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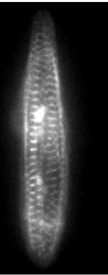
Chapter 1

General Introduction



Human pluripotent stem cells (hPSC)

Human embryonic stem cells (hESC), derived from the inner cell mass (ICM) of late blastocyst stage embryos, were first isolated and maintained in culture in 1998 [1]. HESCs have the ability to undergo unlimited self-renewal and are pluripotent, i.e. have the capacity to differentiate into all ~220 somatic cell types in the adult organism and germ cells [2],[3]. hESC are typically characterized by the expression of transcription factors OCT3/4 [4], NANOG [5] and SOX2 [6], which play an important role in the maintenance of their pluripotent state. Additional cell surface markers such as SSEA3, SSEA4, TRA-1-60, TRA-1-80 and TRA-2-49/6E (alkaline phosphatase) are used to distinguish pluripotent hESC from other cell-types [1],[7],[8]. Although, many studies have shown the utility of hESC in biomedical research after their differentiation to specialized cell-types, ethical issues on their origin limited their widespread use for both research and clinical applications.

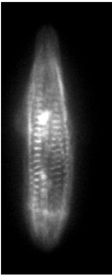
In 2006, groundbreaking work of the group of Shinya Yamanaka demonstrated the generation of pluripotent stem cells by reprogramming mouse adult cells *in vitro* through overexpression of only 4 transcription factors associated with pluripotency, i.e. Sox2, Oct3/4, c-Myc, and Klf4 [9]. In 2007, the groups of Yamanaka and Thomson independently described this reprogramming for the first time in adult human skin fibroblasts using a similar approach [10,11]. It was demonstrated that these reprogrammed cells, called induced pluripotent stem (iPS) cells, were comparable to hESC, with respect to their morphology, molecular markers and functional characteristics, including self-renewal and differentiation capacity. In addition, human iPS cells resemble the hESC in other aspects, including proliferation, teratoma formation, and promoter- and telomerase activities [10]. Differences in gene expression and methylation state between hiPS cells and hESC have been described [10] when small samples have been compared but overall they seem very similar. The first approaches used integrating retroviral vectors to introduce the reprogramming transcription factors, but later induced pluripotency without the integration of transgenes [12–14]. Banks of hiPS cells from multiple individuals could support *in vitro* studies at the population level for disease modeling, tissue engineering and drug toxicity. Due to their human origin, the tools created with hiPS technology are expected to be more predictive and relevant to human conditions than existing animal or cell line based models.

hPSC cultures

hPSCs self-renewal *in vitro* is rather complex and still under intensive study. Initially hPSC cultures were maintained on mouse embryonic fibroblast (MEF) feeder layers in fetal calf serum (FCS); however, FCS-based media introduced variation in culture and differentiation. Some advances have been made regarding the use of defined medium for sustaining the hPSC self-renewal in culture. Several factors have been reported to be important for the maintenance of pluripotent stem cells *in vitro*. In particular, basic fibroblast growth factor 2 (bFGF) has a pivotal role in hPSC stability and maintenance [15]. Activin A, a member of the transforming growth factor-b family is thought to be important in combination with bFGF for self-renewal of hPSC [16,17]. It has been suggested that the mechanism consists of promoting the expression of the transcription factors needed for self-renewing, such as Oct3/4 and Nanog, through the induction of Smad2/3 [18]. Furthermore, data have been

published demonstrating a crucial role for Phosphoinositide 3-kinases (PI3K) signaling in hESC self-renewal. PI3K appears to be involved in inhibiting differentiation of hESCs as well as in the self-renewal regulatory pathway. Therefore, the presence of PI3K agonists, such as insulin/IGF type molecules, in the culture medium is important for cell survival and self-renewal by inhibiting differentiation [19]. In addition, PI3K suppresses the activity of glycogen synthase kinase-3 (GSK3). Suppression of GSK3 is mandatory for pluripotency maintenance [20]. Furthermore, the production of a completely defined culture medium is essential in the development of large scale culture systems for differentiation purposes and for enabling high throughput *in vitro* screening. The first fully defined medium was made commercially available in 2006 . However, the costs of some components in the medium made it too expensive for daily research use [21]. Therefore modifications were made to develop an alternative, named mTeSR1. Five years later, based on the mTeSR1 formulation Chen et al. decreased the components necessary for hPSC maintenance to 8, the E8 medium containing insulin, selenium, transferrin, L-ascorbic acid, FGF2 and TGFβ in DMEM/F12 [22]. This fully defined medium is now widely used as the starting point for differentiation protocols.

Cardiac differentiation



Initially cardiomyocytes were generated from hESC during "spontaneous" differentiation that takes place when the cells are grown as aggregates in suspension called embryoid bodies (EBs) [23] . A more robust and efficient protocol was later described using END-2 co-culture to differentiate hESC into cardiomyocytes [24]. This protocol was further improved by removal of serum and insulin [25,26]. Presently, there are several methods described for efficient differentiation of human pluripotent stem cells into cardiomyocytes often tailored to the particular hESC or hiPSC. Most efficient are based on defined, serum free medium (methods reviewed in Mummery et al 2012 [27]). Among the more widely used are those based on the serum-free defined media called APEL (albumin, polyvinyl alcohol, essential lipids)[28] or BPEL, a variant of APEL where albumin is replaced by BSA [29]. Since signaling events during early cardiogenesis are highly conserved between species, lessons from cardiac development have been very important for stepwise efficient differentiation of hPSCs to cardiomyocytes. The major signaling pathways involved in cardiogenesis are initiated by ligands of the Transforming growth factor beta family (TGF-β), the wingless/INT proteins (WNTs) and the fibroblast growth factors (FGFs) (reviewed in [30]). Studies on early development have demonstrated that the TGF-β family signaling proteins Activin or nodal are important in the induction of mesoderm specification prior to gastrulation in Xenopus and chick embryos [31,32]. Activin/Nodal signaling pathway activates downstream SMAD proteins type 2 and 3, which have an important role in embryonic development and organ growth. Bone morphogenic proteins (BMPs) also play a role. Among its members BMP4 has a pivotal role in cardiac development, acting via the SMAD (type1,3,5) and MAPK pathways to promote cardiogenesis [33]. The Wnt family are signaling proteins that play a regulatory role in many different developmental processes, including cell survival, migration, proliferation, cell-fate specification and adhesion [34]. Wnt signaling can be divided into two distinct pathways, referred to as canonical and non-canonical. Initial findings suggested a clear functional separation of these signaling pathways, where β-cat-

enin-dependent canonical Wnt pathway is thought to play an inhibitory role on cardiac specification [35,36], as opposed to the cardiovascular development promoting role of the β-catenin-independent non-canonical Wnt signaling [37],[38]. Currently, different studies suggest more complex function of the canonical Wnt pathway in the temporal specification of cardiac commitment. Expression of canonical Wnt ligands before gastrulation promotes mesoderm induction [39], whereas activation of the canonical Wnt pathway during late cardiac differentiation blocks the expression of cardiac genes [40]. Recent completely defined protocols were developed using cytokines BMP4 and Activin A together with a GSK3 inhibitor that acts as a potent Wnt activator to initiate cardiomyocyte differentiation and later removed in a time dependent manner, recapitulating the development cardiac induction signaling *in vivo* [28]. An alternative format of differentiation have been developed using monolayer culture on a specific extracellular matrix (ECM) (e.g. Matrigel) in serum free medium (RPMI/B27 or APEL) in combination with similar cytokine cocktail and timing as the EB methods; cardiomyocyte yields of 30% have been reported [28,41]. More recent advances however, now enable the production of cultures containing 85% or more cardiomyocytes across different hESC and hiPSC lines. This was achieved through the use of RPMI medium in combination with defined chemicals (Wnt activator Chir99021 and Wnt inhibitor Wnt-C59) on hPSC cultures maintained in animal component-free medium (E8) [42]. Further increases in purity of the cardiomyocytes culture have been achieved by taking advantage of the cardiomyocyte's ability to use l-Lactate as an energy source. The use of l-Lactate as a replacement of Glucose as energy substrate eliminates the non-cardiomyocytes from the culture, increasing the cardiomyocytes yield to 95% [42,43]. Other selection methods include the use of cardiomyocte cell surface markers like Sirpα in combination with VECAM [44,45] or cell type specific reporters (review den Hartogh and Passier 2016 [46])

hPSC derived cardiomyocyte phenotype

These recent advances in robust cardiomyocyte differentiation procedures enables the use of hPSC-derived cardiomyocytes for (hPSC-CM) in the fields of disease modeling or drug toxicity screening, which are of interest for both the academic and the industrial research communities. HPSC-CMs based models have the inherent advantage of being human, making them closer to the clinic reality then the established animal models. Moreover, these cells can be generated from any individual as hiPSC, enabling the disease modeling of particular individuals, bridging the *in vitro* phenotype and the clinical symptoms in order to understand the disease mechanism. Despite the advantages of this model, the use of hPSC-technology is still limited by the immature phenotype of the cardiomyocytes. Although hPSC-CMs express cardiac-specific genes, which are necessary for action potential (AP) induction and contractile function, their electrophysiological and contractile properties resemble those of human fetal cardiomyocytes [24]. In particular the APs of hPSC-CM have low resting membrane potentials (~-63mV), low upstroke velocities (~20V/s) and short AP amplitudes (~90mV) in comparison to the adult situation (-85mV, 270V/s and 106mV, respectively)[47]. This immature phenotype is also observed in cell morphology with a variable shape, partially disarrayed sarcomeres and a smaller cell size [48],[49] (**Figure 1**). Additionally, the T-tubules, which are key components of excitation–contraction coupling

mechanism in adult cardiomyocytes, are not present in hPSC-CM, making this feature a hallmark of cardiomyocyte maturation [50]. Mitochondrial structure and function play an important role during development. hPSC-CM have long and slender mitochondria located around the nucleus [51] and use glycolysis as a major energy source, which is comparable to fetal cardiomyocytes, as opposed to the crystal-like lattice pattern distributed throughout the cell [52] and use of fatty acids as energy source in adult cardiomyocytes [53]. Different studies have shown that the gene profiles of hPSC-CM have a higher degree of similarity with fetal than with adult cardiomyocytes [54],[55].

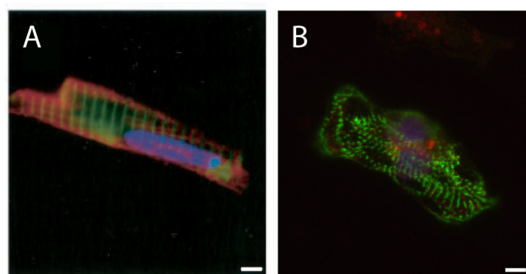


Figure 1- Cardiomyocyte structure. **A.** Human adult cardiomyocytes (Adapted from C. Mummery et. al 2003). **B.** Human stem cell derived cardiomyocytes. Both stained for α-actinin (green), MLC-2v (red) and nuclei (blue).

Maturation of hPSC-CM *in vitro*

Although several studies have shown the potential of hPSC-CM for disease modeling, drug toxicity screening and cell-based therapies, it is crucial to drive hPSC-CM towards a mature phenotype. During cardiac development *in vivo*, cardiomyocytes are exposed to different maturation cues, varying from circulating factors in the blood, to cell-cell interactions, extracellular matrix components to electrical and mechanical signals for a prolonged time allowing cardiomyocytes to develop an adult phenotype and function. Different approaches have been applied to mimic the conditions present in the development of adult heart. The effect of long term culture on hPSC-CM maturation have been described with variable outcomes [56–58]. The most striking change has been reported by Lundy et al. in 2013 by culturing hPSC-CM on glass for periods up to 120 days, leading to improved morphology, sarcomeric structure and length, calcium handling and electrophysiological properties [56]. Interestingly, the commonly used cell culture surfaces such as plastic or glass for cell culture have Young's modulus of ≈2 GPa and ≈50 GPa, respectively, which are 5 to 6 orders of magnitude stiffer than ≈24.4kPa measured on the native heart tissue [59]. Different studies have described that culturing neonatal rat ventricular myocytes (NRVM) on substrates with physiological-like stiffness improved their sarcomere structure, cell aspect ratio and contractile forces [60,61]. Moreover, cardiomyocyte length/width ratio has been proposed as a measure of maturity and disease [62,63]. Cell shape plays an important role in cardiomyocyte function since changes in cytoskeletal architecture have been described to regulate gene expression [64], impulse propagation [65], excitability [66] and contraction [62,67]. Morphology of healthy adult ventricular cardiomyocytes is characterized by an elongated shape with a length/width ratios of 7:1 *in vivo* [68]. Accordingly, restraining cardiomyo-

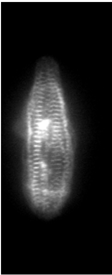
cyte shape to an adult like length/width ratio, with techniques like microcontact-printing or defined topology, can enhance its sarcomere structure and contractile performance[62]. Furthermore, 2D monolayer cultures lack endogenous 3D biophysical properties crucial for cardiomyocyte development such as electical impulse and mechanical loading. Electrical stimulation have been shown to improve NRVM alignment and ultrastructural organization and to increase contraction amplitude [69]. In 2011 Tulloch et al. were the first to show improvements of hPSC-CM sarcomere alignment and physiological hypertrophy by mechanically stretching cells cast in a 3D collagen gel [70]. Later, a combination of collagen type I and a suture wire in a PDMS channel template created a 3D microenvironment with architectural and electrical cues conducive for hPSC-CM maturation. This so-called 3D biowire platform induced physiological hypertrophy with maturation of contractile apparatus, improvements in calcium handling and electrophysiology properties of hPSC-CM *in vitro* [71]. Although 3D structures are a better representatives of the environment *in vivo* they are still deprived of the biochemical cues present in the blood during development and adult stages. Despite its complexity some components present in human serum such as hormones, growth factors and adrenergic receptor agonists have been described to play a role in cardiomyocyte maturation. Triiodothyronine (T3) is a thyroid hormone involved in numerous physiological processes throughout the body, related to growth and development. More specifically for the development of the heart, T3 has been shown to affect heart rate and metabolism [72]. In addition, T3 has been shown enhance the expression of cardiac genes such as NKX2.5, MYH6, MLC2v, SERCA, RYR2, KCND3 and PGC1α [73,74], which have pivotal roles in cardiomyocyte differentiation and function including electrophysiology [75], calcium handling and contraction force [76]. Furthermore, Insulin-like growth factor 1 (IGF-1) is known to promote cell growth, proliferation, survival and metabolism (reviewed in Troncoso et al. 2014[77]). IGF-1 activates the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, Ras/Raf/ Mitogen-activated protein kinase (MEK) and consequently the extracellular signal-regulated kinase (ERK) pathway, which are major pathways involved in protein production leading to metabolic changes, cell growth and at a later stage cardiomyocyte hypertrophy [78]. Although IGF-1 have been shown to inhibit cardiomyocyte formation [26], its role in promoting migration, proliferation, and survival of cardiac progenitor cells has been established [79,80], making it a potential effector in hPSC-CM maturation.

Cardiac hypertrophy

Cardiac hypertrophy is a process characterized by an increase in cell size with increased protein synthesis and sarcomere assembly. Since mammalian cardiomyocytes rapidly fail to retain their ability to divide, cardiomyocyte hypertrophy accompanied with fibroblast and endothelial proliferation is the major process responsible for the development of the adult size heart. This developmental enlargement of cardiomyocytes is referred to as physiological hypertrophy [48]. During postnatal physiological hypertrophy the contractile apparatus matures, there is a switch to a fatty acid based metabolism and T-tubules develop, improving the excitation contraction coupling efficiency [81]. Physiological hypertrophy also occurs in adult hearts during female pregnancy or extreme exercise by increasing 10–20% heart weight, in order to handle the necessary cardiac output, in a fully reversible man-

ner[82,83]. In contrast, pathological hypertrophy is defined as an overall increase in the size of cardiomyocytes compensating for impaired cardiac function where there is loss of cardiomyocytes (as occurs after myocardial infarction) or pressure overload (aortic stenosis, high blood pressure) or genetic causes. In addition to an increase in cardiomyocyte size, pathological cardiac hypertrophy is characterized by fibroblast proliferation and interstitial fibrosis leading to worsened cardiac function and an increased mortality. Cardiomyopathies can be classified in two different categories: Concentric (hypertrophic cardiomyopathy) or Eccentric (dilated cardiomyopathy). Concentric hypertrophy is evidenced by thicker/wider cardiomyocytes with length/width ratio of 5:1 or lower, which generates a thickening of the cardiac wall and consequently a decrease in ventricular volume [68]. On the other hand, cardiomyocytes in eccentric hypertrophy display an elongated shape with a length/width of 10:1 or greater, leading to a disproportionally thin cardiac walls and ventricular volume overload [63]. Despite the differences between these two extreme phenotypes, both conditions lead to the same outcome: a decrease in cardiac output leading to cardiac failure. An important molecular marker of pathological hypertrophy is the induction or reactivation of genes such as c-jun, c-fos, c-myc, atrial natriuretic peptide (ANP), α-myosin heavy chain (α-MHC) and skeletal alpha actin (SKA), which are also important during development[84].

Molecular pathways in hypertrophy



Cardiac hypertrophy is originated from the cardiomyocyte response to mechanical and neurohormonal stimuli such as angiotensin II, IGF-1, endothelin-1, adrenaline, etc. Although there is some insight on the initial triggers of hypertrophy, the mechanisms underlying progression of pathological hypertrophy are still intensively studied. Several molecular pathways have been described to be involved in the initiation and progression of cardiac hypertrophy. The Phosphoinositide 3-kinase (PI3K)/Akt pathway plays a major role in cardiac hypertrophy and serves as a nodal point that is directly activated by tyrosine kinase receptors such as IGF-R, TGFβ-R (transforming growth factor β-receptor), FGF-R (fibroblast growth factor-receptor), as well as β-adrenergic G protein coupled receptors [85]. The 3-kinase (PI3K)/Akt pathway promotes hypertrophy through mediation of two direct target proteins, mTOR (mammalian target of Rapamycin) and glycogen synthase kinase 3β (GSK3β)[86]. Another important pathway in hypertrophy is the MAPK signalling cascades (mitogen activated protein kinases). These cascades are involved in protein production, cell proliferation, growth and survival, and can be activated by different stimuli as mechanical stress and growth promoting factors (ET-1, AngiotensineII, Phenylephrine and TGF-β). Downstream of these cascades are the Extracellular signal receptor-Regulated Kinase (ERKs), c-jun NH2-terminal Kinase (JNKs) and p38 MAPK, which are known to induce cell growth, increase in protein synthesis and hypertrophy specific gene expression [87,88]. The calcineurin/nuclear factor of activated T-cells (NFAT) was one of the first pathways described as playing a role in hypertrophy. In this pathway, calcineurin is induced by mechanical and/or neurohormonal stimuli to regulate NFAT dephosphorylation [89], leading to nuclear translocation where it can activate transcription factors involved in hypertrophy such as GATA-4 and MEF2 [90]. Furthermore, this process can be inhibited by a class of histone deacetylases (HDACIIa) as they repress transcription of GATA-4, MEF2 and NFAT. On the other hand the class I HDACs are known for their pro-hypertrophic role in the heart

as they are involved in the regulation of growth proliferation and differentiation [91]. The interleukin-6 family of cytokines are known to induce cardiac hypertrophy by binding to a common receptor unit glycoprotein 130 (gp130) and consequently activating the (PI3K)/Akt, MAPK and Janus Associated Kinase/signal transducers and activators of transcription (JAK/STAT) pathways [92]. The JAK/STAT signalling pathway has been implicated as important key in cardiac pathophysiology[93] (**Figure 2**). Dysregulation of this pathway has been described in pressure overload induced hypertrophy [94].

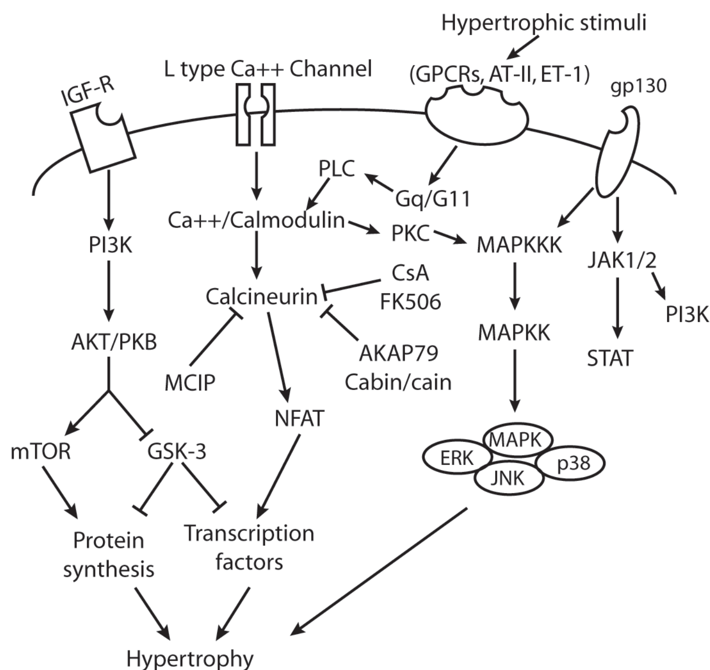


Figure 2- Different mechanisms leading to hypertrophy. Activation of the IGF receptor, L-Type calcium channel or G protein coupled receptors by hypertrophic stimuli leads to the activation of PI3K, Calcineurin and MAP kinase pathways, respectively. The activation of these pathways result in an upregulation or activation of transcription factors involved in protein synthesis and cell growth, the building blocks of hypertrophy. (Figure adapted from Agrawal et al 2010, with permission)

MYBPC3 in Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disease with a prevalence of 1:500 in general population [95] (**Figure 3**). The clinical signs and symptoms for HCM include palpitations, dizziness, syncope and sudden cardiac death (SCD), with the latter often being the first symptom. Genetic linkage studies revealed nine different chromosomal loci, with the preponderance of genes encoding cardiac sarcomeric proteins [96–99] inherited in a dominant autosomal manner. The genes most frequently mutated encode β-myosin heavy chain (35%), myosin binding protein C (20%), troponin T (5%) and α-tropomyosin (<3%), which are proteins with pivotal roles in the cardiomyocyte contraction mechanism [100]. Such mutations can arise de novo or be familial and passed

on from each generation. In the Netherlands nearly 35% of the familial hypertrophic cardiomyopathy are caused by founder mutations in the MYBPC3 gene, which encompass a Guanine duplication in exon 25 (2373dupG) or a Cysteine and threonine deletion from exon 29 (2864_2865delCT)[101]. Although these mutations are predicted to encode a truncated MYBPC3 version, such protein is not found in patient material. Moreover, the amount of the wild-type MYBPC3 has been shown to be reduced by 33% in these patients, suggesting haploinsufficiency as a trigger of the disease mechanism [101]. The cardiac MYBPC3 is present in the C zones of the A band as 9 transverse stripes 43nm apart [102]. This protein is composed of 11 domains named C0-C10 from the N- to the C-terminus and by a linker amino acid sequence (MYBPC motif) between C1 and C2 with four phosphorylation sites. The MYBPC3 interacts with different sarcomeric proteins via de different protein domains. The C0 and C1 are anchored on the actin filaments, allowing the MYBPC motif to interact with the myosin head during contraction. The C2 to C4 domains work as an arm connecting the thin to the thick filament. Furthermore, two predictive models have been suggested describing the interaction between C5 and C10 domains and components of the thick filament [103],[104]. The most accredited model describes a trimeric collar constituted by the C5 to C10 domains of three different MYBPC3 wrapped around the myosin, titin and light meromyosin (LMM). This anchoring is further stabilized by the interaction between domains of the different MYBPC3 (C5:C8 and C7:C10)[105]. Functionally, the MYBPC3 restrains the distance between the thick and the thin filaments and acts as a physical holder of the myosin heads at a specific distance of the actin filament, reducing the probability of their interaction during diastole. Importantly, low levels or absence of MYBPC3 leads to a hypertrophic cardiomyopathy phenotype characterized by an eccentric left ventricular hypertrophy with myocardial disarray, diastolic dysfunction and interstitial fibrosis[106,107].

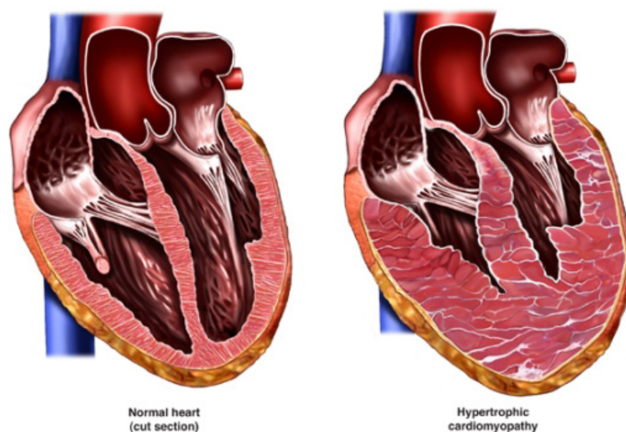


Figure 3- Hypertrophic cardiomyopathy illustration. The hypertrophic heart (right) displays thicker ventricular walls due to the increase in interstitial fibrosis and cardiomyocyte size, decreasing the ventricular volume capacity. (http://www.extremehealthradio.com/i-no-longer-have-hypertrophic-cardiomyopathy)

Scope of this thesis

This thesis describes different approaches for studying human stem cell cardiomyocyte differentiation and their maturation in health and disease using state-of-the-art technologies. By integrating stem cell biology with other disciplines of molecular biology, imaging, biomaterials and biophysics, we were able to address different biological questions. In **Chapter 2** we tested different biocompatible materials for hPSC-CM culture in order to partially mimic the *in vivo* microenvironment using soft substrate stiffness and controlling cell shape. Moreover, we describe the combination of cell traction force measurement technique with μcontact-printing and its application in the assessments of contraction force of single hPSC-CM. In **Chapter 3** a comparative analysis on contraction force was performed between hPSC-CM and primary human fetal cardiomyocytes on the same substrate. Moreover, by adjusting the culture conditions we assessed changes in maturation stage of hPSC-CM by measuring contraction force and electrophysiology in identical cardiomyocytes. In Chapter 4 we describe a combination of different molecules to induce maturation of hPSC-CM *in vitro*. We further use this maturation technology to assess contraction differences on hiPSC-CM derived from patients with a Dutch founder mutation in the MYBPC3 protein. In Chapter 5 we develop a genetically engineered α-actinin-mCherry fusion to study sarcomere development, structure and function of hPSC-CM with live imaging. In Chapter 6 we apply the technology and initial findings of chapters 4 and 5 to develop a hESC-CM based disease model, where we assess changes in calcium kinetics, contraction force and sarcomeric structure caused by the absence of the MYBPC3 protein. In Chapter 7 we make use of an inducible cMYC overexpression to arrest cardiac progenitors in their multipotent state. We were able to expand and differentiate these cardiac progenitors to cardiomyocytes, smooth muscle and endothelial cells. Moreover, within the cardiomyocyte lineage we were able to specifically differentiate the cardiac progenitors to nodal-like or working myocardium *in vitro*. Finally, in Chapter 8 the content of the experimental chapters is discussed and future perspectives in this field are proposed.

References

1. Thomson JA, Itskovitz-eldor J, Shapiro SS, et al. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science (80-). 1998;282(5391):1145-1147.

2. Shimosato D, Shiki M, Niwa H. Extra-embryonic endoderm cells derived from ES cells induced by GATA factors acquire the character of XEN cells. BMC Dev Biol. 2007;7(Icm):80. doi:10.1186/1471-213X-7-80.

3. Fujikura J, Yamato E, Yonemura S, et al. Differentiation of embryonic stem cells is induced by GATA factors. Genes Dev. 2002;16(7):784-789. doi:10.1101/gad.968802.

4. Nichols J, Zevnik B, Anastassiadis K, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell. 1998;95(3):379-391. http://www.ncbi.nlm.nih.gov/pubmed/9814708.

5. Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003;113(5):631-642. http://www.ncbi.nlm.nih.gov/pubmed/12787504.

6. Avilion A a, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003;17(1):126-140. doi:10.1101/gad.224503.

7. Brimble SN, Sherrer ES, Uhl EW, et al. The cell surface glycosphingolipids SSEA-3 and SSEA-4 are not essential for human ESC pluripotency. Stem Cells. 2007;25(1):54-62. doi:10.1634/stemcells.2006-0232.

8. Reubinoff BE, Pera MF, Fong CY, Trounson a, Bongso a. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol. 2000;18(4):399-404. doi:10.1038/74447.

9. 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.

10. Takahashi K, Tanabe K, Ohnuki M, 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.

11. Yu, Vodyanik, Smuga-Otto, et al. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. Science (80-). 2007;318(5858):1917-1920. doi:10.1126/science.1151526.

12. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009;324(5928):797-801. doi:10.1126/science.1172482.

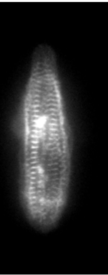
13. Nishimura K, Sano M, Ohtaka M, et al. Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. J Biol Chem. 2011;286(6):4760-4771. doi:10.1074/jbc.M110.183780.

14. González F, Boué S, Belmonte JCI. Methods for making induced pluripotent stem cells: reprogramming à la carte. Nat Rev Genet. 2011;12(4):231-242. doi:10.1038/nrg2937.

15. Levenstein ME, Ludwig TE, Xu R-H, et al. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. Stem Cells. 2006;24(3):568-574. doi:10.1634/stemcells.2005-0247.

16. Wang G, Zhang H, Zhao Y, et al. Noggin and bFGF cooperate to maintain the pluripotency of human embryonic stem cells in the absence of feeder layers. Biochem Biophys Res Commun. 2005;330(3):934-942. doi:10.1016/j.bbrc.2005.03.058.

17. Vallier L, Alexander M, Pedersen R a. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J Cell Sci. 2005;118:4495-4509. doi:10.1242/



jcs.02553.

18. Babaie Y, Herwig R, Greber B, et al. Analysis of Oct4-dependent transcriptional networks regulating self-renewal and pluripotency in human embryonic stem cells. Stem Cells. 2007;25(2):500-510. doi:10.1634/stemcells.2006-0426.

19. McLean AB. Activin A Efficiently Specifies Definitive Endoderm from Human Embryonic Stem Cells Only When Phosphatidylinositol 3-Kinase. Stem Cells. 2007:29-38. doi:10.1634/stemcells.2006-0219.S.

20. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med. 2004;10(1):55-63. doi:10.1038/nm979.

21. Ludwig TE, Levenstein ME, Jones JM, et al. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol. 2006;24(2):185-187. doi:10.1038/nbt1177.

22. Chen G, Gulbranson DR, Hou Z, et al. Chemically defined conditions for human iPSC derivation and culture. Nat Methods. 2011;8(5):424-429. doi:10.1038/nmeth.1593.

23. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest. 2001;108(3):407-414. doi:10.1172/JCI12131.

24. Mummery C, Ward-van Oostwaard D, Doevendans P, 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.

25. Mummery CL, Ward D, Passier R. Differentiation of Human Embryonic Stem Cells to Cardiomyocytes by Coculture with Endoderm in Serum-Free Medium. In: Current Protocols in Stem Cell Biology. John Wiley & Sons, Inc.; 2007. doi:10.1002/9780470151808.sc01f02s2.

26. Freund C, Ward-van Oostwaard D, Monshouwer-Kloots J, 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.

27. Mummery CL, Zhang J, Ng ES, Elliott D a, Elefanty AG, Kamp TJ. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. Circ Res. 2012;111(3):344-358. doi:10.1161/CIRCRESAHA.110.227512.

28. Elliott D a, Braam SR, Koutsis K, et al. NKX2-5(eGFP/w) hESCs for isolation of human cardiac progenitors and cardiomyocytes. Nat Methods. 2011;8(12):1037-1040. doi:10.1038/nmeth.1740.

29. Ng ES, Davis R, Stanley EG, Elefanty AG. A protocol describing the use of a recombinant protein-based, animal product-free medium (APEL) for human embryonic stem cell differentiation as spin embryoid bodies. Nat Protoc. 2008;3(5):768-776. doi:10.1038/nprot.2008.42.

30. Olson EN, Schneider MD. Sizing up the heart: development redux in disease. Genes Dev. 2003;17(16):1937-1956. doi:10.1101/gad.1110103.

31. Johansson BM, Wiles M V. Evidence for involvement of activin A and bone morphogenetic protein 4 in mammalian mesoderm and hematopoietic development. Mol Cell Biol. 1995;15(1):141-151.

32. Ladd a N, Yatskievych T a, Antin PB. Regulation of avian cardiac myogenesis by activin/TGFbeta and bone morphogenetic proteins. Dev Biol. 1998;204:407-419. doi:10.1006/dbio.1998.9094.

33. van Wijk B, Moorman AFM, van den Hoff MJB. Role of bone morphogenetic proteins in cardiac differentiation. Cardiovasc Res. 2007;74(2):244-255. doi:10.1016/j.cardiores.2006.11.022.

34. Nusse R. Wnt signaling in disease and in development. Cell Res. 2005;15(1):28-32. doi:10.1038/sj.cr.7290260.

35. Marvin MJ, Di Rocco G, Gardiner A, Bush SM, Lassar AB. Inhibition of Wnt activity induces heart formation from posterior mesoderm. Genes Dev. 2001;15:316-327. doi:10.1101/gad.855501.

36. Schneider V a., Mercola M. Wnt antagonism initiates cardiogenesis in Xenopus laevis. Genes Dev. 2001;15:304-315. doi:10.1101/gad.855601.

37. Eisenberg C a., Eisenberg LM. WNT11 promotes cardiac tissue formation of early mesoderm. Dev Dyn. 1999;216(November 1998):45-58. doi:10.1002/(SICI)1097-0177(199909)216:1<45::AID-DVDY7>3.0.CO;2-L.

38. Pandur P, Lasche M, Eisenberg LM, Kuhl M. Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. Nature. 2002;418(August):17-19. doi:10.1038/nature00909.1.

39. Ueno S, Weidinger G, Osugi T, et al. Biphasic role for Wnt/beta-catenin signaling in cardiac specification in zebrafish and embryonic stem cells. Proc Natl Acad Sci U S A. 2007;104(23):9685-9690. doi:10.1073/pnas.0702859104.

40. Naito AT, Shiojima I, Akazawa H, et al. Developmental stage-specific biphasic roles of Wnt/beta-catenin signaling in cardiomyogenesis and hematopoiesis. Proc Natl Acad Sci U S A. 2006;103:19812-19817. doi:10.1073/pnas.0605768103.

41. Laflamme M a, Chen KY, Naumova A V, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007;25(9):1015-1024. doi:10.1038/nbt1327.

42. Burridge PW, Matsa E, Shukla P, et al. Chemically defined generation of human cardiomyocytes. Nat Methods. 2014;11(8):855-860. doi:10.1038/nmeth.2999.

43. Tohyama S, Hattori F, Sano M, 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.

44. Elliott DA, Braam SR, Koutsis K, et al. for isolation of human cardiac progenitors and cardiomyocytes. Nat Methods. 2011;(october):1-7. doi:10.1038/nmeth.1740.

45. Dubois NC, Craft AM, Sharma P, et al. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nat Biotechnol. 2011;29(11):1011-1018. doi:10.1038/nbt.2005.

46. Den Hartogh SC, Passier R. Concise Review: Fluorescent Reporters in Human Pluripotent Stem Cells: Contributions to Cardiac Differentiation and Their Applications in Cardiac Disease and Toxicity. Stem Cells. 2016;34(1):13-26. doi:10.1002/stem.2196.

47. Koncz I, Szél T, Bitay M, et al. Electrophysiological effects of ivabradine in dog and human cardiac preparations: potential antiarrhythmic actions. Eur J Pharmacol. 2011;668(3):419-426. doi:10.1016/j.ejphar.2011.07.025.

48. Maillet M, van Berlo JH, Molkentin JD. Molecular basis of physiological heart growth: fundamental concepts and new players. Nat Rev Mol Cell Biol. 2013;14(1):38-48. doi:10.1038/nrm3495.

49. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res. 2014;114(3):511-523. doi:10.1161/CIRCRESAHA.114.300558.

50. Ziman AP, Gómez-Viquez NL, Bloch RJ, Lederer WJ. Excitation-contraction coupling chang-

es during postnatal cardiac development. J Mol Cell Cardiol. 2010;48(2):379-386. doi:10.1016/j.yjmcc.2009.09.016.

51. Gherghiceanu M, Barad L, Novak A, et al. Cardiomyocytes derived from human embryonic and induced pluripotent stem cells: Comparative ultrastructure. J Cell Mol Med. 2011;15(11):2539-2551. doi:10.1111/j.1582-4934.2011.01417.x.

52. Schaper J, Meiser E, Stämmler G. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice, and from human hearts. Circ Res. 1985;56:377-391. doi:10.1161/01.RES.56.3.377.

53. Lopaschuk GD, Jaswal JS. Energy metabolic phenotype of the cardiomyocyte during development, differentiation, and postnatal maturation. J Cardiovasc Pharmacol. 2010;56(2):130-140. doi:10.1097/FJC.0b013e3181e74a14.

54. Synnergren J, Améen C, Jansson A, Sartipy P. Global transcriptional profiling reveals similarities and differences between human stem cell-derived cardiomyocyte clusters and heart tissue. Physiol Genomics. 2012;44(4):245-258. doi:10.1152/physiolgenomics.00118.2011.

55. Xu XQ, Soo SY, Sun W, Zweigerdt R. Global expression profile of highly enriched cardiomyocytes derived from human embryonic stem cells. Stem Cells. 2009;27(9):2163-2174. doi:10.1002/stem.166.

56. Lundy SD, Zhu W-Z, Regnier M, Laflamme M a. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev. 2013;22(14):1991-2002. doi:10.1089/scd.2012.0490.

57. Snir M, Kehat I, Gepstein A, et al. Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. Am J Physiol Heart Circ Physiol. 2003;285(6):H2355-H2363. doi:10.1152/ajpheart.00020.2003.

58. Kadota S, Minami I, Morone N, Heuser JE, Agladze K, Nakatsuji N. Development of a reentrant arrhythmia model in human pluripotent stem cell-derived cardiac cell sheets. Eur Heart J. 2013;34(15):1147-1156. doi:10.1093/eurheartj/ehs418.

59. Mirsky I, Parmley WW. Assessment of passive elastic stiffness for isolated heart muscle and the intact heart. Circ Res. 1973;33:233-243. doi:10.1161/01.RES.33.2.233.

60. Jacot JG, McCulloch AD, Omens JH. Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. Biophys J. 2008;95(7):3479-3487. doi:10.1529/biophysj.107.124545.

61. Chopra A, Tabdanov E, Patel H, Janmey PA, Yasha J, Kresh JY. Cardiac myocyte remodeling mediated by N-cadherin-dependent mechanosensing Cardiac myocyte remodeling mediated by N-cadherin-dependent mechanosensing. Changes. 2011;(January). doi:10.1152/ajpheart.00515.2010.

62. Kuo P-L, Lee H, Bray M-A, et al. Myocyte shape regulates lateral registry of sarcomeres and contractility. Am J Pathol. 2012;181(6):2030-2037. doi:10.1016/j.ajpath.2012.08.045.

63. Gerdes a. M, Kellerman SE, Moore J a., et al. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. Circulation. 1992;86(2):426-430. doi:10.1161/01.CIR.86.2.426.

64. Pijnappels D a, Schalij MJ, Ramkisoensing A a, et al. Forced alignment of mesenchymal stem cells undergoing cardiomyogenic differentiation affects functional integration with cardiomyocyte cultures. Circ Res. 2008;103(2):167-176. doi:10.1161/CIRCRESAHA.108.176131.

65. Kléber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. Physiol Rev. 2004;84(2):431-488. doi:10.1152/physrev.00025.2003.

66. Walsh KB, Parks GE. Changes in cardiac myocyte morphology alter the properties of voltage-gated ion channels. Cardiovasc Res. 2002;55(1):64-75. http://www.ncbi.nlm.nih.gov/pubmed/12062709.

67. Feinberg AW, Feigel A, Shevkoplyas SS, Sheehy S, Whitesides GM, Parker KK. Muscular thin films for building actuators and powering devices. Science. 2007;317(5843):1366-1370. doi:10.1126/science.1146885.

68. Gerdes a M, Capasso JM. Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure. J Mol Cell Cardiol. 1995;27:849-856.

69. Radisic M, Park H, Shing H, et al. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc Natl Acad Sci U S A. 2004;101(52):18129-18134. doi:10.1073/pnas.0407817101.

70. Tulloch NL, Muskheli V, Razumova M V., et al. Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ Res. 2011;109(1):47-59. doi:10.1161/CIRCRESAHA.110.237206.

71. Nunes SS, Miklas JW, Liu J, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods. 2013;10(8):781-787. doi:10.1038/nmeth.2524.

72. Brent G a. Science in medicine Mechanisms of thyroid hormone action. Sci Med. 2012;122(9):3035-3043. doi:10.1172/JCI60047.three.

73. Klein I, Danzi S. Thyroid disease and the heart. Circulation. 2007;116(15):1725-1735. doi:10.1161/CIRCULATIONAHA.106.678326.

74. Weitzel JM, Radtke C, Seitz HJ. Two thyroid hormone-mediated gene expression patterns in vivo identified by cDNA expression arrays in rat. Nucleic Acids Res. 2001;29(24):5148-5155. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=97559&tool=pmcentrez&rendertype=abstract.

75. Lee Y-K, Ng K-M, Chan Y-C, et al. Triiodothyronine promotes cardiac differentiation and maturation of embryonic stem cells via the classical genomic pathway. Mol Endocrinol. 2010;24(9):1728-1736. doi:10.1210/me.2010-0032.

76. Yang X, Rodriguez M, Pabon L, et al. Tri-iodo-l-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. J Mol Cell Cardiol. 2014;72:296-304. doi:10.1016/j.yjmcc.2014.04.005.

77. Troncoso R, Ibarra C, Vicencio JM, Jaimovich E, Lavandero S. New insights into IGF-1 signaling in the heart. Trends Endocrinol Metab. 2014;25(3):128-137. doi:10.1016/j.tem.2013.12.002.

78. Song Y-H, Godard M, Li Y, Richmond SR, Rosenthal N, Delafontaine P. Insulin-like growth factor I-mediated skeletal muscle hypertrophy is characterized by increased mTOR-p70S6K signaling without increased Akt phosphorylation. J Investig Med. 2005;53:135-142. doi:10.2310/6650.2005.00309.

79. Kawaguchi N, Smith AJ, Waring CD, et al. c-kitpos GATA-4 high rat cardiac stem cells foster adult cardiomyocyte survival through IGF-1 paracrine signalling. PLoS One. 2010;5(12). doi:10.1371/journal.pone.0014297.

80. Linke A, Müller P, Nurzynska D, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc Natl Acad Sci U S A. 2005;102:8966-8971. doi:10.1073/pnas.0502678102.

81. Hopkins SF, McCutcheon EP, Wekstein DR. Postnatal changes in rat ventricular function. Circ Res. 1973;32:685-691. doi:10.1161/01.RES.32.6.685.

82. Schannwell CM, Zimmermann T, Schneppenheim M, Plehn G, Marx R, Strauer BE. Left ventricular hypertrophy and diastolic dysfunction in healthy pregnant women. Cardiology. 2002;97:73-78. doi:10.1159/000057675.

83. Sugishita Y, Koseki S, Matsuda M, Yamaguchi T, Ito I. Myocardial mechanics of athletic hearts in comparison with diseased hearts. Am Heart J. 1983;105:273-280.

84. Rohini A, Agrawal N, Koyani CN, Singh R. Molecular targets and regulators of cardiac hypertrophy. Pharmacol Res. 2010;61(4):269-280. doi:10.1016/j.phrs.2009.11.012.

85. Oudit GY, Crackower M a., Eriksson U, et al. Phosphoinositide 3-Kinase γ-Deficient Mice Are Protected From Isoproterenol-Induced Heart Failure. Circulation. 2003;108:2147-2152. doi:10.1161/01.CIR.0000091403.62293.2B.

86. Lupu F, Terwilliger JD, Lee K, Segre G V, Efstratiadis a. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev Biol. 2001;229:141-162. doi:10.1006/dbio.2000.9975.

87. Lamortes VJ, Thorburns J, Abshers D, et al. Gq- and Ras-dependent Pathways Mediate Hypertrophy of Neonatal Rat Ventricular Myocytes following cw , -Adrenergic Stimulation *. Biol Chem. 1994;269(18):13490-13496.

88. Wang Y. Signal transduction in cardiac hypertrophy - Dissecting compensatory versus pathological pathways utilizing a transgenic approach. Curr Opin Pharmacol. 2001;1:134-140. doi:10.1016/S1471-4892(01)00029-7.

89. De Windt LJ, Lim HW, Bueno OF, et al. Targeted inhibition of calcineurin attenuates cardiac hypertrophy in vivo. Proc Natl Acad Sci U S A. 2001;98(6):3322-3327. doi:10.1073/pnas.031371998.

90. Morimoto T, Hasegawa K, Kaburagi S, et al. Phosphorylation of GATA-4 is involved in a1-adrenergic agonist- responsive transcription of the endothelin-1 gene in cardiac myocytes. J Biol Chem. 2000;275(18):13721-13726. doi:10.1074/jbc.275.18.13721.

91. Kee HJ, Kook H. Roles and targets of class I and IIa histone deacetylases in cardiac hypertrophy. J Biomed Biotechnol. 2011;2011:928326. doi:10.1155/2011/928326.

92. Kunisada K, Hirota H, Fujio Y, et al. Activation of JAK-STAT and MAP Kinases by Leukemia Inhibitory Factor Through gp130 in Cardiac Myocytes. Circ . 1996;94 (10):2626-2632. doi:10.1161/01.CIR.94.10.2626.

93. Harada M, Qin Y, Takano H, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med. 2005;11(3):305-311. doi:10.1038/nm1199.

94. Pan J, Fukuda K, Kodama H, et al. Role of Angiotensin II in Activation of the JAK/STAT Pathway Induced by Acute Pressure Overload in the Rat Heart. Circ Res . 1997;81 (4):611-617. doi:10.1161/01.RES.81.4.611.

95. Maron BJ. Hypertrophic cardiomyopathy: A Systematic Review. Clin Cardiol. 2002;287(10):1308-1320. doi:10.1016/j.cpcardiol.2004.01.001.

96. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med. 1989;321:1372-1378.

97. Geisterfer-Lowrance AAT, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: A β cardiac myosin heavy chain gene missense mutation. Cell. 1990;62(5):999-1006. doi:10.1016/0092-8674(90)90274-I.

98. Watkins H, MacRae C, Thierfelder L, et al. A disease locus for familial hypertrophic cardiomyo-

pathy maps to chromosome 1q3. Nat Genet. 1993;3(4):333-337. http://dx.doi.org/10.1038/ng0493-333.

99. Thierfelder L, Watkins H, MacRae C, et al. α-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of the sarcomere. Cell. 1994;77(5):701-712. doi:10.1016/0092-8674(94)90054-X.

100. Maron BJ, Maron MS. Hypertrophic cardiomyopathy. Lancet. 381(9862):242-255. doi:10.1016/S0140-6736(12)60397-3.

101. van Dijk SJ, Dooijes D, Remedios C Dos, et al. Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. Circulation. 2009;119(11):1473-1483. doi:10.1161/CIRCULATIONAHA.108.838672.

102. Luther PK, Bennett PM, Knupp C, et al. Understanding the Organisation and Role of Myosin Binding Protein C in Normal Striated Muscle by Comparison with MyBP-C Knockout Cardiac Muscle. J Mol Biol. 2008;384(1):60-72. doi:10.1016/j.jmb.2008.09.013.

103. Squire JM, Luther PK, Knupp C. Structural evidence for the interaction of C-protein (MyBP-C) with actin and sequence identification of a possible actin-binding domain. J Mol Biol. 2003;331(03):713-724. doi:10.1016/S0022-2836(03)00781-2.

104. Moolman-Smook J, Flashman E, De Lange W, et al. Identification of novel interactions between domains of myosin binding protein-C that are modulated by hypertrophic cardiomyopathy missense mutations. Circ Res. 2002;91:704-711. doi:10.1161/01.RES.0000036750.81083.83.

105. Schlossarek S, Mearini G, Carrier L. Cardiac myosin-binding protein C in hypertrophic cardiomyopathy: mechanisms and therapeutic opportunities. J Mol Cell Cardiol. 2011;50(4):613-620. doi:10.1016/j.yjmcc.2011.01.014.

106. Harris SP, Bartley CR, Hacker T a., et al. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. Circ Res. 2002;90:594-601. doi:10.1161/01.RES.0000012222.70819.64.

107. Carrier L, Knöll R, Vignier N, et al. Asymmetric septal hypertrophy in heterozygous cMyBP-C null mice. Cardiovasc Res. 2004;63:293-304. doi:10.1016/j.cardiores.2004.04.009.