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CONTEMPORARY REVIEW

Brugada Syndrome: Different Experimental Models and the Role of Human Cardiomyocytes From Induced Pluripotent Stem Cells

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ABSTRACT: Brugada syndrome (BrS) is an inherited and rare cardiac arrhythmogenic disease associated with an increased risk of ventricular fibrillation and sudden cardiac death. Different genes have been linked to BrS. The majority of mutations are located in the *SCN5A* gene, and the typical abnormal ECG is an elevation of the ST segment in the right precordial leads V1 to V3. The pathophysiological mechanisms of BrS were studied in different models, including animal models, heterologous expression systems, and human-induced pluripotent stem cell–derived cardiomyocyte models. Currently, only a few BrS studies have used human-induced pluripotent stem cell–derived cardiomyocytes, most of which have focused on genotype–phenotype correlations and drug screening. The combination of new technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR associated protein 9)-mediated genome editing and 3-dimensional engineered heart tissues, has provided novel insights into the pathophysiological mechanisms of the disease and could offer opportunities to improve the diagnosis and treatment of patients with BrS. This review aimed to compare different models of BrS for a better understanding of the roles of human-induced pluripotent stem cell–derived cardiomyocytes in current BrS research and personalized medicine at a later stage.

Key Words: Brugada syndrome ■ human-induced pluripotent stem cell-derived cardiomyocytes ■ model systems ■ precision medicine

Brugada syndrome (BrS) is an inherited arrhythmic disorder that is characterized by an increased risk of sudden cardiac death. It was first described in 1992 by research on 8 patients who were resuscitated from sudden cardiac death because of ventricular fibrillation and/or suffered from atrial fibrillation.[1] Patients can have symptoms of nocturnal breathing, syncope, seizures, and in worse cases ventricular fibrillation.[2] Fever can increase the risk of ventricular fibrillation[3]. In a 2012 expert consensus report, 2 types of ECG patterns of BrS were described, type 1 (coved type) and type 2 (saddleback type).[4] However, only the type 1 ECG pattern is regarded as the diagnostic criterion for BrS, and type 2 is only suggestive of BrS. The typical type 1 ECG pattern of BrS is an ST-segment elevation ≥2 mm in 1 or more leads among right precordial leads V1 to V3[2] (Figure 1A). To date, the definition of diagnosis for BrS is mainly based on its typical ECG changes or the application of different sodium channel blockers as described in 2015 by the European Society of Cardiology guidelines.[5] Although the incidence of BrS appears to be low, only 0.12% to 0.8%, it is responsible for 4% to 12% of sudden cardiac deaths in the general population at a young age. According to a recent study, men are more likely to suffer from BrS than women.[6]

Approximately 11% to 28% of patients with BrS were found to be carriers of genetic mutations, the majority of which are located in the *SCN5A* gene.[7] This gene encodes the α subunit of the cardiac sodium channel Nav1.5, which plays a significant role in phase 0 of the action potential in cardiomyocytes. In addition,

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Nonstandard Abbreviations and Acronyms

BrS	Brugada syndrome
HEK	human embryonic kidney
hiPSC-CMs	human-induced pluripotent stem cell–derived cardiomyocytes
I_{Na}	sodium current
I_{to}	transient outward potassium current

>50 genes that were also reported to be associated with BrS were responsible for 2% to 5% of diagnosed cases[8] (Figure 1B). However, a recent study indicated that most of the putative genes associated with BrS had no sufficient evidence for causality in BrS, which indicated that BrS is likely polygenic rather than being influenced by mutations of a single gene.[9] To date, thousands of mutations have been reported to be related or possibly related to BrS, but only a small proportion of them have been experimentally investigated.

Most pathogenic or likely pathogenic variants were detected in the *SCN5A* gene, such as E1784K, p.S1812X, and p.Val1429Met.[10-12] In other rare genes, few pathogenic and benign variants as well as variants with unknown significance were identified.[13] In the study by Chen,[14] *SCN5A* accounted for 87.5% of pathogenic and likely pathogenic variants, whereas others accounted for only 12.5%. Despite advances in understanding the genotype association with the clinical phenotype, there is a reappraisal of reported genes with an evidence-based evaluation of gene validity for BrS.

Although loss of function of the Nav1.5 channel in BrS is not generally controversial, 2 main hypotheses about the pathophysiological mechanism of BrS are disputed. The repolarization hypothesis indicated that the stronger expression of transient outward potassium current (I_{to}) in the epicardium led to the transmural dispersion of repolarization in the right ventricle, which could cause ST-segment elevation in BrS[15] (Figure 1C); the depolarization hypothesis suggested that a right ventricular outflow tract conduction delay caused an intercellular current from the right ventricle to right ventricular outflow tract and a back current from the right ventricular outflow tract to the right ventricle, which caused ST-segment elevation in right precordial leads in BrS[16] (Figure 1D). In recent years, conventional ECG analysis has been commonly applied to explore the features and mechanisms of BrS, but it could provide limited information for researchers. Because of the development of next-generation sequencing, the identification of pathogenic genes of BrS has also become possible and is performed in clinical practice. However, these technologies are not able to study the detailed molecular mechanisms of the syndrome in vivo. Therefore, experimental models are commonly used in BrS studies. Although the human heart is the best choice for building models, animal and cellular models have been chosen by most researchers because of the difficulty in obtaining human heart tissues and ethical limitations. Several models have been used to simulate the disease, including transgenic mice and porcine, canine, and rabbit heart preparations, and various cellular models with expression of mutant *SCN5A* and human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs)[17] (Table 1). Another difficulty is the current technological inability to study the detailed impulse propagation mechanisms of the syndrome in vivo. In this regard, mapping analysis could be helpful. In the study by Calvo et al,[18] the 3-dimensional (3D) electroanatomic mapping system, which can create 3D endocardial reconstructions, were used to study the mechanism of ventricular tachycardia and early ventricular fibrillation in patients with BrS. 3D mapping showed that *SCN5A*-positive patients with BrS exhibited a larger epicardial arrhythmogenic substrate area. In addition, more prolonged electrograms and a higher frequency of noninvasive late potentials were detected. The analysis showed a predictable sequence in the frequency-phase domain, which is consistent with the anatomic location of the arrhythmogenic substrate.[19]

Compared with other models, hiPSC-CMs from patients who carry the desired genetic profile have unique advantages. These are relatively easy to obtain from somatic cells, including peripheral blood mononuclear cells or fibroblasts of patients.[20] Although animal models can allow integral studies from whole animals, organs, and tissues, there are some fundamental differences in physiology between humans and animals. Patient-specific hiPSC-CMs carry the exact genetic background of patients. Recent studies have indicated that hiPSC-CMs can effectively recapitulate the electrophysiological pattern in several cardiac disorders, including BrS,[21,22] long-QT syndrome,[23] arrhythmogenic right ventricular cardiomyopathy,[24] catecholaminergic polymorphic ventricular tachycardia,[25] Timothy syndrome,[26] short-QT syndrome,[27] dilated cardiomyopathy,[28] and Noonan syndrome,[29] at the cellular level. Other cellular models of BrS, such as human embryonic kidney (HEK) cells or *Xenopus* oocytes, can easily explore the impact of mutations on ion channels, but these cells lack many cardiac proteins. Previous studies[30,31] suggested that hiPSC-CMs possess basic features of human cardiomyocytes and express a variety of cardiac-specific genes, including those that can encode ion channels in cardiomyocytes.[32] This circumstance makes it possible for investigators to use hiPSC-CMs combined with other techniques to recapitulate disease phenotypes in vitro and conduct various studies to explore the underlying mechanism of the disease or new therapeutic strategies (Figure 2).

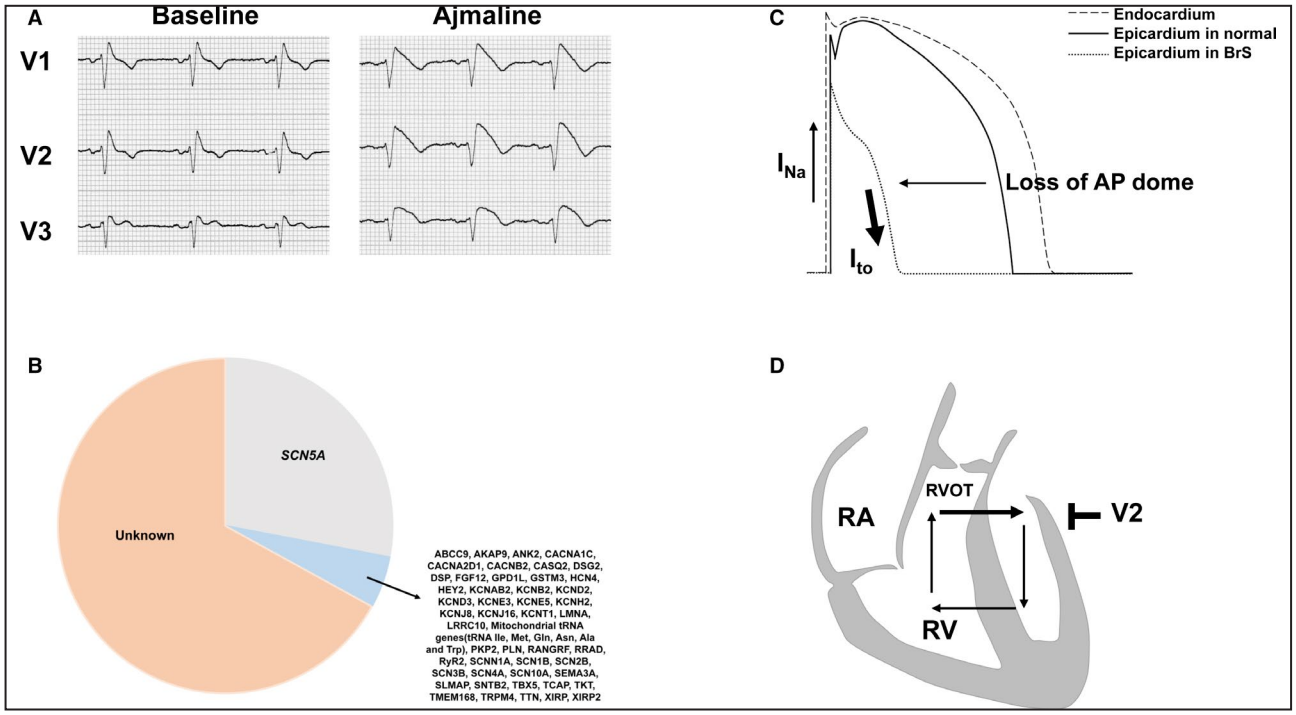

Figure 1. Main characteristics and possible mechanism of Brugada syndrome (BrS).
A, The Ajmaline test unmasked the typical cover-type ST-segment elevation in a BrS patient. **B**, All reported genes related to Brugada syndrome. **C**, Repolarization hypothesis of the BrS. **D**, Depolarization hypothesis of the BrS. I_{Na} indicates sodium current; I_{to}, transient outward potassium current; RA, right atrium; RV, right ventricle; and RVOT, right ventricular outflow tract.

This review aimed to summarize studies that use different BrS models and focuses on the application of hiPSC-CMs in BrS studies.

EXPERIMENTAL MODELS OF BRS

Animal Models

Several animal models have often been used for BrS research, including murine models, porcine models, canine models, and rabbit models. Each model has its own unique advantages and limitations. The murine model has been commonly used because mice are easier to feed than other animals, which can allow researchers to study BrS in its physiological environment and different developmental stages. However, there are significant differences in the electrophysiology and profile of ion channel expression between the human heart and murine heart. Therefore, this type of model is not perfect for BrS studies. Alternatively, porcine, canine, and rabbit models show electrophysiological properties and profiles of ion channel expression similar to those of the human heart, and these models allow investigation of cells in the epicardium and endocardium with intact structural organization in the heart. Nevertheless, they still have their own limitations. Porcine and canine animals are expensive and have long reproductive cycles. The canine and rabbit models of BrS were induced by ion channel blocking agents,[33,34] which could be different from the disease caused by mutations in ion channel genes.

Intact Animal Models

Two methods are commonly used in murine models of BrS: *Scn5a*$^{+/-}$ mice[35] and *Scn5a*1798insD mice.[36] In 2002, Papadatos and colleagues generated a mouse model by targeted disruption of the cardiac sodium channel gene *Scn5a*. Homozygous knockout mice showed severe defects in ventricular morphogenesis and eventually died. However, heterozygous knockout mice survived and exhibited similar cardiac morphology compared with the wild type. Furthermore, whole-cell patch clamp analyses of ventricular myocytes showed an ≈50% reduction in sodium conductance and impaired conduction compared with the wild type.[35] Subsequent studies used this model to investigate the drug response and underlying mechanisms of the phenotypes observed before. Several studies recapitulated the effect of pro- and antiarrhythmic drugs, including flecainide and quinidine, in a *Scn5a*$^{+/-}$ mouse model.[37,38] Nav1.5 messenger RNA and protein expression levels in the mouse model were found to be lower than those in the wild type, which was further related to the presence of fibrosis.[39,40] In 2006, Remme and colleagues generated a transgenic model of mice containing the

Table 1. Experimental Models for BrS

Model	Methods	Advantages	Disadvantages
Animal	*Scn5a*+/– mouse; *Scn5a*(1798insD/+) mouse	Easier to feed than other animals Allows investigators to study BrS in physiological environment and different developmental stages	Different electrophysiological properties and cardiac markers between human and mouse heart Hard to evaluate the typical ECG pattern of BrS in the model
	Canine	Similar electrophysiology properties and profile of ion channel expression compared with the human heart Allows investigation in epicardium and endocardium with intact structural organization in the heart	Transgenic model is difficult to obtain and drug could be required for inducing the phenotype Not easier to feed and relatively expensive
	Porcine	Similar electrophysiology properties compared with the human heart Allows investigation in epicardium and endocardium with intact structural organization in the heart	High price and long reproductive cycles of cells Transgenic model is difficult to obtain and a drug may be required for inducing the phenotype Did not display the typical ECG pattern of BrS
	Rabbit	Similar electrophysiology properties and profile of ion channel expression compared with the human heart Allows investigation in epicardium and endocardium with intact structural organization in the heart	Transgenic model is difficult to obtain and a drug could be required for inducing the phenotype
Heterologous expression	HEK-293 cells; *Xenopus* oocytes; tsA201 cells; CHO cells	Easier to feed than animal models Allows investigation by more detailed experiments in cellular mechanism of the disease	Lack of cardiac markers and electrophysiological properties of cardiomyocytes Results will be various because of the different cell lines
Native human cardiomyocytes	Native human cardiomyocytes from right atrial tissue	Carries patient's exact genetic background, cardiac markers and electrophysiological properties of cardiomyocytes	Difficult to obtain cardiomyocytes from ventricular tissue Ethical limitations
hiPSC-CMs	hiPSCs derived from human somatic cells	Carries patient's exact genetic background; expression profile is similar to cardiomyocytes Relatively easier to feed and combine with other advanced technologies	Immature phenotype of cells Composed of a mixture of ventricular-, atrial-, and nodal-like cells Hard to conduct studies in a physiological environment

BrS indicates Brugada syndrome; CHO cells, Chinese hamster ovary; HEK, human embryonic kidney; hiPSCs, human-induced pluripotent stem cells; and hiPSC-CMs, human-induced pluripotent stem cell–derived cardiomyocytes.

equivalent mutation 1798insD.[36] *Scn5a*(1798insD/+) heterozygous mice showed lower heart rates and abnormal ECG patterns than wild-type mice. Whole-cell patch-clamp analysis exhibited action potential prolongation, a ≈39% reduction in peak sodium current density, and a reduction in action potential upstroke velocity. This mutation could cause common symptoms of long-QT syndrome type 3 or BrS or progressive cardiac conduction defects.[41] A recent study showed that conduction defects were related to the strain in a *Scn5a*(1795insD/+) mouse model.[42] However, the 12-lead ECG morphology in mice is quite different from humans, so it is hard to evaluate whether the typical ECG pattern of BrS was present in the mouse model.

In 2015, Park and collaborators[43] generated a porcine model by introducing a nonsense mutation into the orthologous position (E558X) in the pig *SCN5A* gene. *SCN5A*(E558X/+) pigs displayed conduction abnormalities with no structural abnormalities. However, the porcine model did not display a BrS-type ECG. Although the electrophysiology of porcine hearts is similar to that of humans, few studies of BrS use this model because of its high price.

In addition, although the majority of genetic mutations of BrS are located in the *SCN5A* gene, other factors or genetic mutations can also cause BrS. These animal models contained only *SCN5A* mutations and only displayed a part of BrS's features, mainly the loss of function of sodium current and conduction slowing, rather than the characteristic ECG pattern of BrS. This may reflect the difference between humans and animals on cardiac structure and electrophysiology, and also an important limitation of animal models.

Tissue Models

The canine model first used for BrS was in the study conducted by Yan and Antzelevitch in 1996, which explored the underlying mechanism of the J wave.[44] In the canine model, arterially perfused wedges in the left or right ventricles of dogs and drugs were used to induce the BrS phenotype. Several drugs were used for the canine model, including sodium channel blockers, calcium channel blockers, and potassium channel agonists.[45-47] ST-segment elevation in drug-induced BrS in a canine model was caused by a transmural gradient across the right ventricular wall, which was generated by loss of the epicardial action potential dome.[44] Further studies showed that ventricular repolarization abnormalities that were located in the right ventricular outflow tract could lead to ST-segment elevation.[48] In a recent study, radiofrequency

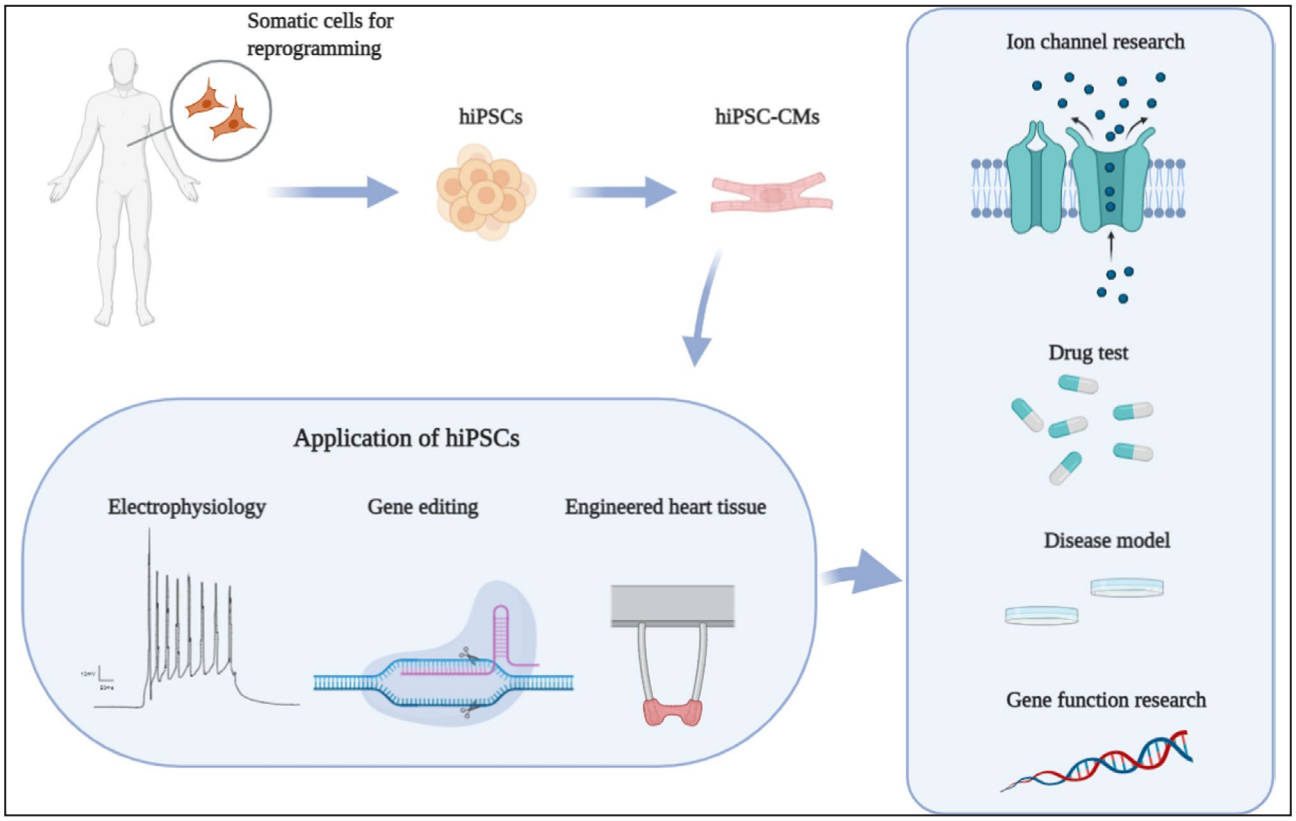

Figure 2. Applications of human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) in disease research by using different technologies.
hiPSCs were generated by somatic reprogramming from Brugada syndrome patients and then were differentiated into patient-specific hiPSC-CMs. hiPSC-CMs combined with other advanced technologies have been used for disease modeling of inherited cardiomyopathies and channelopathies, drug testing, and studying gene function. Created with http://BioRender.com/.

catheter ablation technology was used in a canine model of BrS, and the results showed that radiofrequency catheter ablation could eliminate abnormal repolarization sites and the substrate for ventricular tachyarrhythmias.[49] Chen and colleagues[34] generated a rabbit model for J-wave syndrome by using cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine, which decreased sodium current (I_{Na}) and action potential upstroke as well as intracellular calcium transients. At the same time, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine triggered a significant J-wave elevation and frequent spontaneous ventricular fibrillation.

Heterologous Expression Systems

Transfecting plasmids with mutant genes identified in patients with BrS into HEK cells, SV40 transformed (tsA201), or Chinese hamster ovary cells or other cells could help to understand the underlying molecular mechanisms and explore the biophysical properties of associated mutations in BrS. This type of model is relatively easy to use compared with animal models, and it is more suitable for studying the effect of mutations on ion channels. Casini and colleagues[50] used HEK-293 cells to study the effect of a missense *SCN5A* mutation called G1319V and found that the G1319V mutation could decrease voltage-dependent activation and channel availability and hence reduce the peak amplitude of I_{Na}. In a recent study, HEK-293 cells were also used to identify the relationship between BrS and the *SCN1B* variant A197V. The results showed that this mutation could cause a reduction in the activation velocity and current density.[51] In addition, *Xenopus laevis* oocytes are also commonly used for heterologous expression of ion channels. In a recent study, *SCN5A* variants of 13 families were functionally characterized in *Xenopus laevis* oocytes by the 2-electrode voltage-clamp technique. The results showed that patients carrying variants S216L, K480N, A572D, F816Y, and G983D were more likely to develop long-QT syndrome, and variants R222stop and R2012H were new mutations for BrS genetic causes.[52] In the study by Watanabe and colleagues,[53] Chinese hamster ovaries or tsA201 cells with the D1275N *SCN5A* mutation did not show a significant difference in sodium current amplitudes and gating, whereas the mouse model with the same variant displayed sodium channel dysfunction.

Although expression systems are better methods to investigate the molecular mechanism of associated mutations in BrS than animal models, the results of these experiments could differ because of the variety of cell types. In a study by Dumaine et al, transfection of the Thr1620Met mutation into a mammalian cell line showed that Thr1620Met current decay kinetics were faster, and that the recovery from inactivation was slower than that of the wild type at 32 °C, whereas the electrophysiological data failed to explain the electrocardiographic phenotype when the mutant was heterologously expressed in frog oocytes.[54] A novel mutation T1620M in the *SCN5A* gene also displayed different phenotypes when expressed in *Xenopus* oocytes and mammalian tsA201 cells.[55] The mutation led to a faster recovery from inactivation and a shift of steady-state inactivation to more positive voltages when expressed in *Xenopus* oocytes, whereas it showed a slower recovery from inactivation and no effect on steady-state inactivation when expressed in tsA201 cells. Although all of the cell models express sodium channels, the difference in Nav1.5 morphology and function among these models could cause the discrepant results. Nevertheless, heterologous expression systems are still suitable for investigating the effects of individual mutations.

Native Human Cardiomyocytes

A recent study investigated the functional roles of a mutation in *SNTB2* (β-2-syntrophin) (*SNTB2*-N167K) in native cardiomyocytes obtained from right atrial tissue of a patient with BrS.[17] Cardiomyocytes with the *SNTB2*-N167K mutation showed reduced peak I_{Na} and late I_{Na} and shortened action potential duration. The results were consistent with findings in the coexpression of Nav1.5 with WT *SNTB2* and *SNTB2*-N167K or with *SNTB2*-N167K alone in *Xenopus* oocytes, which showed a significant reduction in I_{Na} in both homozygous and heterozygous conditions. Although cardiomyocytes were obtained from atrial tissue because of the difficulty of obtaining them from ventricular tissue, these results can be extrapolated to ventricular electrophysiology because of the similar electrophysiological properties between atrial and ventricular tissues.

hiPSC-CM Model

Human-induced Pluripotent Stem Cell–Derived Cardiomyocytes

Embryonic stem cells are a type of cells that have properties of self-renewal and pluripotency. They were first shown in the study by Evans and Kaufman[56] in 1981 and were derived from the inner cell mass of murine blastocysts. Human embryonic stem cells were successfully derived from human blastocysts in 1998.[57] Although embryonic stem cells have a unique advantage for studying cell fate and tissue development, destroying early-stage embryos in the process of embryonic stem cell preparation makes this technology ethically controversial. Yamanaka's research group improved the situation by the production of induced pluripotent stem cells. They found that somatic cells from mice[58] and humans[59] could be induced to a pluripotent state through the introduction of 4 reprogramming factors (Octamer-binding transcription factor 4, Sex-determining region Y-box 2, Kruppel-like factor-4, and Cellular-myelocytomatosis viral oncogene). The induced cells could also maintain the properties of unlimited self-renewal and pluripotency characteristics, and they avoid the ethical issues caused by embryo destruction. Currently, induced pluripotent stem cells that can differentiate into specific cell types are commonly used in extensive studies with different differentiation methods. Induced pluripotent stem cells have considerable advantages in disease modeling and personalized medicine. To date, clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing technologies combined with induced pluripotent stem cells can create patient-specific isogenic controls.[60] Using this method, a raised limitation of comparing diseased hiPSC-CMs with independent healthy cells with their individual variability could be overcome.

In previous studies, several signaling pathways, such as bone morphogenetic protein (BMP), wingless/integrated (Wnt) and Activin/Nodal/transforming growth factor-β (TGF-β), have been indicated to play important roles in cardiovascular development.[61-63] Protocols of hiPSC-CM differentiation aim to simulate these developmental signaling cues and generate cardiomyocytes that are highly similar to cells in the human heart. Therefore, different methods for hiPSC-CM differentiation have been developed. Initially, hiPSC-CMs were obtained from embryoid bodies with serum-containing media, but the differentiation efficiency of this method was low, only 5% to 10%.[64] To date, several endogenous growth factors and small molecules of related signaling pathways have been used in different hiPSC-CM differentiation protocols to improve reproducibility and efficiency.[30] By various methods for purification of cardiomyocytes, hiPSC-CMs could be further purified, and more beating cardiomyocytes expressing human cardiac-specific proteins could be obtained.[65-67] The expression of specific genes in several stages of normal cardiac development was also detected in hiPSC-CMs, including genes for mesoderm formation (*BRY* and *MIXL1*), cardiogenic mesoderm (*MESP1* and *ISL1*), cardiac-specific progenitors (*NKX2.5*, *GATA4*, and *TBX5*), and mature cardiomyocyte structure (*MYL2*, *MYH7*, and *TNNT2*).[68]

Recently, atrial-specific differentiation has been reported after recently published techniques for the

differentiation of human-induced pluripotent stem cells into hiPSC-CMs generated a mixture of cell types with a higher presence of ventricular cardiomyocytes.[69,70] Fluorescent activated cell sorting could be a useful method to distinguish subtype-specific hiPSC-CM populations by specific markers, but the cells would be exposed to additional influencing factors that could affect their functions. Previous studies showed that pluripotent stem cells could be directed into atrial-like or ventricular-like cardiomyocytes by modulating retinoic acid and Wnt signaling pathways.[71,72] In more recent years, Liu and colleagues directed hiPSC-CMs into sinoatrial node-like cells by combined modulation of the BMP, fibroblast growth factor, and retinoic acid signaling pathways.[73] In the study by Cyganek,[74] a protocol that can direct hiPSC-CMs into either atrial-like or ventricular-like cardiomyocytes was provided. These methods will be useful in further BrS studies to explore the underlying mechanisms of the disease by the hiPSC-CM model and to improve our understanding of lineage-specific development or chamber-specific remodeling of some heart diseases.

hiPSC-CM Model for Precision Medicine

Use of hiPSC-CMs for Studying the Pathogenicity of Gene Variants

Precision medicine is an emerging approach for disease treatment and prevention that accounts for individual variability in genes, environment, and lifestyle for each person.[75] In this regard, there is no doubt that hiPSCs can provide a good model for studying BrS. The key point for studying BrS by hiPSC-CMs with respect to precision medicine is to identify personal-specific mutations and their effect on the phenotypes of diseases. The main accomplishments of using hiPSC-CMs in the BrS study are shown in Table 2.[11,21,22,31,76,77,78,79] To date, hundreds of mutations in *SCN5A* have been reported, including missense mutations, nonsense mutations, nucleotide insertion/deletions, and splice site mutations, which can cause loss-of-function in sodium channels by different mechanisms, such as reduced expression of sodium channel proteins, expression of channels with no function, and changed gating properties of sodium channels.[80] tion in hiPSC-CMs was conducted by Davis and collaborators in 2012.[41] They used both hiPSC-CMs derived from *SCN5A*(1795insD/+) and *Scn5a*(1798insD/+) heterozygous mice to investigate the electrophysiological properties of sodium channel mutations, and the results demonstrated that hiPSC-CMs are suitable for use as models of cardiac sodium channel disease. In 2014, Cerrone et al showed that missense mutations in plakophilin-2 (*PKP2*) could cause sodium current deficits, including decreased I_{Na} and Nav1.5 at the site of cell contact, and the deficit could be restored by transfection of wild-type *PKP2*, which indicated that *PKP2* mutations could be a molecular substrate of BrS.[78] Later, Liang and colleagues conducted another study of the hiPSC-CM model for BrS.[31] Two patients with BrS carrying 2 variants in the *SCN5A* gene were recruited, and patient-specific hiPSC-CMs showed a reduction in I_{Na} and maximal upstroke velocity (Vmax) of the action potential compared with healthy control hiPSC-CMs. Increased triggered activity and abnormal Ca^{2+} handling were also observed in patient-derived hiPSC-CMs. Furthermore, genomic correction of the causative variant in hiPSC-CMs from 1 patient with BrS by CRISPR/Cas9 (CRISPR associated protein 9)-mediated genome editing technology showed a rescue of triggered activity and a reversion of abnormal Ca^{2+} transients.

In addition to *SCN5A*, other genes that encoded subunits of the sodium channel have also been reported to be associated with sodium channel dysfunction in BrS. For example, 3 genes (*SCN1B*, *SCN2B*, and *SCN3B*) that encoded the β subunits of sodium channels, which can modify the channel function, have been discovered to cause abnormal electrical features, as in BrS. Recently, hiPSC-CM models of BrS with double variants in *SCN1B* (c.629T > C and c.637C > A) and *SCN10A* (c.3803G>A and c.3749G>A) were established by our research group.[21,22] Cardiomyocytes from induced pluripotent stem cell from patients with BrS showed a significantly reduced peak I_{Na} and late I_{Na}. The action potential amplitude and Vmax were also reduced, which was consistent with the reduced peak I_{Na}.

The loss of function of sodium channels can cause conduction defects, which are important phenotypic features in BrS. To date, many studies have displayed conduction slowing in several models with a reduction in I_{Na}. In a study of BrS using the *Scn5a*(1798insD/+) mouse model, the hearts of mice showed prolonged atrioventricular conduction[81] and lower expression of genes that encoded proteins for conduction in the right ventricular outflow tract during cardiac development.[82] Pigs with mutations in *SCN5A* showed similar results, and HEK-293 cells transfected with the *SCN5A* gene mutation (p.1493delK) recapitulated the loss of function of sodium channels.[43,83] Several studies of the hiPSC-CM model for BrS showed that mutations in genes associated with sodium channels could reduce Vmax and peak I_{Na} amplitude with prolonged action potentials.[11,79] However, the conduction-slowing effect of mutated sodium channels has not been investigated in hiPSC-CMs.

BrS-associated genes were also found in calcium channels and potassium channels. The calcium channel is composed of the α su84 bunit and accessory proteins. Mutations in α1 (*CACNA1C*), β2 (*CACNB2*), and α2/δ (*CACNA2D1*) subunit genes of cardiac

Table 2. Current Accomplishments of Using hiPSC-CMs in BrS

Mechanism study			Drug testing	
Mutations	**Variants**	**Major findings**	**Drugs**	**Major findings**
SCN5A	p.S1812X[11]	Reduction of I_{Na} density and NaV1.5 expression, impaired localization of NaV1.5 and connexin 43 at the cell surface, reduced action potential upstroke velocity and conduction slowing in BrS-CMs	Carbachol[21]	Increasing arrhythmia events and the beating frequency in BrS
	p.R620H & p.R811H[31]	BrS-CMs displayed reductions in I_{Na} density, reduced maximal upstroke velocity of action potential, increased burden of triggered activity, abnormal calcium transients and beating interval variation		
	p.A226V & p.R1629X[76]	BrS-CMs displayed reductions in I_{Na} density, and reduced maximal upstroke velocity and amplitude of action potential		
SCN1B	p.L210P & p.P213T[21]	BrS-CMs displayed significantly reduced peak and late sodium channel current as well as reduced amplitude and upstroke velocity of action potentials	Ajmaline[11,21,77]	Reduced amplitude and Vmax of action potential; blocking effect on both depolarization and repolarization in hiPSC-CMs of BrS and control
SCN10A	p.Arg1250Gln & p.Arg1268Gln[22]	BrS-CMs displayed significantly reduced I_{Na} as well as reduced amplitude and upstroke velocity of action potentials	Milrinone and cilostazol[11]	Inhibited I_{to} and alleviated the arrhythmic activity in BrS
PKP2	p.D26N[78]	Loss of PKP2 caused decreased I_{Na} and NaV1.5 at the site of cell contact		
RRAD	p.R211H[79]	BrS-CMs displayed reduced action potential upstroke velocity, prolonged action potentials and increased incidence of early after depolarizations, with decreased Na+ peak current amplitude and increased Na+ persistent current amplitude as well as abnormal distribution of actin and fewer focal adhesions		

BrS indicates Brugada syndrome; BrS-CMs, cardiomyocytes form Brugada syndrome-derived human-induced pluripotent stem cell; hiPSC-CMs, human-induced pluripotent stem cell–derived cardiomyocytes; I_{Na}, sodium current; I_{to}, transient outward potassium current; PKP2, plakophilin-2; and Vmax, maximal upstroke velocity.

long-lasting (L)-type channels can cause shortened action potential duration (representing shorter QT intervals) in patients with BrS[84,85]. A previous study showed that Chinese hamster ovary K1 Chinese hamster ovary K1 cells transfected with mutations of these genes could cause a reduction in Calcium current.[86] Because mutations in calcium channels commonly occur in patients with overlapping phenotypes of BrS and short-QT syndrome, and it is rare to see patients with BrS without a short QT interval, functional analysis of mutant L-type calcium channels is limited. Gain-of-function mutations in genes encoding potassium channels have also been reported in a few BrS studies. Mutations in *KCND3* and *KCNE3* identified in patients with BrS increased transient outward potassium current and loss of the action potential dome.[87,88] Genes that encoded adenosine triphosphate-sensitive potassium channels, including *KCNJ8* and *ABCC9*, also showed gain-of-function mutations in patients with BrS.[89,90] However, these mutations have not been investigated in the hiPSC-CM model. It is meaningful to explore the effect of these mutations on the electrophysiological properties of BrS with an hiPSC-CM model in the future.

Use of hiPSC-CMs for Drug Screening

To date, limited numbers of drugs, including quinidine,[91] disopyramide,[92] β-adrenergic agonists,[93,94] phosphodiesterase inhibitors,[95] and bepridil,[96] have been studied for pharmacological therapy of BrS[97]. Some of them exhibited beneficial effects in preventing arrhythmic events in BrS by augmenting I_{Na} and I_{Ca} or blocking I_{to}. However, some drugs also showed ineffective effects in BrS studies, such as amiodarone,[98] β-blockers, calcium channel blockers,[99] and some contraindicated drugs[92,98,100] (ajmaline, procainamide, flecainide, propafenone, and pilsicainide). All of the aforementioned animal and cellular models were used to explore the effects of those drugs on BrS[1] (Table 3). To date, with the development of hiPSC-CM models, several research groups have already tested the effects of some drugs on BrS patient-specific hiPSC-CMs. Phosphodiesterase inhibitors (cilostazol and milrinone) are phosphodiesterase III inhibitors that can increase I_{Ca} and suppress I_{to} by increasing the heart rate. Previous studies showed that cilostazol had a beneficial effect on preventing ventricular fibrillation storms in some patients with BrS.[96,101] In recent studies, cilostazol and milrinone also reduced ST elevation and arrhythmogenesis in a canine model.[46,102] In the study by Li and colleagues,[11] hiPSC-CMs from patients with BrS with the *SCN5A* mutation p. S1812X showed a reduction in I_{Na} and the expression of sodium channels. At the same time, a significant increase in I_{to} was

*References 11,21,34,35,36,49,77,86,101,103,104,105,106,107,108,109,110.

Table 3. Drug Research in BrS Experimental Models

Model	Drugs	Major findings	Reference
Murine	Quinidine	Increased regional VERPs and increased corresponding APD(90)s	35
		Fewer ventricular arrhythmias, but variable effects on ST segments and worsened conduction abnormalities	36
	Flecainide	Increased regional VERPs and decreased corresponding APD(90)s	35
		Accentuated ventricular arrhythmias, ST elevation, and conduction disorders	36
	Ajmaline	Prolonged QRS interval and conduction defects	103
Canine	Wenxin Keli, quinidine	Suppressed P2R and pVT	104
	Isoproterenol	Spontaneous VF following premature ventricular beats was induced by vagal nerve stimulation	105
	Milrinone, cilostazol	Restored the epicardial AP dome, reduced dispersion, and abolished phase 2 reentry–induced extrasystoles and ventricular tachycardia	101
	Acacetin	Reduced I_{to} density, AP notch, and J-wave area and totally suppressed the electrocardiographic and arrhythmic manifestation	106
	Ajmaline	Accentuation of epicardial AP notch and ECG J waves resulting in characteristic BrS	106
		Increased maximal J-wave area, AP notch area, and interval between the peak	49
		Generating polymorphic VT when combined with verapamil	107
	Verapamil, NS5806	Accentuation of epicardial AP notch and ECG J waves resulting in characteristic BrS	106
		Increased maximal J-wave area, AP notch area, and interval between the peak and the end of the T wave	49
	Terfenadine	Loss of the epicardial AP dome and resulting ST-segment elevation	107
	Flecainide, procainamide	Generating polymorphic VT when combined with verapamil	107
	Pilsicainide, pinacidil	Coved-type ST elevation in the ECG and longer APD in the epicardium than in the endocardium	108
Porcine	Ajmaline	Total conduction blocked	109
Rabbit	CyPPA	Significant J-wave elevation, frequent spontaneous ventricular fibrillation, and conduction delay	34
Heterologous expression	Quinidine	Normalized the QT interval and prevented stimulation-induced ventricular tachycardia	86
	Lidocaine	More negative shift of steady-state inactivation	110
hiPSC-CMs	Ajmaline	More reduced APA and Vmax than healthy control cells	11,21
		Blocking effect on both depolarization and repolarization in hiPSC-CMs in BrS and control	77
	Milrinone, cilostazol	Inhibited I_{to} and alleviated the arrhythmic activity	11

AP indicates action potential; APD, action potential duration; APD(90)s, action potential duration at 90% repolarization; APA, action potential amplitude; BrS, Brugada syndrome; CyPPA, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine; hiPSC-CMs, human-induced pluripotent stem cell–derived cardiomyocytes; I_{to}, transient outward potassium current; P2R, phase 2 reentry; pVT, polymorphic ventricular tachycardia; VERPs, ventricular effective refractory periods; VF, ventricular fibrillation; VT, ventricular tachycardia and Vmax, maximal upstroke velocity.

detected, and cilostazol and milrinone dramatically inhibited I_{to} and alleviated arrhythmic activity, which indicated the therapeutic potential of phosphodiesterase inhibitors for patients with BrS.

A recent study by Miller et al[77] showed that hiPSC-CMs from patients with BrS with no identified pathogenic mutations and control hiPSC-CMs had similar action potential parameters. Although ajmaline generated blocking effects on both depolarization and repolarization in hiPSC-CMs, there was no difference between BrS and control cells. Furthermore, hiPSC-CMs from patients with BrS with variants in the *SCN1B* and *SCN10A* proteins, which are subunits of the sodium channels, showed reduced I_{Na}, action potential

amplitude and Vmax, and disease cells displayed a greater reduction in action potential amplitude and Vmax than healthy control cells after the application of ajmaline.[21,22]

In the study by Kosmidis and colleagues,[111] hiPSC-CMs were used to investigate the effect of several compounds, such as gentamicin and PTC124, which could increase the translational readthrough of premature stop codons in patients who carry loss-of-function *SCN5A* gene mutations. Although the hiPSC-CMs derived from patients who carry the *SCN5A* mutations R1638X and W156X displayed reduced I_{Na} and action potential upstroke velocities, gentamicin and PTC124 showed no significant influence on reversing the effect of either mutation. This study not only used BrS patient-specific hiPSC-CMs as an experimental model but also used them to investigate novel therapeutic tools for specific mutations associated with BrS.

Latest Advances in Applications of hiPSC-CMs

Currently, an increasing number of investigators have applied CRISPR/Cas9-mediated genome editing technology combined with hiPSC techniques to target specific genes that cause disease to study pathophysiology, conduct drug screening, and improve cell therapeutic potential.[112]

Gene editing technology is used as a tool that can modify the genome, including correcting, writing, or removing genetic information in specific DNA sequences. The CRISPR/Cas9 system, acting as the latest tool for gene editing, has shown unprecedented ease and precision compared with previous technologies. It is a system of adaptive immunity to viruses and plasmids in bacteria and archaea. Three types of CRISPR/Cas9 exist, whereas type 2 is the best known in genome editing.[113] The CRISPR/Cas9 system can target many genomic sequences through gene knockout or knock-in, gene interference or activation, and other applications related to chromosomes with unchanged genetic backgrounds except for the editing part. The combination of CRISPR/Cas9 technology and hiPSC-CMs has been widely used in recent years. It can explore the effect of variants with unknown significance mutations or variants in cardiac channelopathies by inserting the variant into wild-type cell lines or correcting the variant in patient-specific cell lines.[114] Furthermore, these advanced technologies have also contributed to drug screening and repurposing, which showed potential effects for precision medicine.[115] In most gene-editing studies of BrS, the CRISPR/Cas9 system was used to investigate the effect of a mutation on the electrophysiological properties of disease after genomic correction in hiPSC-CMs.[31,79] There is no doubt that the CRISPR/Cas9 system together with hiPSC-CMs will be widely used in studies of BrS for different purposes in the future, including studying specific gene functions, identifying new associated gene mutations and drug screening.

Although most of the hiPSC-CM models reported to date of human arrhythmias have focused on single-cell phenotypes, studying arrhythmias at the 2-dimensional cell culture or tissue level would be a better way to understand the mechanism of disease. In recent studies, hiPSC-CMs were cultured by the 2-dimensional method and provided reliable drug risk stratification in drug screening applications. Furthermore, the inclusion of conduction velocity studies improved the ability of prediction models to test proarrhythmia risk.[116] The 2-dimensional culture of hiPSC-CMs was also applied in another study, which indicated that mutation of MYPBC3 played a critical role in inherited hypertrophic cardiomyopathy.[117]

To date, tissue engineering systems have been developed to enhance electrical coupling, promote physiological maturation, and make it possible to establish cardiac tissues with hiPSC-CMs together with multiple cell types.[118] The main purpose of tissue engineering is to create an environment that is closely similar to that in vivo. In 1997, a type of 3D model heart tissue was generated that could directly measure isometric contractile force, and collagen played an important role in hydrogels for 3D cardiomyocyte culture.[119] In a study by Zimmermann and colleagues,[120] 3D engineered heart tissue from rat cardiac myocytes in vitro improved tissue formation and inhibited dedifferentiation and overgrowth of noncardiomyocytes. These results indicated that engineered heart tissue retained many physiological characteristics of rat cardiac tissue and allowed efficient gene transfer with subsequent force measurement. This technology could also be used for drug discovery. Although previous single-cell studies are useful for initial screening and toxicity testing, they could not organize differentiated cells into the organ-like structures that are necessary for high-content analysis of candidate drugs. The combination of 3D (engineered heart tissue) and 2D models using hiPSC-CMs has significant potential for advancing preclinical drug screening and has also promoted the development of cardiovascular tissue engineering in the field of precision medicine. A particular microphysiological system using hiPSC-CMs has been established to test the effect of pharmacological agents, including isoproterenol (β-adrenergic agonist), E-4031 (human ether-a-go-go-related gene blocker), verapamil (multi-ion channel blocker), and metoprolol (β-adrenergic antagonist). The beat rate and mechanical motion were used as metrics of cardiac tissue function.[121] Advanced technologies combined with hiPSC-CMs could be used in BrS studies to further investigate the molecular and cellular mechanisms that underlie the disease, develop new potential target drugs, and test drug toxicity.

Limitations of hiPSC-CMs

Although hiPSC-CMs have great strengths in current BrS studies compared with other systems, there are several limitations for research. First, the main limitation of hiPSC-CMs is the immature phenotype. This property leads to different expression profiles in ion channels and sarcomeric proteins, such as less organized sarcomeric structures and calcium-handling machinery.[122] However, multiple efforts to overcome this limitation have been used recently,[123-141] including biochemical stimulation, electrical stimulation, mechanical stimulation, surface topography, substrate stiffness modification, 3D culture, long-term culture of hiPSC-CMs, and extracellular matrix culture (Table 4). All of these methods have been reported to improve hiPSC-CM maturation.

Second, differentiated hiPSC-CMs can be composed of a mixture of ventricular-, atrial-, and nodal-like cells, each of which showing different expression profiles of cardiac proteins and electrophysiological

Table 4. Maturation Methods for hiPSC-CMs

Methods		Major findings	Reference
Biochemical stimulation	Tri-iodo-l-thyronine treatment	Increased cardiomyocyte size, anisotropy, and sarcomere length	123
	Fatty acid treatment	Higher myofibril density and alignment, enhanced contractility and improved calcium handling	125
	Centrosome reduction	Increased postmitotic transitions and aspects of cardiomyocyte maturation	126
	Polyinosinic-polycytidylic acid	Increased cell size, greater contractility, faster electrical upstrokes, increased oxidative metabolism, and more mature sarcomeric structure and composition	127
Electrical stimulation	Biowire	Enhanced degree of structural and electrophysiological maturation	128
	Silicon nanowire	Enhanced contractility and expression of contractile protein α-SA and cTnI	129
	Chronic electrical stimulation	Increased connexin-43 abundance and sarcomere ultrastructure	130
Mechanical stimulation	Static stress	Increased contractility, construct alignment, cell size, and expression of RYR2 and SERCA2	131
	Uniaxial stress	Enhanced alignment cells/matrix fiber, myofibril genesis, and sarcomeric banding	132
	Cyclic stress	Increased expression of β-myosin heavy chain and cardiac troponin T, and the tissue showed enhanced calcium dynamics and force production	133
Surface topography	Bioinspired onion epithelium-like structure	High level of genes relating to sarcomere proteins, ion channels, and calcium handling proteins	134
Substrate stiffness	Softest fibronectin-coated PDMS surface	Increased expression of key mature sarcolemmal (SCN5A, Kir2.1, and connexin43) and myofilament markers (cTnI)	135
	Undiluted Matrigel surface	More rod-shape morphology, increased sarcomere length, cTnI and Vmax	136
	Carbon nanotube and pericardial matrix	Improved contraction amplitude, cellular alignment, connexin 43 expression and sarcomere organization	137
3D culture	3D CMTs with biochemical factors	Higher fidelity of adult cardiac phenotype, including sarcoplasmic reticulum function and contractile properties	138
	Scaffold-based tissue engineering with electrical stimulation	Enhanced contractility and expression of contractile protein α-SA and cTnI	129
	EHT with electrical stimulation	Increased connexin-43 abundance and sarcomere ultrastructure	130
	hiPSC-CMs spheroids with cyclic, uniaxial stretch in PDMS channels	Enhanced protein expressions of cTnI, MLC2v, and MLC2a, along with improved ultrastructure, fibril alignment, and fiber number	139
	Microfluidic platform with surface topography	Increased sarcomeric striations, highly synchronous contractions, and upregulation of several maturation genes	140
Long-term culture of hiPSC-CMs	200 d	Upregulation of cellular metabolism and increased cell contractility	141
ECM culture	Human perinatal stem cell derived ECM	More rod-shape morphology, highly organized sarcomeres, elevated cTnI expression, mitochondrial and electrophysiological function	142

3D indicates 3-dimensional; α-SA, α-sarcomeric actinin; CMTs, cardiac microtissues; cTnI, cardiac troponin I; ECM, extracellular matrix; EHT, engineered heart tissue; hiPSC-CMs, human-induced pluripotent stem cell–derived cardiomyocytes; Kir2.1, the dominant subunit of I(K1) channel in ventricle; MLC2a, myosin light chain 2a; MLC2v, myosin regulatory light chain; PDMS, polydimethylsiloxane; RYR2, ryanodine receptor 2; SCN5A, sodium voltage-gated channel alpha subunit 5; SERCA2, Sarco/Endoplasmic Reticulum Calcium ATPase 2 and Vmax, maximal upstroke velocity.

properties.[142] Fortunately, many studies have shown effective methods to direct hiPSC-CMs into subtype-specific cardiomyocytes.

Third, the human heart consists of various cell types, including smooth muscle cells, endothelial cells, leukocytes, and fibroblasts. Cardiomyocytes account for only ≈30% of the heart cell numbers. Therefore, a single-cell model of hiPSC-CMs cannot fully reflect the physiological and pathological conditions of the heart. The 3D tissue engineering platforms established in recent years have improved this situation. Tissue engineering systems can also promote the maturation of hiPSC-CMs and make it possible for investigators to conduct studies of hiPSC-CMs from patients with BrS at a complex organ level.

FUTURE PERSPECTIVES

In recent years, hiPSC-CMs have been widely applied in disease models, drug screening, studying pathological mechanisms, and developing novel treatments. With the development of precision medicine, hiPSCs have received increasing attention from researchers in this field. The main purpose of precision medicine is to provide an individual patient or a group of patients with more effective treatment or preventive actions.[75] hiPSCs with individual patient genetic profiles are ideal tools for precision medicine. They can differentiate into specific cell types and be used in related studies to better understand the underlying mechanisms of disease, improve risk stratification, and develop a more personalized treatment. To date, cardiomyocytes derived from hiPSCs have been used to investigate the pathogenicity of gene variants and drug screening in BrS studies, which has made large contributions to mechanistic studies and personalized medicine. With the identification of genetic variants, it is possible to more specifically identify genotype–phenotype links of BrS to generate more precise risk stratification and to fulfill more exact clinical management for patients with BrS. The results of drug screening could also help to evaluate the response of specific genetic variants to specific pharmacological treatments. Furthermore, in combination with novel technologies, such as genome editing technology and tissue engineering systems, hiPSC-CMs would play a more important role in the study of BrS in the future.

CONCLUSIONS

BrS is a heterogeneous disease, and its clinical course cannot be predicted. Numerous studies have been conducted to explore its underlying mechanism, but the exact pathophysiological mechanism of BrS remains disputed. Different experimental models possess different advantages and disadvantages, leading to different relevance for BrS study. Animal models can model arrhythmias and some ECG changes but are different from humans in gene background. Heterologous expression in cells is ideal for examining the effects of mutations or variants on ion channel functions but cannot simulate action potential changes and arrhythmias. HiPSC-CMs can overcome the shortcomings of animal and heterologous expression models but cannot model the ECG phenotype of BrS. Nevertheless, HiPSCs possess their own advantages in BrS studies, especially in the identification of pathogenicity of gene variants and drug screening.

The hiPSC-CM model derived from human somatic cells shows unique advantages, which makes it possible for investigators to conduct studies of BrS in cell models with desired genetic profiles. The immaturity of hiPSC-CMs is the main limitation of this model. Fortunately, different efforts have been made to solve this problem, and most of them have been shown to be effective in promoting the maturation of hiPSC-CMs. Furthermore, several advanced technologies, including CRISPR/Cas9-mediated genome editing and tissue engineering technology, have been combined with hiPSC-CMs, which can improve mechanistic and therapeutic studies on BrS.

From the available research data in the field of iPSC studies, we believe that studies using patient-specific hiPSC-CMs with unique advantages will provide novel insights into the mechanisms and pathophysiology of the disease and could offer opportunities to improve the diagnosis and treatment of patients with BrS.

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