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Chapter 12

Modeling Hypertrophic Cardiomyopathy with Human Induced Pluripotent Stem Cells

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Abstract

Research of genetic cardiovascular diseases has lacked of good disease models because rodents, which are primarily used, differ greatly from humans. The ability to derive human induced pluripotent stem cells (hiPSCs) from patients carrying inherited cardiac diseases has revolutionized research in the cardiovascular field. The aim for this chapter is to review the current hiPSC reprogramming methods and methods for differentiating human pluripotent stem cells (hPSCs) into cardiomyocytes. The chapter focuses on the published hiPSC models for hypertrophic cardiomyopathy (HCM) and discusses the challenges related to modeling this interesting disease using hiPSC technology.

Keywords: cardiac differentiation, cardiomyocyte, disease modeling, human induced pluripotent stem cell, hypertrophic cardiomyopathy

1. Introduction

The derivation of human embryonic stem cells (hESCs) [1] and, more recently, the invention of human induced pluripotent stem cells (hiPSCs) [2] have opened new opportunities for research and cellular therapies in regenerative medicine. These cells, collectively called human pluripotent stem cells (hPSCs), have the ability to self-renew indefinitely and to differentiate into derivatives of all three germ layers [1, 2]. Thus, hPSCs provide a potential source of cells for regenerative medicine applications as well as in vitro modeling of genetic diseases and drug screening.

Traditionally, hiPSCs have been reprogrammed from skin fibroblasts by virally transferring four pluripotency factors, specifically octamer-binding transcription factor 3/4 (*OCT3/4*), sex-

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determining region Y-box 2 (*SOX2*), Kruppel-like factor 4 (*KLF4*), and myelocytomatosis viral oncogene homolog (*c-MYC*), which integrate into the genome of the target cell [2]. More recent methods have aimed to produce hiPSCs using non-integrative viral transfection [3], integrative vectors that can be excised after reprogramming [4], or non-viral delivery methods, such as the introduction of episomal vectors into target cells using electroporation [5].

hPSCs can be differentiated into cardiomyocytes under laboratory conditions. During the recent years, the cardiac differentiation methods have developed from embryoid body (EB) formation [6] and co-culturing hPSCs with mouse endodermal-like cells (END-2) [7] to more direct differentiation on a monolayer using different growth factors and small molecules [8–10].

Hypertrophic cardiomyopathy (HCM) is one of the most common genetic cardiac diseases, with a worldwide prevalence of 1:500. In HCM, the cardiac muscle tissue mainly in the interventricular septum is thickened. The most severe symptoms of HCM are progressive heart failure and sudden cardiac death [11]. HCM is caused by more than 1400 mutations, which reside primarily in genes coding for sarcomeric proteins [12]. The clinical phenotype of the disease is variable, and most of the patients carrying the mutations live their lives without any symptoms [11]. Currently, there have been five reports published using HCM patient-specific hiPSCs for modeling the disease [13–17]. Results from these reports will be reviewed thoroughly in this chapter.

2. Human pluripotent stem cells

hPSCs are defined as undifferentiated cells which have the ability to self-renew indefinitely and to differentiate into derivatives of all three germ layers: endoderm, mesoderm, and ectoderm [1, 2]. The first hESC line was derived by Thomson and co-workers in 1998 [1]. Traditionally, hESCs are derived from the inner cell mass (ICM) of the blastocyst but early blastomeres or morula stage embryos have also been used [18, 19].

For a long time, the scientific community believed that cell differentiation was a one-way route and that there was no turning back when a cell had passed specific differentiation stages or reached the fully differentiated state. However, in 2006, Yamanaka and co-workers were able to reprogram already fully differentiated mouse cells back into the pluripotent stage via retroviral induction with specific pluripotency factors: *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc*. These four transcription factors, so called "Yamanaka factors," were able to force endogenous pluripotency genes to be turned on in the transfected cell, changing the cell back to a pluripotent state [20]. In 2007, the team repeated the reprogramming using human fibroblasts, creating hiPSCs [2]. Similar to hESCs, hiPSCs also have the ability to self-renew and give rise to all somatic cell types. Although the discovery of hiPSCs has been a revolutionary invention in the stem cell field, it would not have been possible without earlier research, including somatic cell nuclear transfer (SCNT) [21–23], which suggested that the nuclear status of a differentiated cell could be reverted back to totipotency by factors in the cytoplasm of an oocyte [24].

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Because of their unique properties, hPSCs can be maintained in the laboratory for extended periods of time in their undifferentiated state and differentiated into various cell types, including cardiomyocytes [6], retinal pigmented epithelial (RPE) cells [25], neural cells [26], and hepatocytes [27] (**Figure 1**). Thus, hPSCs represent a limitless cell source for regenerative medicine applications as well as for studying developmental processes and genetic diseases.

The groundbreaking discovery of hiPSCs has opened completely new opportunities for disease modeling and drug screening also in the cardiac field. Conventionally, cardiac diseases have been modeled using animal models or genetically engineered cell lines. These models often correlate poorly with the results from human studies. In addition, obtaining cardiac tissue

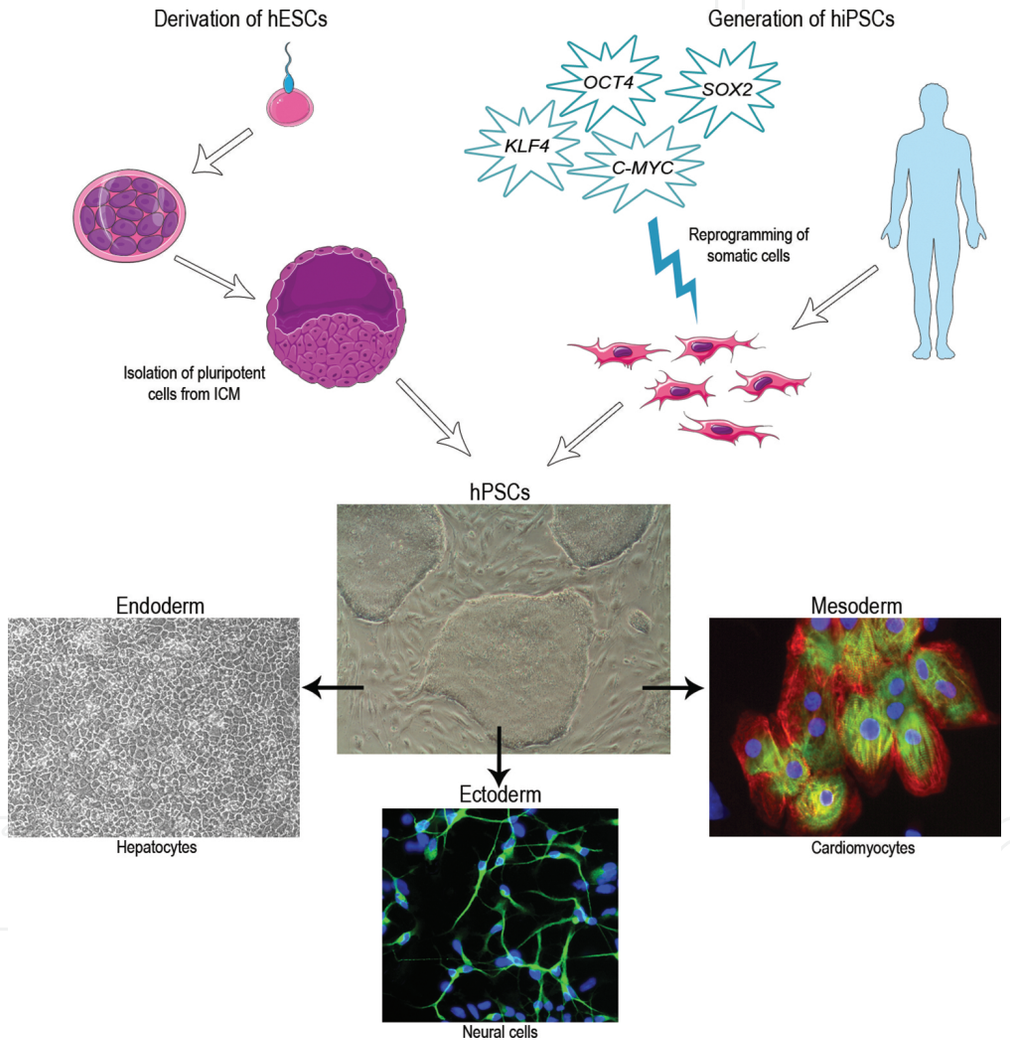


Figure 1. Human pluripotent stem cells (hPSCs). Human embryonic stem cells (hESCs) are derived from the inner cell mass (ICM) of a blastocyst. Somatic cells from adult individual are reprogrammed into human induced pluripotent stem cells (hiPSCs) by transferring exogenous pluripotency factors into the cells. hESCs and hiPSCs, collectively called hPSCs, have the ability to form all three germ layers. In the laboratory, hPSCs can be cultured for extended periods of time and differentiated into derivatives of different germ layers, such as hepatocytes, neural cells and cardiomyocytes. The figure was composed of images from the Servier Medical Art image bank (www.servier.com/Powerpont-image-bank) and cell pictures from BioMediTech.

directly from patients for research purposes is difficult, and adult human cardiomyocytes dedifferentiate rapidly under cell culture conditions and lose their characteristic properties. With the hiPSC technique, we are able to derive cells directly from a patient and transfer the same genetic information and mutations into hiPSC-derived cardiomyocytes. Therefore, hiPSCs have great potential to revolutionize the research of cardiovascular diseases.

2.1. Generation of human induced pluripotent stem cells

In the first conversion of mouse and human fibroblasts into iPSCs, Yamanaka's group used the viral transduction of four transcription factors (*OCT3/4*, *SOX2*, *KLF4*, and *c-MYC*) [2, 20]. This method involved the integration of viral genes into the host cell genome, which involves a risk of tumorigenicity due to the insertional mutagenesis and uncontrolled gene expression as well as potential reactivation of the virus [28, 29]. To circumvent these problems, a variety

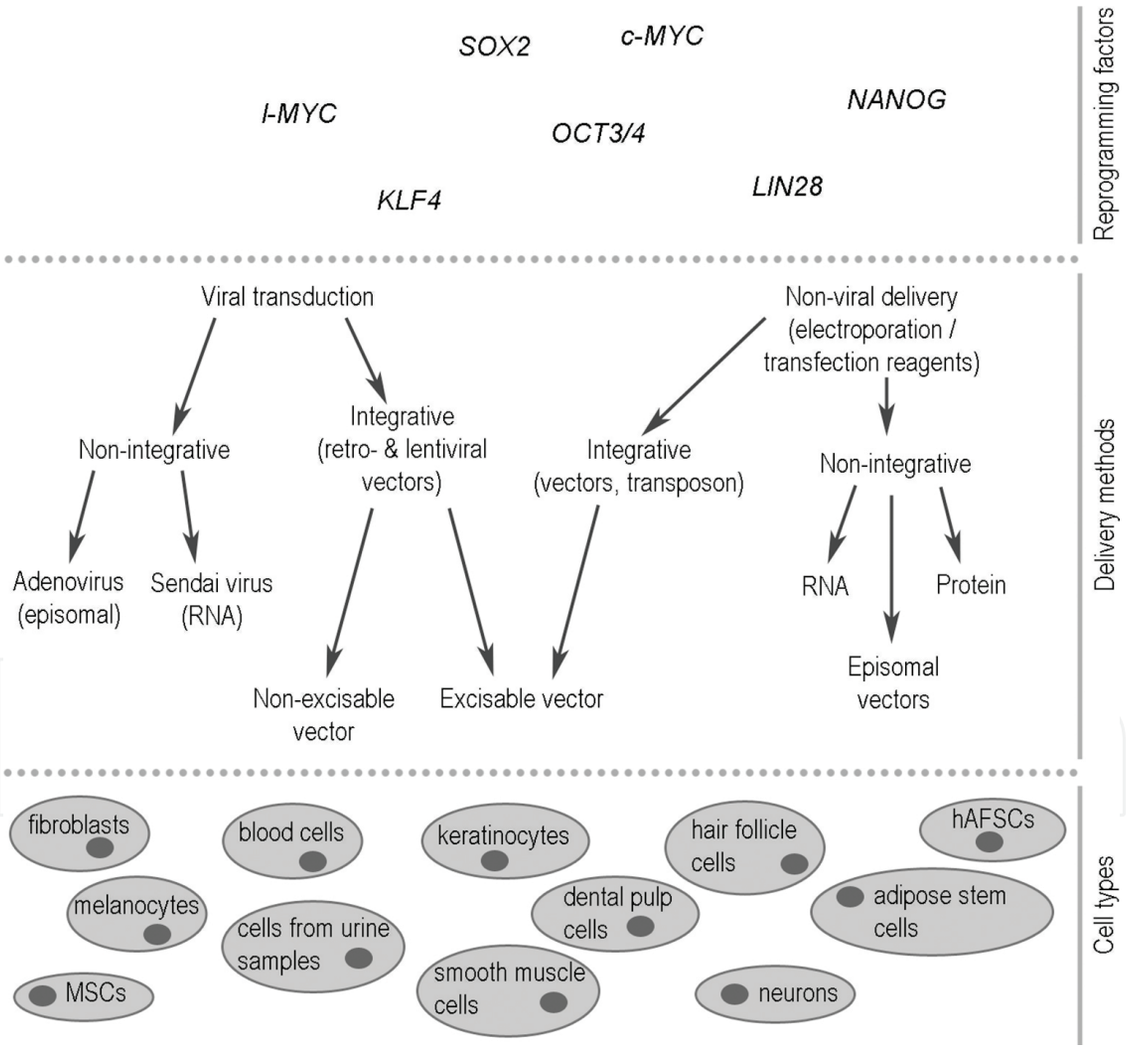


Figure 2. Reprogramming factors, different delivery methods, and donor cell types used in hiPSC generation. The data presented in the figure were collected from review articles [28, 30]. MSCs, mesenchymal stem cells; hAFSCs, human amniotic fluid stem cells.

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of new methods using different non-viral and non-integrative methods have been developed. Technological options for hiPSC transduction are presented in **Figure 2**.

Viral transduction can be achieved using either integrating or non-integrating viral vectors. In 2007, two distinct research groups published the first generation of hiPSCs. In the first paper, the delivery was accomplished using retroviral pMXs vectors [2], while in the second paper, the transduction was performed using lentiviruses [31]. The proteins, which are needed for the additional rounds of virus replication and packaging, are deleted from the pMXs vectors. Retroviral vectors are able to target cells according to their envelope pseudotype and they only transduce actively dividing cells. Lentiviruses, in contrast, can also transduce non-dividing cells [28]. Both retro- and lentivirally transferred genes are expected to be silenced during the reprogramming process through methylation and epigenetic modification [32]. Sometimes, however, the process is incomplete, which results in partially reprogrammed hiPSC lines [20, 33, 34].

To overcome the problems related to transgene integration into the host genome, excisable vectors have been engineered based on, for example, Cre-recombinase-mediated excision [4]. In this method, the sequence of the gene to be integrated into the genome is inserted between two loxP sites in the LTR (long terminal repeat) region of the vector. The integrated transgenes can be afterwards excised from the genome by transfecting the hiPSCs with Cre-recombinase [4]. Reprogramming factors in distinct vectors are integrated at independent sites in the genome, which can lead to genomic instability and genome reorganization when Cre-recombinase is introduced into the cells. Therefore, polycistronic vectors, which express all reprogramming factors in one vector separated by 2A sequences, are favored when using this method [35, 36].

Non-integrative viral methods include the use of Sendai viral and adenoviral vectors. With these two non-integrative viral methods, the Sendai virus has turned out to be more efficient for the generation of hiPSCs. Sendai virus vectors replicate their single-stranded RNA in the cytoplasm without entering the nucleus of the infected cell [3]. In addition, they are able to infect a wide variety of cell species and tissues by attaching to sialic acid receptors, which are present on the surface of various cell types [3]. Adenoviral vectors, in contrast, contain DNA, which is transported to the nucleus of the target cell. However, this adenoviral DNA is not integrated into the genome, and the expression of the adenoviral genes is thus transient [37, 38].

Non-viral methods are based on delivering genes (DNA), RNA copies of the genes or proteins to target cells. Different delivery carriers, such as transposons, and methods, including electroporation and transfection reagents, have been reported. The previously mentioned polycistronic vectors can also be transferred into target cells without viral delivery through electroporation [4, 36]. The PiggyBac transposon and transposase system is another integrative non-viral method used in hiPSC generation [39]. In this method, the transposase enzyme cleaves the delivered genes from specific cleavage sites in the PiggyBac vector and transfers them into the target genome. The same enzyme can be used to excise exogenous genes from the genome after reprogramming [39].

Methods based on episomal vectors, messenger RNA (mRNA) molecules, or purified protein have been developed for non-integrative non-viral hiPSC production. Episomal vectors derived from Epstein–Barr virus (oriP/EBNA1) can be used to introduce reprogramming factors into cells without a need for viral packaging [5, 40]. The expression of the exogenes is transient and they disappear from the transfected cells during culture [5]. Lastly, nanoparticles have been used to improve the efficiency of reprogramming, particularly with mRNA molecules and proteins [41, 42].

3. Differentiation of human pluripotent stem cells into cardiomyocytes

The heart is the first functional organ that develops during embryogenesis. It develops from the mesoderm, although signals from adjacent cell populations, especially from the endoderm, have a significant role in cardiogenesis. The entirety of the development of the human heart is not yet fully understood; however, many molecular events and factors taking part in the early stages of cardiomyogenesis have been identified. The three main growth factor families thought to participate in early mesodermal induction and cardiomyogenesis are the wingless/INT proteins (WNTs), the fibroblast growth factors (FGFs), and members of transforming growth factor-beta (TGFβ) superfamily, which include bone morphogenetic protein 4 (BMP4), Nodal and Activin A [43]. The expression of these factors or their inhibitors in the adjacent

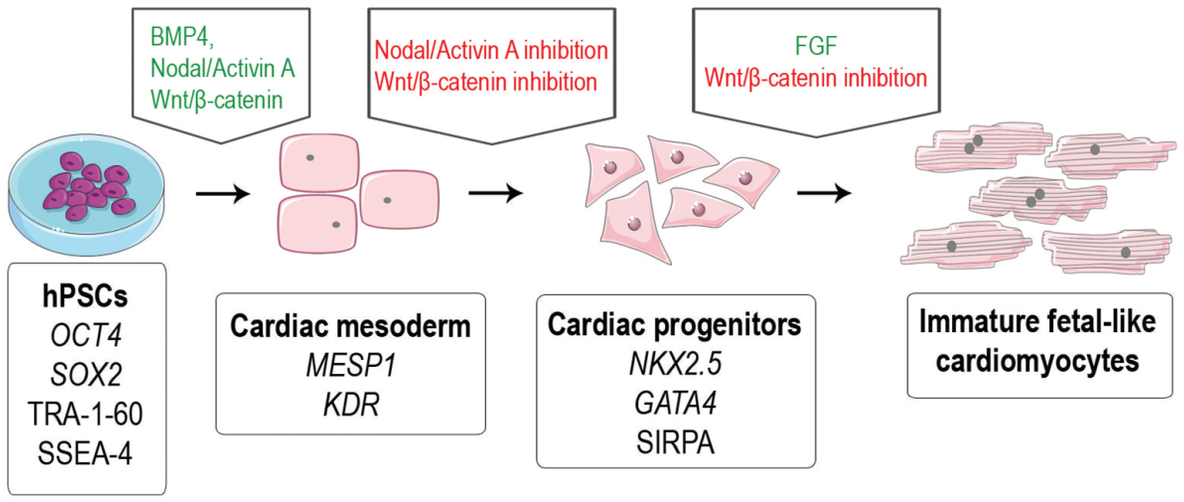


Figure 3. Model of different stages in cardiac differentiation. Transcription factors are presented in cursive and cell surface markers in normal text. The first precardiac mesoderm cells express the T-box factor Brachyury T and the homeodomain protein Mixl1 (*MIXL1*). These cells subsequently activate the mesoderm posterior 1 protein (*MESP-1*), and the cardiac mesoderm is formed. NK2 transcription factor-related gene, locus 5 (*NKX2.5*) and GATA-bonding protein 4 (*GATA4*) direct the development of embryonic cardiomyocytes. Finally, *NKX2.5*, together with certain other growth factors, activates cardiac structural genes, including actin, myosins, and troponins. By activating (green) and inhibiting (red) signaling pathways, hPSCs can be directed toward cardiac differentiation in vitro [43, 44]. The figure was composed of images from the Servier Medical Art image bank (www.servier.com/Powerpont-image-bank).

endoderm occurs at different times, and their combination eventually leads to the induction of the cardiac mesoderm. After receiving these initial signals, development is directed to more specific and highly conserved cardiogenesis [43–46].

In vitro differentiation methods mimic the phases of heart development, from the mesoderm to the cardiac mesoderm, cardiac progenitors, and finally, cardiomyocytes [44]. This process is regulated by alternating activation and inhibition of the signaling pathways participating in the cardiomyogenesis (**Figure 3**). Current cardiac differentiation methods are based on EB differentiation in suspension, co-culturing the cells with END-2 cells or inducing cardiac differentiation with different growth factors on a monolayer. The details of the most important differentiation methods are collected in **Table 1**.

Platform	Basal medium	GFs/SMs	Description/breakthrough	Efficiency	Reference
EB-based differentiation methods					
Low-attachment	DMEM + ko-SR	–	First generation of EBs from hESCs	–	Itskovitz-Eldor et al. [6]
Low-attachment	DMEM + FBS	–	First functional cardiomyocytes from EBs	30% (cTnI, FC)	Kehat et al. [47]
V-96 well	RPMI + FBS/HSA	PVA, BMP4, bFGF, insulin, AA	Forced aggregation strategy	60–90% (cTnI, FC)	Burridge et al. [48]
3D microwell	DMEM/F-12 + FBS	–	Engineered 3D microwell coated with Matrigel	18–20% (beating EBs)	Mohr et al. [49]
Low-attachment	StemPro34	AA, BMP4, bFGF, Activin A, VEGF, DKK1	Serum-free, GF-based EB method	40–50% (cTnT, FC)	Yang et al. [50]
Low-attachment	StemPro34	BMP4, AA, IWR-1, blebbistatin, Activin A	Serum-free, GF-based EB method	90% (cTnT, FC)	Karakikes et al. [51]
Methods based on END-2 cells					
END-2	DMEM + FBS	–	First co-culture with END-2 cells	35% (beating wells)	Mummery et al. [7]
END-2	DMEM + ko-SR	AA	Addition of AA and serum-free medium in END-2 co-culture	5–20% (α-actinin, ICC)	Passier et al. [52]
Low-attachment	END-2 CM	SB203580	Differentiation with medium conditioned on END-2 cells	22% (α-MHC, cytospin)	Graichen et al. [53]
Low-attachment	END-2 CM	Prostaglandin I2, AA, SB203580	Differentiation of EBs in END-2 CM, inhibitory	6–9%	Xu et al. [54]

Platform	Basal medium	GFs/SMs	Description/breakthrough	Efficiency	Reference
			effect of insulin	(α-MHC, cytospin)	
Monolayer methods					
Matrigel	RPMI + B27	Activin A, BMP4	First monolayer differentiation method	30% (β-MHC, ICC)	Laflamme et al. [9]
Matrigel	RPMI + B27	Activin A, BMP4, bFGF	Sandwhich method, Matrigel is applied below and on top of cells	40–98% (cTnT, FC)	Zhang et al. [55]
Matrigel	RPMI + B27	CHIR99021, IWP2/ IWP4	Protocol exploiting small molecules modulating Wnt signaling pathway	80–95% (cTnT, FC)	Lian et al. [10]
Vitronectin	RPMI + albumin	AA, CHIR99021, WNT-C59	Serum- and animal-component free, defined differentiation protocol	80–90% (cTnT, FC)	Burridge et al. [8]

GFs, growth factors; SMs, small molecules; ko-SR, knockout serum replacement; FC, flow cytometry; HSA, human serum albumin; PVA, polyvinyl alcohol; AA, ascorbic acid; ICC, immunocytochemistry; CM, conditioned medium.

Table 1. Overview of the development of cardiac differentiation methods.

The first hESC-derived cardiomyocytes were isolated from spontaneously formed EBs [6, 47]. EBs are spherical, multicellular, three-dimensional aggregates, which are formed when hPSCs are detached from the feeder cell layer or the culture matrix and cultured in suspension. In these EB structures, the hPSCs spontaneously differentiate into all three germ layers. In more recent versions of EB differentiation protocols, the aim has been to generate more uniformly-sized EBs, for example, via forced-aggregation using centrifugation [48, 56] or by culturing hPSCs in microwells coated with Matrigel prior to EB formation [49]. Due to the increased knowledge of the heart development in recent years, more guided EB differentiation methods using different growth factors and serum-free basal medium have been developed. Yang and co-workers were able to generate a population of cardiac progenitor cells by inducing EB differentiation with Activin A, BMP4, bFGF, vascular endothelial growth factor (VEGF), and Dickkopf homolog 1 (Dkk-1) [50]. With the protocol of Karakikes et al. [51], almost 100% of the EBs were beating and 90% of the cells were cTnT positive. In this protocol, the EB formation is induced by BMP4 and blebbistatin, which inhibits the actin-myosin contraction and suppress the dissociation-induced apoptosis. After this initial step, the EBs are induced to cardiac lineages by ascorbic acid, BMP4, Activin A, and finally with IWR-1 [51].

As discussed above, anterior endoderm is located directly posterior to mesoderm in the early development and the signals from adjacent endoderm initiate the early cardiogenesis. Based on this fact, Mummery et al. developed a differentiation method in 2003, in which the hPSCs are plated on top of mitotically inactivated END-2 cells [7, 52]. END-2 cells are derived from mouse P19 embryonal carcinoma cells, and they provide cell-to-cell contacts and produce

factors that induce cardiac differentiation. However, medium conditioned on END-2 cells alone has been used in cardiac differentiation, which suggest that cell-to-cell contacts might not be needed in the cardiac induction [53, 54]. The exact mechanism how END-2 cells induce the cardiac differentiation is still unclear. However, a valuable details of cardiac differentiation has been discovered using these cells. For example, END-2 cells have been shown to remove insulin from the medium and insulin has been shown to inhibit the differentiation of hPSCs into cardiac lineages [54, 57]. Although, the efficiency of END-2 co-culture method is quite low, the method has turned out to be reliable in generating cardiomyocytes from various hPSC lines.

The increase in understanding the regulatory signaling pathways related to cardiogenesis in conjunction with advances in hPSC culture methods has enabled the development of more defined and guided monolayer-based differentiation methods. Monolayer methods begin with the feeder cell-free culture of hPSCs, in which the feeders are substituted with Matrigel or Geltrex, or matrix composed of different extracellular matrix (ECM) proteins. The advantage of monolayer differentiation methods is that the cells are in uniform monolayers and there are no diffusional barriers, which would prevent the function of growth factors. Thus, differentiation should be easier to control and reproduce, than in EB or in co-culture methods [44]. In the first monolayer method published in 2007 by Laflamme and co-workers [9], cells were directed toward cardiac differentiation by a combination of Activin A and BMP4. One of the more recent methods, the so-called sandwich method, is based on the combination of ECM with growth factor signaling [55]. Cells were seeded on Matrigel matrix, and after reaching 90% confluence, Matrigel was added on top of the cells. The sequential application of Activin A, BMP4, and bFGF on the matrix sandwich resulted in 40–90% pure cardiomyocyte populations [55].

As in the case of hPSC culture development, cardiac differentiation is also moving toward easily scalable, chemically defined, and xeno-free conditions. A group of small molecules has been identified and applied to replace the recombinant cytokines and unknown factors in the serum. These molecules either activate or inhibit the WNT and TGFβ-signaling pathways. Two recent publications are based on the sequential activation and inhibition of the WNT signaling pathway. At first, the formation of the mesoderm is induced using small molecules, such as CHIR99021 and BIO for WNT signaling activation [10, 58]. After that, more specific cardiac differentiation is induced by inhibiting the WNT signaling pathway using small molecules, such as KY02111 and IWP2 [10, 58].

In 2014, Burridge and co-workers [8] published a chemically defined cardiac differentiation method. The medium consisted of Roswell Park Memorial Institute (RPMI) basal medium supplemented with HSA and L-ascorbic acid. Cardiac differentiation was further induced by the sequential activation and inhibition of WNT signaling by CHIR99021 and WNT-C59, respectively. They also tested various defined matrices (E-cadherin, vitronectin, vitronectin peptide, laminin-521, laminin-511, fibronectin, and fibronectin peptide) in combination with differentiation medium. Laminins were the most promising, but because they are extremely expensive for large-scale applications, vitronectin was selected for further studies. The final

protocol resulted in 80–90% pure cardiomyocyte populations for the multiple hPSC lines tested [8].

Although the differentiation methods for hPSC-derived cardiomyocytes have developed greatly, the efficiency of differentiation varies to a large degree when using different methods and cell lines and none of the methods result in a homogenous population of cardiomyocytes [8, 44, 59]. Homogenous cell populations would be needed, for example, to obtain reliable results from drug-screening assays [44]. Thus, there is a need for efficient purification methods.

Manual dissection [7] and Percoll gradient separation [60] were the first published methods for cardiomyocyte purification. However, the yield of pure cardiomyocytes with these methods was quite low. There are a few cell surfer markers that can be used for fluorescence activated cell sorting (FACS)-based purification of cardiomyocytes. These markers include signal regulatory protein α (SIRPA), which is expressed both in cardiac progenitor cells and in hPSC-derived cardiomyocytes [61], and vascular cell adhesion molecule 1 (VCAM1), which functions in leukocyte-endothelial cell adhesion but is also expressed in hPSC-derived cardiomyocytes [62]. One of the most recent method takes advantage of cardiomyocytes' ability to use lactate as an energy source. When culturing cells in glucose-depleted and lactate-abundant conditions other cells than cardiomyocytes will not survive [63]. Another interesting option is the microRNA (miRNA) switch technology, in which the cells are purified based on their miRNA activity. Heterogenous cell population is transfected with synthetic mRNA, which comprise cell type specific miRNA target site and fluorescent protein. Cells in which the target miRNA is absent will translate the fluorescent protein and can be sorted out from the cardiomyocyte population. Alternatively, miR-Bim-switch can be used, which selectively induces the apoptosis of cells in which the miRNA is not present [64].

3.1. Characterization of hPSC-derived cardiomyocytes

hPSC-derived cardiomyocytes can be characterized by their structural and biochemical, as well as by their functional features. The most apparent characteristic of hPSC-derived cardiomyocytes is their ability to contract spontaneously in culture [7, 47]. The organization of the internal structures can be studied and analyzed after immunolabeling the sarcomeric proteins [65]. The ultrastructural features of hPSC-derived cardiomyocytes can be studied in more detail using electron microscopy (EM). Laurila et al. has recently published a thorough review on different methods used in functional analysis of hiPSC-derived cardiomyocytes [66]. Shortly, the electrical properties of hPSC-derived cardiomyocytes can be studied on a cell cluster level using the microelectrode array (MEA) platform approach [47, 67] or on the single-cell level using the patch clamp-method [68–70]. Calcium plays a major role in excitation-contraction coupling process by which an electrical signal is transformed into a mechanical contraction [71]. The intracellular Ca^{2+} signaling in hPSC-derived cardiomyocytes on a single-cell level is studied in vitro using specific fluorescent probes for Ca^{2+} ions, such as fura-2 [72]. The mechanical beating behavior of hPSC-derived cardiomyocytes can be analyzed using methods based on video imaging [73].

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4. Hypertrophic cardiomyopathy

An adult cardiomyocyte is composed of evenly distributed and organized myofibrils, which are divided into approximately 2.2-μm-long contractile units called sarcomeres. Sarcomeres are composed of thin actin and thick myosin filaments and Z-discs. The thin filaments are attached to Z-discs, which separate the sarcomeres from each other. The thin filament is composed of repeating actin molecules, troponin (Tn) complexes, and α-tropomyosin (TPM1) molecules. Tn complexes consist of TnT, TnI, and TnC. The Tn complex works together with TPM1 during cardiac contraction. The thick filament consists of myosin molecules, which are built upon two units of α- and β-myosin heavy chain (α-MHC, β-MHC) and four myosin light chain (MLC) molecules. Among the other proteins of the thick filament, myosin-binding protein C (MYBPC) plays the most important role in the contraction. It contributes to actin-myosin interactions and cross-bridge formation [74].

When the Ca^{2+} concentration in the cytosol increases, the Ca^{2+} binds to TnC leading to a conformational change in the Tn complex. This leads to TPM1 moving from its inhibitory position, allowing the head region of the MHC to bind to actin, forming a cross-bridge. Then, myosin hydrolyzes adenosine triphosphate (ATP), causing the sliding of actin and myosin filaments and muscle contraction. In addition to the structural proteins mentioned above, the sarcomere consists of many other important proteins, which together form a stabilized and organized structure [71].

Cardiomyopathies are diseases that affect the heart muscle and can lead to progressive heart failure and cardiac death. Cardiomyopathies can either be genetic or acquired, and they can be divided into groups based on their morphological and functional characteristics. Cardiomyopathies include, among others, HCM, dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) [75]. HCM is one of the most common genetic cardiac diseases, with a worldwide prevalence of 1:500, and is the most common cause of sudden cardiac death among young competing athletes. HCM is inherited in an autosomal dominant pattern, and the mutations are mainly located in the sarcomeric proteins, which are responsible for the contraction and relaxation of the cardiomyocyte. The clinical manifestation of the disease is extremely variable: it has age-related penetrance, and the clinical symptoms can vary within the same family having the same gene mutation. Together, these facts indicate that there might be other factors in addition to the actual gene mutation, for example, epigenetic and environmental factors, that determine the clinical outcomes of the disease. Although a large number of mutations have now been identified and related to HCM, the pathophysiological mechanisms of the disease are still largely unknown [11].

In HCM, the cardiac muscle is thickened (≥15 mm) most commonly in the interventricular septum separating the right and left ventricles. This thickening, i.e., hypertrophy, can lead to outflow tract obstruction, which indicates that the passage to the aorta from the left ventricle becomes narrow and obstructive, disturbing the blood flow. This narrowed outflow induces a massive overload to the heart and can lead to progressive heart failure. Other severe complications related to HCM include arrhythmias and sudden cardiac death. However, most individuals remain asymptomatic for their whole lives, and it has been estimated that the

actual prevalence of the disease might even be 1:200 in the general population [11]. Penetrance indicates the percentage of mutation carriers who experience the phenotype of the disease. In HCM, the penetrance is highly variable: it can be age-related or even incomplete and related to gender [76].

There is no specific cure for this disease and all the complications related to HCM should be treated individually. Patients are typically asymptomatic for a long time. Often, the first sign is diastolic heart failure, while systolic heart failure can develop later. Treatments include beta-blockers or Ca^{2+}-channel blockers for relieving symptoms such as chest pain and shortness of breath and implantable cardioverter defibrillators (ICDs) for those patients who have survived cardiac arrest. The actual hypertrophy can be treated by surgical myectomy, which indicates removing a small portion of the thickened cardiac tissue; via ethanol ablation, in which a myocardial infarction is induced in the septal area; or at the end-stage via heart transplantation [11]. Histologically, HCM is characterized by myocyte hypertrophy (diameter >40 μm); disorganization of myocyte bundles, individual myocytes, and sarcomeres; and fibrosis of heart tissue. Nuclei are hyperchromatic (contain an abundance of chromatin), pleomorphic (vary in size and shape), and often enlarged [77].

HCM is inherited in an autosomal dominant pattern and is caused by over 1400 mutations found in eleven or more genes coding primarily for sarcomeric proteins. Approximately 70% of patients have a gene mutation either in the *MYH7* or *MYBPC3* genes while mutations in other genes are far less common [11, 12]. Rarer mutations are located in Z-disc genes or calcium handling and regulation genes. One interesting feature of HCM mutations is that they are almost all identified only in one or a few families [76]. Typically, patients carry only one heterozygous mutation in a single allele. However, lately, double or even greater numbers of mutations have been reported to be found in one patient, which might affect the clinical variance related to the disease [76, 78]. Most pathogenic mutations are missense mutations in which a single nucleotide is changed, resulting in an amino acid substitution. Missense mutations are thought to act in a dominant negative manner. Thus, the mutated protein is produced and interferes with the normal function of the sarcomere. Nonsense mutations, in contrast, lead to a premature stop codon and truncated proteins. These mutations are thought to result in haploinsufficiency, in which the mutated protein is either degraded or not produced at all. The majority of the gene mutations in *MYBPC3* are thought to act through this mechanism [74, 76].

In Finland, two founder mutations in genes coding for MYBPC and TPM1 proteins account for approximately 18% of Finnish HCM cases, and these *MYBPC3-Gln1061X* and *TPM1-Asp175Asn* mutations are relatively uncommon in other countries besides Finland [79]. *MYBPC3-Gln1061X* is a nonsense mutation leading to a premature stop-codon and a truncated MYBPC protein lacking the binding sites for both myosin and titin [80]. This mutation, similar to other mutations in *MYBPC3*, is characterized by age-related penetrance and late onset of the disease [80–82]. *TPM1-Asp175Asn* is a missense mutation that leads to the substitution of aspartic acid with asparagine in codon 175 [83]. Originally, *MYBPC3-Gln1061X* was associated with a clinically mild phenotype, and *TPM1-Asp175Asn* was associated with a clinically intermediate phenotype with a substantial risk for sudden cardiac death. Additionally,

patients with *MYBPC3-Gln1061X* were suggested to be more prone to cardiac dilation and heart failure [84]. However, in the most recent studies, both mutations were associated with variable left ventricular hypertrophy, and no clear genotype-phenotype correlations could be verified in clinical studies [79, 85].

Different animal models have been used to study HCM in vitro. These animals include cats, which have certain naturally occurring HCM mutations, and genetically engineered mice, rats, rabbits, and Drosophila [86]. Additionally, HCM patient tissues obtained from myectomy samples have been studied [87]. One of the major difficulties in studying the pathophysiological mechanisms of HCM has been the lack of tissue samples at early stages of disease development. While animal models have provided valuable insight into disease mechanisms, they contain only the mutated gene and not the rest of the genome, which might have effects on disease phenotype and progression. Thus, given that they contain the whole genomes of HCM patients, in addition to the fact that they are cell of human origin, hiPSC-derived cardiomyocytes represent a valuable new tool for modeling HCM in vitro.

4.1. Human induced pluripotent stem cell models for studying hypertrophic cardiomyopathy

To date, morphological and functional characteristics of cardiomyocytes, derived from HCM patient-specific hiPSCs, have been studied in five different reports [13–17]. The results presented in these publications are collected in **Table 2**. Cardiomyocytes have been obtained with different cardiac differentiation methods in each publication. In our report, we have differentiated hiPSCs into cardiomyocytes using END-2 co-culture method [15]. In addition, HCM hiPSC-derived cardiomyocytes carrying *MYBPC3-c.2373dupG* mutation were used in one report, in which the effects of serum on hypertrophic phenotypes were studied [88]. In this particular study, serum was found to mask the hypertrophic phenotype of hiPSC-derived cardiomyocytes with the *MYBPC3-c.2373dupG* mutation [88]. Serum increased the cell size of neonatal rat cardiomyocytes as well as cardiomyocytes derived from hESCs and control hiPSCs. However, the size of the hiPSC-derived cardiomyocytes with the *MYBPC3-c.2373dupG* mutation was smaller than that of hiPSC-derived control cardiomyocytes in the presence of serum. Under serum-free conditions, hiPSC-derived cardiomyocytes with the *MYBPC3-c.2373dupG* mutation were significantly larger than hiPSC-derived control cardiomyocytes [88]. Conversely, in our study, cardiomyocytes carrying the *MYBPC3-Gln1061X* mutation were enlarged, despite the 20% FBS used in the culture medium, challenging the role of serum in masking the hypertrophic phenotype of hiPSC-derived cardiomyocytes [15].

Patients, mutations and hiPSCs	HCM phenotype in hiPSC-CMs	Drug treatments/other findings
Lan et al. [16]	**Morphological properties**	**Calcineurin-NFAT signaling**
Family with HCM	Cellular enlargement, multinucleation,	Blockade by cyclosporin A and FK506
–5 pers. with MYH7-Arg663His	increased myofibril content, disorganized sarcomeres	reduced hypertrophy
–5 pers. without mutation	**Biochemical properties**	**β-adrenergic stimulation**
		Isoproterenol increased cell size and

Patients, mutations and hiPSCs	HCM phenotype in hiPSC-CMs	Drug treatments/other findings
(used as control) Timepoints: 20, 30, 40 days	Upregulation of *ANF*, *TNNT2*, *MYL2*, *MYH7*, *GATA4* and *MEF2c* Elevation of *MYH7*/*MYH6* ratio Nuclear translocation of NFAT **Ca^{2+} handling properties** Ca^{2+} transient irregularities Elevation of intracellular [Ca^{2+}] Smaller SR Ca^{2+} release **Electrophysiological and mechanical properties** Arrhythmic waveforms including frequent DADs Irregular beating observed in video recordings	Amount of irregular Ca^{2+} transients and arrhythmia Isoproterenol together with propanolol abolished Ca^{2+} abnormalities, arrhytmia and hypertrophy **Blockade of L-type Ca^{2+} channel** Treatment with verapamil for 5 days ameliorated HCM phenotype Diltiazem abolished Ca^{2+} abnormalities and arrhythmia
Han et al. [14] One patient: - *MYH7-Arg442Gly* Two control hiPSC lines from unrelated donors	**Morphological changes** Cellular enlargement, disorganized sarcomeres Disorganized Z lines in TEM **Biochemical changes** Changes in (whole transcriptome sequencing): wnt/β-catenin pathway Notch signaling pathway FGF pathway Nuclear translocation of NFAT Decreased level of *RYR2*, *SERCA2* **Ca^{2+} handling properties** Ca^{2+} transient irregularities elevation of intracellular [Ca^{2+}] Smaller SR Ca^{2+} release Delayed Ca^{2+} transient decay time **Electrophysiological properties** Prolonged and dispersed interspike intervals and increase of arrhythmogenic events in MEA Irregular contractility in real-time cell analyzer APD prolongation Changes in the shape of AP Increased Ca^{2+}, Na^{+} and outward K^{+} currents	**β-adrenergic stimulation** Isoproterenol elevated premature beats and irregular beating rates Isopoterenol together with metoprolol decreased beating irregularity and arrhythmia **Blockade of L-type Ca^{2+}channel** Treatment with verapamil for 4 days reduced arrhythmia and Ca^{2+} handling abnormalities **K_{ATP} channel opener** Antihypertensive drug pinacidil induced irregular interspike intervals **Inhibition of histone deacetylase activity** Treatment with trichostatin A for 3 days decreased cell size, nuclear translocation of NFAT, suppressed Ca^{2+} abnormalities and decreased resting [Ca^{2+}]
Tanaka et al. [17] Three patients:	**Morphology without stimulation** Mildly but significantly larger cell size	**Stimulation with hypertrophic factors** Angiotensin II, IGF-1, phenylephrine

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Patients, mutations and hiPSCs	HCM phenotype in hiPSC-CMs	Drug treatments/other findings
–*MYBPC3-GLY999-Gln1004del* –*TMP1-Arg91Cys* (identified later) –Mutation unknown Two control hiPSC lines from Unrelated donors Timepoints: 30, 60, 90 days culture as EBs	No time-dependent changes in cell size Myofibrillar disarray in EM and cTnT staining (>60d, >90d) cTnT and ANP protein levels higher MYBPC level lower in hiPSC-CMs with *MYBPC3* mutation Mildly disorganized contractile form in video analysis	No difference to non-stimulated cells Endothelin 1 (ET-1) Increased cell size and disarray Nuclear translocation of NFAT Disorganized contraction Similar response in mouse MYBPC$^{+/-}$ **Blocking of ET-1 signaling** ETA-b was able to block ET-1 induced hypertrophic phenotype ETB-b had no effect
Birket et al. [13] (hiPSC lines published in Dambrot et al. [88] Three patients: –*MYBPC3-c.2373.dupG* –One hiPSC line from each One control hiPSC line from unrelated donor	**Biochemical properties** Decreased level of MYBPC relative to α-actinin	**Stimulation with (T3, IGF-1, Dex) TID** Decreased traction stress Decreased traction force No difference in the cell size or contraction frequencies when compared to controls Similar findings in MYBPC knockdown hESC-derived cardiomyocytes
Ojala et al. [15] Four patients: –2 pers. with *MYBPC3-Gln1061X* –2 pers. with *TPM1-Asp175Asn* Two control hiPSC lines from unrelated donors Timepoints: 1w, 3w, 6w (as single cells)	**Morphological properties** Cellular enlargement, multinucleation **Biochemical properties** Upregulation of *MYBPC3*, *TNNT2*, *ACTN2*, *TTN* *MYL7* and *MYL9* MYBPC level slightly reduced in *MYBPC3-Gln1061X* cells **Ca^{2+} handling properties** Ca^{2+} transient irregularities **Electrophysiological and mechanical properties** Arrhythmic waveforms including frequent EADs and DADs	**Differences between mutations** *MYBPC3-Gln1061X* cells significantly larger Differences in expressions of various cardiac genes More abnormal Ca^{2+} signals in *TPM1-Asp175Asn* mutation More prolonged APD in *TPM1-Asp175Asn* mutation *MYBPC3-Gln1061X* mutation was not detected in mRNA or protein level

hiPSC-CM, human induced pluripotent stem cell-derived cardiomyocyte; SR, sarcomeric reticulum; DAD, delayed after depolarization; EM, electron microscopy; MEA, microelectrode array; APD, action potential duration; AP, action potential; T3 triiodothyronine hormone; IGF, insulin-like growth factor 1; Dex, dexamethasone; EAD, early after depolarization.

Table 2. Summary of results from published reports using HCM patient-specific hiPSCs.

Lan et al. published the first report of HCM hiPSC-derived cardiomyocytes in 2013. In their publication, hiPSCs were established via the lentiviral infection of fibroblasts derived from five patients carrying the *MYH7-Arg663His* mutation and from five related healthy individuals. Although the two youngest patients had not developed the clinical phenotype of HCM, the hiPSC-derived cardiomyocytes from all patients were significantly larger than the control cardiomyocytes [16]. In 2014, Han et al. published a report in which hiPSCs were derived from a single patient carrying a *MYH7-Arg442Gly* mutation. Control hiPSCs were derived from two unrelated healthy individuals. Fibroblasts were used as a cell source for all established hiPSC lines, and the infection was performed using retroviruses [14]. Tanaka et al. [17] derived hiPSCs from three unrelated HCM patients and three healthy volunteers in 2014. One of the HCM patients carried the *MYBPC3-Gly999-Gln1004del* mutation, while in the two other patients, the mutations were unknown. Two control hiPSC lines were generated from dermal fibroblasts using retroviruses, while all patient hiPSC lines and one control hiPSC line were derived from T lymphocytes or peripheral blood with Sendai viruses. They used a mixture of EBs derived from all three patients in their experiments [17]. In our publication, we derived hiPSC lines from two patients carrying the *MYBPC3-Gln1061X* mutation and from two patients carrying the *TPM1-Asp175Asn* mutation. The ages and clinical symptoms of our patients varied from asymptomatic to a patient suffering from atrial fibrillation accompanied with substantial thickening of interventricular septum. Two unrelated hiPSC lines derived from healthy volunteers were used as control cells. hiPSCs were derived from fibroblasts using either retro- or Sendai viruses. All hiPSC-derived HCM cardiomyocytes were significantly larger than hiPSC-derived control cardiomyocytes [15].

In addition to cell size, myofibrillar disarray has been studied in three of the publications. However, in all publications, different methods and criteria have been used to quantify the disarray, and the results can be subjective. Han et al. showed that HCM hiPSC-derived cardiomyocytes have more disrupted sarcomeres than cardiomyocytes derived from control hiPSCs. However, the authors did not present any criteria to determine how the disruption was qualified [14]. In Tanaka et al. [17], cardiomyocytes were qualified with myofibrillar disarray if over 50% of the myofibrils intersected with each other. In Lan et al. [16], cardiomyocytes that had more than 25% of their cell area exhibiting punctate TnT distribution were considered disorganized. The overall morphology of hiPSC-derived cardiomyocytes is not mature, and their structure is often unorganized; this is also the case in cardiomyocytes derived from control hiPSCs. We did not report myofibrillar disarray due to the lack of proper quantitation criteria for this phenomenon.

Electrophysiological properties of hiPSC-derived cardiomyocytes have been studied in three of the publications. In our study, the APD90 was significantly increased in hiPSC-derived cardiomyocytes carrying the *TPM1-Asp175Asn* mutation or the *MYBPC3-Gln1061X* mutation. Additionally, the action potential duration 90 (APD90) was significantly longer in cardiomyocytes carrying the *TPM1-Asp175Asn* mutation compared to cardiomyocytes carrying the *MYBPC3-Gln1061X* mutation. Although the numbers of cardiomyocytes exhibiting arrhythmias (delayed after depolarizations (DADs) and early after depolarizations (EADs) were similar for both mutations, the DAD rate was higher in cardiomyocytes carrying the *MYBPC3-*

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Gln1061X mutation than in control cardiomyocytes [15]. Lan et al. observed more cardiomyocytes exhibiting DADs in hiPSC-derived HCM cell populations carrying the *MYH7-Arg663His* mutation than in control cardiomyocyte populations. In addition, the DAD rate was significantly higher in these cells. Significant differences were observed only 30 days after the initiation of cardiac differentiation [16]. Han and co-workers [14] demonstrated a marked prolongation of APD in HCM hiPSC-derived cardiomyocytes carrying the *MYH7-Arg442Gly* mutation but no increased DAD ratio.

Changes in Ca^{2+} handling properties are considered to be one of the earliest pathophysiological mechanisms in HCM. In our study, the proportion of cardiomyocytes with abnormal Ca^{2+} transients was approximately 20% for hiPSC-derived control cardiomyocytes, 42% for cardiomyocytes carrying the *TPM1-Asp175Asn* mutation and 21% for cardiomyocytes carrying the *MYBPC3-Gln1061X* mutation at basic conditions. Lan and co-workers [16] reported that approximately 20% of hiPSC-derived HCM cardiomyocytes carrying the *MYH7-Arg663His* mutation had irregularities in their Ca^{2+} handling properties on day 30 and approximately 30% on day 40 after initiating cardiac differentiation, while in control cardiomyocytes, the proportion was approximately 5% at all timepoints. Han et al. [14] reported that approximately 20% of hiPSC-derived cardiomyocytes (*MYH7-Arg442Gly*) had abnormal Ca^{2+} handling properties, while no control hiPSC-derived cardiomyocytes had abnormalities. Similar to the characterization of disrupted sarcomeres, these data are also subjective, and the qualification or quantification criteria are rarely stated in publications. Thus, the results obtained in different publications are not directly comparable. For example, the portion of control hiPSC-derived cardiomyocytes, which have abnormalities in their Ca^{2+} handling properties varies from 0% to 20% in different publications. However, it is apparent that cardiomyocytes with HCM mutation have more abnormalities in their Ca^{2+} transients than control cardiomyocytes.

The publication from Birket et al. is to date the only one focused on studying the contraction forces of HCM hiPSC-derived cardiomyocytes. They studied cardiomyocytes derived from three individual patient hiPSC lines carrying *MYBPC3-c.2373dupG* mutation and two unrelated control hiPSC lines [13]. These hiPSC lines, derived from fibroblasts by lentiviruses, have been published earlier by Dambrot et al [88]. To be able to detect the beating forces from hiPSC-derived cardiomyocytes, Birket et al. [13] used triiodothyronine hormone (T3), insulin-like growth factor 1 (IGF-1), and dexamethasone to enhance their bioenergetics and contractile force generation. In these optimized conditions, decreased force generation was observed in hiPSC-derived cardiomyocytes carrying the *MYBPC3-c.2373dupG* mutation. The impact of mutation was confirmed by studying the generation of force in cardiomyocytes derived from *MYBPC3* knockdown hESCs [13].

4.2. Variable phenotype of hypertrophic cardiomyopathy

The primary cause of HCM is a mutation in a sarcomeric gene, while changes in Ca^{2+} handling properties, energy deficiency, ion channel remodeling, and microvascular dysfunction are thought to be the earliest pathophysiological mechanisms that play a role in disease progression [89]. However, the reasons why progressive changes initiated by these primary mutations occur in one individual and not in others are still largely unknown. Many mechanisms related

to the variable phenotype of HCM have been proposed, including additional mutations, genetic modifiers, epigenetic factors, environment and function of protein quality systems. These additional disease modifiers are thought to impact on the development of mild HCM phenotype to end-stage, in which the functionality of the heart is disrupted [90]. Conventionally, HCM has been studied with animal models or patient samples obtained from surgical myectomy. However, animal models carry only the mutated gene, and although they contain the entire genome of an HCM patient, myectomy samples are obtained from patients in the late stage of HCM development. While HCM leads to progressive heart failure, the major difficulty in studying the pathophysiological mechanisms leading to HCM has been the lack of tissue at the early stages of disease development. Thus, hiPSC models represent a valuable tool to study HCM in vitro.

In our study, we could not detect the mutated MYBPC protein in hiPSC-derived cardiomyocytes carrying the *MYBPC3-Gln1061X* mutation, which is in line with previous studies using human myectomy samples and hiPSC-derived cardiomyocytes [17, 87, 91, 92]. The total amount of the MYBPC protein was slightly reduced in our study in cardiomyocytes carrying the *MYBPC3-Gln1061X* mutation compared to hiPSC-derived control cardiomyocytes [15]. Similarly, Birket et al. [13] observed a decrease in MYBPC protein relative to α-actinin in their hiPSC-derived cardiomyocytes carrying *MYBPC3-c.2373dupG* mutation, which they suggested to be the reason for the decreased traction force. The dosage of the mutated gene is one of the factors that has been proposed to affect the severity of the observed clinical phenotype. HCM is inherited in an autosomal dominant pattern, and thus, both mutated and wildtype proteins are expected to be incorporated into the sarcomere [90]. However, the expression of the mutated protein can be regulated on many levels. In particular, the *MYBPC3* mutations leading to truncated proteins can be directed toward degradation by nonsense-mediated mRNA decay, ubiquitin proteasome system (UPS), and the autophagy/lysosomal pathway, which leads to haploinsufficiency [93]. The age-related decline of these protein quality systems has been suggested to effect the progression of HCM [90]. The amount of the wildtype MYBPC protein varies in patient tissue samples, which might correlate with the clinical phenotypes observed in patients [92, 92]. Another mechanism affecting the dosage of the mutated gene and thus disease severity could be allelic imbalance, which indicates differences in the expression levels of mutated and wildtype alleles [93, 94].

Protein phosphorylation is one of the most important post-translational modifications, which has also been suggested to affect disease development. For example, the reduced phosphorylation of TnI and MYBPC has been related to increased myofilament Ca^{2+} sensitivity, which is a common feature of HCM [95]. However, Ca^{2+} sensitivity has also been suggested to be a primary consequence of HCM. Additionally, Ca^{2+} sensitivity has been proposed to increase arrhythmia sensitivity either by increasing the Ca^{2+} binding affinity in the cytosol, which could lead to remodeling of action potentials and thus trigger arrhythmias, or by affecting energy consumption and increasing arrhythmia susceptibility via stress [90, 96, 97].

Some HCM patients have been shown to carry more than just one mutation in their genotype, and patients carrying multiple mutations have been associated with more severe symptoms or earlier onset of disease [76, 78]. Other genetic mechanisms include genetic modifiers, which

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can be either near or distantly located DNA variants that influence the expression of the mutated gene [76]. Additionally, epigenetic changes, which cannot be explained by the DNA sequence itself, have been suggested to contribute to the progression of HCM. These mechanisms include the methylation of GpC islands by DNA methyltransferases, histone modification, miRNAs and long non-coding RNAs (lncRNAs), which can lead to the altered regulation of genes [76].

4.3. Challenges in modeling hypertrophic cardiomyopathy with human induced pluripotent stem cells

The major limitation when using hiPSC-derived cardiomyocytes in disease modeling is the immature nature of these cells. The characteristics of hPSC-derived cardiomyocytes and the differences between these and adult human cardiomyocytes have recently been reviewed [98]. Compared to adult cardiomyocytes, the sarcomeric structures of hPSC-derived cardiomyocytes are unorganized, they lack clear T tubules, they have different ion channel profiles, and their Ca^{2+} handling is immature. In addition, the shapes of hPSC-derived cardiomyocytes vary from circular to star-shaped, while adult human cardiomyocytes are rod-shaped in vivo. Overall, the phenotype of hPSC-derived cardiomyocytes is thought to more closely resemble that of fetal cardiomyocytes than adult cardiomyocytes [98]. At the moment, much effort is being expended to obtain more mature cardiomyocytes. In addition to culture conditions and the long-term culture of cardiomyocytes, other techniques such as electrical and mechanical stimulation, as well as engineered heart tissue (EHT) structures, have been developed [99–102].

Another issue related to the study of HCM with hiPSC-derived cardiomyocytes is related to the assumption that cell types other than cardiomyocytes might be directly involved in the progression of HCM. For example, microvascular dysfunction is thought to be the primary reason for replacement-type fibrosis observed in HCM patients [90]. Thus, it is important to consider whether studying HCM at the single cell level is sufficient to obtain an exact picture of the disease mechanisms. EHT structures and cardiovascular constructs, consisting of various cell types could be useful in this context [102, 103].

As discussed above, in addition to primary HCM gene mutations, gene modifiers and epigenetic changes might also have an effect on disease development and progression. In all published reports, the phenotypes of HCM hiPSC-derived cardiomyocytes have been compared to control hiPSC-derived cardiomyocytes established from related or non-related healthy individuals [13–17]. Currently, useful gene-editing approaches, including clustered regularly interspaced short palindromic repeats (CRISPR) and transcription activator-like effector nuclease (TALEN) techniques, are available, and they can be used either to create mutations or correct existing mutations in an hiPSC line, thus generating genotype-matched isogenic control lines [104]. When considering the variable phenotypes of HCM patients, these isogenic control hiPSCs would be useful when further studying HCM disease mechanisms.

5. Conclusions

Although, the current methods for studying cardiomyopathies in vitro with patient-specific cardiomyocytes are far from optimal, we and others have been able to create hiPSC models with HCM phenotype. hiPSC-derived cardiomyocytes can also be utilized to study the effects of genetic modifiers and epigenetic factors on disease progression between different individuals, which has been difficult when using animal models or samples from surgical myectomy.

However, particularly in the case of HCM associated with highly variable phenotypes, it would be important to optimize cardiac differentiation and cell culture conditions. The study design becomes highly valuable, and the culture conditions should be similar for both control and disease-specific cardiomyocytes. When experiments are thoroughly designed, the results obtained from these studies would be more robust and reliable. Nonetheless, hiPSC-derived HCM in vitro models represent a valuable tool to study the pathophysiological mechanisms of HCM as well as to test novel drug therapies developed to prevent disease progression and potentially optimize treatments in a mutation-specific manner.

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References

[1] Thomson J.A., Itskovitz-Eldor J., Shapiro S.S., Waknitz M.A., Swiergiel J.J., Marshall V.S., et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–1147.

[2] Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K., et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–872. DOI: 10.1016/j.cell.2007.11.019

[3] Fusaki N., Ban H., Nishiyama A., Saeki K., Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proceedings of the Japan Academy Series B, Physical and Biological Sciences. 2009;85(8):348–362.

[4] Soldner F., Hockemeyer D., Beard C., Gao Q., Bell G.W., Cook E.G., et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell. 2009;136(5):964–977. DOI: 10.1016/j.cell.2009.02.013

[5] Yu J., Hu K., Smuga-Otto K., Tian S., Stewart R., Slukvin I.I., et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009;324(5928): 797–801. DOI: 10.1126/science.1172482

[6] Itskovitz-Eldor J., Schuldiner M., Karsenti D., Eden A., Yanuka O., Amit M., et al. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Molecular Medicine. 2000;6(2):88–95.

[7] Mummery C., Ward-vanOostwaard D., Doevendans P., Spijker R., van den Brink S., Hassink R., et al. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation. 2003;107(21):2733–2740. DOI: 10.1161/01.CIR.0000068356.38592.68

[8] Burridge P.W., Matsa E., Shukla P., Lin Z.C., Churko J.M., Ebert A.D., et al. Chemically defined generation of human cardiomyocytes. Nature Methods. 2014;11(8):855–860. DOI: 10.1038/nmeth.2999

[9] Laflamme M.A., Chen K.Y., Naumova A.V., Muskheli V., Fugate J.A., Dupras S.K., et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nature Biotechnology. 2007;25(9):1015–1024. DOI: 10.1038/nbt1327

[10] Lian X., Hsiao C., Wilson G., Zhu K., Hazeltine L.B., Azarin S.M., et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(27):E1848–E1857. DOI: 10.1073/pnas.1200250109

[11] Maron B.J., Ommen S.R., Semsarian C., Spirito P., Olivotto I., Maron M.S. Hypertrophic cardiomyopathy: present and future, with translation into contemporary cardiovascular medicine. Journal of the American College of Cardiology. 2014;64(1):83–99. DOI: 10.1016/j.jacc.2014.05.003

[12] Roma-Rodrigues C., Fernandes A.R. Genetics of hypertrophic cardiomyopathy: advances and pitfalls in molecular diagnosis and therapy. The Application of Clinical Genetics. 2014;7:195–208. DOI: 10.2147/TACG.S49126

[13] Birket M.J., Ribeiro M.C., Kosmidis G., Ward D., Leitoguinho A.R., van de Pol V. Contractile defect caused by mutation in MYBPC3 revealed under conditions optimized for human PSC-cardiomyocyte function. Cell Reports. 2015;13(4):733–745. DOI: 10.1016/j.celrep.2015.09.025

[14] Han L., Li Y., Tchao J., Kaplan A.D., Lin B., Li Y. Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. Cardiovascular Research. 2014;104(2):258–269. DOI: 10.1093/cvr/cvu205

[15] Ojala M., Prajapati C., Pölönen R-P., Rajala K., Pekkanen-Mattila M., Rasku J., et al. Mutation-specific phenotypes in hiPSC-derived cardiomyocytes carrying either myosin-binding protein C or α-tropomyosin mutation for hypertrophic cardiomyopathy. Stem Cells International. 2016; Article ID: 1684792. DOI: 10.1155/2016/1684792

[16] Lan F., Lee A.S., Liang P., Sanchez-Freire V., Nguyen P.K., Wang L., et al. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. Cell Stem Cell. 2013;12(1):101–113. DOI: 10.1016/j.stem.2012.10.010

[17] Tanaka A., Yuasa S., Mearini G., Egashira T., Seki T., Kodaira M., et al. Endothelin-1 induces myofibrillar disarray and contractile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-derived cardiomyocytes. Journal of the American Heart Association. 2014;3(6):e001263. DOI: 10.1161/JAHA.114.001263

[18] Klimanskaya I., Chung Y., Becker S., Lu S-J., Lanza R. Human embryonic stem cell lines derived from single blastomeres. Nature. 2006;444(7008):481–485. DOI: 10.1038/nature05142

[19] Strelchenko N., Verlinsky O., Kukharenko V., Verlinsky Y. Morula-derived human embryonic stem cells. Reproductive Biomedicine Online. 2004;9(6):623–629.

[20] Takahashi K., Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–676. DOI: 10.1016/j.cell.2006.07.024

[21] Briggs R., King T.J. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. PNAS. 1952;38(5):455–463.

[22] Gurdon J.B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. Journal of Embryology and Experimental Morphology. 1962;10:622–640.

[23] Wilmut I., Schnieke A.E., McWhir J., Kind A.J., Campbell K.H. Viable offspring derived from fetal and adult mammalian cells. Nature. 1997;385(6619):810–813. DOI: 10.1038/385810a0

[24] Yamanaka S. Pluripotency and nuclear reprogramming. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2008;363(1500):2079–2087. DOI: 10.1098/rstb.2008.2261

[25] Vaajasaari H., Ilmarinen T., Juuti-Uusitalo K., Rajala K., Onnela N., Narkilahti S., et al. Toward the defined and xeno-free differentiation of functional human pluripotent stem cell-derived retinal pigment epithelial cells. Molecular Vision. 2011;17:558–575.

[26] Nat R., Nilbratt M., Narkilahti S., Winblad B., Hovatta O., Nordberg A. Neurogenic neuroepithelial and radial glial cells generated from six human embryonic stem cell lines in serum-free suspension and adherent cultures. Glia. 2007;55(4):385–399. DOI: 10.1002/glia.20463

[27] Hay D.C., Fletcher J., Payne C., Terrace J.D., Gallagher R.C.J., Snoeys J., et al. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. PNAS. 2008;105(34):12301–12306. DOI: 10.1073/pnas.0806522105

[28] Bayart E., Cohen-Haguenauer O. Technological overview of iPS induction from human adult somatic cells. Current Gene Therapy. 2013;13(2):73–92.

[29] Park H-J., Shin J., Kim J., Cho S-W. Nonviral delivery for reprogramming to pluripotency and differentiation. Archives of Pharmacal Research. 2014;37(1):107–119. DOI: 10.1007/s12272-013-0287-z

[30] Hu K. All roads lead to induced pluripotent stem cells: the technologies of iPSC generation. Stem Cells and Development. 2014;23(12):1285–1300. DOI: 10.1089/scd.2013.0620

[31] Yu J., Vodyanik M.A., Smuga-Otto K., Antosiewicz-Bourget J., Frane J.L., Tian S., et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318(5858):1917–1920. DOI: 10.1126/science.1151526

[32] Matsui T., Leung D., Miyashita H., Maksakova I.A., Miyachi H., Kimura H., et al. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature. 2010;464(7290):927–931. DOI: 10.1038/nature08858

[33] Mikkelsen T.S., Hanna J., Zhang X., Ku M., Wernig M., Schorderet P., et al. Dissecting direct reprogramming through integrative genomic analysis. Nature. 2008;454(7200):49–55. DOI: 10.1038/nature07056

[34] Sridharan R., Tchieu J., Mason M.J., Yachechko R., Kuoy E., Horvath S., et al. Role of the murine reprogramming factors in the induction of pluripotency. Cell. 2009;136(2):364–377. DOI: 10.1016/j.cell.2009.01.001

[35] Chang C-W., Lai Y-S., Pawlik K.M., Liu K., Sun C-W., Li C., et al. Polycistronic lentiviral vector for "hit and run" reprogramming of adult skin fibroblasts to induced pluripotent stem cells. Stem Cells. 2009;27(5):1042–1049. DOI: 10.1002/stem.39

[36] Kaji K., Norrby K., Paca A., Mileikovsky M., Mohseni P., Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature. 2009;458(7239):771–775. DOI: 10.1038/nature07864

[37] Stadtfeld M., Nagaya M., Utikal J., Weir G., Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science. 2008;322(5903):945–949. DOI: 10.1126/science.1162494

[38] Zhou W., Freed C. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells. 2009;27(11):2667–2674. DOI: 10.1002/stem.201

[39] Woltjen K., Michael I.P., Mohseni P., Desai R., Mileikovsky M., Hämäläinen R., et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009;458(7239):766–770. DOI: 10.1038/nature07863

[40] Okita K., Matsumura Y., Sato Y., Okada A., Morizane A., Okamoto S., et al. A more efficient method to generate integration-free human iPS cells. Nature Methods. 2011;8(5):409–412. DOI: 10.1038/nmeth.1591

[41] Khan M., Narayanan K., Lu H., Choo Y., Du C., Wiradharma N., et al. Delivery of reprogramming factors into fibroblasts for generation of non-genetic induced pluripotent stem cells using a cationic bolaamphiphile as a non-viral vector. Biomaterials. 2013;34(21):5336–5343. DOI: 10.1016/j.biomaterials.2013.03.072

[42] Lee C.H., Kim J-H., Lee H.J., Jeon K., Lim H., Choi H., et al. The generation of iPS cells using non-viral magnetic nanoparticle based transfection. Biomaterials. 2011;32(28):6683–6691. DOI: 10.1016/j.biomaterials.2011.05.070

[43] Später D., Hansson L., Zangi L., Chien K.R. How to make a cardiomyocyte. Development. 2014;141(23):4418–4431. DOI: 10.1242/dev.091538

[44] Mummery C., Zhang J., Ng E.S., Elliott D.A., Elefanty A.G., Kamp T.J. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. Circulation Research. 2012;111(3):344–358. DOI: 10.1161/CIRCRESAHA.110.227512

[45] Rajala K., Pekkanen-Mattila M., Aalto-Setälä K. Cardiac differentiation of pluripotent stem cells. Stem Cells International. 2011; Article ID: 383709. DOI: 10.4061/2011/383709

[46] Verma V., Purnamawati K., Manasi S.W. Steering signal transduction pathway towards cardiac lineage from human pluripotent stem cells: a review. Cellular Signalling. 2013;25(5):1096–1107. DOI: 10.1016/j.cellsig.2013.01.027

[47] Kehat I., Kenyagin-Karsenti D., Snir M., Segev H., Amit M., Gepstein A., et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. The Journal of Clinical Investigation. 2001;108(3):407–414. DOI: 10.1172/JCI12131

[48] Burridge P.W., Thompson S., Millrod M., Weinberg S., Yuan X., Peters A., et al. A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PloS One. 2011;6(4):e18293. DOI: 10.1371/journal.pone.0018293

[49] Mohr J.C., Zhang J., Azarin S.M., Soerens A.G., de Pablo J.J., Thomson J.A., et al. The microwell control of embryoid body size in order to regulate cardiac differentiation of human embryonic stem cells. Biomaterials. 2010;31(7):1885–1893. DOI: 10.1016/j.biomaterials.2009.11.033

[50] Yang L., Soonpaa M.H., Adler E.D., Roepke T.K., Kattman S.J., Kennedy M., et al. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. Nature. 2008;453(7194):524–528. DOI: 10.1038/nature06894

[51] Karakikes I., Senyei G.D., Hansen J., Kong C-W., Azeloglu E.U., Stillitano F., et al. Small molecule-mediated directed differentiation of human embryonic stem cells toward ventricular cardiomyocytes. Stem Cells Translational Medicine. 2014;3(1):18–31. DOI: 10.5966/sctm.2013-0110

[52] Passier R., Oostwaard D.W., Snapper J., Kloots J., Hassink R.J., Kuijk E., et al. Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures. Stem Cells. 2005;23(6):772–780. DOI: 10.1634/stemcells.2004-0184

[53] Graichen R., Xu X., Braam S.R., Balakrishnan T., Norfiza S., Sieh S., et al. Enhanced cardiomyogenesis of human embryonic stem cells by a small molecular inhibitor of p38 MAPK. Differentiation. 2008;76(4):357–370. DOI: 10.1111/j.1432-0436.2007.00236.x

[54] Xu X.Q., Graichen R., Soo S.Y., Balakrishnan T., Rahmat S.N.B., Sieh S., et al. Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells. Differentiation. 2008;76(9):958–970. DOI: 10.1111/j.1432-0436.2008.00284.x

[55] Zhang J., Klos M., Wilson G.F., Herman A.M., Lian X., Raval K.K., et al. Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: the matrix sandwich method. Circulation Research. 2012;111(9):1125–1136. DOI: 10.1161/CIRCRESAHA.112.273144

[56] Burridge P.W., Anderson D., Priddle H., Barbadillo Muñoz M.D., Chamberlain S., Allegrucci C., et al. Improved human embryonic stem cell embryoid body homogeneity and cardiomyocyte differentiation from a novel V-96 plate aggregation system highlights interline variability. Stem Cells. 2007;25(4):929–938. DOI: 10.1634/stemcells.2006-0598

[57] Freund C., Ward-vanOostwaard D., Monshouwer-Kloots J., van den Brink S., van Rooijen M., Xu X., et al. Insulin redirects differentiation from cardiogenic mesoderm and endoderm to neuroectoderm in differentiating human embryonic stem cells. Stem Cells. 2008;26(3):724–733. DOI: 10.1634/stemcells.2007-0617

[58] Minami I., Yamada K., Otsuji T.G., Yamamoto T., Shen Y., Otsuka S., et al. A small molecule that promotes cardiac differentiation of human pluripotent stem cells under defined, cytokine- and xeno-free conditions. Cell Reports. 2012;2(5):1448–1460. DOI: 10.1016/j.celrep.2012.09.015

[59] Osafune K., Caron L., Borowiak M., Martinez R.J., Fitz-Gerald C.S., Sato Y., et al. Marked differences in differentiation propensity among human embryonic stem cell lines. Nature Biotechnology. 2008;26(3):313–315. DOI: 10.1038/nbt1383

[60] Xu C., Police S., Rao N., Carpenter M. K. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circulation Research. 2002;91(6): 501–508.

[61] Dubois N.C., Craft A.M., Sharma P., Elliott D.A., Stanley E.G., Elefanty A.G., et al. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nature Biotechnology. 2011;29(11):1011–1018. DOI: 10.1038/nbt.2005

[62] Uosaki H., Fukushima H., Takeuchi A., Matsuoka S., Nakatsuji N., Yamanaka S., 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):e23657. DOI: 10.1371/journal.pone.0023657

[63] Tohyama S., Hattori F., Sano M., Hishiki T., Nagahata Y., Matsuura T., et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell. 2013;12(1):127–137. DOI: 10.1016/j.stem.2012.09.013

[64] Miki K., Endo K., Takahashi S., Funakoshi S., Takei I., Katayama S., et al. Efficient detection and purification of cell populations using synthetic microRNA switches. Cell Stem Cell. 2015;16(6):699–711. DOI: 10.1016/j.stem.2015.04.005

[65] Kartasalo K., Pölönen R-P., Ojala M., Rasku J., Lekkala J., Aalto-Setälä K., et al. CytoSpectre: a tool for spectral analysis of oriented structures on cellular and subcellular levels. BMC Bioinformatics. 2015;16:344. DOI: 10.1186/s12859-015-0782-y

[66] Laurila E., Ahola A., Hyttinen J., Aalto-Setälä K. Methods for in vitro functional analysis of iPSC derived cardiomyocytes—special focus on analyzing the mechanical beating behavior. Biochimica et Biophysica Acta. 2015. DOI: 10.1016/j.bbamcr.2015.12.013

[67] Reppel M., Pillekamp F., Lu Z.J., Halbach M., Brockmeier K., Fleischmann B.K., et al. Microelectrode arrays: a new tool to measure embryonic heart activity. Journal of Electrocardiology. 2004;37:104–109.

[68] Hamill O., Marty A., Neher E., Sakmann B., Sigworth F. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Archiv: European Journal of Physiology. 1981;2(391):85–100.

[69] Sakmann B., Neher E. Patch clamp techniques for studying ionic channels in excitable membranes. Annual Review of Physiology. 1984;46:455–472. DOI: 10.1146/annurev.ph.46.030184.002323

[70] Zilberter Y.I., Timin E.N., Bendukidze Z.A., Burnashev N.A. Patch-voltage-clamp method for measuring fast inward current in single rat heart muscle cells. Pflügers Archiv: European Journal of Physiology. 1982;394(2):150–155.

[71] Bers D.M. Calcium cycling and signaling in cardiac myocytes. Annual Review of Physiology. 2008;70:23–49. DOI: 10.1146/annurev.physiol.70.113006.100455

[72] Grynkiewicz G., Poenie M., Tsien R.Y. A new generation of Ca2+ indicators with greatly improved fluorescence properties. The Journal of Biological Chemistry. 1985;260(6): 3440–3450.

[73] Ahola A., Kiviaho A.L., Larsson K., Honkanen M., Aalto-Setälä K., Hyttinen J. Video image-based analysis of single human induced pluripotent stem cell derived cardiomyocyte beating dynamics using digital image correlation. Biomedical Engineering Online. 2014;7:13–39. DOI: 10.1186/1475-925X-13-39

[74] Lopes L.R., Elliott P.M. A straightforward guide to the sarcomeric basis of cardiomyopathies. Heart. 2014;100(24):1916–1923. DOI: 10.1136/heartjnl-2014-305645

[75] Elliott P., Andersson B., Arbustini E., Bilinska Z., Cecchi F., Charron P., et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. European Heart Journal. 2008;29(2):270–276. DOI: 10.1093/eurheartj/ehm342

[76] Ho C.Y., Charron P., Richard P., Girolami F., van Spaendonck-Zwarts K.Y., Pinto Y. Genetic advances in sarcomeric cardiomyopathies: state of the art. Cardiovascular Research. 2015;105(4):397–408. DOI: 10.1093/cvr/cvv025

[77] Kocovski L., Fernandes J. Sudden cardiac death: a modern pathology approach to hypertrophic cardiomyopathy. Archives of Pathology & Laboratory Medicine. 2015;136(3):413–416. DOI: 10.5858/arpa.2013-0489-RS

[78] Maron B.J., Maron M.S., Semsarian C. Double or compound sarcomere mutations in hypertrophic cardiomyopathy: a potential link to sudden death in the absence of conventional risk factors. Heart Rhythm. 2012;9(1):57–63. DOI: 10.1016/j.hrthm.2011.08.009

[79] Jääskeläinen P., HeliöT., Aalto-Setälä K., Kaartinen M., Ilveskoski E., Hämäläinen L., et al. Two founder mutations in the alpha-tropomyosin and the cardiac myosin-binding protein C genes are common causes of hypertrophic cardiomyopathy in the Finnish population. Annals of Medicine. 2013;45(1):85–90. DOI: 10.3109/07853890.2012.671534

[80] Jääskeläinen P., Kuusisto J., Miettinen R., Kärkkäinen P., Kärkkäinen S., Heikkinen S., et al. Mutations in the cardiac myosin-binding protein C gene are the predominant cause of familial hypertrophic cardiomyopathy in eastern Finland. Journal of Molecular Medicine. 2002;80(7):412–422. DOI: 10.1007/s00109-002-0323-9

[81] Maron B.J., Niimura H., Casey S.A., Soper M.K., Wright G.B., Seidman J.G., et al. Development of left ventricular hypertrophy in adults in hypertrophic cardiomyop-

athy caused by cardiac myosin-binding protein C gene mutations. Journal of the American College of Cardiology. 2001;38(2):315–321.

[82] Niimura H., Bachinski L.L., Sangwatanaroj S., Watkins H., Chudley A.E., McKenna W., et al. Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. The New England Journal of Medicine. 1998;338(18): 1248–1257. DOI: 10.1056/NEJM199804303381802

[83] Thierfelder L., Watkins H., MacRae C., Lamas R., McKenna W., Vosberg H.P., et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell. 1994;77(5):701–712.

[84] Jääskeläinen P., Miettinen R., Kärkkäinen P., Toivonen L., Laakso M., Kuusisto J. Genetics of hypertrophic cardiomyopathy in eastern Finland: few founder mutations with benign or intermediary phenotypes. Annals of Medicine. 2004;36(1):23–32.

[85] Jääskeläinen P., Heliö T., Aalto-Setälä K., Kaartinen M., Ilveskoski E., Hämäläinen L., et al. A new common mutation in the cardiac beta-myosin heavy chain gene in Finnish patients with hypertrophic cardiomyopathy. Annals of Medicine. 2014;46(6):424–429. DOI: 10.3109/07853890.2014.912834

[86] Duncker D.J., Bakkers J., Brundel B.J., Robbins J., Tardiff J.C., Carrier L. Animal- and in silico models for the study of sarcomeric cardiomyopathies. Cardiovascular Research. 2015;105(4):439–448. DOI: 10.1093/cvr/cvv006

[87] Marston S., Copeland O., Jacques A., Livesey K., Tsang V., McKenna W.J., et al. Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency. Circulation Research. 2009;105(3):219–222. DOI: 10.1161/CIRCRESAHA.109.202440

[88] Dambrot C., Braam S.R., Tertoolen L.G.J., Birket M., Atsma D.E., Mummery C.L. Serum supplemented culture medium masks hypertrophic phenotypes in human pluripotent stem cell derived cardiomyocytes. Journal of Cellular and Molecular Medicine. 2014;18(8):1509–1518. DOI: 10.1111/jcmm.12356

[89] Tardiff J.C., Carrier L., Bers D.M., Poggesi C., Ferrantini C., Coppini R., et al. Targets for therapy in sarcomeric cardiomyopathies. Cardiovascular Research. 2015;105(4): 457–470. DOI: 10.1093/cvr/cvv023

[90] Van der Velden J., Ho C.Y., Tardiff J.C., Olivotto I., Knollmann B.C., Carrier L. Research priorities in sarcomeric cardiomyopathies. Cardiovascular Research. 2015;105(4):449–456. DOI: 10.1093/cvr/cvv019

[91] Rottbauer W., Gautel M., Zehelein J., Labeit S., Franz W.M., Fischer C., et al. Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy: Characterization of cardiac transcript and protein. The Journal of Clinical Investigation. 1997;100(2):475–482. DOI: 10.1172/JCI119555

[92] Van Dijk S.J., Dooijes D., Remedios C.D., Michels M., Lamers J.M.J., Winegrad S., et al. Cardiac myosin-binding protein C mutations and hypertrophic ardiomyopathy

haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. Circulation. 2009;119(11):1473–1483. DOI: 10.1161/CIRCULATIONAHA.108.838672

[93] Helms A.S., Davis F.M., Coleman D., Bartolone S.N., Glazier A.A., Pagani F., et al. Sarcomere mutation-specific expression patterns in human hypertrophic cardiomyopathy. Circulation. Cardiovascular Genetics. 2014;7(4):434–443. DOI: 10.1161/CIRCGENETICS.113.000448

[94] Tripathi S., Schultz I., Becker E., Montag J., Borchert B., Francino A., et al. Unequal allelic expression of wild-type and mutated β-myosin in familial hypertrophic cardiomyopathy. Basic Research in Cardiology. 2011;106(6):1041–1055. DOI: 10.1007/s00395-011-0205-9

[95] Sequeira V., Wijnker P.J.M., Nijenkamp L.L.M., Kuster D.W.D., Najafi A., Witjas-Paalberends E.R., et al. Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric gene mutations. Circulation Research. 2013;112(11):1491–1505. DOI: 10.1161/CIRCRESAHA.111.300436

[96] Huke S., Venkataraman R., Faggioni M., Bennuri S., Hwang H.S., Baudenbacher F., et al. Focal energy deprivation underlies arrhythmia susceptibility in mice with calcium-sensitized myofilaments. Circulation Research. 2013;112(10):1334–1344. DOI: 10.1161/CIRCRESAHA.113.301055

[97] Schober T., Huke S., Venkataraman R., Gryshchenko O., Kryshtal D., Hwang H.S., et al. Myofilament Ca sensitization increases cytosolic Ca binding affinity, alters intracellular Ca homeostasis, and causes pause-dependent Ca-triggered arrhythmia. Circulation Research. 2012;111(2):170–179. DOI: 10.1161/CIRCRESAHA.112.270041

[98] Van den Heuvel N.H.L., van Veen T.A.B., Lim B., Jonsson M.K.B. Lessons from the heart: mirroring electrophysiological characteristics during cardiac development to in vitro differentiation of stem cell derived cardiomyocytes. Journal of Molecular and Cellular Cardiology. 2014;67:12–25. DOI: 10.1016/j.yjmcc.2013.12.011

[99] Kreutzer J., Ikonen L., Hirvonen J., Pekkanen-Mattila M., Aalto-Setälä K., Kallio P. Pneumatic cell stretching system for cardiac differentiation and culture. Medical Engineering & Physics. 2014;36(4):496–501. DOI: 10.1016/j.medengphy.2013.09.008

[100] Kujala K., Ahola A., Pekkanen-Mattila M., Ikonen L., Kerkelä E., Hyttinen J., et al. Electrical field stimulation with a novel platform: effect on cardiomyocyte gene expression but not on orientation. International Journal of Biomedical Science: IJBS. 2012;8(2):109–120.

[101] Nunes S.S., Miklas J.W., Liu J., Aschar-Sobbi R., Xiao Y., Zhang B., et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nature Methods. 2013;10(8):781–787. DOI: 10.1038/nmeth.2524

[102] Stoehr A., Neuber C., Baldauf C., Vollert I., Friedrich F.W., Flenner F., et al. Automated analysis of contractile force and Ca2+ transients in engineered heart tissue. AJP: Heart

and Circulatory Physiology. 2014;306(9):H1353-H1363. DOI: 10.1152/ajpheart.00705.2013

[103] Vuorenpää H., Ikonen L., Kujala K., Huttala O., Sarkanen J.R., Ylikomi T., et al. Novel in vitro cardiovascular constructs composed of vascular-like networks and cardiomyocytes. In vitro Cellular & Developmental Biology. Animal. 2014;50(4):275–286. DOI: 10.1007/s11626-013-9703-4

[104] Li M., Suzuki K., Kim N.Y., Liu G-H., Izpisua Belmonte J.C. A cut above the rest: targeted genome editing technologies in human pluripotent stem cells. The Journal of Biological Chemistry. 2014;289(8):4594–4599. DOI: 10.1074/jbc.R113.488247

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