Induced pluripotent stem cell-based therapies for inherited arrhythmias: Opportunities and challenges involved (Review)

GUOLIANG LI^* , XIN HE^* and CHAOFENG SUN

Department of Cardiovascular Medicine, The First Affiliated Hospital of Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi 710061, P.R. China

Received April 4, 2014; Accepted September 24, 2014

DOI: 10.3892/mmr.2014.2668

Abstract. The identification of induced pluripotent stem cell (iPSC) technology represents great potential for recapitulating complex physiological phenotypes, probing toxicological testing and screening candidate drugs, demonstrating novel mechanistic insights and, in particular, applying iPSC-based therapeutic strategies for inherited disorders. Inherited arrhythmias are caused by various genetic abnormalities and harbor similar clinical outcomes. Clinically, the poorest outcomes are fatal arrhythmias and sudden cardiac death. However, the current therapeutic options for inherited arrhythmias are inadequate and problematic. In this review, we summarize the advances of the iPSC technique in the field of inherited arrhythmias and discuss the possibility of iPSC-based therapies for inherited arrhythmias. Additionally, we highlight the key challenges faced in the field of iPSC and the emerging strategies used to address these concerns before the novel technique can be used safely and efficiently in clinical practice. It is likely that the iPSC technique will present opportunities and further challenges in the future.

Contents

- 1. Introduction
- 2. Advances in iPSC technology
- 3. Advances in the iPSC technique in the field of inherited arrhythmias

Correspondence to: Professor Chaofeng Sun, Department of Cardiovascular Medicine, The First Affiliated Hospital of Xi'an Jiaotong University College of Medicine, 277 Yanta West Road, Xi'an, Shaanxi 710061, P.R. China E-mail: cfsun1@mail.xjtu.edu.cn

*Contributed equally

Key words: induced pluripotent stem cell, inherited arrhythmias, long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, therapies

- 4. iPSC-based therapeutic strategies for inherited arrhythmias
- 5. Challenges
- 6. Perspective
- 7. Conclusion

1. Introduction

Inherited arrhythmias are caused by certain genetic abnormalities, and the majority result in similar clinical outcomes. The worst of these are fatal arrhythmias and sudden cardiac death (SCD) (1-3). Inherited arrhythmic disorders consist of inherited long QT syndrome (LQTS; Table I), catecholaminergic polymorphic ventricular tachycardia (CPVT), J wave syndrome (JWS) and other rare arrhythmic disorders, including familial atrial fibrillation resulting from gain-of-function mutations (3-5). Inherited LQTSs are inherited electrical heart diseases characterized by prolongation of the QT interval on the surface electrocardiogram. Clinically, patients with LQTS are at increased risk of torsade de pointes, a malignant polymorphic ventricular tachycardia that either self-terminates or progresses into cardiac arrest or SCD. To date, hundreds of mutations responsible for LQTS have been identified in at least 13 genes (Table I) (6). JWS may be inherited or acquired (7,8). To date, inherited JWSs have been associated with hundreds of mutations in multiple genes encoding the cardiac ion channels or genes associated with the regulation of channel function, including SCN5A, CACNA1C, CACNB2b, CACNA2D1, glycerol-3-phosphate dehydrogenase 1-like enzyme gene, SCN1B, KCNE3, SCN3B, KCNJ8 and KCND3 (9). CPVT, also known as familial polymorphic ventricular tachycardia, is a familial arrhythmogenic syndrome caused by unstable sarcoplasmic reticulum calcium storage leading to exercise- or emotion-induced ventricular tachyarrhythmias and SCD (10-12). The majority of CPVT cases are associated with dominant mutations in the cardiac ryanodine receptor gene (RyR2), with variable penetrance, whereas the minority of cases result from recessive mutations in the cardiac calsequestrin isoform 2 gene (12-14). Clinically, CPVT is characterized by the most common symptoms of dizziness and syncope, which often occur during exercise or as a response to emotional stress. Individuals affected by CPVT have a notably increased risk of SCD. The first syncope

Туре	Affected gene	Chromosome	Protein	Current	Trigger	Proportion
LQT1	KCNQ1	11p15	$I_{\rm Ks} \alpha$ -submit	$I_{ m Ks}\downarrow$	Exercise	42%
LQT2	KCNH2	7q35-36	$I_{\rm Kr} \alpha$ -submit	$I_{\rm Kr}\downarrow$	Ring, exercise, waking	45%
LQT3	SCN5A	3p21-23	$I_{\rm Na} \alpha$ -submit	$I_{\rm Na}$	Rest, sleep	8%
LQT4	ANK2	4q25-27	Ankyrin-B	$I_{Ca2+}\uparrow$	Exercise	<1%
LQT5	KCNE1	21p22	$I_{\rm Ks}\beta$ -submit	$I_{\rm Ks}\downarrow$	Rage, exercise	3%
LQT6	KCNE2	21p22	$I_{\rm Kr}\beta$ -submit	$I_{\rm Kr}\downarrow$	Rest, exercise	2%
LQT7	KCNJ2	17p23-24	I _{K1}	$I_{\text{K2.1}}\downarrow$	Rest, exercise	<1%
LQT8	CACNA1	12p13	$I_{Ca} \alpha$ -submit	$I_{Ca}\uparrow$	Exercise, nervousness	<1%
LQT9	CAV3	3p25	Caveolin	$I_{\rm Na}$	Rest, sleep	Rare
LQT10	SCN4B	11q23	NAVβ4	$I_{\rm Na}$	Exercise	N/A
LQT11	AKAP-9	7q21-22	Yotiao	$I_{\rm Ks}\downarrow$	N/A	N/A
LQT12	SNTA1	20q11	α1 syntrophin	I _{Na} ↑	N/A	N/A
LQT13	KCNJ5	11q24	Kir4.3	$I_{\text{K-ACH}}\downarrow$	N/A	N/A
JLN1	KCNQ1	11p15	$I_{\rm Ks} \alpha$ -submit	$I_{\rm Ks}\downarrow$	Rage, exercise	1-7%
JLN2	KCNE1	21p22	$I_{\rm Kr}$ β -submit	$I_{ m Ks} \downarrow$	Rage, exercise	<1%

Table I. Inherited long QT syndrome.

LQT, long QT syndrome; JLN, Jervell and Lange-Nielsen syndrome.

in untreated patients often manifests around the age of 40, and the mortality rate is 30 to 50% (15,16).

Conventional studies have provided great insight into the mechanisms underlying inherited arrhythmias. For example, Delisle et al summarized the classification scheme for mutations leading to long QT type 2 (LQT2) (17): 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; and Class 5 mutations cause a decrease in mutant mRNAs through nonsense-mediated decay, thereby altering the amount of mRNA available for subsequent human ether-à-go-go-related gene (hERG) protein generation (17,18). More recently, Wu et al initially identified the molecular mechanisms underlying the adrenergic-induced QT prolongation associated with KCNQ1 mutations (19). However, previous insight into inherited arrhythmias mainly relied on particular cell types or animals, which do not always accurately reproduce the human phenotypes. With respect to the therapeutic strategies, conventional treatments are problematic in reducing the frequency of SCD in patients with inherited arrhythmias (20-22). The current situation has prompted the search for alternative therapeutic strategies. The development of a model system in which a mutant channel can be studied in human cardiomyocytes (CMs) may provide an ideal platform for better understanding inherited arrhythmias and evaluating newer therapeutic approaches.

Cellular reprogramming has successfully shown that patient profiles and response to drugs may be truly reflected through patient-derived induced pluripotent stem cell (iPSC) cultures, promising notable potential in the field of regenerative medicine (23,24). iPSCs differentiate into various somatic cell lines of the human body, demonstrate the capacity of unlimited replication and, notably, bypass conventional ethical and technical issues. Additionally, iPSCs represent a more effective source of producing patient-specific and disease-specific adult cells for therapeutic applications in the field of inherited arrhythmias.

In this review, we summarize the advances in the iPSC technique in the field of inherited arrhythmia and discuss the possibility of using iPSC-based therapies for its treatment. Additionally, we highlight the key challenges the iPSC field has faced and the emerging strategies to address these concerns before the iPSC technique can be used both safely and efficiently in clinical practice, providing a potential information resource for researchers who intend to exploit the iPSC technique in the area of inherited arrhythmias. This will be of great significance, as the detection of inherited arrhythmias is increasing and they are emerging as a critical threat to public health.

2. Advances in iPSC technology

Takahashi and Yamanaka were the first to reprogram fully differentiated adult mouse cells into iPSCs in 2006 (24). Since then, a number of studies have reported notable advances in iPSCs over recent years. iPSCs are generated most commonly from fibroblasts but also from other cell types, including hepatocytes, gastric epithelial cells, gastric epithelial cells, B lymphocytes, keratinocytes and pancreatic cells (25,26). Additionally, researchers have used various approaches to improve the drawbacks of low efficiency and the relatively long process of reprogramming. A tenfold more efficient generation of iPSCs has been reported using a polycistronic vector to transfer the reprogramming factors (27). Moreover, small molecules have also been reported to significantly improve reprogramming efficiency (28). Researchers have reported

Syndrome	Species	Affected gene	Methodology
SMA	Humans	SMN2	Single-stranded oligonucleotides
Tyrosinemia type 1	Mice	FAH	Tetraploid embryo complementation method, transduced FAH cDNA into the FAH ^{-/-} -iPSCs using a third-generation lentiviral vector to generate gene-corrected iPSCs.
FH	Humans	LDLR	A plasmid vector carrying the normal receptor ORF to genetically transform human iPSCs
AAT deficiency	Humans	AAT	Transcription activator-like effector nuclease technology
Hemophilia	Mice	F9	ZFNs
DMD	Mice and humans	Dystrophin	Transferring the DYS-HAC via MMCT
β-thalassemia major	Mice and humans	β-globin	Homologous recombination
LQT	Humans	KCNH2 (hERG)	Allele-specific RNAi
SCN	Humans	HAX1	Lentiviral transduction

Table II. Representative examples of induced pluripotent stem cell-based therapies.

SMA, spinal muscular atrophy; iPSC, induced pluripotent stem cell; FH, familial hypercholesterolemia; AAT, alpha-1 antitrypsin; ZFNs, zinc finger nucleases; DMD, Duchenne muscular dystrophy; MMCT, microcell-mediated chromosome transfer; DYS-HAC, human artificial chromosome with a complete genomic dystrophin sequence; LQT, long QT syndrome; hERG, human ether-à-go-go-related gene; RNAi, RNA interference; SCN, severe congenital neutropenia.

that iPSCs may be generated from mouse somatic cells using a combination of small-molecule compounds (29).

The majority of reprogramming strategies include delivering exogenous genes into somatic cells using retroviral vectors or non-integrating vectors. However, multiple reprogramming vectors possess the potential to induce pluripotency in somatic cells (25,26,30). The advances in RNA- and transcriptome-based techniques may overcome the safety issues involved with vector-based gene alterations (31).

Although primarily derived from mice, iPSCs are also derived from other species, including humans, rats, pigs and monkeys (23,32-34). Cellular reprogramming of the adult cells of larger animals to iPSCs represents a great achievement in the area of regenerative medicine. Larger animals, such as pigs and monkeys, share the same physiological and morphological properties as human beings, providing an ideal model similar to humans for preclinical trials (32-34).

iPSCs hold great promise, primarily due to their powerful potential for modeling human diseases. Multiple disease-specific iPSCs have been cloned from patients with various diseases, including amyotrophic lateral sclerosis, Parkinson's, muscular dystrophy, Huntington's disease and familial hypercholesterolemia (35-40). The potential to restore pluripotency to somatic cells of patients has created a new abundance of opportunities for modeling diseases without an appropriate research model, offering the possibility of personalized regenerative therapies (12,41-46). This method may revolutionize the current treatment strategies for human disease in the future, particularly in the case of inherited arrhythmias, for which we discuss the possibilities below.

iPSC-based therapies involve two aspects. The first, which presents a greater number of challenges, is to create

patient-specific iPSCs or derived body cells to achieve therapeutic potential using autologous gene correction (47-55). iPSC-based therapies created by editing disease-associated mutations are a promising therapeutic strategy for inherited disorders, leading to the restoration of normal gene function. Spinal muscular atrophy (SMA) is caused by homozygous mutations of the survival motor neuron 1 gene (56). Corti et al initially demonstrated that generating genetically corrected iPSCs obtained from SMA patients and differentiating them into motor neurons may provide a source of motor neurons for therapeutic transplantation in SMA (54). To date, a number of studies involving iPSC-based gene correction therapies have been reported (Table II). An alternative method is allogeneic cell replacement therapy. Somatic cells derived from healthy iPSCs are implanted into the pathological organs and then integrate and improve the condition in the disease model (57,58).

3. Advances in the iPSC technique in the field of inherited arrhythmias

Current models of cardiovascular disease are problematic. The lack of sufficient sources of CMs from patients for use *in vitro* and the inability to accurately model patient-specific disease variations significantly hamper the development of novel therapeutic strategies for inherited arrhythmias. With advances in cellular reprogramming techniques, the potential of iPSC-derived CMs (iPS-CMs) to model cardiovascular disorders may improve our understanding of the cellular and molecular mechanisms underlying inherited arrhythmias and promote our use of iPSC-based therapeutic strategies.

To date, multiple iPSC models of inherited arrhythmias have been established (Table III) (12,59-69). Itzhaki *et al*

ias
E
¥t.
arrhy
p
erite
hh
fi
0
e
ро
Ē
IF
č
ű
ste
nt
te
j
Ξŋ.
ηd
p
ğ
ιpι
.H
a u
sti
.X
Ξ
le
ab
E

Syndrome	Species	Affected gene	Mutation	Somatic cells	Vector	Transcription factors	Methodology	Drug testing
CPVT	Humans	RyR2	M4109R	Fibroblasts	Retroviruses	OCT4, SOX2 and KLF4	Immunostainings, whole-cell patch-clamp recordings, microelectrodearray recordinos calcium imaoino	Yes
LQT1	Humans	KCNQ1	R190Q	Fibroblasts	Retroviruses	OCT3/4, SOX2, KLF4 and c-MYC	Whole-cell patch clamp, immunofluorescence	Yes
LQT2	Humans	KCNH2 (hERG)	A614V	Fibroblasts	Retroviruses	SOX2, KLF4 and OCT4	Whole-cell patch clamp, extracellular multielectrode recordings	Yes
Timothy syndrome	Humans	CACNAIC	G406R	Fibroblasts	Retroviruses	SOX2, OCT3/4, KLF4 and c-MYC	Whole-cell patch clamp, calcium imaging	Yes
LQT2	Humans	KCNH2 (hERG)	G1681A	Fibroblasts	Lentivirus	OCT4, NANOG, SOX2 and LIN28	Whole-cell patch clamp, extracellular multielectrode recordings	Yes
LQT2	Humans	KCNH2 (hERG)	R176W	Fibroblasts	Retroviruses	OCT4, SOX2, KLF4 and MYC	Whole-cell patch clamp, extracellular multielectrode recordings	Yes
LQT1	Humans	KNCQ1	1893delC (P631fs/33)	Fibroblasts	Lentivirus	OCT3/4, SOX2, KLF4, and c-MYC	Extracellular multielectrode recordings, patch-clamp analysis and immunostaining	Yes
LQT2	Humans	KCNH2 (hERG)	G603D	T lymphocytes	Sendai virus vectors	OCT3/4, SOX2, KLF4 and c-MYC	Immunocytochemistry	No
LQT3	Mice	SCN5A	AKPQ	Fibroblasts	Retroviruses	OCT4, SOX2 and KLF4 or additionally with the fourth factor c-MYC	Patch-clamp technique, immunostaining	Yes
LQT2	Humans	KCNH2 (hERG)	G1681A	Fibroblasts	Lentivirus	OCT4, NANOG, SOX2 and LIN28	Allele-specific RNA interference, whole-cell patch-clamp, multi-electrode arrav analvsis	Yes
LQT2	Humans	KCNH2 (hERG)	W1001X	Fibroblasts	Episomal vectors	OCT4, SOX2, NANOG, LIN28, c-MYC and KLF4	Confocal imaging, voltage clamp	Yes
CPVT, catecho	laminergic poly	morphic ventricular tach	ıycardia; RyR2, ryan	odine receptor isoform	1 2; LQT, long QT s	yndrome; hERG, human ether-à-go	-go-related gene.	

reported the development of disease-specific human iPS-CMs obtained from a patient with LQT2 due to the A614V missense mutation in the hERG gene (62). Detailed whole-cell patch-clamp and extracellular multi-electrode recordings revealed evident prolongation of the action-potential duration (APD) in LQTS human iPS-CMs when compared with healthy control iPS-CMs. Further voltage-clamp analysis confirmed that this APD prolongation was the result of a significant reduction of a rapidly activating component of the delayed rectifier K^+ current (I_{Kr}). Significantly, LQTS-derived iPS-CMs also demonstrate marked arrhythmogenicity, characterized by early afterdepolarization (EAD) and triggered arrhythmias. These authors also used a human iPSC-derived cardiac tissue LQTS model to evaluate the potency of existing and novel pharmacological compounds that may either aggravate or improve the disease phenotype (62). Additionally, these authors also initially demonstrated the potential of human iPSCs to model CPVT (12). They demonstrated the development of delayed afterdepolarizations (DADs) in approximately 70% of the CPVT iPS-CMs compared with approximately 10% in healthy control iPS-CMs. They further illustrated that adrenergic stimulation by isoproterenol or forskolin increased the frequency and magnitude of afterdepolarizations and led to the development of triggered activity in the CPVT iPS-CMs. In contrast, flecainide and thapsigargin eliminated all afterdepolarizations in these cells (12). Laser-confocal calcium imaging revealed significant whole-cell calcium transient irregularities (frequent local and large-storage calcium-release events, broad and double-humped transients, and triggered activity) in the CPVT CMs, which worsened with adrenergic stimulation and calcium overload and improved with β-blockers. Store overload-induced calcium release was also identified in the iPS-CMs, and the threshold for such events was significantly reduced in the CPVT cells.

Currently, the significant advances in iPSC technology reveal the transformative potential of iPS-CMs in patients with inherited arrhythmias, which offer a potentially unlimited source of materials for biomedical study. These iPS-CMs may be used to recapitulate complex physiological phenotypes, probe toxicological testing and drug screening, clarify novel mechanistic insights and also potentially rectify gene defects at the cellular and molecular levels (12). Despite the emerging challenges, iPSC technology has been increasingly recognized as a valuable and growing tool kit for modeling inherited arrhythmias. These achievements indicate that human iPSC technology enables the modeling of the abnormal functional phenotype of inherited arrhythmias and the screening of new potential therapeutic agents, representing a promising paradigm to study disease mechanisms, optimize patient care and aid in the development of new therapies.

4. iPSC-based therapeutic strategies for inherited arrhythmias

With respect to the treatment of inherited arrhythmias, the current strategies include β -blockers, surgery for the implantation of pacemakers, cardioverter defibrillators and left cardiac sympathetic denervation (70). However, the current therapeutic options are empirical and do not eliminate the risk of

SCD. For example, an implantable cardioverter defibrillator (ICD) is highly effective in terminating malignant ventricular arrhythmias and appears to be the most effective treatment available in the prevention of SCD in a variety of other clinical disorders. However, in the field of inherited arrhythmias, inappropriate therapy and even exacerbation of the arrhythmic disorders by ICD therapy is possible (21,71-73).

As for the therapeutic advantages of iPSC-CMs for individuals with arrhythmic disorders, the most significant is the provision of a high-throughput model system in which to screen more conventional pharmacological approaches. However, the present review will mainly focus on the anticipation of translating cell-based therapies for inherited arrhythmias into reality following initial application of the iPSC technique in these diseases (64). The recent application of iPSC technology for the treatment of LOTS was the first step. By coupling iPSC technology with RNA interference (RNAi), Matsa et al produced corrected iPS-CMs from LQT2 patients carrying a hERG G1681A mutation (68). Allele-specific RNAi directed towards the mutated hERG mRNA resulted in its knockdown while leaving the wild-type mRNA unaffected. Electrophysiological analysis revealed normalized APDs and K⁺ currents with the concurrent rescue of spontaneous and drug-induced arrhythmias in patientderived iPS-CMs treated with mutation-specific siRNAs. These findings provide initial in vitro evidence that the iPSC technique combined with other gene-editing methods may rescue the diseased phenotypes of iPS-CMs obtained from patients with inherited arrhythmias.

This is a potentially novel route for the treatment of numerous autosomal dominant-negative inherited disorders, extending our capacity to develop new therapeutic strategies for inherited arrhythmias. There are two notable characteristics of this type of research. First, the ability to 're-supply' the iPS-CMs and overcome their limited source will be possible with further research. Second, gene-corrected therapeutic iPS-CMs could be patient- and disease specific, overcoming issues concerning the immune rejection of therapeutic cells.

However, there are certain issues and concerns that need to be addressed with further research. For example, the steps following the rectification of mutant iPS-CMs in vitro must be determined. Here, we propose several possible schemes. The in vitro-corrected iPS-CMs could be injected into the heart with mutant CMs. However, this method involves a number of challenges, including how to replace or dispose of local mutant iPS-CMs and how to ensure that the corrected CMs delivered are integrated into the heart. The second proposal is based on the more recent development involving a notable step forward in regenerative medicine. Takebe et al initially demonstrated the generation of vascularized and functional human liver from human iPSCs following the transplantation of liver buds created in vitro (iPSC-LBs) (74). The formation of a functional vasculature stimulated the maturation of iPSC-LBs into tissue resembling the adult liver. These results highlight the immense therapeutic potential of using in vitro-grown organ-bud transplantation to treat organ failure. Based on this new idea, the same strategies used for the liver could potentially be applied to the heart in cases of malignant inherited arrhythmias, enabling the replacement of the whole end-stage heart with gene-edited iPSC-CMs.

5. Challenges

It is clear that iPS-CMs could be of benefit for diagnostics, research and therapeutic approaches. However, it may prove challenging to promote large quantities of iPSC-based therapies in the field of inherited arrhythmias in clinical practice since a number of obstacles, as discussed below, need to be overcome before the novel iPSC-based treatment strategies for inherited arrhythmias can be achieved safely and economically. These issues include the dynamic and complex properties of arrhythmic disorders in the clinic, the risk of teratoma formation, incomplete reprogramming, tissue-inappropriate differentiation, insertional mutagenesis caused by the reprogramming factors, the potential for genetic and epigenetic abnormalities and the immunogenicity of the transplanted cells. All major limitations preclude the use of this novel technique.

The first point to consider is the presence of the dynamic and complex characteristics of inherited arrhythmic disorders in clinical practice. The *in vitro* screening of isolated CMs has substantial limitations. Arrhythmias are initiated when a trigger encounters an appropriate arrhythmogenic substrate. EADs and DADs are accepted triggers for arrhythmias, although they are able to sustain the arrhythmia if they occur repetitively in high frequency. EADs and DADs may be studied and predicted in iPS-CM cultures. In contrast, reentry is the primary mechanism underlying cardiac arrhythmias, and it is impossible to study reentry in isolated cells as this is a multi-cellular phenomenon. This may be a significant limitation in all research approaches dealing with isolated cells, and also applies to iPS-CMs used for studying inherited arrhythmias.

The second concern to highlight is the presence of residual undifferentiated iPSCs, which may lead to tumorigenicity following delivery into patients. Evidence indicates that the pluripotent potential of iPSCs is associated with rapid cellular growth, which is one of the properties of cancer (75,76). Wu *et al* demonstrated that iPSCs remained pluripotent following the correction of genetic defects (53). In accordance with this, other studies also confirmed that iPSCs have similarities with malignant cancer cells (77). Therefore, the primary impediment to the use of the iPSC technique in regenerative medicine is likely to be preventing tumorigenicity.

The third issue of concern is that generating iPSCs may introduce unexpected mutations and genomic alterations, which may induce cancer in the host (57). Despite the improvements leading to virus-free and transgene-free reprogramming in recent research, the potential for inducing genetic and epigenetic abnormalities remains. Therefore, the iPSC lines used in pre-clinical trials need to be validated not only in small animal models but also in large animal models that are more physiologically similar to humans, ensuring their safe application in future trials (32,78-80).

iPSCs harbor other problems as *in vitro* models of various diseases. For example, it is known that chromosomal instability and molecular changes exist in iPSC lines. Therefore, a disease model using iPSCs is not yet a perfect model for analyzing the pathophysiology of a disease. Additionally, there is no current evidence supporting the use of iPSC-based applications *in vivo* in the field of inherited arrhythmias. Animal models may be useful for providing this evidence in the future.

Animal models have traditionally been considered more informative than cell-based *in vitro* approaches, and mice have become the gold standard for human disease modeling *in vivo*. For example, animal models are used to evaluate the therapeutic potential of candidate compounds in the presence of an intact heart *in vivo* as well as the adverse drug reactions of other organ systems. Such issues need to be further examined before iPSC-based therapies are able to contribute to the field of inherited arrhythmias.

6. Perspective

The recent advances in iPSC technology have set the stage for devising alternative strategies for the treatment of inherited arrhythmias. The generation of patient-specific iPS-CMs derived from individuals with inherited arrhythmias will offer the possibility to characterize these inherited disorders, screen new therapeutics and, in particular, improve the limited treatment strategies currently available to patients. Furthermore, iPSC technology may ultimately provide clinical trials of personalized therapies not only for inherited arrhythmias including LQTS, CPVT, JWS and symptomatic atrial fibrillation, but also other inherited disorders. For researchers, however, iPSC technology is currently a black box. iPSC-based therapies are still in their infancy, and there are likely to be numerous challenges ahead.

The future use of iPSC technology in clinical practice primarily depends on how accurately iPSCs are able to differentiate into the affected cells and on the development of safe and effective methods to deliver them into the human body. For instance, although it is necessary to establish methods to generate functional CMs from gene-corrected iPSCs *in vitro*, it remains to be determined whether the findings of *in vitro* and *in vivo* models are also valid for human iPSCs. Moreover, cell derivation protocols and transplantation procedures still need to be optimized, and further research is required to advance our knowledge of the mechanisms underlying cellular reprogramming. With regard to the limitations of studying multi-cellular preparations, iPSC technology in combination with other modern techniques, including computational modeling, may be helpful as a supplemental tool.

It may prove challenging to safely produce sufficient quantities of clinical-grade, transplantable iPSCs to treat inherited arrhythmias; however, the development of a global iPSC bank may boost productivity, thus furthering the use of the iPSC technique and creating additional clinical applications (81). Due to the speed and extent of iPSC technological advances in recent years, particularly in cardiovascular regenerative medicine, iPSC therapy is likely to play a major role, providing a strong foundation on which to build and expand our knowledge and identify new opportunities for iPSC-based therapeutic strategies and personalized medicine in the field of inherited arrhythmias.

7. Conclusion

iPSC technology represents a substantial promise in the field of regenerative medicine. In this review, the advances in iPSC technology and iPSC-based therapies for inherited arrhythmias were summarized. Several hypotheses on the use of iPSC-based therapies in the field of inherited arrhythmias in further clinical practice were also discussed. Finally, the key challenges that the iPSC field has faced were highlighted, as well as the emerging strategies used to address these concerns before the iPSC technique can be used both safely and efficiently in the clinical practice. The iPSC technique will likely present opportunities and challenges in the future.

Acknowledgements

The authors are grateful for the advice of Changcong Cui with regard to this manuscript. This study was supported by the National Natural Science Foundation of China (81270236) and Xi'an Jiaotong University Graduate Independent Innovation Research (CX1116).

References

- Schwartz PJ, Stramba-Badiale M, Crotti L, *et al*: Prevalence of the congenital long-QT syndrome. Circulation 120: 1761-1740, 2009.
- Nader A, Massumi A, Cheng J and Razavi M: Inherited arrhythmic disorders: long QT and Brugada syndromes. Tex Heart Inst J 34: 67-75, 2007.
- Obeyesekere MN, Klein GJ, Modi S, *et al*: How to perform and interpret provocative testing for the diagnosis of Brugada syndrome, long-QT syndrome, and catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol 4: 958-964, 2011.
- 4. Bartos DC, Duchatelet S, Burgess DE, *et al*: R231C mutation in KCNQ1 causes long QT syndrome type 1 and familial atrial fibrillation. Heart Rhythm 8: 48-55, 2011.
- Chen YH, Xu SJ, Bendahhou S, *et al*: KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 299: 251-254, 2003.
- Tester DJ and Ackerman MJ: Genetic testing for potentially lethal, highly treatable inherited cardiomyopathies/channelopathies in clinical practice. Circulation 123: 1021-1037, 2011.
- 7. Antzelevitch C and Yan GX: J wave syndromes. Heart Rhythm 7: 549-558, 2010.
- Antzelevitch C, Yan GX and Viskin S: Rationale for the use of the terms J-wave syndromes and early repolarization. J Am Coll Cardiol 57: 1587-1590, 2011.
- 9. Antzelevitch C: Genetic, molecular and cellular mechanisms underlying the J wave syndromes. Circ J 76: 1054-1065, 2012.
- Hayashi M, Denjoy I, Extramiana F, et al: Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation 119: 2426-2434, 2009.
- Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD and Coumel P: Catecholaminergic polymorphic ventricular-tachycardia in children. A 7-year follow-up of 21 patients. Circulation 91: 1512-1519, 1995.
- Itzhaki I, Maizels L, Huber I, *et al*: Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. J Am Coll Cardiol 60: 990-1000, 2012.
- Laitinen PJ, Brown KM, Piippo K, *et al*: Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. Circulation 103: 485-490, 2001.
 Priori SG, Napolitano C, Tiso N, *et al*: Mutations in the cardiac
- Priori SG, Napolitano C, Tiso N, *et al*: Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 103: 196-200, 2001.
- Kauferstein S, Kiehne N, Neumann T, Pitschner H and Bratzke H: Cardiac gene defects can cause sudden cardiac death in young people. Dtsch Arztebl Int 106: 41-47, 2009.
- Mohamed U, Napolitano C and Priori SG: Molecular and electrophysiological bases of catecholaminergic polymorphic ventricular tachycardia. J Cardiovasc Electrophysiol 18: 791-797, 2007.
- Delisle BP, Anson BD, Rajamani S and January CT: Biology of cardiac arrhythmias: ion channel protein trafficking. Circ Res 94: 1418-1428, 2004.

- Gong Q, Zhang L, Vincent GM, Horne BD and Zhou Z: Nonsense mutations in hERG cause a decrease in mutant mRNA transcripts by nonsense-mediated mRNA decay in human long-QT syndrome. Circulation 116: 17-24, 2007.
- Wu J, Naiki N, Ding WG, *et al*: A molecular mechanism for adrenergic-induced long QT syndrome. J Am Coll Cardiol 63: 819-827, 2014.
- 20. Schwartz PJ, Spazzolini C, Priori SG, *et al*: Who are the long-QT syndrome patients who receive an implantable cardioverter-defibrillator and what happens to them?: data from the European Long-QT Syndrome Implantable Cardioverter-Defibrillator (LQTS ICD) Registry. Circulation 122: 1272-1282, 2010.
- Goel AK, Berger S, Pelech A and Dhala A: Implantable cardioverter defibrillator therapy in children with long QT syndrome. Pediatr Cardiol 25: 370-378, 2004.
- 22. Benito B, Guasch E, Rivard L and Nattel S: Clinical and mechanistic issues in early repolarization of normal variants and lethal arrahythmia syndromes. J Am Coll Cardiol 56: 1177-1186, 2010.
- 23. Takahashi K, Tanabe K, Ohnuki M, *et al*: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872, 2007.
- Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676, 2006.
- Sun N, Longaker MT and Wu JC: Human iPS cell-based therapy: considerations before clinical applications. Cell Cycle 9: 880-885, 2010.
- Miyazaki S, Yamamoto H, Miyoshi N, *et al*: Emerging methods for preparing iPS cells. Jpn J Clin Oncol 42: 773-779, 2012.
- Carey BW, Markoulaki S, Hanna J, et al: Reprogramming of murine and human somatic cells using a single polycistronic vector. Proc Natl Acad Sci USA 106: 157-162, 2009.
- Desponts C and Ding S: Using small molecules to improve generation of induced pluripotent stem cells from somatic cells. Methods Mol Biol 636: 207-218, 2010.
- Hou P, Li Y, Zhang X, *et al*: Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 341: 651-654, 2013.
- Deng W: Induced pluripotent stem cells: paths to new medicines. A catalyst for disease modelling, drug discovery and regenerative therapy. EMBO Rep 11: 161-165, 2010.
- 31. Zhang R, Zhang L-H and Xie X: iPSCs and small molecules: a reciprocal effort towards better approaches for drug discovery. Acta Pharmacol Sin 34: 765-776, 2013.
- 32. Liu H, Zhu F, Yong J, *et al*: Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 3: 587-590, 2008.
- 33. Morizane A, Doi D, Kikuchi T, Okita K, Hotta A, Kawasaki T, et al: Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a nonhuman primate. Stem Cell Reports 1: 283-292, 2013
- 34. Okamoto S and Takahashi M: Induction of retinal pigment epithelial cells from monkey iPS cells. Invest Ophthalmol Vis Sci 52: 8785-8790, 2011.
- Mackay-Sim A: Patient-derived stem cells: pathways to drug discovery for brain diseases. Front Cell Neurosci 7: 29, 2013.
- Dimos JT, Rodolfa KT, Niakan KK, et al: Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321: 1218-1221, 2008.
- Ebert AD, Yu J, Rose FF Jr, *et al*: Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457: 277-281, 2009.
- Lee G, Papapetrou EP, Kim H, *et al*: Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461: 402-406, 2009.
- 39. Park I-H, Arora N, Huo H, *et al*: Disease-specific induced pluripotent stem cells. Cell 134: 877-886, 2008.
- 40. Amenduni M, De Filippis R, Cheung AYL, *et al*: iPS cells to model CDKL5-related disorders. Eur J Hum Genet 19: 1246-1255, 2011.
- 41. Webb S: iPS cell technology gains momentum in drug discovery. Nat Rev Drug Discov 8: 263-264, 2009.
- 42. Gunaseeli I, Doss MX, Antzelevitch C, Hescheler J and Sachinidis A: Induced pluripotent stem cells as a model for accelerated patient- and disease-specific drug discovery. Curr Med Chem 17: 759-766, 2010.
- Zhou Q: Progress and prospects in stem cell therapy and drug discovery. Acta Pharmacol Sin 34: 717-718, 2013.

- 44. Mordwinkin NM, Burridge PW and Wu JC: A review of human pluripotent stem cell-derived cardiomyocytes for high-throughput drug discovery, cardiotoxicity screening, and publication standards. J Cardiovasc Transl Res 6: 22-30, 2013.
- 45. Merkle FT and Eggan K: Modeling human disease with pluripotent stem cells: from genome association to function. Cell Stem Cell 12: 656-668, 2013.
- 46. Siller R, Greenhough S, Park IH and Sullivan GJ: Modelling human disease with pluripotent stem cells. Curr Gene Ther 13: 99-110, 2013.
- 47. Morishima T, Watanabe K, Niwa A, *et al*: Genetic correction of HAX 1 in induced pluripotent stem cells from a patient with severe congenital neutropenia improves defective granulopoiesis. Haematologica 99: 19-27, 2014.
- Wang Y, Žheng CG, Jiang Y, *et al*: Genetic correction of beta-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice. Cell Res 22: 637-648, 2012.
 Choi SM, Kim Y, Shim JS, *et al*: Efficient drug screening and
- 49. Choi SM, Kim Y, Shim JS, *et al*: Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. Hepatology 57: 2458-2468, 2013.
 50. Raya A, Rodriguez-Piza I, Guenechea G, *et al*: Disease-corrected
- Raya A, Rodriguez-Piza I, Guenechea G, *et al*: Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature 460: 53-59, 2009.
- 51. Kazuki Y, Hiratsuka M, Takiguchi M, *et al*: Complete genetic correction of iPS cells from Duchenne muscular dystrophy. Mol Ther 18: 386-393, 2010.
- 52. Li H, Haurigot V, Doyon Y, *et al*: In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature 475: 217-221, 2011.
- 53. Wu G, Liu N, Rittelmeyer I, *et al*: Generation of healthy mice from gene-corrected disease-specific induced pluripotent stem cells. PLoS Biol 9: e1001099, 2011.
- 54. Corti S, Nizzardo M, Simone C, et al: Genetic correction of human induced pluripotent stem cells from patients with spinal muscular atrophy. Sci Transl Med 4: 165ra162, 2012.
- 55. Fattahi F, Asgari S, Pournasr B, et al: Disease-corrected hepatocyte-like cells from familial hypercholesterolemia-induced pluripotent stem cells. Mol Biotechnol 54: 863-873, 2013.
- 56. Lunn MR and Wang CH: Spinal muscular atrophy. Lancet 371: 2120-2133, 2008.
- 57. Robinton DA and Daley GQ: The promise of induced pluripotent stem cells in research and therapy. Nature 481: 295-305, 2012.
- 58. Wernig M, Zhao J-P, Pruszak J, et al: Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci USA 105: 5856-5861, 2008.
- 59. Malan D, Friedrichs S, Fleischmann BK and Sasse P: Cardiomyocytes obtained from induced pluripotent stem cells with long-QT syndrome 3 recapitulate typical disease-specific features in vitro. Circ Res 109: 841-847, 2011.
- 60. Matsa E, Rajamohan D, Dick E, *et al*: Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. Eur Heart J 32: 952-962, 2011.
- Mehta A, Sequiera GL, Sudibyo Y, *et al*: Derivation and characterization of transgene free induced pluripotent stem cell derived cardiomyocytes from asian patient with long QT syndrome. Eur Heart J 33: 22, 2012.
- 62. Itzhaki I, Maizels L, Huber I, *et al*: Modelling the long QT syndrome with induced pluripotent stem cells. Nature 471: 225-229, 2011.

- 63. Lahti AL, Kujala VJ, Chapman H, *et al*: Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis Model Mech 5: 220-230, 2012.
- Moretti A, Bellin M, Welling A, et al: Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 363: 1397-1409, 2010.
- 65. Sadguna YB, Jianhua Z, Amanda H, *et al*: Loss of ikr in lqt2 patient iPS-derived cardiomyocytes: nonsense mediated decay as a potential mechanism? Abstract 15643, 2011. http://circ.ahajournals.org/cgi/content/meeting_abstract/124/21_MeetingAbstracts/A15643.
- 66. Yazawa M, Hsueh B, Jia X, *et al*: Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 471: 230-234, 2011.
- 67. Egashira T, Yuasa S, Suzuki T, *et al*: Disease characterization using LQTS-specific induced pluripotent stem cells. Cardiovasc Res 95: 419-429, 2012.
- 68. Matsa E, Dixon JE, Medway C, *et al*: Allele-specific RNA interference rescues the long-QT syndrome phenotype in human-induced pluripotency stem cell cardiomyocyte. Eur Heart J 35: 1078-1087, 2013.
- 69. Okata S, Yuasa S, Yamane T, Furukawa T and Fukuda K: The generation of induced pluripotent stem cells from a patient with KCNH2 G603D, without LQT2 disease associated symptom. J Med Dent Sci 60: 17-22, 2013.
- 70. Schimpf R, Veltmann C, Wolpert C and Borggrefe M: Arrhythmogenic hereditary syndromes: Brugada Syndrome, long QT syndrome, short QT syndrome and CPVT. Minerva Cardioangiol 58: 623-636, 2010.
- Kron J, Öliver RP, Norsted S and Silka MJ: The automatic implantable cardioverter-defibrillator in young-patients. J Am Coll Cardiol 16: 896-902, 1990.
- 72. Groh WJ, Silka MJ, Oliver RP, Halperin BD, McAnulty JH and Kron J: Use of implantable cardioverter-defibrillators in the congenital long QT syndrome. Am J Cardiol 78: 703-706, 1996.
- Perry GY and Kosar EM: Problems in managing patients with long QT syndrome and implantable cardioverter defibrillators: a report of two cases. Pacing Clin Electrophysiol 19: 863-867, 1996.
- 74. Takebe T, Sekine K, Enomura M, et al: Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 499: 481-484, 2013.
- 75. Lee AS, Tang C, Cao F, *et al*: Effects of cell number on teratoma formation by human embryonic stem cells. Cell Cycle 8: 2608-2612, 2009.
- Cao F, Drukker M, Lin S, *et al*: Molecular imaging of embryonic stem cell misbehavior and suicide gene ablation. Cloning Stem Cells 9: 107-117, 2007.
- Riggs JW, Barrilleaux BL, Varlakhanova N, Bush KM, Chan V and Knoepfler PS: Induced pluripotency and oncogenic transformation are related processes. Stem Cells Dev 22: 37-50, 2013.
- Esteban MA, Xu J, Yang J, et al: Generation of induced pluripotent stem cell lines from Tibetan miniature pig. J Biol Chem 284: 17634-17640, 2009.
- Ezashi T, Telugu BPVL, Alexenko AP, Sachdev S, Sinha S and Roberts RM: Derivation of induced pluripotent stem cells from pig somatic cells. Proc Natl Acad Sci USA 106: 10993-10998, 2009.
- Wu Z, Chen J, Ren J, *et al*: Generation of pig induced pluripotent stem cells with a drug-inducible system. J Mol Cell Biol 1: 46-54, 2009.
- Stacey GN, Crook JM, Hei D and Ludwig T: Banking human induced pluripotent stem cells: lessons learned from embryonic stem cells? Cell Stem Cell 13: 385-388, 2013.