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Chiseling away at the multiple mechanisms of aortic stenosis

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Don't call it "degenerative!"

Non-rheumatic aortic stenosis has assumed center stage in contemporary cardiology due to the aging of the population and the prodigious advances in percutaneous approaches to its treatment. Many refer to this disease dismissively as "degenerative." That term implies an inevitable, ineluctably progressive, and inherent process that defies slowing or regressing. Use of the term "degenerative" cloaks our ignorance regarding mechanisms, much as do the designations "idiopathic" cardiomyopathy or "essential" hypertension. Yet, in step with the remarkable advances in addressing the mechanical aspects of aortic stenosis, researchers continue to stride toward better mechanistic understanding of the pathophysiology of this condition. Rather than "degenerative" aortic stenosis, we advocate use of the terms "fibrocalcific" or "sclerocalcific" aortic valve disease: nomenclature that captures the hardening of the valve tissue and its mineralization. We now recognize a number of active biological mechanisms that pave the pathway to alterations in the structure and function of the aortic valve (Figure 1).

New insights into the mechanisms of aortic stenosis

A healthy aortic valve maintains its physiologic functions by means of highly organized tissue architecture. The aortic valve leaflets contain three distinct extracellular matrix layers: a collagen-rich fibrosa, an elastin-rich ventricularis, and a spongiosa, a middle layer rich in glycosaminoglycans (Figure 2A). This tri-laminar structure determines the biomechanical properties of the valve leaflets. Disease progression involves simultaneous thickening and stiffening of the valve leaflets due to fibrosis and formation of calcific nodules that originate on the base of the fibrosa/aortic aspect of the leaflet (Figure 2B).

The resident cell populations responsible for maintenance of valve homeostasis and structural integrity include valvular endothelial cells (VECs) and valvular interstitial cells (VICs). Healthy adult cardiac valves contain mostly VICs that resemble quiescent fibroblasts. During disease progression, these cells can undergo activation and become myofibroblast–like cells that express metalloproteinases (e.g., MMP-1, MMP-2, MMP-9, MMP-13) and pro-inflammatory cytokines (e.g., IL-1β), mediators that can promote tissue remodeling[1] and fetal valve development.[2] The extent of VICs heterogeneity (including progenitor cells,) and how VIC subpopulations might contribute to aortic stenosis requires further investigation.

Multiple mechanisms lead to the evolution of the normal to the diseased valve. Two distinct forms of calcification predominate in calcific aortic valve disease (CAVD): (1) inflammatory/oxidative stress-driven and (2) hyperphosphatemia-related. These processes involve distinct mechanisms and may operate simultaneously in the same tissue.

Inflammation and immunity: Newly recognized drivers of aortic stenosis

In addition to the resident cells, the diseased valve harbors different denizens, including immune cells such as macrophages and CD8-positive T lymphocytes, likely recruited through an abnormal endothelium. Emerging evidence suggests that these immune cells contribute to the formation of osteoclast-like cells, although they remain dysfunctional as they lack calcium resorptive activity.[3] During the early pro-inflammatory phases of calcification, activated macrophages and other inflammatory cells likely drive disease progression through pathological matrix remodeling and the release of osteogenic cytokines.[4] TNF-α or IL-6 can trigger biomineralization and osteogenic signaling through activation of bone morphogenetic protein family members (BMP) and Wnt signaling. Foci of incipient calcification/microcalcification may then activate macrophages and promote a positive calcification-inflammation feedback loop that drives disease progression. Indeed, chronic inflammation contributes to valve calcification in mice, a phenomenon now established by Fluoride and fluorodeoxyglucose imaging in humans.[5]

Generation of reactive oxygen species (ROS) often accompanies inflammation, and has particularly pathogenic properties in CAVD.[6] ROS activate osteogenic reprogramming in human CAVD and in cultured VICs evident by DNA damage and elevation of the transcriptional factors Runx2 and Msx2. Increased intracellular ROS accumulation causes mitochondrial dysfunction in type 2 diabetes mellitus and the metabolic syndrome, conditions that both associate with CAVD. Therefore, targeting ROS-associated osteogenic signaling cascade provides one potential avenue for modifying the progression of aortic stenosis.

Elevated levels of oxidized LDL associate with heightened fibrocalcific responses in aortic valvular tissue, possibly through pro-inflammatory pathways that involve Toll-like receptors. Strong human genetic evidence implicates Lipoprotein (a) [Lp(a)] as a causal factor in aortic stenosis. Lp(a) has many physiological and pathological actions including transport of oxidized phospholipids and binding to LDL and very low-density lipoprotein receptors. Lp(a) also transports lysophosphatidic acid secreted by VICs and promotes mineralization of aortic valves.[7] In view of the strong association between the *LPA* gene variant (rs10455872) with the increased risk of developing aortic stenosis by >50%, Lp(a)-targeted interventions (including anti-sense oligonucleotide and anti-PCSK9 therapies) warrant further exploration in the context of CAVD.

Renal dysfunction, hyperphosphatemia, and uremic toxins also contribute to aortic stenosis
Approximately 50% of individuals with chronic kidney disease (CKD) die from cardiovascular complications.[8] In addition to classic risk factors such as age and dyslipidemia, patients with CKD have hyperphosphatemia, an independent risk factor for cardiovascular death. Recent preclinical studies show that macrophage-derived MMPs or cathepsins degrade elastin fibers, producing biologically active elastin peptides that can initiate calcification of VICs, which together with elevated phosphate concentrations could accelerate valve mineralization. Emerging evidence suggests that high serum concentrations of uremic toxins, including indoxyl sulfate, increase with the progression of CKD, particularly in patients undergoing hemodialysis. *In vitro*

studies using vascular cells further demonstrate that uremic toxins promote cell proliferation, and inflammatory cytokine and ROS production. In addition, administration of indoxyl sulfate to rats induced aortic calcification and fibrosis.

Microvesicles can contribute to calcification

Elevated extracellular calcium and phosphate, as found in patients with CKD, can induce the release of calcifying extracellular vesicles (microvesicles) from cardiovascular cells, including macrophages and vascular smooth muscle cells. Calcifying vesicles can carry cargo that promotes calcification of neighboring cells and extracellular matrix. The content of extracellular vesicles dictates their function. Intracellularly, phosphorylated sortilin transports tissue-nonspecific alkaline phosphatase, a molecule required for calcification, from the Golgi to the intracellular membrane resulting in the formation of calcification-prone vesicles.[9] Upon their release these vesicles tend to aggregate, merge, nucleate hydroxyapatite, generate microcalcifications, and thus foster the formation and growth of advanced calcific nodules.[10] Proteomic studies have shown that circulating extracellular vesicles contain multiple mediators implicated in cardiovascular calcification (e.g., sortilin, annexins, S100A9, tissue-nonspecific alkaline phosphatase, microRNAs, oxidized LDL.)

Monogenic disorders contribute to cardiovascular calcification

Bicuspid aortic valve, the most common congenital heart valve disease, occurs in 1-2% of the population. A combination of factors, including genetic susceptibility, abnormal mechanical forces, and environmental risk likely contributes to the pathobiology and earlier onset of symptoms in patients with BAV.[11] BAV contributes to 50% of aortic stenosis and associates with structural abnormalities of the aortic wall such as aortic aneurysm and aortic dissection. Genetic variants may promote abnormal expression of proteins regulating extracellular matrix and alter different signal transduction cascades during valvulogenesis. After birth, severe disruption of valve structure can create anomalous blood flow, which in turn may alter cell signaling and tissue remodeling. Mutations in the Notch signaling pathway associate with BAV.[12] In addition, studies indicate that eNOS-deficient mice develop BAV and eNOS-derived nitric oxide modulates the Wnt/Lrp5 pathway, which may mediate aortic valve calcification.

Dipeptidyl peptidase-4: A new link between aortic stenosis and pathways involved in diabetes?

In this issue of *Circulation* Song and colleagues[13] propose a novel mechanism that promotes aortic valve calcification, action of the enzyme dipeptidyl peptidase-4 (DPP4), a multifunctional enzyme, found in plasma in the catalytically active soluble form and on the surface of many cells. DDP-4 inhibitors, oral hypoglycemic drugs, enjoy broad use for the treatment of type 2 diabetes mellitus. The authors found that VEC dysfunction gauged by nitric oxide depletion promotes DPP-4 expression in human VICs. DPP-4 in turn induces osteogenic differentiation of VICs through increasing degradation of insulin-like growth factor-1 (IGF-1). Hence, the DPP-4-

IGF-1 axis may mediate VEC-VIC interaction. *In vitro*, the inhibition of DPP-4 enzymatic activity blocked the osteogenic changes in VICs. DPP-4 inhibition reduced calcified lesion formation in eNOS-deficient mice and prevented experimental CAVD in rabbits. This study underscores again a critical role for the endothelium, particularly eNOS, in the initiation of CAVD through paracrine regulation of VICs. These novel findings raise the exciting possibility that currently available DPP-4 inhibitors might mitigate aortic stenosis, an issue ripe for formal investigation.

Therapeutic horizons for medical modification of fibrocalcific aortic valve disease

Pharmacologic modification of valvular heart disease has lagged behind other aspects of cardiovascular medicine. Strong association between lipids and CAVD led to trials of statins in treatment of CAVD. SEAS (the Simvastatin and Ezetimibe in Aortic Stenosis trial) and other clinical studies showed a lack of the therapeutic benefits of statins in CAVD and further emphasized the need to focus on valve-specific therapeutic targets.[14] The studies of Song et al.[13] indicate the need to evaluate DPP-4 inhibitors in this regard, and examine whether they have adverse effects on bone metabolism or promote heart failure, the latter of particular concern in the CAVD population.[15] The recent advances in the mechanistic understanding of CAVD such as the provocative results with DPP-4 presented by Song et al. should reveal more novel targets for non-interventional approaches to modulating valve disease.

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Figure Legends:

Figure 1. Multifactorial mechanisms contribute to fibrocalcific aortic valve disease.

Figure 2. Structure of a normal and diseased aortic valve. (2A) A healthy aortic valve leaflet contains VECs and quiescent fibroblast-like VICs, and three distinct extracellular matrix layers: a collagen-rich fibrosa, a glycosaminoglycan-enriched spongiosa, and an elastin-rich ventricularis. (2B) Disease progression involves VIC activation, recruitment of immune cells, and subsequent thickening and stiffening of the valve leaflets due to fibrosis and formation of calcific nodules that originate on the fibrosal surface of the leaflet. Histological staining of normal (C) and diseased (D) valve leaflets. Movat's staining; collagen – yellow; proteoglycans – blue-green; elastin and calcium – black. (C) Modified from Aikawa E. Circulation 2006.[2]

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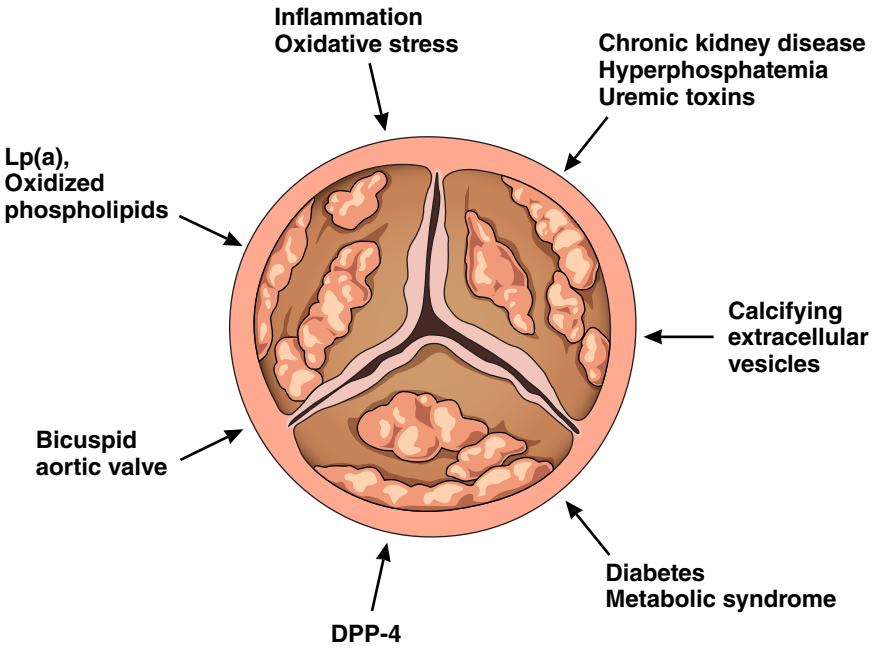

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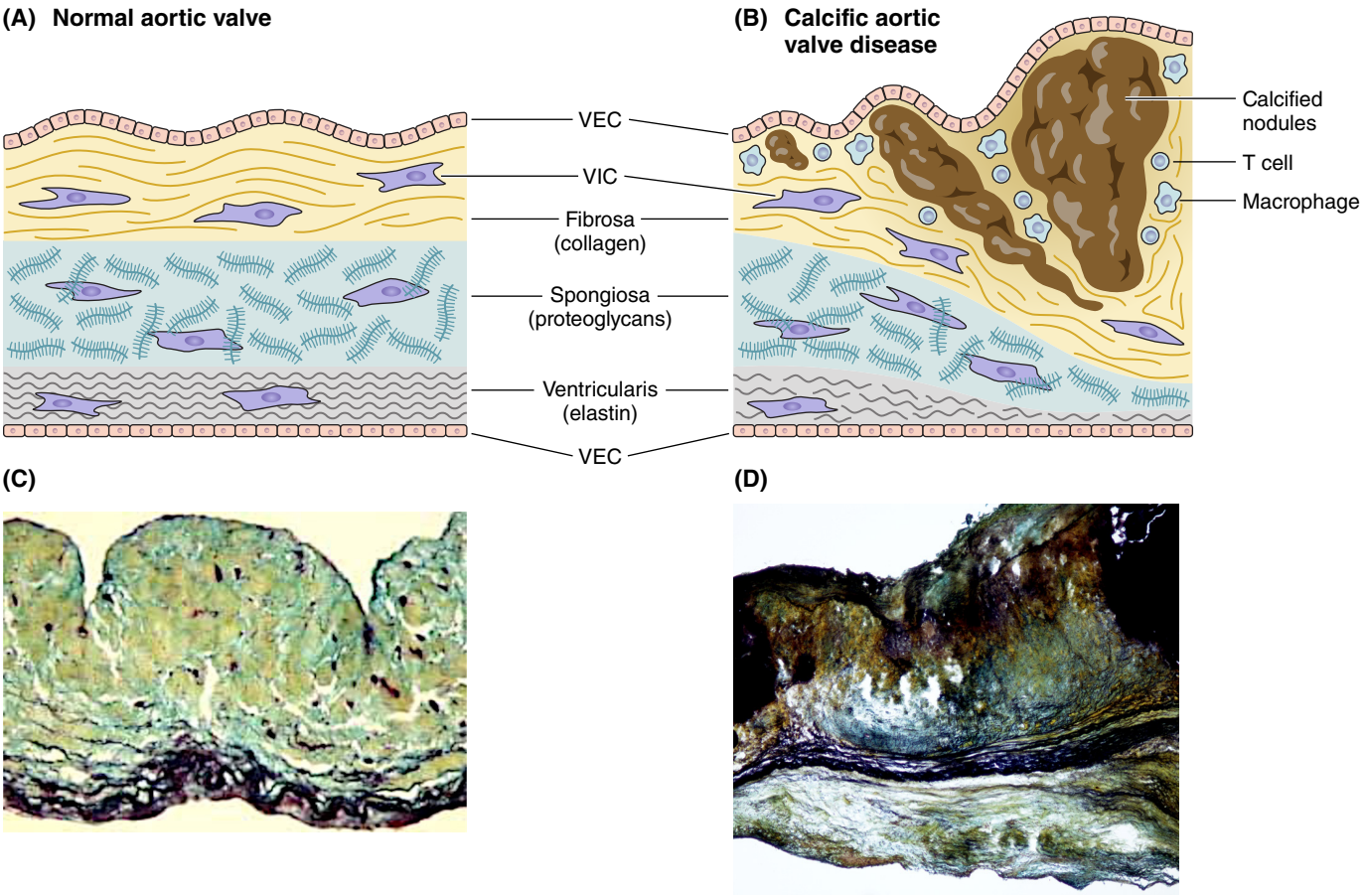

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