyocytes (10,11). More recent proof-of-concept studies demonstrated the ability to establish patient- and disease-specific hiPSCs-derived cardiomyocytes (hiPSCs-CMs) that could recapitulate the abnormal cardiac phenotypes of Leopard and long-QT syndromes (12–14). In the current report, our goal was to establish an hiPSCs model of CPVT. We hypothesized that the generated CPVT-hiPSCs-CMs will recapitulate the disease phenotype in vitro, providing insights into disease mechanisms and offering a unique platform to evaluate patient-specific therapies.

Abbreviations and Acronyms

AP = action potential
CASQ2 = cardiac calsequestrin isoform 2
CPVT = catecholaminergic polymorphic ventricular tachycardia
DADs = delayed afterdepolarizations
EB = embryoid body
hiPSC = human-induced pluripotent stem cell
hiPSCs-CMs = human-induced pluripotent stem cells–derived cardiomyocytes
PCR = polymerase chain reaction
RT-PCR = real-time polymerase chain reaction
RyR2 = ryanodine receptor isoform 2
SOICR = store-overload–induced Ca^{2+} release
SR = sarcoplasmic reticulum

Methods

hiPSCs generation and cardiomyocyte differentiation. The patient-specific hiPSCs clones were generated by retroviral reprogramming of dermal fibroblasts with *Oct-4*, *Sox-2,* and *Klf-4* followed by valproic acid treatment as previously described (13,15). Cardiomyocyte differentiation was induced using the embryoid body (EB) differentiating system. Briefly, undifferentiated hiPSCs were removed from the mouse embryonic fibroblast feeder-layer, dispersed into cell-clumps using collagenase type IV (300 U/ml [Life Technologies Corporation, Grand Island, New York]), cultivated in suspension for 10 days as EBs, and plated on gelatin-coated culture dishes (11).

Immunostainings. Specimens were fixed with 4% paraformaldehyde, permeabilized with 1% Triton, blocked with 5% horse serum, and incubated with primary antibodies targeting: Oct4, Tra1-60 (Santa-Cruz Biotechnology, Santa Cruz, California), Nanog (Peprotech, Rehovot, Israel), SSEA-4 (R&D Systems, Minneapolis, Minnesota), troponin I, ryanodine receptor (Chemicon, Merck Millipore, Billerica, Massachusetts), and sarcomeric alpha actinin (Sigma-Aldrich, St. Louis, Missouri). The preparations were incubated with secondary antibodies and examined using a laser-scanning confocal microscope (Zeiss LSM-510-PASCAL). Alkaline phosphatase staining of hiPSCs colonies was performed using a Sigma detection kit.

Teratoma formation. Undifferentiated hiPSCs were dissociated with collagenase-IV and injected subcutaneously into SCID/beige mice. Tumor samples were collected at 6 to 8 weeks, fixed in 4%-formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Genomic sequencing. Genomic DNA was isolated by using the high-pure polymerase chain reaction (PCR) template preparation kit (Roche, Basel, Switzerland). The relevant DNA fragment of the *RYR2* gene was amplified by PCR using 100-ng genomic DNA. Primers are detailed in Online Table 1. PCR products were then sequenced.

Gene expression analysis. RNA was isolated by using the RNeasy Plus Mini Kit (Qiagen Inc., Valencia, California). Reverse transcription into cDNA was conducted with the high-capacity cDNA reverse-transcription kit (Applied Biosystems). Real-time polymerase chain reaction (RT-PCR) studies were performed by using the DreamTaq Master Mix (Ferments Molecular Biology Tools, Thermo Fisher Scientific, Waltham, Massachusetts). Primers are listed in Online Table 1. Each RT-PCR included 2 min at 93°C followed by 27 to 35 cycles of 30 s at 93°C, 60 s at 60°C, and 40 s at 72°C.

RT-PCR studies were conducted in triplicate by using the Fast SYBR Green Master Mix (Applied Biosystems, Life Technologies Corporation). Primers are detailed in Online Table 2. Samples were cycled by using the Fast ABI-7500 sequence detector. Conditions were as follows: 20 s at 95°C followed by 40 cycles of 3 s at 95°C and 30 s at 60°C. Cycle threshold was calculated by using default settings for the real-time sequence detection software (Applied Biosystems).

Whole-cell patch-clamp recordings. Contracting EBs were enzymatically dispersed (1 mg/ml collagenase B [Roche]) and attached to fibronectin-coated glass coverslips. Action potentials (APs) were recorded from spontaneously contracting and quiescent hiPSCs-CMs at 32°C under spontaneous and paced (1 Hz) rhythms. The tyrode solution included (in mmol/l: NaCl 140; KCl 4; $CaCl_2$ 2; $MgCl_2$ 1; HEPES 10; and glucose 5 (pH 7.4; NaOH). The pipette solution contained (in mmol/l): K-aspartate 120; KCl 20; $MgCl_2$ 1; Na_2ATP 4; GTP 0.1; HEPES 10; and glucose 10 (pH 7.2; KOH). APs were recorded in the current-clamp mode with Axopatch 200B, Digidata 1322A, and pClamp 9 (Axon Instruments, Sunnyvale, California).

Microelectrodearray recordings. Microelectrodearray recordings (Multichannels Systems, Reutlingen, Germany) were performed as previously described (11,16). Local activation times were determined at each electrode and used for generation of color-coded activation maps.

Ca^{2+} imaging. Cells were loaded with 5μM fluo-4 fluorescent Ca^{2+} indicator (Molecular Probes, Life Technologies Corporation) to allow recordings of whole-cell $[Ca^{2+}]_i$ transients as previously reported (15). Experiments were