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Physiology of Cardiac Development: From Genetics to Signaling to Therapeutic Strategies

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Abstract

The heart is one of the first organs to form and function during embryonic development. It is comprised of multiple cell lineages, each integral for proper cardiac development, and include cardiomyocytes, endothelial cells, epicardial cells and neural crest cells. The molecular mechanisms regulating cardiac development and morphogenesis are dependent on signaling crosstalk between multiple lineages through paracrine interactions, cell-ECM interactions, and cell-cell interactions, which together, help facilitate survival, growth, proliferation, differentiation and migration of cardiac tissue. Aberrant regulation of any of these processes can induce developmental disorders and pathological phenotypes. Here, we will discuss each of these processes, the genetic factors that contribute to each step of cardiac development, as well as the current and future therapeutic targets and mechanisms of heart development and disease. Understanding the complex interactions that regulate cardiac development, proliferation and differentiation is not only vital to understanding the causes of congenital heart defects, but to also finding new therapeutics that can treat both pediatric and adult cardiac disease in the near future.

Keywords

signaling; development; congenital; heart; disease; genetics

Introduction

The heart consists of multiple cell lineages, including cardiomyocytes, endothelial cells, fibroblasts, and smooth muscle cells, that together orchestrate a sophisticated network of crosstalk in cardiac development, disease and regeneration [1]. The process of development

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itself, and the interactions between cell-autonomous and non-autonomous events, are required for cardiac cell proliferation, differentiation, migration, and survival [2]. Growth factors, signaling pathways and transcription factors all play a critical role in facilitating these events. In this review, we focus on the discoveries of recent studies that help unveil the critical role of genetic mutations, signaling pathways and lineage-specific contributions to cardiovascular development, physiology, and disease.

Heart anatomy and function

The heart is a vital, multi-chambered organ that pumps blood to maintain proper pulmonary and systemic circulation, mediating oxygenation of the body's vital tissues. The mammalian heart is made up of four chambers, the left atrium (LA), left ventricle (LV), right atrium (RA), and right ventricle (RV), each divided by septal and valvular structures [3] (Figure 1). The heart relaxes (dilates) or pumps (contracts) according to electrical signals that stem from the cardiac conduction system. Importantly, it also functions to transport oxygen, nutrients, and signaling molecules to the entire body [3, 4].

Briefly, de-oxygenated blood returning from the body through the inferior and superior vena cava enters the heart through the RA and is pumped to the RV through the tricuspid valve. Subsequently, blood from the RV is propelled into the lungs through the pulmonary arteries, where it becomes enriched with oxygen [3]. Pulmonary veins return oxygenated blood to the LA, which contracts through the mitral valve to fill the LV, the main pumping chamber of the heart, where it is then ejected through the aorta and into the major circulatory network of the body [4]. The process repeats and begins all over again. The contraction of the ventricles is referred to as systole, whereas diastole is the term used to describe the relaxation, or dilation, of the heart muscle [5]. (Figure 1)

Process of cardiac development

The heart is the first fully developed and functional organ in embryogenesis. During gastrulation, a single-layered blastula is re-organized into three germ layers: a dorsal ectoderm, a ventral endoderm and a mesoderm layer [6]. The heart tissue, consisting of myocardial, endocardial and epicardial cells, is predominantly derived from the mesodermal layer [7]. In addition, ectoderm-derived cardiac neural crest cells are involved in the development of the cardiac cushions of the outflow tract [8].

Prospective cardiogenic cells originate from the posterior epiblast, precursor cells derived from mesodermal tissues [9]. At human embryonic day 12 (E12) (E5.5 in mice), the embryo becomes an elongated cylinder, divided into 2 portions: the proximal and distal regions [10]. Subsequently, formation of bilaterally symmetric cardiac primordia occurs in early gestation, at E18 (E7.5 in mice), which is derived from lateral plate mesoderm [10]. These cells then migrate medially and fuse at E20 (E8.0 in mice), generating the initial heart tube, whose composition is comprised of an outer myocardial layer and an inner endocardial layer [10]. The myocardial layer gives rise to cardiomyocytes, the fundamental unit of rhythmic beating; the endocardial layer leads to the development of cardiac valves [11]. By E25 (E10.5 in mice), in response to myocardial signals deposited in the cardiac jelly, the

endocardial cells undergo epithelial–mesenchymal transition (EMT), delaminating from the endocardial surface and differentiating into mesenchymal cells, which proliferate and invade the cardiac jelly [11]. This, in turn, gives rise to the endocardial cushion in the atrioventricular (AV) canal and to the outflow tract (OFT), the precursor to future valvular structures [12]. (Figure 2)

At E34 (E11.5 in mice), epicardium, derived from proepicardium, proliferates and covers the entire embryonic heart. It is derived from mesodermal cells positioned dorsal to the initial heart tube and give rise to cardiac fibroblasts, coronary vascular smooth muscle cells, coronary endothelial cells, and even to an additional small population of cardiomyocytes [13, 14]. Finally, migrating neural crest cells (NCC) integrate into the circumpharyngeal ridge and invade endocardium derived cushion tissue in OFT to generate asymmetric growth, allowing for septation of the myocardial wall [15]. In the end, the heart becomes a fully formed, functional organ by E55 (E15.5 in mice) [10]. (Figure 2)

Developmental signals and cardiogenesis

The process of cardiac development is controlled by a complex network of signaling pathways that include NODAL [a member of the transforming growth factor beta superfamily (TGF β)], bone morphogenic protein (BMP), wingless-type MMTV integration site family member 3 (WNT3A), and fibroblast growth factor (FGF) [16, 17]. Signaling pathway regulation of transcription factors, including the T-box transcription factor eomesodermin, the basic-helix-loop-helix transcription factors mesoderm posterior-1 (MESP1) and Heart and Neural Crest Derivatives Expressed 1/2 (HAND1/2), NK2 Homeobox 5 (NKX2.5), zinc-finger transcription factor GATA, ISL homeobox 1 (ISL1), myocyte enhancer factor 2c (MEF2c), and T-Box (TBX) [2], are also involved in mediating proper development of the heart, the stability of which can be negatively regulated by microRNAs (miRs), a class of small single stranded noncoding RNAs [18, 19].

The cardiac genetic program is initiated following expression of MESP1+ precardiac mesoderm [18]. As MESP1+ progenitors migrate towards the anterolateral plate mesoderm, MESP1 becomes downregulated and a subunit of the SWI/SNF (switch/sucrose non-fermentable) chromatin remodeling complex, SMARCD3, becomes induced, mediating GATA4 binding to enhancer regions of transcription factors required for cardiogenesis, including GATA4, NKX2-5, ISL1, TBX5, and MEF2C [19–21]. These factors each play a key role in the initiation of cardiac development and differentiation. For instance, NKX2.5, the most frequently mutated gene in CHD, is an integral transcriptional regulator that specifies cardiac mesoderm [22–24]. GATA factors too play a central role in early cardiac development; indeed, GATA4/6 double mutant mice lack hearts entirely [25].

The cardiac primitive streak

Signaling networks induce cardiac development through regulation of early mesoderm, generating the cardiac primitive streak, the site where epiblast cells begin to ingress into the embryo and differentiate into the three embryonic germ layers [26]. Regulated by canonical Wnt/ β -catenin signaling, eomesodermin expression is induced and increases at the anterior

primitive streak, giving rise to both definitive endoderm and cardiac mesoderm [27]. These eomesodermin-positive cells then activate MESP1, which regulates the activation of cardiac and mesodermal genes to mediate migration of mesodermal precursor cells through the primitive streak and become the anterior lateral plate mesoderm [18, 26, 27]. By week 3 of human development (E7.5 in the mouse), these migratory cells become the first and second heart fields (FHF and SHF), the first cell populations of distinct cardiac progenitors to have a defined cardiac fate [28]. (Figure 3)

Development of the first heart field

FHF progenitors are the first to reach the anterolateral plate following mesodermal cell migration, at around E7.5 in mouse development. These cells become spatially organized in a crescent-like shape, now termed the cardiac crescent. FHF progenitors receive BMP2 [29], FGF8 [30], and non-canonical WNT [31] signals from the underlying endoderm to promote their differentiation and to activate TBX5, a regulator of cell fate [25]. TBX5, in turn, interacts with GATA4 and NKX2.5 to drive cardiac muscle development and specification of the LV through induction of several integral cardiac genes, including natriuretic peptide A (*Nppa*) and the gap junction protein connexin 40 (*Gja5*) [26, 32, 33]. GATA4 and NKX2.5 repress the hemangiogenic gene program, concomitantly upregulating cardiac-specific genes that include *Hand1*, *Mef2c*, and myosin light chain-2v (*Myl2*, also known as *Mlc2v*), all components of the necessary machinery required for normal cardiomyocyte structure and function [26, 34, 35]. Ultimately, the FHF cells give rise to the LV free wall, part of the septum, and a portion of the atria [36].

Aberrant regulation of FHF progenitors in the cardiac developmental process leads to severe cardiac defects. In mice, loss of NKX2.5 results in embryonic lethality due to failure of both cardiac looping and left ventricular formation [37]. In humans, mutations in NKX2.5 result in multiple CHDs, including cardiac conduction defects, atrial septal defects, and ventricular septal defects [24]. Similarly, deletion of GATA4 in murine hearts leads to embryonic lethality by E11.5 due to insufficient cardiomyocyte proliferation [38]. Moreover, knock-down of GATA4 causes septation, valvular and functional defects in mouse heart [39–41]. Finally, aberrant expression of TBX5 in the FHF leads to improper positioning (or even absence) of the interventricular septum [33]. (Figure 3)

Development of the second heart field

SHF progenitors give rise to the RV, part of the septum, the outflow tracts, and a portion of the atria [36]. SHF cells are located medially and dorsally to FHF cells. Compared with FHF, SHF progenitors have delayed commitment to the cardiomyocyte lineage. They receive signals from FGF [42], sonic hedgehog [43] and canonical WNT/ β -catenin [44] to promote proliferation and multi-lineage differentiation [26]. SHF progenitors contribute to the growth of the heart tube and form the inflow and outflow tracts, ultimately migrating and differentiating into cardiomyocytes, endothelial cells, and smooth muscle cells [45, 46]. SHF is marked by expression of ISL1 [45, 47], although FHF progenitors also transiently express this as well [48]. ISL1 activates FGF and BMP, gene pathways to modulate cardiac progenitor cell proliferation and differentiation [47]. Together with GATA4, ISL1 activates

MEF2c to induce expression of HAND2, a transcription factor important for RV development [38, 49, 50]. As SHF progenitors continue to differentiate, NKX2.5 is induced, repressing ISL1 and transitioning progenitor cells from a state of proliferation to one of differentiation [51]. Importantly, direct repression of ISL1 by NKX2.5 is necessary for development of ventricular cardiomyocytes [52] (Figure 3). Significantly, aberrant SHF regulation also leads to severe cardiac developmental defects. ISL1 knockout mice exhibit cardiac looping, RV, and outflow tract abnormalities [47, 53]. HAND2 knockout mice display varying degrees of RV hypoplasia [54].

BMP signaling in cardiomyogenesis

BMP signaling is mediated, at least in part, by induction of GATA4, MEF2c, SRF, and NKX2.5 [55, 56]. Specifically, BMP2 and BMP4, secreted by underlying endoderm, induce cardiomyogenesis of the overlying lateral plate mesoderm [29, 57, 58]. Within SHF, BMP is required for upregulation of TBX2 and TBX3 to maintain slow conduction velocity and to reduce proliferation of myocardium within the outflow tract, atrioventricular canal, and sinus horns [59].

Wnt/ β -catenin signaling

Canonical WNT/ β -catenin signaling is critical for maintaining proliferation of the SHF progenitors [60]. At the same time, this pathway inhibits differentiation of cells toward more terminal lineages [61, 62]. Therefore, in developing mouse hearts, as SHF progenitors migrate into the developing outflow tract, canonical WNT signaling is downregulated, facilitating concomitant activation of cardiomyocyte-specific genes [58, 63].

Interestingly, non-canonical WNT signaling is required to inhibit canonical WNT/ β -catenin pathway regulation [64]. Through calcium-dependent pathways, non-canonical WNT signaling is crucial for normal cardiomyocyte specification [65]. For instance, two non-canonical WNT ligands, WNT5A and WNT11, have demonstrated roles in cardiac development; mouse embryos lacking both WNT5a and WNT11 have dramatically reduced numbers of SHF progenitor cells [64].

HOPX signaling

HOPX, a homeodomain-containing transcriptional repressor, is a recently identified regulator of canonical WNT/ β -catenin and BMP signaling pathways in cardiac development [58]. HOPX expression initiates in FHF and in SHF derivatives that are exclusively committed to the cardiomyocyte lineage [26]. Notably, HOPX promotes cardiomyocyte differentiation via inhibition of WNT, which is mediated by direct interaction of HOPX with SMAD4, a transcription factor essential for transducing BMP signals [58]. In essence, as SHF cells migrate into the outflow tract and become exposed to increased concentrations of BMP4 and HOPX, canonical WNT/ β -catenin signaling is reduced, facilitating continuation of the cardiomyocyte differentiation process [26, 58].

Role of MicroRNAs in Cardiac Differentiation

MicroRNAs (miRs) are single-stranded, noncoding RNA molecules that negatively affect gene expression at the post-transcriptional level, either by guiding mRNA degradation or by preventing protein translation [26, 66]. In cardiogenesis, miRs mediate transcription factor expression to modulate cell fate, proliferation, and function of cardiac cells [2, 67].

Several miRs play a role in cardiac development. For example, miR-1 and miR-133 are regulated by SRF and MEF2 [68, 69]. Indeed, homozygous deletion of miR-1 is embryonic or perinatal lethal, with defects that include ventricular septal defect, heart failure, and dysrhythmias [70]. Overexpression of miR-1 in fetal cardiomyocytes is also deleterious, resulting in thinning of the ventricular wall and heart failure [70]. Like miR-1, deletion of miR-133a in mice also causes severe heart failure due to ventricular septal defects and dilated cardiomyopathy [71].

Embryonic myocardial growth and differentiation

Once cardiac progenitor cells differentiate into cardiomyocytes, additional cardiomyocyte growth is facilitated only through active proliferation of existing cells [26]. Several signaling pathways control proliferation of cardiomyocytes. For example, Hippo/YAP signaling is required to regulate the size of the heart through activation of Hippo pathway kinases (MST1/2 and LATS1/2), which inhibit cardiomyocyte proliferation through inhibition of transcriptional coactivators YAP and TAZ (formally known as WWTR1) [72, 73]. YAP interacts with TEAD1, a transcription factor that drives activation of downstream signaling pathways, including PI3K-AKT [74–76]. YAP likely also upregulates cardiomyocyte proliferation through interaction with β -catenin and direct modulation of WNT signaling [77].

Differentiation of myocardium leads to generation of an inner trabeculated cardiomyocyte layer adjacent to the endocardium, coupled with an outer compact layer of cells. Interestingly, cardiomyocytes in the compact myocardium proliferate more rapidly than those in the trabecular region [78–80]. Changes in signaling pathway regulation and/or gradients of mitogenic or environmental factors may be important factors in this difference in proliferation [78–80]. In addition, cardiomyocyte proliferation during trabeculation may also be tightly regulated by both cell-autonomous and non-autonomous processes; for example, cross-communication between myocardium and endocardium, regulated through NOTCH1, BMP10, ephrin B2 (EFNB2), HAND2, and neuregulin-1 (NRG1) signaling, is critical for proper proliferation and growth of myocardial cells [78–81].

Endocardium and valve development

Cardiac endothelium is required for establishing the cardiac cushion and valve structures during development, as well as for maintaining valvular homeostasis in the adult heart [11, 82]. Valves are highly organized structures generated to withstand the constant blood pressure driven by heart beats; they are critical for proper blood flow. They are comprised of an outer layer of endothelium and an inner mixture of extracellular matrix (ECM) and interstitial cells [11, 83, 84]. During the initial stages of cardiac looping, ECM is rapidly

generated in the atrioventricular canal and OFT. Local endocardium then undergoes EMT, proliferating into a pool of mesenchymal cells that populate this newly derived ECM, thereby forming the endocardial cushions [85]. During EMT, paracrine signals, including those from TGF β , WNT, VEGF, and Notch, mediate induction of cardiac-specific transcription factors, including SNAIL, MSX2, and TBX20, to facilitate cardiac remodeling, cell proliferation and apoptosis of the endocardial cushions and valves [86]. (Figure 4)

Specifically, Notch determines endocardium competence through binding of Delta/Jagged [87], initiating EMT and regulating expression of EMT related genes, including ACTA2, SNAIL2, SMAD3, and RUNX3 [88]. Accordingly, inhibition of Notch signaling leads to collapsed endocardium, EMT abnormalities and impaired volume in the cushion mesenchyme [88]. In contrast, increased Notch activity induces elevated expression of mesenchyme genes, including SNAIL1/2, TWIST2, and TGF β 2, as well as mediates ectopic EMT in the endocardial chamber [88]. (Figure 4)

Several pathways play a critical role in valvular development. WNT/ β -catenin has a demonstrated role in restricting endocardial competence. Indeed, increasing WNT signaling expands the number of endothelial cells, causing abnormally enlarged valves; conversely, impairing WNT signaling blocks cushion formation [89]. BMP and TGF β signals induce EMT and promote cushion mesenchyme to proliferate [11]. Cushion mesenchyme also locally represses production of VEGF to permit EMT [90]. Intriguingly, both too much and too little VEGF cause similar cardiac phenotypes; diminishing VEGF signaling evokes increased cushion mesenchyme, whereas increased VEGF increases valve size [11]. Regulation of FGF and MAPK signaling, through expression of Scleraxis (SCX), are also important regulators of proper valvular development; loss of SCX leads to aberrant differentiation of valvular cell lineages and disrupted ECM organization [91]. Finally, our group recently demonstrated that the protein tyrosine phosphatase SHP2 is also an important regulator of valvular development through its modulation of the Ras-MAPK signaling cascade in embryonic cardiac endothelium; loss of SHP2 phosphatase activity disrupts AKT mediated Notch and FOXP1 signaling, leading to enlarged and amorphic valves, endocardial cushions, and valve leaflets [92].

Neural crest cells and migration

Neural crest cells (NCCs) migrate to the heart and facilitate remodeling, particularly septation of the developing OFT [93, 94]. Dysregulated gene expression in cardiac NCCs causes OFT and aortic arch defects in the developing heart. For example, disruption of Hippo-Notch signaling results in impaired smooth muscle differentiation [93].

Epicardium

The epicardium is a continuous sheath of cells derived from mesoderm that covers the entire heart and is essential for growth of the compact myocardium [95]. Epicardium secretes IGF2, which activates IGF1R, and subsequently ERK, in cardiomyocytes to induce their proliferation [96, 97]. In mice, the pro-epicardium appears at the venous pole at E9.5. These cells expand and ultimately form a single cell layer of epicardium. Like pre-cardiac

mesoderm, FGF and canonical BMP signaling are major players in epicardial specification; however, FGF is thought to be the dominant signal that determines epicardial as opposed to myocardial fate [98]. Intriguingly, the epicardium contains valve progenitors, and participates, at least in part, in the development of atrioventricular valves [99]. In the adult heart, epicardial-derived cells modulate immune responses [100], through Hippo-YAP/TAZ signaling, to prevent fibrosis in myocardial tissue following cardiac injury [101]. (Figure 5)

Cardiac fibroblasts

During development, murine embryonic fibroblasts appear at E12.5 and increase in number throughout the myocardial proliferation process [102]. Cardiac fibroblasts promote cardiomyocyte proliferation through expression and activation of several factors, including transcriptional regulators FN1 and COL3a1, growth factors HB-EGF and β 1 integrin, as well as upregulation of ERK and PI3K/AKT signaling pathways [102]. Thus, embryonic cardiac fibroblasts synthesize specific ECM components and growth factors to promote myocardial proliferation. Activation of cardiac fibroblasts in adult myocardium typically occurs in response to stress, including hypertrophic cardiomyopathy (HCM) and/or other adult-onset cardiac diseases. (Figure 6)

Role of macrophages in cardiac development

Macrophages are well known inhabitants of most organs and are necessary to maintain homeostasis, repair, and immunity of the local tissue [103]. The heart, specifically, is populated by a large number of resident macrophages, and their function in response to postnatal pathogenic stimuli, such as inflammation or injury of the heart, has been the primary focus of several previous studies [104]. More recently, however, effort has been placed on identifying a potential function for these cells in neonatal, proliferating hearts; principally, it has been documented that macrophages play a critical role in cardiac regeneration here, likely by promoting angiogenesis [105]. Interestingly, we know little about what role macrophages play in cardiac development. Previously, it was suggested that macrophages were derived from Myb-dependent hematopoietic stem cells delineated from fetal liver and, later on in development, from bone marrow. More recently, however, a group of Myb-independent yolk sac derived macrophages was identified [106]. Subsequently, these cells, identified as early as E8.5 in mice, get recruited to the developing heart by WT1-dependent epicardial signals [100]. At around E13.5, these now classified chemokine (C-C motif) receptor type 2 (CCR2)-negative primitive yolk sac-derived Myb-independent macrophages, but not the CCR2-positive macrophages derived from Myb-dependent fetal liver, are required to exclusively regulate coronary blood vessel patterning in the heart through IGF-dependent signals [107], suggesting that the function of specific subsets of macrophages during cardiogenesis is likely determined by ontogeny. Together, these studies suggest macrophages are integral for the highly coordinated events that occur in the developing heart.

Postnatal Cardiac Growth

Shortly after birth, cardiomyocytes exit the cell cycle and become terminally differentiated, polyploid cells (a single polyploid nucleus in humans or two diploid nuclei in rodents) [26]. They now have a need to become highly specialized for contraction, shifting to oxidative phosphorylation and developing accompanying ultrastructural specializations and changes in gene expression that enable efficient and coordinated cardiomyocyte contraction [26]. Interestingly, the same signaling pathways, transcription factors, and miRs that play a role in the developing heart also modulate postnatal maturation, mediating adult cardiac function and immuno-reactive activities [101, 108]. However, the function of these networks in adult heart is uniquely different from that in development. For example, GATA4, one of central regulators of cardiomyocyte differentiation, significantly alters its chromatin occupancy between fetal and adult stages to activate or suppress different subsets of genes [40]. In addition, re-expression of fetal genes (MHC, ANF, BNP, SERCA2a, etc) is driven by pathological or stress conditions in the adult heart, potentiating onset of myocardial disease, hypertrophy, and heart failure [109].

Several factors are involved in adult cardiomyocyte cell cycle exit. First, mitogenic signaling pathways that drive fetal cardiomyocyte proliferation are attenuated after birth [110]. For example, expression of ERBB2 quickly decreases in cardiomyocytes after birth, reducing the proliferative potency of NRG1 [111]. Second, expression and function of cell cycle machinery is actively inhibited in adult cardiomyocytes. Transcriptional regulators, epigenetic modifiers and miRs actively repress core cell-cycle activators and/or activate cell-cycle inhibitors [26, 112]. Third, activation of targeted signaling pathways, such as p38-MAPK, also inhibits cardiomyocyte proliferation [113, 114]. Finally, increased oxidative stress in postmitotic cardiomyocytes causes DNA damage response-mediated cell-cycle arrest [115].

Congenital Heart Disorders

Congenital heart disorders (CHDs) are the most common type of birth defect, with ~1/100 live births, and are the major cause of birth-related deaths [116]. Moreover, because of major advances in medical and surgical procedures, there are now more adults living with CHD than children [117]. CHD phenotypes range from mild atrial septal defects to severe LV outflow obstructions that are directly attributed to genetic abnormalities. Among numerous CHD phenotypes, abnormal valves, septation defects, and cardiomyopathies are presented in a majority of patients. While recent studies have established a causal relationship between genetic defects to cardiac abnormalities, the underlying molecular mechanisms remain unclear [4]. The aggregate of genetic contributions to CHD are likely to not only underlie structural CHDs, but also contribute to CHD comorbidities as well, including heart failure, arrhythmia, neurocognitive outcomes, and even cancer [117].

Mendelian and inherited forms of CHD have recently been identified using linkage analyses, positional cloning and targeted sequencing of CHD candidate genes [118]. Indeed, dysregulation of multiple integral developmental components lead to CHDs, including

aneuploidy, copy number variation, inherited or de novo mutations, dysregulation of transcription factors, and disruption of signaling pathways [117].

Mutations and/or alterations in cardiac transcription factors, including NKX2.5, GATA, TBX and MEF2, have also been implicated in CHD [119–121]. For example, individuals with isolated ASDs, as well as individuals with ASDs along with abnormalities of the conduction system, were identified to have NKX2.5 mutations that were causal to both these defects [23]. In addition, GATA4 mutations have been implicated in at least two families with CHD with cardiac septal defects [122]. Indeed, the GATA4 Del8p23 mutation manifests with a range of CHDs, along with developmental delay [123]. Mutations in TBX5 are likewise implicated in two families with Holt–Oram Syndrome, a disease characterized by upper limb malformations and cardiac septation and conduction defects [124, 125]. Evidence of causality is also demonstrated in heterozygous TBX5 null mice, which have limb abnormalities, septal defects, deformed hearts, and other complex cardiac malformations [33, 126]. Another example, Del22q11 (DiGeorge Syndrome), is caused by haploinsufficiency in TBX1 [127].

The genetics underlying CHD have identified critical biological pathways involved in CHD, including chromatin remodeling, Notch signaling, cilia function, sarcomere structure and function, and RAS-MAPK signaling [117, 128]. These pathways provide insights into the mechanisms of heart development, as well as identify potential CHD comorbidities, such as the ventricular dysfunction phenotype observed in patients with sarcomeric and RAS-MAPK pathway mutations. Another example is Notch signaling; NOTCH1 mutations, together with its downstream pathway effectors, have been demonstrated in multiple CHD pedigrees [129–132].

New technologies and potential therapeutic approaches

Over the last decade, technological approaches and analytical tools for next-generation sequencing has provided a greater opportunity for us to understand the genetics of complex cardiac diseases. In particular, whole exome sequencing (WES) has allowed identification of mutations that were undefinable through traditional genomic methods, such as *de novo* variation, variants without clear Mendelian inheritance patterns, variants with marked reduced penetrance, and somatic alterations, among others [26, 133].

In addition, the establishment of inducible pluripotent stem cells (iPSCs) has made it possible to study various disease mutations, now “in a dish.” Here, human somatic cells (blood or skin) can be dedifferentiated and then reprogrammed to generate specified cardiac lineages using a panoply of growth factors to study genetic mutations, developmental differentiation processes, onset of heart disease, cardiac tissue regeneration and even replacement therapy for heart failure patients. We now have the capacity to generate iPSC-derived cardiomyocytes, endothelial cells, cardio-fibroblasts, and smooth muscle cells, all with high efficiency, to determine functional, mechanistic and phenotypic properties of various genetic, developmental, and disease properties [134]. Moreover, there is significant growing interest in developing and understanding the unique roles of various cardiomyocyte

subtypes (eg, atrial, ventricular, and nodal), in particular for consideration of therapeutic purposes.

As well, there are limitations to consider when using iPSCs and other technologies. For example, we are limited in understanding mechanisms driving cardiomyocyte maturation [135]; iPSC-derived CMs do not yet become fully differentiated adult cardiomyocytes. Moreover, use of only one cell lineage *in vitro* to understand the role of the entire functional heart *in vivo* is indeed limiting (and a bit concerning). In this regard, approaches have been considered to more closely match the *in vivo* microenvironment, including use of the “heart-on-a-chip” technology, administration of a 3-dimensional (3D) tissue engineering process, mechanical loading procedures, modulation of substrate stiffness on the heart, and electric stimulation of the tissue [26]. In addition, induction of signaling pathways, hormonal supplements, and longer-term differentiation processes in culture have also been considered in understanding the differentiation process of cardiomyocytes in particular. In addition, it may be feasible to combine iPSC technology with three-dimensional printed scaffolds to generate cardiac muscle patches that can be used to treat cardiac disease and/or be used for regeneration of diseased heart tissue [136]. Finally, current advances in genome editing may soon make it feasible to correct genetic anomalies, providing an additional therapeutic opportunity to actually cure CHDs and heart disease in the very near future. [137]. Further studies will be necessary to reproduce, validate, and further advance findings from all these studies.

Conclusions and Future Perspectives

In the past few decades, cardiovascular research efforts have been instrumental in uncovering the role that signaling pathways, transcriptional factors, and genetics play in the cardiomyogenic process. Consequently, we have a better understanding of the regulatory networks needed to drive differentiation and proliferation of various cell lineages involved in cardiac development [26]. Indeed, during the course of writing this review, a new method for time-induced tailored embryonic repair of an autosomal dominant *MYBPC3* mutation that causes hypertrophic cardiomyopathy was developed, using CRISPR-Cas9 technology that targeted metaphase II in the oocyte cell cycle [138]. Compared to previous studies using genome editing in early human embryos, this technique offers significantly increased genomic correction efficiency, with minimal mosaicism, undetectable off-target mutations, and no signs of early developmental defects [138]. Although undesired random DNA repair still occurred at considerable frequency using this new methodology, it remains promising that genome editing-based therapeutic approaches can be used to treat and/or cure congenital heart diseases in the near future. Clearly, this needs to be carefully addressed in subsequent studies before proceeding to clinical applications. In summary, the knowledge we have gained in recent years will ultimately enable us to continue to identify new methods and technologies that will advance our understanding of complex genetic and pathological mechanisms, allow for more accurate diagnoses, increase therapeutic precision, and cure many of the cardiac diseases not possible to treat today.

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Gli1 and Ptch1 expression are downregulated, and Foxf1a and Foxf2 are the direct downstream targets of SHH signaling, all necessary actions that mediate atrioventricular septation. This work further demonstrates that Foxf1a is regulated by joint Tbx5 and Hedgehog activity via a cis-regulatory element. While the phenotypic changes of Foxf1a and Foxf2 double heterozygotes are similar to those of a previously described Tbx5-Hedgehog deficiency model, this study expands on the understanding of Tbx5-Hedgehog mediated genetic axis and determines its critical role in atrioventricular septal development. Using this method could facilitate discovery of new risk factors for atrioventricular septal (AVS) defects. [PubMed: 25356765]

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targets, and the molecular functions of HOPX can be critical for elucidating the signaling axis mediating specification of early myocardial progenitor cells. HOPX, a factor that plays a critical role in converting other cardiac lineages into cardiomyocytes, may therefore be considered in cardiac regeneration therapies. [PubMed: 26113728]

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109:2394–9. The Hippo pathway has been well characterized for its functions in organ size determination during embryogenesis. YAP1 is the downstream target of Hippo signaling. In this study, the role of YAP1 in myocardium was carefully dissected at both the fetal and postnatal stages. During embryogenesis, ablation of YAP1 caused marked, lethal myocardial hypoplasia and decreased cardiomyocyte proliferation. In contrast, fetal activation of YAP1 stimulated cardiomyocyte proliferation. Interestingly, at the postnatal stage, YAP1 gain-of-function mutations were able to induce cardiomyocyte proliferation, but the alteration of YAP1 activity did not seem to affect cell size. The authors found YAP1 binding of TEAD transcription factors is required to induce expression of cell-cycle genes that enhance proliferation in the myocardium. Together, these results suggest that YAP1-TEAD may be a good candidate for future myocardial regeneration strategies. [PubMed: 22308401]

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- 82*. Anstine LJ, Bobba C, Ghadiali S, Lincoln J. Growth and maturation of heart valves leads to changes in endothelial cell distribution, impaired function, decreased metabolism and reduced cell proliferation. *J Mol Cell Cardiol*. 2016; 100:72–82. The authors identified that cardiac valvular endothelium undergoes regressive changes in morphology, metabolism, proliferation and physiological properties of the heart as a consequence of aging. This observation identifies the critical importance and impact that the age of the valvular structures have on development of disease, a huge cost and social burden worldwide for this, as well as many other disease etiologies. Future genetic studies of valvular endothelium in animal model systems would lead to better understanding of the molecular mechanisms and potential therapeutic approaches that could be taken to offset these effects. As well, the signaling and genetic markers associated with these pathologies need to be identified to combat the effects of aging and diseased heart. [PubMed: 27756541]
- 83*. Yzaguirre AD, Padmanabhan A, de Groh ED, Engleka KA, Li J, Speck NA, Epstein JA. Loss of neurofibromin Ras-GAP activity enhances the formation of cardiac blood islands in murine embryos. *Elife*. 2015; 4:e07780. Normally, NF1 activity is required for suppression of the overgrowth of neural crest-derived tissues and for the prevention of abnormalities in the developing outflow tract. In this study, authors showed that loss of the GAP function of NF1 leads to ectopic formation of blood islands and to malfunctioning endothelium. Functionally, the mechanisms for the loss of GAP activity was caused by an elevated downstream ERK-MAPK activity. In the future, Ras/ERK-MAPK signaling needs to be carefully evaluated as a determinant of erythroid/myeloid cell fate, as well as an integral signaling pathway needed for the proper development of functional endocardium. [PubMed: 26460546]
- 84*. Aghajanian H, Cho YK, Manderfield LJ, Herling MR, Gupta M, Ho VC, Li L, Degenhardt K, Aharonov A, Tzahor E, Epstein JA. Coronary vasculature patterning requires a novel endothelial ErbB2 holoreceptor. *Nat Commun*. 2016; 7:12038. The authors identified a novel role for ERBB2 in the coronary endothelial cells in heart development. The disruption of ERBB2-NRP1-Sema3d complex in the endocardium resulted in abnormal connection of the coronary veins to the right atrium and to impaired AKT signaling. This novel role of ERBB2 signaling may lead to further investigation for its possible mechanisms in controlling other endocardial tissue functions, both during cardiac development and in adult heart. In addition, ERBB2 is a common mutation and target for cancer therapies. This research suggests the reasons why, at least in part, ERBB2 inhibition may result in cardiac toxicity during cancer treatment. [PubMed: 27356767]

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- 92*. Lauriol J, Cabrera JR, Roy A, Keith K, Hough SM, Damilano F, Wang B, Segarra GC, Flessa ME, Miller LE, Das S, Bronson R, Lee KH, Kontaridis MI. Developmental SHP2 dysfunction underlies cardiac hypertrophy in Noonan syndrome with multiple lentigines. *J Clin Invest*. 2016; 126:2989–3005. The majority of Noonan syndrome with multiple lentigines (NSML) patients have characteristic congenital heart disease (CHDs), particularly hypertrophic cardiomyopathy (HCM). It was unclear which cardiac lineage and signaling pathway was responsible for the myocardial hypertrophic phenotype until this work. Moreover, it was unclear whether the HCM phenotype was primary during development or secondary, as a consequence of added pressure overload in the adult. The authors here exerted tissue specific Cre alleles to investigate the effect of SHP2 loss of function mutation in cardiomyocytes, cardiac endothelium, or neural crest cells (NCC). Interestingly, while the cardiomyocyte and NCC specific mutants of SHP2 fail to replicate the myocardial defect in conventional NSML heart, the endothelial NSML mutant was able to recapitulate nearly all of the ubiquitous-expressing NSML cardiac abnormalities. These data indicate that signaling crosstalk between myocardium and endocardium is the key regulator of myocardial differentiation and proliferation in cardiac embryogenesis. Consequently, dysfunction of the crosstalk events during development leads to onset HCM in the adult. Although the underlying mechanism, particularly for trabecular differentiation, needs to be further evaluated, this model well illustrates the complexity of cardiac signaling regulation. This knowledge could promote further understanding of the role of paracrine signaling from endocardium to myocardium needed to facilitate developmental events, as well as to maintain cardiac homeostasis in the adult heart. [PubMed: 27348588]
- 93*. Phillips HM, Mahendran P, Singh E, Anderson RH, Chaudhry B, Henderson DJ. Neural crest cells are required for correct positioning of the developing outflow cushions and pattern the arterial valve leaflets. *Cardiovasc Res*. 2013; 99:452–60. Bicuspid aortic valve (BAV) is a very common type of congenital heart defect, but the regulatory mechanisms underlying its development remain poorly understood. Interestingly, endocardium had been previously thought to contribute to most of the embryonic valvular structures during the developmental process. Here, however, the authors provide evidence to suggest that neural crest cells (NCCs) also are required in early OFT valve formation and regulation. NCCs are known to give rise to multiple cell lineages, integral to the development of the outflow tract (OFT) and for OFT septation. In this study, authors found that the NCCs were critical for proper positioning of the outflow tract cushions, the precursor cells that differentiate to later OFT valvular structures, through regulation of Rho GTPases and ROCK signaling. Further dissection of the ROCK signaling cascade is needed to screen for genetic risk factors that may be causal to valvular disease and/or to identify novel mechanisms or markers that could improve cardiac function in these BAV patients. [PubMed: 23723064]
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Development. 2015; 142:2962–71. Interestingly, in development, Hippo signaling is known to be involved in the regulation of organ size; little, however, was known about whether it may have additional functions and/or regulate cell fate during this process. Here, authors identified that Hippo-Notch signaling functions to regulate smooth muscle differentiation through a Yap-NICD-Rbp-J transcriptional complex in neural crest-derived cardiac tissues. Disruption of Hippo-Notch resulted in impaired smooth muscle differentiation, as well as to disrupted molecular marker expression in the aortic arch artery during embryogenesis. Taken together, these data suggest that the role of Hippo signaling extends far beyond just organ size, and that the pathway regulation of Yap needs to be further evaluated for its potential role in cardiac differentiation and/or cell fate decisions. [PubMed: 26253400]

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- 97*. Huang Y, Harrison MR, Osorio A, Kim J, Baugh A, Duan C, Sucov HM, Lien CL. IGF Signaling is Required for Cardiomyocyte Proliferation during Zebrafish Heart Development and Regeneration. *PLoS One*. 2013; 8:e67266. In contrast to mammalian hearts, cardiomyocytes in adult zebrafish undergo robust proliferation to compensate for the loss of myocardial cells following cardiac injury. However, the paracrine signaling mechanisms underlying this regeneration capacity is poorly understood. The authors showed that IGF2 expression, which is required for myocardial proliferation during normal development, is also upregulated in response to cardiac injury and mediates proliferation of adult cardiomyocytes in zebrafish. In contrast, however, it is likely that the transduction of IGF signaling may be more limited in the adult mammalian heart; while, like zebrafish, it is required in embryogenesis, the metabolic, structural and genetic differences between fish and mammals suggest its role in adult heart may be to induce hypertrophy, not proliferation. Further genetic and pharmacological studies on mouse models are needed to further elucidate the role of IGF signaling in mammalian myocardial regeneration. [PubMed: 23840646]
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- 99*. Wessels A, van den Hoff MJ, Adamo RF, Phelps AL, Lockhart MM, Sauls K, Briggs LE, Norris RA, van Wijk B, Perez-Pomares JM, Dettman RW, Burch JB. Epicardially derived fibroblasts preferentially contribute to the parietal leaflets of the atrioventricular valves in the murine heart. *Dev Biol*. 2012; 366:111–24. Epicardium and epicardial derived cells (EPDCs) are known to give rise to multiple cardiac lineages, particularly cardiac fibroblasts during cardiogenesis. In this study, the authors carefully conducted lineage tracing experiments to identify EPDC-derived cells. They found that while EPDCs contribute to a majority of the embryonic cardiac fibroblasts, these fibroblasts do not populate the trabecular myocardial tissue until E17. Moreover, the authors asked whether EPDCs contribute to the valvular fibroblast (or valve interstitial cell) population. In contrast to what was previously reported, they found that a considerable number of

valvular fibroblasts, particularly in lateral atrioventricular cushions, were in fact epicardially derived. While the mechanism regulating the migration pattern of epicardial-derived fibroblasts in the myocardium remains to be determined and additional methods need to be assessed to confirm the EPDC contribution to valvular fibroblasts, this study provides interesting and important findings regarding the contribution of this lineage to the leaflet mesenchyme of the cardiac valve. Indeed, these cells may have very different origins with distinct signaling networks critical for normal cardiac valve development. [PubMed: 22546693]

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- 107*. Leid J, Carrelha J, Boukarabila H, Epelman S, Jacobsen SE, Lavine KJ. Primitive Embryonic Macrophages are Required for Coronary Development and Maturation. *Circ Res.* 2016; 118:1498–511. The role of macrophages has been extensively studied in adult tissues. However, the ontogeny and function of macrophages in the developing heart remain largely unknown. In this study, the authors identified two groups of macrophages, CCR2– primitive yolk sac derived macrophages and CCR2+ fetal liver monocyte derived macrophages, both of which are required to populate the embryonic heart. They found that only CCR2– macrophages are required for normal coronary blood vessel formation, through specific regulation of IGF signaling. While the authors have yet to explore the role of embryonic CCR2+ macrophages in the development of trabeculation and endocardium, this work provides valuable insight into yolk sac macrophage development and its role in establishing coronary structures in the fetal heart. Together with other recent studies, this work suggests that the function of specific subsets of macrophages is determined by ontogeny, particularly during cardiogenesis. [PubMed: 27009605]

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- 110**. Lin Z, Guo H, Cao Y, Zohrabian S, Zhou P, Ma Q, VanDusen N, Guo Y, Zhang J, Stevens SM, Liang F, Quan Q, van Gorp PR, Li A, Dos Remedios C, He A, Bezzerides VJ, Pu WT. Acetylation of VGLL4 Regulates Hippo-YAP Signaling and Postnatal Cardiac Growth. *Dev Cell.* 2016; 39:466–79. During development, Hippo signaling mediates the regulation of organ size. Recently Hippo-YAP signaling was also shown to be involved in the proliferation of the fetal heart. Here, the authors found that acetylation of VGLL4 serves as a switch to activate Hippo-YAP signaling through TEAD. During heart maturation, however, the level of VGLL4 acetylation is downregulated, resulting in a more modest proliferative capacity of adult cardiomyocytes. This work focuses on the newly discovered function of Hippo-YAP pathway and unveils its upstream regulator. In the future, the regulators and cofactors of Hippo signaling are worthy of being investigated. This knowledge will give us insight into the cardiac maturation process, which is currently poorly understood, as well as identify potential mechanisms that allow adult myocardium to continue dividing, particularly following stress and/or injury. [PubMed: 27720608]
- 111*. D'Uva G, Aharonov A, Lauriola M, Kain D, Yahalom-Ronen Y, Carvalho S, Weisinger K, Bassat E, Rajchman D, Yifa O, Lysenko M, Konfino T, Hegesh J, Brenner O, Neeman M, Yarden Y, Leor J, Sarig R, Harvey RP, Tzahor E. ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol.* 2015; 17:627–38. In postnatal mammalian heart, the proliferation capacity of cardiomyocytes is gradually shut down, terminally differentiating cardiomyocytes and posing a major issue for cardiac repair after injury. In this study, the oncogene ERBB2 was found to be involved in the regulation of postnatal myocardial proliferation through NRG1 signaling. The authors showed that in mice, a loss-of-function model of myocardial ERBB2 leads to reduced proliferation and dysfunction in the heart, whereas constitutively active ERBB2 signaling causes enlarged cell size, dedifferentiation, and significantly increased proliferation of cardiomyocytes, even in the adult heart after cardiac injury. The therapeutic purposes for this finding remains unclear; activation of ERBB2 in adults is still considered a risk factor for cardiomegaly and for development of cancer. However, this data may identify possible cell-cycle control mechanisms in the postnatal myocardium, and may therefore help identify possible approaches for treatment of cardiac injury. [PubMed: 25848746]
- 112*. Aix E, Gutierrez-Gutierrez O, Sanchez-Ferrer C, Aguado T, Flores I. Postnatal telomere dysfunction induces cardiomyocyte cell-cycle arrest through p21 activation. *J Cell Biol.* 2016; 213:571–83. Heart disease remains the top cause of death worldwide, largely due to the very limited proliferation capacity of adult cardiomyocytes in response to cardiac injury. To explore the mechanism of the postnatal transition between proliferation and cell-cycle arrest, the authors here investigated the role of telomere shortening in response to cellular events. They identify that telomere dysfunction/shortening results in premature cardiomyocyte cell-cycle arrest. Moreover, they find that the upregulation of the cell-cycle inhibitor and tumor suppressor p21 is likely responsible for these effects. These findings are validated in Terc and p21 knock-out mice. It is interesting that disruption of telomerase does not appear to have any significant effect on cardiac phenotype in embryogenesis, suggesting that other, perhaps additional or parallel mechanisms may be involved in maintaining the rapid cell proliferation of these cells during development. This work give us insight into the molecular mechanisms that regulate cell-cycle control during cardiac maturation, a process which is still poorly understood in the heart. Moreover, these data

provide us with targets to consider for potential regenerative therapies, for improving recovery of patients following cardiac injury. [PubMed: 27241915]

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- 118*. McKean DM, Homsy J, Wakimoto H, Patel N, Gorham J, DePalma SR, Ware JS, Zaidi S, Ma W, Patel N, Lifton RP, Chung WK, Kim R, Shen Y, Brueckner M, Goldmuntz E, Sharp AJ, Seidman CE, Gelb BD, Seidman JG. Loss of RNA expression and allele-specific expression associated with congenital heart disease. *Nat Commun.* 2016; 7:12824. The authors utilized next generation RNA sequencing analyses of tissues from congenital heart diseases (CHD) to identify candidates of pathogenic genetic mutations. While molecular mechanisms have yet to be determined and validated in animal models, this pioneer research provides us with potentially important gene candidates that can be used to confirm association in CHDs. Currently, whole genomic sequencing, which has become cheaper and easier to use in research, is used to identify additional mutations and regulatory elements involved in the development of CHDs. This approach is instrumental in the discovery of novel genes, which in time, can be used to identify targets and test therapeutics for the treatment of CHDs. [PubMed: 27670201]
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- 131*. Preuss C, Capredon M, Wunnemann F, Chetaille P, Prince A, Godard B, Leclerc S, Sobreira N, Ling H, Awadalla P, Thibeault M, Khairy P, M.L. consortium. Samuels ME, Andelfinger G. Family Based Whole Exome Sequencing Reveals the Multifaceted Role of Notch Signaling in Congenital Heart Disease. *PLoS Genet*. 2016; 12:e1006335. The role of Notch signaling pathway in heart development and CHD is well established in mouse models. However, the mutations that disrupt Notch signaling in many left-ventricular outflow tract obstructions (LVOTO) have not been fully evaluated. The authors utilized whole exome sequencing on families with LVOTOs, and discovered a caustic link between a group of rare mutations in conserved members of Notch signaling and congenital heart defect patients. While this study is limited by the requirement for large sample sizes and the difficulties associated with filtering environmental factors, it provides valuable insights that can help future understanding of critical biological pathways and genetic causes involved in these and many other types of CHDs. [PubMed: 27760138]
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- 134**. Zhou H, Dickson ME, Kim MS, Bassel-Duby R, Olson EN. Akt1/protein kinase B enhances transcriptional reprogramming of fibroblasts to functional cardiomyocytes. *Proc Natl Acad Sci U S A*. 2015; 112:11864–9. The evolutionary conserved transcription factors GATA4, HAND2, MEF2, and TBX5 (GHMT) have been shown to be key players involved in cardiomyocyte differentiation. In this work, the authors found that activation of AKT significantly increases the efficiency of reprogramming of fibroblasts into cardiomyocytes through presence of GHMT. While physiological studies should be conducted to further strengthen this discovery, the authors demonstrate that fibroblasts can be a source for new cardiomyocytes and that they could be used for therapeutics. Importantly, the mechanism that underlies this process in fibroblasts appears to be AKT signaling; AKT facilitates the fibroblast-derived cardiomyocytes to develop binuclear or multinuclear phenotypes, indicating AKT signaling is integral to the cardiomyocyte maturation process. [PubMed: 26354121]
- 135*. Guo Y, VanDusen NJ, Zhang L, Gu W, Sethi I, Guatimosim S, Ma Q, Jardin BD, Ai Y, Zhang D, Chen B, Guo A, Yuan GC, Song LS, Pu WT. Analysis of Cardiac Myocyte Maturation Using CASA AV, A Platform for Rapid Dissection of Cardiac Myocyte Gene Function In Vivo. *Circ Res*. 2017 Researchers are facing numerous challenges in studying cardiomyocyte maturation, including not yet knowing all the signaling and transcription factors involved, as well as not yet having all the technical expertise needed to properly address the questions at hand. Here, the authors utilized a novel CRISPR/Cas9-AAV9-based somatic mutagenesis (CASA AV) method to identify a new candidate gene RYR2 that is critical for T-tubule maturation. According to the presented data, CASA AV is a powerful tool that can be used to dissect postnatal heart maturation. Interestingly, using this method, mosaic knockout of Jph2, which is considered to be important in postnatal cardiac maturation, shows no major cell-autonomous role in T-tubule maturation, indicating that the CASA AV mosaic model is valuable in ruling out organ level secondary effects in this process. Indeed, this technology may be a feasible method to use in re-evaluating several of the genes previously thought to be critical in the heart maturation process.
- 136**. Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, Da Sie Y, Tran Q, Ajeti V, Freeman BT, Fast VG, Campagnola PJ, Ogle BM, Zhang J. Myocardial Tissue Engineering With Cells Derived From Human-Induced Pluripotent Stem Cells and a Native-Like, High-Resolution, 3-Dimensionally Printed Scaffold. *Circ Res*. 2017; 120:1318–25. Traditional 3-dimensional (3D) printing techniques are not capable of building structures sufficient to harbor a fully functional and contractile cardiac tissue. The authors here utilized newly developed multiphoton-excited 3D printing technology to construct a cardiac extracellular matrix-like scaffold, with higher resolution to facilitate the integration of seeded cardiac cells. Combined with induced pluripotent stem cell (iPSC) technology, they were able to produce functional cardiac patches that contain iPSC derived cardiomyocytes, smooth muscle cells, and endothelial cells. They were able to further test these in context of cardiac injury mouse models to unveil their efficacy and potency in

prevention of cardiac tissue damage. While the survival rate of cardiac cells, particularly cardiomyocytes, is relatively low at 4 weeks after implantation, this cardiac patch model has great potential for future optimization and use in cardiac disease treatment. Indeed, it may be possible to obtain artificial cardiac tissue with higher cell viability and electromechanical properties. [PubMed: 28069694]

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Highlights

- heart consists of multiple cell lineages
- cellular crosstalk facilitates cardiac development, disease and regeneration
- growth factors, signaling pathways and transcription factors play a role in cardiac development
- review focuses on role of genetic mutations, signaling pathways and lineage-specific contributions to cardiovascular development, physiology, and disease

Figure 1. Schematic representation of the physiology, major vessels, and circulation of the heart
 The heart structures, major blood vessels, and directions of blood flow are implicated. The oxygenated (red) and deoxygenated (blue) blood exchanges occur through systemic and pulmonary circulations. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

Figure 2. Schematic representation of murine heart development

At E7.5, the cardiac mesodermal cells form the crescent like structure comprised of the first heart field (FHF) and the second heart field (SHF). The FHF cells fuse at the midline, forming the primitive heart tube that mostly contributes to left ventricle (LV) by E8.0. The lumen of this tubule structure is covered by a layer of endocardial cells which is required for the development of atrioventricular (AV) canal and outflow tract (OFT), the precursor cells that give rise to valvular structures. Meanwhile, the SHF cells migrate and integrate into the heart, giving rise to right ventricle (RV), parts of the left and right atrium (LA and RA), and the outflow tract (OFT). After the initiation of heart looping at E8.5, the epicardium expands from the venous pole and covers the entire embryonic heart by E11.5. The epicardial derivatives give rise to multiple cardiac cell lineages including fibroblasts and coronary vascular smooth muscle cells. In addition, cardiac neural crest cells (NCCs) migrate from the neural tube (NT), invade the OFT, mediating the development of endocardium derived cushion tissue and the septation of the myocardial wall. By E15.5, septation is completed, enclosing the ventricular chambers and generating the fully functional four-chambered heart.

Figure 3. Differentiation of the myocardial cell lineage during development

Schematic representation shows the genetic regulation in stagewise commitment of cardiac mesodermal cells. The inhibition of WNT/ β -catenin is required for the MESP1 positive cardiac mesoderm to undergo further specification. While the FHF and SHF cells share the expression of some of the same core transcription factors, the two lineages have differences in signaling effects and give rise to distinct myocardial cell types. AV, atrioventricular; SA, sinoatrial.

Figure 4. Schematic representation of cardiac valve development and the crosstalk between cardiac cell lineages

In response to myocardial signals, the endocardial cells undergo epithelial–mesenchymal transition (EMT), delaminating from the endocardial surface and differentiating into mesenchymal cells, which proliferate and invade the cardiac jelly to form the endocardial cushion in the atrioventricular (AV) canal and outflow tract (OFT). Signaling from endocardium thus regulates myocardial proliferation and specification, as well as pathological events that include hypertrophic cardiomyopathy (HCM). In later stages of valvular maturation, signaling from endocardium directs remodeling events that include apoptosis and differentiation of mesenchymal cells.

Figure 5. Schematic representation of signaling pathways regulating the differentiation of epicardium during cardiogenesis

TGF β and FGF are the predominant signaling pathways required to promote epithelial–mesenchymal transition (EMT). Mediated by TGF β , PDGF, and WNT/ β -catenin, epicardial derived cells (EDCs) undergo specification and differentiate into cardiac fibroblasts and vascular smooth muscle cells.

Figure 6. Distinct roles of cardiac fibroblasts between embryonic and adult stages

Embryonic cardiac fibroblasts promote cardiomyocyte proliferation through expression and activation of transcriptional regulators FN1 and COL3a1, growth factors HB-EGF and β 1 integrin, as well as upregulation of ERK and PI3K/AKT signaling pathways. Activation of cardiac fibroblasts in adult myocardium typically occurs in response to stress, including hypertrophic cardiomyopathy (HCM) and/or other adult-onset cardiac diseases.