Dysregulation of hyaluronan homeostasis during aortic valve disease

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Abstract

Aortic valve disease (AVD) is one of the leading causes of cardiovascular mortality. Abnormal expression of hyaluronan (HA) and its synthesizing/degrading enzymes have been observed during latent AVD however, the mechanism of impaired HA homeostasis prior to and after the onset of AVD remains unexplored. Transforming growth factor beta (TGFβ) pathway defects and biomechanical dysfunction are hallmarks of AVD, however their association with altered HA regulation is understudied. Expression of HA homeostatic markers was evaluated in diseased human aortic valves and TGFβ1-cultured porcine aortic valve tissues using histology, immunohistochemistry and Western blotting. Further, porcine valve interstitial cell cultures were stretched (using Flexcell) and simultaneously treated with exogenous TGFβ1 ± inhibitors for activated Smad2/3 (SB431542) and ERK1/2 (U0126) pathways, and differential HA regulation was assessed using qRT-PCR. Pathological heavy chain HA together with abnormal regional expression of the enzymes HAS2, HYAL1, KIAA1199, TSG6 and IαI was demonstrated in calcified valve tissues identifying the collapse of HA homeostatic machinery during human AVD. Heightened TSG6 activity likely preceded the end-stage of disease, with the existence of a transitional, pre-calcific phase characterized by HA dysregulation. TGFβ1 elicited a fibrotic remodeling response in porcine aortic valves similar to human disease pathology, with increased collagen and HYAL to HAS ratio, and site-specific abnormalities in the expression of CD44 and RHAMM receptors. Further in these porcine valves, expression of HAS2 and HYAL1 was found to be differentially regulated by the Smad2/3 and ERK1/2 pathways, and CD44 expression was highly responsive to biomechanical strain. Leveraging the regulatory pathways that control both HA maintenance in normal valves and early postnatal dysregulation of HA homeostasis during disease may identify new mechanistic insight into AVD pathogenesis.

1. Introduction

Surgical intervention for aortic valve disease (AVD) is the second most common cardiothoracic procedure in the US. Over 100,000 valve surgical procedures are performed each year in the US, and AVD results in more than 25,000 deaths annually [1–4]. Aging is a significant clinical risk factor, and calcific AVD is the most common type of AVD occurring in 2% of the aged population [5–7]. Structurally, the aortic valve consists of three leaflets, each composed of three layers of organized extracellular matrix (ECM) with collagen in the fibrosa layer (outflow side), elastic fibers in the ventricularis layer (inflow side), and proteoglycans and glycosaminoglycans (GAGs) separating these in the intermediate spongiosa layer [8]. Valve ECM is primarily synthesized by valve interstitial cells (VICs), the predominant, mechanosensitive cells. The GAG hyaluronan (HA) is an abundant valve

ECM component that maintains tissue hydration and flexibility, and impacts tissue viscoelasticity. Studies have shown that HA plays diverse biological roles in ECM assembly, cell proliferation and migration, and tissue development [9]. However, the molecular roles of HA in aortic valves are understudied.

HA is regulated through a homeostatic balance between enzymes that produce HA (HA synthases; HAS), enzymes that degrade HA (hyaluronidases; HYAL), and receptors that bind HA for retention and/or signaling, such as CD44 and RHAMM (the receptor for HA mediated motility) [10]. Any alterations in HA maintenance during disease are potentially indicative of coordinated pathogenesis involving these molecules. Most human valve HA studies have focused on mitral valve prolapse in which HA and proteoglycan accumulations lead to myxomatous change and valve dysfunction [11,12]. However, calcific AVD results in a fibrotic aortic valve phenotype with increased collagen expression and associated GAG reduction at advanced age, and since aortic valves demonstrate tissue collagen deposition even with normal aging [13], analysis of collagen usually takes precedence over GAGs in AVD studies. As a result, GAG expression during AVD is largely underappreciated [14]. Previous studies from our group and others have shown abnormal expression of HA and its homeostatic markers in calcified aortic valve tissues [15–17], however the mechanistic regulation of HA homeostasis in aortic valves at early postnatal or diseased/end stages is poorly understood.

Development of novel therapies for AVD requires improved understanding of molecular pathways, such as transforming growth factor beta (TGFβ) signaling, in regulating valve ECM composition prior to disease. The TGFβ cytokine signaling pathways regulate valve cell phenotype and ECM remodeling through their ligands TGFβ1/2/3 via the activation of Smad2/3 by the TGFβ receptor complex (canonical pathway) [18]. The importance of other (non-canonical) pathways including the activation of mitogen-activated protein kinase (MAPK) cascades, notably the phosphorylation of ERK1/2 to pERK1/2 by TGFβ in valves, has only been recently established [19,20]. Diseased aortic valves demonstrate increased VIC activation, as well as increased expression of TGFβ and its pathway activation factors such as pSmad2/3 and pERK1/2 [21–24]. Further, TGFβ promotes VIC proliferation and activation and is known to regulate GAG fine structure as well as HA synthesis in cultured VICs [23,25–27]. Therefore these molecular pathways may play a significant role in both pre-calcific and late-stage calcified valves.

AVD manifests as stenosis and/or regurgitation and causes hemodynamic perturbations that often affect VIC phenotype initiating a maladaptive ECM remodeling response and resulting in a progressive disease process. Biomechanical studies on cultured VICs have shown that both calcific and myxomatous changes in valves are strain dependent. In particular, mechanical strain together with TGFβ enhances the activation, remodeling, and calcification potential of cultured VICs [28,29]. HA secretion by VICs is mechanosensitive [30–32]; however, the mechanical regulation of its homeostatic factors is unclear. Along with biomechanics, aging provides an environmental factor that amplifies the early genetic predisposition associated with aortic valve malformation, resulting in AVD later in life [5–7]. Significant age-induced alterations in GAG content and biomechanical properties have been identified in aortic valves from humans and other animals [33,34]. Therefore, simultaneous investigation of HA homeostasis, TGFβ, biomechanics, and aging is important to understand their mechanistic interactions during AVD and to elucidate structure–function relationships in native as well as diseased valves.

Overall, the goal of this study was to combine molecular and biomechanical approaches to determine the differential effects of Smad2/3 and ERK1/2 pathways on markers of HA homeostasis (regulatory enzymes/receptors) particularly in the presence of biomechanical stretch, and in the framework of postnatal AVD. We tested the hypothesis that Smad2/3 and ERK1/2 pathway inhibitors rescue the defective regulation of HA homeostasis in VICs exposed to TGFβ1 and biomechanical strain, and that the rescue effect of these inhibitors is additive. We show that TGFβ1 caused fibrotic remodeling and regional abnormalities in the expression patterns of HA homeostatic regulators in porcine aortic valves, similar to diseased human valves. Further, the Smad2/3 and ERK1/2 pathways differentially regulated HA enzymes and there is an imbalance of HAS to HYAL ratio in valves exposed to TGFβ1 and biomechanical stretch. These results advance our understanding of HA homeostasis in AVD pathogenesis and may provide insight for future mechanistic studies.

2. Methods

2.1. Human tissue acquisition

Human hearts with normal (young and aged) and sclerotic (aged) aortic valves were obtained from donors through the National Disease Research Interchange program. The donors were under the age 20 (N = 4) or over the age 50 (N = 7; 4 normal, 3 sclerotic), for the young and aged stages respectively. They demonstrated normal cardiac structure and function, and died of non-cardiac causes with a warm ischemia time of less than 6 h. As revealed by patient autopsy information reports, healthy donors