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Genome Editing and Induced Pluripotent Stem Cell Technologies for Personalized Study of Cardiovascular Diseases

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Abstract

Purpose of Review—The goal of this review is to highlight the potential of induced pluripotent stem cell (iPSC)-based modeling as a tool for studying human cardiovascular diseases. We present some of the current cardiovascular disease models utilizing genome editing and patient-derived iPSCs.

Recent Findings—The incorporation of genome-editing and iPSC technologies provides an innovative research platform, providing novel insight into human cardiovascular disease at molecular, cellular, and functional level. In addition, genome editing in diseased iPSC lines holds potential for personalized regenerative therapies.

Summary—The study of human cardiovascular disease has been revolutionized by cellular reprogramming and genome editing discoveries. These exceptional technologies provide an opportunity to generate human cell cardiovascular disease models and enable therapeutic strategy development in a dish. We anticipate these technologies to improve our understanding of cardiovascular disease pathophysiology leading to optimal treatment for heart diseases in the future.

Keywords

Induced pluripotent stem cells; Cardiovascular disease; Genome editing; Cardiomyocytes; Personalized medicine

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Conflict of Interest Young Wook Chun, Matthew D. Durbin, and Charles C. Hong declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Papers of particular interest, published recently, have been highlighted as:

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•• Of major importance

Introduction

While heart disease remains a leading cause of morbidity and mortality in the world, the discovery of fundamental disease insights and transformative new therapies have not kept up with steady increase in disease prevalence. For instance, congenital heart disease is one of the most complicated and lethal diseases, yet its etiology remains mostly unknown, due in part to limitation in current disease models. Here, we review new findings in human cardiovascular disease, with a focus on patient-derived stem cell models and genome-editing technology.

Historical Cardiovascular Disease Models

According to the American Heart Association, cardiovascular disease accounted for one in three deaths in the USA. To meet the tremendous need for transformative new therapies for heart disease, it is essential to improve our understanding of its pathophysiology. Because human cardiovascular pathology is complex, with multiple contributing factors, both genetic and environmental, it is important to develop disease models that optimally recapitulate key aspects of cardiovascular disease in human. In the past three decades, important insights into cardiovascular disease pathophysiology have been provided through animal models. For example, the use of transgenic and genetic knockout mice allows for examination of the effects of genetic manipulation of a single or very small number of genes involved in heart development or physiology. However, major differences in human and rodent cardiovascular systems present important limitations [1, 2]. Due to species-specific differences, a genetic perturbation in mice may not accurately reflect human disease processes. Also, many adult-onset diseases or those having polygenic contributions are not faithfully recapitulated using mouse models. Thus, the direct relevance of many mouse models to human cardiovascular diseases is often uncertain [3]. Alternatively, large animal models of human heart disease have been utilized based on the premise that they are more physiologically and clinically similar to humans than the mice. For instance, a dog’s cardiovascular physiology is similar to humans in many respects, including the heart rate, blood pressure, and the contributions of sympathetic and parasympathetic nervous system [4]. Humans and dogs are also highly similar at the cellular and molecular levels, including the transmural and apicobasal distribution of key ion channels in the heart [5, 6]. However, there are several important limitations to large animal models including much higher maintenance costs and longer gestation time, making large animals less optimal as genetic models [7, 8]. For obvious reasons, the use of human heart tissue for experimentation is highly restricted. Lastly, isolated human or animal cardiomyocytes are in a highly catabolic state, rapidly approaching death within a few days of culture.

Given the limitations of current models, the emergence of human pluripotent stem cells, capable of multiplying indefinitely and differentiating to all tissue types such as the cardiovascular cells, has raised substantial interest in using them to study human cardiovascular diseases. This revolutionary technology, together with equally revolutionary advances in genomic editing, has provided an unprecedented opportunity for modeling human cardiovascular diseases.

Induced Pluripotent Stem Cells as Cardiovascular Disease Models

Pluripotent embryonic stem cells (ESCs) were first discovered in the blastocyst of developing mouse embryos [9, 10] and subsequently in human embryos [11]. The ESCs possessed the ability to continuously replicate themselves and to differentiate to all tissue types. Despite their vast potential, however, the human ESCs were not widely adopted due to significant technical limitations and ethical concerns regarding the destruction of human embryos. In 2006, by introducing the four transcription factors *OCT4*, *SOX2*, *LKF4*, and *c-MYC*, Shinya Yamanaka and colleagues perfected the method to reprogram terminally differentiated somatic cells into pluripotent stem cells, which they termed induced pluripotent stem cells (iPSCs) [12–14]. Like ESCs, the iPSCs are pluripotent, capable of differentiating to virtually all cell types [15]. However, as opposed to ESCs, iPSCs could be generated from readily accessible tissue samples, including skin, blood, and urine [16–18]. While the early efforts involved the delivery of the reprogramming transcription factors via retroviruses, which posed tumorigenic potential by genome integration, more recent reprogramming efforts involve vectors that do not integrate into the genome [19, 20]. Based on these and other advances, reprogramming a patient's cells to generate iPSCs as a renewable source of autologous donor cells and tissues has become commonplace.

The iPSC technology, along with advances in techniques for directed differentiation into cardiovascular cells, has already made important impact in cardiovascular biology. Among the first cardiac diseases to be modeled using this technology were the inherited arrhythmia syndromes since electrical abnormalities can be readily detected in hiPSC-CMs and because mouse, whose heart beats 10× faster than human heart, is suboptimal for modeling human arrhythmias [21, 22]. For example, long QT syndrome (LQTS) is a class of arrhythmias associated with delayed repolarization of cardiomyocytes that increases the risk of Torsade de Pointes (TdP), a ventricular arrhythmia which can cause sudden death [23–26]. While most LQTS is congenital, LQT and TdP can be triggered by certain drugs that can delay repolarization in susceptible individuals [27]. In 2010, Moretti and colleagues [22] successfully generated iPSC-CMs from patients with type 1 LQTS (LQTS1), caused by an autosomal dominant missense mutation (R190Q), in the *KCNQ1* gene, which encodes the repolarizing potassium channel responsible for the slow delayed rectifier I_{KS} current. Consistent with a defective repolarization, the action potential duration was prolonged in LQTS1 iPSC-CMs compared to controls from healthy donors. At the cellular level, LQTS1 iPSC-CMs exhibited a dominant negative trafficking defect in the potassium channel that caused a 70–80% diminution of the I_{KS} current, resulting in defective repolarization. In another study, Gaborit and colleagues generated iPSCs from squamous epithelial cells in urine samples of patients with type 2 LQTS (LQTS2), caused by a A561P mutation in the *KCNH2* gene, which encodes the potassium channel responsible for the rapid delayed rectifier I_{Kr} current [28]. The electrophysiological analysis of iPSC-CMs demonstrated that I_{Kr} was decreased by 50–60%, leading to a prolonged action potentials compared to controls. Additionally, LQTS2 iPSC-CMs exhibited significantly increased arrhythmogenicity when treated with clobutinol, an antitussive drug associated with drug-induced TdPs. Another model of LQTS utilized iPSC-CMs derived from an LQTS patient with an F142L mutation in the *CALM1* gene, encoding calmodulin1 [29]. This study found

that the mutant iPSC-CMs exhibited impaired calcium-dependent inactivation of the L-type calcium channel current (I_{CAL}), resulting in prolonging repolarization associated with I_{Ks} inhibition and delayed sodium channel (I_{Na}) inactivation. Interestingly, the electrical abnormalities in the mutant *CAML1* iPSC-CMs were exacerbated by β-adrenergic stimulation, consistent with what is observed in these patients [29]. Lastly, a study examining the effects of drugs associated with LQT and TdP on human iPSC-CMs supports the growing body of evidence for the role of the late sodium current in drug-induced arrhythmias [27]. Thus, patient-derived iPSC-CMs have proven valuable for studying pathogenesis of both inherited and drug-induced arrhythmias in man.

iPSC-CMs models have been developed for various cardiomyopathies, including familial dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). Wu and colleagues demonstrated that iPSC-CMs derived from familial DCM patients with a mutation in the *TNNT2* gene, encoding sarcomeric protein cardiac troponin T, recapitulated much of the morphological and functional defects seen in these patients. The mutant iPSC-CMs had altered beating rates and contraction, along with sarcomere disarray seen in the heart of DCM patients [30]. The Wu group's study of left ventricular non-compaction cardiomyopathy utilizing patient-derived iPSC-CMs determined that the mutation in the transcription factor TBX20 recapitulates pathological phenotypes at the single-myocyte level and demonstrated the relationship between defective TBX20 function and the aberrant regulation of its down-stream target *RDM16* via altered TGF-β signaling [31].

Patient-derived iPSC-CMs have also contributed to a better understanding of HCM pathogenesis. For example, the Wu group demonstrated that iPSC-CMs derived from familial HCM patients due to a R663H mutation in the MYH7 gene, encoding the β-myosin heavy chain, exhibited many hallmarks of HCM such as hypertrophy, abnormal calcium handling, increased arrhythmia propensity, increased β-myosin/α-myosin ratio, increased atrial natriuretic factor expression and calcineurin activation [32]. Blockage of Ca^{2+} entry by L-type Ca^{2+} channel blocker verapamil abrogated much of the HCM phenotype in iPSC-CMs, suggesting that elevated intracellular calcium plays a central role in HCM pathogenesis [33]. Importantly, this study provides intriguing insights to a potential pharmaceutical treatment of HCM. Taken together, these examples exhibit the importance of human cardiovascular models and the potential of iPSC technology for modeling diseases and developing therapeutic strategies.

Genome Editing for the Study of Cardiovascular Biology

Revolutionary advances in the ability to precisely edit genomic DNA will greatly aid cardiovascular research. Genome-editing techniques are classified based on the site-specific nucleases utilized: the meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPRs)-associated nuclease Cas9 (CRISPR/Cas9) [34–36]. These nucleases create sequencespecific double-stranded breaks (DSB), which are then repaired in the cell by two distinct endogenous repair systems: non-homologous end-joining (NHEJ) or homology-directed repair (HDR). NHEJ repair often results in insertions or deletions (indels) at the DSB sites, typically resulting in frameshift mutations [37]. Thus, NHEJ can generate loss-

of-function mutations in the targeted genes. By contrast, in HDR, a single- or double-stranded DNA template is utilized to repair the break site. Using template repair of DSB, specific changes or tag sequences can be introduced to any target gene. The endonucleases can be categorized into two groups by the mode of DNA recognition. Meganucleases, ZFNs, and TALENs bind specific DNA sequence through a protein-DNA interaction [38–43] whereas CRISPR-Cas9 is targeted to specific DNA sequence through a complementary base pairing involving a guide RNA (gRNA) [34, 44, 45]. The CRISPR-Cas9 genome editing has rapidly become the dominant technique given the relative efficiency of repair and the ease of genome targeting with site-specific gRNA.

While initial efforts at CRISPR-Cas9 genome editing resulted in low rates of HDR [34, 46], optimization efforts have led to substantial improvements in precise editing efficiencies [47]. Nonetheless, indels are common undesired byproduct of CRISPR-Cas9 genome editing since this approach depends on inducing DSBs. To reduce the formation of undesired indels during editing and to obviate the need for donor repair templates, a number of newer CRISPR-mediated base-editing methods, collectively called base editing, have been developed [48]. For example, Liu and colleagues developed Base Editor (BE) system, which utilizes a catalytically deactivated Cas9 (dCas) fused to a cytidine deaminase [49••, 50]. Basically, dCa9 binds to the target sequence in the genomic DNA without causing a DSB, and then the attached deaminase converts cytidine (C) into uridine (U). The resulting C:U mismatch is repaired in the cell by the mismatch base excision repair (BER) response, generating U:A base pair, which is subsequently converted to T:A [50]. Transfection of BE and a targeted gRNA can successfully convert C to T near the target sequence. Further refinements, such as the attachment of uracil DNA glycosylase inhibitor (UGI) to promote BER response, and the conversion of Cas9 to a Cas9 with nickase activity, which nicks the strand opposite the deaminated cytidine, have significantly increased the base-editing efficiency of the C > T substitution at the desired sites in the genomic DNA [48]. In summary, the ongoing revolution in the genome-editing technology, coupled to the equally revolutionary iPSC technology, offers an unprecedented opportunity for exploration of gene-disease relationships relevant to human cardiovascular diseases and development of new paradigms for treating cardiovascular diseases.

Potential of Combining Genome Editing and hiPSC Technologies in Cardiovascular Biology

Mutations in the *PRKAG2* gene, encoding the gamma-2 sub-unit of adenosine monophosphate kinase (AMPK), an enzyme central to cellular energy homeostasis, is known to a variant of familial HCM associated with an aberrant atrioventricular conduction [51]. These HCM mutations are known to augment glycogen storage in myocytes [52]. Recently, Binah and colleagues [53] utilized iPSC-CMs derived from a patient with the R302Q *PRKAG2* mutation to examine the association between glycogen storage and arrhythmias seen in these patients. On TEM, the mutant iPSC-CMs displayed significant glycogen accumulation near mitochondria, myofibrils, and the nucleus, similar to what was observed in the hearts of patients with the mutant AMPK. Moreover, the mutant iPSC-CMs exhibited electrophysiological abnormalities, including delayed afterdepolarizations, which

resulted in triggered beats similar to tachyarrhythmias seen in these patients. Importantly, correction of the PRKAG2 mutation by CRISPR/Cas9 in the patient's iPSCs resulted in iPSC-CMs that exhibited significant amelioration of both glycogen accumulation and electrophysiological abnormalities. This and similar studies demonstrate the power of combining the iPSC and the genome-editing technology for the study of human cardiovascular disease.

Marfan syndrome is a heritable genetic disorder of the connective tissue caused by mutations in the extracellular matrix protein fibrillin-1(*FBN1*). Sinha and colleagues generated iPSC-derived smooth muscle cells (iPSC-SMCs) from two Marfan patients and found that the mutant iPSC-SMCs recapitulated pathologic characteristics seen in Marfan syndrome aortas, including the degradation of extracellular matrix and abnormal fibrillin-1 accumulation [54•]. Furthermore, CRISPR-Cas9 mediated correction of the *FBN1* mutation resulted in correction of the Marfan disease phenotypes in iPSC-SMC, including normalization of fibrillin-1 accumulation, matrix degradation, and TGF-β signaling. Importantly, these studies demonstrated that p38, KLF4, and β_1-integrin play an important role in the apoptosis observed in Marfan iPSC-SMCs, which suggested that p38 and KLF4 may be new therapeutic targets for Marfan syndrome [54•].

While this review is primarily focused on the use of the iPSC technology for modeling monogenic diseases, vast majority of cardiovascular diseases are multifactorial, with complex genetic and environmental contributions. In the past two decades, genome-wide association studies (GWASs) have led to hundreds of genomic loci associated with human diseases. However, the functional impact of individual gene variant at the individual level is quite small, indicating disease predisposition or susceptibility is influenced by the summation of multiple genetic influences. Here, patient-derived iPSCs, together with the CRISPR-Cas9 genomic-editing technology, will have significant impact on assessing functional impact of individual and groups of gene variants [55]. Another area of potential impact of the iPSC technology is the study of drug-induced cardiotoxicity, such as arrhythmias (QT prolongation and polymorphic ventricular tachycardia torsade de pointes). Recent study demonstrated that common gene variants associated with long QT in GWAS are associated with drug-induced QT prolongation and torsade de pointes, forming a basis for a genetic QT risk score [56]. The iPSC technology may prove valuable for functionally validating the contributions of these proarrhythmic risk variants and for evaluating proarrhythmic effects of new drugs on iPSCs reflecting genetic susceptibility in the general population and not necessarily in only rare proarrhythmic cohorts.

Conclusions

The two revolutions in cellular reprogramming and genome editing have opened up tremendous opportunities for the study of human cardiovascular disease, which had been hampered by limited access to live tissues. Besides the few examples discussed here, there are now numerous studies which have combined patient-specific iPSC-based models with genome editing for the personalized study of variety of cardiovascular diseases. Numerous hurdles remain, particularly to ensure that cardiovascular cells and tissues generated from human iPSCs are relevant to the study of human diseases [57, 58•, 59]. The rapid rate of

advances in these two revolutionary technologies suggest that patient-derived and genome-edited iPSC models may become the gold standard approach for studying cardiovascular diseases enabling discovery of new therapies in the future.

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Compliance with Ethical Standards

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