MODERN APPROACHES TO THE SEARCH FOR DRUG THERAPY FOR AORTIC VALVE CALCIFICATION

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ABSTRACT

Calcific aortic valve stenosis is the most common valvular heart disease. No medical therapies are proven to be effective in holding or reducing disease progression. Therefore, aortic valve replacement remains the only available treatment option. This study discusses the application of multi-omics approaches, proteomics, epigenomics, transcriptomics to the study of valvular heart disease and how these emerging insights might translate into potential novel treatments. Moreover, a machine learning approach that could identify small molecules that correct gene networks seems to shed new light on the pathogenesis of calcification.

Key words: aortic valve calcification, aortic valve stenosis, metabolomics, microRNA, multi-omics approaches, proteomics, transcriptomics.

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LIST OF ABBREVIATIONS:

AS — aortic stenosis,
ACEi — inhibitors of angiotensin-converting enzyme

INTRODUCTION

Over the past few decades, significant changes have occurred in the structure of valvular heart defects, which are characterized by a decrease in the frequency of heart defects of rheumatic etiology and an increase in the prevalence of sclerodegenerative lesions of the valves. The increase in the life expectancy of the population and the improvement of medical technologies has led to the fact that aortic stenosis (AS) is recognized the most common valvular heart disease today. The frequency of detection of AS among persons aged 65 years is about 25%, and after reaching the age of 75 years it increases to 48%, although among persons under the age of 65 it is only 4-5% [1]. To date, there is no drug therapy that can stop the progression of aortic stenosis, so the only radical method is still aortic valve replacement. However, taking into account the life expectancy of the population, accompanied by an annual increase in the number of patients with degenerative aortic stenosis, it becomes obvious that there is a need to actively search for potential targets for therapeutic effects on the processes of calcification of the aortic valve. This review is devoted to the current state of research aimed at finding drug therapy for aortic valve calcification, including the multiomics approach, the achievement of proteomics, genomics and transcriptomics. This approach allows, firstly, to take a more comprehensive look at the problem, and secondly, to identify key points in the process of valve calcification which is necessary for the search and testing of chemical calcification inhibitors.

MACRO- AND MICROSTRUCTURE OF THE AORTIC VALVE

1.1 Tricuspid aortic valve

The aortic valve is part of the aortic root. Most often, the aortic valve consists of three cusps (called depending on their location): the left semilunar (coronary) cusp, the right semilunar (coronary) cusp and the posterior (non-coronary) cusp of the aortic valve [2, 3]. The cusps are thin (1 mm), flexible, avascular structures consisting of three layers: ventricular (*ventricularis*), aortic (*fibrosa*) and spongy (*spongiosa*) [4]. On the aortic and ventricular sides, the valves are covered with a monolayer of endothelial cells. It has been proved that endothelial cells of the aortic valve differ from endothelial cells lining arteries and veins in their ability to respond to changes in hemodynamics, which confirms their unique morphology [4, 5]. The spongy *spongiosa* layer contains a large amount of glycosaminoglycans and proteoglycans. Between all extracellular components in three layers there are interstitial valve cells. VIC are necessary to maintain valve function and homeostasis through proliferation, secretion of matrix metalloproteinases and extracellular matrix components. VIC is a heterogeneous group of cells with unique characteristics. In the aortic valves of adults, VIC are predominantly represented by fibroblasts ("silent cells"), only 2-5% of interstitial cells are in the activated state normally. Their phenotype normally changes with age and when environmental conditions change (for example, sudden changes in blood pressure) [5]. Interstitial cells of the aortic valve are the basis of pathological differentiation either into osteoblast-like cells or into myofibroblasts. The interaction of cells with each other plays a significant role in determining the differentiation of cells. Endothelial and interstitial cells must interact with each other to ensure proper development and homeostasis in the valve. It seems that the disruption of this interaction may contribute to the development of valve pathology. Apparently, in the proper functioning of the community of these cells lies the mechanism of maintaining the integrity of the aortic valve. Imbalance between these cells may, apparently, lead to impaired differentiation and changes in the valve, in particular to calcification [6, 7].

1.2 Bicuspid aortic valve

Bicuspid aortic valve is a widespread variant of valve development, which occurs in 2% of the population with a ratio between men and women of 4:1 [8, 9]. Morphologically, the bicuspid aortic valve consists of 2 cusps, which can be either of the same or unequal size (the difference can reach 1.5-2 times). In people with a bicuspid aortic valve, the manifestation of aortic stenosis usually occurs 20 years earlier than in people with a tricuspid valve, and by the age of 45, more than half of people with a bicuspid valve have a pronounced aortic stenosis.

MOLECULAR AND CELLULAR MECHANISMS OF AORTIC STENOSIS DEVELOPMENT

The main cause of AS development remains calcification of the initially normal tricuspid or congenital bicuspid aortic valve. The early formation of AS was considered as a passive degenerative process as a result of mechanical wear of the cuspids in the sixth and seventh decades of life or as an atherosclerotic process, taking into account the connection with traditional risk

factors such as hypertension, diabetes mellitus, smoking, high cholesterol level [10, 11]. However, attempts to use standard approaches aimed at suppressing the processes of atherogenesis did not lead to a restraint in the rate of progression of AS.

During the formation of aortic stenosis, it is customary to distinguish 3 stages: the first is caused by inflammatory changes in the valve, the second by the development of fibrosis, the third by the formation of calcification of the aortic valve. In turn, in the process of development of calcification of the aortic valve, it is also customary to distinguish several stages: aortic sclerosis (mild calcification stage) — sealing and thickening of the valve cusps with local calcification areas, without commissures being fused and without pronounced obstruction of the left ventricular outflow tract. In the future, aortic sclerosis may pass into the stage of moderate calcification and, finally, into the third stage — severe calcification of the valves, accompanied by obstruction of the left ventricular outflow tract [12, 13].

Currently, it has been proven that calcification of the aortic valve is an active process, the molecular and cellular mechanisms of which are poorly understood [14]. It has been shown that calcification and ossification regulators, such as osteopontin, osteonectin, osteocalcin and bone protein *BMP (bone morphogenetic protein*), are involved in the calcification process. Osteoprotegerin and its ligand (*RANKL — receptor activated of nuclear factor- kB ligand*) are also involved in valve calcification. The RANK — RANKL — OPG (osteoprotegerin) signaling chain is controlled by *RUNX2*, which is controlled by the Notch signaling pathway [14-24]. The spectrum of action of the Notch signaling pathway affects a large number of different genes, including genes responsible for differentiation and proliferation. It is known that the mutation of the *NOTCH1* gene is associated with calcification of the aortic valve. It is important that the results of preclinical studies have shown that specific blocking of the Notch signaling pathway significantly suppresses calcification of the vessel and aortic valve [25-29]. It has been shown that normally *NOTCH1* inhibits the activation of Runx2, thereby blocking calcium deposits on the valve. Mutations in the *NOTCH1* gene lead to the activation (derepression) *of* RUNX2, thus leading to the differentiation of interstitial cells into osteoblasts [30-32].

Aortic stenosis is a complex, multifactorial disease [33]. Among the components that affect the development of aortic stenosis are the following factors: renin-angiotensin-aldo-sterone system, the influence of the sympathetic nervous system [34], lipid accumulation, osteogenesis, myofibrogenesis, biosynthesis and aggregation of extracellular vesicles, platelet activation, osteochondrogenesis [35], cellular aging, disorganization of the intercellular matrix by metalloproteinases, inflammatory elements (macrophages, T-cells lymphocytes, mast cells and molecules characteristic of typical inflammation, such as IL-2, HLA-DR, TNF alpha) [36-40], endothelial dysfunction, impaired phosphate processing [26, 27, 33]. To date, with the development of severe aortic stenosis in a patient, either aortic valve replacement or transcatheter aortic valve implantation remain the only method of treatment. These surgical interventions are associated with a high risk of complications, technical nuances, moreover, in our country their implementation is possible only in large specialized cardiac centers. The high cost of these surgical interventions is certainly a burden on the healthcare system. Taking into account the tendency to aging of the population and the increase in the number of patients with sclerodegenerative aortic stenosis, there is an obvious urgent need for chemical inhibition of calcification processes, which will avoid or delay surgical intervention. The lack of drug therapy is largely due to problems in studying the pathogenesis of this disease.

THERAPEUTIC TARGETS

1.3. Statins

There is a large number of studies dedicated to the effect of statins on the development of aortic stenosis. It has been shown that oxidized lipoproteins, apo A, apo B and apo E lipoproteins, macrophages, T-lymphocytes, foam cells, growth factors and proinflammatory cytokines, which are involved in the initiation of the pathological process, are present in the stenotic valve. At the same time, not only the hypolipidemic properties of statins, but also their pleiotropic effects are well known [41].

However, one of the largest studies, SEAS (Simvastatin and Ezetimib in Aortic Stenosis), did not confirm the ability of statins to restrain the progression of AS. The report on this study, published on July 21, 2008, clearly states that there are no differences in either primary (death, aortic valve replacement, decompensation of chronic heart failure, stroke, myocardial infarction and unstable angina) or secondary (progression of stenosis, assessed by EchoCG criteria) efficacy points between groups taking medications and placebo [42, 43]. Small retrospective studies were conducted evaluating the relationship between the intake of biphosphonates and slowing the progression of aortic stenosis. However, further detailed studies did not reveal a positive effect of drugs on the rate of progression [44, 45].

1.4. RANK/RANKL inhibitors

Interstitial cells of the aortic valve differentiate into osteoblast-like cells by activating the kappa-beta nuclear factor receptor (RANK — receptor activator of

nuclear factor kappa-B). During osteoblastic differentiation, alkaline phosphatase, osteopontin, metalloproteinases, Runx2 and bone protein BMP (*bone morphogenetic protein*) significantly increase. RANKL is a transmembrane glycoprotein, a cytokine of the tumor necrosis factor family, produced by osteoblastic cells and activated T-lymphocytes, which, by binding to the RANK receptor, sends a signal for the differentiation of progenitor cells and the maturation of osteoclasts.

Osteoprotegerin also belongs to the cytokines of the superfamily of tumor necrosis factor and is produced by osteoblasts. As a receptor to RANKL, it blocks its interaction with its own receptor RANK, preventing osteoclastogenesis. RANKL in the aortic valve tissues promotes the transition of myofibroblasts to osteoblasts. Denosumab is a human monoclonal antibody (IgG2) created to bind the RANKL receptor (to prevent joining of RANKL and RANK), thereby mimicking the effect of osteoprotegerin. Denosumab is used in the treatment of osteoporosis, multiple myeloma and other conditions accompanied by bone resorption. To date, a study has not been completed to assess the effectiveness of taking denosumab in order to inhibit the progression of AS (SALTIRE II). However, the results of a study conducted on pig interstitial cells have been published, showing that denosumab is able to partially block calcification [46].

1.5 Angiotensin converting enzyme inhibitors

There are several works that have studied the effect of angiotensin converting enzyme inhibitors (ACE inhibitors) on the stenosis process, as it has been proven that ACE, angiotensin II and angiotensin II type I receptors are present in calcified valves. Angiotensin II potentiates inflammation, accumulation of lipoproteins, oxidative stress and stimulates the expression of lipoprotein-binding proteoglycans and biglycans by fibroblasts. Angiotensin II receptors are present on stenotic valve fibroblasts. Angiotensin II type I receptors, constantly expressed smooth muscle cells, appear on the interstitial cells of the valve only when stenosis begins. Thus, until the moment when the cells begin to express type I angiotensin II receptors, the valve is protected from the effects of angiotensin II. Based on the fact that the pro-inflammatory and profibrogenic features of angiotensin II are known, attempts have been made to block the process of stenosis by taking ACE inhibitors. It has been proved that ACE inhibitors have an antiproliferative effect (reduce hypertrophy of vascular and myocardial walls and proliferation of extracellular matrix), improve endothelial function (enhance the production of NO), inhibit the progression of atherosclerosis (since they block the formation of angiotensin and lead to an increase in the level of bradykinin and NO, which, in turn, leads to suppression of migration and proliferation of vascular smooth muscle cells, taxis and activation of inflammatory cells, decrease of oxidative stress and improvement of endothelial function). Retrospective analysis showed that ACE inhibitors somewhat slow down calcium deposits on the valves, but do not prevent the progression of stenosis. It is quite difficult to prove the effect of ACE inhibitors on the stenosis process, since ACE inhibitors alter intracardiac hemodynamics, which probably masks all other effects of drugs [47].

1.6. Vitamin K

To date, the effect of vitamin K on the process of calcification of the aortic valve is also being studied. Vitamin K is a fat-soluble vitamin that exists in 2 forms: vitamin K1 (phylloquinone) and vitamin K2 (menaquinone). Vitamin K2 is involved in the processes of inhibition of arterial calcification through the maintenance of matrix Gla protein carboxylation processes. The carboxylation process is necessary to maintain optimal absorption of calcium by the cell. It is known that a sufficient amount of vitamin K is required for optimal performance and posttranslational carboxylation of matrix Gla protein. Thus, it has been suggested that sufficient intake of vitamin K may prevent the progression of aortic stenosis. In the only small randomized study (a group of 38 people), it was shown that taking vitamin K can slow the progression of AS. However, given the short follow-up period (1 year), such a small group of patients obviously requires further, more in-depth study of this fact [48].

Taking into account the complexity of the components of the pathogenesis of aortic stenosis, ACE inhibitors, statins, vitamin K, bisphosphonates, etc. were studied to inhibit calcification processes. However, either most drugs did not prove their effectiveness in restraining the rate of development of aortic stenosis, or further study of the drug with a larger sample of patients or prospective follow-up is required.

PROSPECTS FOR STUDYING THE CALCIFICATION PROCESS AND SEARCHING FOR A NEW THERAPY TARGET

To date, the target is being actively pursued, the impact on which will inhibit or slow down calcification. There is an assumption that calcification has different mechanisms in women and in men. In men, interstitial cells go into osteodifferentiation, and in women into myofibroblastic differentiation [49]. Perhaps this will explain the effectiveness of various drugs for containing the calcification process.

Various laboratories have carried out many studies and experiments on cultures of interstitial and endothelial cells of the valve of humans, pigs, sheep, rats and mice. Of course, for accurate verification, it is preferable to work with human cells, but this is far from always being possible due to technical difficulties in taking material from operating rooms and cultivating these cells. The cultivation of cells in three-dimensional space with the reproduction of the biomechanics of valve behavior (simulating blood flow on the valve) is a promising focus area today.

Previously, it has been repeatedly shown that a significant change in the expression level of a number of genes of the Notch signaling pathway and other signaling pathways in cells from patients with bicuspid and tricuspid valves plays an important role in the development of aortic stenosis [22]. However, it has now become clear that analyzing the expression level of several genes is only a superficial and one-sided view of the problem.

Today, the "omics" approach to the study of the pathogenesis of aortic valve calcification is relevant. It is customary to refer to technologies that use methods of genomics, transcriptomics, proteomics, metabolomics, that is, sciences that study how the genome is made, how the information encoded in it is realized and how it is converted into the structure of proteins and later into some signs of the organism. Unlike genes and proteins, which are predisposed to epigenetic and posttranslational changes, metabolites give a true idea of cellular activity [50]. The metabolomic analysis of the affected valve provides "molecular" information about metabolites and metabolic pathways that are active at different stages of the calcification process.

However, it must be understood that the detection of only metabolites circulating in the blood can serve as a marker for diagnosing and detecting the severity of stenosis. One of the candidates for the role of a disease marker is lysophosphatidic acid. It has been shown that the level of lysophosphatidic acid is significantly

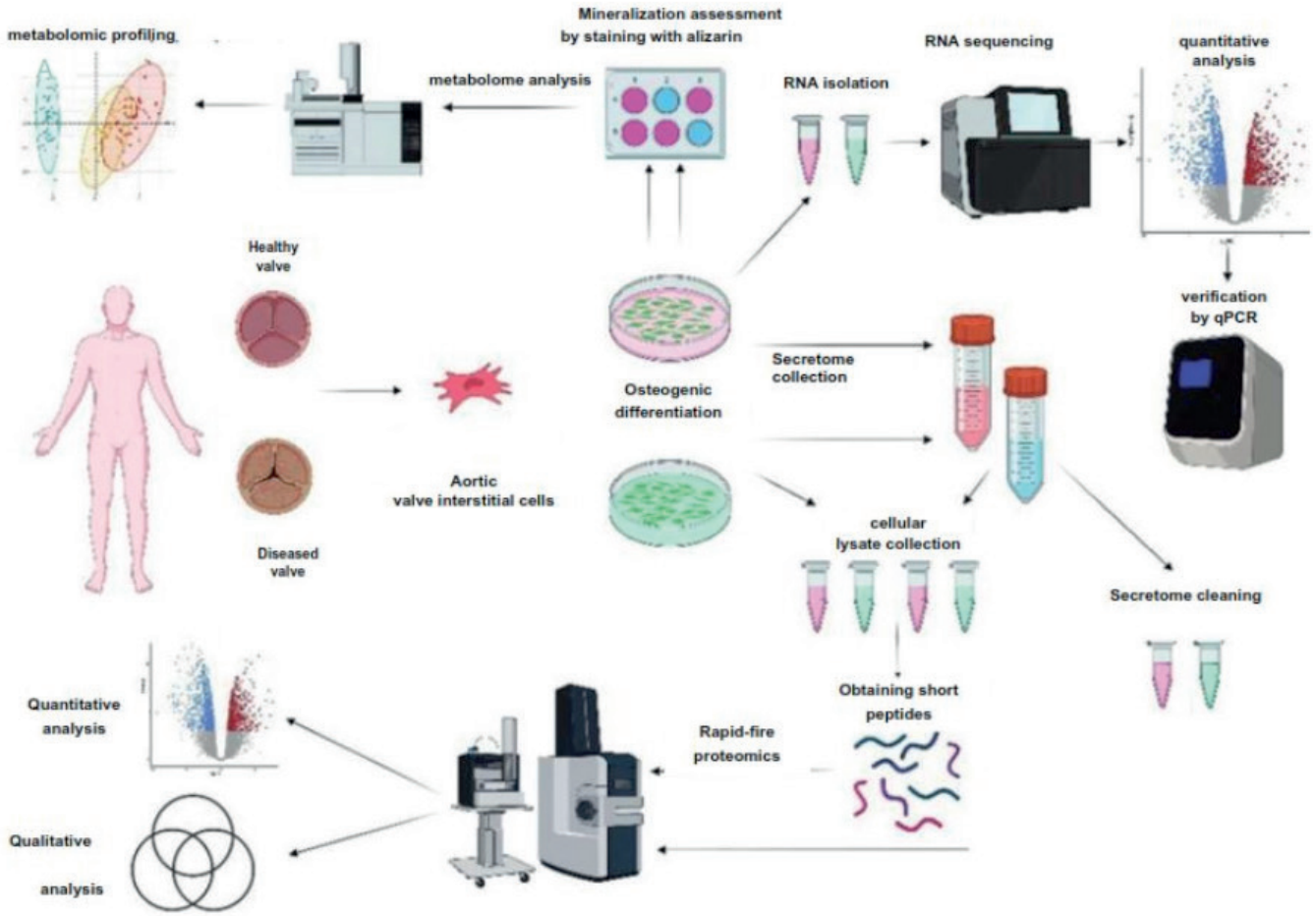

Fig. 1. The multiomics approach to the study of the pathogenesis of calcification: obtaining cell cultures from the human aortic valve, osteogenic differentiation, analysis of transcriptome/ proteome/ metabolome/ secretome

increased in blood serum in patients with rapidly progressing AS, in contrast to patients with slowly progressing disease. Nevertheless, the integration of multiomics data in the aortic valve should be approached with caution due to the low density of valve cells [51].

Studying the role of microRNAs in the process of valve calcification looks promising [52]. MicroRNAs are regulators of many genes at the translational level. Each microRNA regulates many transcripts. There are studies that have shown that microRNA inhibitors can suppress calcification of the aortic valve. A group of scientists led by T.Tashima has demonstrated that microRNA34a potentiates calcium deposition in the valve cusp by acting on the Notch-Runx2 signaling pathway, and that inhibition of this microRNA significantly suppressed calcification (myofibrogenesis) in interstitial valve cells in comparison with the control [53-54].

Today, the approaches of "network medicine" are in great demand, when a large amount of transcriptomic, proteomic and other data is integrated and used for prioritization, that is, highlighting key interactions that are important in the development of a particular pathology [50]. A group of scientists, including specialists from the Almazov National Medical Research Centre completed a work aimed at finding potential targets for inhibiting calcification processes during the development of aortic stenosis. A machine learning approach was used to find molecules capable of normalizing the genotype of a cell with a mutation in the *NOTCH1* gene. The study was performed on the model of induced pluripotent cells, which deprives the study of the effect of subjectivity when working with cell lines isolated from the affected valve. About 1500 potential molecules were analyzed, and only one substance, XCT 790, completely led to the complex normalization of the genetic network of endothelial cells. XCT 790 is a reverse agonist of the orphan nuclear receptor ERRa involved in WNT signaling. Moreover, administration of XCT 790 to mice significantly reduced the percentage of cells expressing RUNX2, which means a weakening of osteogenesis processes in the valve [55].

Currently, the research laboratory of diseases with excessive calcification of the world-class Scientific Centre "Centre for Personalized Medicine" of the Almazov National Medical Research Centre is conducting an active research aimed at finding therapeutic agents that can prevent pathological calcification — in particular, using multiomics approaches — to analyze the pathological osteogenic differentiation/calcification of interstitial cells of the aortic valve (Fig. 1).

Today, the search for targeted therapy is about creating a detailed molecular portrait of the affected and healthy valve. And only an understanding of the key and secondary fundamental cellular and molecular mechanisms involved in the development of calcification can shed light on understanding the problem.

Conflict of interest

The authors declare no conflict of interest.

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