Pathological Mechanism and Treatment of Calcified Aortic Stenosis

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Calcified aortic stenosis (AS) is one of the most common valvular heart diseases worldwide, characterized by progressive fibrocalcific remodeling and thickening of the leaflets, which ultimately leads to obstruction of blood flow. Its pathobiology is an active and complicated process, involving endothelial cell dysfunction, lipoprotein deposition and oxidation, chronic inflammation, phenotypic transformation of valve interstitial cells, neovascularization, and intravalvular hemorrhage. To date, no targeted drug has been proven to slow down or prevent disease progression. Aortic valve replacement is still the optimal treatment of AS. This article reviews the etiology, diagnosis, and management of calcified aortic stenosis and proposes novel potential therapeutic targets.

Key Words: aortic valve stenosis, heart valve disease, inflammation, osteogenic differentiation, aortic valve replacement

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INTRODUCTION

Calcified aortic stenosis (AS) is a common heart valve disease in the elderly, usually manifested as angina, dizziness or syncope, and dyspnea. The incidence of calcified aortic stenosis has been increasing, affecting >2% of individuals over 60 years and up to 10% of patients over 80, with high morbidity and mortality. The 2-year mortality rate of symptomatic severe AS is approximately 50%.[1]

The aortic valve is an avascular, tricuspid valve that is connected to the aorta by the valve annulus. Bicuspid aortic valve (BAV) is a common congenital valvular abnormality with an estimated incidence of 1% to 2%.[2] Under normal circumstances, the thickness of the leaflet is less than 1mm, mainly composed of valve endothelial cells (VECs) and valve interstitial cells (VICs). VECs form the outer layer, maintaining valve homeostasis by regulating permeability and inflammatory cell adhesion. VICs and a small number of smooth muscle cells and fibroblasts(<5%) constitute the inner layer, including fibrosa, spongiosa, and ventricularis, which provide biomechanical strength to withstand a constant oscillating hemodynamic environment.[3]

The incidence of calcified aortic stenosis increases exponentially with age, so it has long been regarded as an age-related degenerative disease with calcium deposition. However, growing evidence proved that calcified aortic stenosis is an active cellular process involving complex pathogenesis.[4] In the initial stage, the aortic valve thickens focally with formation of calcium nodules without any obstruction to blood flow. As the disease progresses, it gradually develops into severe aortic valve calcification, valve leaflet dyskinesia, massive blood flow obstruction, and increased transvalvular pressure gradient.[5]

At present, TTE is the standard diagnostic test for patients with calcified aortic stenosis. The main observation indicators include peak velocity, mean pressure gradient, and aortic valve area.[6] However, when the results of noninvasive examination are uncertain or the clinical symptoms of patients are inconsistent with the results of noninvasive examination, cardiac catheterization should still be considered for a definite diagnosis.[7] Currently, no drugs have been found to prevent the progression of aortic valve calcification, including statins.[8–10] Aortic valve replacement is still the only effective treatment, and transcatheter aortic valve replacement has emerged as an alternative to surgical aortic valve replacement.[11,12]

Here we review the pathophysiological mechanism, diagnosis, and standard treatment of calcific aortic stenosis. In addition, we highlight several innovative therapeutic targets that have the potential to limit aortic valve calcification.

MECHANISM

Endothelial Cells Dysfunction

The normal aortic valve usually consists of three leaflets, which have an outer layer composed of VECs and an inner layer composed of VICs.[3] VECs are located on the surface of the valve and usually maintain the stability of the valve by regulating permeability, adhesion of inflammatory cells, and paracrine signals.[13] Surface-covered VECs keep the silence of VICs and prevent osteogenic differentiation by secreting "protective" growth factors and molecules.[14] Long-term mechanical and oscillating shear stress may damage endothelial function, leading to lipoprotein deposition. Activated VECs express adhesion molecules and promote the recruitment of monocytes and macrophages and their transendothelial migration[15] (Figure 1). The bicuspid aortic valve is considered the most frequent congenital aortic valve malformation that causes AS. Patients with congenital bicuspid aortic valves suffer from greater mechanical stress and more severe endothelial damage, so the progression of their disease tends to be earlier and faster.[16,17] In addition, abnormal endothelial cells also undergo endothelial mesenchymal transformation (EndMT), which could be inhibited by VICs. The transformed endothelial cells can promote proliferation, migration, synthesis of extracellular matrix, and inflammation. Cells coexpressing endothelial markers and mesenchymal markers are a marker of EndMT, in which endothelial markers (CD31, VEcadherin, vWF) are downregulated while mesenchymal markers (a-SMA, CDH2) are upregulated. Current studies found that EndMT exists both in vivo and in vitro, which may be caused by inflammatory stimuli, hemodynamic shear stress, and altered ECM composition.[18]

Lipid Deposition and Oxidative Stress

The infiltration of lipoproteins, mainly including low-density lipoproteins and lipoprotein a, in the aortic valve is of strategic importance to calcified aortic stenosis.[19] Studies showed that single nucleotide polymorphisms (SNPs) of the LPA gene locus, which encodes Lp(a), are associated with the occurrence of AS.

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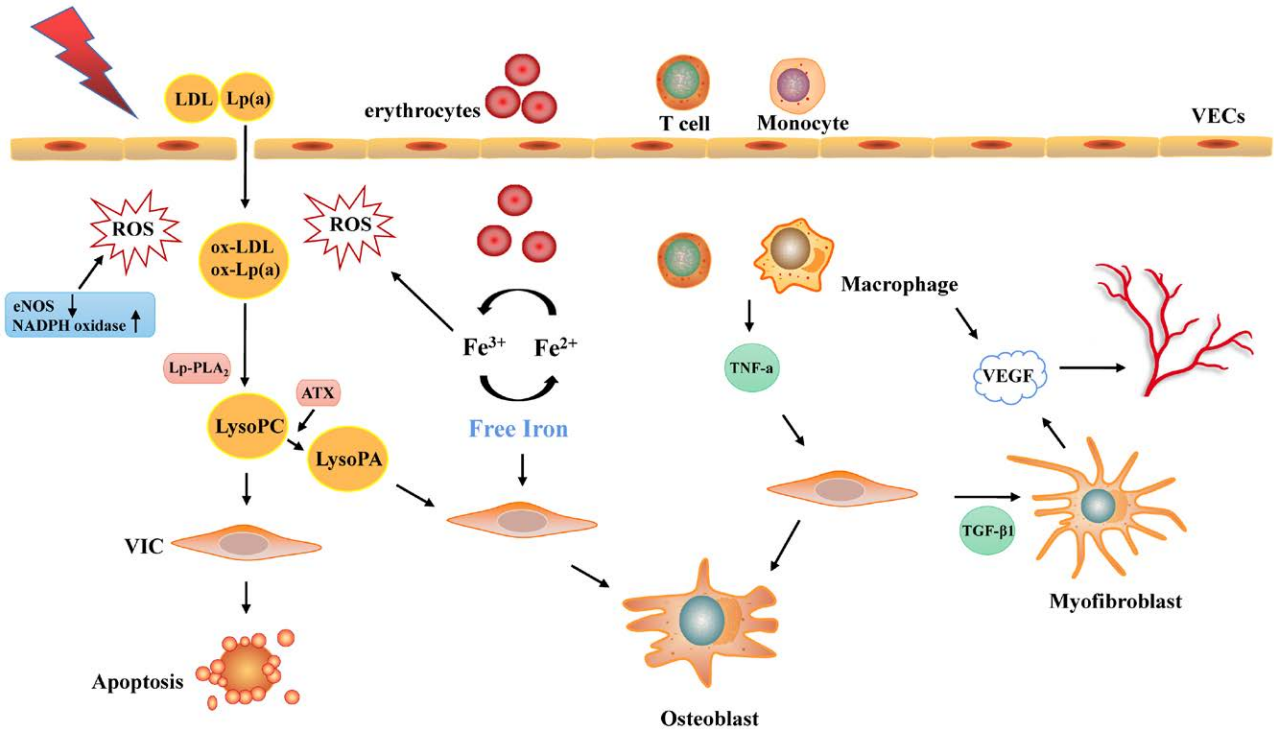

FIGURE 1. Pathological process of calcified aortic valve stenosis. The pathological mechanism of the occurrence and development of CAVS is an active process involving multiple factors, including endothelial dysfunction, lipid deposition, oxidative stress, chronic inflammation, intravalvular hemorrhage, and neovascularization. External stimuli, such as mechanical/shear stress, induce dysfunction of valve endothelial cells, causing the infiltration of lipoprotein into the interstitium of the valve leaflets, especially LDL and Lp(a). The imbalance of the eNOS pathway and excessive iron deposition caused by intravalvular hemorrhage induces the production of ROS, thereby stimulating the oxidation of infiltrating lipids. Ox-LDL and ox-PLs are further converted into lysoPC under the stimulation of Lp-PLA2, which promotes the apoptosis of VICs and releases apoptotic bodies, leading to diffuse calcification. With the oxidation of lipids, immune cells (T cells, monocytes) infiltrate tissues and are activated. Activated macrophages and T cells promote the osteogenic transformation of VICs by secreting TNF-α. Meanwhile, TGF-β1 induces VICs to differentiate into myofibroblasts. Myofibroblasts and macrophages also secrete VEGF together to induce neovascularization. These factors together promote the occurrence and development of CAVS.

Enome-wide association (GWA) studies and mendelian randomized studies found that the rs10455872SNP mutation reduced the duplication of the KIV2 loop domain by affecting the LPA copy number, resulting in increased Lp(a) levels.[20] The combination of LPA and LPAR triggers an intracellular signal cascade by activating the NFκB pathway as well, thereby exacerbating valve calcification.[21] At the same time, growing evidence supported that PCSK9 may also participate in aortic valve calcification. The level of PCSK9 negatively correlates with the progression of calcified aortic valve disease (CAVS). Loss of function due to PCSK9 R46L mutation reduces the risk of CAVS.[22] A clinical trial showed that the use of PCSK9 inhibitors decreased the hazard of new or worsening aortic stenosis.[23] But PCSK9 inhibitors mainly reduce LDL-C but not Lp(a). Therefore, in addition to regulating lipoprotein levels, PCSK9 must affect the occurrence and development of CAVS by regulating other pathways, such as inflammation, local adaptive immune regulation, apoptosis, and autophagy.[24]

Meanwhile, the aortic valve calcification is also accompanied by oxidative stress. The increased oxidative stress is related to the dysfunction of endothelial nitric oxide synthase (eNOS) and increased expression of NADPH oxidase, both contributing to the production of reactive oxygen species (ROS).[25] ROS oxidize infiltrated lipids and promote the formation of oxidized low-density lipoproteins (Ox-LDL) and oxidized phospholipids (Ox-PLs).[26] Ox-LDL and Ox-PLs are further converted into lysophosphatidylcholine (LysoPC) by increased Lipoprotein-associated phospholipase A2 (Lp-PLA2) in the valve. LysoPC induces mineralization of aortic valve through the loss of mitochondrial membrane potential and VICs apoptosis.[26,27] ATX, encoded by ENPP2, is a ubiquitous lysophospholipase D enzyme that converts LysoPC to lysophosphatidic acid (LysoPA), a highly active metabolite that promotes inflammation, fibrosis, and osteogenesis[28] (Figure 1).

Inflammation

As described before, invasion and activation of immune cells is also important cue in aortic valve calcification.[29] Firstly, activated VECs express adhesion molecules to promote the recruitment of monocytes and macrophages and transendothelial migration[30] (Figure 1). Second, there is an interaction between lipid deposition and inflammation. Ox-LDL upregulates cell adhesion molecules ICAM-1 and VCAM-1, leading to greater immune cell adhesion. Oxidized lipids also activate macrophages, triggering the release of proinflammatory mediators and inflammatory responses. Activated macrophages secrete TNF-a, which triggers osteogenic differentiation of VICs via activating NFκB and inducing the release of IL-1β and IL-6.[31] IL-1β could adjust the remodeling of extracellular matrix by increasing MMP1 and MMP2 expression.[32] IL-6 promotes the calcification of aortic valve by upregulating BMP2, a major factor

that induces bone and cartilage formation.[33] Thirdly, VICs produce leukotrienes by expressing 5-lipoxygenase to amplify the inflammation.[34,35] Surprisingly, recent studies found that Piezo1, a stress-sensing mechanoreceptor on the cell surface, senses the high shear forces generated by severe aortic stenosis, which in turn activates circulating monocytes and further exacerbates inflammation, and this proinflammatory response can be resolved by TAVI.[36]

Myofibroblastic and Osteoblastic Differentiation

As previously mentioned, lipid deposition and chronic inflammation are initiating factors for CAVS. However, during the propagation phase, the phenotypic transformation of VICs becomes the main driving force.[37] VICs have the great plasticity to differentiate into myofibroblasts and osteoblasts (Figure 1). Among various factors, TGF-β1 is the main factor to promote the differentiation of VICs into myofibroblasts, which may be inhibited by FGF-2.[38] This signal stimulates the expression of cadherin 11 through activating the noncanonical ERK pathway to induce the formation of myofibroblasts, which is often accompanied by a marked increase in aSMA expression. Cadherin 11 also promotes the formation of nodules by combining with α-SMA. Finally, the central cells of nodules undergo dystrophic changes and apoptosis, leading to diffuse calcification.[39] In addition to this, toll-like receptor 4 (TLR4) also drives the phenotypic transformation of VICs and collagen deposition through upregulating type IV collagen and MMP2[40,41] (Figure 2).

Compared with the above, the mechanism involved in the osteoclastic differentiation of VICs is more complicated. Osteogenic differentiation of VICs leads to valvular calcification, usually manifested as increased expression of osteoblast markers, such as BMP2, Runx2, osterix, and elevated ALP activity. The specific mechanisms are as follows. First of all, the Notch signal plays a key role in many stages of aortic valve development, such as the initiation of EMT, the formation of the endocardial cushion, and the subsequent aortic valve remodeling process. The mutant of Notch has been shown to cause aortic valve hypoplasia, such as BAV. Regarding the role of Notch 1 in the osteoblastic differentiation of VICs, previous studies fully demonstrated that Notch 1 inhibits this process.[42] First, Notch 1 activates the transcription of Hey1 and Hey2, thereby repressing the expression of Runx2.[43] Then, Notch 1 directly inhibits the expression of BMP2 and indirectly inhibits BMP2 by upregulating matrix Gla protein (MGP) as well, a vitamin K-dependent inhibitor of endochondral ossification.[44] Thirdly, Notch1 haploinsufficiency leads to

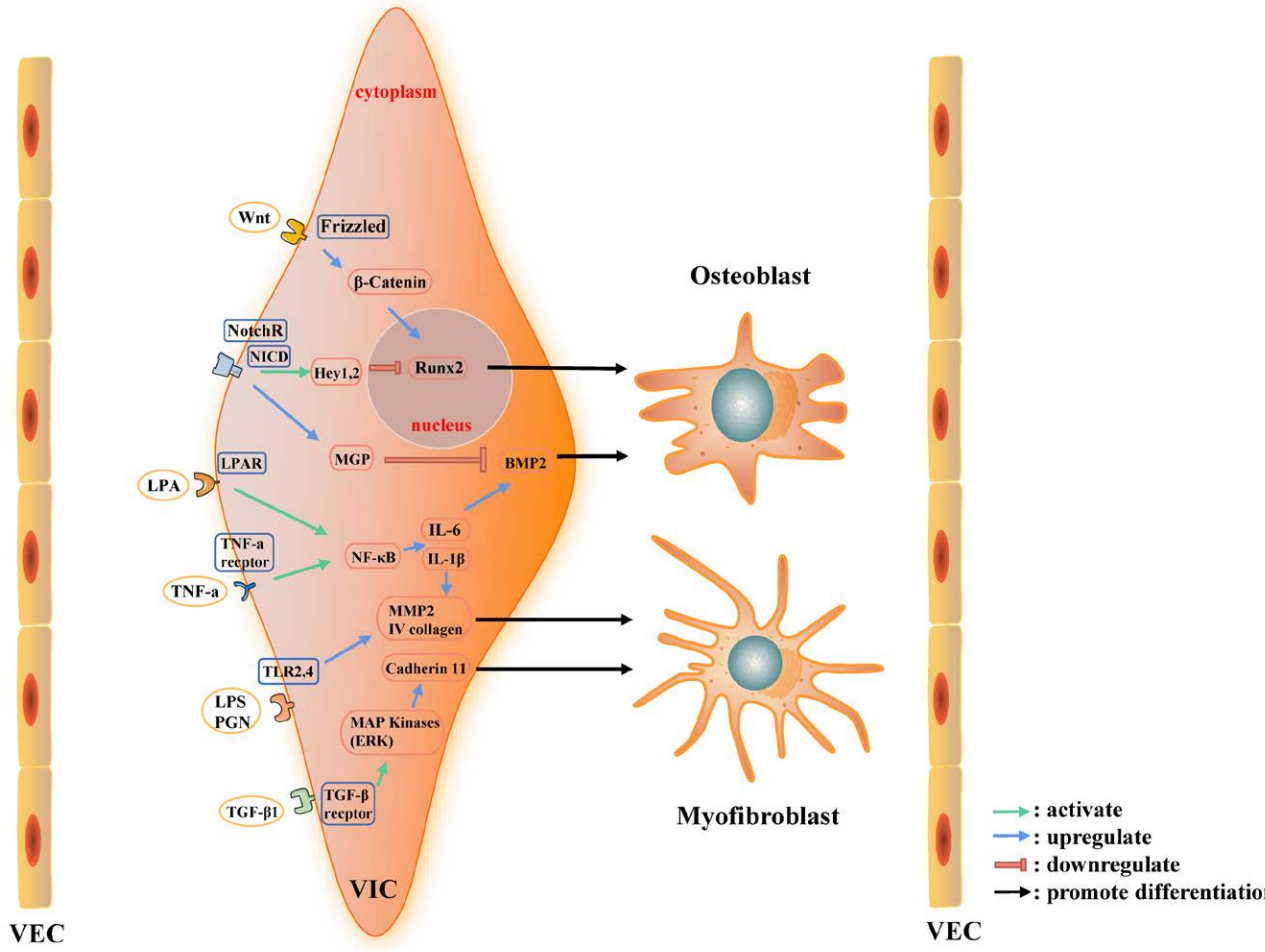

FIGURE 2. Molecular mechanisms of calcified aortic valve stenosis. VICs can differentiate into myofibroblasts or osteoblasts. TGF-β1 stimulates the expression of cadherin-11 to promote the differentiation of myofibroblasts. TLR also promotes this process by inducing the expression of MMP2 and IV collagen. Osteoblast differentiation of VICs is induced by TNF-a, LPA, and Wnt/β-catenin, while Notch1 inhibits this process.

telomere shortening, which is sufficient to cause premature calcification by affecting the network of pro-osteogenic and proinflammatory genes potentially.[45] In addition to Notch, the Wnt signal pathway also participates in regulating heart valve formation. But contrary to the protective effect of Notch, Wnt/β-catenin acts as a procalcific factor. In the absence of a signal, β-catenin is present in the cytoplasm and forms a complex with glycogen synthase kinase 3β, Axin, and APC to maintain β-catenin at very low levels in the cytoplasm by promoting β-catenin phosphorylation and ubiquitination. However, binding of the ligand Wnt to cell surface Frizzled receptors and lipoprotein-related peptide co-receptors disrupts this complex, resulting in β-catenin instability and nuclear translocation.[46] Activation of the Wnt/β-catenin promotes the expression of Runx2 and other osteogenic genes such as OPN, OSX, and BGLAP, thereby stimulating the osteogenic differentiation of VICs.[47] Interestingly, there is also an interaction between Notch1 and Wnt. The methylation of the Notch1 promoter mediates decreased nuclear translocation of NICD, which promotes the activation of the Wnt/β-catenin pathway and the expression of osteogenic factors.[48] Moreover, in calcified aortic valves, the high expression of RANKL and the low level of OPG suggest that they also participate in this disease. In VICs, binding of the receptor-activator of nuclear factor kappa B ligand (RANKL) and RANK induce their osteogenic differentiation. Osteoprotegerin (OPG), a member of the TNFα superfamily, acts as a decoy receptor of RANKL, preventing RANKL-RANK binding.[49]

It is worth mentioning that microRNAs (miRNAs) and lncRNAs are potent regulators in the phenotypic transformation of VICs. For example, MiR30, especially miRNA-30b, effectively represses BMP2 and alkaline phosphatase activities as negative regulators of osteogenic differentiation.[50] In addition to miRNAs, lncTUG1 reverses the repression of Runx2 expression by directly interacting with miR-204-5p to downregulate its expression, thereby promoting the osteogenic differentiation of VICs[51] (Figure 2).

Stem and Progenitor Cells

The recruitment of bone marrow cells is believed to be a common mechanism for tissue remodeling and regeneration of damaged tissues. The interaction between aortic valve matrix components and cells and bonemarrow-derived mesenchymal stem cells is crucial in inhibiting the differentiation of MSCs into osteoblasts. The damage and mineralization of the valve may stimulate the osteogenesis of MSCs.[52]

Endothelial progenitor cells present in normal and calcified aortic valves. The damaged aortic valve cannot regenerate, and endothelial progenitor cells contribute to the recovery of damaged endothelium and the maintenance of endothelial function after injury. However, previous studies found that in the calcified aortic valve, the number of circulating endothelial progenitor cells is significantly reduced, resulting in the integrity of the endothelial cell layer may no longer be maintained.[53,54] The main mechanism is that on the one hand, the increase in the activity of the proapoptotic gene caspase-3 in the cells leads to increased cells apoptosis; on the other hand, the low expression of TFR2, controlling the length and function of telomeres, leads to increased cell senescence.[55] At the same time, TRF2 also reduces the migration of endothelial progenitor cells and their ability to repair damaged endothelium.[56]

Angiogenesis and Hemorrhage

Normal heart valves are avascular and supply oxygen through the diffusion of blood flow. However, under pathological conditions, such as rheumatic valvular disease and CAVS, the heart valve expresses multiple angiogenic factors that lead to neovascularization.[57,58]

As we all know, angiogenesis plays an important role in the progression of atherosclerosis. Similar to atherosclerosis, recent studies demonstrated that angiogenesis is also associated with the pathogenesis of aortic valve disease. In calcified valves, angiogenesis occurs near the calcified nodules, under the edge of the valve leaflets, or in areas where inflammatory cells infiltrate.[59] Under pathological conditions, the balance between angiogenic and antiangiogenic factors in the valve may be destroyed by mast cells and myofibroblasts, thus leading to neovascularization. In calcified stenotic valves, both vascular endothelial growth factor (VEGF) and its receptors VEGFR-1 and VEGFR-2 are upregulated.[57] Conversely, antiangiogenic factors, such as chondromodulin-I and endostatin, are downregulated, which results in a greatly enhanced vascular generation.[60] The mechanism is rough as follows. First of all, activated mast cells VEGFpositive cytoplasmic granules degranulation. At the same time, TNF-α secreted by mast cells causes myofibroblasts to secrete VEGF. Furthermore, tryptase derived from mast cells can degrade antiangiogenic endostatin. By the way, HIF-2 axis signaling activated by the NFκB signaling pathway also participates in angiogenesis by promoting the expression of VEGF.[61]

Interestingly, intravalvular hemorrhage, mainly in areas adjacent to neovascularization, has also been shown in relation to the rapid progression of aortic valve stenosis.[62] Excessive accumulation of hemoglobin induces iron overload and oxidative stress. Iron from erythrocyte heme effectively catalyzes the generation of toxic ROS through Fenton redox reaction, to accelerate the oxidative modification of lipids, activate pro-inflammatory transcription factors.[63] Previous study further clarified that excessive iron deposition caused by senescent erythrocyte infiltration is not only an important part of the progress of calcified aortic valve disease but also related to the occurrence of it by detecting the iron content in aortic valves at different disease stages (from noncalcified to calcified valves).[64] Ferroptosis is a new type of programmed cell death, which is associated with abnormal activation of iron-dependent reactive oxygen species.[65] Therefore, clarifying the relationship between valvular interstitial cell ferroptosis and valve calcification may be a breakthrough in further understanding the pathogenesis of calcific aortic stenosis.

DIAGNOSIS

According to the latest definition of ACC/AHA guidelines and ESC EACTS guidelines, TTE is the standard diagnostic test in the initial evaluation of patients with suspected AS, which accurately assesses valve anatomy and hemodynamic severity, measures LV size and systolic function and determines prognosis and timing of valve intervention. The key indicators for the clinical diagnosis of AS are the peak velocity, mean pressure gradient, and aortic valve area.[6,66] The classification of aortic stenosis severity includes mild AS (aortic Vmax 2.0–2.9 m/s or mean pressure gradient <20 mmHg or AVA 1.5–2.9 cm^2), moderate AS (aortic Vmax 3.0–3.9 m/s or mean pressure gradient 20–39 mmHg or AVA 1.0–1.4 cm^2) and severe AS (Aortic V_{max} ≥4 m/s or mean pressure gradient ≥ 40 mmHg or AVA < 1.0 cm^2). When these measurements are inconsistent with other clinical or imaging data, the ratio of the velocity in the LV outflow tract near the aortic valve to the velocity in the narrowed aortic orifice ≤ 0.25, is consistent with severe AS and is a predictor of symptom onset and adverse outcomes.[67] It is worth mentioning that for asymptomatic AS patients, regular TTE is necessary to give timely treatment for the irreversible consequences of severe AS.[68]

However, noninvasive tests cannot provide an accurate diagnosis for every patient. When results of noninvasive are inconsistent with physical examination, invasive examination, such as transesophageal echocardiography or cardiac catheterization, is still required.[6,66] In asymptomatic patients with severe AS, exercise testing is reasonable to assess physiological changes with exercise and to confirm the absence of symptoms. However, in symptomatic patients with severe AS, exercise testing is not suitable. In patients with suspected

low-flow, low-gradient severe AS with normal or decreased LVEF, the severity can be further determined by calculating the ratio of outflow tract to aortic flow velocity or by measuring the aortic valve calcification score by CT imaging.[67,69]

BIOMARKERS OF CAVS

In recent years, research on biomarkers of CAVS has become more and more popular, which is important for disease diagnosis.[70] Previous case-control studies demonstrated a strong association between Lp(a) and CAVS. Genetic variation in the LPA locus resulting in elevated Lp(a) is an independent risk factor for CAVS independent of coronary artery disease and bicuspid aortic valve. Lp(a) levels greater than 90 mg/dl indicate a three-fold increased risk of AS.[71] Multiple studies support BNP as a prognostic biomarker, with high levels of BNP indicating poor prognosis.[72] Especially in asymptomatic patients with severe stenosis, monitoring BNP provides useful prognostic information. BNP is also used as an indicator to assessment of CAVS severity. Several studies support that NT-proBNP increases with CAVS severity and NYHA grade.

Incidentally, the detection of BNP helps to differentiate between low-flow, low-gradient severe aortic stenosis (LFLGAS) and pseudo-severe aortic stenosis, those corrected on dobutamine stress testing.[73] In addition, two large case-control cohort studies suggest that high serum phosphorus may be used as a biomarker of CAVS. High serum phosphate levels were positively correlated with aortic valve calcification, independent of serum calcium, vitamin D, FGF-23, or parathyroid hormone related to calcium and phosphorus homeostasis.[74,75] Finally, the latest study found that patients with aortic stenosis with high levels of circulating cardiac $CD172a^+$ EVs had a higher survival rate than patients with low levels, indicating circulating heart-derived $CD172a^+$ EVs is a promising prognostic biomarker.[76]

STANDARD TREATMENT

To date, no drug has been shown to inhibit the occurrence and progression of CAVS. Several clinical trials found statins are effective in controlling blood lipid levels, but they produced disappointing results in treating CAVS. One possible reason is that statins may increase Lp(a).[77] Fortunately, the FOURIER Trial demonstrated that the use of PCSK9 inhibitors in patients with atherosclerotic cardiovascular disease reduced the risk of new and worsening aortic stenosis by reducing blood lipid levels. For example, the PCSK9 inhibitor evolocumab can reduce circulating LDL-C concentration by 50% to 60% and Lp(a) by 20% to 30%.[23]

Meaningfully, in patients who have undergone TAVI, the use of ACEI or ARB significantly reduces the 1-year mortality of patients, especially for patients with normal LVEF.[78]

Recommended according to ACC/AHA guidelines and ESC EACTS guidelines, aortic valve replacement remains the only effective treatment for patients with severe aortic stenosis currently. AVR is recommended for patients with symptomatic severe high-gradient aortic stenosis who have experienced exertional dyspnea, heart failure, angina pectoris, syncope, or pre-syncope symptoms in previous or exercise tests.[79,80] For asymptomatic patients with LVEF > 55% and normal exercise test results, when the risk of intervention is low and one of the following conditions is met, (1) very severe aortic stenosis (mean pressure gradient ≥ 60 mmHg or V_{max} ≥ 5 m/s); (2) severe valve calcification and V_{max} progression ≥ 0.3 m/s/year; (3) BNP level >3 normal times by multiple tests without other explanation, AVR also should be considered.[81–83] However, for symptomatic severe AS conservative treatment, is recommended if the quality of life after AVR will not be improved significantly and the postoperative survival time is less than 12 months.[84]

For patients with AVR indications, the choice of a bioprosthetic valve or a mechanical valve should according to the specific situation. For patients younger than 50 years old and without contraindications to anticoagulation, a mechanical aortic valve is recommended. However, for patients over 65 years, a bioprosthetic valve is superior to a mechanical valve. For patients with AVR indications at any age, if anticoagulation with vitamin K antagonists is contraindicated or cannot be monitored or managed well, bioprosthetic valve is considered.[85] Meanwhile, for patients with AVR indication of biological valve, age has become the main reference factor for selecting SAVR and TAVR. SAVR is recommended for patients with severe AS under 65 years old with a life expectancy of more than 20 years. For AS patients over 80 years old or with a life expectancy of fewer than 10 years and without anatomical restrictions, TAVR is superior to SAVR. For patients between 65 and 80 years old, the surgical method should be selected according to specific conditions. However, there are some special circumstances, such as in patients with asymptomatic severe AS and very severe AS with abnormal exercise test, in the case of rapid progression or elevated brain natriuretic peptide levels, SAVR is recommended over TAVR. By the way, TAVR is recommended for symptomatic severe AS patients of any age group with high surgical risk or contraindication.[86] At present, several clinical studies have been conducted to compare the prognosis of TAVR and SAVR in patients with low surgical risk and severe AS. PARTNER 3 Clinical Trials found that even among patients with severe aortic stenosis at low surgical risk, TAVR effectively reduced the mortality, stroke, or rehospitalization rate at 1 year compared with surgery.[87] Evolut Low-risk trial also proved that TAVR was not inferior to SAVR in the primary endpoint of all-cause mortality or disabling stroke in the 2-year follow-up of patients with low surgical risk.[88] Given the results of these clinical studies and the minor surgical trauma, short hospitalization, and rapid improvement in the quality of life of TAVR, TAVR is a suitable choice for such patients. However, the mid-term and long-term prognosis of patients treated with TAVR still needs to be further observed.

With the progress of technology, percutaneous aortic valvuloplasty also provides a choice for patients.[89] Percutaneous balloon aortic valvuloplasty is not able to change the pathological state and progression of valve calcification, which only temporarily improves the patient's hemodynamic instability and clinical symptoms.[90] As a consequence, it is usually regarded as a bridge to SAVR or TAVR.

POTENTIAL NOVEL THERAPEUTIC TARGETS

Despite the relevant research on the molecular mechanism of CAVS being relatively sufficient, how to translate molecular mechanism into clinical application is still facing many difficulties. Based on the current research, we propose the following novel possible therapeutic targets. First of all, NADPH oxidase 2 (NOX2), a ROS-generating enzyme, is upregulated in calcified aortic valve disease. Celastrol, a selective NOX2 inhibitor, is effective in reducing ROS production and attenuating valve fibrosis and calcium nodule formation in rabbit models, indicating its potential efficacy.[91] Second, a recent study showed that the DDP4 inhibitor evogliptin effectively inhibited the expression of inflammatory cytokines and valve fibrosis and calcification in mice models, suggesting it may be used as a selective drug.[92] Third, Purinergic receptor 2Y2 (P2Y2R) regulates NFκB pathway and its downstream target IL-6. Applying 2thioutp (P2Y2R agonist) to mice effectively improved calcification and fibrosis of aortic valve, proving that P2Y2R is a potentially effective target.[93] Fourthly, Notch1 is an important participant in this disease. XCT790 corrected the changes in the proinflammatory and pro-calcification gene network caused by NOTCH1 haploinsufficiency. This effect was verified in a mouse model, and XCT790 effectively inhibited the progression of valve calcification.[94] Fifth, as a regulator of myofibroblast

differentiation, cadherin-11 is identified as a potential therapeutic target for calcified aortic valve disease. The application of SYN0012 (an anti-cadherin 11 blocking antibody) significantly attenuated the thickening and stiffening of leaflet in mice, which plays a protective role in CAVS.[95] Sixth, MGP activity was negatively correlated with aortic valve calcification, and vitamin K could effectively increase MGP activity. A small clinical study found that exogenous vitamin K1 supplementation slowed the progression of aortic valve calcification, which may be achieved by affecting MGP activity.[96] Seventhly, as described earlier, Lp(a), as a biomarker of CAVS, is undoubtedly an important therapeutic target. AKCEA-APO(a)-Lrx, an antisense oligonucleotide targeting LPA, was shown to effectively reduce Lp(a) levels (>95%) in a dose-dependent manner in patients with established cardiovascular disease.[97] AMG 890 is a siRNA targeting Lp(a), which is currently in clinical trials and is expected to be used in the treatment of CAVS in the future. Finally, a new study demonstrated that PCSK9 is highly expressed in the calcified aortic valve, and PCSK9 variants maintain low cholesterol levels and show a protective effect on valve calcification. Therefore, PCSK9 is considered an effective target for CAVS. The FOURIER trial proved that evolocumab, a PCSK9 inhibitor, downregulated circulating concentrations of LDL-C and Lp(a), which reduced the incidence of CAVS in patients with cardiovascular disease.[23]

CONCLUSION

In conclusion, calcified aortic stenosis is a complex disease involving multiple pathological mechanisms. To date, due to the partial understanding of its molecular mechanism, no drugs have been developed to prevent or treat calcified aortic stenosis. Valve replacement is still the gold standard treatment for severe aortic stenosis, and transcatheter aortic valve replacement has become an alternative to surgical aortic valve replacement. By establishing comprehensive research in the future, we may hopefully discover and develop effective drugs for battling CAVS.

AUTHOR CONTRIBUTIONS

CSY wrote the manuscript; KXQ. prepared the figures; ZJJ provided the idea and revised the manuscript. All authors have agreed to the published version of the manuscript.

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