Rebuilding a broken heart: lessons from developmental and regenerative biology

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ABSTRACT
In May 2016, the annual Weinstein Cardiovascular Development and Regeneration Conference was held in Durham, North Carolina, USA. The meeting assembled leading investigators, junior scientists and trainees from around the world to discuss developmental and regenerative biological approaches to understanding the etiology of congenital heart defects and the repair of diseased cardiac tissue. In this Meeting Review, we present several of the major themes that were discussed throughout the meeting and highlight the depth and range of research currently being performed to uncover the causes of human cardiac diseases and develop potential therapies.

KEY WORDS: Congenital heart defects, Heart development, Regeneration

Introduction
Congenital heart disease (CHD) is the most common form of structural birth defect observed in humans and represents a leading cause of infant mortality worldwide (van der Linde et al., 2011). For modern biologists studying the ontogeny and molecular genetics of CHDs, it is sometimes easy to forget how far research in this field has progressed in a relatively short period of time. Indeed, as recently as three decades ago, many of the fundamental processes regulating cardiovascular development and the manifestation of congenital heart malformations were not well understood.

The impetus for increased research into CHDs was initially spearheaded in the 1970s by pathologists and pediatric cardiologists. Anatomical descriptions of these disorders had existed for decades; however, those who confronted CHDs in the clinic realized that their underlying etiologies were largely unknown. This lack of understanding was the primary motivation behind a workshop organized by Dr Glenn Rosenquist titled ‘Morphogenesis and Malformation of the Cardiovascular System’, which was held in Arizona, USA in 1978 (Pexieder, 1978). This was also the focus of a subsequent meeting held in 1984 titled ‘Selective Topics in Cardiac Morphogenesis’ and hosted by Dr Constance (Connie) Weinstein of the National Heart, Lung, and Blood Institute (NHLBI) (Ferrans et al., 1985). The goal of these conferences was to initiate dialog between pathologists, pediatric clinicians and experimental embryologists and to discuss CHDs in the context of developmental biology.

Many of the attendees of these early meetings soon began to realize that basic sciences could play an important role in identifying the root causes of CHDs. Connie Weinstein, in particular, was instrumental in focusing the attention of the NIH on the need for increased funding for basic research related to CHDs. As a result, a series of funding opportunities was initiated in the mid 1980s that included, as part of their awarding system, an annual meeting. Bringing together the members of the supported laboratories allowed for frank discussions of results and emerging technologies, and fostered scientific collaboration. The first of these meetings was a simple gathering held in a basement conference room at the NIH in Bethesda, Maryland, in 1986. The meeting grew in size and influence over subsequent years as larger numbers of projects were funded and more researchers became aware of the collegial spirit of the conference. Following the expiration of these initial NHLBI funding programs in 1993, there was strong motivation among regular attendees to continue the meeting in an independent and extended format. The first of these independent meetings was organized by Professor Roger Markwald at the Medical University of South Carolina, USA, in 1994. The following year, during a meeting organized by Dr Ed Clark at the University of Rochester, USA, the conference was formally named the ‘Weinstein Cardiovascular Development Conference’ to honor Dr Connie Weinstein upon her retirement from the NIH.

The most recent Weinstein Conference, which was held in May 2016 in Durham, North Carolina, USA, and co-organized by Drs Kenneth Poss and Frank Conlon, brought together more than 350 investigators from around the world. In keeping with tradition, the training and development of junior scientists was a central priority of the conference, and nearly every presentation at the meeting was given by a student, postdoc or junior investigator. Rather than describing each talk in detail, this Meeting Review focuses on several of the overriding topics that spanned the entire conference.

Cardiac development
Cardiovascular development has long been a primary focus of Weinstein Conferences. The scope of the developmentally oriented talks at this year’s meeting was, as always, quite large, ranging from very eloquent mechanistic advances to the identification of novel cellular processes, and several themes were prominent across a variety of presentations.

A cell population of particular focus at this year’s meeting was the epicardium and its developmental rudiment, the proepicardium (PE). The PE is a multipotent, extra-cardiac progenitor population that gives rise to a variety of cell types in the heart. To date, much remains unknown about the processes that govern early epicardial/PE formation in the embryo. To this end, Panna Tandon (a member of Frank Conlon’s lab, University of North Carolina at Chapel Hill, USA) presented a system-based proteomics approach to identify key factors that influence PE development. This talk served as a strong proof-of-principle for an experimental pipeline combining
proteomics with the genetically tractable Xenopus model system to identify novel PE-specific cellular processes necessary for heart development. Looking at the events that control PE expansion and translocation to the myocardium, Wasay Mohiuddin Shaikh Qureshi (from Mingfu Wu’s lab, Albany Medical College, USA) presented work highlighting the role of Cdc42 in the primary outgrowth of the PE and spreading of the epicardium. Finally, Michael Dressan (University of North Carolina at Chapel Hill, USA) described a novel function of PE-derived cells in the morphogenetic patterning of the heart’s pacemaker complex, the sinoatrial node. This work demonstrated that PE-derived cells invade the developing pacemaker region over the course of cardiac development, and that PE ablation results in abnormal pacemaker cellular architecture with severe electrical dysfunction.

Although the PE was once considered the primary source of endothelial cells that comprise the coronary vasculature, growing evidence suggests that, in the mouse model, multiple sources can contribute, including the PE, the endothelium of the sinus venosus and the endocardial endothelium (Red-Horse et al., 2010; Katz et al., 2012; Wu et al., 2012). How these sources interact to form a functional cardiac circulatory loop is largely unknown, and several groups are actively investigating this topic. Kazuaki Maruyama (Tokyo University, Japan) presented data indicating that proper coronary vascular formation requires the initial cardiac vascular plexus to fuse with a population of aortic subepicardial vessels. Bikram Sharma (a member of Kristy Red-Horse’s lab, Stanford University, USA) further elaborated on this topic, demonstrating the plasticity of coronary vascular formation. He reported that the genetically induced disruption of one source of coronary vascular endothelial cells has only minor defects, as cells from a separate source can expand and compensate for their loss.

Primary atrial septal defects (ASD) are common in a variety of CHDs. However, the etiology of these developmental defects is not fully understood. A number of talks at this year’s Weinstein Conference highlighted significant progress in understanding the mechanisms that control atrial patterning and septation. For example, Linglin Xie (Texas A&M University, USA) reported the identification of an interactive gene regulatory network including Tbx5, Osr1 and Pesh6 that operates in the second heart field and contributes to the ontogeny of ASDs. Furthermore, Jeffrey Steimle (from Ivan Moskowitz’s lab, University of Chicago, USA) presented data suggesting that Tbx5 is involved in regulating mesoderm and endoderm communication in secondary heart field progenitors prior to atrial septation. Jeffer’s presentation further indicated that several factors, including sonic hedgehog, are involved in a complex system of tissue-tissue cross-talk that seems to be crucial for proper atrial septation and the formation of the entire cardio-pulmonary circuit. Continuing on the theme of Hedgehog signaling in the second heart field, Tara Burns (a member of Andrew Wessels’ lab, Medical University of South Carolina, USA) spoke about the disruption of Hedgehog signal reception through the genetic ablation of primary cilia. This resulted in a broad variety of morphogenetic malformations, including atrioventricular septal defects. Taking a more evolutionary approach, Sven Reischauer, working in collaboration with Didier Stanier (both at the Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany), presented findings suggesting that, even in the absence of a defined atrial septum, the single zebrafish atrium displays an inherent left-right asymmetry reminiscent of that seen in higher vertebrates. Furthermore, this study linked atrial asymmetry to spatially restricted gene expression patterns in the early precardiac mesoderm, suggesting that the molecular identity of the left versus right atrium might have preceded the evolutionary adaption of an atrial septum. Collectively, these compelling presentations highlighted an emerging model of atrial septation in which molecular events that, in many cases, occur well before the formation of a physical septum are crucial for functional division right and left atria.

**Cardiac regeneration**

The myocardium of the adult mammalian heart was once believed to be incapable of proliferative repair post injury. However, it is now becoming clear that adult mammalian hearts do have a limited capacity to regenerate, and that this capacity can be amplified experimentally. In addition, the ability of urodele amphibians, teleosts and neonatal mouse hearts to regenerate following significant ventricular resection (Oberpriller and Oberpriller, 1974; Posse et al., 2002; Porrello et al., 2011) has inspired a number of laboratories to examine the molecular mechanisms of myocardial regeneration, with the aim of leveraging these mechanisms to develop novel therapeutics that can be used to repair heart damage caused by ischemic heart disease.

A major barrier towards these goals has been the fact that, in most vertebrates including mammals, terminally differentiated cardiomyocytes display extremely low rates of proliferation. However, several groups at this year’s Weinstein Conference presented mechanistic insights into the genetic and cellular events that functionally limit myocardial division. Honghui Lui (from Bernard Kuhn’s lab, Children’s Hospital of Pittsburgh, USA) described how postnatal cardiomyocytes lose competency to produce a successful cleavage furrow. He discussed how failure of full cleavage results in binucleation rather than full cytokinesis and appears to inhibit the ability of mature cardiomyocytes to undergo complete cell division. Similarly, Michaela Patterson (from Henry Sucov’s lab, University of Southern California, USA) presented a systematic genome-wide association study that aimed to identify gene loci associated with increased percentages of embryonic-like mononucleated diploid cardiomyocytes (MNDCMs) in the adult heart. Following the logic that animals harboring more MNDCMs may have a greater capacity for full myocyte cell division, she was able to identify a genetic locus associated with an increased ratio of MNDCMs. Certain SNPs within this locus correlate with higher myocardial DNA synthesis (presumably related to cell division) and improved metrics of cardiac function post injury. Work is ongoing to understand how genes in the region contribute to cardiomyocyte renewal and variability of response and recovery after adult cardiac injury.

Myocardial infarction is a major cause of cardiac muscle necrosis and heart failure in humans. Therapeutic approaches that could potentially regenerate or replace damaged cardiac muscle are therefore a major area of investigation. John Leach (from James Martin’s lab, Baylor College of Medicine and the Texas Heart Institute, USA) described how manipulation of the Hippo signaling pathway may provide a tractable system for cardiac muscle repair in a model of ischemic heart failure. His presentation suggested that altering the Hippo pathway substantially increases the functional recovery of cardiac muscle following ischemic damage.

Investigating further mechanisms of cardiomyocyte self-renewal and repair, Yi-Li Min (from Eric Olson’s lab, University of Texas Southwestern Medical Center, USA) examined the role that the transcription factors Twist1 and Twist2 play in the adult mouse heart. Although Twist2 is primarily expressed in epicardial and interstitial cells, genetic fate mapping experiments demonstrated that Twist2-expressing cells are capable of contributing to
cardiomyocytes through cell fusion. The deletion of both Twist1 and Twist2 results in poor cardiac function and cardiomyopathy, suggesting that Twist-expressing cells might contribute to the long-term renewal and maintenance of adult heart tissue. Following a similar line of investigation in zebrafish, Ashley Smith (from Voot Yin’s lab, MBI Biological Laboratory, USA) examined the mechanisms by which non-muscle, epicardial-derived cells contribute to cardiac repair. Her presentation specifically focused on how post-transcriptional modifiers influence the migratory capacity and localization of epicardial-derived cells following injury. Jingli Cao (a member of Kenneth Poss’ lab, Duke University School of Medicine, USA) also explored the cell biology of epicardial cell-mediated regeneration. His presentation included interesting data indicating that cells spatiotemporally go through different cell cycle behaviors during regeneration. These findings reveal previously unknown and largely unexpected behaviors in regenerating epicardial cells, and identify a potentially novel paradigm for both epicardial-mediated repair and development.

Overall, these presentations focusing on cardiac regeneration underscored the rapid progress being made in this field, with many talks highlighting new mechanisms that regulate cardiomyocyte proliferation, specifically in the context of cardiac injury. Not surprisingly, many parallels between regeneration and development were also touched upon, emphasizing how studies of heart development and regeneration can complement one another as we work towards the ultimate goal of treating human heart diseases.

Cardiovascular disease

Many talks at this year’s Weinstein Conference focused on modeling human disease via state-of-the-art genome editing or the use of patient-specific induced pluripotent stem cells (iPSCs). These experimental systems are powerful tools that can be used to better understand the molecular mechanisms involved in cardiac disease formation and progression.

Yen-Sin Ang (from Deepak Srivastava’s lab, Gladstone Institutes, USA) generated patient-specific iPSCs that were subsequently differentiated into cardiomyocytes in order to examine the ontology of this individual’s cardiac dysfunction. The cardiomyopathy-causing mutation in this patient was found to occur in a gene encoding a key cardiac transcription factor. It was shown that, under normal circumstances, interactions with additional cardiac transcriptional regulators guide the correct chromosomal localization of this transcription factor, whereas the mutation prevents assembly of the proper transcriptional complex, resulting in ectopic enhancer recruitment and gene expression defects.

Cardiomyopathy in patients with Duchenne’s muscular dystrophy (DMD) was modeled by Alex Chang (from Helen Blau’s lab, Stanford University, USA) using DMD patient-specific iPSC-derived cardiomyocytes. In a provocative talk, Alex showed that telomere shortening occurs in cardiomyocytes in a non-proliferation-dependent manner. This, in turn, triggers mitochondrial dysfunction, aberrant calcium transients and elevated oxidative stress contributing to cardiomyopathy in DMD patients.

Studying a version of cardiomyopathy resulting from left ventricular non-compaction, Casey Gifford (a member of Deepak Srivastava’s group, Gladstone Institutes, USA) used genome editing to generate mice that harbor patient-specific mutations in two crucial cardiac regulatory genes. This study demonstrated that compound heterozygous mice exhibit developmental defects mimicking the human disease. In addition, by examining human iPSC-derived cardiomyocytes from patients harboring these mutations, Casey identified a network of genes that were dysregulated during cardiomyocyte differentiation, demonstrating the combinatorial contribution of both mutations to disease formation.

Leslie Kennedy (from Frank Conlon’s lab, University of North Carolina at Chapel Hill, USA) further elaborated on the multifactorial regulation of cardiac transcriptional networks, describing a novel interaction that affects the function of the transcription factor TBX20. The Tbx20 gene has been identified as a disease-related gene; it is mutated in patients with CHDs and cardiomyopathy (Posch et al., 2010; Liu et al.; Liu et al., 2008; Liu et al., 2014; Kirk et al., 2007; Chen et al., 2015; Qiao et al., 2012). Using a proteomics-based approach, Leslie identified the transcription factor Castor (CASZ1) as a binding partner for TBX20. Compound double-heterozygous mice with mutations in Tbx20 and Casz1 exhibit reduced ejection volume, enlarged cardiomyocytes and interstitial fibrosis, and these mice die postnatally due to severe cardiomyopathy.

In an effort to develop a model for the etiology of heart valve defects, Florian Wuenenmann (Gregor Andelfinger’s group, CHU Sainte-Justine Research Centre, Université de Montréal, Canada) described the identification of a rare homozygous deletion in the ADAMTS19 gene that was present in two siblings with multiple valve abnormalities. Adamts19 knockout mice phenocopy the clinical presentation of these patients, displaying aortic stenosis and regurgitation due to a partially fused bicuspid aortic valve. This study determined that ADAMTS19 is expressed in valvular interstitial cells and is important for late stage valve maturation. Similarly, Maiko Matsui (from Geoffrey Pitt’s lab, Duke University, USA) discussed a genome-wide association study that identified Cav1.2 (Cacna1c) as an aortic valve stenosis susceptibility gene. Their further studies using a mouse model that exhibits a gain-of-function mutation in Cav1.2 shed light on a potential mechanism for progressive aortic valve stenosis.

Cardiac arrhythmias are the leading cause of sudden death (Khairy, 2016; Priori et al., 2016; Saffitz and Corradi, 2016), and many talks focused on the molecular mechanisms underlying such arrhythmias. Examining a post-injury paradigm that occurs in many human tissues, Catherine Lipovsky (from Stacey Rentschler’s lab, Washington University in St Louis, USA) found that transient activation of Notch1 in the adult mouse myocardium leads to sinus bradycardia (low heart rate) and slow atrial conduction velocity, suggesting a direct role for Notch activation in long-term maintenance of the atrial electrical phenotype. Cardiac sympathetic nerve dysfunction also causes arrhythmias and sudden death in humans (Huang et al., 2015; Li and Li, 2015). Emmanuil Tampakakis (from Chulian Kwon’s group, Johns Hopkins School of Medicine, USA) presented data indicating that cardiac sympathetic neurons with proper electrophysiological properties and gene expression patterns can be generated using human pluripotent stem cells (hPSCs). Importantly, hPSC-derived neurons form physical and functional connections with cardiomyocytes and are capable of influencing their beating rates in a co-culture system (Oh et al., 2016). These exciting findings might provide new platforms for neuromodulation to treat arrhythmias and sudden cardiac death. Finally, Chaitali Misra (from Auinash Kalsotra’s group, University of Illinois, USA) aimed to define mechanisms responsible for lethal arrhythmias observed in patients with myotonic dystrophy 1. She found that an embryonic spliced isoform of an RNA-binding protein is aberrantly upregulated in adult myotonic dystrophy patient hearts. When this fetal splice
variant was expressed in adult mouse cardiomyocytes it induced arrhythmias, which was likely to be due to defective splicing patterns in transcripts encoding excitation:coupling proteins.

Altogether, these presentations highlighted the importance of precise gene regulation via epigenetic, transcriptional and post-transcriptional mechanisms during cardiovascular development, and emphasized how misregulation of these processes can lead to malformations.

**Emerging technologies and approaches**

In the last section of this Meeting Review, we highlight emerging new technologies that were presented at the meeting that aim to overcome limitations affecting current cardiovascular biology research. For example, cardiomyocytes can be generated from human iPSCs and are an excellent candidate for potential stem cell-based therapies; however, human iPSC-derived cardiomyocytes do not fully mature on their own. To address this, Ilya Shadrin (a member of Nenad Bursac’s lab, Duke University, USA) presented novel approaches for the in vitro culture of human iPSC-derived cardiomyocytes that display physiological characteristics of mature cardiomyocytes. He discovered that human iPSC-derived cardiomyocytes can be rapidly matured in a specialized 3D hydrogel environment. Using this method, human iPSC-derived cardiac tissue patches that display a variety of near-adult human myocardial characteristics could be created.

While a number of studies have identified individual factors that can influence cardiac regeneration, the changes that occur on a more global scale to activate tissue regeneration programs are unclear. Taking advantage of the fact that continuous exchange of histone variant H3.3 is associated with increased transcription rates (Deal et al., 2010; Chow et al., 2005; Venkatesh and Workman, 2015), Joseph Goldman (from Kenneth Poss’ group, Duke University, USA) generated transgenic zebrafish that express biotinylatable H3.3 in cardiomyocytes and obtained a cell-specific profile of histone exchange. This system was specifically used to identify novel enhancers that are active during cardiac regeneration.

Finally, a significant challenge in the study and surgical correction of CHDs is that not all patients present with the same structural anomalies. Isao Shiraishi (National Cerebral and Cardiovascular Center, Osaka, Japan) described the generation of superflexible heart replicas using stereolithography 3D printing technology and a vacuum casting technique. Based on clinical imaging modalities, patient-specific heart replicas can be fabricated and provided to pediatric cardiologists so that the internal structure of the complex malformations can be examined in more detail. It was stressed that this technology can be used to design patient-specific surgical interventions as well as for the training of cardiac researchers and clinicians.

**Concluding remarks**

Altogether, this year’s Weinstein Conference covered a wide area of research in cardiovascular development and disease with a specific emphasis on cardiac regeneration. It also highlighted the complexity of cardiovascular development and the underlying causes of human heart diseases. Importantly, the meeting underlined just how multidisciplinary and integrative the field is becoming (Fig. 1), combining new technologies from molecular/cellular biology, genome-wide studies, computational studies, genome editing, tissue engineering and stem cell biology to unlock unknown aspects of human CHDs. Based on the intriguing new findings presented at this meeting, the prospect for future translational studies to treat human heart diseases is promising.

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**Competing interests**

The authors declare no competing or financial interests.

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