Minireview

New iPSC for old long QT syndrome modeling: Putting the evidence into perspective

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Abstract

Induced pluripotent stem cells (iPS cells or iPSCs) are typically derived by transfection of certain stem cell-associated genes into non-pluripotent cells, such as adult fibroblasts (typically adult somatic cells). Various diseases can be modeled through iPSC technology. The important implication of iPSCs to offer an unprecedented opportunity to recapitulate pathologic human tissue formation *in vitro* has generated great excitement and interest in the whole biomedical research community. Long QT syndrome (LQTS), an inherited heart disease, is characterized by prolonged QT interval on a surface electrocardiogram. LQTS presents with life-threatening cardiac arrhythmias, which can lead to fainting, syncope, and sudden death. The iPSC-derived cardiomyocytes from LQTS patients offer a potentially unlimited source of materials for biomedical study. They can be used to recapitulate complex physiological phenotypes, probe toxicological testing and drug screening, clarify the novel mechanistic insights and may also rectify gene defects at the cellular and molecular level. Despite the emerging challenges, iPSC technology has been increasingly recognized as a valuable and growing toolkit for modeling LQTS over other various models of human diseases.

Keywords: Induced pluripotent stem cells, long QT syndrome, drug development, regenerative medicine

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Introduction

According to the World Health Organization, cardiovascular diseases (CVD) are the leading cause of death worldwide and have increased at a fast rate in many countries.[1] CVD consist of a broad range of disorders extending from myocardial infarction to congenital heart disease, some of which are heritable. There is therefore an enormous effort in understanding the genes that are responsible for heritability.[2] The heritable long QT syndrome (LQTS) occurs when a genetic defect tips the balance of inward and outward currents to delay repolarization of the ventricular action potential (AP). The resulting increase in AP duration (APD) is reflected by a prolongation of QT interval on a 12-lead ECG. The prolonged QT interval is responsible for early afterdepolarizations (EADs), which present with the potentially fatal ventricular arrhythmias under some circumstances.[3–6] So far, hundreds of mutations in at least 13 genes encoding cardiac ion channels or channel-related proteins have been indentified to be responsible for LQTS (Table 1).[7]

The mechanisms of LQTS are not fully illustrated because of the lack of human ventricular cardiomyocytes (CMs), which cannot be directly obtained from LQTS patients. Previous insights into the mechanisms underlying LQTS come primarily from heterologous expression and genetic animal models.[8] However, heterologous systems do not faithfully reveal the impact of known and unknown accessory channel subunits, which may exist in natural CMs and interact with the mutated ion channels.[9–11] Animal models have traditionally been considered more informative than cell-based *in vitro* approaches and mice have become the gold standard for human disease models.[12–14] For example, we can use animal models *in vivo* with the ability to evaluate the therapeutic potential of candidate compounds to the intact heart and the drug adverse reactions to other organ systems as well. However, animals cannot precisely reveal the characteristics of human LQTS due to the dramatic differences between animals and humans in the biochemical, physiological and anatomical features of CMs, such as very different and heart rate and AP characteristics in mouse and human hearts.[15,16] In addition, creating the causal mutation in an animal to reproduce the human genotype requires complex genetic engineering, which is technically challenging and may introduce unwanted effects into the models.[8] Therefore, as a

Table 1 Congenital long QT syndrome

Type	Affected gene	Chromosome	Protein	Current	Incentive	Ration
LQT1	KCNQ1	11p15	I_{Ks} α-submit	$I_{Ks}\downarrow$	Exercise	42%
LQT2	KCNH2	7q35-36	I_{Kr} α-submit	$I_{Kr}\downarrow$	Ring, exercise, awaken	45%
LQT3	SCN5A	3p21-23	I_{Na} α-submit	$I_{Na}\uparrow$	Rest, asleep	8%
LQT4	ANK2	4q25-27	Ankyrin-B	$I_{Ca2+}\uparrow$	Exercise	<1%
LQT5	KCNE1	21p22	I_{Ks} β-submit	$I_{Ks}\downarrow$	Rage, exercise	3%
LQT6	KCNE2	21p22	I_{Kr} β-submit	$I_{Kr}\downarrow$	Rest, exercise	2%
LQT7	KCNJ2	17p23-24	I_{K1}	$I_{K2.1}\downarrow$	Rest, exercise	<1%
LQT8	CACNA1	12p13	I_{Ca} α-submit	$I_{Ca}\uparrow$	Exercise, nervous	<1%
LQT9	CAV3	3p25	Caveolin	$I_{Na}\uparrow$	Rest, asleep	Rare
LQT10	SCN4B	11q23	NAVβ4	$I_{Na}\uparrow$	Exercise	N/A
LQT11	AKAP-9	7q21-22	Yotiao	$I_{Ks}\downarrow$	N/A	N/A
LQT12	SNTA1	20q11	α1syntrophin	$I_{Na}\uparrow$	N/A	N/A
LQT13	KCNJ5	11q24	Kir4.3	$I_{K\text{-}ACH}\downarrow$	N/A	N/A
JLN1	KCNQ1	11p15	I_{Ks} α-submit	$I_{Ks}\downarrow$	Rage, exercise	1–7%
JLN2	KCNE1	21p22	I_{Kr} β-submit	$I_{Ks}\downarrow$	Rage, exercise	<1%

substantial amount of variants in genes for ion channels associated with LQTS are emerging, it is urgent to develop an effective and accurate alternative system to illustrate the mechanisms of LQTS.

Induced pluripotent stem cells and its advances

Induced pluripotent stem cell (iPSC) technology, whereby a patient's somatic cells can be reprogrammed into an embryonic pluripotent state by the forced expression of a defined set of transcription factors, has achieved great progress since it was firstly established in 2006.[17] Currently, iPSCs have been generated from several lines of human tissues. New approaches to improve the safety and efficiency have also been suggested. As to the various strategies of reprogramming, adenoviruses, transposons, plasmids, recombinant proteins, synthetic mRNAs, mature microRNAs, and virus RNA have the potential to be tested for inducing pluripotency in somatic cells. Most methods employed so far to generate iPSCs with consistent efficiency include the transfer of foreign DNA into the target cell by retroviral vectors or non-integrating vectors. Other research groups worked on the issue of cancer risk by using non-integrating adenoviral or episomal vectors. The RNA- and transcriptome-based technology might overcome the safety concerns related with vector-based DNA transfer. A recent innovative approach to increase the efficiency of cell reprogramming has been the inactivation or inhibition of p53 by lentivirus-mediated RNA interference.[18] Compared with iPSC technology, direct reprogramming technologies can use patient-specific cells for the rapid production of models of various human degenerative ''diseases in a dish'', without the challenges of time-consuming and labor-intensive iPSCs. Two alternative methods have been developed that enable to insert a foreign gene sequence into a live cell by triggering the cells to DNA repair processes.[19,20] Additionally, the presence of small molecules such as valproic acid, a histone deacetylase inhibitor, and Vitamin C has been reported to improve reprogramming efficiency significantly.[18] Further screening identified more small molecules that can increase the efficiency of reprogramming as well as the completeness, including RG108 and AZA (DNA methyltransferase inhibitors), BayK8644 (a calcium channel agonist), dexamethasone (a steroid glucocorticoid).[21] Recently, researchers from China revealed that complete small-molecule reprogramming is possible.[22] Hockemeyer et al.[19] showed that pluripotent stem cells can be generated from mouse somatic cells at a frequency up to 0.2% using a combination of seven small-molecule compounds. Currently, further optimization of this technology in reprogramming somatic cells to iPSCs is still needed in order to approach a similar efficiency level as obtained using viral protocols.

iPSC technology provides a potential solution to overcome the drawbacks of using heterologous cell lines or animals to model human diseases, especially for investigating the consequences of human genetic variation on cellular phenotypes.[23] The establishment of disease models through patient-specific reprogramming involves two steps. The first step is derivation of iPSCs from somatic cells of a patient and the second step is differentiating the iPSCs into cell types affected by the patient's disease. To date, several lines of groups have generated patient-specific iPSCs and differentiated them into relevant cell types, which still harbor the responsible mutations and manifest the morphologic characteristics of the corresponding disease.[24–28] The ability to generate disease-relevant iPSC types to manifest cellular disease phenotypes shows tremendous potential of iPSC technology in studying *in vitro* human disease. First, the range of specific cell types available for study has been greatly expanded. By starting with materials from affected individuals, if mutant genes involved in the pathogenesis of the disease are expressed in the iPSCs, transcriptional abnormalities in the somatic

cells affected from the patient can be investigated in iPSC lines. This will reduce the dependence on complex genetic engineering strategies. Second, tissue culture of immortal cell strains from patients is an invaluable resource for basic research. The cost of working with human iPSCs compares favorably with those of working with large animal models. Third, disease-specific iPSCs could provide new insight into the pathophysiology of various diseases by permitting the analysis of a system that is close to that of humans. Thus, the introduction of the iPSC models into preclinical pipelines may substantially lower the risk of failure of clinical trials.[8]

CMs derived from iPSCs

Experimentally, iPSCs have been shown to differentiate into each of the major cardiovascular components, including smooth muscle cells, endothelial cells, vascular mural cells, and CMs.[29–31] Takahashi et al.[32] firstly reported that human iPSCs from adult human dermal fibroblasts can differentiate into beating CMs *in vitro*. Zhang et al.[33] effectively and innovatively described a detailed evaluation of the cardiac differentiation potential of human iPSC lines in comparison with well-studied hESC lines. Like hESC-derived CMs, human iPSC-derived CMs (iPS-CMs) consist of three subtypes of CMs, including sinus nodal, atrial, and ventricular CMs. These subtypes show different forms of AP and can be distinguished by electrophysiological analysis.[33–35]

Differentiation of iPS-CMs derived not only from fibroblasts but also from other somatic cells such as from human cord blood and keratinocytes was demonstrated. As an alternative, iPSCs derived from human cord blood were shown to differentiate into all three germ layers, including spontaneous beating CMs.[36] In addition, due to its advantage of coming from a juvenescent cell source, cord blood is already routinely harvested for clinical use. Moreover, the potential to harvest cells for reprogramming without surgical intervention is attractive. Reprogramming experiments have also been performed using plucked human hair follicle keratinocytes.[37] These iPSCs were able to differentiate into cells from all three germ layers including beating CMs.[37]

To date, extensive studies have been done about iPSC-CMs, including cardiac differentiation,[13,33,36–38] cardiac marker expression profile,[13,39,40] structural properties,[33,37,38] electrophysiological properties,[13,38,40] ion channel expression,[24,40] and excitation–contraction coupling.[24,41] The studies performed so far for cardiac differentiation provide the impression that the cardiac differentiation protocols employed for ESCs are applicable for the differentiation of iPSCs, although slight adjustments may be required. Additionally, given that cardiac differentiation efficiency and yield vary significantly between hESC lines, it seems reasonable to conclude that cardiogenesis from iPSCs is efficient to an extent compared with that of ESCs and make the study of human CMs possible. More importantly, these developed protocols provide an important alternative platform for the production of patient-specific CMs, avoiding the concerns the traditional models face.

Recapitulating LQTS using iPSC technology

Heterologous expression systems and animal models related to LQTS, such as mouse models displaying human LQTS, were generated with success to recapitulate at least in part the phenotype of CMs.[12–14] However, it is questionable how predictive these models are due to the drawbacks mentioned above. The lack of human CMs from patients with the genetic mutations associated with LQTS as well as the inability to model patient-specific disease variations significantly hamper the studies of LQTS. With respect to LQTS, there is a clear relation between genotype and phenotype. That is why inherited LQTS related to mutations in single genes should be the initial preference in order to recapitulate the cardiac disease phenotype, at the CM level, as it manifests in patients.

Several groups have reported successful generation of iPSCs from LQTS patients (Table 2), and differentiated them into iPS-CMs and documented phenotypes that are indicative of LQTS.[13,24,39,40,42–49] iPSCs from LQT1 individuals with a mutation (R190Q) in the KCNQ1 gene showed the disease genotype of LQT1 and generated functional iPS-CMs.[39] The APD was significantly prolonged in ''ventricular'' and ''atrial'' cells from LQT1 individuals, as compared with those from control individuals. Further analysis of the influence of the R190Q–KCNQ1 in the pathogenesis of LQT1 demonstrated a dominant negative trafficking defect related with a 70–80% reduction in I_{Ks} currents and altered channel activation and deactivation properties. LQTS patient-specific iPSCs were also the subject of another study, albeit for studying LQTS type 2 (LQT2).[24] Significant prolongation of the APD in iPS-CMs from patients harboring LQT2 due to the missense mutation in the hERG gene was observed when compared with healthy control counterparts. Further studies confirmed that this APD prolongation stems from a significant reduction of the cardiac potassium current I_{Kr}. Additionally, iPS-CMs from patients showed arrhythmogenicity characterized by EADs and triggered arrhythmias. The disease-specific iPS-CMs from an LQT3 mouse with a human mutation recapitulating the typical pathophysiological phenotype were also demonstrated *in vitro*.[13] LQT3-specific iPS-CMs show the biophysical effects of the human ΔKPQ mutation (Scn5aΔ/+), which is known to display typical electrophysiological features of LQT3, with faster recovery from inactivation and larger late currents than that observed in controls. These iPS-CMs show prolonged APDs and EADs at low pacing rates, and both of which are clearly different from control iPS-CMs and display classic features of the LQT3.[13,14,50]

In general, the iPSC lines derived from LQTS individuals can be differentiated into CMs, elucidating the disease's electrophysiologic characteristics of disease, providing a powerful method for further research (Figure 1).

Table 2 iPSC models of long QT syndromes

Syndromes	Species	Affected gene	Mutation	Somatic cells	Vector	Transcription factors	Methodology	Drug testing	Reference
LQT1	Human	KCNQ1 gene	R190Q	Fibroblasts	Retroviruses	OCT3/4, SOX2, KLF4, and c-MYC	Whole-cell patch clamp, immunofluorescence	Yes	39
LQT2	Human	KCNH2 (hERG) gene	A614V	Fibroblasts	Retroviruses	SOX2, KLF4, and OCT4.	Whole-cell patch clamp, extracellular multi-electrode recordings	Yes	24
LQT8	Human	CACNA1C gene	G406R	Fibroblasts	Retroviruses	SOX2, OCT3/4, KLF4, and C-MYC	Whole-cell patch clamp, calcium imaging	Yes	47
LQT2	Human	KCNH2 (hERG) gene	G1681A	Fibroblasts	Lentivirus	OCT4, NANOG, SOX2, and LIN28	Whole-cell patch clamp, extracellular multi-electrode recordings	Yes	40
LQT2	Human	KCNH2 (hERG) gene	R176W	Fibroblasts	Retroviruses	OCT4, SOX2, KLF4, and MYC	Whole-cell patch clamp, extracellular multi-electrode recordings	Yes	43
LQT1	Human	KNCQ1 gene	1893delC (P631fs/33)	Fibroblasts	Lentivirus	OCT3/4, SOX2, KLF4, and c-MYC	Extracellular multielectrode recordings, patch-clamp analysis and immunostaining	Yes	48
LQT2	Human	KCNH2 (hERG) gene	G603D	T lymphocytes	Sendai virus vectors	OCT3/4, SOX2, KLF4, and c-MYC	Immunocytochemistry	No	45
LQT3	Mice	SCN5A gene	△KPQ	Fibroblasts	Retroviruses	Oct4, Sox2, and Klf4 or additionally with the fourth factor c-Myc	Patch-clamp technique, immunostainings	Yes	13
LQT2	Human	KCNH2 (hERG) gene	G1681A	Fibroblasts	Lentivirus	OCT4, NANOG, SOX2, and LIN28	Allele-specific RNA interference, whole-cell patch-clamp, multi-electrode array analysis	Yes	49
LQT2	Human	KCNH2 (hERG) gene	W1001X	Fibroblasts	Episomal vectors	OCT4, SOX2, NANOG, LIN28, c-Myc, and KLF4	Confocal imaging, voltage clamp	Yes	46

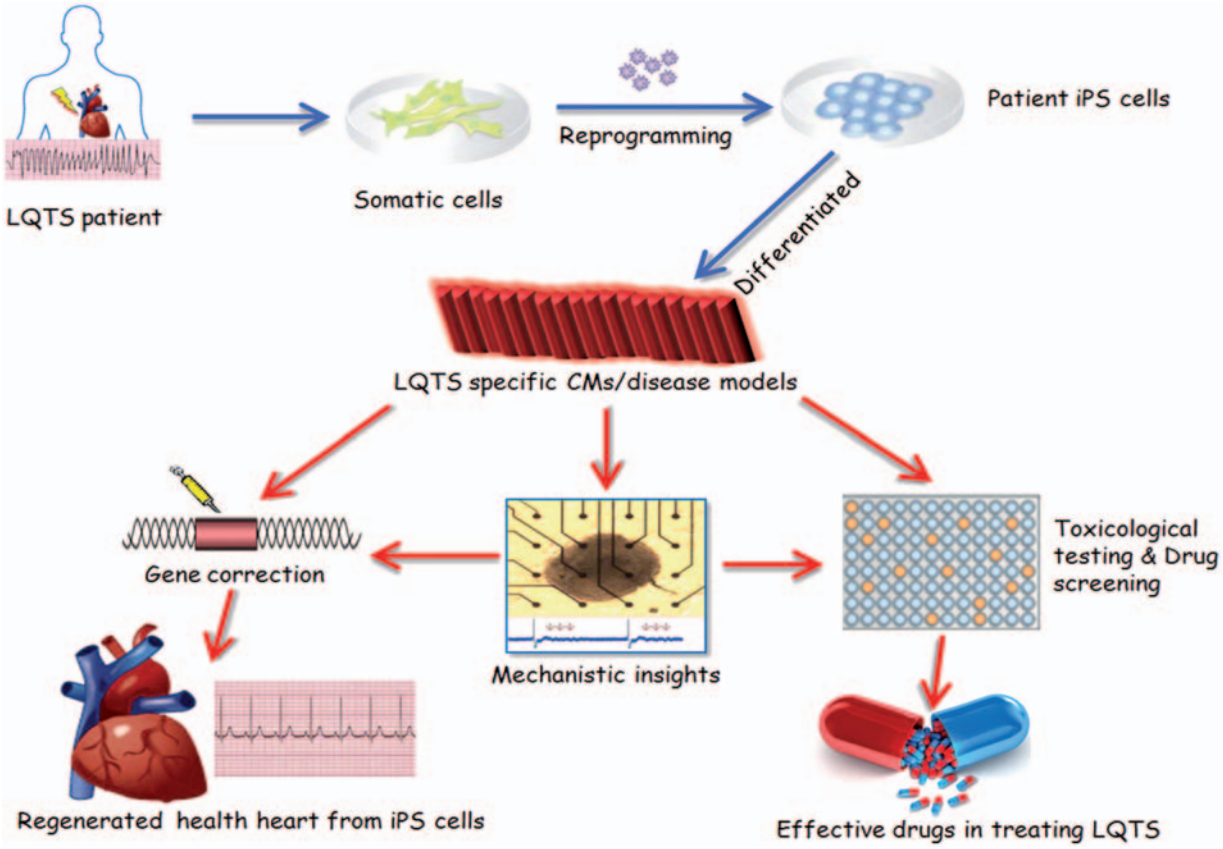

Figure 1 The further application of iPSC technique in LQTS. Modified from Itzhaki I, et al. Nature 2011;471:225–U113.[24] (A color version of this figure is available in the online journal)

The applications of iPSC models in LQTS

Elucidating pathogenesis

For LQTS, which was in 1991 initially described as the hereditary disease due to genetic lesion,[51] the path from the discovery of hERG as the causal gene to a breakthrough in the molecular understanding of LQTS has spanned for more than two decades.[52] Presently, mutations in at least 13 genes are documented to be associated with LQTS.[53,54] Various kinds of mutations cause channel dysfunction by different mechanisms. Herein, hERG, whose frequency in mutation-identified LQTS patients is the highest in China, is given as an example for illustration. The classification scheme reviewed by Delisle et al.[55] and Gong et al.[56] illustrates the mechanisms underlying LQT2 due to mutations. Class 1 mutations cause abnormal protein synthesis by defective transcription or translation. Class 2 mutations lead to defective protein trafficking. Class 3 mutations result in abnormal gating and/or kinetics. Class 4 mutations result in altered or absent channel selectivity or permeability. Class 5 mutations cause a decrease in mutant mRNAs through nonsense mediated decay (NMD) thereby altering the amount of mRNA available for subsequent hERG protein generation.[55,56]

Although traditional studies have provided great insights into the possible mechanisms responsible for channel dysfunction, major questions regarding the mechanisms of LQTS remain to be defined. These issues are more than academic questions, because the answers have broad implications on drug developments and therapy strategies.[44] More importantly, although there are LQTS cases in which single gene mutations lead to straightforward genotype–phenotype associations, other more complex relationships exist as well.[2] This complexity can arise from three distinct genetic phenomena, pleiotropy, penetrance, and expressivity, which together with other non-genetic factors conspire to ensure the complexity of LQTS. Even in the single gene disorders, a genotype is not often consistent with a specific phenotype. Previous studies of LQTS mostly relied on particular cell types or animals carrying the same genetic deficiencies. However, animal models or cell types do not always faithfully reproduce the human phenotype due to the drawbacks described above.[57–59]

Patient-specific cells from human iPSCs are making a human cellular system available for *in vitro* demonstration of mechanisms of LQTS. Particularly, one advantage of iPS-CMs is the possibility to estimate the impact of the individual's genetic defect on the phenotype. For example, iPS-CMs derived from LQTS individuals showed all the conduction-related phenotypes found in LQTS patients.[24,39] Possibilities to study the altered proteins directly in CMs may provide additional important information concerning the effects of the mutations on cell excitability. Moreover, iPSC cellular disease models can be combined with other technologies to systematically perturb cells *in vitro* to elucidate the mechanisms of LQTS. For example, iPSC

technology combination with microelectrode arrays provided insights into the arrhythmias pathogenesis of LQT2 iPS-CMs carrying A614V mutation in the hERG gene, demonstrating that the development of spontaneous EADs can lead to the triggered activity at both the cellular and multicellular levels.[24] Missense mutations in LQT2 generate trafficking-deficient channel protein to cause loss of I_{Kr} by reducing channel density on the cell surface. However, nonsense and frame-shift mutations can introduce a premature termination codon (PTC) resulting in degradation of hERG mRNA through NMD, thus the mutant allele generates little or no protein.[56] Recently, the NMD hypothesis was evaluated using iPS-CMs with the W1001X nonsense mutation.[46] iPS-CMs harboring W1001X in hERG gene demonstrate less expression of Kv11.1 protein on the cell surface compared with control iPS-CMs. The mutant iPS-CMs also display a marked reduction in peak tail current of I_{Kr} compared with control iPS-CMs. There is no increase in I_{Kr} in the W1001X iPS-CMs after PTC124 (a drug shown to read through mRNA PTCs) administration. It is concluded that the W1001X mutation, which is predicted to introduce a PTC in hERG mRNA, causes around a 50% loss of I_{Kr} in LQT2 iPS-CMs consistent with NMD of mutant hERG transcripts.

By all accounts, iPSCs derived from LQTS individuals in combination with other modern tools provide the platform to illustrate the LQTS pathophysiologic characteristics, expanding our understanding of LQTS pathogenesis not readily evident through traditional models.

Drug screening

Currently, the drug screening models are deficient, particularly in detecting cardiac side effects. The average time to develop a typical new drug is 10–15 years with tremendous cost.[60–62] Compounds that experimentally proved to be safe and efficacious in animal models or heterologous cell lines may fail in later clinical trials because of the safety concerns or little efficacy.[63–66] In the conventional target-centric drug discovery models, compounds are typically not tested in a relevant patient population until Phase II clinical trials, thus contributing to a high drug attrition rate: 30% of these failures is due to the lack of efficacy.[67]

iPSC technology represents the ''disease in a dish'' and prospectively affords the opportunity to obtain drug efficacy and toxicity data in a disease-specific condition at the earliest stages of drug development. The strategies will guarantee lower attrition rates at the late stages of the clinical trials. For example, given the clear disease features of LQT2-iPS-CMs, several drugs that may be able to correct the disease phenotype were tested. Treatment of LQT2-iPS-CMs with nicorandil and PD118057 leads to AP shortening and abolished EADs in some cases.[40] Nadolol can reverse EADs induced by combined treatment with isoprenaline.[40] Patient LQT2-iPS-CMs respond appropriately to clinically relevant drugs and will be a valuable human *in vitro* model for testing experimental drug combinations.

This approach is particularly attractive because of the pluripotent nature and potentially unlimited number of iPS-CMs, enabling enough CMs available for candidate compound tests and high-throughput drug development. Moreover, modeling using iPSC technology opens an avenue for testing the drugs on LQTS-specific iPS-CMs, meaning the possibility for personalized drug therapy strategies. This would be a significant advance in the field of targeted health care and has great potential benefit in the further clinic.

Toxicity assessment

Besides a lack of efficacy, drug toxicity has been increasingly recognized as one of the greatest limitations to the pharmaceutical development.[68] Around 40% of the candidate compounds tested in clinical trials are abandoned because of the toxic effects.[67] Furthermore, adverse drug reactions are considered as a major cause of morbidity and mortality, particularly in certain individuals. This failure in early drug toxicity detection is proving extremely time-consuming and costly, which leads people's health to risk. Cardiovascular toxicity is a major cause of drug withdrawal during clinical development, accounting for up to 33% of drug failure.[69]

Variations in genes responsible for ion channels can predispose stable individuals to fatal arrhythmias when administration of agents that block ion channels.[70–72] Current evidences suggest that around 5–10% of people developing torsade de pointes when exposed to QT-interval-prolonging drugs harbor mutations linked to inherited LQTS.[73] Human stem cell-derived CMs could aid early cardiotoxicity detection. Work over the past decade generated embryonic stem cells as the potentially accurate sources of human CMs, but ethical issues and poor efficacy in establishment of cell lines limit their use.[69]

LQTS iPS-CMs have great potential in the prediction of the tendency of agents to exacerbate cardiac defects, thus enabling unexpected cardiotoxicity to be detected during early test, decreasing the risk and the cost of clinical trials, making up the basis for safety assessment. For example, there is a family history of overt LQTS, this individual was asymptomatic except for occasional palpitations and a heart-rate-corrected QT time of 437 ms.[74] However, the unmasking of latent LQTS can occur accidentally. In line with the repolarization reserve hypothesis and the clinical data, iPS-CMs harboring R176W linked to LQT2 were more sensitive to drugs compared with control iPS-CMs.[74] Similarly, characterization with chromanol 293B and E4031 showed that I_{Ks} current densities in LQT1 patient-derived iPS-CMs, as compared with those from control subjects, were decreased.[39] The significant APD prolongation in LQT1 patient-derived iPS-CMs exposed to E-4031 was also observed, manifesting either by the development of new EADs or by an increase in their number and complexity. E-4031 or cisapride significantly prolongs cFPD as well as increases arrhythmogenicity at the multicellular level. These results, together with the I_{Kr} current analysis, offer clues to the increased susceptibility of LQTS patients to malignant arrhythmias in response to culprit agents that may further inhibit the remaining I_{Kr} current.[24] These studies, in which iPS-CMs from LQTS patients were used to reflect the tendency of drugs to exacerbate cardiac

conduction defects, allow a system to screen for the toxic side effect prior to *in vivo* use of new drugs.[2] This approach can practically reduce the cost burden of failed drug development projects and enable the enrichment of projects with the highest probability of success.[75]

Taking advantage of this resource of individuals with the same mutation, it might be possible to evaluate the electrophysiological properties and drug responses of CMs from mutation carriers with and without symptoms. In the future, more reliable assays can be expected to predict the arrhythmogenic risk of drug candidates in humans.[76] The platform of iPSC to evaluate adverse cardiac effects of drugs with the potential to prolong the QT interval and personalize drug development may prove to be a powerful means of reducing drug toxicity, stratifying patient response, and reducing late-stage clinical failures.

Stem cell-based therapies

ß-blockers are routinely prescribed as therapeutic and preventive measures in LQTS.[77–79] ß-blockers are associated with a significant reduction in cardiac events in LQTS patients. However, syncope, aborted cardiac arrest, and LQTS-related death continue to occur while patients are on prescribed ß-blockers, particularly in those who were symptomatic before starting this therapy.[79] Implantable cardioverter defibrillator (ICD) therapy is a safe and useful tool in high-risk LQTS patients. However, some patients remain an increased risk for cardiac events despite ICD implantation. The benefits of ICD implantation in some LQTS patients now appear questionable.[80,81] Current advances in molecular reprogramming set the stage for devising alternative strategies for the treatment of LQTS.

Several therapeutic relevant cell types derived from iPSCs have been tested to treat diseases in animal models.[28,82] Mouse iPSCs can be used for the restoration of physiological function of diseased tissues *in vivo*, as demonstrated by using iPSC-derived hematopoietic cells in a humanized mouse model of sickle cell anemia.[83] Mouse iPSC-derived endothelial injected directly into the liver of irradiated hemophilia A mice extended their survival for more than 3 months and rescued depleted plasma FVIII levels.[84] H9c2 cell-derived iPSCs can repair and regenerate infarcted myocardium with improved cardiac function *in vivo*.[85] iPSCs seem to have better therapeutic efficacy compared with those from adult stem cell sources.[86]

To date, there is no direct evidence showing iPSC-based therapy in the setting of LQTS. However, all the experimental evidence strongly supports the possibilities of genetic correction in LQTS patient-specific iPSCs for stem cell-based therapies. Here, we provide a bold assumption. iPS-CMs derived from mouse model with LQTS can be repaired and then have the similar abilities as healthy iPS-CMs. These repaired iPS-CMs can be cultured to form a healthy heart combination with other modern techniques. Then the healthy heart can be transplanted back into the mouse model with fatal arrhythmias and replace a failing heart. From a practical point of view, the large-scale production of mutation-corrected patient-specific iPSCs will definitely move farther to the promise of the cell-based therapeutic of LQTS.

Perspective

Despite major advances in iPSC technologies, iPSC technologies for modeling LQTS are tempered by a number of obstacles, which need to be overcome before safe and high-quality patient-specific iPSCs can be derived.[23] These obstacles include the low efficiency of iPSC generation, tumorigenicity, incomplete reprogramming, tissue-inappropriate differentiation, the potential for genetic and epigenetic abnormalities, and immunogenicity of transplanted cells.[23] Especially, genetic manipulations such as nuclear transfer used in reprogramming significantly limit the clinical potential of applications. Therefore, a lot of work is needed to ensure the safety and therapeutic applications of iPSC technologies for modeling LQTS before advancing to any clinical trial. Small molecules that modulate stem cell fate and function offer significant opportunities and avoid the random integration of delivery vectors into the genome during the induced process that will allow the full realization of the clinical potential of iPSCs.[87] Recently, Hou et al.[22] revealed that chemical reprogramming strategy has potential use in generating functional desirable cell types for clinical applications. They showed that iPSCs can be generated from mouse somatic cells at a frequency up to 0.2% using a combination of seven small-molecule compounds. These chemically iPSCs resemble embryonic stem cells in terms of their gene expression profiles, epigenetic status, and potential for differentiation and germline transmission.[22]

The efficiency and reproducibility of the differentiation protocols that enable cost-effective production suitable for the industry have to be developed for automated high-throughput platforms. As much as iPS-CMs appear to be a viable model for drug screening, a word of caution must be added. Because the information regarding the properties of different ion channels and their response to hormones, phosphorylation, and other messengers that regulate biology of CMs are only beginning to emerge, more studies are required. Considering the complexity depth and potential of economy of iPSC technology, much more alternative interventions will be necessary. One method for inducing electrophysiological maturation using replanting or 3D culture technique has been published.[88] Lastly, this research will likely ignite a paradigm shift among patients, researchers, and the society at large, and these changes may bring unforeseen challenges to existing governing policies in regard to privacy and ethics involving human materials and data generated. It is imperative to be mindful of such implications as iPSC technology is poised to usher in a new paradigm in clinical practice. This issue appears very significant at this time when recent breakthrough of iPSC-based regenerate medicine has been achieved,[22,89,90] especially when the world's first clinical study using iPSCs has been launched.[91]

In the review, we outlined the revolutionary advances of iPSC technology and the transformative potential of iPS-CMs from LQTS patients. iPSC technology offers a potentially unlimited source of materials for studies and has the

advantage of avoiding the conventional concerns related to the animal or embryonic materials. The crucial experiments performed in monogenic disease LQTS have greatly advanced the field, providing important proof of concept that iPS-CMs from LQTS patients can be used to recapitulate complex physiological phenotypes, probe drug screening, and toxicological testing, clarify the novel mechanism insights and rectify gene defects at the cellular and molecular levels. iPS-CMs in LQTS open a whole new window of research and thinking. We are on the road to the powerful applications of iPSCs someday, though this remains a largely unmapped route that requires us to proceed not only expeditiously but also cautiously.

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