KCNQ1-deficient and KCNQ1-mutant human embryonic stem cell-derived cardiomyocytes for modeling QT prolongation

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Research Article

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Abstract

Background

The slowly activated delayed-rectifier potassium current (I_{Ks}) composed of KCNQ1 is one of the main currents in the process of repolarization. KCNQ1 mutation can lead to the occurrence of long-QT syndrome type 1 (LQT1). I_{Ks} does not participate in repolarization in mice; thus, no good model for the mechanism research and drug screening of LQT1 is currently available. In this study, we established a KCNQ1-deficient human cardiomyocyte (CM) model and performed a series of microelectrode array (MEA) detection using KCNQ1-mutant CMs constructed in other studies to explore the pathogenic mechanism of KCNQ1 deletion and mutation, and conduct drug screening.

Method

KCNQ1 was knocked out in human embryonic stem cell (hESC) H9 line using the CRISPR/cas9 system. KCNQ1-deficient and KCNQ1-mutant hESCs were differentiated into CMs using a chemically defined differentiation protocol. Subsequently, high-throughput MEA analysis and drug intervention were performed to characterize the electrophysiological characteristics of KCNQ1-deficient and KCNQ1-mutant CMs.

Results

In high-throughput MEA analysis, the electric field potential and action potential durations in KCNQ1-deficient CMs were significantly longer than those in wild-type CMs. KCNQ1-deficient CMs also showed an irregular rhythm. Furthermore, KCNQ1-deficient and KCNQ1-mutant CMs have different responses to different drug treatments, reflecting the differences in their pathogenic mechanisms.

Conclusion

We established a human CM model with KCNQ1 deficiency, which showed prolonged QT interval and irregular heart rhythm. Simultaneously, we used various drugs to treat KCNQ1-deficient and KCNQ1-mutant CMs. The three models showed different responses to drugs. These models can be used as important tools for studying different pathogenic mechanisms of KCNQ1 mutation and the relationship between genotype and phenotype of KCNQ1, facilitating drug development.

Background

Long-QT syndrome (LQTS) is a cardiogenetic disorder, causing life-threatening arrhythmias, which is associated with sudden cardiac death[1,2]. Long-QT syndrome type 1 (LQT1) is the most prevalent subtype, accounting for approximately 40–50% of patients with LQTS[3]. Studies have reported that LQT1 syndrome is caused by loss of function mutations in the KCNQ1 gene[4], which encodes the α-subunit of the cardiac Kv7.1 potassium channel mediating the slowly activated delayed-rectifier potassium current

(I_{Ks})[5,6,7,8,9].To date, numerous mutations in KCNQ1 were identified as responsible for hereditary LQT1 syndrome, and the mutated type and location of KCNQ1 are associated with different clinical severities[10,11,12,13]. However, the clinical phenotype caused by different mutations and the underlying mechanisms remain poorly understood.

Studies have shown that mutations at the N-terminus of KCNQ1, such as Y111C, L114P, and P117L, could affect the transport of KCNQ1[14]. Moreover, mutations on the C-loop inactivate the I_{Ks} mediated by protein kinase A (PKA), causing the inward Ca^{2+} current to increase without confrontation during β-adrenergic stimulation12. These studies have provided direct evidence that mutation types are differently related to physiological activities and mechanisms, which provide the theoretical basis for drug target screening. However, most procedures have been performed almost exclusively on animal models[15,16]. Although KCNQ1 knockout mice showed the Jervell and Lange–Nielsen phenotype[17,18], the mouse heart repolarization K+ current is a fast, slow-transient outward currents, and delayed rectified voltage-gated K^+ current, which cannot reflect the effect of KCNQ1 deficiency in humans[19]. Hence, the establishment of KCNQ1-mutant human embryonic stem cell-derived cardiomyocytes (CMs) is necessary for the mechanistic exploration and drug screening of LQT1 caused by KCNQ1 mutations.

Recently, the establishment of disease models and drug screening using human induced pluripotent stem cell-derived CMs (hiPSC-CMs) has become a promising therapeutic approach for cardiovascular diseases[20]. Here we developed KCNQ1-deficient, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ human myocardial models using the CRISPR/Cas9 system to determine a well-defined genotype–phenotype correspondence. KCNQ1-deficient cells showed serious QT prolongation, irregular rhythm, early post-depolarization (EAD), and I_{Kr} current insensitivity. KCNQ1$^{L114P/+}$ CMs showed a significantly longer QT delay than KCNQ1$^{R190Q/+}$ CMs. Our results showed that MgCl2, propranolol, and amiodarone could reverse the abnormal phenotype caused by KCNQ1 deficiency or mutations separately. The results showed that those models can well reflect the disease phenotype and contribute to the drug screening and accurate treatment of KCNQ1 mutation-related diseases.

Methods

Cell culture and Cardiac differentiation of hESC.

The hESC line was purchased from Cellapy (Beijing, China). was routinely maintained in the presence of PSCeasy medium (Cellapy, China) on six-well plates (Corning, USA) coated with 5% Matrigel (Corning, USA) . Medium was changed every day and passaged every 2-3 days with EDTA (Cellapy, China).The cells were grown in a humidified atmosphere of 95% air and 5% CO2 at 37℃.The hESCs were differentiated when they reached 70~80 % confluence. Medium was changed to the basal differentiation medium.For day 0 to day 2, medium was changed to the basal medium C01 (Cellapy, China). For day 2 to day 4, medium was changed to the basal differentiational medium C02 (Cellapy, China). On day 4,Medium was

changed to the basal differentiational medium C03 (Cellapy, China),and changed medium every other day. Contracting cells were noted from day 9.

Genome editing

KCNQ1 single-stranded guide RNA (sgRNA) (ATGCTACACGTCGACCGCCA,CAGCCGCCCCCAGAGGCCCA,GCTCGAGGAAGTTGTAGACG)was designed using an online tool (https://www.synthego.com). We electroporated the epiCRISPR vector and sgRNA (100μl electrotransformation solution(Cellapy, China) plus 2.5μg KCNQ2 gRNA plasmid) into the cells using the 4D nuclear receptor system and the CA137 programme (Lonza, Germany). The transfected cells were seeded in 6-well plates and cultured overnight in PSCeasy medium 10μM of Rho kinase inhibitor Y-27632. The medium was changed the next day. Drug (puromycin) selection was initiated after 72h of transfection at a lower concentration of 0.1μg/ml for the first hour and then at 0.3μg/ml until the transfected lines were stable. The surviving cells were collected in 48-well plates and amplified for polymerase chain reaction (PCR) screening. The point-mutation cells were prepared by epi-ABEmax/epi-AncBE4max/epi-ABEmax-NG/epi-AncBE4 max-NG plasmid. The plasmid was transfected using Lipofectamine 3000, then the transfected cells were selected by blasticidin. The specific methods can refer to the previous research.

Drug treatment

100mM 293B ,100μM Isoproterenol(ISO) ,5μM Propranolol,100μM Amiodarone 100μM $MgCl_2$(Selleck, USA) were diluted in C05(Cellapy, China) . hESC-CMs were treated with 293B, ISO, Propranololfor 12h. hESC-CMs were treated with Amiodarone, $MgCl_2$ for 30mins.

RNA Extraction and RT- PCR

Total RNA from cells was extracted by using TRIZOL Reagent (Invitrogen, USA). An amount of 2 μg total RNA was reversed to cDNA by using the GoScript Reverse Transcription System (Promega, USA). Quantitative RT-PCR involved use of SYBR Green II (Takara, Japan) in the iQ5 system (Bio Rad, Hercules, CA). A comparative CT method was used to analyze the relative changes in gene expression. The results were expressed as relative to the data of GAPDH transcripts (internal control). Primer sequences are listed in **Table S1**.

Immunofluorescent staining (IF) and imaging analyses

The cells were plated on 20 mm coverslips coated with 5% Matrigel, and were fixed with 4% PFA for 15 minutes. Then, after washing with PBS three times for 5 minutes, the cells were permeabilized with 0.2% Triton X-100 (Sigma, USA) for 15 minutes and blocked with 3% BSA (Sigma, USA) for 1 hour at room temperature . After that cells were incubated with primary antibodies, overnight at 4 °C. Then cells were washed by PBS and incubated for 1 hour at room temperature in the dark with secondary antibodies (Invitrogen,USA). Cells were washed again as above, mounted with Fluoroshield Mounting Medium with

DAPI (4, 6 diamino-2-phenylindole). Images were taken under a Confocal Microscope (Leica DMI 4000B, German). The antibody and their appropriate dilution are provided in **Table S2**.

Western blot (WB)analysis

Protein from hESC-CMs was extracted by using a Protein Extraction Kit (Promega, USA). The protein concentration of the supernatant was measured by BCA method. The 30 μg protein was separated on 10% SDS-PAGE and transferred to PVDF membrane at 300 mA for 90 min, which was blocked with 5% albumin bovine (BSA) at room temperature for 1 h, then incubated at 4℃ overnight with the primary antibodies , then with IR dye-conjugated secondary antibodies (LI-COR, USA) for 1 h at room temperature. GAPDH was used as an internal control. Blots were exposed and analyzed with use of an Odyssey infrared imaging system (LI-COR Biosciences, USA). The antibody and their appropriate dilution are provided in **Table S2**.

Flow cytometry

The hESC-CMs under different treatments were singularized with CardioEasy Human Cardiomyocyte Digestive Fluid (Cellapy, China).Observe that most of the clones are detached from the bottom of the plate under the microscope, gently pipette the cells and suck them out, centrifuge, wash three times with PBS. The cells were stained with different antibodies, filtered through the 300 mesh filter and immediately analyzed by FACS (Beckman, USA). The cell count is generally 1-2 million. The results were analyzed with Flow Jo X program.

Microelectrode array (MEA) analysis

hESC-CMs were digested in CardioEasy Human Cardiomyocyte Digestive Fluid (Cellapy, China), after which 2×104 cells were plated on a micro- electrode array (MEA) pre-coated with 5% Matrigel (Cellapy, China). The next day, 300μl medium was added to each well. After the hESC-CMs resumed spontaneous beating, the experimental data were recorded on a Maestro EDGE (Axion Biosystems, Inc., Atlanta, USA) according to the MEA manual. Cardiac Analysis Tool, AxIS Navi- gator, AxIS data export tool and Origin were used to analyse the data.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed with GraphPad Prism 8.00 for Windows. Two-sided unpaired Student's t-test was used to compare 2 groups with normal distribution. One-way ANOVA was used to compare 3 or more groups. $P<0.05$ was considered statistically significant. *P < 0.05; **P < 0.01, ***P < 0.001; NS, not significant.

Results

Establishment of the KCNQ1-knockout and KCNQ1-mutant hESC-CM models

We used the CRISPR/Cas9 system to establish a KCNQ1-deficient H9 hESC cell model. We designed a highly specific sgRNA targeting KCNQ1 and electroporated hESC H9 cells with a plasmid containing sgRNA and Cas9 element. Subsequently, the transfected cells were screened using puromycin, and the genotype of the surviving clones was identified using Sanger sequencing. Sequencing results showed that a homozygous clone with a 2-bp mutation in KCNQ1 was obtained ($KCNQ1^{R190Q/+}$) **(Fig. S1A).** The pluripotency of $KCNQ1^{R190Q/+}$ was identified using the pluripotency markers, and the karyotype and tumorigenic characteristics of stem cells did not change **(Fig.S1B, C, D, and 1E)**. $KCNQ1^{R190Q/+}$ and other hESCs ($KCNQ1^{L114P/+}$ and $KCNQ1^{-/-}$) established in our previous work were induced to differentiate into CMs using small molecules with clear chemical compositions **(Fig. S2A)**[21]. Immunofluorescence staining of CMs for 30 days showed normal expression of troponin T (TNNT2) and α-actin **(Fig. S2B)**. Flow cytometry showed that the TNNT2 positivity rate of H9 hESC wild-type (WT) and $KCNQ1^{-/-}$ CMs was close to 86% **(Fig. S2C and S2D)**. Western blotting confirmed the absence of the KCNQ1 protein in $KCNQ1^{-/-}$ CMs **(Fig. 1A and 1B)**. Furthermore, the expression of KCNQ1 in $KCNQ1^{L114P/+}$ CMs significantly decreased compared with that in WT CMs and $KCNQ1^{R190Q/+}$ CMs, suggesting that the KCNQ1 transport of $KCNQ1^{L114P/+}$ CMs was abnormal.

$KCNQ1^{-/-}$, $KCNQ1^{L114P/+}$, and $KCNQ1^{R190Q/+}$ CM models can reflect the LQT phenotype

Differences between $KCNQ1^{-/-}$, $KCNQ1^{L114P/+}$, and $KCNQ1^{R190Q/+}$ CMs were observed at the multicellular level using the high-throughput Maestro Edge microelectrode array (MEA) system[22,23]. The field potential duration (FPD) was calculated as the time between the depolarization and repolarization, noted by the beat time and the repolarization peak or T-wave, respectively. The FPD can reflect the duration of myocardial QT interval. FPD statistics showed no difference between $KCNQ1^{R190Q/+}$ and WT CMs. Moreover, the FPD of $KCNQ1^{L114P/+}$ CMs was slightly prolonged, whereas that of $KCNQ1^{-/-}$ CMs was significantly prolonged **(Fig. 1C ,D)**. The prolongation of the FPD of $KCNQ1^{L114P/+}$ CMs may be due to the lack of repolarization I_{Ks} caused by abnormal KCNQ1 transport, which is consistent with the decreased expression of KCNQ1 in $KCNQ1^{L114P/+}$ CMs shown in Western blotting results. Irregular rhythm and EADs are precursors of ventricular arrhythmias in LQT; therefore, we also used the MEA system to analyze the rhythm of the three models. The results showed that compared with WT CMs, $KCNQ1^{-/-}$ CMs showed obvious arrhythmia, and the proportion of EADs significantly increased. $KCNQ1^{L114P/+}$ and $KCNQ1^{R190Q/+}$ CMs also showed obvious arrhythmia **(Fig. 1E ,F)**.

Response to I_{Ks}-specific blocker

Moreover, we tested the effects of the I_{Ks}-specific blocker chromanol 293B (293B) on the FPD of the three models[24]. We used the ratio of the FPD after dosing treatment to baseline FPD (FDP'/FDP) to represent the degree of FPD change. A value greater than 1 indicates that the FPD is prolonged, and a value less than 1 indicates that FPD is shortened. Interestingly, the FPD of WT, $KCNQ1^{L114P/+}$, and $KCNQ1^{R190Q/+}$ CMs showed prolongation after treatment with 100-mM 293B. In contrast, the FPD of $KCNQ1^{-/-}$ CMs did

not significantly prolong as that of KCNQ1$^{L114P/+}$ and KCNQ1$^{R190Q/+}$ CMs after treatment with 293B **(Fig. 2A and 2B)**. Meanwhile, the FDP'/FDP of WT, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ CMs was significantly higher than that of KCNQ1$^{-/-}$ CMs; however, the prolongation of the FPD of knockout CMs is the least obvious **(Fig. 2C)**. These results indicated that the KCNQ1 mutation and knockout models were successfully established.

Responses to $MgCl_2$

Mg^{2+} is the main coenzyme for potassium ion transfer inside and outside a cell. Mg^{2+} supplementation can increase potassium ion transport, increase the intracellular potassium concentration, and increase cell membrane and electrocardiogram stability. Therefore, we observed changes in the FPD of four CMs after $MgCl_2$ treatment. $MgCl_2$ treatment can shorten the FPD of all three models, and the FPD shortening of KCNQ1$^{-/-}$ CMs is the most significant **(Fig. 3A,B,C).** This suggests that magnesium supplementation is essential for LQT treatment with different mechanisms. However, EAD cannot be eliminated **(Fig. 3D,E)**.

Responses to Isoproterenol

Since the occurrence of LQT1 is often related to sympathetic nerve excitement (e.g., exercise or emotional agitation), sympathetic nerve excitement was simulated using isoproterenol (ISO) treatment[25]. ISO is a β-agonist that binds β-AR, and activates cAMP–PKA-dependent downstream signals, which can promote the phosphorylation of several target proteins, including L-type calcium channel and lysine receptor. PKA phosphorylates these proteins and increases the calcium concentration in the sarcoplasm. Therefore, the cross-bridge is activated and further enhances the contraction of CMs[26]. After treatment with 100-μM ISO, the FPD of WT, KCNQ1$^{L114P/+}$, and KCNQ1$^{-/-}$ CMs was significantly shortened compared with the baseline **(Fig. 4A, B, C)**. This phenotypic change was caused by the agonistic effect of ISO. However, the FPD of KCNQ1$^{R190Q/+}$ CMs did not appear to be significantly shortened, suggesting that KCNQ1$^{R190Q/+}$ CMs are not sensitive to ISO. This is consistent with the results of previous studies, which confirmed that mutations in the C-loop, such as KCNQ1$^{R190Q/+}$ CMs, may indeed inactivate Kv7.1's response to PKA stimulation[12]. Furthermore, WT CMs did not show arrhythmia after ISO treatment, whereas KCNQ1$^{L114P/+}$ CMs and KCNQ1$^{R190Q/+}$ CMs both showed arrhythmia aggravation **(Fig. 4D, E)**. Both point-mutation cells showed the arrhythmia phenotype under β-adrenergic stimulation, which was consistent with the phenotype in which LQT1 was more easily induced under sympathetic excitation, indicating that the point process model can well reflect the response of the myocardium to sympathetic excitation.

Responses to Propranolol

β-blockers can directly decrease β-adrenergic signaling and have antiarrhythmic effects[26]. Propranolol, a β-blocker, is one of the most common drugs for the clinical treatment of LQT. Therefore, we used propranolol to treat the CM models and examine their responses. The results showed that the three CM models showed prolongation of the FPD **(Fig. 5A, B, C)**. Moreover, the slowing of heart rhythm and the occurrence of arrhythmia in KCNQ1$^{-/-}$, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ CMs were significantly

decreased compared with the baseline **(Fig. 5D, E)**. Propranolol can better improve the arrhythmia phenotype of the three KCNQ1-mutant myocardial models, including KCNQ1$^{-/-}$ CMs, indicating that propranolol has therapeutic effects on LQT1 with multiple mechanisms**.**

Responses to Amiodarone

Subsequently, we tested the response of the three models to other common LQT medications in the clinic. Amiodarone is a type III multi-ion channel blocker, which can selectively prolong the repolarization time of the myocardium and is suitable for various ventricular arrhythmias[27]. After treatment with 100-μM amiodarone, WT, KCNQ1$^{L114P/+}$, KCNQ1$^{R190Q/+}$, and KCNQ1$^{-/-}$ CMs showed significant FPD prolongation; however, KCNQ1$^{-/-}$ CMs had the smallest FPD extension (**Fig. 6A, B, C**). Amiodarone treatment has alleviated the arrhythmia phenotype of KCNQ1$^{L114P/+}$ CMs but caused the pulsation of KCNQ1$^{-/-}$ CMs to become weak **(Fig. 6D,E)**. These results suggest that amiodarone has a good therapeutic effect on LQT1 with different mutations; however, it is not suitable for treating patients with KCNQ1 large fragment deletion.

Discussion

KCNQ1 mutation is strongly correlated with LQT1. In this study, we used the MEA analysis system to study the response of different KCNQ1-mutated genotypes to different drugs to reveal the underlying mechanisms of different KCNQ1 mutations. Our results indicated that KCNQ1-deficient and KCNQ1-mutant cells showed differently serious QT prolongation and irregular rhythm, which can be corrected by I_{Ks}-specific blocker, β-blockers, and multi-ion channel blocker. These results suggested the novel hiPSC-CM models are helpful tools for determining pathogenic mechanisms and drug screening of KCNQ1 mutation-induced LQT1.

Congenital LQTS is a life-threatening arrhythmia syndrome and is the leading cause of sudden death in young people[28]. The typical characteristics of LQTS are prolongation of the QT interval on electrocardiogram and the occurrence of syncope or cardiac arrest, mainly caused by emotional or physical stress. The three main genotypes of LQTS—LQT1, LQT2, and LQT3—account for 80–90% of all 15 gene mutations found in patients with LQTS[14]. As the main genetic genotype of LQT, LQT1 is caused by mutations in the slow potassium (K+) outward current channel encoded by KCNQ1[29]. The KCNQ1 gene encodes the α-subunit of K+ channel Kv7.1, producing a depolarized I_{Ks} current that increases through sympathetic activation and is critical for QT adaptation when heart rate increases[30]. When I_{Ks} is defective, the QT interval cannot be appropriately shortened during tachycardia, resulting in a high-grade arrhythmia. Homozygous mutations or compound heterozygous mutations in KCNQ1 lead to Jervell and Lange–Nielsen syndrome, which is characterized by decreased inner ear I_{Ks} and deafness[31]. There are more than 100 pathogenic heterozygous mutations in KCNQ1, and these mutations have different effects on the polymeric K+ channel. Mutant and WT protein subunits may assemble together and have a significant negative effect on the current. Alternatively, certain mutant subunits may fail to co-assemble

with the WT peptide, resulting in a loss of function that reduces I_{Ks} by 50% or less (haploinsufficiency). The latter may also be the result of mutations that interfere with intracellular subunit transport, preventing the mutant peptide from reaching the cell membrane.

The complex mutation mechanism increases the difficulty of precise treatment of LQT1; therefore, establishing an effective model is essential for mechanism exploration and drug screening. Due to the huge difference in cardiac function between mice and humans, I_{Ks} does not participate in the repolarization of mice, which makes it difficult to construct animal models. HiPSC-CMs can well simulate human cardiac action potential, which is a good application prospect in disease modeling. Models constructed using patient-derived hiPSC-CMs have been shown to be effective[32]. However, patient-derived hiPSC-CMs cannot accurately reflect the gene–phenotype relationship due to the influence of background genes, and obtaining patient-derived cardiac muscles is difficult. Therefore, the use of gene editing to artificially prepare point-mutation myocardium for disease simulation has good application prospects. In previous studies, our research group constructed $KCNQ1^{L114P/+}$ and $KCNQ1^{R190Q/+}$ models using BaseEditor's method.

$KCNQ1^{L114P/+}$ and $KCNQ1^{R190Q/+}$ are serious pathogenic mutations in LQT1 phenotypes. $KCNQ1^{L114P/+}$ is a mutation at the N-terminus of KCNQ1, which affects the upper membrane transport of KCNQ1[14] $KCNQ1^{R190Q/+}$ is a mutation located on the C-ring of KCNQ1. Studies have shown that such mutations may reduce the sensitivity of KCNQ1 to PKA, leading to the inability of I_{Ks} to significantly increase when stimulated by adrenaline, thus inducing arrhythmias. The electrophysiological phenotypes of the two models were preliminarily detected and proved to be able to reflect the disease phenotypes. Furthermore, due to the diverse pathogenic mechanisms of KCNQ1, there is no effective human KCNQ1 deletion model to clarify the direct phenotype of KCNQ1. Large fragment deletion cases of KCNQ1 have been reported, and the development of a KCNQ1 deletion model helps improve the treatment of this disease. Even though KCNQ1 knockout mice were present and exhibited the Jervell and Lange–Nielsen syndrome, the QT prolongation phenotype was thought to be mediated by extracardial factors since I_{Ks} did not participate in the mouse repolarization process[12]. Therefore, we developed an hESC-CM model without KCNQ1.

In this study, the MEA test results showed that the FPD of $KCNQ1^{-/-}$ CMs was significantly prolonged compared with that of WT CMs, indicating that the absence of I_{Ks} prolonged the repolarization time course of CMs. The FPD of $KCNQ1^{L114P/+}$ CMs was prolonged, whereas that of $KCNQ1^{R190Q/+}$ CMs was not different from that of WT CMs, suggesting that the L114P mutation decreases the upper membrane of KCNQ1, while R190Q does not cause QT prolongation under baseline conditions. Furthermore, $KCNQ1^{-/-}$ CMs showed arrhythmias even at baseline, suggesting that the complete loss of I_{Ks} has a severe effect on myocardial action potentials. When treated with 293B, the FPD of $KCNQ1^{-/-}$ CMs was not significantly prolonged, whereas that of $KCNQ1^{L114P/+}$ and $KCNQ1^{R190Q/+}$ CMs was prolonged, indicating that this insensitivity was caused by the deletion of Kv7.1 channel encoded by KCNQ1. The

baseline phenotype initially reflected the success of model construction and the phenotype of the disease.A schematic of the mechanism can be seen in Figure7.

Since LQT1 often occurs during exercise and emotional arousal, we used ISO to simulate sympathetic excitation. After ISO treatment, WT, KCNQ1$^{-/-}$, and KCNQ1$^{L114P/+}$ CMs showed significant shortening of the FPD, indicating that they responded to ISO's excitement on cardiac function. However, KCNQ1$^{R190Q/+}$ CMs showed insensitivity to ISO stimulation, confirming that this mutation indeed causes the passivation of Kv7.1 channel to PKA stimulation. Under ISO stimulation, all models showed aggravation of the arrhythmia phenotype, partially simulated LQT1 caused by KCNQ1 mutation under sympathetic excitation. Subsequently, we used common clinical LQT medications for treatment to explore the response of different mutations to different drugs. The use of propranolol significantly prolonged the FPD of the three types of CMs and maintained cardiac rhythm stability of KCNQ1$^{-/-}$, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ CMs, indicating that β-blockers have good therapeutic effects on LQT with different mechanisms. Amiodarone has a greater prolongation effect on the FPD in the three models; however, treatment with amiodarone may result in reduced pulsation of KCNQ1$^{-/-}$ CMs, suggesting that amiodarone is not be suitable for treating patients with KCNQ1 large fragment deletion. $MgCl_2$ treatment can reduce the FPD of the three models and has a good effect on the stability of heart rhythm, which reflects the importance of magnesium supplementation in treating patients with LQT.

This study established a hESC-CM model of KCNQ1 deletion, clarified the relationship between KCNQ1 gene and phenotype, and made up for the gap in the human model of KCNQ1 deletion. Simultaneously, KCNQ1$^{-/-}$, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ CMs had different responses to different drug interventions, and their phenotypic changes were consistent with the mechanism proposed in previous studies, indicating that the artificial absence and pitting myocardial model can well reflect the LQT phenotype. As a good disease model, it has great application potential in precision treatment and drug screening.

Conclusion

In this study, we developed a KCNQ1 defect model using the CRISPR/Cas9 system. Electrophysiological detection was performed on KCNQ1$^{-/-}$, KCNQ1$^{L114P/+}$, and KCNQ1$^{R190Q/+}$ CMs simultaneously. The KCNQ1$^{-/-}$ CM model showed significant QT interval prolongation, arrhythmia, and sensitivity to other ion channel blockers. It can be used as an important tool to increase our understanding of the basic pathological mechanism of KCNQ1 dysfunction, define the genotype–phenotype correspondence, and promote drug development. Furthermore, under different intervention conditions, the phenotypes of KCNQ1$^{L114P/+}$ and KCNQ1$^{R190Q/+}$ CMs showed different responses, suggesting that 114 and 190 have different pathogenic mechanisms. It provides an effective model for studying the different pathogenic mechanisms of LQT and confirms the feasibility of preparing a single-gene genetic disease model through gene editing.

Abbreviations

IKs：delayed-rectifier potassium current ；hESC：human embryonic stem cell ；LQTS：long-QT syndrome；MEA：microelectrode array；CM：cardiomyocyte ；hiPSC-CMs：human induced pluripotent stem cell-derived CMs；EAD：early post-depolarization；sgRNA：single-stranded guide RNA ；ISO：Isoproterenol；FPD：field potential duration；WT：wild-type.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no conflict of interest

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Authors’ contributions

MC, FL, YXS,TWG,YXJ and WJL conceived the idea and designed the project. YXS, TWG and YXJ performed most of the experiments and analyzed the data.HYW and MQJ provided technical assistance. YXS, TWG and YXJ wrote and revised the paper.

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Figures

Figure 1

The baseline of four CMs.

A, B. Western blotting showed the expression of KCNQ1 in four hESC cells and quantitative analysis.

C. MEA recorded representative FPD waveforms on four CMs.

D. Representative FPD signal and quantitative analysis of four CMs.

E. MEA recorded representative rhythm traces on four CMs.

F. MEA quantitative analysis showed the proportion of EAD in four CMs.

Results are presented as means ± standard error of the mean (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Figure 2

Four CMs in response to 293B.

A, B. Representative FPD and quantitative analysis of four CMs after treatment with 100-mM 293B.

C. Ratio of the FDP to baseline of four CMs after 293B treatment.

Figure 3

Four CMs in response to $MgCl_2$.

A, B. Representative FPD and quantitative analysis of four CMs after treatment with 1-mM $MgCl_2$.

C. Ratio of the FDP to baseline of four CMs after $MgCl_2$ treatment.

D. Representative traces of EAD in the FPD of four CMs after treatment with $MgCl_2$.

E. Quantitative analysis showed the proportion of EAD in four CMs after treatment with $MgCl_2$.

Figure 4

Four CMs in response to ISO.

A, B. Representative FPD and quantitative analysis of four CMs after treatment with 100-uM ISO.

C. Ratio of the FDP to baseline of four CMs after ISO treatment.

D. Representative traces of EAD in the FPD of four CMs after treatment with ISO.

E. Quantitative analysis showed the proportion of EAD in four CMs after treatment with ISO.

Figure 5

Four CMs in response to Propranolol

A, B. Representative FPD and quantitative analysis of four CMs after treatment with 5-uM propranolol.

C. Ratio of the FDP to baseline of four CMs after propranolo treatment.

D. Representative traces of EAD in the FPD of four CMs after treatment with propranolol.

E. Quantitative analysis showed the proportion of EAD in four CMs after treatment with propranolol.

Figure 6

Four CMs in response to Amiodarone.

A, B. Representative FPD and quantitative analysis of four CMs after treatment with 100-uM amiodarone.

C. Ratio of the FDP to baseline of four CMs after amiodarone treatment.

D. Representative traces of EAD in the FPD of four CMs after treatment with amiodarone.

E. Quantitative analysis showed the proportion of EAD in four CMs after treatment with amiodarone.

Figure 7

Schematic illustration of KCNQ1 cell membrane expression in four hESC-CMs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.pdf
- FigureS2.pdf
- KCNQ1supplementarymaterials.docx