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Transcriptional regulation of heart valve development and disease

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Abstract

Aortic valve disease is estimated to affect 2% of the United States population. There is increasing evidence that aortic valve (AoV) disease has a basis in development, as congenital valve malformations are prevalent in patients undergoing valve replacement surgery. In fact, a number of genetic mutations have been linked to valve malformations and disease. In the initial stages of AoV pathogenesis, the valvular interstitial cells become activated, undergo cell proliferation, and participate in extracellular matrix remodeling. Many of these cell properties are shared with mesenchymal progenitor cells of the normally developing valves and bones. Historically, valve calcification was thought to be a passive process reflecting end stage disease. However, recent evidence describes the increased expression of transcription factors in diseased AoV that are common to valvulogenic and osteogenic processes. These studies lend support to the idea that a developmental gene program is re-activated in aortic valve disease and may contribute to the molecular mechanisms underlying valve calcification in disease.

Keywords

valve development; aortic valve disease; transcriptional regulation

Summary

Here we review shared transcription factor gene expression in valve development and aortic valve disease. The processes of valve development, chondrogenesis, and osteogenic mechanisms related to embryonic bone development are compared to molecular mechanisms in aortic valve calcification during disease.

Introduction

Aortic valve disease affects approximately 2% of the United States population, and aortic valve (AoV) replacement surgery is the second most common open heart surgery (1,2). Progressive AoV disease typically results in stenosis, a narrowing of the valve opening, and often includes valve calcification (3). Recent studies suggest that developmental malformations may lead to adult valve disease, however, the molecular regulation of AoV

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disease progression is unknown. In a large study of AoV replacement surgery, 50% of patients had congenitally malformed valves, including bicuspid aortic valve (BAV) (4). BAV, in which the aortic valve has two rather than three cusps, occurs in 0.5% to 2% of the population (5,6). This evidence suggests that a congenitally malformed valve is pre-disposed to valve disease. Mutations in several genes including *NOTCH1*, *COLLAGEN1A1*, and *ELASTIN*, are linked to AoV malformations and disease, supporting the hypothesis that AoV disease is rooted in development (7–9). The linkage of AoV disease with congenital malformations, such as BAV, as well as specific gene mutations, supports the idea that valve disease in adults arises from lesions present at birth. However, the molecular progression of valve pathogenesis is largely unknown.

The molecular pathways governing valve progenitor cell development and valve maturation share some similarities to those of osteogenic cell lineages (10). Overall there is accumulating evidence for early commonalities between valve and bone progenitors that eventually lead to a specialized gene program and unique cell type differentiation in these tissues (10). Transcription factors including Twist1 (a basic helix-loop-helix transcription factor), Msx2 (muscle segment homeobox 2), and Sox9 (SRY-box 9) are important for embryonic valve mesenchyme formation and are also expressed in the mesenchymal progenitor cells of developing cartilage and bone (11–16). Recent pathological reports on AoV disease have described the expression of developmental genes in areas of valve calcification, supporting the idea that valve calcification is not a passive deposition of calcium, but is actively regulated by hierarchies of signaling pathways and transcription factors. For example, bone morphogenetic protein (BMP) signaling is important for valvulogenesis and osteogenesis, and is increased during calcific aortic valve disease (17–19). Furthermore, activation of the osteogenic transcription factors Msx2, Sox9, Runx2, and Osterix has recently been described in diseased AoV (20,21). Overall, this evidence supports a mechanism whereby developmental gene programs related to valvulogenesis and/or bone formation are re-activated during calcific AoV disease.

Transcriptional regulation of heart valve development

During development of vertebrate embryos, the heart is the first organ to form and function. The heart forms initially as a primitive tube with the arterial outflow tract (OFT) located cranially to the posterior venous pole (22). The first evidence of heart valve development is the formation of endocardial cushions in the developing OFT and atrioventricular canal (AVC) (Figure 1A, A'). Endocardial cushion formation is initiated when signals from the AVC and OFT myocardium induce an epithelial to mesenchymal transition (EMT) of adjacent endocardial cells (23). The resulting mesenchymal cells are highly proliferative and migratory, and they comprise the valve progenitors of the semilunar valves in the OFT and AV valves in the AV canal. The primordia of the individual valve leaflets form with the elongation and fusion of the primitive endocardial cushions and are evident by E13.5 in mice and by 5–6 weeks of development in human fetuses (Figure 1B, B') (24). During late fetal and early postnatal development, the valve primordia continue to elongate and remodel into AV and SL valve leaflets which are highly stratified into diversified extracellular matrix layers rich in collagen, elastin and proteoglycans (Figure 1C, D) (25,26).

The valve progenitor cells of the endocardial cushions express several transcription factors associated with a variety of mesenchymal cell populations in the developing embryo as well as with transformed cells of metastatic cancers (27). The bHLH transcription factor Twist1 is preferentially expressed in the highly proliferative, undifferentiated, migratory cells of the endocardial cushions and is down regulated during maturation and differentiation of the valve leaflets (28). In isolated endocardial cushion cells, Twist1 promotes cell migration and proliferation, while inhibiting differentiation (16). Likewise, persistent Twist1 expression in

the developing valves of transgenic mice leads to increased cell proliferation and prolonged expression of primitive ECM genes at late fetal stages (Chakraborty et al. unpublished). The T-box transcription factor Tbx20 also is expressed highly in the developing AV and OFT valves. Studies in cultured endocardial cushion cells and in vivo demonstrate that Tbx20 promotes cell proliferation and migration downstream of Twist1 (16,29) (Chakraborty et al. unpublished). Msx1 and Msx2 are NK-class homeodomain transcription factors expressed in a variety of mesenchymal cell types and are also required for endocardial cushion formation (13). Together, these transcription factors promote cell proliferation, migration, and early ECM expression characteristic of the endocardial cushions and define a molecular profile of valve progenitor cells.

The remodeling and stratification of the AV and SL valve leaflets requires the activity of several transcription factors that are also important for the development of related connective tissue types (24). The initiation of heart valve ECM remodeling requires the transcription factor NFATc1 (Nuclear Factor of Activated T-cells), which regulates expression of *cathepsin K* downstream of RANKL signaling (30–32). Expression of specific components of the stratified ECM is dependent on transcription factors that are also active in cartilage, tendon, and bone development (10). Sox9 is required for cartilage lineage development and also is expressed in the remodeling valves. Targeted loss of Sox9 in mouse heart valve development prevents valve maturation and leads to decreased expression of Col2 and cartilage link protein, which are characteristic of proteoglycan-rich ECM related to cartilage (14). Scleraxis, a bHLH transcription factor first identified in developing tendons, also is expressed in the remodeling valves with the tendinous matrix markers tenascin and collagen 14 (33,34). Overall, there is increasing evidence that each of these transcription factors controls the expression of genes related to specific ECM characteristics of the mature stratified valves. Furthermore, as discussed later, many of these transcription factors are re-activated during aortic valve disease and may play a role in an osteogenic-like process during valve calcification.

Transcription factors and aortic valve pathogenesis

Features of valve sclerosis include disorganization of collagen bundles, loss of valve cusp stratification, and increased activity of ECM remodeling enzymes (35,36). (Figure 2-C). AoV disease is a narrowing of the valve opening, which often includes calcification of the valve cusps (Figure 2-D) (3). Valve calcification occurs primarily in the fibrosa layer, in regions of greatest mechanical stress, and calcium deposits often protrude on the aortic surface of the valve (37). Two patterns of calcification have been described. The first beginning at the valve hinge, where the cusp attaches to the aortic wall, and the second initiating along the line of coaptation, the point at which the AoV cusps meet during diastole (38). Histologically, the majority of valve calcification is thought to be dystrophic (passive) however, cartilaginous nodules and mature lamellar bone have also been described, suggesting an active calcification process has been underappreciated (39).

The valvular interstitial cells (VIC) are the main cellular constituents of the mature valves and contribute to valve homeostasis as well as valve pathogenesis. The VIC in a healthy valve arise from valve progenitor cells of the developing endocardial cushions, are primarily quiescent, and are important for maintaining normal valve structure and function (Figure 2-A) (40). Conversely, in a diseased valve, the VIC become activated to a myofibroblast state and express the marker alpha smooth muscle actin (Figure 2-B) (20,40). Other cell populations including infiltrating immune cells and resident valve stem cells have also been described in diseased AoV (39,41). Quiescent VIC do not proliferate whereas activated VIC have been shown to undergo cell proliferation (42). There is accumulating evidence that the

activated, proliferating VIC initiate a transcriptional program common to heart valve progenitors and osteogenic processes.

Recent studies report the expression of transcription factors associated with valve and bone formation in human diseased AoV. In limb morphogenesis, *Msx2* is important for the proliferation of osteogenic progenitor cells and for bone and cartilage formation through the regulation of the canonical Wnt signaling pathway (15,43). Likewise, in human diseased AoV, *Msx2* expression is increased in calcified areas associated with increased Wnt signaling and cell proliferation (21,42). Similarly, *Twist1* is expressed in the osteoblast progenitors, where it inhibits osteoblast differentiation while promoting chondrogenesis (12). In human diseased AoV, *Twist1* expression is apparent in small clusters of cells near regions of calcification and increased cell proliferation (Wirrig and Yutzey, unpublished). These studies support a role for *Twist1* in promoting VIC proliferation during AoV disease. *Sox9* is expressed in the mesenchyme of the developing limbs and is important for differentiation of the chondrocyte lineage and for the expression of ECM genes characteristic of cartilage, such as *collagen 2a1* (11,44). In diseased AoV, *Sox9* expression is increased in the VIC, consistent with a role in cell proliferation and ECM gene regulation (20). Combined, this evidence supports the idea that transcription factors involved in early valve formation and chondrogenesis are abnormally activated in AoV disease. However, it is not known if this is part of a valve repair process or a maladaptive pathologic mechanism.

In addition to factors involved in chondrogenesis, transcription factors associated with endochondral ossification are reported to have increased expression in diseased aortic valves. *Runx2* (RUNT-related transcription factor 2) is expressed strongly in osteoblasts during bone development, is important for osteoblast maturation, and regulates the expression of osteogenic ECM genes including *collagen 10* and *osteocalcin* (45,46). *Osterix* (transcription factor *Sp7*) functions downstream of *Runx2* and is necessary for bone formation, osteoblast differentiation, and expression of *osteocalcin* (47). Studies on human diseased AoV demonstrate increased expression of *Runx2* and *Osterix* in the VIC surrounding areas of calcification (20). Furthermore, increased expression of *Runx2* target genes *collagen 10* and *osteocalcin* have also been described in stenotic AoV disease (35,42). Therefore, the increased expression of *Osterix*, *Runx2*, and the downstream bone matrix proteins *collagen 10* and *osteocalcin*, is evidence for an active osteogenic process in AoV disease. Progress towards a better understanding of the molecular mechanisms underlying calcific AoV disease is being made, however significant gaps still exist in the areas of VIC activation, valve stem cell contributions, and initiation of an osteogenic gene expression profile.

Conclusions and perspectives

The most common treatment for AoV disease is replacement with either a mechanical or bioprosthetic valve (3). Valve replacement is not optimal because mechanical valves require life-long anticoagulation therapy to avoid thromboemboli, and bioprosthetic valves have limited durability, often requiring additional surgeries (3). Therefore, intervention or reversal of valve pathology would represent a significant advance in the treatment and management of patients with AoV disease. During AoV disease, VIC become activated, assuming a myofibroblast-like phenotype, and can undergo cell proliferation (40,42). Activated VIC express transcription factors involved in developmental processes like valvulogenesis and osteogenesis. In addition to activated VIC, valve stem cell populations from internal and external sources have been reported, which could contribute to pathological valve calcification or may be an important factor in valve repair (41). Significant progress has been made in recent years in understanding passive deposition of calcium versus an active osteogenic process in AoV disease. However, evidence is lacking

in the areas of VIC activation, what steps precede end stage stenosis and calcification, and how valve calcification can be lessened or reversed. Measures to identify AoV disease earlier and treat AoV disease pharmacologically or with less invasive approaches would be a significant improvement over the current standard of care. These advances will only be possible with a better understanding of the molecular mechanisms underlying valve development and disease.

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References

1. Bach DS, Radeva JI, Birnbaum HG, Fournier AA, Tuttle EG. Prevalence, referral patterns, testing, and surgery in aortic valve disease: leaving women and elderly patients behind? *J Heart Valve Dis.* 2007; 16:362–369. [PubMed: 17702360]
2. Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M. Burden of valvular heart diseases: a population-based study. *Lancet.* 2006; 368:1005–1011. [PubMed: 16980116]
3. Bonow RO, Carabello BA, Chatterjee K, et al. 2008 Focused update incorporated into the ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 1998 Guidelines for the Management of Patients With Valvular Heart Disease): endorsed by the Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *Circulation.* 2008; 118:e523–e661. [PubMed: 18820172]
4. Roberts WC, Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. *Circulation.* 2005; 111:920–925. [PubMed: 15710758]
5. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation.* 2009; 121:e46–e215. [PubMed: 20019324]
6. Movahed MR, Hepner AD, Ahmadi-Kashani M. Echocardiographic prevalence of bicuspid aortic valve in the population. *Heart Lung Circ.* 2006; 15:297–299. [PubMed: 16914375]
7. Bonita RE, Cohen IS, Berko BA. Valvular heart disease in osteogenesis imperfecta: presentation of a case and review of the literature. *Echocardiography.* 2010; 27:69–73. [PubMed: 19725849]
8. Garg V, Muth AN, Ransom JF, et al. Mutations in NOTCH1 cause aortic valve disease. *Nature.* 2005; 437:270–274. [PubMed: 16025100]
9. Hallidie-Smith KA, Karas S. Cardiac anomalies in Williams-Beuren syndrome. *Arch Dis Child.* 1988; 63:809–813. [PubMed: 3415298]
10. Lincoln J, Lange AW, Yutzey KE. Hearts and bones: shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development. *Dev Biol.* 2006; 294:292–302. [PubMed: 16643886]
11. Akiyama H, Chaboissier MC, Martin JF, Schedl A, de Crombrughe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. *Genes Dev.* 2002; 16:2813–2828. [PubMed: 12414734]
12. Bialek P, Kern B, Yang X, et al. A twist code determines the onset of osteoblast differentiation. *Dev Cell.* 2004; 6:423–435. [PubMed: 15030764]
13. Chen YH, Ishii M, Sucov HM, Maxson RE Jr. Msx1 and Msx2 are required for endothelial-mesenchymal transformation of the atrioventricular cushions and patterning of the atrioventricular myocardium. *BMC Dev Biol.* 2008; 8:75. [PubMed: 18667074]
14. Lincoln J, Kist R, Scherer G, Yutzey KE. Sox9 is required for precursor cell expansion and extracellular matrix organization during mouse heart valve development. *Dev Biol.* 2007; 305:120–132. [PubMed: 17350610]

15. Satokata I, Ma L, Ohshima H, et al. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat Genet.* 2000; 24:391–395. [PubMed: 10742104]
16. Shelton EL, Yutzey KE. Twist1 function in endocardial cell proliferation, migration, and differentiation during heart valve development. *Dev Biol.* 2008; 317:282–295. [PubMed: 18353304]
17. Kaden JJ, Bickelhaupt S, Grobholz R, et al. Expression of bone sialoprotein and bone morphogenetic protein-2 in calcific aortic stenosis. *J Heart Valve Dis.* 2004; 13:560–566. [PubMed: 15311861]
18. Ma L, Lu MF, Schwartz RJ, Martin JF. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development.* 2005; 132:5601–5611. [PubMed: 16314491]
19. Yoon BS, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, Lyons KM. Bmpr1a and Bmpr1b have overlapping functions and are essential for chondrogenesis in vivo. *Proc Natl Acad Sci U S A.* 2005; 102:5062–5067. [PubMed: 15781876]
20. Alexopoulos A, Bravou V, Peroukides S, et al. Bone regulatory factors NFATc1 and Osterix in human calcific aortic valves. *Int J Cardiol.* 2008; 139:142–149. [PubMed: 19019468]
21. Miller JD, Chu Y, Brooks RM, Richenbacher WE, Pena-Silva R, Heistad DD. Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *J Am Coll Cardiol.* 2008; 52:843–850. [PubMed: 18755348]
22. Srivastava D. Making or breaking the heart: from lineage determination to morphogenesis. *Cell.* 2006; 126:1037–1048. [PubMed: 16990131]
23. Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. *Int Rev Cytol.* 2005; 243:287–335. [PubMed: 15797462]
24. Combs MD, Yutzey KE. Heart valve development: regulatory networks in development and disease. *Circ Res.* 2009; 105:408–421. [PubMed: 19713546]
25. Aikawa E, Whittaker P, Farber M, et al. Human semilunar cardiac valve remodeling by activated cells from fetus to adult. *Circulation.* 2006; 113:1344–1352. [PubMed: 16534030]
26. Hinton RB Jr, Lincoln J, Deutsch GH, et al. Extracellular matrix remodeling and organization in developing and diseased aortic valves. *Circ Res.* 2006; 98:1431–1438. [PubMed: 16645142]
27. Chakraborty S, Combs MD, Yutzey KE. Transcriptional regulation of heart valve progenitor cells. *Pediatr Cardiol.* 2010; 31:414–421. [PubMed: 20039031]
28. Chakraborty S, Cheek J, Sakthivel B, Aronow BJ, Yutzey KE. Shared gene expression profiles in developing heart valves and osteoblast progenitor cells. *Physiol Genomics.* 2008; 35:75–85. [PubMed: 18612084]
29. Shelton EL, Yutzey KE. Tbx20 regulation of endocardial cushion cell proliferation and extracellular matrix gene expression. *Dev Biol.* 2007; 302:376–388. [PubMed: 17064679]
30. de la Pompa JL, Timmerman LA, Takimoto H, et al. Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum. *Nature.* 1998; 392:182–186. [PubMed: 9515963]
31. Ranger AM, Grusby MJ, Hodge MR, et al. The transcription factor NF-ATc is essential for cardiac valve formation. *Nature.* 1998; 392:186–190. [PubMed: 9515964]
32. Combs MD, Yutzey KE. VEGF and RANKL regulation of NFATc1 in heart valve development. *Circ Res.* 2009; 105:565–574. [PubMed: 19661463]
33. Lincoln J, Alfieri CM, Yutzey KE. BMP and FGF regulatory pathways control cell lineage diversification of heart valve precursor cells. *Dev Biol.* 2006; 292:290–302.
34. Levay AK, Peacock JD, Lu Y, et al. Scleraxis is required for cell lineage differentiation and extracellular matrix remodeling during murine heart valve formation in vivo. *Circ Res.* 2008; 103:948–956. [PubMed: 18802027]
35. Bosse Y, Miqdad A, Fournier D, Pepin A, Pibarot P, Mathieu P. Refining molecular pathways leading to calcific aortic valve stenosis by studying gene expression profile of normal and calcified stenotic human aortic valves. *Circ Cardiovasc Genet.* 2009; 2:489–498. [PubMed: 20031625]
36. Fondard O, Detaint D, Iung B, et al. Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors. *Eur Heart J.* 2005; 26:1333–1341. [PubMed: 15827062]

37. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*. 1994; 90:844–853. [PubMed: 7519131]
38. Thubrikar MJ, Aouad J, Nolan SP. Patterns of calcific deposits in operatively excised stenotic or purely regurgitant aortic valves and their relation to mechanical stress. *Am J Cardiol*. 1986; 58:304–308. [PubMed: 3739919]
39. Mohler ER 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001; 103:1522–1528. [PubMed: 11257079]
40. Rabkin-Aikawa E, Farber M, Aikawa M, Schoen FJ. Dynamic and reversible changes of interstitial cell phenotype during remodeling of cardiac valves. *J Heart Valve Dis*. 2004; 13:841–847. [PubMed: 15473488]
41. Pho M, Lee W, Watt DR, Laschinger C, Simmons CA, McCulloch CA. Cofilin is a marker of myofibroblast differentiation in cells from porcine aortic cardiac valves. *Am J Physiol Heart Circ Physiol*. 2008; 294:H1767–H1778. [PubMed: 18263709]
42. Caira FC, Stock SR, Gleason TG, et al. Human degenerative valve disease is associated with up-regulation of low-density lipoprotein-related protein 5 receptor-mediated bone formation. *J Am Coll Cardiol*. 2006; 47:1707–1712. [PubMed: 16631011]
43. Cheng SL, Shao JS, Cai J, Sierra OL, Towler DA. Msx2 exerts bone anabolism via canonical Wnt signaling. *J Biol Chem*. 2008; 283:20505–20522. [PubMed: 18487199]
44. Bell DM, Leung KK, Wheatley SC, et al. SOX9 directly regulates the type-II collagen gene. *Nat Genet*. 1997; 16:174–178. [PubMed: 9171829]
45. Komori T, Yagi H, Nomura S, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*. 1997; 89:755–764. [PubMed: 9182763]
46. Zheng Q, Zhou G, Morello R, Chen Y, Garcia-Rojas X, Lee B. Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. *J Cell Biol*. 2003; 162:833–842. [PubMed: 12952936]
47. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002; 108:17–29. [PubMed: 11792318]

Figure 1. Diagram of embryonic valve development and valve maturation

(A) At mouse E10.5, immature cushions have formed in the atrioventricular (AV) canal and outflow tract (OFT). Endocardial derived cells populate the atrioventricular cushions (AVC) and portions of the OFT cushions (blue). Neural crest cells (NCC) (yellow) also contribute to the OFT cushions and aorticopulmonary septum (APS). (A') Transcription factors including *Twist1*, *Tbx20*, *Msx1*, and *Msx2* are important for AVC development. (B) At E13.5, the valve primordia (blue) begin remodeling and start to elongate. The dorsal mesenchymal protrusion (DMP) (purple) has fused with the AVC to form part of the atrial septum (AS). (B') The transcription factors *Sox9*, *Scleraxis*, and *Nfatc1* are important for valve remodeling. (C, D). The mature valves contain highly specialized extracellular matrix in three distinct layers, the collagenous fibrosa layer, the elastin-rich ventricularis/atrialis layers, and the proteoglycan abundant spongiosa layer. Ao= aorta, EC= endocardial cushions, EP= epicardium, EPDC= epicardial derived cells, IVS= interventricular septum, LA=left atrium, LV= left ventricle, RA= right atrium, RV= right ventricle.

Figure 2. The progression of AoV disease

(A) The normal aortic valve (AoV) is stratified into three highly specialized extracellular matrix (ECM) layers. The fibrosa layer is composed primarily of collagens, the spongiosa contains proteoglycans, and the ventricularis layer is rich in elastin. (B) Valvular interstitial cells (VIC) are activated during AoV disease, assuming a myofibroblast phenotype (red stars). (C) Valve sclerosis (orange) involves an overproduction of ECM molecules and maladaptive ECM remodeling, resulting in valve thickening. (D) Late stages of AoV disease are characterized by valve stenosis, which commonly involves valve calcification (black). Ao= aorta.