REGENERATIVE MEDICINE RESEARCH

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Human induced pluripotent stem cell for modeling cardiovascular diseases

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Abstract

The invention of the induced pluripotent stem cell (iPSC) technology allows patient-specific, mature somatic cells to be converted into an unlimited supply of pluripotent stem cells (PSCs). These iPSCs can then in turn be differentiated into any cell type including neurons, cardiac cells, pancreatic cells, liver cells, blood cells or enterocytes. Although cardiovascular disease (CVD) is a leading cause of death in the world, the limited cell derivation and cell number in cardiac tissue makes it difficult to study the CVDs using the existing cardiac cell model. By differentiating the patient-specific iPSCs into cardiomyocytes, scientists can generate iPSC-based 'disease in a dish' models and use them to better understand disease mechanism. Here we review the current progress in using iPSC-derived cardiomyocytes to model human CVDs.

Keywords: Stem cells, Induced pluripotent stem cells, Cardiovascular disease, Disease modeling

Pluripotent stem cells (PSCs) for translational medicine

Recently, human embryonic stem cells (hESCs), used as pluripotent cells, were widely considered a useful resource in translational medicine. In 1998, hESCs were isolated from inner cells of blastocysts and characterized for their pluripotency and self-renewal [1]. In contrast to adult stem cells, hESCs can be differentiated into any type of functional cells. The applications of hESCs in translational medicine, however, are controversial, and it is difficult to suppress immunologic rejection in hESC-based therapies [2].

IPSC: a game changer

Two groups (Yamanaka group in Kyoto University and Thomson group in University of Wisconsin) separately reported that somatic cells can be successfully reprogrammed into iPSCs, marking a major landmark in stem cell research [3,4]. In each study, four transcription factors were used for induction. Yamanaka's group selected Oct3/4, Sox2, Klf, and c-Myc [3], while. Thomson's group used Oct3/4, Sox2, Nanog, and Lin28 [4]. This allowed researches to reprogram mature somatic cells harvested from patients and generate an unlimited supply of PSCs which in turn could be differentiated into various cell types needed such as neurons, cardiac cells, pancreatic cells, liver cells, blood cells or enterocytes for disease modeling, drug screening and cell therapy [5-10]. iPS cells have a couple of key advantages: they avoid the ethical concerns that have plagued the embryonic stem cell field, and they are patient-specific, thus providing a powerful tool for translational medicine.

iPSC for cardiovascular diseases

A straightforward application of iPSCs is to establish patient-specific genetic disease models *in vitro*. These models are useful for understanding mechanism of physiology and pathology of disease, validating therapeutic targets, and drug screening/discovery. CVD, more commonly known as heart disease, encompasses all heart and blood vessel disease. CVD is the world's leading cause of death; more people die annually from CVD than from any other cause, with an estimated 17.3 million people dying in 2008, accounting for 30 % of all global deaths. CVD encompasses a broad range of disorders such as atherosclerosis, ischemic heart disease, acute myocardial infraction, valvular heart disease, heart failure, cardiomyopathies, arrhythmias, hypertension, and congenital heart disease. By differentiating patient-specific iPSCs into patient-specific cardiomyocytes, researchers

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can generate iPSC-based 'disease in a dish' models and use them to better understand disease mechanism and develop new therapeutics [11,12]. Ion channels are pore-forming membrane proteins whose functions allow them operate like biological micro-machines to establish a resting potential, shape action potentials, and control the excitability of neurons, cardiac cells and muscle cells, thus playing an essential role in functional regulation of the cardiovascular system. The outward currents (such as I_{ks} and I_{kr} encoded by KCNQ1 and human ether-á-go-go–related gene (hERG) gene, respectively) contribute to repolarization of cardiac action potentials, which helps bring membrane potentials back to the resting level and terminate a ventricular contraction. The timing of these repolarizing currents (I_{ks} and I_{kr}) is critical. Failure to repolarize due to inherited mutations in KCNQ1 or hERG gene, or drug-induced I_{ks} and I_{kr} inhibitions, for example, can result in abnormalities in action potentials, thus causing Long QT syndrome (Type 1 or 2). Moretti et al. [13] were an early example, taking skin biopsies from patients with Long QT Syndrome Type 1 (LQT-1), reprogramming their cells into iPSCs, and then differentiating those iPSCs into cardiac cells. These patient-specific cardiomyocytes recapitulated the clinical presentation of the 'Long QT' phenotype [13]. Using a similar approach, [14] successfully modeled Long QT Syndrome Type 2 (LQT-2). These studies clearly established iPSC-derived cardiomyocytes as a powerful tool for drug discovery and personalized medicine [15]. To date, iPSC models have been used to model a large number of genetic arrhythmias including LQT syndromes, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD), and Overlap Syndrome [14,16-24]. A number of genetic cardiomyopathies have also been studied using iPSCs [25]. generated iPSCs from skin cells of patients in a family with inherited dilated cardiomyopathy (DCM) carrying a mutation in the TNNT2 gene, a gene encodes Troponin T type 2 (cardiac) that is the tropomyosin-binding subunit of the troponin complex and mutations in this gene have been associated with dilated cardiomyopathy familial hypertrophic cardiomyopathy as well as restrictive cardiomyopathy [25]. Compared to cardiomyocytes derived from iPSCs of healthy controls in the same family, cardiomyocytes derived from iPSCs of DCM patients exhibited increased heterogeneous myofilament organization, susceptibility to stress, compromised ability to regulate calcium flux, and decreased contractile force. More recently, Lan et al. reported modeling a familiar hypertrophic cardiomyopathy (HCM) [26]. Patient-specific induced pluripotent stem cell cardiomyocytes (iPSC-CMs) from a ten-member family cohort carrying a hereditary HCM missense mutation (Arg663His) in the MYH7 gene were generated. This gene encodes a myosin heavy chain beta (MHC-β) isoform expressed primarily in the heart. Diseased iPSC-CMs recapitulated numerous aspects of the HCM phenotype including cellular enlargement, abnormal Ca^{2+} handling and contractile arrhythmia at the single-cell level. In addition to those early-onset cardiovascular diseases modeled by iPSC-derived cardiomyocytes mentioned above, Kim et al. for the first time demonstrated late-onset disease models using ARVD/C patient-specific iPSCs, in which the patients don't have clinical phenotype until adulthood [27]. Several groups have reported that iPS cells can also be differentiated into functional endothelial cells (ECs) or vascular smooth muscle cells (SMCs), which can shed new light up on understanding the mechanisms of various vascular diseases such as hypertension, pulmonary atrial hypertension (PAH), coronary heart disease, and diabetic cardiomyopathy. There are, however, fewer vascular diseases being modeled by iPSCs thank cardiac diseases [28-32]. Kinnear et al. generated iPSCs from a patient with aortic and pulmonary stenosis who was suffering from Williams-Beuren syndrome (WBS), a rare genetic neurodevelopmental disorder that can cause cardiovascular disease [33]. Interestingly, WBS patient-specific iPS cell-derived SMCs demonstrate an immature proliferative phenotype with reduced functional and contractile properties compared to healthy controls, thereby recapitulating the human disease phenotype. Moreover, they showed that the long-term treatment (5 days) of the antiproliferative drug rapamycin can rescue the disease phenotype, providing an attractive therapeutic candidate for patients with WBS and vascular stenosis.

Review and conclusions

A growing number of research groups have employed iPSCs to model CVDs. It remains unclear whether diseased iPSC-derived cardiomyocytes show disease phenotypes *in vitro* and how similar *in vitro* phenotypes are to their clinical equivalents. It is to be noted that iPSC-derived cardiomyocytes are morphologically and electrophysiologically immature compared to human adult counterparts. Several studies have focused on developing methologies to obtain more mature cardiomyocytes derived from iPSCs [34]. iPSC-derived cardiomyocytes are also a mixture of 3 subtypes of cells (ventricular-like, atrial-like, nodal-like). All three differentiation methods including embryoid body formation, endodermal induction and directed differentiation method reflect these shortcomings. Future challenges will focus on how diseased iPSC models can faithfully reflect disease phenotypes. Published iPSC studies only reproduce disease phenotypes and drugs that have already been reported using other approaches such as transgenetic animal models or primary cells. Moreover, most of the published iPSC disease model works are the ones looking into arrhythmic disorders carried ion channel gene mutations. To demonstrate the power of

iPSCs, scientists have to gain more mechanistic insights, discover novel drug targets, and investigate more complicated cardiovascular diseases. In conclusion, although iPSC-based cardiovascular disease research is in its infancy, further improvement and standardization of the culturing methods can lead to a more comprehensive understanding of mechanisms in cardiovascular disease.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
PL drafted the manuscript, JD discussed the manuscript. Both authors read and approved the final manuscript.

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