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Hemodynamics driven cardiac valve morphogenesis☆

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ABSTRACT

Mechanical forces are instrumental to cardiovascular development and physiology. The heart beats approximately 2.6 billion times in a human lifetime and heart valves ensure that these contractions result in an efficient, unidirectional flow of the blood. Composed of endocardial cells (EdCs) and extracellular matrix (ECM), cardiac valves are among the most mechanically challenged structures of the body both during and after their development. Understanding how hemodynamic forces modulate cardiovascular function and morphogenesis is key to unraveling the relationship between normal and pathological cardiovascular development and physiology. Most valve diseases have their origins in embryogenesis, either as signs of abnormal developmental processes or the aberrant re-expression of fetal gene programs normally quiescent in adulthood. Here we review recent discoveries in the mechanobiology of cardiac valve development and introduce the latest technologies being developed in the zebrafish, including live cell imaging and optical technologies, as well as modeling approaches that are currently transforming this field. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Integration of Developmental and Environmental Cues in the Heart edited by Marcus Schaub and Hughes Abriel.

1. Introduction

Cardiovascular diseases (CVDs) are the leading causes of death in the world. Heart malformations account for as many as 30% of prenatal deaths [54], and the incidence of heart defects in live births varies from 0.4% to 5% in different studies, depending on the severity of the defects included in the statistics. Furthermore, another 2% of newborn babies have bicuspid aortic valves (BAVs) or other valve defects [55], which may cause significant morbidity and mortality later in life [19]. Congenital heart valve malformations, therefore, constitute an important medical issue challenging our society.

Cardiac valves function to ensure unidirectional blood flow through the heart and efficient delivery of blood around the body. In recent years, tremendous progress has been made to characterize the molecular and cellular defects associated with valve disease. It is becoming ever more apparent that most valve pathologies have their origin in embryogenesis, either as signs of abnormal developmental processes or the aberrant re-expression of fetal gene programs normally quiescent in adulthood [74,77]. They are usually associated with genetic mutations in genes essential for heart valve development. Notch signaling, for example, is necessary for the correct patterning of the heart chambers and the valve-forming endocardium [28]. By restricting Bmp2 expression to the atrioventricular canal (AVC) myocardium, as well as being expressed itself in the endocardial cells (EdCs), Notch regulates the epithelial-to-mesenchymal transition (EMT) process necessary for the formation of cardiac cushions in the mouse [95]. The significance of this role is demonstrated by the formation of BAVs in *Notch* mutants due to remodeling defects [42]. BAVs often go unnoticed in childhood, but causes severe dysfunction later in life [19]. The abnormally large mitral valves observed in *filamin*, *dachsous1* and *tensin1* mutant mice [32,33,89] further demonstrate how genetic variants affecting the expression of proteins during valve development can progressively affect mitral valve function in adults [70]. The need to understand the link between impaired developmental processes and pathological disorders that present themselves later in life, therefore, highlights the importance and medical relevance of cardiac development studies.

The role of mechanical forces and cell mechanics is becoming ever more appreciated in developmental [53], cancerous [38] and bio-inspired systems [34]. While its impact on cell behavior during morphogenesis is clear [50,53], the mechanism allowing the integration of the molecular pathways activated by forces [61] and morphogenesis *per se* are still not resolved. The cardiac valves are located in areas of high flow velocity and are one of the most mechanically challenged structures in the body, constituting an extraordinary model for studying the impact of mechanical forces and their diversity during morphogenesis. Key challenges lie in the identification of the mechanotransduction

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machinery and the mechanical forces characterizing the system. In this review we will introduce the mechanobiology associated with cardiac valve morphogenesis, focusing primarily on the zebrafish, and the different approaches that are now available to address these questions.

2. The zebrafish model of cardiovascular development

The zebrafish is one of the most widely used animal models for developmental and regeneration studies. Adults give rise to large numbers of embryos, which can be kept completely translucent for up to 5 days post-fertilization, by which time organogenesis has been completed. This allows high throughput screening of small molecules [68] and unique whole genome analyses to be performed *in vivo* [62]. In addition, TALEN and CRISPR-mediated genome engineering [2,7] and fast, high-resolution imaging of live hearts [58,79] provides unique access to cardiac morphogenesis at the cellular level *in vivo*. With the additional ability to implement optical approaches, such as optogenetics [3,4] and optical tweezing [3,83,105] to probe the local mechanical environment, the zebrafish provides a valuable opportunity to study and measure the impact of hemodynamics and mechanical forces on cardiac development and physiology.

3. Mechanical signals during valve development

At the onset of valvulogenesis, the zebrafish heart is composed of two chambers, an atrium and a ventricle, separated by the atrioventricular canal (AVC), where the AV valve will develop (Fig. 1). Heart contraction begins at around 22 h post-fertilization (hpf) and is required for cardiac morphogenesis [8,11,56]. Altering myocardial function can lead to abnormal intracardiac flow forces, myocardial cell shape changes and trabeculation defects [8,75,84]. With this in mind, the mechanical properties of the embryonic heart need to be considered when describing the behaviors of cells and tissues during cardiac morphogenesis. Recently, the mechanical forces generated during the process of heart contraction have been the subject of several investigations [16]. At embryonic stages, viscosity dominates and the main mechanical cues generated at the heart wall are the tissue strain generated by pressure variations and contraction (which can be assimilated to the cell–cell tension) and Wall Shear Stress (WSS) generated by the flowing blood. Furthermore, it has recently been demonstrated that the red blood cells themselves generate important WSS fluctuations that could represent an additional mechanical trigger in this system [41]. Thus EdCs experience multiple mechanical stimuli during cardiac development (Fig. 2). Interestingly, the very nature of the heart that exposes the EdCs to such a unique environment also impedes our ability to study it *in vivo*. The combination of the dynamics of heart contraction and the accompanying fast flow kinetics within a three-dimensional volume is difficult to resolve by conventional microscopy. Yet, in order to identify the mechanosensors involved in EdC intracellular signaling pathways and an EdC response, it is essential to describe the mechanical forces experienced by the EdCs *in vivo*.

Among the different mechanical cues generated in the developing heart, oscillatory flow is observed in the AVC at the earliest stage of valve morphogenesis (Fig. 1). It has been proposed that it constitutes an essential factor in instructing EdC fate towards valvulogenesis [100]. While oscillatory flows and flow reversals are usually associated with regions developing atherosclerosis or inflammation [51], oscillatory flow is observed in the zebrafish AVC specifically at the onset of valvulogenesis and triggers the expression of the transcription factor *klf2a* [100], a known atheroprotective gene [6]. This observation highlights the need for further understanding how features of this flow profile regulate specific downstream cellular responses and valve morphogenesis. Recently, *in vitro* experiments using endothelial cells (EC) suggested that Haemodynamic Frequency Harmonics (HFH) constitutes a key regulator of the endothelial inflammatory phenotype [37]. This hypothesis is supported by the observation that ECs are extremely responsive to perturbations of the 0th and 1st harmonics, leading to an atherogenic-like cell response, with increased NFκB activity and decreased *klf2* expression [37]. Reversing flow, as found prior to valve formation, can be expected to increase the oscillatory nature of the flow in the AVC during the onset of valvulogenesis and can therefore be expected to significantly alter the WSS harmonics generated, a key mechanical input sensed by ECs. The possibility that the mechanotransduction process involved in valvulogenesis responds to the frequency spectrum of the flow pattern observed *in vivo* has not been fully explored. Yet, initial results suggest that mechanotransduction in response to HFH, in particular the 1st harmonic, directly affects valvulogenesis and the endocardial cell response through *trpv4* and *trpp2* channels [52].

4. Blood flow and *klf2a* expression

As the heartbeat starts early in embryonic development, cardiovascular development is dynamic: ECs reorganize and migrate to form an

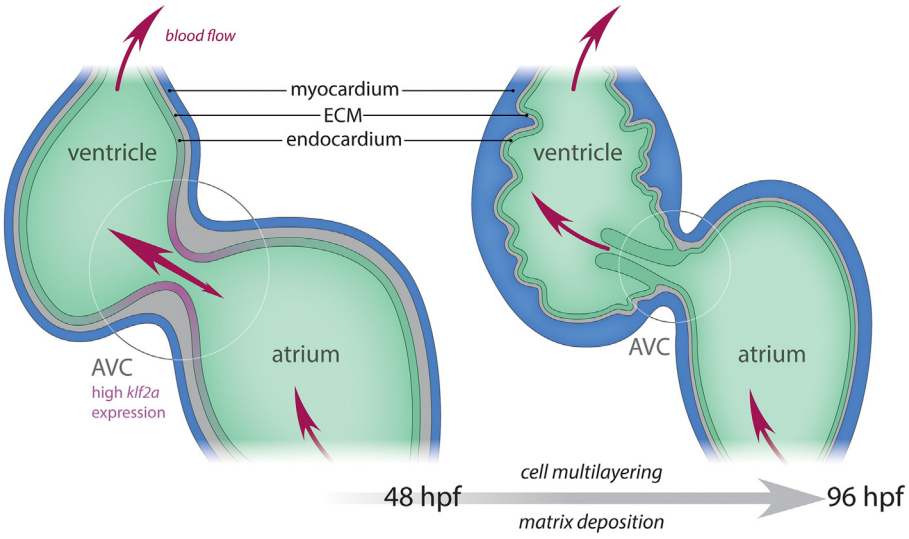

Fig. 1. Schematic representation of heart hemisections before and after atrioventricular valve formation. The endocardial cells lining the lumen of the heart are shown in green. The endocardial wall is highlighted in darker green. The myocardium is shown in blue and the cardiac jelly separating the two cell layers is represented in gray. Red arrows demonstrate the direction of blood flow. The double-headed arrow represents the oscillatory flow profile within the AVC at 48 hpf. This oscillatory flow profile leads to localized expression of *klf2a* (pink) in the AVC endocardium. Valve leaflets form within the AVC by 96 hpf, ensuring unidirectional blood flow through the heart by this stage.

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