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Abstract

The mature heart valves consist of stratified extracellular matrix (ECM) layers, and heart valve disease is characterized by ECM dysregulation and mineralization. There is increasing evidence that regulatory pathways that control heart valve development also are active in disease. In human diseased valves and mouse models, the expression of valve progenitor markers, including *Twist1*, *Msx1/2* and *Snail1/2*, is induced. Additional markers of osteogenesis, including *Runx2*, osteocalcin and bone sialoprotein, also are expressed in calcific aortic valve disease (CAVD) in humans and mice. New mouse models have been developed for studies of valve disease mechanisms. *Klotho*-null mice are a model for premature aging and exhibit calcified nodules in aortic valves with osteogenic gene induction. *Osteogenesis Imperfecta* mice, bearing a *collagen1a2* mutation, develop features of myxomatous valve disease, including thickening, increased proteoglycan deposition and chondrogenic gene induction. Together, these findings demonstrate specific molecular indicators of valve disease progression, including the identification of early disease markers, which represent potential targets for therapeutic intervention.

Keywords

Heart valve • Embryo • Mouse model • Aortic valve disease

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18.1 Introduction

The semilunar and atrioventricular (AV) valves of the heart are made up of highly organized extracellular matrix (ECM) layers populated by quiescent valve interstitial cells (VICs) [1]. In healthy valves, the ECM is compartmentalized into layers composed of collagens, proteoglycans, and elastin, which are maintained by the VICs for proper valve function throughout life (Reviewed in [2]). In diseased valves, the leaflets are thickened as a result of ECM dysregulation and VIC activation. Calcific aortic valve disease (CAVD) includes calcification of the cusps [3], whereas mitral valve prolapse (MVP) is accompanied by increased proteoglycans and myxomatous changes in the leaflets [4]. Currently, the standard treatment for severe heart valve disease is surgical replacement, and new therapies based on molecular mechanisms are needed.

Molecular mechanisms associated with heart valve disease include activation of signaling pathways involved in progenitor specification, cell proliferation, and differentiation of heart valve and bone precursors [5, 6]. We have reported that pediatric and adult diseased valves are characterized by expression of markers of valve mesenchymal and chondrogenic progenitor cells, while adult diseased aortic valves express markers of osteogenic calcification [7]. We also have identified novel mouse models of calcific and myxomatous valve disease [8] that will be useful in determination of the underlying mechanisms driving disease and in development of pharmacologic-based therapies.

18.2 Heart Valve Development

Heart valve development in vertebrate embryos begins with the formation of endocardial cushions in the AV canal and outflow tract of the primitive heart tube [5]. The mesenchymal cells of the endocardial cushions originate from the endocardium after an endothelial-to-mesenchymal transition (EMT). Valve progenitors are highly proliferative and migratory and express transcription factors *Twist1*, *Tbx20*, *Sox9*, *Msx1/2*, and *Snai1*. The endocardial cushion cells diversify into lineages that express distinct ECM profiles regulated by BMP and FGF signaling [9]. *Wnt*/ β -catenin signaling also is active during endocardial cushion maturation, but the specific role for this pathway in valve lineage differentiation is yet to be determined [10]. Valve development continues with the remodeling of the endocardial cushions into thin elongated leaflets composed of stratified ECM, which occurs soon after birth in mice and humans [2]. The ECM layers consist of the collagen-rich fibrosa, proteoglycan-rich spongiosa, and elastin-rich atrialis/ventricularis [2]. These layers are oriented in the semilunar and AV valves with the elastin layer adjacent to blood flow. While it is likely that hemodynamics has a role in leaflet stratification, the regulatory pathways that control ECM organization and compartmentalization during valve maturation are largely unknown.

18.3 Heart Valve Disease

Heart valve disease can result from congenital malformation or gene mutations, or it may be acquired later in life [1]. The prevalence of heart valve disease increases significantly with age, such that ~10 % of people >75 years old have moderate aortic or mitral valve disease [11]. However, the pathogenic mechanisms that drive the development of heart valve disease and that could serve as potential therapeutic targets are not well understood. There is increasing evidence that regulatory pathways that control heart valve and bone development also are active in disease. However, the roles of these pathways in valve pathogenesis and/or repair are not well defined.

18.3.1 Calcific Aortic Valve Disease (CAVD)

CAVD is a progressive disease, initially presenting with aortic valve (AoV) thickening (sclerosis) and resulting in valve stenosis and insufficiency later in life [12]. End-stage disease is characterized by the presence of calcific nodules at the hinge region of the AoV, underlying the pathology of CAVD [3]. In an effort to draw parallels between the progression of disease and the underlying molecular mechanisms, pediatric and adult diseased AoV were analyzed for markers of valve development and endochondral bone formation [7]. Activated VICs in both pediatric and adult valves have increased expression of valvulogenic markers *Twist1*, *Msx2*, and *Sox9*. Strikingly, the formation of calcific nodules was found to be an exclusive feature of adult calcified AoV. Furthermore, phosphorylation of Smads1/5/8, indicative of active BMP signaling, in addition to expression of osteogenic genes, such as *Runx2*, was observed only in adult calcified AoV. These findings demonstrate that both pediatric and adult diseased AoV express valvulogenic markers, while adult calcified AoV also express markers of osteogenic calcification. Differential expression of these markers suggests that an osteogenic regulatory mechanism contributes specifically to CAVD.

The incidence of human CAVD strongly correlates with aging, which is an independent risk factor for AoV disease. We have recently identified *Klotho*^{-/-} mice, a model of premature aging, as a novel mouse model of CAVD [8]. Notably, *Klotho*^{-/-} mice develop calcific nodules at the hinge region of the fibrosa side of the AoV (Fig. 18.1a, b), similar to human CAVD. In these mice, calcification occurs independent of inflammation and cusp thickening, providing initial evidence for a valve-intrinsic molecular mechanism for age-related calcification common in elderly patients. *Klotho*^{-/-} AoV have increased expression of osteogenic factors *Runx2* and *Osteopontin*, in addition to increased expression of chondrogenic factors *Sox9* and *Col10a1*, consistent with an osteochondrogenic-like mechanism of disease (Fig. 18.1e). Increased activation of pSmad1/5/8 also precedes calcification in the *Klotho*^{-/-} mice, and inhibition of BMP signaling represents an attractive new therapeutic approach for CAVD.

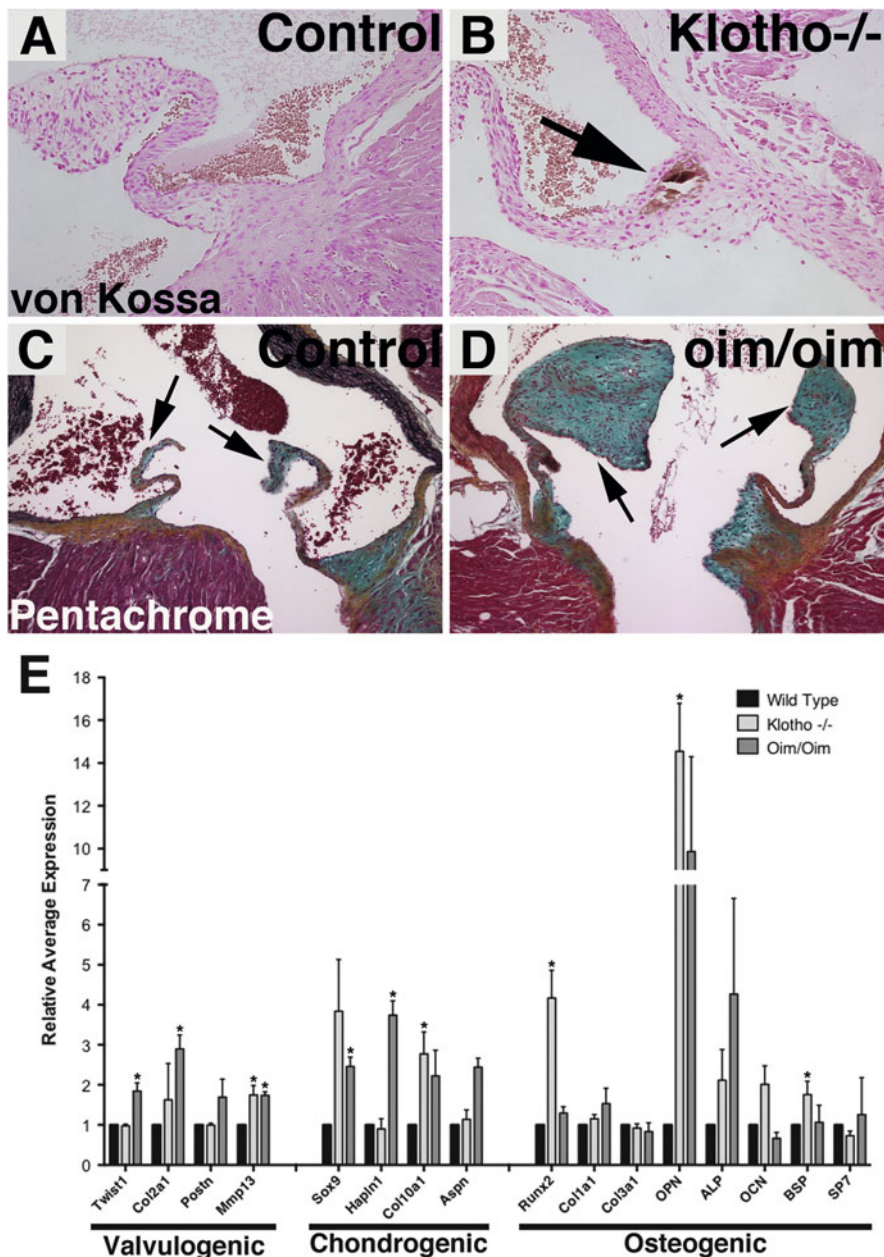


Fig. 18.1 Valvulogenic, chondrogenic, and osteogenic programs are induced in mouse models of calcific (*Klotho*^{-/-}) and myxomatous (*Oim/Oim*) valve disease. *Klotho*^{-/-} mice (b) exhibit AoV nodular calcification (arrows), as compared to wild-type littermate controls (a) at 6 weeks of age, as detected by von Kossa staining. *Oim/Oim* AoV cusps (d) exhibit distal thickening and increased proteoglycan deposition (aqua, arrows), compared to WT littermates (c) at 9 months of age as observed by Movat's pentachrome staining. Expression of genes involved in valvulogenesis, chondrogenesis, and osteogenesis was examined by qRT-PCR of RNA isolated from *Klotho*^{-/-}

18.3.2 Myxomatous Valve Disease

The most common cause of MVP is myxomatous valve disease, which is defined by pathological thickening of the valve leaflets, primarily due to accumulation of proteoglycans [4]. This is accompanied by alterations in the distribution of ECM components, such as disrupted collagen fiber organization and elastic fiber fragmentation. The pathogenesis of MVP is not well understood; however, MVP is often linked to connective tissue disorders or specific mutations in ECM genes, supporting the concept that defects originating during valve development could underlie adult disease.

While myxomatous disease most commonly affects the mitral valves, myxoid AoV have been reported. *Osteogenesis imperfecta murine (Oim)* have a spontaneous mutation in the *Colla2* gene and display bone fragility characteristic of human osteogenesis imperfecta (OI) [13]. Interestingly, humans with OI or *Colla2* mutations have a predisposition to AoV disease [14]. Likewise, the AoV of *Oim/Oim* mice exhibit distal cusp thickening and increased proteoglycan accumulation, characteristic of myxomatous valve disease (Fig. 18.1c, d) [8]. Furthermore, the *Oim/Oim* mice have increased expression of valve progenitor markers *Twist1*, *Col2a1*, *Mmp13*, *Sox9*, and *Hapln1*, in addition to increased *Coll10a1* and *Asporin* expression (Fig. 18.1e). These changes in gene expression are consistent with increased proteoglycan accumulation and cartilage gene induction, which are key features of myxomatous disease.

18.4 Signaling Pathways in Heart Valve Development and Disease

Similar to heart valve and bone development, studies of human explanted valves implicate BMP, Notch, and Wnt signaling pathways in the progression of CAVD. Thus, heart valve disease shares signaling networks with valve and bone developmental pathways. Together, these studies demonstrate that activation of both BMP and Wnt signaling correlates with progression of CAVD [15, 16]. On the other hand, loss-of-function mutations in *NOTCH1* are associated with bicuspid aortic valve (BAV) and CAVD, in humans and mice, suggesting an inhibitory function for the Notch pathway in valve calcification [17]. Human genetic conditions including Marfan syndrome and Loays-Dietz syndrome lead to myxomatous mitral valve disease and are associated with increased TGF- β signaling [18, 19]. However, the specific mechanisms by which these different pathways contribute to the development and progression of heart valve disease remain unknown.

Fig. 18.1 (continued) and *Oim/Oim* mice aortic valves relative to wild-type littermate controls (e). Normalized values are shown as average fold changes compared to wild-type group set at 1.0. * is p -value ≤ 0.05 calculated by paired student's t -test

18.5 Future Directions and Clinical Implications

Klotho^{-/-} and *Oim/Oim* mice are novel mouse models of CAVD and myxomatous valve disease that will be useful for determination of the underlying pathogenic mechanisms driving valve disease. Understanding how signaling networks contribute to disease will likely have a significant impact on clinical outcomes, since knowledge gained from these studies will allow for the development and design of new drugs/treatments for patients with valve disease.

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