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CARDIOMYOCYTES MATURATION: CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN CARDIOMYOCYTES MATURATION

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Abstract

The heart is one of the first organs to be formed in the embryo, underscoring its vital function in supplying oxygen and nutrients to the various tissues of the organism both during development and later in life. The cardiomyocytes within the heart must contract in unison to provide an effective pumping action that ensures adequate blood flow to the various organs and tissues. The critical role of cardiomyocytes in ensuring proper cardiac function persists throughout life. In vitro models suggest that cardiomyocyte development goes through two phases: Initiation and Maturation. In the last decade, remarkable progress has been made in controlling the differentiation of human pluripotent stem cells (hPSCs). Cardiomyocytes can be generated in vitro from stem cells with high throughput and purity to a clinically relevant extent, although their differentiation status resembles an embryonic state. Building on these successes, the next challenge is to understand and control cell maturation. This review describes recent approaches to generate mature hPSC-CMs, including their scientific basis, advantages, and limitations. The structural and functional properties of cardiomyocytes and current approaches to maturation of PSC cardiomyocytes are highlighted in this review. Future advances will result from the identification of developmental factors for maturation and their use to generate more mature cardiomyocytes for research and regenerative medicine.

***Keywords**: Cardiomyocytes (CMs), human pluripotent stem cells (hPSC), human induced pluripotent stem cells (hiPSCs), Cellular maturation.*

INTRODUCTION

Cardiomyocytes (CMs) development presents unique challenges when compared to other cell types. Cardiomyocytes are an important constituent of the heart. Genetic manipulations can lead to abnormal morphogenesis of the heart, which is usually fatal to the embryo. Cardiovascular disease has a high rate of morbidity, mortality, and disability and is the leading cause of death in humans regardless of age, race, or region. According to the World Heart Federation, worldwide one-third of adults over the age of 25 suffer from cardiovascular disease [1]. Each year, cardiovascular disease kills over 17.5 million people worldwide, contributes to 30 percent of annual deaths [2]. Diet plays a crucial role in the development and prevention of cardiovascular disease and is one of the main factors that one can change for a healthy heart. Abnormal blood lipid levels have been shown to correlate strongly with the risk of coronary heart disease, and abnormal blood lipid levels are directly related to diet.

Cardiomyocytes are the cells responsible for generating contractile force in the intact heart. Cardiac muscle cells or cardiomyocytes also known as myocardiocytes [3] or cardiac myocytes are the muscle cells (myocytes) that make up the cardiac muscle (heart muscle). Each myocardial cell

contains myofibrils, which are specialized organelles consisting of long chains of sarcomeres, the fundamental contractile units of muscle cells. Cardiomyocytes show light and dark cross bands similar to those present on skeletal muscle cells. Unlike multinucleated skeletal cells, the majority of cardiomyocytes contain only one nucleus, although they may have as many as four [4]. Cardiomyocytes have a high mitochondrial density, which allows them to produce adenosine triphosphate (ATP) quickly, making them highly resistant to fatigue. Two types of cells are present in the heart: the cardiomyocytes and the cardiac pacemaker cells. Cardiomyocytes make up the atria (the chambers where blood enters the heart) and the ventricles (the chambers in which blood is collected and pumped out of the heart). Both cells must be able to compress and elongate their fibers, and the fibers must be flexible enough to stretch. These functions are critical to the proper form during the beating of the heart [5].

Pacemaker cells transmit the impulses responsible for the beating of the heart. They are distributed throughout the heart and have several functions. First, they must be able to spontaneously generate and transmit electrical impulses. They must also be able to receive and respond to electrical impulses from the brain. Lastly, they must be able to transfer electrical impulses from cell to cell. All these cells are connected by cellular bridges. Permeable junctions, called intercalated discs, form connections between cells. They permit sodium, potassium and calcium to easily diffuse from cell to cell. This facilitates depolarization and repolarization in the myocardium. Because of these junctions and bridges the heart muscle is able to act as a single coordinated unit [6].

MORPHOLOGY

In vivo adult cardiomyocyte has a large, anisotropic, rod-like shape that is approximately 150 μm long, 20 μm wide, 15 μm high and 40,000 μm 3 in volume [7]. Conversely, cultured hPSC-CMs range from circular cells with 5–10 μm in diameters ~5 μm high at the start of spontaneous beating, to oblong cells measuring 30 μm in length, 10 μm in width and 2,000 μm 3 in volume after prolonged culture [8]. Adult cardiomyocyte morphology not only provides the structural framework of the cell but also directly establishes other critical functional properties of the cell such as contractility and electrophysiology. Large, anisotropic shape results in a high length-to-width ratio that allows the presence of long myofibrils with laterally arranged sarcomeres, ensuring efficient cardiac contractility [9]. Although immature cardiomyocytes in vivo can have a similar rod-like morphology to adult cardiomyocytes, when isolated and placed in 2D culture, they rapidly adopt flattened, heterogeneous shapes with no clear alignment of myofibrils [10,11].Therefore, differences in structure between immature and mature cardiomyocytes lead to functional variances that limit the potential of hPSC-CMs to recapitulate model human disease or normal development and thus limiting their use in therapeutic regeneration strategies.

As human cardiomyocytes mature, they increase in size due to physiological hypertrophy [12]. Cardiomyocytes tend to form 3D structures over time in culture, a phenomenon also observed in the differentiation of hPSC-CMs. This has been the basis constructing cardiac tissues with a predetermined 3D structure using either primary cardiomyocytes [13]. Several recent studies have investigated the PSC-CMs maturation towards the adult phenotype [14], but most of these studies have focused primarily on electrophysiological end points. Cardiomyocytes are about 100μm long and 10-25μm in diameter [15]. Cardiac myocytes make up approximately 75% of the total volume of the heart in mammals. Non-myocytes, however, make up over 75% of the total number of cells

in the heart. Non-myocyte cell types observed in the heart include fibroblasts, endothelial cells, pericytes, nerve cell processes, Schwann cells, vascular smooth muscle cells, and fat cells located in the outer layer [16].

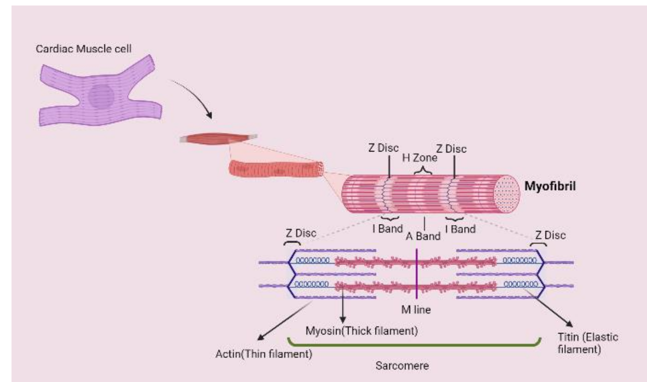

Figure 1: Structure of Myofibril showing the unit of a sarcomere.

Structurally, adult cardiomyocytes display a high length-to-width ratio, may be bi-nucleated, and form sophisticated structures such as T-tubules and the sarcoplasmic reticulum within the sarcomere's Z-line [17]. T-tubules are significant due to their role in contraction propagation [18]. Absent or disrupted T-tubules have been implicated in heart failure in animal models [19] Z-discs, I-, H-, A-, and M-bands are present in adult cardiomyocytes. In addition adult cardiomyocytes have 2.2 µm in length and highly organized. These cells also have large numbers of mitochondria because the heart requires incessant energy. Myocardial mitochondria tend to be evenly distributed throughout the cell and account for 20%-40% of cell size. Contrary to this, immature hiPSC-CMs tend to be round, usually mononuclear, and the sarcomeres are disordered and shorter (1.6 µm). These cells also do not possess T tubules and only have Z-discs and I- bands [17]. As opposed to glycolysis adult cardiomyocytes primarily relay in fatty acid oxidation for energy production and show high levels of oxidative phosphorylation. In contrast, hiPSC-CMs mainly rely on glucose and lactate but do possess some capacity to metabolize fatty acids [20, 21]

CELLULAR MATURATION INVOLVED IN CARDIOMYOCYTES MATURATION

In vitro modelling suggests that cardiomyocyte development has two phases; Initiation and Maturation [21]. During initiation phase, firstly a number of so called pioneer transcription factors expresses by precursor cell, and these pioneer transcription factors lead to significant epigenetic remodeling, the phenotype of the precursor cell is silenced and different genes which are necessary for establishing the cardiomyocyte lineage are activated. The pioneer transcription factor were identified. For the initial establishment of cardiomyocyte lineage combined expression of Tbx5, Gata4m Hand1 and Mef2C is necessary [18].

Which mechanisms are involved in committed cells maturation are unknown. Two recent studies have shown that TLR3 is important for reprogramming fibroblasts to iPS and endothelial cells [22]. Furthermore, TLR3 induces inflammation and inflammation is known play an important role in injury. TLR3 mediates the activation of a number of transcription factors. Of these transcription factors, two mediate the vast majority of the effects of TLR3: AP1 and NF-κB. Consequently, it was hypothesized that TLR3 would influence maturation of reprogrammed cells via AP1 and/or NF-κB

[23]. CMs can be differentiated from human (h) embryonic stem cells (ESCs) that can propagate indefinitely in culture while maintaining their pluripotency. For clinical application and drug testing hESCs may therefore provide an unlimited ex vivo source of cardiomyocytes [24].

The process of cardiomyocyte maturation that starts after birth and proceeds till adulthood is necessary for the efficient contraction and handling of calcium, also for fulfilling the metabolic needs of an adult mammal heart. During the process of maturation the cardiomyocytes increases their length and develop well-coordinated, evenly distributed sarcomeres and dense myofibril structures. A hallmark of cardiomyocyte maturation is isoform switching of contractile genes from the fetal to the adult state [25, 26]. The cultured pluripotent stem cells gives a unique mechanism for study the role of metabolism in the process of maturation of differentiated cells like cardiomyocytes. The well designed protocol helps in helps in differentiation of both hESCs and iPSCs into cardiomyocytes. The characteristics of these cells are highly resembled to those of embryonic cardiomyocyte in the way that they beat spontaneously, show proteins, and also have calcium transients that are highly resembled with cardiac action potentials [27]. Mature cardiomyocytes consume an exorbitant amount of ATP to meet the high metabolic demands placed on these cells by continuous beating. However, because they have a very limited ability to store energy-rich phosphates, they require an efficient ATP production machinery to continuously supply the cell with energy, which is why they make various changes in their metabolic processes as they age to increase their metabolic capacity [28]

One of the most important metabolic changes in cardiomyocytes is the transformation from anaerobic to aerobic metabolism [29]. The fetal heart is adapted to a low-oxygen environment in which circulating fatty acid content is also low, that's why to produce ATP fetal cardiomyocytes are highly dependent on glycolysis [28]. A concomitant increase in circulating levels of free fatty acids. In conjunction with transcriptional regulation of proteins involved in fatty acid oxidation, this leads to a switch in the metabolic profile in cardiomyocytes which as immature cardiomyocytes depend on glycolysis and as mature cardiomyocytes rely predominantly on aerobic metabolism [30]. Immediately after birth, in cardiomyocytes more than half of the ATP is produced through glycolysis, by seven days after birth, glycolysis decreases, accounting for less than10% of ATP production [28]. During development, as lactate levels decrease cardiac tissue is also less reliant on lactate oxidation, resulting in tissue that uses fatty acid as a primary fuel source [30]. As cardiomyocytes mature, the number of mitochondria in each cell increases. The let-7 family of microRNAs work endogenously in cardiomyocytes to accelerate maturation, largely through a significant impact on cardiomyocyte metabolism [31].Comparing the microRNA profile of mature and immature cardiomyocytes, most highly upregulated family of microRNA is let-7 family.

A number of exogenous signals have also been shown to regulate cardiomyocyte development. For example, Thyroid hormone tri-iodo-1-thyronine (T3). It is known that circulating thyroid hormones are important for a number of cardiac processes including the regulation of other developmental switches that are required for cardiomyocyte maturation from fetal to adult [32]. Treatment of cardiomyocytes with T3 led to an increase in a number of maturational markers, including cell size, calcium handling, and contractile kinetics [33]. During cardiomyocytes maturation switching of metabolic profile is not only an accompanying phenomenon, but it is also a potential regulator of cardiomyocytes maturation. Regulation of cardiomyocyte metabolic profile

is a key component of being able to manipulate cardiomyocyte maturation.

MOLECULAR MECHANISMS INVOLVED IN CARDIOMYOCYTES MATURATION

Just over a decade ago, it was first reported that microRNAs (miRs) play a key role in t controlling cardiomyocyte differentiation and proliferation [34]. The heart development is a complex process that requires many temporally and spatially controlled gene regulatory networks [35]. The differentiation of human embryonic and induced pluripotent stem cells (ESCs and iPSCs) into functional CMs can recapitulate different stages of the developing heart and serves as an in vitro model for studying cardiogenesis. Dissection of molecular players involved in cardiomyocytes differentiation and maturation may hold the key to a better understanding of the underlying molecular mechanisms during cardiac development and disease progression. Cardiomyocytes originate from pluripotent stem cells (hPSC) including both embryonic stem cells (ESCs) and human induced pluripotent stem cells (hiPSCs) have received enormous attention as a promising source for cell based cardiac regeneration therapy [36]. The morphology and function of mitochondria is also known as a critical marker for CM maturation. Mature adult mitochondria are the powerhouse of the cell and enable efficient cellular respiration with a large surface area provided by densely packed cristae [37]. However, cristae is absent in both fetal and hPSC-CMs [38]. Maturation is a gradual process which normally requires 6 to 10 years for human neonatal CMs to reach their adult phenotype in vivo. However, functional hPSC-CMs can be generated within 15 days of differentiation [39]. To grow more mature cardiomyocytes, in vitro scientists firstly increased the culturing time of hPSC-CM. As a critical parameter of CM maturation, electrophysiological properties were shown to improved prolonged culture. In another study hPSC-CMs cultured for 90 days and indicated an increase in densities of the inward rectifier current (Ik1) and Ito1, although the densities for IKr and ICaL remained constant [29].

PSC-derived cardiomyocytes can be generated routinely with high yield and purity for disease research and drug development, and gradually these cells are now entering in the clinical research phase to test therapies for cardiac regeneration. In spite of that, considerable obstacle to their application is the mature condition these cardiomyocytes. Most protocols generate cells at embryonic stages or early fetal stages, typically stages just after organogenesis completion. Therefore, the generated cells lack many attributes of adult cells that are desirable for drug screening, modelling of adult-onset diseases or replacing cells lost to disease [40].

At the cardiomyocyte level the developing heart undergoes substantial morphological changes. In the first week of postnatal life in rodents, and in human during the first decade, Cardiomyocytes undergo a mitotic resting phase, and further growth of the heart occurs mainly by enlargement of cardiomyocytes [39]. Standard differentiation of hPSC-CMs in in vitro culture lacks the dynamic physical and environmental cues necessary to induce the degree of physiological hypertrophy observed in vivo from postnatal stages into adulthood, even after prolonged culture [41, 42, 43]. As such, use of standard cell culture protocols results in heterogeneous cell populations of small, misaligned, immature cardiomyocytes of varied shape, generally lacking the well-formed myofibrils and T-tubules, polyploidy, polarized intercalated discs or abundant mitochondria seen in vivo [33].

During heart development, the expression levels, location and function of distinct ion channels in

cardiomyocytes evolve over time into a mature electrophysiological phenotype [44]. The action potentials generated from an immature hPSC-CM and a mature primary cardiomyocyte are quite distinct [45, 46]. Studies in rodents and humans have demonstrated that, whereas embryonic, fetal and early postnatal cardiomyocytes can divide, adult cardiomyocytes are predominately quiescent [47]. This cell cycle arrest coincides with the switch from a hypoxic to oxygen-rich environment postnatally [48]. During cardiomyocyte maturation in vivo, cardiomyocytes become polyploidy in most mammals [49] either via DNA synthesis and nuclear division without cytokinesis to form bi-nucleated cells, as occurs in rodents, or via DNA synthesis without nuclear division to make polyploid nuclei, as observed in humans [50,51]. Early studies demonstrated that polyploidy increases with age in human cardiomyocytes, ranging from 4n to 64n (where n is the haploid content of chromosomes; reviewed previously [39], and the number of polyploid cells increases after injury such as myocardial infarction.

Scientists developed a system in zebrafish in which the ploidy of cardiomyocytes could be genetically tuned. The researchers showed that hearts with genetically increased levels of ploidy had less regenerative potential than wild-type control hearts [52]. Multiple levels of regulation of the cardiac cell cycle have been described, including classic regulators such as cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CKIs), along with microRNAs (miRNAs), transcription factors and metabolic regulators. Cyclin and CDK levels decrease in humans and rodents during fetal to adult cardiomyocyte maturation, reflecting the loss of proliferative capacity. When hPSC-CMs transplanted into a normal or injured heart these cell mature extensively. Our group studied the in vivo maturation of hiPSC-CMs transplanted into rat neonatal and adult hearts [53]. Rat neonatal cardiomyocytes transplanted into rat hosts displayed more mature phenotypes, whereas a human cell graft remained relatively immature. Long-term culture was the first approach used to mature PSC-CMs. In initial experiments, cardiomyocytes derived from differentiation of human embryoid bodies were maintained in culture for up to two months, a number of structural changes were found including changes in cell structure, sarcomeric organization changes and increase in cell size while reduced proliferation [8].

In-depth characterization of culture times for cardiomyocytes that were derived from hPSCs with the use of 2D differentiation protocols (1 month to 3–4 months in culture) showed the development of additional maturation features, like increase in cell size, elongation of cell and its contractions, myofibril alignment, improvement in handling of calcium, action potential, amplitudes and upstroke velocity [41]. Most of these maturation changes occur between 1st week and 4th week, while a minor change is observed after 4th weeks. At this time gene expression trends stabilize [8, 54].

HPSC-CMs are highly responsive to the 3D environment as well as electrical and mechanical stimuli, and this responsiveness can be used to promote maturation. With the advent of next-generation sequencing technology and global 'omics' profiling. The field Microarray analysis demonstrated the similarity of hPSC-CMs with cardiomyocytes in human embryonic hearts in the first trimester of development [55]. Uosaki and colleagues performed an elegant analysis of 200 microarray datasets from wild type mouse hearts across different stages of development (from embryo to adult) to characterize gene regulatory networks [56]. Single-cell RNA sequencing studies have dissected the complexity and heterogeneity among cells during differentiation and have enabled the

identification of maturation-associated transcription factors [57, 58].

Candidate genes that might affect mouse PSC-CM maturation were identified, such as Ppara, Pparg, Ppargc1a, Cebpa, Cebpb (whose expression increased during in vivo cardiomyocyte development but was inactivated in mouse PSC-CMs) and Ctnnb1 (which was inactivated during in vivo cardiomyocyte development but remained active in mouse PSC-CMs [56]. Non-coding RNAs are gene regulators that, by definition, are not translated. miRNA profiling of hESCs, hESC-CMs and human fetal and adult primary ventricular cardiomyocytes revealed a panel of 23 miRNAs that were highly expressed in hESC-CMs and in fetal and adult samples compared with hESCs [59]. Epigenetics provide another layer of gene expression regulation through modifications to and interactions between histones, DNA and regulatory non-coding RNAs. In-depth profiling of human cardiomyocytes (at neonatal to adult stages) shows cooperation between both DNA methylation and histone post-translational modifications in regulating gene expression during cardiac development [51]. The capacity of endothelial cells to induce multiple facets of mouse ESC-CM maturation led to the discovery that co-culture induces the expression of multiple miRNAs in cardiomyocytes and that an miRNA cocktail (comprising miR-125b, miR-199a, miR-221 and miR-222) is sufficient to increase cell size and bi-nucleation, make resting membrane potential more negative, increase action potential amplitude and upregulate GJA1 and KCNJ2 expression in cardiomyocytes [60].

Role for histone and DNA modifications in the regulation of cardiomyocyte maturation, but in-depth characterization of targets and mechanism is still needed in hPSC-CMs. Mass spectrometry analyses show similarities in the proteomes of hPSC-CMs and human fetal primary cardiomyocytes. HPSC-CMs and human fetal primary cardiomyocytes have lowest but adult primary cardiomyocytes have highest levels of contractile proteins. Conversely, the levels of proteins involved in fatty acid metabolism were higher in adult primary cardiomyocytes but similar between fetal primary CMs and hPSC-CMs, persistent with expression pattern of mRNA [51]. Comparison of the metabolic profiles of hPSC-CMs cultured for 1 month and 3 months demonstrated distinct features of cell status and identified the increased levels of glycerol phosphocholine, a product of phospholipid metabolism, as a biomarker for hPSC-CM maturation [61].As mentioned previously, mass spectroscopy metabolite analysis identified the downregulation of nucleotide biosynthesis intervene by the pathway called pentose phosphate pathway as a mediator of low glucose-induced hPSC-CM maturation [62]. Measurements of the flux of carbon sources in PSC-CMs have enabled metabolite tracing and characterization to assess how carbon sources feed into and regulate the maturation programs of the cardiomyocyte [63, 64, 65].

Taken together, the findings from these omics approaches clearly show that cardiomyocyte maturation is controlled at the epigenome, coding and noncoding metabolome and proteome level. These large datasets enable the development of computational networks that generate testable hypotheses about how cardiomyocyte maturation is controlled. Several candidates have been confirmed as major regulators of maturation, but omics analyses and computational modelling are other areas that are ripe for further findings.

Cardiomyocytes and CFs in the heart can communicate through direct cell-cell contacts, soluble paracrine factors, and ECM-mediated interactions [66, 67]. Studies concluded that the neonatal

cardiac fibroblast could change the electrophysiological characteristics of neonatal cardiomyocytes in standard 2D monolayer culture [68, 69, 70, 71]. Further studies have shown that 2Dcultures are unable to completely summarize structural, biochemical and mechanical environment of contracting heart, which includes the complex cell-cell and cell matrix interactions [72]. Particularly, how the age of non-cardiomyocytes in engineered heart tissues affects cardiomyocytes function and maturation remains to be explored. Experiments in 2D co-cultures have already shown the fetal and adult fibroblasts play different roles in hyperplastic and hypertrophic growth of embryonic cardiomyocytes [73]. Human pluripotent stem cells (hPSCs) are capable of prolonged cell division and differentiation into any somatic cell-type including cardiomyocytes. Cardiac diseases are still the most prevalent cause of death in the Western world [74, 75]

In hPSC-CMs maturity can be induced via substrate: (1) By coating with extracellular matrix proteins that can act directly as messenger molecules, either by biochemical or mechanical signaling[76,77,78], (2) changing/ modulating the intrinsic elasticity or stiffness of the substrate, providing mechanical load not present when cells are cultured on rigid glass slides[79,80] , (3) changing/modulating surface topography, both at the nano- and micro-scale, providing mechanical cues and forcing cardiomyocytes to align and elongate [81,82]. Whereas it is not possible to use extended culture for regular biomedical application, it should be performed with the help of useful developmental tool. Thus, hESC-CMs maintained in culture for a year showed molecular signatures similar to those seen for in vivo-derived mature cardiac tissues [31].Tri-iodo-thyronine plays an important role in normal development of heart and during the perinatal period, it controls isoform switching of many myocardial proteins, including MHC and titin. hPSC-CMs incubation with T3 for 1-2 weeks resulted in changes consistent with maturation, including 11-fold upregulation of α-MHC, lower proliferation rates (but not increased bi-nucleation), 1.5-fold increase in twitch force (to ~12nN/cell), higher calcium-derived maximal upstroke and decay velocities enhanced oxygen consumption rates[33].

The expression of cardiac transcription factors, such as GMT (Gata4, Mef2c, and Tbx5), is an early event in the development of cardiomyocytes [83, 84, 85]. The role of the cardiac transcription factors in the later maturation of cardiomyocytes is less known. Genetic ablation of components of the cardiac transcription factor cascade significantly impairs heart development [86] demonstrating their importance. Although these studies do not clearly reveal the role for cardiomyocyte maturation. Over-expression of components of cardiac transcription factor cascade, such as GMT and GMTH (GMT plus Hand2), induces fibroblasts to reprogram into cardiomyocytes [87, 88, 89]. These and other studies [90, 91, 92, 93] suggest that the cardiac transcription factor cascade is involved in cardiomyocyte maturation [17]. Cardiomyocyte maturation can be achieved independently of any effect on cardiac transcription factor expression

Activation of the TLR3-NFκB pathway induced the expression of various sarcomere-related genes and significantly enhanced the maturation of reprogrammed fibroblasts into mature cardiomyocytes [94]. Birth, which is accompanied by significant changes in hemodynamics, oxygenation, and biochemical milieu, has frequently been thought of as a trigger for maturation. The closure of shunts in the fetal circulation during birth leads to significant changes in heart load and output[95,96,97], which in turn promotes structural, contractile, and force generation improvements in CMs. Oxygenation of arterial blood doubles, leading to metabolic and

mitochondrial maturation through reduction in HIF1a signaling [98,99]. In addition to new methods for conducting in vivo studies such as the CASAAV system, improved technologies in transcriptomics will enable a better understanding of regulatory networks in CM maturation.

To date, transcriptomic analysis has been used to: elucidate stage-specific regulatory networks guiding cardiac development and maturation [56]; identify nucleosome and histone-modifying genes in maturation[100] ; identify the role of Let-7 family of microRNAs in guiding CM maturation through metabolic switch [31] ; and identify miR-200c as a regulator of mature ion channel expression and calcium handling [101]. In conjunction with chromatin immunoprecipitation-sequencing (ChIP-seq), transcriptomic analyses were also performed to develop a better understanding of the epigenetic dynamics of maturation [102, 51]. Lastly, transcriptomics has provided a powerful tool to benchmark the maturation status of in vitro-generated CM tissues [31, 55, and 56,103]. Single cell RNA-seq (scRNA-seq) illustrates a great opportunity for better understanding of CM maturation. Performed a seminal study in which they generated scRNA-seq libraries for >1,200 cardiac cells from various developmental time points ranging from e9.5 to p21 [103].

CONCLUSION

Biology of CM maturation continues to be a rapidly evolving and highly interesting area of research, where new technologies are providing new insights. Studying cardiomyocyte development presents unique challenges when compared to other cell types. As cardiomyocytes are an important constituent of the heart. Technological advances in hPSC-CMs maturation have started a new chapter for logical application of hPSC-CMs. Vast studies will be required in future to develop optimal methods for efficient large-scale generation of mature hPSC-CMs. Maturation is likely to be a complex trait, controlled by multiple signaling networks in the cytoplasm and nucleus. With the help of this study we got a solid concept and collect useful data regarding the mechanism taking place with these morphological and functional changes.

A deep study of different maturation strategies in essential to attain progress in cardiomyocyte phenotypes. The advancement in this field has initiated many parallel fields of study which results in new spectrum of therapies for treatment of cardiovascular disease.

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