Chapter 19

Disease Models for the Genetic Cardiac Diseases

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1. Introduction

The ability to reprogram somatic cells into pluripotent stem cells has presented a significant advancement in stem cell research. This technique enables derivation of induced pluripotent stem (iPS) cells from any individual having a unique genotype. iPS cells can be derived from human somatic cells such as fibroblasts, keratinocytes or blood cells. Since, the production of iPS cell lines does not require the destruction of human embryos as in the production of the human embryonic stem cells (hESCs), the legal and ethical issues associated with hESCs can be at least partly avoided. The characteristics of iPS cells are very similar to those of pluripotent hESCs in many respects, including cell morphology, immortal growth characteristics in culture, expression of pluripotent markers, and differentiation potential. The iPS cells combined with the various differentiation protocols developed enable the production of genotype specific cell types. This feature enables also to produce disease-specific iPS cell lines from patients bearing defined genetic mutations. Traditionally, it has been challenging to study genetic cardiac diseases because cardiomyocytes from the heart biopsies of patients are difficult to obtain and the procedure carries a high risk. Additionally these cardiomyocytes do not survive long in culture. Animal models, mostly developed in rodent, have aided in elucidating the basic mechanisms of several genetic cardiac diseases. The disadvantages of small animal models are marked differences in anatomy and physiology of the cardiovascular system in comparison to humans. Rodent models are far from ideal when used in the identification of contractile deficits and signals that initiate pathological growth [1]. Furthermore, the results obtained from neonatal rat cell experiments can be problematic because these cells possess different relative receptor subtypes and cell-signaling mechanisms. It will thus be especially important to investigate functional consequences of genetic cardiac diseases in human cardiomyocytes in which the functional effects of specific proteins have been adjusted to optimize electrical properties, contractile efficiency and power output of larger hearts [2].

Genetic cardiac diseases, such as long QT syndrome, belong to a severe class of diseases which are unpredictable, have variable clinical picture ranging from asymptomatic to sudden cardiac death and lack specific medication. These inherited arrhythmic diseases are caused by single mutations which are relatively common in population. Earlier we did not have *in vitro* models for these diseases, but with the aid of iPS cell derived cardiomyocytes genetic cardiac diseases can now be modeled in cell culture. The patient specific iPS cell derived cardiomyocytes have been demonstrated to manifest the disease-associated electrophysiological abnormalities in a dish [3-6]. Therefore, these cells allow researchers to study and understand disease mechanisms more readily as well as to investigate the effects of different chemical compounds on the electrophysiology of the cardiomyocytes. In addition to basic research, iPS cell derived cardiomyocytes would provide an effective tool for novel drug or treatment discovery. However, before iPS cell derived cardiomyocytes are ready to be considered for use as disease models, the cells produced need to be confirmed to exhibit the essential functional characteristics of human cardiomyocytes.

In this chapter, the production and the characterization of patient specific iPS cell derived cardiomyocytes is described. In addition, we discuss the genetic cardiac disease models so far developed based on iPS technique, their demands, advantages and disadvantages. Furthermore, the future applications for iPS cell derived cardiomyocytes are discussed.

2. Production of disease specific iPS cell lines

The discovery of cellular reprogramming as a technology to generate iPS cells offers a potential solution to the challenge when studying genetic cardiac diseases. In this approach, human adult somatic cells are reprogrammed into stem cells offering comparable function to human pluripotent ESCs in their ability to develop differentiated progeny from all developmental lineages of the human being. When somatic cells are reprogrammed to iPS cells, they shut down the expression of genes specific for that somatic cell type and activate genes that maintain pluripotency. Once reprogramming has occurred, endogenous counterparts of the exogenously supplied reprogramming factors are activated, indicating that exogenous factors are only required for the induction, not for the maintenance of pluripotency [7]. Up to date, various human somatic cell types, including fibroblasts, keratinocytes, and different blood cells have been reprogrammed to iPS cells [7-11].

The initial methods used to generate iPS cells involved the retroviral overexpression of four transcription factors Oct4, Sox2, Klf4, and c-myc observed to be essential in maintaining pluripotency of hESCs [7, 12]. Another set of four transcription factors Oct4, Sox2, Nanog, and Lin-28 was also found to induce pluripotency [9]. Efficient retro- and lentiviral vector systems that have been most widely used to generate iPS cells have several drawbacks including the possibility of proviral genomic integration, which may cause both the reactivation of silenced exogenous genes and the alteration of genomic integrity, thereby increasing the risk for tumorigenesis [12, 13]. Since the seminal discovery the development in this field has been rapid and numerous alternative strategies have been applied to improve the reprogramming safety,

efficiency and kinetics as well as to generate iPS cells without viral integration in the genome (Table 1). The nonintegrating reprogramming methods developed thus far include adeno- and sendai viruses, plasmid- and episomal vector-based approaches, excision systems of integrated transgenes such as Cre/loxP recombination or PiggyBac transposition, and delivery of reprogramming factors directly as RNAs, proteins and chemicals. However, most of these nonintegrating approaches are still highly inefficient when compared to the original retro- or lentiviral reprogramming systems with the exception of nowadays widely used sendai virus reprogramming method.

Methods	Efficiency %	Details	References
Retroviral vectors	Medium, 0.01-0.5	Multiple integration, incomplete silencing, tumorigenicity possible	[7]
Lentiviral vectors	Medium, 0.1-1	Multiple integration, incomplete silencing, tumorigenicity possible	[9]
Adenoviral vectors	Low, 0.001	Non-integrating, however integrated vector-fragment possible	[14]
Sendaiviral vectors	Medium, 0.01-1	Non-integrating, integrated vector-fragment possible, T sensitive Sendai vector allowing removal of the virus	[15]
Plasmids	Low, 0.001	Occasional integration, simple transfection	[16]
OriP/EBNA-1 episomal vectors	Low, 0.0003	Non-integrating, long-term persistent transcription	[17]
Minicircle DNA episomal vectors	Low, 0.005	Non-integrating, multiple transductions needed	[18, 19]
Cre/loxP system	Medium, 0.01-1	Integration but excisable, inefficient loxP site excision, screening needed, tumorigenicity possible	[20]
PiggyBac system	Medium, 0.1	Precise excision possible, screening needed	[21, 22]
RNAs	High, 1	Non-integrating, DNA-free, multiple transfection needed	[23, 24]
Protein	Low, 0.001	Non-integrating, DNA-free, long-term treatment required, genetic abnormality possible	[25]
Factors + small molecules	High, >1	Non-integrating, DNA-free, long-term treatment required, abnormal signaling pathway possible, virus used	[26]

Table 1. Overview of the reprogramming methods for the generation of iPS cells.

3. Cardiomyocyte differentiation

Cardiomyocytes have been differentiated from the hESCs over a decade [27, 28] and multiple cardiac differentiation methods have been developed. The differentiation methods developed for hESC derived cardiomyocytes have been proven to be applicable also for cardiac differentiation of iPS cells.

Overall the differentiation event of hESC and iPS cell derived cardiomyocytes is quite rapid, 10-20 days regardless of the differentiation method used. However, all the differentiation methods share common problems, including uncontrolled differentiation and low differentiation rates. With common differentiation methods the cardiomyocyte yield is between ~1-25 % of the total cell number [28-30]. In addition, the cardiomyocyte differentiation efficiency has been shown to vary markedly between different stem cell lines [31].

All differentiation methods end up with a heterogeneous cell population. In addition to the other cell types, the differentiated population includes all cardiomyocyte subtypes; ventricular, atrial and nodal –like cells [32]. The ventricular cells form usually the majority of differentiated cells (60-80%), atrial cells form usually 10-40 % of the population and only <5% of cells are nodal-like cells [32, 33]. However, these numbers can differ depending on the cell line used [34].

The cardiac differentiation methods are lately reviewed [35] and described in Figure 1.

4. Transdifferentiation of fibroblasts into cardiac cells

Murine fibroblasts can be reprogrammed directly into cardiomyocytes by overexpression of Gata4, Mef2c and Tbx5 (GMT) [36]. This combination of factors has been reported to convert murine cardiac fibroblasts and tail tip fibroblasts into spontaneous beating cells having cardiomyocyte expression profiles. In addition, epigenetic status is typical for cardiomyocytes in these cells. However, Chen and co-workers have shown this method to be inefficient [37]. Overexpression of GMT factors resulted in an increase in cardiac troponin expression but spontaneous action potentials were lacking even though 22% of the cells exhibited voltage-dependent calcium currents.

A lot of effort has been done to transdifferentiate human fibroblasts into cardiomyocytes. So far spontaneously beating human cells have not been obtained. However, with transcription factors mesoderm posterior (MESP) homolog and mammalian v-ets erythoblastosis virus E26 oncogene homolog ETS2 cardiomyocyte progenitors expressing cardiac mesoderm marker KDR have been obtained [38]. It seems that the GMT method alone is not robust enough for direct reprogramming of human cardiomyocytes. Therefore it has been suggested that combination of GMT with other transcription factors, mRNAs or small molecules could provide more efficient reprogramming procedure [39]. In addition, based on animal experiments it can be concluded that cardiac microenvironment has also important role in reprogramming [40].

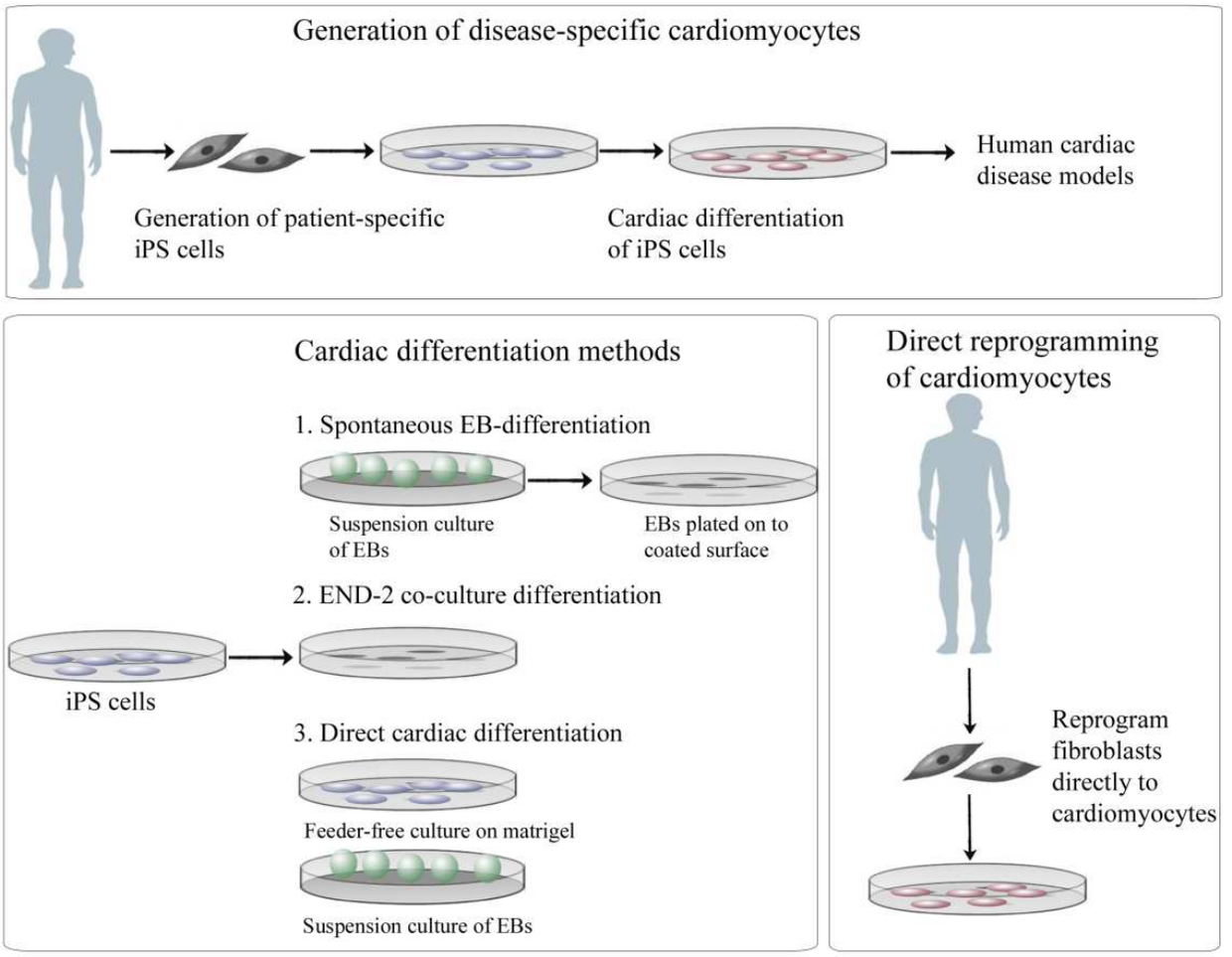

Figure 1. Generation of disease –specific iPS cell lines and cardiomyocytes. Cardiac differentiation methods can be divided into three classes; (1) Embryoid body (EB) based, (2) END-2 coculture based or (3) directed differentiation methods. Traditionally EB method has been based on spontaneous aggregation of EBs and spontaneous differentiation [28]. However, lately multiple methods controlling the EB formation has been developed [41] enhancing the reproducibility and productivity of the cardiac differentiation. END-2 method can be performed in two ways, either co-culturing the hESC or iPS cells in contact with END-2 cells [42] or by using END-2 conditioned media [43]. A lot of effort has been made in enhancing and defining the cardiac differentiation and this has led to the development of directed differentiation methods with growth factors or small molecules. Activin A and BMP-4 has been used in combination with monolayer cultures on matrigel to differentiate cardiomyocytes [44]. A temporal modulation of Wnt signaling by using small molecules has been proven to an even more robust and, in addition, rather inexpensive method for cardiomyocyte differentiation [45]. Directed reprogramming of fibroblasts to cardiomyocytes has been successful with mouse cells. However, this method has not yet been proven to work with human cells.

5. The assesment of the cardiomyocyte functionality

5.1. Cardiomyocyte characterization at gene and protein level

The first characterization step for the differentiated hESC or iPS cell derived cardiomyocytes is the observation of spontaneously beating cells. In addition, cardiomyocyte phenotype can

be assessed at the gene or protein level with cardiomyocyte specific markers such as structural proteins troponin, alpha-actinin or myosins. The commonly used markers in monitoring the cardiac differentiation are listed in Table 2.

Cell stage	Markers
Pluripotent cells	OCT4 Nanog SOX2 Tra-1-60 SSEA-4
Precardiac/cardiac mesodermal cells	Brachyury T FoxC1 Dkk-1 Mesp1 Flk-1
Cardiac precursor cells	KDR Nkx2.5 GATA4 Tbx5 Isl-1 Mef2c Hand1/2
Cardiac cells	Troponin I and T Sarcomeric α-actinin Myosin heavy- and light-chain (MHC and MLC)

Table 2. Markers used in monitoring the cardiac differentiation.

5.2. Electrophysiological methods

5.2.1. Patch clamp

Traditional way to study the functionality and the electrical activity of the cardiomyocytes is the patch clamp technique [46]. Originally patch clamp method has been developed to study ion channels in excitable membranes [47]. In this technique micropipette is attached to the cell membrane by a giga seal and this can be exploited to measure current changes and voltage across the membrane. Due to the unique nature of the cardiomyocyte action potential curve, the ion channel composition and the maturation stage of the cardiomyocyte can be assessed and therefore the method has been widely used with stem cell derived cardiomyocyte studies.

Key cardiac ion channels (and respective current) involved in the human action potential are NaV1.5 (INa), KV4.3 (Ito), CaV1.2 (ICa,L) KV11.1 (IKr), KV7.1 (IKs), and Kir2.X (IK1) [48]. The

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cardiac action potential is composed of co-operation of these channels and the action potential curve can be divided into five different phases (Figure 2). Phase 0 of the action potential is the depolarization phase of the cardiomyocytes from the negative membrane potential to positive, called the upstroke. This is followed by phase 1, the short transient repolarization that is followed by the plateau phase 2. Phase 2 is followed by phase 3, which is the repolarization back to the resting membrane potential. The resting state of the membrane potential is called as the phase 4 [49].

As mentioned, cardiac action potential results from the chain reaction of multiple ion channels. Therefore a malfunction of a single ion channel can be observed from the action potential curve. Figure 2 presents the parameters which are used in analyzing the action potential. In regard to analyzing cardiac disease specific cells, the action potential duration plays an important role because the lengthening of the action potential may lead to severe arrhythmias.

As a method, patch clamp is very informative and provides invaluable data for example for pharmacological and safety pharmacological studies. However, it is very laborious, needs highly specialized machinery and, most importantly, dedicated and specialized users. For these reasons, semi-automated and automated patch clamp machinery are being developed and would be valuable for cardiomyocyte applications [50, 51].

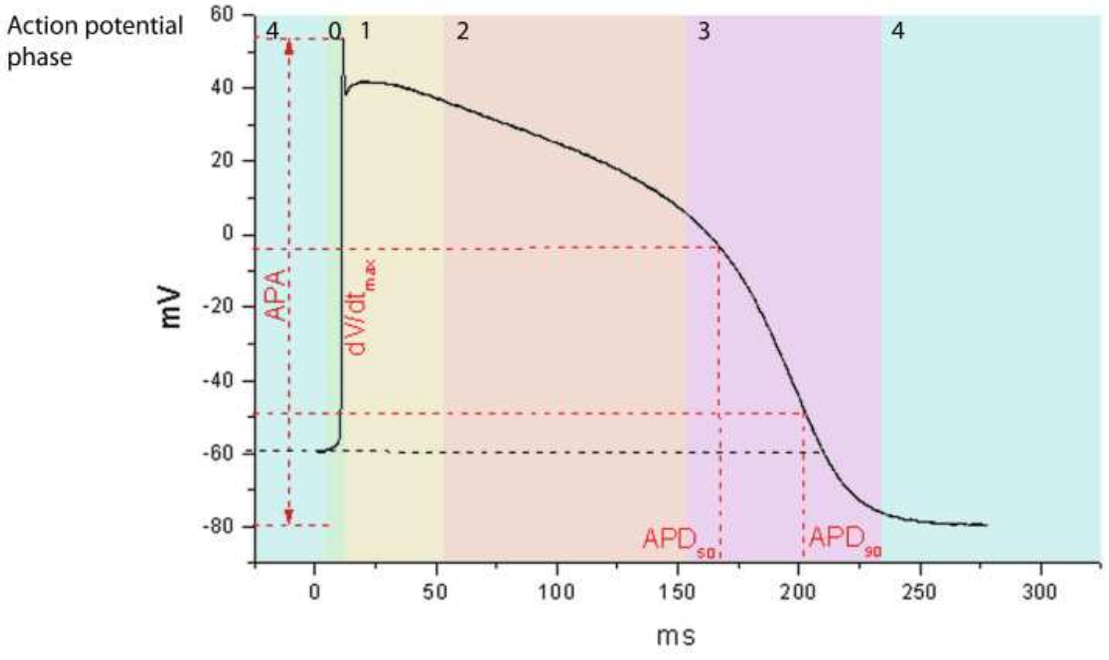

Figure 2. The phases of the cardiac action potential. ADP_{50} and ADP_{90} represent the action potential duration at 50% and 90% of the repolarization and these parameters are used in determining the duration of the action potential. The dV/dt_{max} represents the maximal upstroke velocity and can be used in assessing the electrophysiological phenotype and maturity stage of the cardiomyocytes.

5.2.2. Micro electrode array

In addition to the traditional patch clamp technique [46] the micro electrode array (MEA) – platform [52] offers practical, relative easy and non-invasive technique to assess the electrical properties of the differentiated cardiomyocytes [53]. Contrary to the patch clamp, the MEA system measures the electrical activity of a cell population. Therefore the signal resembles electrocardiogram (ECG) and is called field potential instead of action potential. Even though the ion channel function cannot be studied in the similar accuracy as with patch clamp, it allows examination field potential properties, such as cardiac repolarization, and therefore enables drug effect investigation [53]. During the last years, MEA has been widely used in characterization of hESC- and iPS cell derived cardiomyocytes [31, 54]. MEA has been become a basic electrophysiological tool and in addition to cardiomyocytes, it has been successfully used also with other cell types, such as neurons [55].

The MEA system is also applicable in studying cardiac cell responses to pharmaceutical agents [54]. It also enables cells to be measured repeatedly for longer periods of times e.g. multiple days or weeks. However, the analysis of MEA measurement data is laborious. Therefore, semi-automated and automated systems for data analysis have been developed, which makes MEA system more reliable and efficient tool in research [56].

5.2.3. The assessment of calcium homeostasis

In addition to the unique co-operation of cardiac ion channels, the interaction of calcium-ions with cardiac structure proteins is another crucial feature in cardiomyocytes that is essential for the proper function of the heart. In human cardiomyocytes, calcium ion (Ca2+) influx through L-type calcium channels during the plateau phase triggers the Ca2+-release from the sarcoplasmic reticulum (SR) which is mediated by the ryanodine receptors (RyR2). The Ca2+ influx together with the release raises the free calcium concentration inside the cardiomyocytes. In sytosol, free calcium binds to troponin C in the myofilaments and triggers the machinery which induces the cell contraction. For the cell relaxation to occur, the calcium has to be rapidly removed from the cytosol. The removal is efficient with the aid of four separate pathways; sarcoplasmic reticulum Ca2+-ATPase (Serca2a), sarcolemmal Ca2+-ATPase, sarcolemmal Na+/Ca2+ exchanger and mitochondrial Ca2+ uniport [57].

Similarly as the regular and synchronous chain of action potentials, calcium concentration fluctuates in the cardiomyocytes. Therefore, with the aid of calcium binding dyes and modern fluorescence microscope systems, the function and response to pharmaceutical agents of cardiomyocytes can be monitored. This method is called calcium imaging [58, 59]. The calcium binding dyes, such as Fura-2 and Fluo-4, can be loaded inside the cardiomyocyte cytosol and when the calcium ions are released to the cytosol, the ions bind to the dyes and a fluorescence signal can be detected. When the fluorescence intensity is measured from the single cell, the calcium handling of the single cardiomyocyte can be monitored and analyzed. From the calcium imaging data, the beating rate and the function of the calcium handling machinery in the cardiomyocytes can be assessed. If the calcium is not released or withdrawn from the cardiomyocyte cytosol in a proper way, irregularity or multiple peaks can be seen in the calcium imaging curve.

5.2.4. Force measurement

Recently a lot of effort has been applied to develop measuring systems to understand the mechanobiology of cardiomyocytes. Force measurement technique can be applied to measure isometric cardiomyocyte force contraction. A number of parameters can be determined by using the cardiomyocyte force measurement such as determination of Ca-sensitivity, cooperativity of force production and maximal Ca-activated force. Kinetics of the contractile responses can also be measured such as the actin-myosin turnover kinetics. These parameters can be useful in the characterization of myofibrillar pathologies of various origin and drug effects. Most of the currently existing systems are only suitable for the study of cardiac tissue slices and therefore inappropriate to be used for iPS cell derived cardiomyocytes. Recently, however, cardiomyocyte force measurement system based on atomic force microscopy (AFM) was developed which can also be used to study single cardiomyocytes and small clusters of cardiomyocytes [60]. With the AFM system they were able to measure contractile forces, beat frequencies and durations of single cardiomyocytes and small cardiomyocyte clusters. The AFM-based method is also applicable for the screening of cardiac-active pharmacological agents. Cardiac microtissues have also been constructed using human pluripotent stem cell derived cardiomyocytes and the contraction force of the beating tissues has been analyzed with custom made platforms [61, 62].

6. Diseases modeled with iPS cell technique

Since the revolutionary discovery of iPS cells, multiple genetic diseases including cardiac and neuronal diseases have been modeled with patient specific iPS cell derived cells. Since primary human cardiomyocytes are not available for research in vitro, iPS cell derived cardiomyocytes are invaluable tool to study the pathophysiology of severe cardiac diseases and will undoubtedly provide groundbreaking innovations in the future.

6.1. Long QT-syndrome

Long QT-syndrome (LQTS) appears as a genetic or a drug-induced form. It is characterized by a prolonged cardiac repolarization phase resulting in a prolonged QT interval in the surface electrocardiogram (ECG). The clinical symptoms of LQTS include palpitations, syncope and seizures and even sudden cardiac death.

More than 700 mutations in 12 different genes (LQT1–12) have been found to affect genetic forms of LQTS [63]. However, two of these subtypes account the majority (>90%) of all the genetically identified LQTSs. Both of these mutations affect potassium channels altering their proper function. LQTS type 1 (LQT1) is the most common LQTS subtype, resulting from mutations in the KCNQ1 gene. This gene encodes the α-subunit of the slow component of the delayed rectifier potassium current (IKs) channel [64]. Individuals with LQT1 typically have symptoms when the heart rate is elevated e.g. during exercise [65, 66].

LQTS type 2 (LQT2) is due to non-proper functioning of the α-subunit of the rapid delayed potassium channel (IKr), which is encoded by the human ether-a-go-go-related gene (HERG),

also known as KCNH2-gene [67]. Contrary to type 1, individuals with LQT2 have clinical symptoms when the heart rate is slow [65, 66] and syptoms can be triggered e.g. by an alarm clock during sleep. The drug induced form of LQTS is due to altered function of the HERG-channel by the drug, therefore this channel has a significant importance during drug development and in safety studies.

The prevalence of the genetic form of LQTS is 1:2,000 in the general population [63]. However, the penetrance of the clinical symptoms of LQTS is low and there is considerable variation in phenotypic expression even within families carrying the same mutation [68]. It has also been suggested, that the population prevalence of milder LQTS mutations might be higher. Therefore the prevalence of latent or concealed LQTS, i.e. relatively asymptomatic individuals, would be higher than currently anticipated [69]. Due to this challenging and complex nature of LQTS, in addition, to the great interest of pharmaceutical industry towards this disease, multiple reports of iPS cell- based LQTS cell models have been published since 2007 when the iPS technology was invented.

Moretti and co-workers produced iPS cell derived cardiomycotyes from two patients carrying a KCNQ1 (R190Q) mutation [6]. In this study, the cardiomyocytes possessed the LQT1 genotype and exhibited prolonged action potential duration. The action potential prolongation was determined to be caused by the ion-channel trafficking defect resulting in a 70-80% IKs current density reduction. A β-adrenergic agonist isoproterenol altered the activation and deactivation kinetics of the IKs and this effect was rescued by the β-blockade [6]. Egashira and co-workers also produced a disease model for LQTS type 1 [70]. In their study, the iPS cells were derived from a sporadic patient who did not have a family history of significant QT interval abnormality. The mutation of the patient in the KCNQ1 was novel (1893delC) and the cells exhibited prolonged action potential duration in addition to arrhythmogenity.

Similarly results were found with iPS-CM derived from a patient suffering from the severe LQT type 2 syndrome. The patient had hERG (A614V) mutation and previously presented episodes of torsade de pointes (TdP), a special type of polymorphic ventricular tachycardia which is associated with LQTS [4]. The LQT2-cardiomyocytes derived from the patient's iPS cells demonstrated increased arrhythmogenicity associated with early after depolarizations (EADs) [5]. In addition, significant APD prolongation due to a reduced IKr current density was observed [4]. Arrhytmia and EADs were also induced by a specific HERG-channel blocker E-4031 to iPS cell derived CM having a hERG (G1681A) mutation. In addition, these cells exhibited EADs caused by the isoproterenol treatment and these EADs were rescued by β-blockade [5].

All the aforementioned studies were done with iPS cells derived from the symptomatic LQTS patients. Nevertheless, similar results have been obtained from patients without severe symptoms. In the study made in our institute, iPS cell lines were derived from a patient having a KCNH2 (R176W) mutation and a family history of LQTS. However, this individual was asymptomatic except for occasional palpitations. iPS cell derived cardiomyocytes from this patient manifest the phenotype characteristics to LQT2, such as a prolonged repolarization time and increased arrhythmogenicity [3].

A human cell model for LQT3 has also been produced and its function and characteristics were compared with a mouse models which were based on both mouse ESCs and mouse iPS cells affected with the same disease specific mutation [71]. LQT3 syndrome is due to mutations in the SCN5A gene, which encodes for the α-subunit of the cardiac sodium (Na+) channel. These mutations disrupt the inactivation of the Na+ channel during the action potential plateu phase and this irruption leads to the delay in repolarization and further prolonged QT interval [72]. In addition to LQT3, another kind of cardiac arrhythmia syndromes such as Brugada syndrome and cardiac conduction disease are associated with mutations in the SCN5A gene. In these syndromes the mutations are loss-of-function-type whereas LQT3 syndrome they are gain-of-function-type mutations [72, 73]. The comparison of multiple types of pluripotent stem cell derived cardiomyocytes showed that all of these models manifest the symptoms of the disease and, furthermore, the characteristics are similar within both species [71]. iPS cell models for these loss-of-function diseases have not yet been described.

6.1.1. Timothy syndrome

Timothy syndrome is caused by a single mutation in the CACNA1C-gene. This gene encodes the main L-type calcium channel, $Ca_v1.2$, in the mammalian heart which is essential for the cardiac action potential and also for cardiomyocyte contraction [74-76]. Timothy sydrome characterized by LQTS, syndactyly (webbing of fingers and toes), immune deficiency and autism [77] iPS cell derived cardiomyocytes originating from Timothy syndrome patients exhibited irregular functional properties typical for the disease [78]. Interestingly, these irregularities were restored by roscovitine, a compound which increases the voltage-dependent inactivation of $Ca_v1.2$ [78].

6.1.2. Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac disorder characterized by stress-induced polymorphic ventricular tachycardia in a structurally normal heart. CPVT is a very severe disease and 30-35% of mutation carriers have had symptoms (stress-related syncope, seizures or sudden death) by the age of 30. This disease is caused by mutations in the genes of RyR2 or calsequestrin (CASQ2) which is a regulatory calcium-buffering protein associated with RyR2 in the SR [79-82].

Multiple iPS-based CPVT disease models have been published, most of them having the disease specific mutation in the RyR2 gene while [83-85] and one having the mutation in the CASQ2 gene [86]. The congruent result from these CPVT model studies was the occurrence of delayed after depolarizations (DADs) and arrhythmias which are caused by the aberrant diastolic Ca^{2+} from the SR. Notably the model with RyR2-P2328S mutation also exhibited early after depolarizations (EADs) in addition to DADs suggesting suggesting another pathophysiological mechanism for CPVT [85]. Intriguing finding was also the effect of dantrolene in rescuing the arrhythmogenic phenotype [84].

6.1.3. Cardiomyopathies

Mutations in the genes expressed in the cardiomyocytes can cause heart diseases known as cardiomyopathies. Cardiomyopathies are currently categorized into the following four classes: arrythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, and restrictive cardiomyopathy [87]. Cardiomyopathies that are associated with mutations in genes encoding for sarcomeric proteins are a frequent cause of heart failure.

iPS cells have been used to generate cardiomyocytes from patients in a family with inherited dilated cardiomyopathy (DCM) [88]. The researchers generated a large number of individual-specific cardiomyocytes from a family carrying a deleterious point mutation (R173W) in TNNT2, a gene encoding for a sarcomeric protein cardiac troponin T, which regulates cardiomyocyte contraction. When compared to cardiomyocytes derived from iPS cells of healthy controls within the same family, the researchers showed that cardiomyocytes derived from iPS cells of DCM patients exhibited an increased heterogenous myofilament organization due to abnormal distribution of α-actinin, compromised ability to regulate calcium flux, and decreased contraction force. When DCM specific cardiomyocytes were stimulated with a β-adrenergic agonist, the cells showed characteristics of cellular stress such as reduced beating rates, compromised contraction, and a greater number of cells with abnormal sarcomeric α-actinin distribution. The authors also showed that the function of DCM-specific cardiomyocytes was improved with the treatment with β-adrenergic blockers or overexpression of Serca2a.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is another genetic cardiomyopathy characterized by replacement of cardiomyocytes by adipose and fibrous tissue leading to right ventricular failure, arrhythmias and even sudden death [89]. Twelve different genes have been linked to ARVC and all these encode cardiac cell adhesion proteins resulting in dysfunctional cardiac desmosomes. Cell adhesion proteins resulting in ARVC include plakoglobin (JUP), desmoplakin (DSP) and plakophilin 2 (PKP2). Patient specific iPS cells have been generated from an ARCV patient carrying a PKP2 mutation and having clinical manifestations of the disease [90]. ARVC specific cardiomyocytes revealed reduced amount of desmosomal proteins and more lipid droplets in the cardiomyocytes compared to control cardiomyocytes thus presenting the abnormalities observed in ARCV patients.

The third form of cardiomyopathy, hypertrophic cardiomyopathy (HCM), is a complex autosomal-dominant disease and the affected individuals acquire cardiac hypertrophy without external stimuli. Cardiac hypertrophy can be induced by different exogenous factors such as hypertension and valvular disease and even by severe exercise [91]. Affecting in 1 in 500 individuals within the general population, genetic HCM is the most common inherited cardiovascular disorder and the leading cause of sudden cardiac death in adolescents and young adults, especially in atheletes [92-94]. The majority of gene mutations associated with HCM occur in 13 sarcomere-related genes where several hundred mutations have been identified [94-97]. Typically cardiac hypertrophy affects the left ventricle and the interventricular septum and may eventually lead to left ventricular outflow tract obstruction, arrythmias, diastolic dysfunction, and sudden death. Other hallmark features are myocyte disarray and fibrosis [94-97]. The hypertrophic process in cardiomyocytes is characterized by morphological

changes including increase in protein synthesis, enhanced sarcomere reorganization as well as activation of specific cardiac genes [98-100].

iPS cell technology has not yet been reported to model HCM. However, iPS cells were used to generate cardiomyocytes from two LEOPARD syndrome patients carrying mutation in the PTPN11 gene encoding for the SHP2 phosphatase [101]. LEOPARD syndrome is an autosomal-dominant developmental disorder belonging to inherited RAS-mitogen-activated protein kinase signalling diseases. A major disease phenotype of the LEOPARD syndrome patients is HCM [102]. The iPS cell derived cardiomyocytes from LEOPARD syndrome were larger, had a higher degree of sarcomeric organization as well as preferential localization of NFATC4 in the nucleus when compared to iPS cell derived cardiomyocytes from healthy sibling of the LEOPARD syndrome patient thus presenting some indications of hypertrophy in patient specific cardiomyocytes.

7. Challenges with iPS cell technology and disease modeling

There are still several challenges that need to be carefully considered when designing disease modeling studies with specialized cell types derived from iPS cells. One potential challenge relates to the reactivation of silenced exogenous transgenes in the iPS cells or in their differentiated derivatives leading to the altered genomic integrity which may have unknown effects on the differentiation potential and characteristics of differentiated cell types. Efforts to improve the reprogramming methods have led to the technical development of nonintegrating approaches for iPS cell generation which will eliminate this risk in the future iPS cell lines and their differentiated derivatives. The nonintegrating sendai virus tehnique is already widely used in the generation of iPS cells. Regular monitoring of exogenous genes in iPS cells lines generated by using the integrating techniques is advisable.

Many genetic cardiac diseases are complex demonstrating huge clinical heterogeneity even within families and patients having the same mutation. In addition, reprogrammed cells carry genetic alterations that have accumulated through life, thus there is a risk that the variance overwhelms the ability to detect the authentic mechanisms in the pathophysiology of the disease. Thus, it will be essential to investigate adequate number of iPSC lines and patients to be able to demonstrate the common features of the cardiac disease phenotype. Further, it may be advantageous to initially compare the characteristics of cardiomyocytes from patients having severe symptoms.

Most likely in many genetic cardiac diseases various cell types in the heart contribute to the pathophysiological responses of the disease, thus there is a risk that it is impossible to recapitulate the features of the disorder by using solely cardiomyocytes. A 3D human heart tissue model with proper composition of cardiomyocytes, endothelial cells, fibroblasts, smooth muscle cells as well as neurons has not been developed but in recent years the advancement in this field of research has been rapid and hopefully in future we have besides cell models authentic tissue models to study genetic cardiac diseases.

The current cardiomyocyte differentiation protocols generate cells lacking full maturity when compared to human adult cardiomyocytes. This may lead to a situation where it is impossible to detect some molecular or functional basis of the cardiac disease. To reduce this risk it will be advisable to use control cells to compare diseased cardiomyocytes to healthy cardiomyocytes. For reliable and reproducible modeling of cardiac diseases it is necessary to have preferable multiple iPS lines from healthy controls. For monogenic diseases the use of iPS cells derived from the healthy family members would be favorable for minimizing the effect of genetic variation. However, iPS cells from family members are not always available. On possibility to overcome this challenge is to use genome editing techniques such as zinc finger nuclease technology and transcription activator–like effectors (TALEs) in modifying the iPS cells [103, 104]. With these methods, it is possible to correct a targeted point mutation in human iPS cells and produce control cells for disease specific iPS cells.

8. Conclusions

The most relevant human disease model uses cells of human origin, of the appropriate cell type, and with the identical genetic background as the patients. Traditionally, this approach in cardiac diseases has been out of reach as human cardiomyocytes are not easily procured and their propagation in vitro is extremely problematic. The revolutionary discovery of cellular reprogramming as a technology to generate iPS cells enables the production of patient specific cell types such as cardiomyocytes which can be used as authentic and relevant human cell models to study the pathophysiology of genetic cardiac diseases as well as in drug discovery and safety assays. The most relevant aspects in disease modeling are to show that the produced disease specific cell type bears the disease causing mutation and further to present the functional consequences of the mutant protein. Here we have reviewed the genetic cardiac diseases modeled thus far by using the iPS cell technology. Worthwhile of noticing is that the era of iPS cells in disease modeling is just in the very beginning. As the production of iPS cells and cardiomyocytes with more mature phenotype and the methods available for the functional characterization of cardiomyocytes continue to develop the future looks bright for modeling genetic cardiac diseases. Importantly these models will be extremely valuable for drug discovery and toxicology in the future.

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