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Pluripotent Stem Cells to Model Human Cardiac Diseases

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1. Introduction

For past several decades, laboratory animal models have been the prevailing paradigm for studying human diseases. A classic approach is to study the impact of specific genes through the use of gain- or loss-of-function mutant animals. While the animal models have greatly contributed to our understanding of the etiology and mechanisms of disease, they often fall short of fully recapitulating human pathophysiology and translating to clinical applications due to interspecies physiologic differences. In a review of preclinical studies of animal models published in high-impact scientific journals, approximately one-third translated to the level of human randomized trials and only one-tenth were subsequently approved clinically for patient use [1]. This attrition rate would have been even higher if less frequently cited animal research had been included. These unresolved issues with animal models have set the stage for the emergence of human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) for modeling human diseases.

Laid out in this chapter, we will discuss the development of various stem cell paradigms including mESC, hESC, and hiPSC (Figure 1); examine the utilization of these models via studies of cardiac diseases; assess the current limitations and future challenges; and finally conclude with the prospective outlook and viability of the field holistically in the scope of disease modeling.

2. Human cardiovascular diseases

According to the American Heart Association, cardiovascular diseases (CVD) remains the leading cause of deaths in United States, accounting for 32.8% of all deaths or roughly one of every three deaths [2]. To put into perspective, that is an average of 1 death every 39 sec-

onds. CVD is a generic term that encompasses conditions that affect the circulatory system, including myocardial infarction, angina pectoris, heart failure, stroke, and congenital cardio-vascular defects. Both genetic and environmental factors are implicated in the pathogenesis of CVDs. While some risk factors such as lifestyle habits and family history have been iden-tified for CVDs, much more remains to be learned about the pathophysiology, optimal man-agement, and proper prevention. Moreover, genetic predispositions like abnormalities in specific ion channels and sarcomere proteins pose special diagnostic and therapeutic chal-lenges. In fact, for most heritable forms of heart diseases, current treatment options leave much to be desired.
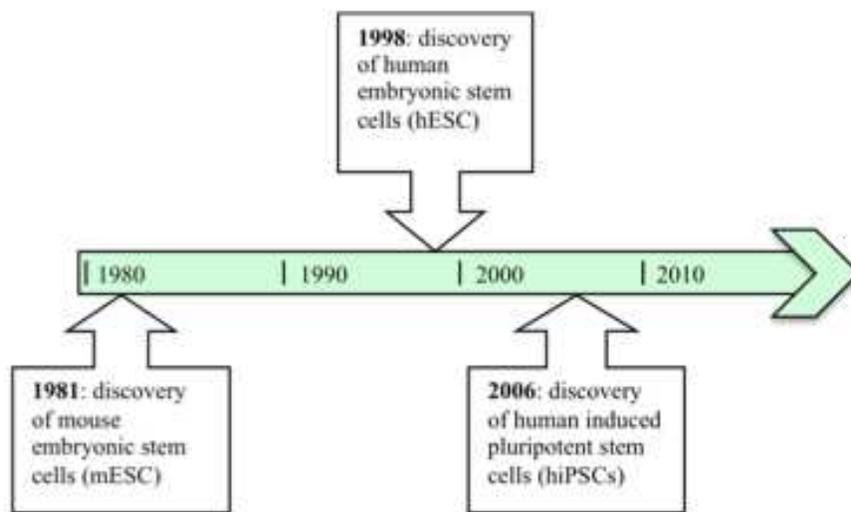

Figure 1. Timeline of stem cell modeling progress. Stem cell platforms are a new technology that was only intro-duced within the last two decades. The most recent breakthrough in hiPSC occurred just six years ago.

3. Stem cell disease modeling

Despite much progress in the past couple decades in the discovery of the molecular and ge-netic causes of many heart diseases, a detailed mechanic understanding of failing heart at the cellular level remains rudimentary. The main reason for this situation is the lack of ac-cess to live human tissues and unproven human cardiomyocyte cell culture models. Post-partum, cardiomyocytes become terminally differentiated and cease to proliferate, thus making isolation and culture of human myocardial cells extremely challenging. One surro-gate for human cardiomyocyte culture is the use of rat neonatal cardiomyocytes, which has been shown to yield 8.4×10^6 cells per heart [3]. However, with both human and rat neonatal cardiomyocytes, the inability to continuously passage cells and scarcity of resource make them unsustainable candidates for disease modeling.
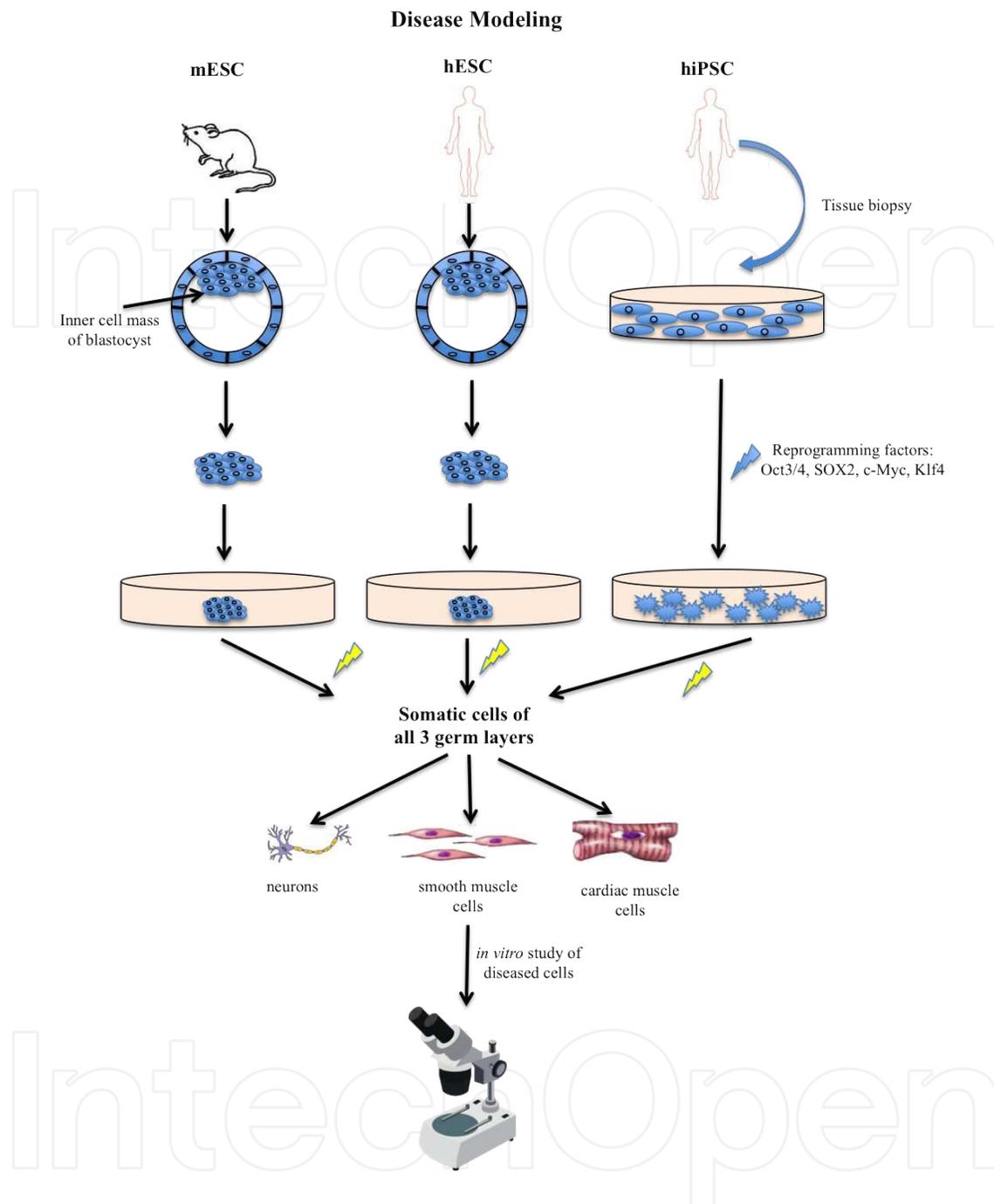
Disease Modeling

Figure 2. Overview of the stem cell disease modeling process. The blue and yellow lightning bolts indicate the addition of reprogramming and directed differentiation factors, respectively.

Furthermore, special considerations must be taken into account for critical differences between animal and human cardiomyocytes, in terms of cell biological, mechanical and electrophysiological properties. The lack of appropriate human heart disease models have hindered development of rational therapies, and the prospects for new therapies to treat heart diseases remain dim despite tremendous advances in various animal models. An alternative human biology based approach for heart disease modeling is to use human stem cells

as a renewable source of cells for cardiomyocytes. In the following section, we will discuss the various stem cell platforms (mESC, hESC, & hiPSC) for disease modeling, with specific focus on cardiovascular diseases (Figure 2).

3.1. mESC paradigm

In 1981, the first pluripotent mouse embryonic stem cells (mESCs) were isolated *in vitro* by culturing the inner cell mass of pre-implantation mouse blastocysts [4, 5]. These cells were capable of self-renewal and pluripotent differentiation into all three germ layers (ectoderm, mesoderm, and endoderm) [6]. The initial studies demonstrated a proof of concept, showing the feasibility of isolating pluripotent cells directly from early embryos. The unique capability of culturing pluripotent cells *in vitro* provided the means for genetic manipulation via selection or transformation of specific DNA fragments, and importantly to develop genetic mouse models of human disease. This platform allowed researchers to begin exploring pathways in cardiac development to dissect underlying molecular and cellular mechanisms causing congenital defects and other abnormalities.

While the general use of mESCs was promising, inherent problems with using animal models remained in the context of studying disease pathogenesis and pathophysiology. One of the crucial points of divergence is the shear difference in size and complexity between humans and mice both macroscopically and genomically [7]. Consequently, disease susceptibility may vary drastically. For instance, a mouse heart is ten thousand times smaller but beats roughly seven times faster than that of a human. The two organisms also differ in their expression of myosin heavy chain (MHC) isoforms. βMHC is the predominant isoform in fetal mouse hearts, whereas mainly αMHC is expressed in adults; conversely, the vice versa is true for humans [8]. Furthermore, mice are resistant to the development of coronary atherosclerosis even on a high-fat, high-cholesterol diet, because they lack cholesteryl ester transfer protein (CETP), an enzyme responsible for the transfer of cholesterol from high-density lipoprotein to low-density lipoprotein [9].

3.2. hESC paradigm

Building on the initial discovery of mESC technologies, increased research focus has been directed towards developing a human-based stem cell approach in anticipation of creating a more accurate disease model. It would be another seventeen years before human embryonic stem cells (hESCs) derived from the inner cell mass of the human blastocyst (stage 4-5 days post-fertilization) were isolated by Thomson *et al.* in 1998 [10]. Many factors hindered the transition from mESC to hESC, such as the limited availability of surplus human embryos, stringent growth requirements for culturing hESC, and the shroud of ethical controversies. Generating hESCs require the destruction of the donor embryo that is considered a potential human life by some ethical and religious groups. The debate revolving around hESC has deterred many researchers, mainly in the United States, from pursuit of this technology. In August of 2001, President Bush became the first President to provide federal funding for embryonic stem cell research, albeit limited to experimenting with only the 15 existing stem

cell lines [11]. Nonetheless, this discovery paved the way for modeling diseases directly on a human-based paradigm.

In a study in 2009, Lu et al. evaluated long-term safety and function of retinal pigment epithelium (RPE) as preclinical models of macular degeneration using hESCs [10]. When hESC-induced RPE were subsequently transplanted into mutant mice, they demonstrated long-term functional rescue, though progressively deteriorating function was noted due to the immunogenic response elicited by the xenografts. The initial data showed promise for future elucidation of macular degenerative disease pathophysiology. However, there were important obstacles to widespread clinical translation. First, transplantation of hESC requires immunosuppression, since the cells are allogeneic. In addition, a well known risk of this technology is the formation of teratomas, tumor-like formations containing tissues belonging to all three germ layers, if some undifferentiated pluripotent cells are transplanted [12]. Finally, perhaps the biggest obstacle to a widespread acceptance of human ESC transplantation is ethical and religious, as derivation of human ESCs typically involves the consumption of a human embryo.

3.3. hiPSC paradigm

Given these obstacles to a widespread use of the human ESCs, a new stem cell technology, human induced pluripotent stem cells (hiPSCs), has rapidly overtaken hESC research. Introduced in 2006 by Takahashi and Yamanaka, hiPSCs have been hailed as "the molecular equivalent of the discovery of antibiotics or vaccines in the last century [13]." The technology revolutionized the stem cell field, and for his achievement, Yamanaka received the 2012 Nobel Prize in Medicine." In a span of just six years, the field has rapidly expanded the repertoire of reprogrammable terminally differentiated tissue into hiPSC (keratinocytes [14, 15], hepatocytes [16], adipose-derived stem cells [17, 18], neural stem cells [19], astrocytes [20], cord blood [21, 22], amniotic cells [23], peripheral blood [24, 25], mesenchymal stromal cells [26], oral mucosa fibroblasts [27], and T-cells [28]). Most recently, the ability to generate hiPSC from Epstein-Barr virus (EBV)-immortalized B cell lines (lymphoblastoid B-cell lines) provides the opportunity to obtain samples from disease cohort repositories such as the Coriell Institute for Medical Research or the UK Biobank [29, 30].

In parallel, tremendous progress has been made towards the directing differentiation of these hiPSCs into various cell fates (neural progenitors [31], [32] motor neurons [33] [34], dopaminergic neurons [35], retinal cells [36], hepatocytes [37], blood cells [25, 38], adipocytes [39], endothelial cells [37, 38], fibroblasts [40, 41], and cardiomyocytes [42]). In theory, these patient-derived hiPSCs should be capable of differentiating into all of the >210 adult cell lineages. Nonetheless, our current growing repertoire sets the stage for studying various disease mechanisms in the laboratory, with the caveat that monogenic diseases such as long-QT syndrome will be much easier to model than complex diseases like Parkinson's.

As alluded to above, the somatic cell reprogramming offers several distinct advantages over embryonic stem cells. In the U.S. particularly, funding may be scarce at times due to the government's political stance regarding stem cell research. Importantly, somatic cells can be obtained from individual patients, enabling the development of truly personalized diagnostics and therapeutics.

4. Modeling cardiovascular diseases

While there is a wide array of cardiovascular diseases, we chose to focus on several with well-defined clinical presentation, strong genetic component, and significant research progress (Long QT syndrome types 1 and 2, Timothy syndrome, LEOPARD syndrome, & dilated cardio-myopathy; see Table 1). As discussed below, the paradigm of using stem cells to model inherit-ed cardiovascular diseases is rapidly being established and validated. Moreover, these advances with the rare inherited conditions may lead to new paradigms to study the much more prevalent acquired heart and vascular diseases at the cellular and molecular levels.

Genetic Disorder	Mutation	Main findings
Long QT syndrome Type 1		
Moretti et al. (2010) [48]	*KCNQ1* R190Q	marked prolonged action potentials; dominant negative trafficking defect associated with a 70 to 80% reduction in I_{ks} current; altered channel activation and deactivation properties; increased susceptibility to catecholamine-induced tachyarrhythmia attenuated by β-blockage
Long QT syndrome Type 2		
Lahti et al. (2012) [83]	*KCNH2* R176W	prolonged action potential; reduced I_{kr} density; more sensitive to potentially arrhythmogenic drugs; more pronounced inverse correlation between the beating rate and repolarization time
Itzhaki et al. (2011) [50]	*KCNH2* A614V	significant reduction of potassium current I_{Kr}; marked arrhythmogenecity; evaluated potency of existing & novel pharmacological agents
Matsa et al. (2011) [84]	*KCNH2* G1681A	prolonged field/action potential duration; I_{kr} blocker & isoprenaline induced arrhythmias presenting as early after depolarizations; attenuated by β-blockers propranolol & nadolol
Timothy syndrome		
Yazawa et al. (2011) [52]	*CACNA1C* G1216A	irregular contraction; excess Ca^{2+} influx; prolonged action potentials; irregular electrical activity; abnormal calcium transients in ventricular-like cells; roscovitine restored electrical and Ca^{2+} signaling properties
LEOPARD syndrome		
Carvajal-Vergara et al. (2010) [57]	*PTPN11* T468M	hypertrophic cardiomyopathy; higher degree of sarcomeric organization; preferential localization of NFATC4 in the nucleus
Dilated cardiomyopathy		
Sun et al. (2012) [67]	*TNNT2* R173W	altered regulation of Ca^{2+}; decreased contractility; abnormal distribution of sarcomeric α-actinin; β-drenergic agonist induced cellular stress; β-adrenergic blockers or overexpression of Serca2a improved function

Table 1. hiPSC studies modeling cardiovascular diseases.

4.1. Long QT syndrome

Long-QT syndrome (LQTS) is a rare congenital channelopathy disease that is characterized by an abnormally prolonged ventricular repolarization phase, inherited primarily in an autosomal dominant manner but sometimes autosomal recessively. It was first described in 1957 in a family with normal parents and two healthy children but also in which three children experienced recurrent syncope and sudden death [43]. Electrocardiography (EKG) studies showed prolonged QT interval due to increased ventricular action potential, hence the name of the disease (Figure 3). The prevalence of LQTS in the U.S. is approximately 1 in 7,000 individuals, causing 2,000 to 3,000 sudden deaths annually in children or adolescents [44]. This abnormality can lead to an increased risk of such reported incidence of sudden death, usually triggered by the resulting ventricular fibrillation or torsade de pointes (polymorphic ventricular tachycardia). Depending on the specific gene mutation, long-QT syndrome can be classified into 12 genetic subtypes [45]. Together, LQT1, LQT2, and LQT3 genotypes account for 97% of the mutations identified to date [46].
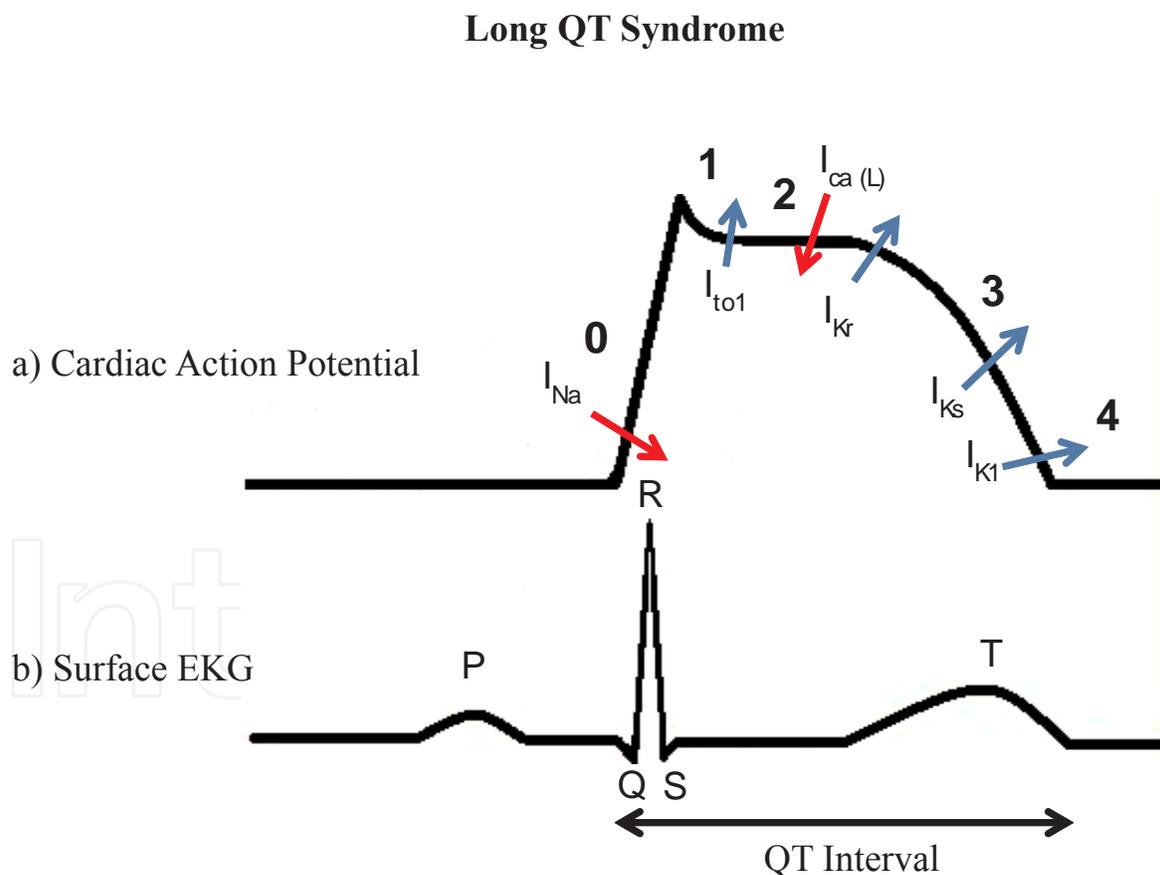
Long QT Syndrome

Figure 3. Long QT Syndrome. a) a visual representation of the cardiac action potential during depolarization and repolarization of the cell. There are 4 phases of the cycle in which various ion channels open and close, causing the flux of charged ions (red: into the cell & blue: out of the cell) and reflecting the change in overall action potential. **b)** an illustration of a normal surface EKG plot, highlighting the QT interval in particular. In long QT syndrome, a clear indication is the prolongation of that interval on an EKG.

Our current understanding of how mutations in ion channels cause disease can only be extrapolated from, at best, mammalian cell lines such as immortalized human embryonic kidney 293 cells or *Xenopus* oocytes using heterologous expression systems designed with the mutant channel of interest [47]. Commonly used mouse models are not apt for studying LQTS because the I_{Kr} current is essentially absent in the mouse heart. With the advent of patient-derived iPSC technology, cardiac induction of these cell lines may recapitulate their respective disease pathophysiology *in vitro*, providing a unique platform for studying cellular and molecular mechanisms and assessing the efficacy of various therapies.

4.1.1. Long QT syndrome type 1

The most common type LQT1, accounting for roughly 45% of genotyped patients, results from mutations of the alpha subunit of the slow delayed rectifier potassium channel KvLQT1, encoded by gene KCNQ1 on chromosome 11 [48]. In a recent study aimed at recapitulating disease phenotype using patient-derived iPSCs, Moretti et al. initially screened a family affected by LQTS type 1 through genotyping and electrophysiology studies, identifying an autosomal dominant missense mutation R190Q of KCNQ1 [48]. Then, they reprogrammed skin fibroblast from two affected family members into iPSCs and directed cardiac induction to yield spontaneously beating cardiomyocytes. Finally, they characterized these heart cells through whole-cell patch clamp, observing reduced I_{ks}, a slow delayed rectifier potassium current, by 70-80%, altered I_{ks} activation and deactivation properties, and an abnormal response to catecholamine stimulation.

Not only were Moretti et al. able to capture characteristics of LQTS type 1 *in vitro*, they were also able to demonstrate physiologically how beta-blockers, clinically administered as a prophylactic therapy for asymptomatic LQTS type 1 patients, had protective effects against catecholamine-induced tachyarrhythmia by reducing early afterdepolarizations [49]. This ability to mimic LQTS type 1 in an *in vitro* human model paved way for similar studies involving other genetic diseases.

4.1.2. Long QT syndrome type 2

Similar to LQTS type 1, LQTS type 2 is another mutation arising from the alpha subunit of a potassium channel, but one with different properties: a KCNH2 (also known as hERG)-encoded rapid delayed rectifier potassium channel [50]. A diagnostic finding in patients is the onset of clinical symptoms such as syncope triggered by sudden loud noises [45].

In a study by Itzhaki et al., A614V missense mutation was identified in the KCNH2 gene in a 28 year old patient with clinically diagnosed type 2 LQTS [50]. Dermal fibroblast samples were obtained, reprogrammed to generate patient-specific human iPSCs, and through retroviral transduction, differentiated into embryoid bodies of spontaneously beating cardiomyocytes. Through the use of these iPSC-generated heart cells, they were able to conduct electrophysiology studies and test the effects of pharmacological intervention. Itzhaki et al. found marked prolonged action potential duration and significantly reduced peak ampli-

tudes of I_{Kr} activation and tail currents in the cells derived from the LQTS patient compared to those generated from a healthy individual, both hallmark signatures of LQTS. They also reported observing early-after depolarizations in 66% of the iPSC-CMs on both cellular and multicellular levels, a key finding suggestive of arrhythmogenicity that explains sudden death in LQTS patients clinically. With the amount of clinical evidence extracted from these patient-derived cardiomocytes, this novel technology can serve as an excellent *in vitro* disease model for understanding cellular & molecular pathogenesis and becomes a very viable option for personalized medicine in the future.

4.2. Timothy syndrome

In contrast to the previously detailed potassium channel defects that lead to LQTS, Timothy syndrome is a form of LQTS caused by a missense mutation in the L-type calcium channel $Ca_v1.2$, encoded by the CACNA1C gene. This is the predominant L-type channel in the mammalian heart, which is essential for normal heart development and excitation-contraction coupling [51]. Mutations in this Ca^{2+} channel cause delayed channel closing and consequently, increased cellular excitability.

Concurrent with Itzhaki et al.'s publication LQTS type 2, Yazawa et al. reported their findings on Timothy syndrome using a patient-derived iPSC-CM disease model [52]. To summarize, using a similar cardiac induction protocol, they successfully reproduced *in vitro* cardiomyocytes exhibiting clinical Timothy syndrome phenotypes. Electrophysiology and calcium imaging studies showed irregular contraction, excess Ca^{2+} influx, prolonged action potentials, irregular electrical activity, and abnormal calcium transients in ventricular-like cells.

One of the key findings in their study was the functional difference between Timothy syndrome and LQTS type 1 cardiomyocytes. Unlike the latter where both ventricular- and atrial-like cells had prolonged action potentials, only ventricular Timothy syndrome cardiomyocytes exhibited this phenotype. Additionally, drug-induced triggering of arrhythmias and delayed depolarizations in LQTS type 1 cells were not necessary, because they were observed spontaneously in Timothy syndrome cells. While a direct correlation has yet to be established to the clinical outcomes (i.e. torsades de points and ventricular fibrillation), this study is another proof-of-concept that iPSC-CMs are invaluable for examining detailed pathogenesis of human diseases.

4.3. LEOPARD syndrome

LEOPARD syndrome is an autosomal-dominant developmental disorder with clinical manifestations described by its acronym: lentigines, electrocardiographic abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth, and deafness [53]. It is caused by a mutation in the PTPN11 gene, which impairs the catalytic region of the encoded SHP2 phosphatase [54]. Currently, drosophila [55] and zebrafish [56] models of LEOPARD syndrome have been described in literature, but the molecular basis of pathogenesis remains to be addressed.

In 2010, Carvajal-Vergara et al. successfully demonstrated the use of iPSC technology to characterize LEOPARD syndrome *in vitro* [57]. One of the clinical hallmarks of the disease is hypertrophic cardiomyopathy. In this study, iPSC-CMs derived from a 25-year old female patient with the condition were compared to those differentiated a healthy brother as a control. Carvajal-Vergara et al. showed, by comparison to the wild-type, larger patient-derived iPSC-CMs with a higher degree of sarcomeric organization and preferential localization of NFATC4 (calcineurin-NFAT pathway is an important regulator of cardiac hypertrophy [58]) in the nucleus [57]. Using antibody microarrays on patient-specific iPSCs, they also noted increased phosphorylation of certain proteins such as MEK1 leading to perturbations in the RAS-MAPK signaling cascade, which can begin to provide some preliminary understanding and elucidation of LEOPARD syndrome's pathogenesis on a molecular level [57].

4.4. Dilated cardiomyopathy

As previously mentioned, cardiovascular disease is the leading cause of morbidity and mortality worldwide, projected to represent 30% of all deaths in 2015 [59]. In the United States alone, heart disease accounts for roughly one-third of all deaths [60]. Of those, dilated cardiomyopathy (DCM) is one of the leading causes of heart failure and is associated with substantial mortality [61]. It leads to progressive cardiac remodeling, characterized by ventricular dilatation, hypertrophy, and systolic dysfunction [62]. In an estimated 20% to 48% of cases depending on the study, DCM is identified as a familial disorder with strong heritability [63]. Mutations in over 30 genes have been shown to be disease causing or disease associated [64].

One of the more common genetic defects causing DCM is a mutation in the cardiac troponin T gene (TNNT2) [65]. Mouse models have already provided invaluable insight to the disease mechanism. For instance, mice still displayed normal phenotype after knockout of one TNNT2 allele, because they only lead to a mild deficit in transcript but not protein [66]. Furthermore, the severity of DCM depends on the ratio of mutant to wild-type TNNT2 transcript, since mutant protein is associated with cardiomyocyte Ca^{2+} desensitization [66]. However, given the differences in electrophysiological and developmental properties, *in vitro* human models of DCM would conceivably provide a more precise platform for understanding molecular basis of pathogenesis.

In Sun et al.'s study published in 2012, they characterized iPSC-CMs from a family carrying a point mutation (R173W) in the TNNT2 gene by comparing to healthy individuals in the same cohort [67]. These patient-specific cardiomyocytes from diseased individuals exhibited dysregulated calcium handling, decreased contractility, and abnormal heterogenous distribution of sarcomeric α-actinin. The overexpression of Serca2a, a gene therapy treatment for heart failure currently in clinical trials [68], significantly improved the contractility force generated by iPSC-CMs derived from DCM patients [67]. Much like the use of hiPSC technology for other cardiovascular diseases discussed previously, it appears to be a robust system for describing pathogenesis of disease that has yielded preliminary positive results.

5. Stem cell disease modeling challenges

In the framework of disease modeling, both hESC and hiPSC technologies still have unresolved issues to address. For instance, hESCs display chromosomal instability with long-term *in vitro* culture [69], and iPSCs undergo dynamic changes in copy number variations during reprogramming, especially in the early passages [70]. In the U.S., research funding for hESC often fluctuates, subjecting to restrictions imposed by Congress and its current stance on the destruction of fertilized human embryos. The advantage of hiPSC over hESC is that it bypasses this controversy and generates autologous cells while maintaining key characteristics: morphology, feeder dependency, surface markers, gene expression, promoter methylation status, telomerase activities, *in vitro* differentiation potential, and *in vivo* teratoma forming capacity [71]. These features heavily favor hiPSC technology as the predominant approach for disease modeling over hESCs.

In the near future, the hiPSC model faces several main challenges. One of the concerns is developing a robust and efficient methodology for yielding large quantities of differentiated and functional cells of a designated lineage. Depending on the protocol and cell lines used, efficiencies can range anywhere from <0.0001% to >50%. Specifically in the case of cardiac induction, the hiPSC-induced cardiomyocytes resemble immature fetal cardiomyocytes in their gene expression profile (key marker is β-tubulin) as well as electrophysiologic and structural properties [72]. Resolving this hindrance will also have great impact on facilitating *in vivo* studies and widespread applications in drug discovery and development.

The practicality of studying disease pathogenesis *in vitro*, especially those with systemic involvement, raises another question. This intrinsic lack of an *in vivo* environment prevents a global understanding of how a disease may impact the body and simplifies interactions of basic signaling pathways. For more complex diseases, it may also be difficult to replicate conditions in a petri dish with a single lineage cell type, even if done via cocultures. Furthermore, the current designation of a control line is arbitrary since it is mainly a criterion of exclusions. In diseases such as Alzheimer's or Parkinson's, there is a long latency period, which would be hard to mimic *in vitro* due to the dynamics of real-time disease progression. Studies are currently underway to assess the possibility of accelerating disease progression *in vitro* via exposure to environmental factors contributing to the disease such as oxidative stress [73].

Finally, not all diseases can be readily modeled using hiPSCs. For example, patients with Fanconi anemia have a defective DNA repair mechanism, and therefore cannot be reprogrammed without antecedent gene correction [74]. For other conditions, some may exhibit low penetrance or do not follow a simple Mendelian form of inheritance and are affected by a multitude of factors ranging from the environment to epigenetics. The latter in diseased state may become an inevitable confounding factor working with iPSCs, because of its contribution to the low efficiency of reprogramming and its stochastic nature. In a study by Meissner et al., sub-clone lineages transfected with an Oct4-GFP reporter were obtained from early appearing iPSC colonies and displayed temporally different expression patterns

of GFP, some never expressing it at all [75]. Because of the sensitivity to epigenetic events, the use of histone deacetylase (HDAC) inhibitors may help promote self-renewal and/or directed differentiation of stem cells [76].

6. Future outlook & research direction

The intent of stem cell technology was to recapitulate, as closely as possible, disease phenotype in the human body for three primary outcomes: disease modeling, drug discovery & development, and regenerative medicine. The first of which will provide the initial platform from which drugs and therapeutic applications can be derived. In some cases, a treatment could be discovered before the underlying disease mechanism is understood, because patient-derived hiPSCs can be differentiated without any genetic modifications *in vitro* into the desired cell type and characterized in drug screenings.

In the context of patient-derived cardiomyocytes, while not a perfect *in vivo* surrogate, they will still be one of the better models currently available due to their identical genomes and phenotype. The complex interactions of normal human physiology is incredibly difficult to mimic outside the host, let alone recapitulating a diseased phenotype. The mouse model is currently the most common mammalian system used to study human physiology for several reasons: 90% genetic homology with comparable genomic sizes, relatively easy maintenance, rapid cost-effective breeding under laboratory settings, and capability for genetic manipulation. It is great for initial studies and insight into basic understanding and elucidation of the mechanisms underlying the disease.

Building on the gradual advancement from mESC to hESC to the current hiPSC technology, one of the technical goals remains to be removing all extrinsic factors with the goal of mimicking *in vivo* conditions. Most established mESC and hESC protocols relied on a fibroblast feeder-cell layer for culture and proliferation, which secrete undefined substrates into the medium and cause batch-to-batch variation [77]. Similarly, initial hiPSC protocols used a mouse embryonic fibroblast (MEF) feeder-cell layer that had similar problems [78]. In 2011, Yu et al. developed a feeder-free system with chemically defined medium and also replaced conventional transfection of somatic cells with footprint-less episomal reprogramming using small molecules to generate hiPSCs [79].

Furthermore, mESC and hESC-directed differentiation formed embryoid bodies (EBs), which are spheroids with an inner layer of ectoderm and a single outer layer of endoderm. These EBs differentiate to derivatives of all 3 primary germ layers, leaving a very low yield of spontaneously contracting cardiomyocytes. While this was sufficient for initial studies, larger quantities of pure cardiomyocytes are necessary to establish a scalable system for disease modeling and drug development. In 2007, Laflamme et al. reported the use of a monolayer cardiac induction system based on activin A and BMP4 with a 30-fold higher yield of pure cardiomyocytes than through the formation of EBs [80]. Most recently, Lian et al. of the Wisconsin stem cell group identified temporal modulation of canonical Wnt signaling as a key step for robust cardiomyocyte differentiation reporting efficient yields of up to 98% [81].

Further studies are needed to evaluate the optimal cardiac induction protocol. Once a robust, universal, and scalable system for directed differentiation of iPSCs into cardiomyocytes is established, we can provide an inexhaustible supply of patient-derived cells for research and therapeutic purposes.

6.1. Zinc finger nucleases

With some host-specific modifications, currently available technologies such as zinc finger nucleases can be applied as the next step in disease modeling after understanding the pathogenesis, developing a cure. Zinc finger nucleases are enzymes that manipulate specific sites of the host genome, generating transgenic lines via knocking-in and knocking-out of genes. The homologous recombination pathway, naturally occurring at DNA replication forks and repairing double stranded breaks, can be exploited to selectively target a locus for modification while leaving the rest of the genome in tact [82]. Through this method, we have been able to identify new gene function in mouse and other homologous mammalian models. The same concept can be applied to gene therapy for humans. For example, with patient-specific cardiomyocytes, constructs can be created and tested *in vitro* to restore wild-type function.

6.2. High-throughput screening

High-throughput screening is another means of advancing disease therapy, but it hinges on its scalability; in other words, whether or not cells of the disease model can be mass-produced. With current protocols for directed cardiac differentiation, every round of experiments would take at least 2 weeks [81]. If hiPSC-derived cardiomyocytes could be consistently generated in 96-well plates, then these high-throughput screenings that could propel translational research from a cellular and molecular level of disease directly to therapeutic applications.

7. Conclusion

In the new era of personalized medicine, the stem cell platform for disease modeling appears very viable, especially given the rapid advancements in the field over the past several decades. We have thoroughly discussed the advantages and disadvantages of using mESC, hESC, and hiPSC, all of which have the common end goal of best recapitulating disease phenotype *in vitro*. Of those, we strongly believe that hiPSC-derived cells can eventually be the gold standard for personalized medicine. Using a heritable cardiovascular class of diseases as an example, we endeavored to convey the potential benefits of harnessing iPSC technology to study the pathogenesis of various disorders. One of the most difficult challenges currently is establishing a robust, universal, and scalable cardiac induction protocol. Combined with the genetic tools available, we will be able to break the barriers to disease modeling with the limitless supply of human cells *in vitro*.

Acknowledgements

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References

[1] Hackam, D.G. and D.A. Redelmeier, *Translation of research evidence from animals to humans.* JAMA: the journal of the American Medical Association, 2006. 296(14): p. 1731-1732.

[2] Members, W.G., et al., *Heart Disease and Stroke Statistics‚Äî2012 Update.* Circulation, 2012. 125(1): p. e2-e220.

[3] Fu, J., et al., *An optimized protocol for culture of cardiomyocyte from neonatal rat.* Cytotechnology, 2005. 49(2): p. 109-116.

[4] Martin, G.R., *Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells.* Proceedings of the National Academy of Sciences, 1981. 78(12): p. 7634.

[5] Evans, M.J. and M.H. Kaufman, *Establishment in culture of pluripotential cells from mouse embryos.* Nature, 1981. 292(5819): p. 154-156.

[6] Urbach, A., M. Schuldiner, and N. Benvenisty, *Modeling for Lesch‚ÄêNyhan Disease by Gene Targeting in Human Embryonic Stem Cells.* Stem Cells, 2004. 22(4): p. 635-641.

[7] Musunuru, K., I.J. Domian, and K.R. Chien, *Stem cell models of cardiac development and disease.* Annual review of cell and developmental biology, 2010. 26: p. 667-687.

[8] Krenz, M. and J. Robbins, *Impact of beta-myosin heavy chain expression on cardiac function during stress.* Journal of the American College of Cardiology, 2004. 44(12): p. 2390-2397.

[9] Thomson, J.A., et al., *Embryonic stem cell lines derived from human blastocysts.* science, 1998. 282(5391): p. 1145-1147.

[10] Lu, B., et al., *Long‚ÄêTerm Safety and Function of RPE from Human Embryonic Stem Cells in Preclinical Models of Macular Degeneration.* Stem Cells, 2009. 27(9): p. 2126-2135.

[11] Walters, L.R., *Human embryonic stem cell research: an intercultural perspective.* Kennedy Institute of Ethics Journal, 2004. 14(1): p. 3-38.

[12] Zhang, Q., et al., *Stem cells and cardiovascular tissue repair: Mechanism, methods, and clinical applications.* Journal of Cardiothoracic Renal Research, 2006. 1(1): p. 3-14.

[13] Wu, S.M. and K. Hochedlinger, *Harnessing the potential of induced pluripotent stem cells for regenerative medicine.* Nature cell biology, 2011. 13(5): p. 497-505.

[14] Aasen, T., et al., *Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes.* Nature biotechnology, 2008. 26(11): p. 1276-1284.

[15] Carey, B.W., et al., *Reprogramming of murine and human somatic cells using a single polycistronic vector.* Proceedings of the National Academy of Sciences, 2009. 106(1): p. 157-162.

[16] Liu, H., et al., *Generation of endoderm‚Äêderived human induced pluripotent stem cells from primary hepatocytes.* Hepatology, 2010. 51(5): p. 1810-1819.

[17] Sun, N., et al., *Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells.* Proceedings of the National Academy of Sciences, 2009. 106(37): p. 15720-15725.

[18] Aoki, T., et al., *Generation of induced pluripotent stem cells from human adipose-derived stem cells without c-MYC.* Tissue Engineering Part A, 2010. 16(7): p. 2197-2206.

[19] Kim, J.B., et al., *Direct reprogramming of human neural stem cells by OCT4.* Nature, 2009. 461(7264): p. 649-643.

[20] Ruiz, S., et al., *High-efficient generation of induced pluripotent stem cells from human astrocytes.* PLoS One, 2010. 5(12): p. e15526.

[21] Haase, A., et al., *Generation of induced pluripotent stem cells from human cord blood.* Cell Stem Cell, 2009. 5(4): p. 434-441.

[22] Giorgetti, A., et al., *Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2.* Cell Stem Cell, 2009. 5(4): p. 353.

[23] Li, C., et al., *Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells.* Human molecular genetics, 2009. 18(22): p. 4340-4349.

[24] Loh, Y.H., et al., *Generation of induced pluripotent stem cells from human blood.* Blood, 2009. 113(22): p. 5476-5479.

[25] Ye, Z., et al., *Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders.* Blood, 2009. 114(27): p. 5473-5480.

[26] Oda, Y., et al., *Induction of pluripotent stem cells from human third molar mesenchymal stromal cells.* Journal of Biological Chemistry, 2010. 285(38): p. 29270-29278.

[27] Miyoshi, K., et al., *Generation of human induced pluripotent stem cells from oral mucosa.* Journal of bioscience and bioengineering, 2010. 110(3): p. 345-350.

[28] Seki, T., et al., *Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells.* Cell Stem Cell, 2010. 7(1): p. 11.

[29] Rajesh, D., et al., *Human lymphoblastoid B-cell lines reprogrammed to EBV-free induced pluripotent stem cells.* Blood, 2011. 118(7): p. 1797-1800.

[30] Choi, S.M., et al., *Reprogramming of EBV-immortalized B-lymphocyte cell lines into induced pluripotent stem cells.* Blood, 2011. 118(7): p. 1801-1805.

[31] Chambers, S.M., et al., *Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling.* Nature biotechnology, 2009. 27(3): p. 275-280.

[32] Neely, M.D., et al., *DMH1, a highly selective small molecule BMP inhibitor promotes neurogenesis of hiPSCs: Comparison of PAX6 and SOX1 expression during neural induction.*

[33] Dimos, J.T., et al., *Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons.* science, 2008. 321(5893): p. 1218-1221.

[34] Ebert, A.D., et al., *Induced pluripotent stem cells from a spinal muscular atrophy patient.* Nature, 2008. 457(7227): p. 277-280.

[35] Soldner, F., et al., *Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors.* Cell, 2009. 136(5): p. 964-977.

[36] Osakada, F., et al., *In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction.* Journal of cell science, 2009. 122(17): p. 3169-3179.

[37] Sullivan, G.J., et al., *Generation of functional human hepatic endoderm from human induced pluripotent stem cells.* Hepatology, 2010. 51(1): p. 329-335.

[38] Choi, K.D., et al., *Hematopoietic and endothelial differentiation of human induced pluripotent stem cells.* Stem Cells, 2009. 27(3): p. 559-567.

[39] Taura, D., et al., *Adipogenic differentiation of human induced pluripotent stem cells: comparison with that of human embryonic stem cells.* FEBS letters, 2009. 583(6): p. 1029-1033.

[40] Hockemeyer, D., et al., *A drug-inducible system for direct reprogramming of human somatic cells to pluripotency.* Cell Stem Cell, 2008. 3(3): p. 346-353.

[41] Maherali, N., et al., *A high-efficiency system for the generation and study of human induced pluripotent stem cells.* Cell Stem Cell, 2008. 3(3): p. 340-345.

[42] Uosaki, H., et al., *Efficient and scalable purification of cardiomyocytes from human embryonic and induced pluripotent stem cells by VCAM1 surface expression.* PLoS One, 2011. 6(8): p. e23657.

[43] Jervell, A. and F. Lange-Nielsen, *Congenital deaf-mutism, functional heart disease with prolongation of the QT interval, and sudden death.* American heart journal, 1957. 54(1): p. 59-68.

[44] Vincent, G.M., *The long QT syndrome.* Indian pacing and electrophysiology journal, 2002. 2(4): p. 127.

[45] Roden, D.M., *Long-QT Syndrome.* New England Journal of Medicine, 2008. 358(2): p. 169-176.

[46] Goldenberg, I., W. Zareba, and A.J. Moss, *Long QT syndrome.* Current problems in cardiology, 2008. 33(11): p. 629-694.

[47] Kamp, T.J., *An electrifying iPSC disease model: long QT syndrome type 2 and heart cells in a dish.* Cell Stem Cell, 2011. 8(2): p. 130-131.

[48] Moretti, A., et al., *Patient-specific induced pluripotent stem-cell models for long-QT syndrome.* New England Journal of Medicine, 2010. 363(15): p. 1397-1409.

[49] Vincent, G.M., et al., *High Efficacy of Œ≤-Blockers in Long-QT Syndrome Type 1.* Circulation, 2009. 119(2): p. 215-221.

[50] Itzhaki, I., et al., *Modelling the long QT syndrome with induced pluripotent stem cells.* Nature, 2011. 471(7337): p. 225-229.

[51] Xu, M., et al., *Enhanced expression of L-type Cav1. 3 calcium channels in murine embryonic hearts from Cav1. 2-deficient mice.* Journal of Biological Chemistry, 2003. 278(42): p. 40837-40841.

[52] Yazawa, M., et al., *Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome.* Nature, 2011. 471(7337): p. 230-234.

[53] Legius, E., et al., *PTPN11 mutations in LEOPARD syndrome.* Journal of medical genetics, 2002. 39(8): p. 571-574.

[54] Kontaridis, M.I., et al., *PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects.* Journal of Biological Chemistry, 2006. 281(10): p. 6785-6792.

[55] Oishi, K., et al., *Phosphatase-defective LEOPARD syndrome mutations in PTPN11 gene have gain-of-function effects during Drosophila development.* Human molecular genetics, 2009. 18(1): p. 193-201.

[56] Jopling, C., D. Van Geemen, and J. Den Hertog, *Shp2 knockdown and Noonan/LEOPARD mutant Shp2‚Äìinduced gastrulation defects.* PLoS genetics, 2007. 3(12): p. e225.

[57] Carvajal-Vergara, X., et al., *Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome.* Nature, 2010. 465(7299): p. 808-812.

[58] Heineke, J. and J.D. Molkentin, *Regulation of cardiac hypertrophy by intracellular signalling pathways.* Nature Reviews Molecular Cell Biology, 2006. 7(8): p. 589-600.

[59] Fuster, V. and B.B. Kelly, *Epidemiology of Cardiovascular Disease*. 2010.

[60] Roger, V.L., et al., *Heart Disease and Stroke Statistics--2012 Update: A Report From the American Heart Association*. Circulation, 2011.

[61] Refaat, M.M., et al., *Genetic Variation in the Alternative Splicing Regulator, RBM20, is associated with Dilated Cardiomyopathy*. Heart Rhythm, 2011.

[62] Maron, B.J., et al., *Contemporary definitions and classification of the cardiomyopathies an American heart association scientific statement from the council on clinical cardiology, heart failure and transplantation committee; quality of care and outcomes research and functional genomics and translational biology interdisciplinary working groups; and council on epidemiology and prevention*. Circulation, 2006. 113(14): p. 1807-1816.

[63] Brauch, K.M., et al., *Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy*. Journal of the American College of Cardiology, 2009. 54(10): p. 930-941.

[64] Li, D., et al., *Identification of novel mutations in RBM20 in patients with dilated cardiomyopathy*. Clinical and translational science, 2010. 3(3): p. 90-97.

[65] Willott, R.H., et al., *Mutations in Troponin that cause HCM, DCM AND RCM: What can we learn about thin filament function?* Journal of molecular and cellular cardiology, 2010. 48(5): p. 882-892.

[66] Ahmad, F., et al., *The role of cardiac troponin T quantity and function in cardiac development and dilated cardiomyopathy*. PLoS One, 2008. 3(7): p. e2642.

[67] Sun, N., et al., *Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy*. Science Translational Medicine, 2012. 4(130): p. 130ra47-130ra47.

[68] Jessup, M., et al., *Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Clinical Perspective A Phase 2 Trial of Intracoronary Gene Therapy of Sarcoplasmic Reticulum Ca2+-ATPase in Patients With Advanced Heart Failure*. Circulation, 2011. 124(3): p. 304-313.

[69] Hanson, C. and G. Caisander, *Human embryonic stem cells and chromosome stability*. Apmis, 2005. 113(11‚Äê12): p. 751-755.

[70] Hussein, S.M., et al., *Copy number variation and selection during reprogramming to pluripotency*. Nature, 2011. 471(7336): p. 58-62.

[71] Takahashi, K., et al., *Induction of pluripotent stem cells from adult human fibroblasts by defined factors*. Cell, 2007. 131(5): p. 861-872.

[72] Josowitz, R., et al., *Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders*. Current Opinion in Cardiology, 2011. 26(3): p. 223.

[73] Saha, K. and R. Jaenisch, *Technical challenges in using human induced pluripotent stem cells to model disease*. Cell Stem Cell, 2009. 5(6): p. 584.

[74] Raya, Å., et al., *Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells.* Nature, 2009. 460(7251): p. 53-59.

[75] Meissner, A., M. Wernig, and R. Jaenisch, *Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells.* Nature biotechnology, 2007. 25(10): p. 1177-1181.

[76] Kretsovali, A., C. Hadjimichael, and N. Charmpilas, *Histone deacetylase inhibitors in cell pluripotency, differentiation, and reprogramming.* Stem Cells International, 2012. 2012.

[77] Boheler, K.R., et al., *Differentiation of pluripotent embryonic stem cells into cardiomyocytes.* Circulation research, 2002. 91(3): p. 189-201.

[78] Zhang, J., et al., *Functional cardiomyocytes derived from human induced pluripotent stem cells.* Circulation research, 2009. 104(4): p. e30-e41.

[79] Yu, J., et al., *Efficient feeder-free episomal reprogramming with small molecules.* PLoS One, 2011. 6(3): p. e17557.

[80] Laflamme, M.A., et al., *Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts.* Nature biotechnology, 2007. 25(9): p. 1015-1024.

[81] Lian, X., et al., *Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling.* Proceedings of the National Academy of Sciences, 2012. 109(27): p. E1848-E1857.

[82] Lombardo, A., et al., *Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery.* Nature biotechnology, 2007. 25(11): p. 1298-1306.

[83] Lahti, A.L., et al., *Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture.* Disease Models & Mechanisms, 2012. 5(2): p. 220.

[84] Matsa, E., et al., *Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation.* European heart journal, 2011. 32(8): p. 952-962.