Developmental Mechanisms of Aortic Valve Malformation and Disease

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Abstract

Normal aortic valves are composed of valve endothelial cells (VECs) and valve interstitial cells (VICs). VICs are the major cell population and have distinct embryonic origins in the endocardium and cardiac neural crest cells. Cell signaling between the VECs and VICs plays critical roles in aortic valve morphogenesis. Disruption of major cell signaling pathways results in aortic valve malformations, including bicuspid aortic valve (BAV). BAV is a common congenital heart valve disease that may lead to calcific aortic valve disease (CAVD), but there is currently no effective medical treatment for this beyond surgical replacement. Mouse and human studies have identified causative gene mutations for BAV and CAVD via disrupted VEC to VIC signaling. Future studies on the developmental signaling mechanisms underlying aortic valve malformations and the pathogenesis of CAVD using genetically modified mouse models and patient-induced pluripotent stem cells may identify new effective therapeutic targets for the disease.

INTRODUCTION

CAVD: calcific aortic valve disease

BAV: bicuspid aortic valve

VEC: valve endothelial cell

VIC: valve interstitial cell

EMT: epithelial to mesenchymal transformation

NCC: neural crest cell

OFT: outflow tract

ECM: extracellular matrix

AVC: atrioventricular canal

The aortic valve ensures the unidirectional systemic circulation of blood flow from the left ventricle to the aorta during the cardiac cycle. Homeostasis of aortic valves is critical for durable valve function throughout life. Disruption of valve maintenance may lead to calcific aortic valve disease (CAVD). CAVD is a major health problem with risk of severe morbidity and mortality that has no effective medical treatment beyond surgical valve replacement (1–5). Aortic valve malformations, including the bicuspid aortic valve (BAV), are present in ~2% of newborns (6). Malformed aortic valves are associated with the high incidence of CAVD later in life, suggesting developmental and genetic origins of aortic valve disease.

The aortic valve is made of valve endothelial cells (VECs) and valve interstitial cells (VICs), with the latter derived from embryonic endocardial cells through epithelial to mesenchymal transformation (EMT) during development (7–10). The cardiac neural crest cells (NCCs) also contribute to a subpopulation of the aortic VICs (11). The EMT process gives rise to the valve mesenchymal progenitor cells in the endocardial cushions of the proximal cardiac outflow tract (OFT), whereas the cushion mesenchyme of the distal OFT originates from NCCs. These endocardial cushions function as the valve primordium for unidirectional blood flow in the early embryonic heart. The cushions then undergo a complicated and underappreciated remodeling process that sculptures the primitive valve primordia into the mature aortic valve leaflets with three distinct extracellular matrix (ECM) layers necessary for normal valve structure and function (12–14).

Both endocardial cushion formation and valve remodeling require proper cell functions and interactions supported by ECM and regulated by signals from multiple molecular pathways (TGF-β/BMP, NOTCH, WNT, VEGF, FGF, and EGFR) (14, 15). Evidence emerging from mouse studies indicates that aberrant signaling in VECs or VICs or between the two cell populations can account for abnormal formation of the aortic valve and may lead to CAVD over time (16–19). Human genetic studies have also uncovered genetic mutations in major signaling as well as ECM genes that are strongly associated with BAV, a predisposition of CAVD (20, 21). Advanced mouse genetics, induced pluripotent stem cells (iPSCs), and tissue engineering technologies offer unprecedented opportunities for scientists and physicians to reveal the molecular signals underlying aortic valve development and disease. These combined approaches may identify the disease-specific targets that can be used to develop novel therapies for aortic valve malformations and calcification.

AORTIC VALVE DEVELOPMENT

Overview of Cardiogenesis

During embryogenesis, the heart is the first organ to develop and function. The embryonic heart arises from mesodermal cells located in the anterior part of the primitive streak (22, 23). These cardiac progenitor cells migrate from the streak to the splanchnic mesoderm to form the cardiac crescent or the first heart field (24, 25). As the embryo grows, the crescent of the first heart field fuses at the ventral midline to form a heart tube composed of an external myocardial cell layer surrounding an internal endocardial layer (14, 26). The primitive heart tube elongates at the arterial and venous pools through the addition of progenitor cells from the secondary heart fields located medially and posteriorly to the first heart field (27–30). The elongated heart tube then undergoes rightward looping that forms morphologically distinct atria, the atrioventricular canal (AVC), ventricles, and the OFT. It is during this looping stage that the cardiac valves begin to develop concurrently with cardiac septation to eventually form a four-chamber heart with the separation of systemic and pulmonic circulations.

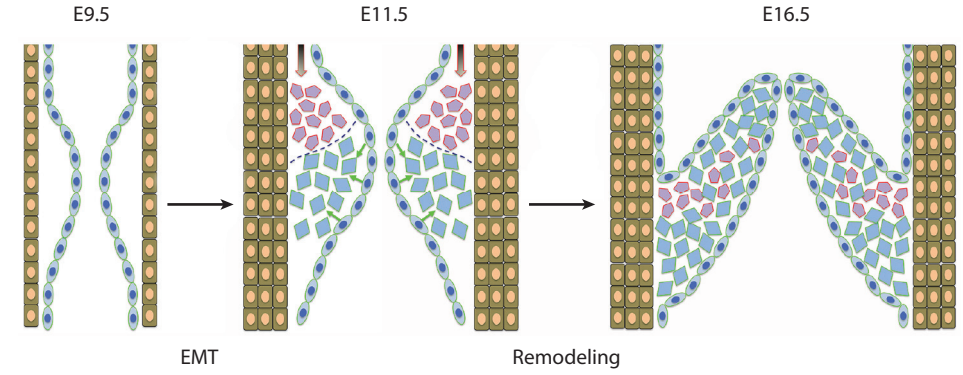

Figure 1

Schematic summary of aortic valve morphogenesis. Aortic valve morphogenesis in mouse embryo starts at embryonic day (E) 9.5 with the formation of matrix-rich and acellular tissue swellings, named endocardial cushions. Both myocardial (*yellow*) and endocardial (*blue*) cells of the proximal cardiac OFT region are the sources of ECM proteins. At approximately E10.5, a subpopulation of endocardial cells begins to delaminate from the epithelial layer of the cushions, in response to myocardial signals such as BMP4, and invade the ECM-rich cushions via a classic process of EMT. By E11.5 the OFT cushions are already fully cellularized. This EMT process occurs mainly in the proximal OFT cushions, whereas the distal end is populated by cardiac neural crest cells (*purple*). The OFT cushion mesenchyme formation is controlled by signals from myocardium, endocardium, and neural crest cells. Subsequently, these cushions undergo complicated remodeling through balanced proliferation and apoptosis, as well as ECM rearrangement, which shape the mesenchymal cushions into mature valves with fine defined leaflets. Abbreviations: ECM, extracellular matrix; EMT, epithelial to mesenchymal transformation; OFT, outflow tract.

Aortic Valve Morphogenesis

The first sign of valve development during vertebrate embryogenesis is the formation of tissue swellings, termed endocardial cushions, in the OFT and AVC regions of the looped heart tube (**Figure 1**). These cushions are formed by the accumulation of hyaluronan-rich cardiac jelly between the endocardium and myocardium, which is initially acellular. The cushions are then populated by mesenchymal cells derived from the endocardial cells by EMT (7, 9, 31). The EMT process is induced by myocardial signals to the endocardial cells (32, 33). The mesenchymal progenitor cells from EMT contribute to most VICs of adult valves (9, 34). In the OFT, the cushion mesenchymal cells come from two distinct origins. The endocardial cells contribute to the proximal cushions by EMT, whereas the distal cushions contain mesenchymal cells derived from the cardiac neural crest that has migrated into the OFT from the dorsal neural tube (11).

The EMT process gives rise to the mesenchymal progenitor cells that are initially highly proliferative in a loosely organized ECM (35). This allows these cells to quickly cellularize the cushions. The mesenchymal cushions perform the valve-like function as primitive valves to drive unidirectional blood flow in the embryonic heart (36). The cushions of the AVC then fuse medially and contact the ventricular septum from below and the protruding dorsal mesocardium from above that completes the septation of the heart into left and right sides (37–39).

The OFT contains the proximal and distal cushions according to their location. The two parts of cushions have distinct cellular origins and spatiotemporal configurations, and they undergo septation in different ways (40, 41). The septation of the OFT into the aortic and pulmonic outlets requires correct fusion of the two main OFT cushions (42). The downward migration of cardiac NCCs from the aortic sac and their expansion give rise to the aorticopulmonary septum

that divides the distal OFT (43) and separates the distal OFT cushions into two sets of three-leaflet primordia (40–42), which later remodel into respective mature aortic and pulmonic valves, each with three leaflets. The process involves the proper contributions of two mesenchymal progenitor populations (44–46), elongation of the distal cushions by balanced proliferation and apoptosis (16, 47), and remodeling of the ECM into layers rich in elastin (ventricularis), fibrillar collagen (fibrosa), and proteoglycans (spongiosa) (13, 48). The fibrosa side of the aortic valve cushions gets sculpted through an invagination process driven by apoptosis (49), and the free leading edge of the valve cushion is elongated by highly proliferative VECs (45). The correct formation and function of the aortic valve ensure that blood flows in one direction from the left ventricle to the aorta in the adult heart.

Embryonic Origins of Aortic Valve Cells

Multiple cells of distinct embryonic origins contribute to the formation of the aortic valve. The VECs that surround the aortic valve leaflets are part of the cardiac endocardium and form a continuous epithelial cell layer with the ventricular endocardium on the ventricular side and with the aortic endothelium on the aortic side. Cell lineage tracing in mice shows that the progenitors of OFT VECs are derived from the secondary heart field (50). In vivo lineage tracing in mouse embryos and ex vivo culture of early chick and mouse valve tissues also show the existence of two distinct aortic valve mesenchymal lineages arising separately from the endocardium and the cardiac NCCs (34, 51). As outlined above, the endocardium-derived lineage contributes primarily to the mesenchymal cushions of the proximal OFT endocardial cushions via EMT, whereas the cardiac NCC lineage contributes to the mesenchyme of the distal OFT cushions through invasion and transdifferentiation. Overall, lineage-tracing studies in mice demonstrate that most VICs of the mature aortic valve arise from endocardially derived mesenchymal cells in the OFT endocardial cushions, whereas the NCC-derived valve mesenchymal cells contribute to only a small part of VICs in the adult aortic valve leaflets (13, 45, 46, 52) (**Figure 1**). These observations suggest specific spatiotemporal functions of distinct valve progenitor cells in the morphogenesis of the aortic valve. In the adult aortic valve, VICs from the VEC origin are important for valve homeostasis. Whether the VICs of different origins may have specific roles in the homeostasis and function of adult valves is currently unknown. Of additional note, as well as the two embryonic origins of VICs discussed above, circulating CD45-positive hematopoietic stem cells are recruited to the adult valves and become part of VICs, thus serving potential functions in valve homeostasis (53).

MECHANISMS OF AORTIC VALVE DEVELOPMENT

Molecular Mechanisms of Endocardial Cushion Formation

Previous studies have focused on EMT in the AVC, with an assumption that similar mechanisms may underlie the formation of OFT cushions. These studies show that multiple molecular and cellular signaling pathways have critical functions in the EMT process. In mice early tissue swelling from the production of hyaluronic acid–rich ECM in the OFT and AVC requires localized signals from myocardial VEGF (54–57). In addition, BMP4 (and BMP2) signaling from the myocardium to the endocardium in the OFT and AVC is required for the initial induction of EMT (32). Canonical WNT (58, 59) and NOTCH signaling pathways (60–64) are also required for EMT and the proliferation of mesenchymal endocardial cushion cells. Notably, recent studies show that VEGF and WNT signaling pathways differentially regulate EMT in the AVC and OFT

(65, 66). Only a small subset of endocardial cells undergoes EMT, with the majority requiring NFATC1 to maintain an endocardial endothelial phenotype and also to promote proliferation of the epithelial monolayer covering the growing endocardial cushions (45). The mesenchymal cells of the endocardial cushions also are highly proliferative and express TWIST1, MSX1/2, SOX9, and TBX20 transcription factors that promote cell proliferation, migration, and ECM synthesis in the endocardial cushions (64, 67–69). Together, the proliferation of transformed mesenchymal cells with increased ECM production rapidly cellularizes the endocardial cushions, which project into the lumens of OFT and AVC and control the direction of blood flow.

Molecular Mechanisms of Aortic Valve Remodeling

Shortly after EMT ceases, the mesenchymal cushions undergo a series of changes, including condensation and extension into the mature leaflets. The molecular mechanisms underlying this transition and subsequent changes are understudied compared to the EMT process. Hence, the process of valve maturation is broadly called remodeling. The formation of OFT valve leaflets occurs by the downward invagination of the distal OFT cushions and a selective growth of free edges to form a characteristic semilunar shape (49). As outlined above, it is known that the OFT cushions include progenitor cells from the secondary heart field endocardium and cardiac neural crest. Both mesenchymal lineages coordinately apply spatiotemporal cellular and molecular mechanisms in OFT valve remodeling (**Figure 2*b***) (44–46, 70). The cardiac NCC-derived mesenchymal progenitors in the distal OFT cushions form a tissue boundary with the endocardially derived proximal OFT cushions that likely delineates the anatomic location where the OFT valves will eventually form. Correct tissue contact is regulated by endocardial NFATC1 (45, 46) and NOTCH signaling in the second heart field as well as cardiac neural crest lineages (44, 70).

The transition from endocardial cushion to remodeling valves requires the NFATC1 transcription factor, which promotes the expression of the ECM-remodeling gene cathepsin-K in VECs during valve elongation (66, 71). Cathepsin K is also expressed by the osteoclasts in remodeling bone. Indeed, cushion mesenchymal progenitors are able to undergo osteogenic-like differentiation (72). Subsequent valve maturation requires cushion condensation, involved in remodeling of ECM and changes in mesenchymal cell phenotypes. This process begins with a tissue-specific gene programming driven by SOX9 and TWIST1, which also promote the expression of cartilage genes. BMP2 signaling activates the SOX9 transcription factor, aggrecan gene expression in cartilage precursors, and the proliferation of valve progenitors (67, 73). BMP/TGF-β signals also regulate the maturation of the embryonic valve mesenchymal cells from an activated myofibroblast-like progenitor to a quiescent adult-like fibroblast phenotype of VICs in vitro (74). FGF and WNT signaling is activated in the developing valves and promotes expression of genes characteristic of the collagen-rich fibrosa layer in cultured VICs (73, 75). It is worth noting that the expression of osteogenic genes is present in the calcified adult aortic valves, suggesting a reactivation of embryonic gene programming underlying the pathogenesis (76). In addition, mouse genetic studies show that disruption of key signaling factors such as SMAD6, an inhibitor of BMP signaling (77), and NOTCH1 in the NOTCH pathway (16) can predispose valves to bone-like formation and early calcification. Together, these observations show that the signaling molecules and transcription factors that control differentiation of connective tissues also regulate the expression of ECM genes in the developing valves. Besides ECM remodeling and transdifferentiation of cushion mesenchymal progenitor cells, the process of cushion elongation requires VEGF and NFATC1 expressed in the endocardium to maintain a high proliferation of endocardial cells at the leading edge while suppressing EMT (12, 45, 65).

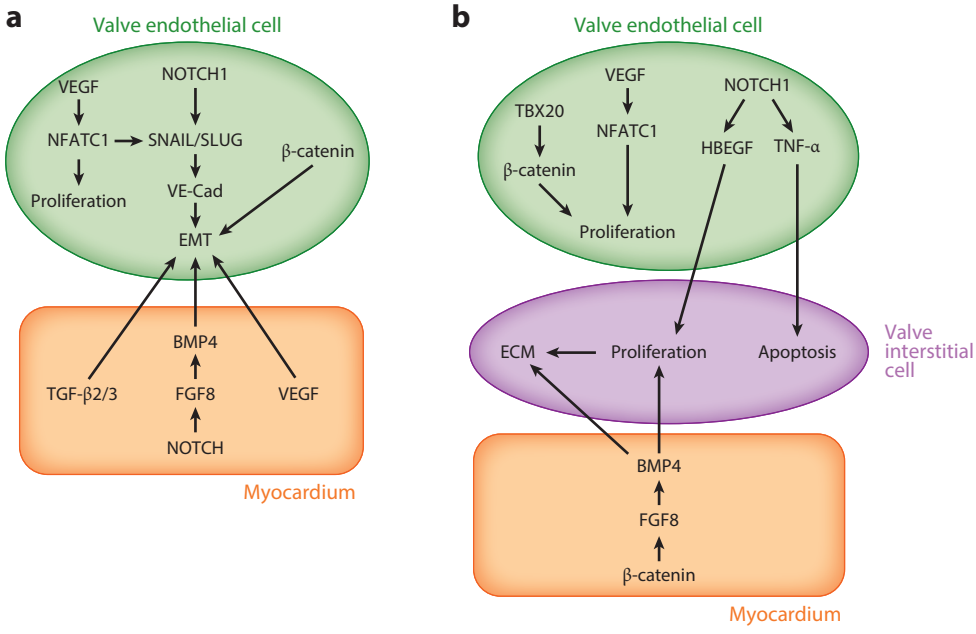

Figure 2

Schematic summary of molecular pathways underlying aortic valve development. (*a*) Molecular pathways regulate EMT during outflow tract cushion development. After receiving a signal from the adjacent myocardium, the VECs located within the outflow tract region start to undergo EMT at approximately embryonic day (E) 10.5 in mouse embryo. The EMT process is regulated by myocardial signals, including TGF-β, BMP4, VEGF, FGF, and NOTCH. TGF-β induces EMT, and FGF signaling can induce BMP4 expression that then promotes VEC EMT. Later, VEGF secreted by cardiomyocytes represses EMT but promotes VEC proliferation. Within VEC, NOTCH1 signaling promotes EMT by repressing VE-cadherin expression via SNAIL/SLUG. In contrast, NFATC1 represses EMT by inhibiting SNAIL/SLUG expression. NFATC1 also mediates VEGF function to promote VEC proliferation. WNT/β-catenin signaling in VECs is required for EMT. (*b*) Molecular pathways regulate post-EMT cushion remodeling into mature heart valves. During valve remodeling, VICs receive multiple signals from VECs and myocardium that collectively regulate the balanced proliferation and apoptosis of VIC. VICs also produce ECM proteins regulated by myocardial BMP4 and critical for stratifying the valve leaflets. NOTCH1 in VECs coordinates the aortic valve remodeling by controlling the balanced proliferation and apoptosis of VICs through HBEGF and TNF-α, respectively. VEGF in VECs regulates VEC proliferation through NFATC1. In addition, TBX20 promotes VEC proliferation through regulating WNT/β-catenin signaling. A WNT/β-catenin-FGF8-BMP4 signal in the myocardium regulates VIC proliferation and differentiation. Abbreviations: EC, valve endothelial cell; ECM, extracellular matrix; EMT, epithelial to mesenchymal transformation; HBEGF, heparin-binding epidermal growth factor; VE-Cad, vascular endothelial-cadherin; VIC, valve interstitial cell.

Hemodynamic Regulation of Valve Formation

The proper formation of valves requires hemodynamic stimuli. During heart looping, shear stress is greatest in the inner curvature and sites of lumen constrictions in the AVC and OFT where endocardial cushions form and valves arise (78). Later in development, the cushions elongate to form thin fibrous leaflets with stratified ECM proteins and greater mechanical stiffness (79, 80). Regulation of the valve leaflet formation is likely heavily influenced by mechanotransduction. VECs lining the endocardial cushion surface may promote valve morphogenesis by coupling mechanical stimuli and molecular signaling pathways (81). A number of studies demonstrate that

the shear stress controls the valve morphogenesis in vivo. Shear stress and shear stress–induced molecular signals regulate the remodeling of the endocardial cushions (82). The expression of signaling molecules critical for valve formation, such as TGF-β, BMP, and VEGF, is spatiotemporally regulated by hemodynamic forces (74, 83). Altered hemodynamic flow patterns during critical periods of development are shown to lead to a variety of cardiac abnormalities, many of which influence valve formation (83).

AORTIC VALVE COMPONENTS AND FUNCTION

Valve Endothelial Cells

The endothelial cells of the aortic valve form an endothelium surface covering the valve leaflets. The VECs are a special endothelial population with unique functions in aortic valve development and disease. They play critical roles in valve homeostasis, and their dysfunction is the initial step in a cascade of events leading to CAVD, with some similarities to the process that occurs in atherosclerosis (84). Recent studies indicate that the VECs have unique functions compared to other vascular endothelial cell populations. For example, the aortic VECs align perpendicularly to the direction of flow (85), whereas the vascular endothelium aligns in parallel to the flow direction (86). The different cellular orientations relative to the flow direction are associated with different mechanotransduction pathways in each type of endothelium. The luminal surface of VECs senses the flow change and communicates it to the cytoskeleton to activate several signaling pathways, such as the secretion of nitric oxide (NO) and endothelin-1 (ET-1) in response to sheer stress (87, 88). Transcription profiling for the VECs versus vascular endothelium shows a set of genes with differential expression, and those genes with a higher expression in the VECs are transcription factors associated with higher proliferation rates (89, 90). In addition, VECs express more genes involving chondrogenesis, whereas vascular endothelial cells express more genes associated with osteogenesis. Shear stress reduces the expression of osteogenic genes (90). Therefore, signaling molecules released from the VECs can transmit the flow change to the underlying VICs and impact valve function.

Valve Interstitial Cells

The VICs are the main cellular component of the aortic valve and consist of at least two different subtypes: smooth muscle α-actin (SMA)-positive and SMA-negative cells. SMA-expressing VICs are activated myofibroblasts that express contractile proteins, whereas the SMA-negative VICs are the quiescent fibroblasts. The active VICs are involved in valve calcification (91, 92). The change in the phenotypes of VICs is thought to be part of normal valve homeostasis as well as the pathogenesis of calcifying valves, as aortic VICs are able to activate gene profiles related to osteoblasts, adipocytes, and chondrocytes during calcification (93, 94). Cell–cell communication among VICs is essential to regulate their phenotypes and function (95, 96). For example, overexpression of cadherin-11 in the mouse heart valves results in hemodynamically significant CAVD that is associated with upregulation of the osteoblastic and myofibroblastic markers in VICs and extensive pathogenic ECM reorganization (97).

Extracellular Matrix

The aortic valve is a delicate, yet strong and durable tissue due partly to its precise ECM composition and organization. Different hemodynamic stresses contribute to valve deformation or

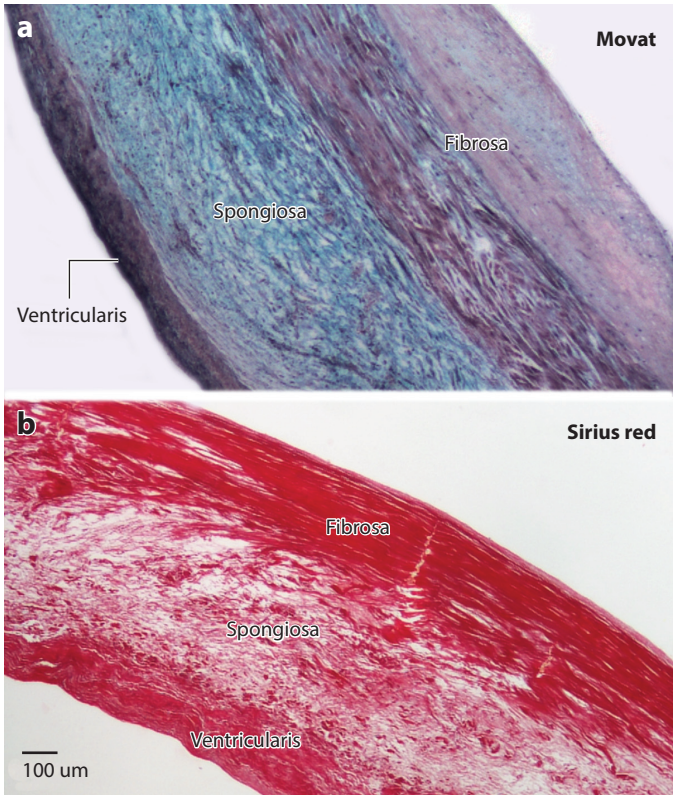

Figure 3

(*a*) Movat's pentachrome staining of one leaflet of human aortic valve shows valve extracellular matrix (ECM) structure and composition. Three stratified ECM layers compose the collagen-rich fibrosa layer, the elastin-rich ventricularis layer, and the proteoglycan-rich spongiosa layer. (*b*) Sirius red staining for collagens shows the three layers of a human aortic valve leaflet.

displacement of the valve tissue (98). These deformation forces are counterbalanced by different ECM deposition. The aortic valve is thicker with increased collagen fibers compared to the pulmonic valve (99). Clinically, aortic valve disease may result from predisposing genetic variants and valve malformations that alter its response to hemodynamic stress, thus impacting valve maintenance. Histologically, the aortic valve is composed of three distinct layers: the fibrosa, spongiosa, and ventricularis (**Figure 3**). The fibrosa lies on the aortic side of the leaflet and the ventricularis on the ventricular-facing side with the spongiosa between these two layers. The fibrosa represents nearly half of the thickness of the leaflet and is rich in collagens, mainly types I and III, providing structural strength to the leaflet and stretching stiffness to the leaflet (100). In contrast, the ventricularis is the thinnest layer of the leaflet and composed mainly of elastin fibers that confer elasticity on the valve and facilitate valve motion by allowing extension and recoil during the cardiac cycle (101). The spongiosa layer is rich in proteoglycans and glycosaminoglycans with a high hydrous content that provides tissue compressibility, allowing smooth sliding of the fibrosa and ventricularis layers during the cardiac cycle (102). The annulus, composed mainly of collagens, fastens the leaflets to the aortic root and supports the movement of the leaflets (13). The mature aortic valve requires a fine balance between stiffness and flexibility to close and open properly. This function relies on proper stoichiometry and the distribution of ECM components. The VICs

express genes that encode collagens, chondroitin sulfate proteoglycans, and elastin, associated with the stratified ECM of the valve leaflets (47, 48), as well as ECM-remodeling enzymes, such as matrix metalloproteases and tissue inhibitors of matrix metalloproteases (47, 91). Note that VICs are largely quiescent in normal adult valves; they are not proliferative and only produce basal levels of ECM synthesis needed for valve homeostasis (47).

In addition to defining physical characteristics of the valve leaflets, the ECM also provides microenvironments that modulate the function of VICs. Thus, the interaction between the ECM and VICs, as well as with the surface VECs, is crucial to valve homeostasis. The ECM is responsible for transmitting the mechanical forces experienced by the VECs to the VICs. The VICs express integrin proteins known to have dual functions in cellular anchoring to the ECM and signal transduction (103). Indeed, mice lacking major ECM proteins have developmental defects in valve formation and function (13). For example, mice lacking elastin do not survive after birth due to vascular obstruction, and heterozygous elastin mice have aortic valve abnormalities in adulthood (104–106). Therefore, the expression and organization of diverse ECM components are essential to the morphogenesis and structural integrity of the valves. Mature aortic valve composition and biomechanics reflect underlying hemodynamics (107, 108).

DEVELOPMENTAL AND GENETIC ORIGINS OF AORTIC VALVE DISEASE

Aortic Valve Disease

The prevalence of aortic valve disease is 2.5% of the general population in the United States (109). CAVD is present in more than 25% of the aged population in the United States and may cause significant complications, ultimately compromising cardiac function when the disease progresses irreversibly (110). Currently, there is no effective medical treatment for CAVD, and the present standard of care is surgical replacement of the diseased valves, with potential complications or contraindications, especially in elderly patients. Therefore, the public health impact of aortic valve disease is quite significant.

In the past, the progressive degeneration of the valve due to "wear and tear" was thought to cause the structural changes in the stenotic aortic valve. The diseased valve is characterized by increased fibrosis with collagen accumulation, proteoglycan degradation, and elastic fiber fragmentation. CAVD is further characterized by sclerosis, progressive fibrosis, and calcification. These changes result in a stiff valve with restricted movement. In recent years, CAVD has been considered as an active cell-driven process that shares some similarities with atherosclerosis, such as endothelial dysfunction, lipid deposition, and inflammatory cell infiltration. However, lipid lowering by statin therapy that is used for atherosclerosis does not impact the valve calcification or prevent the need for aortic valve replacement (111). CAVD is now recognized as a disease entity different from atherosclerosis (112). Therefore, research to better understand the developmental, genetic, and molecular bases of the disease may identify new disease-specific targets for effective therapeutics.

Cellular Mechanisms of Aortic Valve Disease

VECs and VICs of the aortic valve were recently the focus of research because their biology plays important roles in valve development and maintenance. At the cellular level, CAVD is characterized by VIC activation, increased ECM production, and ECM-remodeling enzyme activity (47, 48, 92). Activated VICs are proliferative and express the myofibroblast marker SMA,

resembling valve progenitor cells during development. These observations indicate that activated VICs in diseased valves represent a developmental phenotype. In addition, TWIST1, critical in endocardial cushion mesenchyme, is expressed in human diseased valves (68). Similarly, calcific human aortic valves express transcription factors SOX9 and RUNX2 as well as osteoblastic cell markers involved in osteogenesis and valve development (2, 113, 114). The origins and inductive mechanisms of activated VICs in valve disease are not identified. Activated VICs may arise from quiescent VICs resident in the valve leaflets. Valve progenitors arising during development are present in the adult valves, but there is evidence that circulating CD45-expressing hematopoietic stem cells are also recruited to the valves during disease (53, 115–117). Regardless of their origins, VICs with properties of osteogenic cells activate the expression of genes involved in bone mineralization. This worsens valve sclerosis and stiffness, which in turn exacerbates the faulty differentiation of VICs into osteoblast-like cells (118). In addition, damage to VECs, which is understudied compared to VICs, may result in a loss of the maintenance mechanism necessary for modulating VIC function via the secretion of paracrine mediators. One well-studied example is the dysregulation of antioxidant mechanisms by hemodynamic changes that contributes to increased oxidative stress in the calcified human aortic valves (119–123). Together, accumulating evidence suggests that both VECs and VICs are actively involved in the pathogenesis of CAVD.

Developmental and Genetic Mechanisms of Aortic Valve Disease

Studies show that the majority of patients with aortic valve disease have congenital valve malformation, indicating a genetic and developmental basis of adult aortic valve disease in many cases (124–127). BAV is frequently associated with CAVD and regurgitation later in life. Studies suggest a mechanosensory mechanism of CAVD in individuals with BAV due to the hemodynamic abnormalities caused by abnormal valve leaflet structure (128).

Genetics studies have identified multiple gene mutations and genetic variants associated with human BAV, and many of these affected genes have previously known roles in valve development (**Table 1**) (129, 130). Early studies demonstrate that mutations in a variety of ECM genes are associated with genetic syndromes that include aortic valve malformations and progressive valve dysfunction. Mutations in the *FIBRILLIN-1* gene cause Marfan syndrome (131, 132), whereas Williams syndrome is associated with heterozygous *ELASTIN* mutations (133, 134). Mutations in *COL1A1* are associated with the prolapse of aortic and mitral valves in patients with osteogenesis imperfecta (135). Mutations in the *NOTCH1* gene are associated with some cases of BAV and CAVD (20, 136). Notably, a recent study using targeted, combinatorial next-generation sequencing identified a large number of putative disease-causing variants in a cohort of patients with BAV. Many of these genes are known to be involved in valve developmental signaling and transcriptional programming, including NOTCH, EGFR, NFATC, SOX9, and NOS (**Table 1**) (125). These findings support the notion that a developmental pathogenesis underlies BAV and valve calcification.

Mouse and Inducible Human Pluripotent Stem Cell Models of Human Bicuspid Aortic Valve and Aortic Valve Disease

The human genetic syndromes with aortic valve malformations and disease were phenocopied in genetically modified mouse models using gene-specific targeted mutagenesis (**Table 2**). For example, mice with deficient ELASTIN and NOTCH1, as well as with transcriptional programming, are associated with aortic valve malformation and disease in mouse genetic models. Of note,

Table 1 Human gene mutations associated with aortic valve disease, including bicuspid aortic valve

Gene	Syndrome	Valve phenotype(s)	Reference
FIBRILLIN1	Marfan	ARD, BAV, MVP	132
ELASTIN	Williams	SVAS, BAV, MVP	138
COLLAGEN3	Ehlers-Danlos	ARD, BAV, MVP	139
NOTCH1	ND	BAV, CAVD, CVM	20
ACTA2	ND	AA, BAV	140
MYH11	ND	AA, BAV	141
FLN-A	ND	BAV, MVP	142
GATA5	ND	BAV	143
NKX2.5	ND	BAV, ASD, TOF	144
NOS3	ND	BAV	145
EGFR	ND	BAV	141
TGFBR2	Marfan, Loeys-Dietz	BAV, AA, ARD	146
AXIN1	ND	BAV	147
ENG	ND	BAV	143
PDIA2	ND	BAV	147
TEX26	ND	BAV	148
APC	ND	BAV	129
AXIN2	ND	BAV	129
FLT1	ND	BAV	129
GATA4	ND	BAV	129
GLI1	ND	BAV	129
JAG1	ND	BAV	129
MCTP2	ND	BAV	129
MSX1	ND	BAV	129
NFATC1	ND	BAV	129
NOS1	ND	BAV	129
NOTCH2	ND	BAV	129
NOTCH3	ND	BAV	129
PAX6	ND	BAV	129
PIGF	ND	BAV	129
PPP3CA	ND	BAV	129
PTCH1	ND	BAV	129
PTCH2	ND	BAV	129
SLC35B2	ND	BAV	129
SNAI3	ND	BAV	129
SOX9	ND	BAV	129
TBX5	ND	BAV	129
VEGFB	ND	BAV	129
VEGFC	ND	BAV	129
WNT4	ND	BAV	129
ZNF236	ND	BAV	129

Abbreviations: AA, aortic aneurism; ARD, aortic root dilation; ASD, atrial septal defect; BAV, bicuspid aortic valve; CAVD, calcific aortic valve disease; CVM, cardiovascular malformation; MVP, mitral valve prolapse; ND; not defined; SVAS, supravalvular aortic stenosis; TOF, Tetralogy of Fallot.

Table 2 Mouse models of aortic valve disease, including bicuspid aortic valve

Gene	Genotype	BAV and valve phenotypes	Reference
GATA5	*Gata5*$^{-/-}$	BAV	149
NKX2.5	*Nkx2.5*$^{-/-}$	ASD, stenotic BAV	150
eNOS	*eNOS*$^{-/-}$	BAV	151
HDAC3	*Mef2C-AHF-Cre*, *Cdh5-Cre*	AAD, BAV, VSD	152
Brg1	*Endocardial Brg1-deficient*	Thickened semilunar valves	153
Robo1	*Robo1*$^{-/-}$, *Robo2*$^{-/-}$	VSD, BAV	154
Robo2	*Robo1*$^{-/-}$, *Robo2*$^{-/-}$	VSD, BAV	154
Matr3	*Matr3Gt-ex13-mutant mice*	BAV, CoA, PDA, VSD	155
Smad2	*Adamts5*$(^{-/-})$, *Smad2*$(^{+/-})$	BAV, BPV	156
Alk2 (Acvr1)	*Alk2KOFX/Gata5-Cre*$^{+}$	BAV, VSD	157
Rac1	*Mef2c-Cre*, *Rac1 f/f*	OFT defects	158
ADAMTS9	*Adamts9*$^{+/-}$	Aortic valve malformations	159
Elastin	*Eln*$^{+/-}$	Aortic valve malformations	106
Fibulin-4	*Fibulin4-R/R*	Thickened aortic valves	160
Periostin	*Postn*$^{-/-}$	Valve defects	161
Notch1	*Notch1*$^{flox/flox}$, *Nfatc1-enCre/+*	BAV, enlarged valve cusps	16
Jag1	*Jag1*$^{flox/flox}$, *Tie2-Cre/+*	BAV, enlarged valve cusps	162
RBPJ	*RBPJ*$^{flox/flox}$, *Nkx2.5-Cre/+*	BAV, enlarged valve cusps	162
Fgf8	*Fgf8*, *MesP1Cre mutants*	BAV, BPV	163
Pbx1/2/3	*Pbx1*$^{+/-}$, *Pbx2*$^{+/-}$, *Pbx3*$^{+/-}$, *Pbx2*$^{-/-}$	BAV	164

Abbreviations: AAD, ascending aortic dilatation; ASD, atrial septal defect; BAV, bicuspid aortic valve; BPV, bicuspid pulmonary valve; CAVD, calcific aortic valve disease; CoA, coarctation of the aorta; OFT, outflow tract; PDA, patent ductus arteriosus; VSD, ventricular septal defect.

mouse inactivation of *Notch1* in VECs, but not VICs, exhibits developmental defects in aortic valve remodeling, BAV, and valve calcification that recapitulate human calcific BAV with *NOTCH1* mutations (16). This study supports a direct causal link between NOTCH1 deficiency and human BAV and CAVD. Similarly, VEC-specific inactivation of *Tgfβ1* results in reduced SOX9 nuclear localization in VICs and increased calcification (17). These findings point to a crucial role in VEC signaling necessary for VIC function in valve development and homeostasis.

In addition to advanced mouse genetics, human iPSCs and CRISPR genome editing technologies were recently used to identify a direct causal relationship between *NOTCH1* mutations and the progressive calcification of VICs (137). Human iPSC-derived VECs with heterozygous nonsense mutations in *NOTCH1* that cause aortic valve calcification have a disrupted epigenetic architecture, resulting in the activation of osteogenic and inflammatory gene networks known to be part of the pathogenesis of human disease. Together, these human and mouse studies suggest that abnormal signaling pathways in valve cells interplay with ECM dysregulation as a pathogenic mechanism underlying the aortic valve malformation and progressive valve calcification later in life. Therefore, research focused on the common developmental signaling pathways and transcriptional programs shared in the pathogenesis of aortic valve malformations and disease may identify new effective therapeutic targets. The full elucidation of genetic and developmental bases of valve malformation and disease will enhance the targeted development of therapies based on specific mechanisms of disease in individual patients to intervene in the disease progression.

CONCLUSIONS

Aortic valve morphogenesis via the interaction of VECs and VICs as well as ECM synthesis is tightly regulated by major developmental and mechanosensory signaling pathways. Normal aortic valve development establishes the fine valve structure and function that are also maintained by VECs, VICs, and ECM in adults. Abnormal aortic valve development results in aortic valve malformations that are highly associated with CAVD, which is characterized by overactive VICs and abnormal ECM with the reactivation of developmental valve programs and induction of osteogenic processes. Therefore, a better understanding of molecular and cellular mechanisms underlying aortic valve development may identify effective therapeutic targets for maintaining valve homeostasis and preventing disease progression.

SUMMARY POINTS

1. The aortic valve is composed of VECs and VICs derived from endocardial cells via EMT during development.
2. Aortic valve development is regulated by interactions of VECs, VICs, and cardiac NCCs.
3. Key developmental cell signaling modulates aortic valve development as well as homeostasis.
4. Congenital BAV underlies CAVD, which has no effective medical therapy and often requires surgery.
5. BAV has a clear genetic basis characterized by aberrant developmental programs resulting from mutations in genes necessary for proper cell signaling and ECM production.
6. Understanding the developmental origins and genetics of aortic valve development may lead to effective new therapies for aortic valve disease.

FUTURE ISSUES

1. Molecular and mechanical links between congenital aortic valve malformations and adult CAVD need to be better defined.
2. Whether a subset of adult VECs reacquire embryonic functions for EMT to generate a unique population of VICs that is involved in calcification should be empirically addressed.
3. Additional markers to distinguish VICs of embryonic or postnatal arising lineages are needed to fully characterize normal (quiescent) and diseased (active) VICs.
4. Single-cell genetics and genomics may reveal cell type-specific makers to develop effective therapeutics for early intervention.
5. Improved new durable valve bioprostheses based on a combination of developmental signaling pathways, hemodynamics, and tissue engineering approaches need to be developed.

DISCLOSURE STATEMENT

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