Conserved Transcriptional Regulatory Mechanisms in Aortic Valve Development and Disease

Elaine E. Wirrig, Katherine E. Yutzey

Abstract—There is increasing evidence for activation of developmental transcriptional regulatory pathways in heart valve disease. Here, we review molecular regulatory mechanisms involved in heart valve progenitor development, leaflet morphogenesis, and extracellular matrix organization that also are active in diseased aortic valves. These include regulators of endothelial-to-mesenchymal transitions, such as the Notch pathway effector RBPJ, and the valve progenitor markers Twist1, Msx1/2, and Sox9. Little is known of the potential reparative or pathological functions of these developmental mechanisms in adult aortic valves, but it is tempting to speculate that valve progenitor cells could contribute to repair in the context of disease. Likewise, loss of either RBPJ or Sox9 leads to aortic valve calcification in mice, supporting a potential therapeutic role in prevention of disease. During aortic valve calcification, transcriptional regulators of osteogenic development are activated in addition to valve progenitor regulatory programs. Specifically, the transcription factor Runx2 and its downstream target genes are induced in calcified valves. Runx2 and osteogenic genes also are induced with vascular calcification, but activation of valve progenitor markers and the cellular context of expression are likely to be different for valve and vascular calcification. Additional research is necessary to determine whether developmental mechanisms contribute to valve repair or whether these pathways can be harnessed for new treatments of heart valve disease. (Arterioscler Thromb Vasc Biol. 2014;34:737-741.)

Key Words: aortic valve stenosis ■ bicuspid aortic valve ■ extracellular matrix ■ fetal heart ■ transcription factors

Heart valve disease, most commonly aortic valve stenosis, is prevalent in the aged population, with ≥2% of the population >65 years being affected.1 The current standard of care is surgical replacement, and there are no pharmacologically based treatments in use clinically.2 The molecular and cellular processes that contribute to aortic valve stenosis are not fully characterized, but could provide insights into the development of new therapeutic approaches. There is increasing evidence that regulatory pathways that control heart valve development also are active with valve pathogenesis later in life. Calcific aortic valve disease (CAVD) includes activation of valve interstitial cells, as well as increased expression of transcription factors that regulate the earliest events of valvulogenesis in the developing embryo.3 In addition to valve developmental pathways, regulatory proteins that promote the development of cartilage and bone lineages also are active in diseased valves.4,5 Thus, knowledge of the molecular regulatory pathways that control valve development will likely be informative in determining the molecular mechanisms of valve pathogenesis. In addition, abnormal valve development before birth can lead to increased or accelerated valve disease later in life. The majority of aortic valves that are replaced as a result of stenosis are congenitally malformed with an abnormal number of leaflets, usually bicuspid rather than tricuspid aortic valves.6 In addition, these abnormal valves require replacement at an earlier age compared with normal tricuspid aortic valves.5 Here, we review transcriptional regulatory pathways that control heart valve development and their contributions and implications for heart valve disease, specifically CAVD.

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Overview of Heart Valve Development

During embryogenesis, heart valve development begins with the formation of endocardial cushions (ECs) in the outflow tract and atrioventricular canal of the primitive heart tube (see the Figure). The molecular and cellular mechanisms of EC formation are conserved in birds and mammals and begin at embryonic day (E) 9 to E10 in mice and E31 to E35 in humans.5 The ECs consist of endocardial endothelial cells that undergo an endothelial-to-mesenchymal transition (EMT), giving rise to mesenchymal valve progenitor cells that reside in a hyaluronic-rich extracellular matrix (ECM). Valvulogenesis continues with elongation and thinning of the ECs to form valve primordia of the semilunar and atrioventricular valves. During fetal and postnatal stages, the ECM of the valve leaflets continues to remodel and compartmentalize into the collagen-rich fibrosa, proteoglycan-rich spongiosa, and elastin-rich atrialis/
ventricularis layers. The ECM composition and organization of the valve leaflets is critical for normal valve function, and dysregulation of ECM remodeling or structural components can lead to valve malformations or pathogenesis, which may be apparent soon after birth or later in life.

Cells from multiple embryonic origins contribute to mature semilunar and atrioventricular valve leaflets as determined by Cre-based cell lineage studies in mice. The predominant source of interstitial cells in all 4 mature valves is the endothelial layer–derived EC cells, as indicated by Tie2–Cre lineage tracing. In adult semilunar valves, neural crest-derived cells, marked with Wnt1–Cre, are present and preferentially localize in the leaflets adjacent to the aorticopulmonary septum. Recent studies using Wilms tumor 1–Cre demonstrate the presence of epicardial-derived cells in the parietal, but not septal, leaflets of the atrioventricular valves. Additional bone marrow–derived cells have been reported to be present in aortic valve leaflets during development and after birth. There is increasing evidence for diversity in cellular origins of individual valve leaflets during development and in adults. Currently, it is not known whether these subpopulations have distinct functions in normal valve homeostasis or have specific roles in valve pathogenesis.

Transcriptional Regulation in ECs and Valve Progenitor Cells

The gene regulatory network of valve progenitor cells in the ECs includes several transcription factors involved in EMT and mesenchymal progenitor populations in other organ systems, including bone and cartilage lineages in the developing skeleton (Table). EC EMT requires Notch signaling acting through the transcription factor RBPJ, which activates expression of the zinc finger transcription factor Snai1. Snai1 is a transcriptional repressor of VE-cadherin (Cdh5), and loss of endothelial cell junctions is required for the transition to a mesenchymal phenotype. Proliferative and migratory mesenchymal cells populate the EC with undifferentiated valve progenitor cells. There is increasing evidence for diversity in cellular origins of individual valve leaflets during development and in adults.

Table. Transcription Factors in Heart Valve Development and Disease

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Role in Development</th>
<th>Role in Disease</th>
<th>Target Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twist1*</td>
<td>EC proliferation, migration</td>
<td>Active in CAVD</td>
<td>Tbx20, Cdh11, Col2a1</td>
</tr>
<tr>
<td>Msx2</td>
<td>EMT, proliferation</td>
<td>Active in CAVD</td>
<td>None identified</td>
</tr>
<tr>
<td>Snai1</td>
<td>EMT</td>
<td>Unknown</td>
<td>Cdh5</td>
</tr>
<tr>
<td>RBPJ</td>
<td>EMT</td>
<td>Loss leads to CAVD</td>
<td>Snai1, Hey1, Hey2</td>
</tr>
<tr>
<td>Hey2</td>
<td>EMT</td>
<td>Inhibits Runx2</td>
<td>Bglap</td>
</tr>
<tr>
<td>Sox9</td>
<td>Proliferation, PG expression</td>
<td>Inhibits CAVD</td>
<td>Col2a1, Acan, Hapln1</td>
</tr>
<tr>
<td>Nfatc1</td>
<td>EC growth, remodeling</td>
<td>Reported in CAVD</td>
<td>CtsK</td>
</tr>
<tr>
<td>Gata5</td>
<td>EC growth</td>
<td>Loss linked to BAV</td>
<td>Nos3</td>
</tr>
<tr>
<td>Runx2</td>
<td>Not present</td>
<td>Osteogenesis</td>
<td>Bglap, ALP</td>
</tr>
</tbody>
</table>

BAV indicates bicuspid aortic valve; CAVD, calcific aortic valve disease; EC, endocardial cushion; EMT, endothelial-to-mesenchymal transition; and PG, proteoglycan.

*See text for details and citations.
Thus, the study of cardiac valve ECM regulatory mechanisms has been greatly facilitated by knowledge obtained from cartilage, tendon, and bone lineages.

**Regulation of Semilunar Valve Leaflet Number and Morphogenesis**

Congenital malformations of the aortic valve, predominantly bicuspid aortic valve (BAV), are the most common congenital heart malformations, occurring with a frequency of \( \approx 1\% \) to 2\% of live births.\(^{31}\) There is little known of molecular and cellular mechanisms that control the formation of individual semilunar valve leaflets from ECs after septation of the cardiac outflow tract in the embryo. The Notch signaling pathway is important in this process because mutations in the Notch1 receptor are associated with human BAV.\(^{32}\) However, mice heterozygous for Notch1 or the downstream transcription factor RBPJ do not have a significant incidence of BAV.\(^{33}\) Notch1 and RBPJ are required for EMT during EC formation; thus, BAV could result from abnormalities present at the earliest stages of heart valve development.\(^{37}\) It is possible that other Notch receptors contribute to valve leaflet formation, but these regulatory interactions have not been fully defined. Mutations in the zinc finger transcription factor Gata5 also have been identified in patients with BAV, and mice with heterozygous loss of Gata5 have BAV with incomplete penetrance.\(^{34,35}\) The heterogeneity of valve malformations in Gata5 heterozygous mice makes it difficult to determine the developmental anomalies that lead to BAV, but defects in EC formation were noted in these animals.\(^{35}\) The paucity of mouse models with BAV at high frequency has limited our understanding of the late stages of semilunar valve morphogenesis and leaflet number determination.

**Developmental Transcription Factors in CAVD**

Many transcriptional regulatory mechanisms of heart valve development also are active in diseased aortic valves. In addition to being critical for EC EMT and valve leaflet formation, Notch signaling has a direct role in inhibition of aortic valve calcification.\(^{32}\) Adult mice with heterozygous loss of Notch1 or RBPJ fed a high-fat diet exhibit aortic valve calcification at several months of age.\(^{33}\) Likewise, adult mice with endothelial deletion of the Notch ligand Jag1 exhibit valve calcification late in life.\(^{36}\) The Notch pathway downstream transcription factor Hesr-2/Hey2 can directly repress osteogenic gene induction by the transcription factor Runx2.\(^{32}\) Similarly, loss of Hey2 in mice leads to aortic valve stenosis and osteogenic gene induction, supporting a direct regulatory role for Notch pathway transcriptional effectors in inhibiting adult valve calcification.\(^{37}\) Together, these studies support an inhibitory role for Notch signaling and its downstream transcriptional effectors in aortic valve calcification. Sox9, required for cartilage lineage development, also has been implicated as a protective factor in CAVD. Mice heterozygous for Sox9 in Col2a1-expressing lineages exhibit aortic valve calcification, and ectopic expression of Sox9 can inhibit valve leaflet mineralization and osteogenic gene induction.\(^{22,38}\) Sox9 expression is increased in pediatric diseased aortic valves that are not calcified and also in adult calcified aortic valves; thus, its roles in the initiation and progression of human aortic valve disease

**Valve Leaflet Morphogenesis and ECM Stratification/Remodeling**

Valve leaflet morphogenesis includes the elongation and thinning of ECs accompanied by remodeling and stratification of the ECM. EC mesenchymal cells are highly proliferative, but proliferation is subsequently reduced during leaflet elongation, and valve interstitial cells remain relatively quiescent in the mature valve leaflets.\(^{7,4}\) The reduction of EC proliferation coincides with the expression of mature ECM proteins, including elastin and fibrillar collagen. The transition from EC to remodeling valve leaflet is regulated by the transcription factor Nfatc1. Loss of Nfatc1 in mice leads to embryonic death at midgestation with apparent heart valve remodeling defects.\(^{25,26}\) Nfatc1 is expressed in EC endothelial cells where it promotes cell proliferation and limits EMT in response to VEGF signaling.\(^{27}\) In the elongating valve primordia, Nfatc1, regulated by RANKL signaling, activates expression of cathepsin K and promotes ECM remodeling.\(^{27}\) Additional transcriptional regulatory mechanisms that control ECM remodeling enzyme gene expression or valve interstitial cell cycle withdrawal are relatively undefined.

The ECM compartments of the stratified leaflets share conserved regulatory mechanisms with other connective tissue cell lineages.\(^{10}\) The spongiosa layer is composed predominantly of proteoglycans, and loss of Sox9, a transcription factor required for cartilage development, leads to decreased proteoglycan expression in the remodeling valves.\(^{22}\) Scleraxis, a bHLH protein required for tendon development, also is expressed in remodeling chordae tendineae of the atrioventricular valves, and loss of scleraxis leads to valve remodeling defects and myxomatous phenotypes in adult animals.\(^{29,30}\) Upstream regulators and downstream targets of these transcription factors defined in cartilage and tendon lineages also are active in the developing valves. In avian valve progenitor cultures, BMP2 treatment promotes Sox9 expression and target gene Aggrecan induction, whereas PFG4 treatment promotes scleraxis expression and tenasin gene induction.\(^{29}\) Osteogenic transcription factors, such as Runx2, are not active in normal developing valves, although they are induced with CAVD.\(^{4,5}\) Thus, the study of cardiac valve ECM regulatory mechanisms
are not fully known.\textsuperscript{3} Msx2 is similarly expressed in human diseased valves and is sufficient to induce vascular calcification through induction of Wnt signaling,\textsuperscript{3,39} but its role in aortic valve calcification has not been defined.\textsuperscript{Nfatc1} also has been reported to be expressed in calcified aortic valves, and the RANKL antagonist osteoprotogerin inhibits aortic valve calcification in hypercholesterolemic mice.\textsuperscript{40,41} Osteogenic genes, including Runx2 and its downstream targets osteocalcin (Bglap) and alkaline phosphatase (ALP), also are expressed in CAVD, but the regulatory mechanisms by which they are induced have not been fully defined.\textsuperscript{42} Thus, it is becoming increasingly clear that many developmental pathways are active in adult valve disease. However, the pathological or reparative functions of these regulatory mechanisms are not known and their therapeutic potential is yet to be exploited.

**Future Directions and Clinical Perspectives**

Much remains to be determined regarding the cellular and molecular mechanisms of aortic valve disease. Although developmental pathways are clearly activated, the cells that express valve progenitor gene regulatory networks or the mechanisms by which these pathways are activated remain unknown. It has been proposed that new valve progenitors arise by induction of EMT in adult valves, but this has not yet been demonstrated in the context of disease in vivo.\textsuperscript{43} The activation of resident valve interstitial cells has been studied extensively as an underlying mechanism of disease, but the origins of cells expressing valve progenitor markers in diseased aortic valves has not yet been determined.\textsuperscript{44} Likewise, collagen remodeling occurs with development of the fibrosa layer of the valve leaflets and also is increased with aortic valve disease, but the relationship between valve fibrosis and mineralization is not completely clear.\textsuperscript{10,45} Infiltrating mesenchymal stem cells and immune cells also have been detected in diseased aortic valves, but their specific contributions to valve thickening or mineralization are not currently known.\textsuperscript{16,44} Together, extensive progress has been made in identifying molecular and cellular processes active in valve disease. However, specific pathogenic or reparative functions are less well defined.

Molecular mechanisms of aortic valve and vascular calcification both include activation of osteogenic factors, such as Runx2\textsuperscript{22,46} However, the cell types involved are likely to be different in that smooth muscle cells, not present in aortic valve leaflets, are predominant in vascular calcification, and inflammation has a significant role in vascular calcification that may not be fully recapitulated in aortic valve disease. In addition, the activation of valve developmental regulatory programs, although apparent in CAVD, has not been reported for vascular calcification. Thus, the therapeutic approaches effective in preventing vascular calcification may not be effective in treating CAVD. It has been demonstrated that statin therapies, widely in use for atherosclerosis, were not successful in inhibiting or preventing the progression of CAVD in clinical trials.\textsuperscript{47} Therefore, the development of effective therapies for CAVD may need to take into account molecular mechanisms or cellular contributions unique to aortic valve development or pathogenesis.

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**Disclosures**

None.

**References**


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