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Mouse Models of Congenital Heart Disease

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Introduction

Congenital heart disease (CHD), a highly prevalent human disorder occurring in approximately 1% of live births, results from abnormal embryonic cardiac morphogenesis and usually involves defects in structural components of the developing heart and vessel [1]. Additionally, in infants there is a wide spectrum of congenital heart defects and 3 per 1000 live births will require an intervention (such as surgical) during the first year of life. Although many advances have been made with palliative and corrective surgery which has increased manifold the survival of children with congenital heart defects, CHD still remains the leading cause of death in children with congenital malformation. Separation of the pulmonary circulation from the systemic circulation is a crucial step in the development of the mature heart and its failure to properly separate results in cardiac outflow tract (OFT) defects and account for up to one third of congenital heart disease cases [1]. Therefore understanding the regulatory events and signaling pathways that regulate OFT formation is a major goal of research into the etiology of CHD.

Various different mouse models have been employed to dissect the cellular and molecular basis of congenital cardiovascular defects. Since mouse cardiovascular structure, physiology and development are highly conserved with humans, these models represent the multiple spectrums of defects seen in human from early stages in life to later stages. Targeted gain-of-function, loss-of function and conditional mutagenesis approaches for the gene-of-interest allows the study of consequences of the mutation of gene function in whole animal as well as in a tissue-and a time-specific manner for distinct aspects of heart development. In following few examples it is described here how identification of genes that cause familial or syndrome associated cardiovascular defects in humans has led to the creation of mouse models using gene ablation and alterations of gene function to understand how the gene products play a role in development of the cardiovascular system.

The vertebrate heart originates from four separate progenitor cell lineages, the mesoderm-derived first (FHF) and second heart fields (SHF) and the cardiac neural crest (CNCC) [2,3]. In addition, the proepicardium (PE), another extracardiac cell population, contributes significantly to the morphogenesis of heart [4]. Gene expression studies have revealed that CNCCs and SHF contribute towards the OFT formation and perturbation of either CNC or SHF leads to a spectrum of congenital heart defects, ranging from a failure of heart tube extension, arterial pole alignment defects, double outlet right ventricle (DORV), ventricular septal defect (VSD), persistent truncus arteriosus (PTA) and tetralogy of fallot [5,6].

DiGeorge Syndrome and Deletion 22q11 Syndrome

The deletion 22q11 has typically a three megabase deletion in chromosome 22 (Del22q11) which accounts for vast majority of patients with DiGeorge [7] and velocardiofacial syndromes (VCFS) [8]. The DiGeorge syndrome (DGS) patients display abnormalities of the thymus and in the neonatal stages life threatening congenital heart defects. OFT malformations are more common in these patients with observations of PTA in association of aortic arch. Molecular defects are hypothesized to affect cardiac neural crest function during cardiac and pharangeal arch development. A T-box family gene, TBX1 was identified to be a critical player in the DGSphenotype, and Tbx1 null mutant mice displayed PTA associated with abnormal aortic arch [9-11]. Tissue specific conditional mutants have shown the cell autonomous requirement of Tbx1 in SHF for normal OFT development [12].

Holt-Oram Syndrome

The cardiovascular defects associated with Holt-Oram syndrome (HOS) was described as atrial septal defect (ASD) coupled with conduction defect, ventricular septal defect (VSD) and alignment defect [13,14]. TBX5 gene was found to be affected in human where nonsense mutation, insertion and deletion mutations presumed to cause haploinsufficiency [15]. In mouse, Tbx5is expressed in the regions of the developing heart and heterozygote mutations in Tbx5 display ASD, VSD, left ventricular malformation and cardiac conduction defects [16,17].

Marfan Syndrome

Marfan syndrome (MS) is an autosomal dominant genetic disorder and affects the structure and function of multiple organs including heart, lungs, skeleton, eyes and blood vessel. The major complications are defects of heart valves, aortic aneurysms and arrhythmia due to progressive cardiovascular disease [18]. The spectrum of Marfan syndrome is caused by mutationsin the FBN1 gene, which encodes a structural connective protein called fibrillin-1 [19]. The phenotype of MS is a result of both altered microfibrillar structure and dysregulated TGFβ signaling as a consequence to haploinsufficiency of Fbn1 [20]. Mouse models of Fbn1 mutation display features of MS with various severities and expression of a wildtypeFbn1 transgene in a mutant mouse model rescued the histological and aortic phenotype of the mutants. Fibrillin-1 is thought to bind TGFβ and regulate its expression. However, Fbn1 mutations display increased TGFβ activity in the heart and lungs which can be reversed/rescued with administration of angiotensin II receptor blocker Losartan. Losartan decreases expression of TGFβ ligands and receptors resulting in decreased TGFβ activity [21]. A NIH trial is currently undergoing to test the effect of Losartan in affected MS patients.

Loeys-Dietz Syndrome

Loeys-Dietz Syndrome (LDS) has close parallels with Marfan

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syndrome although there are additional characteristics. LDS is characterized by an enlarged aortic root and associated with degeneration of the aortic wall aneurysms of the ascending or descending aorta, or both, and a high risk of potentially fatal aortic dissection [22,23]. Although the severity of LDS progresses with age, aortic root dilatation can be present at birth, and additional clinical features include hypertelorism (wide-set eyes) and cleft palate. LDS is caused by mutation in the genes encoding the TGFβ receptor 1 and 2 (TGFBR1 and TGFRB2) and is characterized by aggressive aortic/arterial disease. Heterozygote mutations in either TGFBR1 and/or TGFRB2 results in missense alterations in the highly conserved kinase domain in the receptors. As a consequence TGFβ activity increases and there is an increased nuclear accumulation of phosphorylated Smad2 [24]. LDS is associated with increased TGFβ signaling in the vessel wall and aortic aneurysms and a NIH trial currently undergoing is directed at testing the effect of Losartan at lowering aortic aneurysms. Therefore, losartan also holds promise for the treatment of Loeys-Dietz syndrome.

Noonan Syndrome

Noonan syndrome (NS) is an inherited multifaceted disease that includes craniofacial abnormalities and various heart defects, Defective pulmonary valves is the most common defect, but atrioventricular septal defects and hypertrophic cardiomyopathy are also seen. Mutations in a set of genes that encodes members of RAS/MAPK signaling pathway which regulate various aspects of cellular function cause NS [25-28]. Majority of the patients of NS have gain-of-function mutations in PTNP11, which encodes SHP2, a protein tyrosine phosphatase, resulting in increased basal phosphatase activity. A mouse model expressing mutant SHP2 resulted in SHP2 hyperactivity and recapitulated the features of NS including valve and septal defects due to hyperproliferation of outflow tract cushions and OFT alignment defect [29].

Alagille Syndrome

Alagille syndrome (AS) is an autosomal dominant disorder that affects many organs including liver, kidney and heart. The most common heart defects are cardiac valves and pulmonary artery stenosis with occasional tetralogy of fallot. Notch signaling pathway genes are affected in these patients with mutations in JAG1 (Jagged1), the ligand for the Notch receptor family, being the most predominant [30,31]. Additionally, recent studies have also identified mutations in NOTCH2 in patients with AS [32]. Mouse models of AS have been developed with mouse mutant for Jag1 and Jag1/Notch2, which phenocopy the liver and heart defects seen in human AS [33,34], reinforcing the link between this disease and defective Notch signaling mechanism.

Studies with the mouse embryo have provided extensive information about how different genes and signaling pathways regulate cardiogenesis. These discoveries have revealed the potential mechanism and opened avenues for the diagnosis and prognosis of human congenital heart disease as well as fundamental understanding of mammalian heart development. Complex genetic networks, transcriptional regulation and signaling pathways involved in cardiogenesis have been documented. Therefore the next challenge is to utilize this information and determine how mutations cause human malformations and disease, understand the risk and predict therapeutic outcomes. This will allow us to better apply our understanding of normal and diseased heart development to improve human health.

Although there are many unique aspects of childhood congenital heart disease conditions, some of the new innovative therapeutic approaches that are being developed for the care of adults with heart failure will be applicable for treatment for childhood conditions. The therapeutic interventions that show great promise include small molecule therapies, therapies with proteins and modified peptides; approaches such as RNA interference, gene and cell therapy; and regenerative therapy based on a variety of stem-cell-based methods.

Small molecule drugs have major advantages as they can be produced with low cost, have good oral bioavailability and can be designed to target intracellular targets. However, it is very expensive and difficult to design and identify new small molecules that have specific positive effects on disease but do not bind off-target sites and exhibit undesired side effects and toxicity. To further improve drug discovery efforts recently a new fragment-based drug discovery approach has been widely utilized which has yielded discovery of new small molecule drugs [35]. Proteins serve dynamic and complex set of functions in the body and thus approaches utilizing protein therapeutics offers a tremendous opportunity to alleviate disease. Protein therapeutics has been successfully utilized for effective replacement treatment for genes that have been deleted or mutated and cannot be treated with gene therapy. Protein therapeutics also offer advantages over small molecule drugs since the action of protein is more specific, the time taken for clinical development and approval is shorter with far reaching patent protection [36]. However the limitation for this therapeutics is that intracellular and membrane protein cannot be targeted by this approach.

In the last few years therapeutic strategies have been developed based on non-coding regulatory RNAs such as RNA interference (RNAi) for silencing of specific genes, and microRNA (miRNA) modulations to alter complex gene expression patterns. Targeting small RNA molecules to the heart has been very challenging and with the breakthrough discovery of highly cardiotropicadeno-associated virus systems (AAV), it has led to successful regulatory RNA therapy [37]. miRNAs, were identified as important transcriptional and posttranscriptional inhibitors of gene expression thought not to control on–off switching but to fine-tune the protein expression that controls cardiovascular physiology and development. miRNAs are implicated in the pathogenesis of various cardiovascular diseases such as myocardial remodeling, hypertrophy, cardiomyocyte apoptosis and regeneration, cardiac conduction and fibrosis, and vascular diseases. miRNAs offer exciting opportunities to modulate cardiac function therapeutically by manipulating protective or pathogenic miRNAs. Development of antisense oligonucleotide mediated (antagomiRs) knockdown have been used successfully in small laboratory animals to prevent or treat the disorder state by normalizing the deregulated miRNAs [38-40] and presents a big hope for future miRNA cardiovascular diagnostics and therapeutic strategies.

In recent past, development of cardiac regenerative therapy has been pursued vigorously with the promise that stem and progenitor cells may be useful in treating conditions of heart failure. Researchers are currently using “Disease in a dish”, a cutting-edge stem cell-based strategy to create and efficiently model different diseases observed in patients. Disease in a dish harnesses the power of stem cell to self-renew and to differentiate into different cell types. More recently, a special kind of stem cell called an induced pluripotent stem cell (iPS cell) has made the process quicker, more efficient and broadened the range of diseases that can be modeled. Currently, there are a variety of stem cell based approaches that have been taken for cardiac regeneration. In

one method stem cells were transplanted directly in the injured heart where myoblasts were transplanted during coronary artery bypass grafting [41]. An alternative approach of stimulating endogenous stem/progenitor cells have been used to generate new cardiomyocytes in adult heart. Studies using neuregulin and periostin have shown that this can be a viable strategy [42,43]. Another promising approach to generate functional cardiac tissue is by using tissue engineering using either adult or embryonic stem cells or growth factors [44-46]. In this technology, biopolymers can be used to prepare patches containing cells and/or growth factors which can be surgically implanted in the recipient heart. Under the conditions of heart injury in myocardial infarction, these factors can be released in response to the injured area of the heart and show improvements of cardiac function after implantation. Although there has been some progress in developing the technology and offer a lot of promise to the future, it is still at an early stage.

Additionally better imaging technologies are being developed and implemented to visualize the body to assist doctors in their efforts to diagnose certain diseases or conditions. Unprecedented image accuracy can help in evaluating prenatal detection of serious congenital heart malformations which can lead to providing a better chance of diagnosis and treatment. The imaging techniques commonly used for such detections are echocardiography, computerized tomography (CT scan), MRI (Magnetic Resonance Imaging), ultrasound, and interventional radiology. Echocardiography, a widely used routine test in cardiology, allows detecting the size and shape of the heart and blood flow through the heart's chambers and valves. Echocardiographic examination best defines the morphologic features of intracardiac chambers, cardiac valves, and intracardiac shunts. CT allows additional visualization from different angles and is used as an adjunct to echo to evaluate extracardiac vascular morphology. MRI provides images with excellent contrast that allow clinicians to clearly see details of tissue. Using interventional MRI, which is a developing field; surgeons are able to carry out interventional procedures to correct congenital defects and thus reducing the invasiveness of surgical diagnosis and therapy.

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