Cardiovascular Diseases and Stem Cells

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This entry provides an update on previous and current research in the field of Cardiovascular diseases (CVDs), a class of disorders affecting the heart or blood vessels. Despite progress in clinical research and therapy, CVDs still represent the leading cause of mortality and morbidity worldwide. The hallmarks of cardiac diseases include inflammation, fibrosis, scar tissue, hyperplasia, hypertrophy, abnormal ventricular remodeling, and cardiomyocyte death, which is an irreversible process that induces heart failure with progressive and dramatic consequences. Both genetic and environmental factors pathologically contribute to the development of CVDs, but the precise causes that trigger cardiac diseases and their progression are still largely unknown. In this scenario, the possibility to generate patient-specific cardiac cells from induced pluripotent stem cells (iPSCs) represents a powerful platform for the investigation of these life-threatening disorders.

Keywords: cardiovascular diseases ; human induced pluripotent stem cells ; cardiac differentiation ; iPSC-derived cardiomyocytes ; cardiac disease modeling

1. Discussion

Cardiovascular diseases (CVDs) include a number of disorders affecting the heart functionality many of which entail a massive loss of cardiomyocytes, leading to heart attack, stroke or even death. Thus, the investigation of CVDs pathophysiology is crucial for the discovery of new curative therapies alternative to heart transplantation. Stem cell-based therapy for CVDs is one of the most promising approaches as it provides important molecular understanding of CVD's mechanisms and bears many advantages for the identification of personalized, cell-based, therapies. Cardiomyocytes derived from patient-specific human induced pluripotent stem cells (hiPSCs-CMs) have the quality to carry the same mutations of the patient's cardiac cells, representing the best platform for the creation of cardiac "disease-in-a-dish" models which are advantageous for in vitro high throughput drug screening, tissue engineering and regenerative medicine.

2. Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality and morbidity worldwide and the development of novel therapeutic treatments still remains a major research goal. The contribution of risk factors, such as cigarette smoking, diabetes, hypertension, and hyperlipidaemia, are well recognized as important players for the initiation of cardiac diseases, for which atherosclerosis is commonly shared by all CVDs [1]. Many cardiac disorders, such as heart failure (HF), ischemic/reperfusion (I/R) damage, and myocardial infarction (MI), are characterized by massive cardiac myocytes death [2]. On the other hand, adult human heart has limited capacity to replenish the loss of cardiomyocytes, having an extremely low regenerative ability after cardiac injury, despite some studies having suggested that human heart owns a certain degree of regenerative capacity [3]. Therefore, the loss of cardiomyocytes irreversibly damages the heart with a progressive decrease of its functionality and eventually develops into heart failure. In the context of such a scenario, the identification of the molecular mechanisms underlying cardiac diseases becomes mandatory and the possibility to capture the early events of disease development is crucial for the unraveling of mechanisms and/or markers that can act as potential targets against which to develop new therapeutic strategies. For decades animal models, especially rodents, have represented the model of election for studying human biology, development, and disease, based on the genetic and physiological similarities between the two species. Nonetheless, their phylogenetic relatedness has developed differently for humans and mice, making the experimental results obtained from animal models strikingly different from what is instead true for humans. Thus, although animal models have offered important contributions in understanding human biology and disease, they do not fully mirror the complexity of diseases as they are present in human systems and fail in the intent to translate the results of mice research to humans [4]. Moreover, many promising chemical compounds and drugs that perform well in preclinical animal studies fail in humans due to lack of safety and/or efficacy. Therefore, the use of human tissues would be the most reliable way for a precise understanding of the molecular underpinnings of human

biology and pathology, resulting in more accurate and targeted therapies for many human disorders. The use of primary cells from living affected individuals is limited by the amount of tissue and its lifespan in culture, as well as the technical feasibility of accessing particular cell types such as neurons and cardiomyocytes. Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the capability to self-renew indefinitely and to differentiate into derivatives of the three germ layers (ectoderm, mesoderm, and endoderm). These features make them very promising in a variety of basic research and clinical applications such as developmental biology [5], drug screening, disease modelling, and regenerative medicine. Unlike non-human animal models, tissue specific-derived PSCs offer an unprecedented platform for a comprehensive understanding of the molecular basis of CVDs [6]. The revolutionary discovery that somatic cells can be reprogrammed, via overexpression of a set of specific transcription factors, to induced pluripotent stem cells (iPSCs) [7][8][9], paved the way for the generation of patient-specific iPSCs [8][10]. iPSCs derived from healthy individuals or diseased patients carry the genome of their cell of origin and can be differentiated into any cell type, including cells not otherwise accessible, representing a powerful cell-based model system for human diseases, genetic investigations, drug screening, and personalized therapy [11]. Their human origin, pluripotency and ultimately their capability to differentiate into any disease-relevant cell type, and their epigenetic and genetic matching with the patient they are derived from are all features that make iPSCs the most reliable candidate for studying human disorders at cellular level. Moreover, genome-editing approaches can be used to repair and thus to rescue the disease phenotype in patient-derived iPSCs or to introduce pathologically relevant mutations in wild-type lines. To date, a wide number of different monogenic and complex human cardiac disorders have been modeled in vitro using iPSC technology providing new insights into disease mechanisms. The aim of this work is to address the most relevant scientific advances with respect to the use of iPSCs for cardiac disease modeling and to summarize the revolutionary potential of iPSC-derived cardiomyocytes (iPSC-CMs) for cardiac regenerative medicine.

3. Differentiation of Cardiac Cells from iPSCs

For iPSCs to dominate a wide spectrum of biomedical fields, their effective differentiation into specific cell types is of extreme importance. In vitro CM differentiation from iPSCs is achieved by modulation of signaling pathways known to be involved in cardiac development during normal embryogenesis [12]. To date, there have been three main strategies developed to obtain functional CMs from iPSCs: (1) co-culture with visceral endoderm-like cells (END-2); (2) embryoid body (EB)-based differentiation, and (3) two-dimensional culture. During embryonic development in vivo, visceral END-2 releases factors that lead to cardiac differentiation of the nearby mesoderm [13]; this discovery was the basis of the co-culture strategy in which PSCs cultured either in the presence of END-2 cells or in END-2-conditioned medium enter cardiac fate. Although this protocol was successfully applied to both ESCs [14] and iPSCs [15], the CM yield is very low (less than 10%) [16]. EB-based differentiation is a serum-mediated three-dimensional method relying on the capability of PSCs to form floating cell aggregates when cultivated as single cells in low attachment substrate. These aggregates, known as embryoid bodies (EBs), spontaneously produce derivative cells of all the three germ layers [17]. However, the EB-differentiation procedure, due to the presence of serum, suffers from low reproducibility and inter-line variability [18]. Serum was later replaced by cytokines and growth factors known to be involved in heart development such as Wnt proteins [19], bone morphogenetic proteins (BMPs) and activin A [12][20], and Notch signals [21], together with their corresponding inhibitors [22][23]. Different small molecules have been tested for their ability to promote in vitro cardiac differentiation; activators (CHIR99021) and inhibitors (IWR, XAV, IWP2) of the Wnt pathway have been proved to increase cardiac differentiation [24]. However, this strategy requires a high number of starting cells and has a low efficiency. To overcome the limitations of methods based on EB formation, differentiation protocols have been developed based on cell monolayers but with the use of the same molecular factors described for EB differentiation [25][26]. A monolayer-based strategy allowed significant improvement of the yield of cardiac differentiation and the phenotype of derived cardiac cells exhibiting typical features of ventricular, atrial, or nodal cardiomyocytes [27]. The “matrix sandwich” method is a modification of monolayer assay, consisting of covering confluent iPSCs, previously treated with specific growth factors and cytokines to induce cardiac differentiation, with an overlay composed of matrix (i.e., Matrigel) and culture medium. This method relies on the pivotal role that the extracellular matrix plays in the differentiation process leading to high CM purity and yield [28]. Other differentiation methods require two steps: during the first step iPSCs are induced to differentiate into cardiac progenitor cells (CPCs), which in turn can be further differentiated into different cellular fates including CMs, smooth muscle cells (SMCs), and endothelial cells [29][30]. Moreover, it was recently demonstrated that induced CPCs can be directly generated using mouse fibroblasts, skipping the intermediate stage of iPSCs [31][32]. Although there are currently available protocols providing a differentiation efficiency of up to 80% or more in terms of CM purity [27][30][33], all the so far reported strategies show major limitations such as heterogeneity and immaturity of the cardiac population [34]. The low purity and high heterogeneity of the differentiated CM population constitutes an important obstacle for their use in cell-based therapy that requires efficient purification methods to enrich the cardiac population. So far, several studies have developed efficient isolation methods based on the identification of specific cardiac markers such as SIRPA and VCAM1

[35][36][37]. Other studies have instead developed protocols to differentiate human iPSCs into specific subtypes of functional cardiac cells, such as atrial-, ventricular- [38][39][40], nodal-like [41] and pacemaker cells [42]. Although the cardiac cells obtained from iPSCs can start beating very early during differentiation, they resemble, morphologically and functionally, fetal cardiomyocytes. iPSC-CMs display a disorganized morphology, reduced contractile capacity, alteration of glycolytic metabolism, abnormal electrophysiological properties, and reduced automaticity [43]. This immaturity renders adult-onset heart disease modeling very challenging, owing to the uncertainty regarding the ability of relatively immature iPSC-CMs to fully recapitulate adult disease phenotypes or as a function of aging, while the understanding of early-stage pathological events is not affected by low iPSC-CM maturity. Moreover, the incomplete maturity of iPSC-derived CMs could narrow the effectiveness of these cells in mimicking the pathology, e.g., if this is caused by a gene mutated postnatally, with negative impact on their usefulness for studies on drug effect/toxicity. In order to improve the differentiation strategy of iPSCs toward the generation of a high mature and homogeneous cardiomyocyte population, new differentiation methodologies and technical modifications have been proposed. A long culture period (80–120 days) results in multinucleated iPSC-CMs exhibiting mature sarcomeres and increased electrophysiological properties compared to 20–40-day-old CMs [44]. This higher grade of maturity of long-term culture CMs is strictly related to mitochondrial metabolism regulation, which is necessary for energy production and increased cell contractility [45]. Other methods aimed to improve CM maturation include addition of T3 hormone [46] or dexamethasone [47] in culture medium and stressing CMs with mechanical and electrical stimuli [48]. Among the methods developed to increase the maturation of iPSC-CMs, in vivo environments provided the most mature phenotype of iPSC-CMs [49][50]. Currently, nanotechnology-based approaches offer new perspectives in many fields of biomedical research, including cardiovascular research [51]. Three-dimensional scaffolds produced starting from natural or synthetic materials and functionalized to reach specific mechanical and chemical features can be used for direct iPSC differentiation into cardiomyocytes. Scaffolds designed for cardiac differentiation should possess specific properties such as good elasticity to allow cardiac cell contraction and properties to allow cardiomyocytes to arrange in a polarized and organized structure typical of native myocardium. These properties are retained for example by poly (vinyl alcohol) [52], polyethylene oxide [53], poly(lactic-co-glycolic acid) [54], and poly(caprolactone) [55]. CMs cultured on 3D structures show an enhanced calcium signaling respect to monolayer culture [56]; however, it is mandatory to combine this method with electrical and physiological stimulation to obtain cardiac cells with a complete degree of maturity [57]. The number of differentiated cells obtained using classical culture methods represents an additional shortcoming for the application of iPSCs in cell therapy, given that up to one billion CMs need to be transplanted within the infarcted myocardium to replace damaged tissue [58]. Large-scale production of CMs from iPSCs can be achieved using bioreactors that make the process scalable and reproducible via the continuous control and stabilization of culture parameters [59]. Bioreactors create a dynamic suspension culture in which there is a constant flow of nutrients and homeostasis of pH and oxygen levels [60]. iPSCs cultured in a spinner flask form aggregates that can be used as starting materials for CM production after treatments with molecules acting on the Wnt pathway. The spinner flask methods allow critical variables to be tightly supervised, such as aggregate size and cytokine release, augmenting differentiation efficiency [61]. The culture in the suspension of cells that are adhesion-dependent for survival and proliferation can be obtained through the use of supporting matrices known as microcarriers [62]. Laco et al. (2020) developed a microcarrier culture system in a tank bioreactor that allowed scalable iPSC expansion and CM differentiation and purification, reaching a yield of ~40 CMs per iPSC seeded after 22 days in culture [63]. A future intension of bioreactor use will be the production of large amounts of high quality CMs in GMP manufacturing, to improve their use in clinical practice.

4. iPSCs in Cardiac Disease Modeling

In vitro disease modeling is one of the most speculated about fields using iPSC technology. Modeling human cardiac disorders enables definition of the functional and molecular mechanisms underlying a disease and creates the possibility to develop new therapies. The first lines of iPSCs from patients harboring monogenic and complex genetic diseases were established in 2008; one year later, iPSCs were generated from a human specimen [10]. This pioneering publication has rapidly been followed by a growing body of scientific literature. iPSC-based disease modeling has dramatically influenced cardiovascular medicine, offering the opportunity to understand the pathological mechanisms of cardiac diseases and to develop novel effective therapies. This has greatly attracted the scientific community, providing an unprecedented opportunity to recapitulate human monogenic and complex cardiac diseases in vitro. Before the advent of iPSC technology, the severity of CVDs together with the lack of efficient treatments rather than transplantation pushed researchers to develop model systems of cardiac diseases comprising animal models for in vivo studies, in vitro cellular models based on the use of stem cells, primary cells, and various cell lines, and computational studies [64]. The potential of iPSCs and their capability to differentiate into cardiac relevant cell types, including cardiomyocytes, smooth muscle cells, and vascular endothelial cells, as well as and their genetic match to the patient they are derived from, offers a large spectrum of possibilities for the establishment of a robust in vitro model of the disease. iPSC-derived CMs share the same

genetic and molecular blueprints as primary human CMs, along with mechanical and electrophysiological properties. To date, a wide range of cardiac diseases including long QT syndrome [65], Leopard syndrome [66], Brugada syndrome [67], catecholaminergic polymorphic ventricular tachycardia [68], arrhythmogenic right ventricular cardiomyopathy/dysplasia [69], dilated cardiomyopathy [70], left ventricular non-compaction [71], hypertrophic cardiomyopathy [72], Andersen-Tawil syndrome [73], and Timothy syndrome [74] have been modeled using iPSC technology. Cardiac diseases are traditionally divided into three main groups: channelopathies, structural cardiomyopathies, and others disorders that do not fit in as channelopathies or structural cardiomyopathies. Among cardiac diseases that are not recognized as channelopathies and/or structural cardiomyopathies, there are several metabolic disorders with cardiac phenotypes. Some of them, such as Friedreich's ataxia [75], Barth syndrome [76][77], fatty acid oxidation disorders, and Pompe diseases [78] have successfully been translated to iPSC-CM-based models. Ion channelopathies are perhaps the form of cardiac disease with the most well-established iPSC-based disease models.

5. Conclusions

Cardiovascular diseases represent the leading cause of morbidity and death worldwide. Current therapies are mostly focused on relieving symptoms and preventing complications. Despite the progress in clinical research, many HF patients become refractory to standard and/or palliative medical therapies. Therefore, invasive cardiac transplantation remains the only choice for end-stage HF. Nevertheless, the procedure is highly risky and strikingly dependent on access to suitable donors. A comprehensive understanding of the molecular mechanisms underlying human cardiac diseases has been hampered by the lack of reliable model systems that mirror the human disease phenotype. ESCs have been considered the milestone for studying cardiac diseases since they can proliferate indefinitely and can give rise to any cell type, including cardiomyocytes. However, the destruction of the human embryo necessary for the derivation of ESCs has raised important ethical issues preventing and limiting their use. In addition, an ESC-based cardiac disease model cannot be considered totally reliable because of its misleading outcomes due to the individual-specific genetic and epigenetic background. The extraordinary advance accomplished in stem cell biology with the discovery of human iPSCs has completely reshaped our approach to studying human diseases. iPSC technology, through the generation of specific cellular models carrying pathogenetic mutations responsible for the disease phenotype, has allowed novel molecular targets and signaling pathways to be uncovered for the development of new therapeutic strategies. Outstanding advances in differentiation methods, in combination with new impressive genome editing tools like CRISPR-Cas9, have allowed the generation of patient-specific CMs models and their respective isogenic controls. Although iPSCs have generated great enthusiasm within the scientific community, concerns have been raised regarding their real equivalence to ESCs, but little conclusive evidence has been reported regarding iPSC and ESC cardiac derivatives, strengthening the applications of iPSCs in basic and clinical cardiac research. Concerns around iPSC technology are not just about their similarity to ESCs, but also concern the intrinsic properties of iPSC-derived cardiomyocytes. (1) iPSC-CMs typically exhibit immature structural and functional properties resembling phenotypically and functionally fetal cardiomyocytes: while this feature may even be advantageous to model early disease stages, particularly relevant for diseases showing early onset, the low maturity may create problems with regard to the use of iPSC-CMs in clinically relevant settings such as novel drug testing or evaluating their efficacy/or toxicity; moreover, the relatively immature phenotype of iPSC-CMs may mask important pathological mechanisms typical of adult-onset cardiac diseases; (2) iPSC-CMs present batch wise variations in differentiation; (3) major limitations of iPSC technology are associated with the reprogramming process: reprogrammed cells might retain the epigenetic signature of the somatic cell from which they were derived, chromosomal aberrations and/or accumulation of mutations, and genomic instability. Therefore, international standard processes are required to characterize and validate these cells at every stage, with special attention to their use in clinics to ensure safety. Despite the mentioned limitations, the power of iPSC technology for clinical and basic cardiovascular research remains undisputed.

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